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Final report

Analysis of Polyquaternium Compounds in Environmental Samples through Non-Target Screening

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On behalf of the German Environment Agency

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Abstract: Analysis of Polyquaternium Compounds in Environmental Samples through Non-Target Screening

Polyquaternium (PQ) compounds are a highly variable sub-class of water-soluble polymers (WSPs) that share a quaternary ammonium substructure as their common structural element. Due to their high production volumes, wide-spread use and assumed persistence in the environment information on their occurrence and environmental concentrations is essential to assess the risk these chemicals may pose and determine if regulatory action is required. To this end, it was assessed if PQs can be retrospectively analyzed in non-target screening data, which was deemed unfeasible due to the lack of characteristic high intensity signals in mass spectrometry. Consequently, an alternative analytical method for PQ-compounds based on size-exclusion chromatography and detection through diagnostic fragments formed by in-source fragmentation was developed. This approach enabled for the detection of PQs in surface water, but did not allow for reliable quantification due to the lack of high-quality analytical standards and complex interactions with inorganic anions and negatively charged components of natural organic matter influencing signal intensities. A monitoring in 15 surface water samples taken in September and October 2024 in Saxony and Saxony-Anhalt revealed the widespread presence of PQ-2 (13/15 samples) and led to the detection of PQ-7 (3/15) and PQ-10 (4/15) after freeze drying. Concentrations for PQ-2 were estimated in the 10^1-10^3 µg/L range. Especially the upper end of this concentration range seems remarkably high and requires confirmation. Based on these results it can be concluded that PQs do occur in the aquatic environment at seemingly high concentrations. Consequently, further analytical improvements are required to widen the scope of PQ-compounds analysed, reduce limits of detection for PQs other than PQ-2, and enable a more reliable quantification. A larger scale monitoring with such an improved method may than be used to determine if regulatory action is required.

Kurzbeschreibung: Analyse von Polyquaternium-Verbindungen in Umweltproben durch Non-Target-Screening

Polyquaternium (PQ)-Verbindungen sind eine sehr variable Unterklasse wasserlöslicher Polymere (WSP), deren gemeinsames Strukturelement eine quaternäre Ammonium-Substruktur ist. Aufgrund ihres hohen Produktionsvolumens, ihrer weit verbreiteten Verwendung und ihrer angenommenen Persistenz in der Umwelt sind Informationen über ihr Vorkommen und ihre Konzentrationen in der Umwelt unerlässlich, um das Risiko, das diese Chemikalien darstellen können, zu bewerten und festzustellen, ob Regulierungsmaßnahmen erforderlich sind. Zu diesem Zweck wurde geprüft, ob PQs rückwirkend in Non-Target-Screening-Daten analysiert werden können, was jedoch aufgrund der fehlenden charakteristischen Signale hoher Intensität in der Massenspektrometrie als nicht durchführbar erachtet wurde. Daher wurde eine alternative Analysemethode für PQ-Verbindungen entwickelt, die auf der Größenausschlusschromatographie und dem Nachweis durch diagnostische Fragmente basiert, die durch Fragmentierung in der Quelle gebildet werden. Dieser Ansatz ermöglichte den Nachweis von PQs in Oberflächengewässern, erlaubte jedoch keine zuverlässige Quantifizierung, da es an hochwertigen Analysestandards fehlte und komplexe Wechselwirkungen mit anorganischen Anionen und negativ geladenen Komponenten natürlicher organischer Stoffe die Signalintensität beeinflussten. Ein Monitoring von 15 Oberflächenwasserproben, die im September und Oktober 2024 in Sachsen und Sachsen-Anhalt entnommen wurden, ergab das weit verbreitete Vorhandensein von PQ-2 (13/15 Proben) und führte nach Gefriertrocknung zum Nachweis von PQ-7 (3/15) und PQ-10 (4/15). Die Konzentrationen für PQ-2 wurden im Bereich von 10^1-10^3 µg/L geschätzt. Insbesondere das obere Ende dieses Konzentrationsbereichs erscheint bemerkenswert hoch und bedarf der Bestätigung. Aus diesen Ergebnissen lässt sich schließen, dass PQs in der aquatischen Umwelt in scheinbar hohen Konzentrationen vorkommen. Folglich sind weitere analytische Verbesserungen erforderlich, um den Umfang der analysierten PQ-Verbindungen zu erweitern, die Nachweisgrenzen für andere PQs als PQ-2 zu senken und eine zuverlässigere Quantifizierung zu ermöglichen. Eine Überwachung in größerem Maßstab mit einer solchen verbesserten Methode kann dann genutzt werden, um festzustellen, ob Regulierungsmaßnahmen erforderlich sind.

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List of abbreviations

Abbreviation	Explanation
ESI	Electrospray Ionisation
HLB	Hydrophilic-Lipophilic-balanced
HRMS	High-Resolution Mass Spectrometry
MW	Molecular Weight
NOM	Natural organic matter
PEO	Polyethylene oxide
PQ	Polyquaternium
REACH	Registration, Evaluation, Authorization of Chemicals
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
SEC	Size Exclusion Chromatography
SPE	Solid Phase Extraction
WSP	Water-soluble Polymer

Summary

Polyquaternium (PQ) compounds are a highly variable sub-class of water-soluble polymers (WSPs) that share a quaternary ammonium substructure as their common structural element. Due to their high production volumes, wide-spread use and assumed persistence in the environment information on their occurrence and environmental concentrations is essential to assess the risk these chemicals may pose and determine if regulatory action is required. The aim of this study was to evaluate if PQ-compounds can be retrospectively analyzed in non-target screening data and to utilize this data to generate first insights into environmental exposure levels. Commonly, non-target screening workflows consist of chromatographic separation with reversed phase high performance liquid chromatography (RP-HPLC) followed by detection with high-resolution mass spectrometry (HRMS) in full-scan mode. Analysis of PQ-standards with HRMS after direct infusion demonstrated that this approach cannot be applied for PQ-compounds since the total intensity of the polymer in electrospray-ionization (ESI) mass spectrometry is spread out over countless signals originating from the polymer's polydispersity, charge states, and adduct formation which are nearly indistinguishable from background noise even in high concentrations and pure standards. Based on these findings, retrospective analysis of PQ-compounds in nontarget screening data was deemed infeasible.

An alternative analytical approach for WSPs first pioneered by Huppertsberg et al. (Huppertsberg et al., 2020) and later applied for the environmental monitoring of polyethylene oxide by Pauelsen et al. (Pauelsen et al., 2023) was adopted to the investigated PQ-compounds. In this approach WSPs are separated from low molecular mass matrix components by size-exclusion chromatography (SEC). In-source fragmentation of WSPs is provoked to facilitate the formation of low molecular mass diagnostic fragments, thus converting the myriad of low intensity polymer ions into a few higher intensity fragments. Adopting this analytical approach to the four selected PQ-compounds (PQ-2, 6, 7, and 10) resulted in the identification of one to four diagnostic fragments for each PQ. Separation of PQs from low molecular weight matrix components with SEC was feasible, but determination of the molecular weight could not be realized due to a lack of PQ-standards with a well-defined molecular weight. Validation of the developed method focused on selectivity to assure that even the structurally similar PQs (6 and 7) can be detected in the same sample. Analyzing individual PQ-standards, none of the diagnostic fragments showed signals of significant intensity for any other PQ-standard than the one it was selected for.

Wastewater treatment plants (WWTPs) were the assumed main environmental exposure pathway for PQs, and thus the developed method was tested in this matrix by analyzing native and spiked wastewater treatment plant effluent samples. However, even when spiking WWTP effluent with high concentrations no signals for any of the PQs were observed. A full scan in the elution range of PQs did not show signals of significant intensity what might indicate ion suppression in the electrospray ionization process, and thus it was assumed that other matrix components may interact with PQs in solution. Inorganic anions and anionic components of natural organic matter were hypothesized to be most like to interact with the polycationic PQs. The interaction of these anionic compounds with PQs was investigated by spiking the PQ-mix standard into solutions of ammonium sulphate (as representative for inorganic ions) and Suwanee River natural organic matter, its fractions Suwanee River fulvic acids and Suwanee River humic acids, as well as pore water form a swamp. While all these anionic compounds resulted in a reduction of intensities for the PQ compounds it was most pronounced for the humic acids and the swamp pore water, which led to a complete suppression of the PQ-signals, indicating that anionic matrix components can massively influence PQ analysis and quantification.

Based on these findings PQ analysis in WWTP effluent was deemed infeasible with the current iteration of the method and surface water, in which humic acids only comprise a small fraction of the natural organic matter was identified as potential matrix of interest. A first experiment with sample from the Elsterbecken in Leipzig revealed that while quantification is still hampered PQs can be detected in surface water. Thus, a monitoring campaign took place between September and October 2024 in Saxony and Saxony-Anhalt, sampling a 15 surface waters that varied in size from 0.09 m^3/s (Parthe) to 1360 m^3/s (Elbe) and were influenced by a variety of potential contamination sources. Direct analysis of these samples resulted in a positive detection of PQ-2 in 13 surface water samples with the highest intensities being observed in rivers in which urban wastewater is discharged and the Pleiße, which is influenced by mining and rehabilitation of mining areas as potential contamination sources. After freeze drying PQ-7 and PQ-10 were detected in 3 and 4 samples with a strong urban influence, respectively. In light of the unavailability of high-quality analytical or isotope-labelled standards and the widespread use of PQs, reduction of false positives was the main goal of quality assurance measures during the sampling campaign. All sampling bottles were carefully rinsed before use and the final wash step of the rinsing procedure was collected and analysed, revealing no signals that coincide with elution times of PQs in the analysed samples. Field blanks were taken regularly during the sampling campaign and were negative as well. Due to the indirect nature of the detection (only fragments instead of the intact molecule) ratios of all diagnostic fragments for PQs in the samples were compared to those in the standard and shows good agreement. Consequently, the likelihood of false positives was deemed low.

While exact quantification is not feasible with the current method due to the lack of well-described standards and complex and not yet fully understood interactions with various anionic matrix components concentrations for PQ-2 were estimated in the 10^1-10^3 µg/L range. Especially the upper end of this concentration range seems remarkably high and requires confirmation. While toxicity data for PQ-2 is scarce studies investigating PQ-6 have shown effects on aquatic organisms in high µg/L to low mg/L range.

Based on these results it can be concluded that PQs do occur in the aquatic environment at seemingly high concentrations. Consequently, further analytical improvements are required to widen the scope of PQ-compounds analysed, reduce limits of detection for PQs other than PQ-2, and enable a more reliable quantification. A larger scale monitoring with such an improved method may than be used to determine if regulatory action is required.

Zusammenfassung

Polyquaternium (PQ)-Verbindungen sind eine sehr variable Unterklasse wasserlöslicher Polymere (WSP), deren gemeinsames Strukturelement eine quaternäre Ammonium-Substruktur ist. Aufgrund ihres hohen Produktionsvolumens, ihrer weit verbreiteten Verwendung und ihrer angenommenen Persistenz in der Umwelt sind Informationen über ihr Vorkommen und ihre Konzentrationen in der Umwelt unerlässlich, um das Risiko, das diese Chemikalien darstellen können, zu bewerten und zu bestimmen, ob regulatorische Maßnahmen erforderlich sind. Ziel dieser Studie war es, zu untersuchen, ob PQ-Verbindungen in Non-Target Screening Daten retrospektiv analysiert werden können und diese Daten zu nutzen, um erste Erkenntnisse über die Umweltexposition zu gewinnen. Üblicherweise bestehen Non-Target Screening Workflows aus einer chromatographischen Trennung mit Umkehrphasen-Hochleistungsflüssigkeits-chromatographie (RP-HPLC) und anschließender Detektion mit hochauflösender Massenspektrometrie (HRMS) im Full-Scan-Modus. Die Analyse von PQ-Standards mit HRMS nach direkter Infusion zeigte, dass dieser Ansatz für PQ-Verbindungen nicht anwendbar ist, da sich die Gesamtintensität des Polymers in der Elektrospray-Ionisations (ESI) Massenspektrometrie auf unzählige Signale verteilt, die von der Polydispersität, den Ladungszuständen und der Adduktbildung des Polymers herrühren und selbst bei hohen Konzentrationen und reinen Standards kaum vom Hintergrundrauschen zu unterscheiden sind. Auf der Grundlage dieser Erkenntnisse wurde die retrospektive Analyse von PQ-Verbindungen in Non-Target Screening Daten als nicht durchführbar erachtet.

Ein alternativer analytischer Ansatz für WSPs, der zuerst von Huppertsberg et al. (Huppertsberg et al., 2020) entwickelt und später von Pauelsen et al. (Pauelsen et al., 2023) für die Umweltüberwachung von Polyethylenoxid angewendet wurde, wurde auf die untersuchten PQ-Verbindungen übertragen. Bei diesem Ansatz werden die WSPs durch Größenausschlusschromatographie (SEC) von den niedermolekularen Matrixkomponenten getrennt. Durch die Fragmentierung der WSP in der Ionenquelle wird die Bildung von diagnostischen Fragmenten mit niedriger Molekülmasse gefördert, wodurch die unzähligen Polymer-Ionen mit niedriger Intensität in einige wenige Fragmente mit höherer Intensität umgewandelt werden. Die Anwendung dieses analytischen Ansatzes auf die vier ausgewählten PQ-Verbindungen (PQ-2, 6, 7 und 10) führte zur Identifizierung von bis zu vier diagnostischen Fragmenten für jedes PQ. Die Abtrennung der PQs von den niedermolekularen Matrixkomponenten mit SEC war möglich, aber die Bestimmung des Molekulargewichts konnte nicht durchgeführt werden, da keine PQ-Standards mit einem genau definierten Molekulargewicht verfügbar waren. Die Validierung der entwickelten Methode konzentrierte sich auf die Selektivität, um sicherzustellen, dass sogar die strukturell ähnlichen PQs (6 und 7) in derselben Probe nachgewiesen werden konnten. Bei der Analyse der einzelnen PQ-Standards zeigte keines der diagnostischen Fragmente Signale von signifikanter Intensität für einen anderen PQ-Standard als dem, für den es ausgewählt wurde.

Da man davon ausgeht, dass Kläranlagen der Hauptexpositionspfad für PQ-Verbindungen in der Umwelt sind, wurde die entwickelte Methode in dieser Matrix getestet, indem native und aufgestockte Kläranlagenablaufproben analysiert wurden. Doch selbst bei der Dotierung des Kläranlagenabwassers mit hohen Konzentrationen wurden keine Signale für eine der PQ-Verbindungen beobachtet. Ein Scan im Elutionsbereich der PQs zeigte keine Signale von signifikanter Intensität die auf eine Ionensuppression im Elektrospray-Ionisationsprozess hindeuten könnten, und daher wurde angenommen, dass andere Matrixkomponenten mit den PQ-Verbindungen in Lösung interagieren. Es wurde angenommen, dass anorganische Anionen und anionische Komponenten natürlicher organischer Stoffe am ehesten mit den polykationischen PQs wechselwirken. Die Wechselwirkung dieser anionischen Verbindungen

mit den PQs wurde untersucht, indem der PQ-Mischstandard in Lösungen von Ammoniumsulfat (stellvertretend für anorganische Ionen) und natürlichem organischem Material aus dem Suwanee River, seinen Fraktionen Fulvosäuren und Huminsäuren aus dem Suwanee River sowie Porenwasser aus einem Sumpf dotiert wurde. Während alle diese anionischen Verbindungen zu einer Verringerung der Intensitäten für die PQ-Verbindungen führten, war dies bei den Huminsäuren und dem Porenwasser aus dem Sumpf am stärksten ausgeprägt und führte zu einer vollständigen Unterdrückung der PQ-Signale. Dies weist darauf hin, dass anionische Matrixkomponenten die PQ-Analyse und -Quantifizierung massiv beeinflussen können.

Aufgrund dieser Erkenntnisse wurde die PQ-Analyse in Kläranlagenabwässern mit der derzeitigen Iteration der Methode als undurchführbar erachtet und Oberflächenwasser, in dem Huminsäuren nur einen kleinen Teil der natürlichen organischen Substanz ausmachen, als potenzielle Matrix von Interesse identifiziert. Ein erster Versuch mit einer Probe aus dem Elsterbecken in Leipzig zeigte, dass PQs im Oberflächenwasser nachgewiesen werden können, auch wenn die Quantifizierung noch erschwert ist. Daher fand zwischen September und Oktober 2024 eine Überwachungskampagne in Sachsen und Sachsen-Anhalt statt, bei der 15 Oberflächengewässer beprobt wurden, die in ihrer Größe zwischen 0,09 m^3/s (Parthe) und 1360 m^3/s (Elbe) variierten und von einer Vielzahl potenzieller Kontaminationsquellen beeinflusst wurden. Die direkte Analyse dieser Proben ergab einen positiven Nachweis von PQ-2 in 13 Oberflächenwasserproben, wobei die höchsten Intensitäten in Flüssen beobachtet wurden, in die kommunale Abwässer eingeleitet werden, sowie in der Pleiße, die durch den Bergbau und die Sanierung von Bergbaugebieten als potenzielle Kontaminationsquellen beeinflusst wird. Nach Gefriertrocknung wurden PQ-7 und PQ-10 in 3 bzw. 4 Proben mit starkem städtischem Einfluss nachgewiesen. Angesichts der mangelnden Verfügbarkeit hochwertiger analytischer oder isotopenmarkierter Standards und der weit verbreiteten Verwendung von PQs war die Reduzierung falsch positiver Ergebnisse das Hauptziel der Qualitätssicherungsmaßnahmen während der Probenahmekampagne. Alle Probenahmeflaschen wurden vor der Verwendung sorgfältig gespült, und der letzte Spülschritt wurde gesammelt und analysiert, wobei keine Signale festgestellt wurden, die mit den Elutionszeiten der PQs in den analysierten Proben übereinstimmten. Während der Probenahmekampagne wurden regelmäßig Feldblanks genommen, die ebenfalls negativ waren. Aufgrund des indirekten Charakters des Nachweises (nur Fragmente statt des intakten Moleküls) wurden die Verhältnisse aller diagnostischen Fragmente für PQs in den Proben mit denen im Standard verglichen und zeigten eine gute Übereinstimmung. Folglich wurde die Wahrscheinlichkeit von falsch-positiven Ergebnissen als gering eingestuft.

Eine exakte Quantifizierung ist mit der derzeitigen Methode zwar nicht möglich, da es keine gut beschriebenen Standards gibt und die Wechselwirkungen mit verschiedenen anionischen Matrixkomponenten komplex und noch nicht vollständig verstanden sind, doch wurden die Konzentrationen für PQ-2 im Bereich von 10^1-10^3 µg/L geschätzt. Insbesondere das obere Ende dieses Konzentrationsbereichs erscheint bemerkenswert hoch und bedarf einer Bestätigung. Während für PQ-2 nur wenige Toxizitätsdaten vorliegen, haben Studien, die PQ-6 untersuchten, Auswirkungen auf Wasserorganismen im Bereich von hohen µg/L bis niedrigen mg/L gezeigt.

Aus diesen Ergebnissen lässt sich schließen, dass PQ-Verbindungen in der aquatischen Umwelt in scheinbar hohen Konzentrationen vorkommen. Folglich sind weitere analytische Verbesserungen erforderlich, um den Umfang der analysierten PQ-Verbindungen zu erweitern, die Nachweisgrenzen für andere PQs als PQ-2 zu senken und eine zuverlässigere Quantifizierung zu ermöglichen. Eine Überwachung in größerem Maßstab mit einer solchen verbesserten Methode kann dann genutzt werden, um festzustellen, ob Regulierungsmaßnahmen erforderlich sind.

1 Introduction

The field of water-soluble, gel-forming and liquid polymers (hereinafter collectively referred to as water-soluble polymers (WSPs)) comprises neutral, anionic and cationic polymers with annual production volumes in the range of hundreds to hundreds of thousands of tons (Huppertsberg et al., 2020). Despite these large quantities and a multitude of applications that favour their entry into the environment, there is hardly any information on the environmental occurrence of water-soluble polymers. This is partly due to analytical challenges that have not yet been solved (Huppertsberg et al., 2020). In order to support the envisaged better integration of polymers in REACH (Registration, Evaluation, Authorization of Chemicals) and to obtain a more detailed understanding of the occurrence, behaviour and effects of water-soluble polymers in the environment, which is prerequisite for effective and targeted regulation, methodological improvements and a resulting improved data situation are essential.

Polyquaternium compounds (PQ compounds) are a diverse group of water-soluble polymers that have a quaternary nitrogen in the repeating unit as a common structural element. For some PQ compounds there are at least indications of ecotoxicity (Gomes et al., 2014; Hansen et al., 2023), which is why this group of water-soluble polymers deserves special attention. Due to their sometimes-high production volumes and widespread use in personal care products (e.g. shampoos), in the paper industry and in wastewater treatment, PQ compounds have a high potential for environmental emissions and contamination. Environmental discharge via the aqueous phase is currently expected to be rather low, as PQ compounds are polycations and thus may have a high sorption tendency to the negatively charged components of sewage sludge or natural organic matter (NOM) (Schramm et al., 2022). For neutral polyethylene oxide (PEO), it has been shown that the chain length can certainly have an influence on the release via the wastewater treatment plant effluent. PEO was predominantly detected in the wastewater treatment plant effluent with a molecular weight of 1 - 4 kDa, which was in clear contrast to the much broader molecular weight distribution in the influent (Pauelsen et al., 2023). According to the current state of knowledge, it cannot be ruled out that the release of PQ compounds via the wastewater treatment plant effluent may also be dependent on molecular weight, which is why not only a concentration estimate but also a consideration of the molecular weight distribution is relevant. This report is intended to show whether further work on PQ compounds is necessary in the analytical and regulatory context and, if so, to provide information on relevant environmental exposure situation in different compartments and analytical methodology. In addition, a more specific objective can be agreed with the client.

2 Analyte Selection

In the context of this project 5 of the over 50 PQ-compounds on the market were selected for chemical analysis based on the following criteria:

- **Relevance/production volumes:** as with most polymers, production volumes of PQ-compounds are not readily assessible and thus the estimation for the most used PQ-compounds based on Schramm et al. (2022) was used
- **Application/use categories:** with the focus of the project on urban effluent as expected main emission pathway of PQ-compounds only analytes with applications that facilitate a release through the urban effluent were prioritized
- **Charge density:** sorption to sludge, sediment, and particulate matter is expected to play a major role in the distribution of PQ-compounds between environmental compartments. The molecular weight and the charge density are the two major chemical properties determining the sorption of PQ-compounds
- **Chemical structure:** to extrapolate if the method used herein may be transferred to a broader range of PQ-chemicals, chemically diverse analytes are required
- **Analytical standard availability**

Based on the first four criteria seven PQ-compounds were prioritized (Table 1) and the availability of standards was checked. Generally, only few PQ-standards are commercially available and only for PQ-6 molecular weight (MW) information is provided. Consequently, PQ-2, PQ-6 (<100 kDa and 200-350 kDa), PQ-7, and PQ-10 were selected.

Table 1: Prioritized PQ compounds and standard availability

Name	Justification for selection	Standard availability	Comments
PQ-2	Toxic for aquatic organisms; aliphatic	yes	No MW information for standard
PQ-6	One of the three most used PQ-compounds; high charge density; aromatic	yes	Available in 3 MW ranges: < 100 kDa, 200-350 kDa, 400-500 kDa
PQ-7	Structurally similar to PQ-6 - to investigate selectivity of method	Yes	No MW information for standard
PQ-10	Low charge density, structurally complex	Yes	No MW information for standard
PQ-33	One of the three most used PQ-compounds	No	
PQ-39	Structurally complex	No	
PQ-52	One of the three most used PQ-compounds	No	

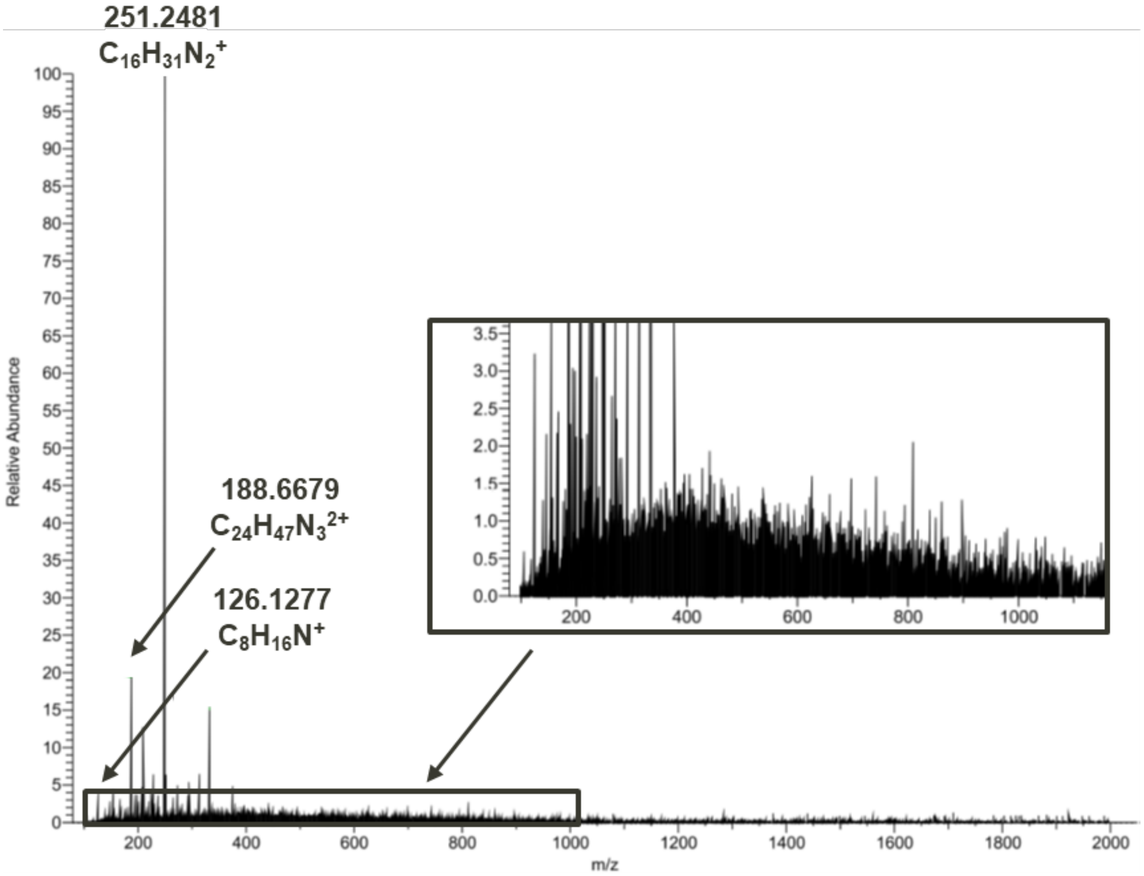
3 Feasibility of retrospective non-target screening

To ensure a retrospective non-target screening for the selected PQ-compounds is feasible, the applicability of a generic non-target screening workflow was investigated with the selected analytes. This workflow included sample enrichment with solid phase extraction (SPE) cartridge, chromatographic separation with reversed phase high-performance liquid chromatography (RP-HPLC) and detection with high-resolution mass spectrometry (HRMS). The amenability of the analytes to all steps of this workflow is a prerequisite for a successful retrospective non-target screening.

3.1 Amenability to detection with HRMS

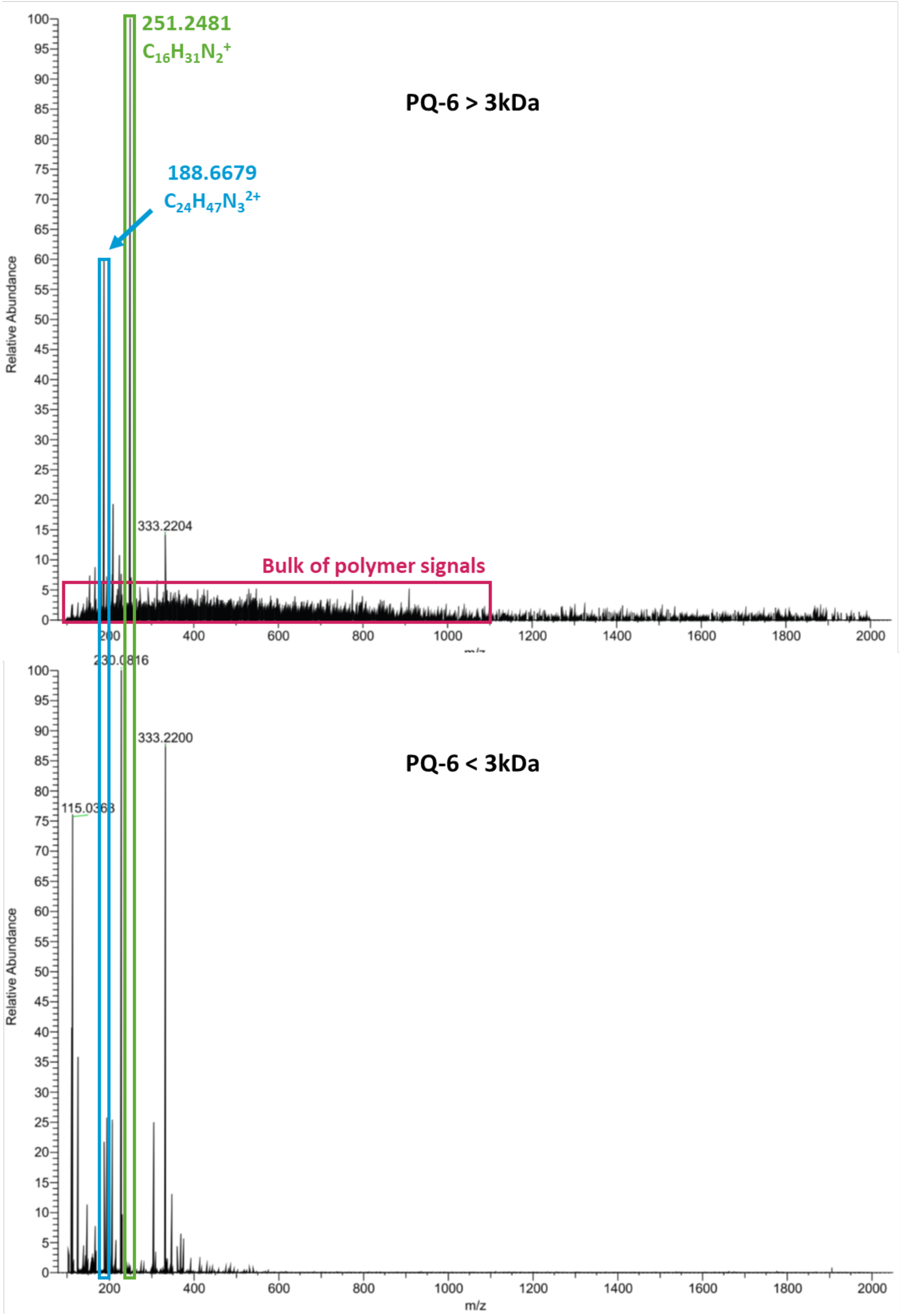
All analytes were dissolved in water, individually diluted to a concentration of 10 mg/L in methanol/water (1:1), and analysed with direct infusion (5 mL/min) in positive electrospray ionization (ESI) mode (Orbitrap mass spectrometer; nominal resolution of 1,000,000 at m/z 200). A solvent blank was analysed in parallel. For PQ-6 several signals of interest were observed: a low intensity signal at m/z 126.1277 which corresponds to a PQ-6 monomer, a signal at m/z 188.6679 which corresponds to a doubly charged trimer, a signal at m/z 251.2481which corresponds to a singly charged dimer, and a bulk of signals between m/z 100 and 1000 which is assumed to be caused by the polymer (Figure 1). To investigate if the signals were caused by the polymer or low molecular weight impurities the PQ-6 standard was passed through a molecular weight filter with a cut-off of 3 kDa and the resulting mass fractions were analysed with direct infusion under the same conditions (Figure 2).

Figure 1: HRMS-spectrum of PQ-6



Source: own illustration, Helmholtz-Centre for Environmental Research

Figure 2: **HRMS-spectra of low and high mass fraction of PQ-6 after separation with a molecular weight filter**



Source: own illustration, Helmholtz-Centre for Environmental Research

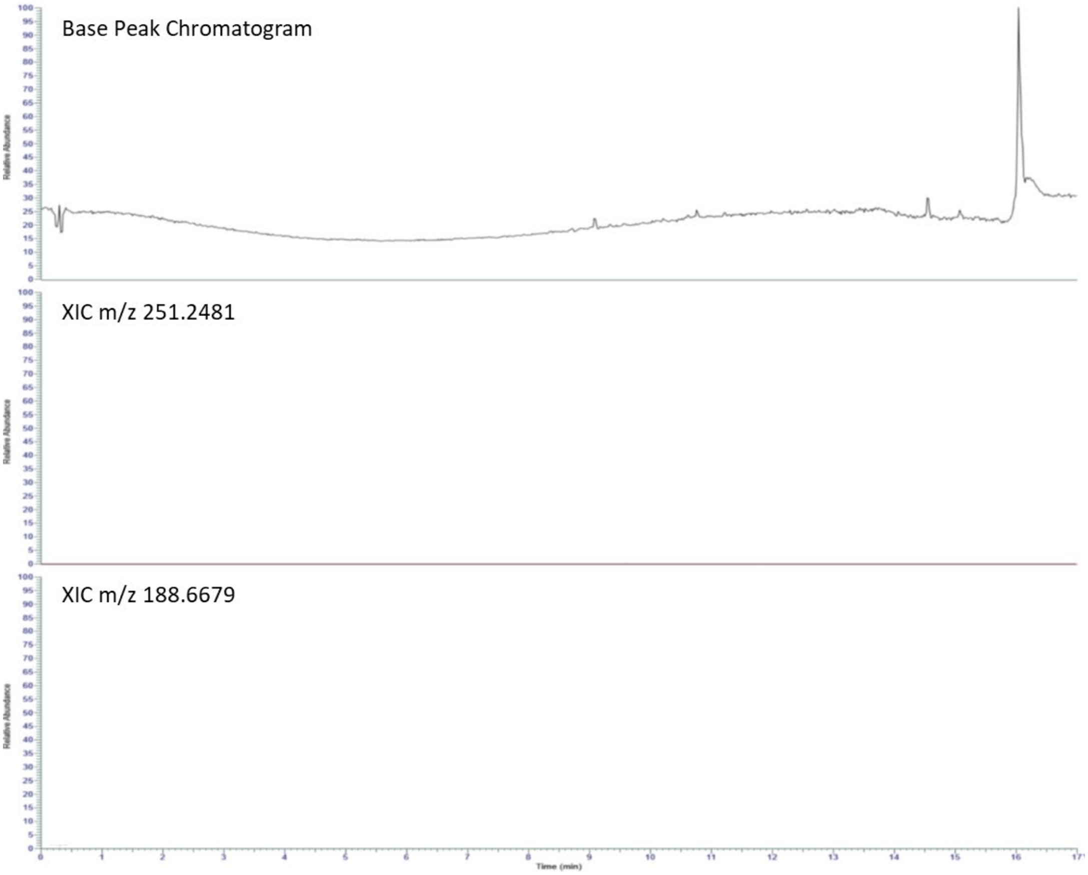
Analysis of the individual mass fractions showed that the bulk of polymer signals, as well as the dimer and trimer signals were only observed in the high mass fractions and must thus originate from compounds with a mass above 3 kDa. For the dimer and trimer signals it was hypothesized that these are in-source fragments of the polymer formed during the ESI process. While the bulk of polymer signals cannot feasibly be used for detection, the dimer and trimer in-source fragments are of significant intensity and thus may allow detection of PQ-6.

Potential in-source fragments were also detected for PQ-2 and PQ-7. However, since these signals were present in both MW fractions and the MW-range of the standard is unknown it is yet unclear if they may be used for detection. For PQ-10 no in-source fragment was observed. In conclusion, these results imply that the formation of in-source fragments during the ESI process may allow the detection of some but not all PQ-compounds, presenting a first limitation for a generic non-target screening workflow.

3.2 Amenability to separation with RP-HPLC

To investigate the feasibility of RP-HPLC separation for PQ-compounds a PQ-6 (<100 kDa) solution with a concentration of 1 mg/L in methanol/water (1:1) was prepared and injected for separation with an RP-HPLC column. PQ-6 (<100 kDa) was chosen as it is the analyte with the most reliable detection and the only analyte with a defined MW-range. High molecular weights are assumed to be the major limiting factor for PQ-analysis with RP-HPLC. Extracted ion chromatograms (XICs) for m/z 188.6679 (trimer) and m/z 251.2481(dimer) were used to evaluate the amenability to RP-HPLC (Figure 3). Despite the high concentrations used no peak was observed in either of the XICs, demonstrating that RP-HPLC is not feasible for the detection of PQ-compounds in the relevant MW-range.

Figure 3: Base peak chromatogram and XICs of in-source fragments of PQ-6 (<100 kDa) after separation on an RP-HPLC column.

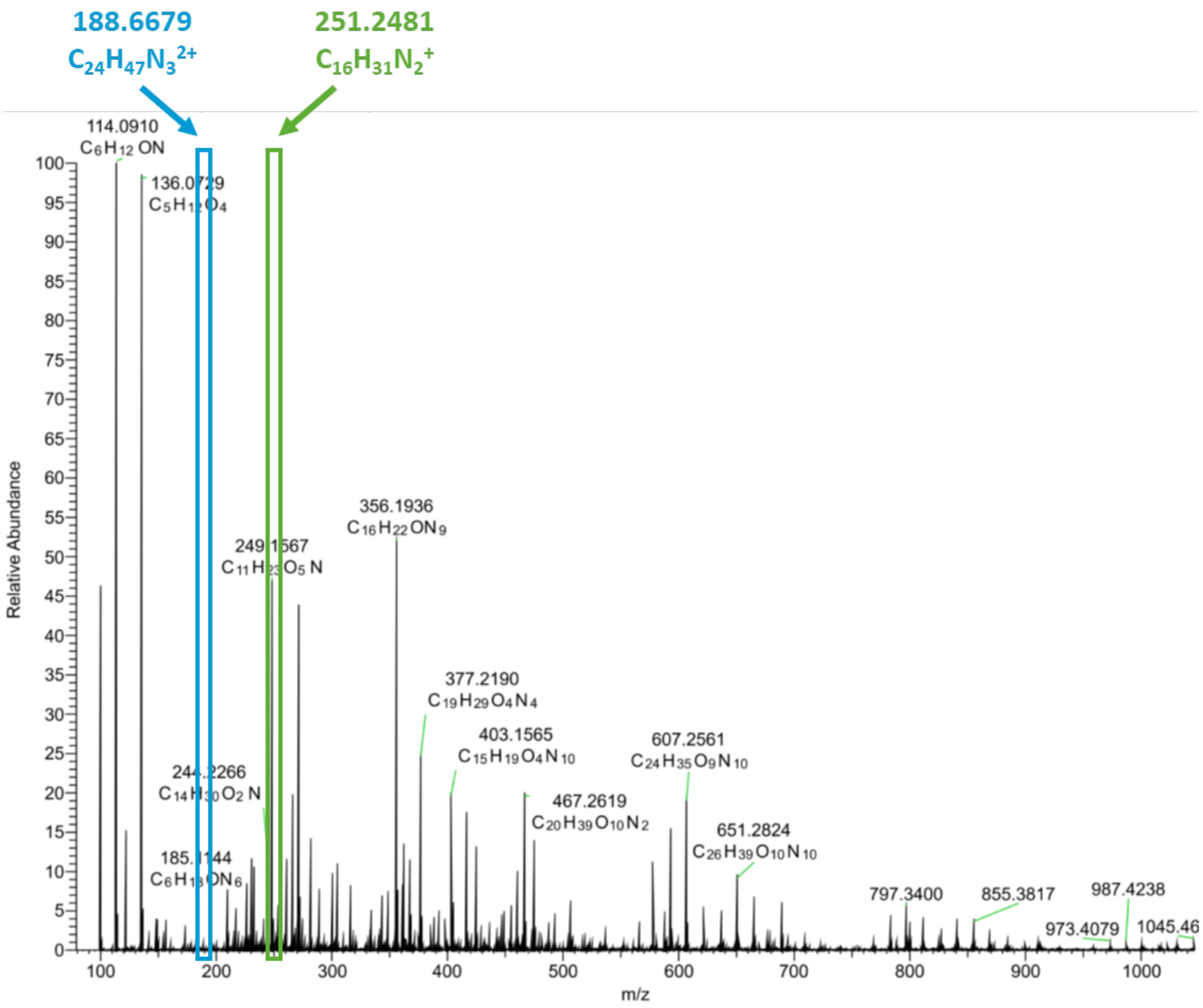


Source: own illustration, Helmholtz-Centre for Environmental Research

3.3 Amenability to enrichment with SPE

SPE is the most widely used enrichment technique in the environmental trace analysis of organic chemicals. To investigate the amenability of PQ-compounds to it 100 ml of water were fortified with 10 µg PQ-6 (<100 kDa), passed through a hydrophilic/lipopilic-balanced (HLB) cartridge, and eluted with methanol. After evaporation the residue was reconstituted in 1 mL methanol/water (1:1), which corresponds to a target concentration of 10 µg/mL. The reconstituted extracts were analysed via direct infusion and compared to standards (Figure 4). From this comparison it was evident that neither the in-source fragments nor the bulk of polymer signals could be detected after SPE.

Figure 4: HRMS-Spectrum of PQ-6 (<100 kDa) after enrichment with HLB-SPE



Source: own illustration, Helmholtz-Centre for Environmental Research

3.4 Impact on project plan and proposed alternative

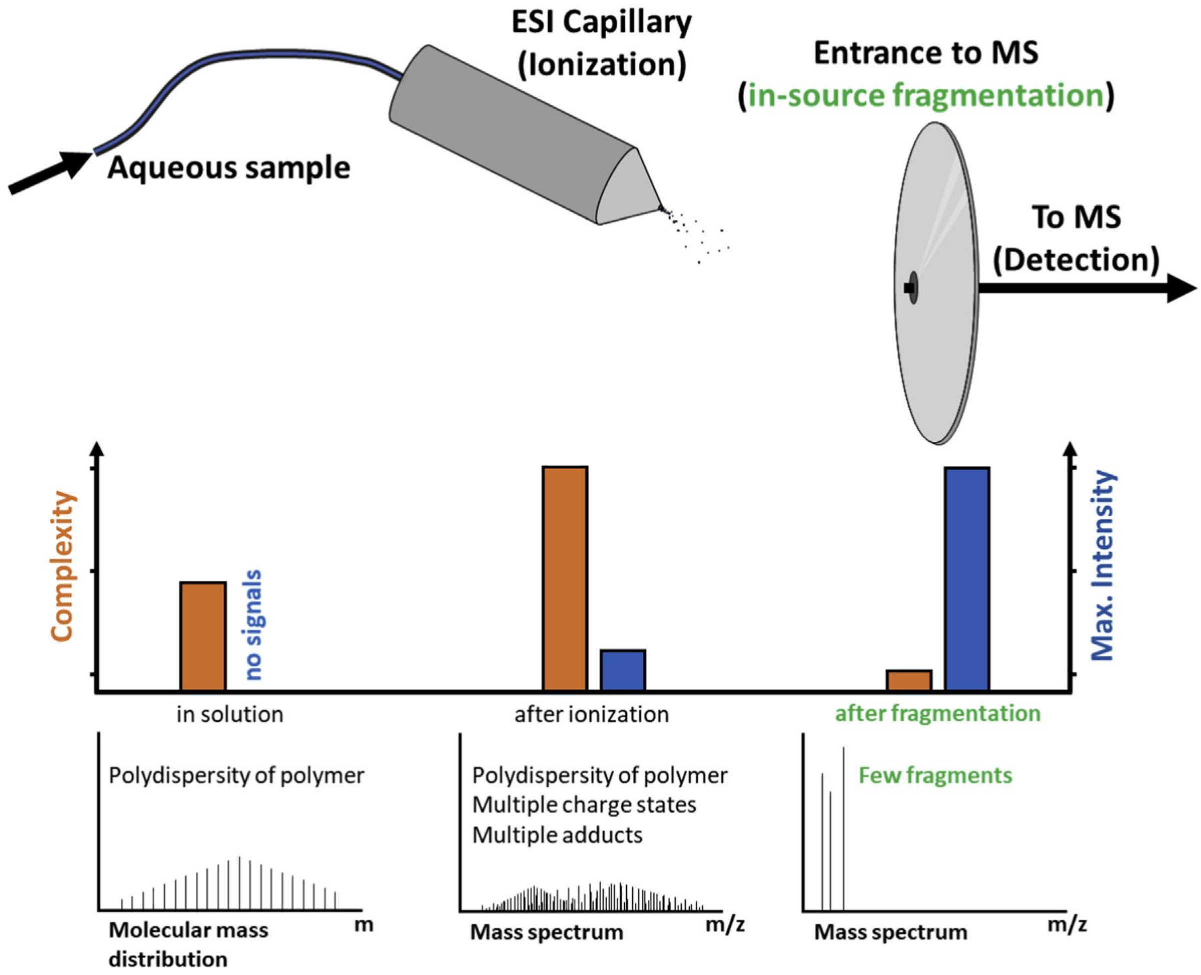
In light of the observed limitations that render PQ-compounds in the relevant MW-range likely undetectable with generic non-target screening workflows a retrospective suspect screening in data of the NORMAN Digital Sample Freezing Platform was deemed unfeasible. Consequently, a method development is necessary to detect PQ-compounds. If this method development is successful, sampling will be performed with a narrower focus on wastewater treatment plants to investigate their role as emission pathway for PQ-compounds.

4 Development of an analytical method for PQ compounds

4.1 Analytical principle

RP-HPLC/MS is often the method of choice for trace analysis of organic substances, which are detected through one of few well characterized masses (often the protonated or deprotonated molecule). Analysis of WSPs at trace levels in complex matrices, however, is hampered by the lack of such a well-defined mass representing the analyte. Polymers are commonly present in a wide range of chain lengths, charge states, and adducts, resulting in hundreds to tens of thousands of masses representing one WSP, and thus being present in much lower intensities. To overcome these problems an analytical approach first developed by Huppertsberg et al. (Huppertsberg et al., 2020) and successfully applied to analyse polyethyleneoxide in surface waters by Pauelsen at al. (Pauelsen et al., 2023) was adopted for PQs. In this approach in-source fragmentation of the WSPs is forced to form diagnostic fragments, thus severely reducing the complexity of the WSP mass spectra and resulting in higher individual intensities since the same fragments are formed from WSPs of various chain lengths and charge states (Figure 5).

Figure 5: Analytical principle of WSP analysis after in-source fragmentation.

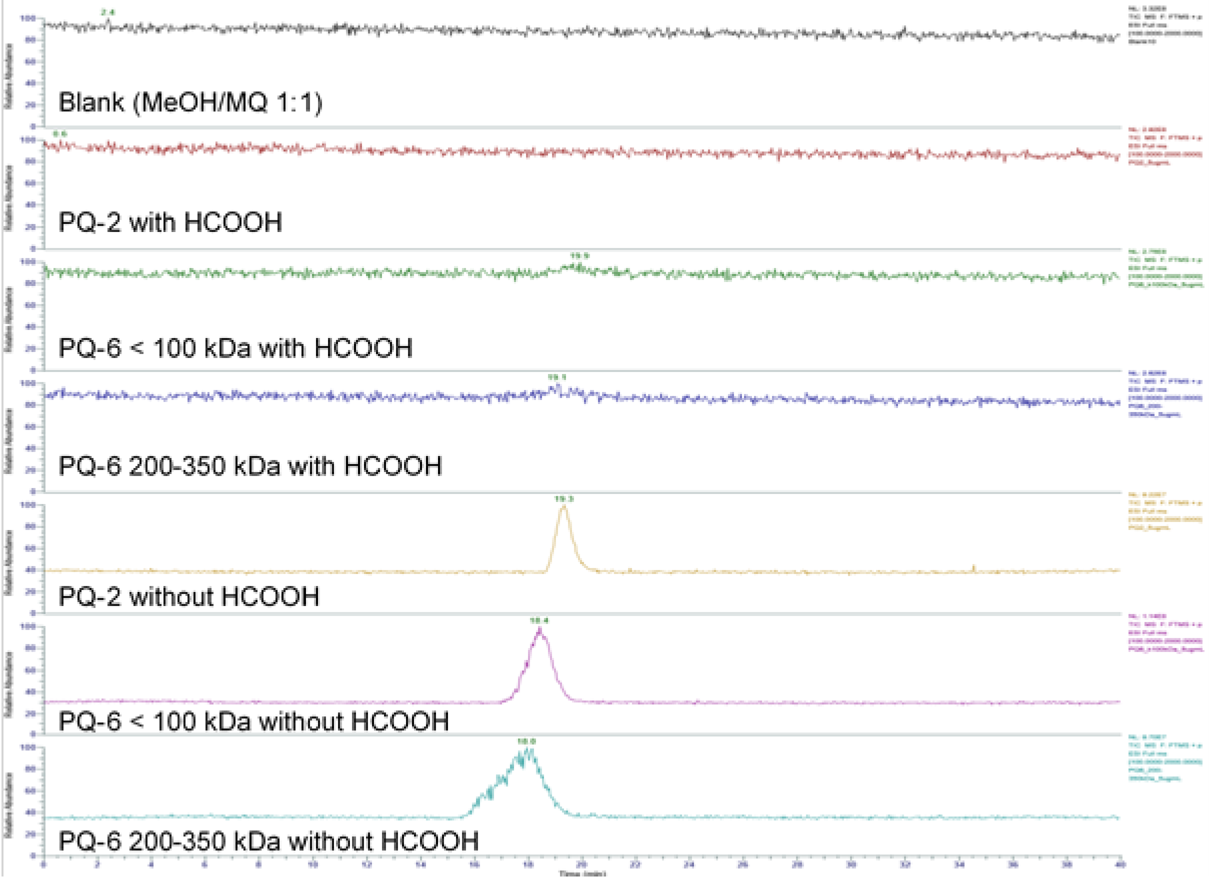


Source: Adopted from Huppertsberg & Zahn et al.(Huppertsberg et al., 2020)

4.2 Development of separation method

Size exclusion chromatography (SEC) is a method of choice for the molecular weight dependent separation of polymers. In SEC it is essential to ensure that no interaction between the polymer and the stationary phase takes place. PQs are polycations and thus stationary phase with a positive surface charge (Pre-Column, PSS, NOVEMA Max., 8 x 5 mm, 5 µm, guard (Agilent Technologies Inc., Santa Clara US) and GPC/SEC Columns, PSS-Polymer, NOVEMA Max. 1000 Å, 8 x 300 mm, 5 µm (Agilent Technologies, Inc., Santa Clara US)) was used. Compared to manufacturer recommendations the column had to be operated at lower flow rates to compensate for the increased backpressure of the used UHPLC instrument compared to SEC systems. While SEC is usually performed with completely aqueous phases, 30 % methanol was added to improve solvent evaporation in the electrospray ionization process. Formic acid, which was recommended to add to the eluent, could not be used since it resulted in severe ion suppression of the PQ compounds (Figure 6). At this point SEC is solely used to separate PQs from low molecular weight matrix components. A determination of PQ molecular weights is not feasible since well-defined standards are not available and electrostatic repulsion between the analyte and stationary phase cannot be guaranteed without formic acid.

Figure 6: Influence of formic acid on PQ signals



Source: own illustration, Helmholtz-Centre for Environmental Research

4.3 Development of mass spectrometric method

Detection of PQs with the chosen methodology requires the identification of diagnostic fragments for each PQ. Since it was demonstrated that the available PQ standards contain low molecular weight contaminations that may be remnants of the synthesis process fragments were identified after SEC separation. Therefore, a SEC-HRMS method was set up, that alternated between a full scan with and without in-source fragmentation (collision energy = 100 eV). The resulting spectra of PQ standards after in-source fragmentation were compared to the standards without in-source fragmentation to exclude masses not derived by fragmentation and to a blank after in-source fragmentation to exclude signals resulting from matrix components. The elution profiles of the highest intensity fragments were compared to the elution profile of the PQs in the total ion chromatogram for confirmation, which resulted in one to four high-intensity diagnostic fragments for each PQ (Table 2). For further optimization the experiment was repeated with collision energies of 10, 20, 30, 40, 50, 60, 70, 80, and 90 eV and fragment intensities were compared. A collision energy of 100 eV was shown to result in the highest sensitivity.

Table 2: List of diagnostic fragments

Analyte	Fragment	Molecular formula	Theoretical mass	Δ in [ppm]
PQ-2	141.1022 186.1600	$C_7H_{13}ON_2^+$ $C_9H_{20}ON_3^+$	141.10224 186.16009	-1.55 -1.72
PQ-6	260.2373 303.2796 345.3264	$C_{18}H_{30}N^+$ $C_{20}H_{35}N_2^+$ $C_{23}H_{41}N_2^+$	260.23728 303.27948 345.32643	-1.26 -1.17 -1.46
PQ-7	197.1647	$C_{11}H_{21}ON_2^+$	197.16484	-2.23
PQ-10	134.1175 278.1596 322.1858 366.2119	$C_6H_{16}O_2N^+$ $C_{12}H_{24}O_6N^+$ $C_{14}H_{28}O_7N^+$ $C_{16}H_{32}O_8N^+$	134.11756 278.15981 322.18603 366.21224	-2.16 -2.53 -2.45 -2.74

4.4 Method performance

After method development a method validation (Table 3) was performed investigating the limit of detection (LOD), linearity (linear regression coefficient R^2), reproducibility (relative standard deviation), and selectivity. The LOD was determined from the lowest calibration standard that could still be clearly distinguished from background noise (signal to noise > 3). The reproducibility was tested through analysis of six replicates of a 1 mg/L standard. Since exact quantification is not possible at this point LODs were only estimated to be in the 10s (PQ-2) to 100s (PQ-6, 7, and 10) µg/L range. These high LOD can partially be explained by the molecular weights of several 100 kDa of the PQs which exceeds that of many micropollutants by a factor of 10^3 and thus molecular responses seem comparable. Such increased LODs were already observed for PEG with a similar analytical approach (Pauelsen et al., 2023). Linearity (all $R^2 > 0.95$) and reproducibility (RSD < 5%) are well within expectation for environmental trace analysis.

Table 3: Analytical figures of merit.

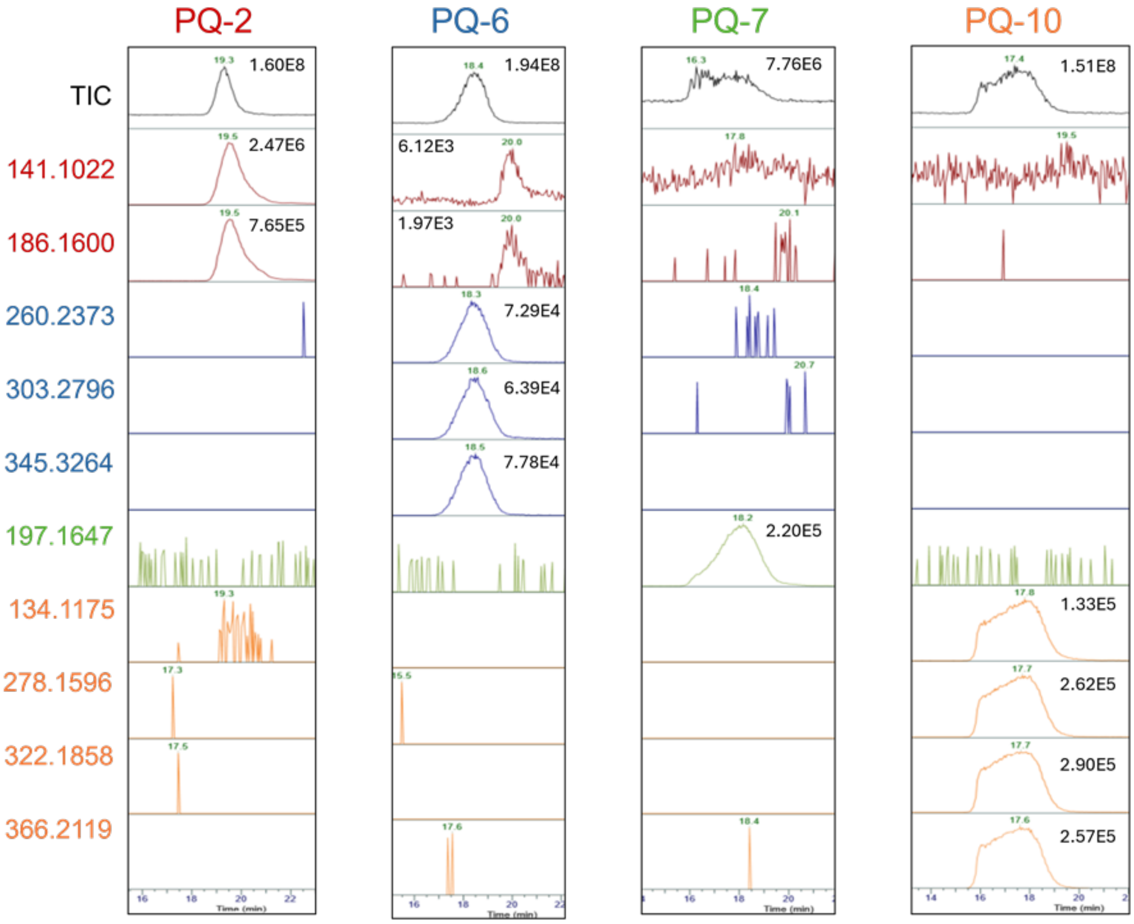
Analyte	Fragment	tRa in [min]	Regression Coefficient R2	Relative Standard Deviation RSD in [%]
PQ-2	141.1022	18.80	0.9683	4.3
	186.1600	18.75	0.9750	4.8
PQ-6	260.2373	17.49	0.9917	0.8
	303.2796	17.44	0.9979	2.8
	345.3264	17.39	0.9981	2.0
PQ-7	197.1647	17.58	0.9994	2.7
PQ-10	134.1175	16.84	0.9986	1.2
	278.1596	16.34	0.9983	1.8
	322.1858	16.30	0.9973	3.3
	366.2119	16.11	0.9966	1.4

a calculated as a mean value of the six replicates

b attained from the lowest concentration with S/N > 3

Since only fragments of PQ-compounds can be observed with the developed method and some PQ-compounds are structurally similar special emphasis was put on the selectivity of the method. To evaluate the selectivity, individual standards of all four PQ-compounds were analysed and checked for the presence of all fragments (Figure 7). None of the selected diagnostic fragments resulted in any significant signals for any of the other investigated PQ-compounds, indicating a good sensitivity of the method.

Figure 7: **Cross-examination of selectivity of diagnostic PQ-fragments and the total ion chromatogram (TIC).**

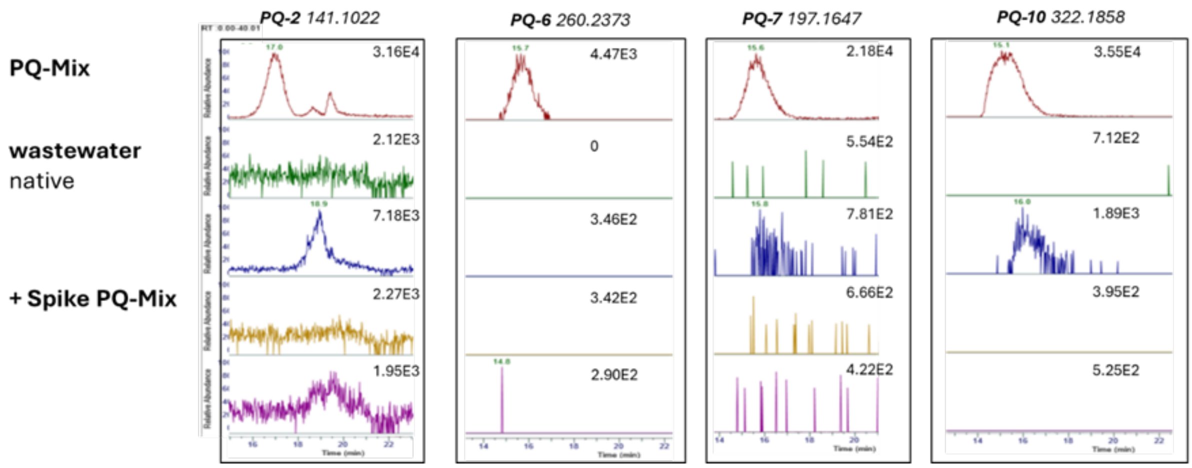


Source: own illustration, Helmholtz-Centre for Environmental Research

4.5 Applicability to environmental matrices

In light of the high LODs wastewater treatment plant effluent was seen as the most promising matrix for a monitoring since concentrations are expected to be higher than in surface water. To test the amenability of the method, wastewater treatment plant effluent (Rosenthal, Leipzig, 24h mixed sample) was spiked with a PQ-mix standard and analysed with the developed method (Figure 8). In the spiked wastewater PQ signals were reduced by at least one order of magnitude compared to the standards or not detected at all. Since the full scan of the elugram showed no coeluting compounds of relevant intensity in this high molecular mass range matrix effects during ionization were deemed unlikely.

Figure 8: **Elugrammes of PQ-compounds (highest intensity fragment) in a standard mixture, native wastewater (duplicate), and spiked wastewater (duplicate)**



Source: own illustration, Helmholtz-Centre for Environmental Research

As polycations PQs may interact with negatively charged dissolved organic matter (DOM) compounds and multivalent anions. To investigate if these compounds may be responsible for the loss of PQ signal intensities in wastewater, ammonium sulphate ($(NH_4)_2SO_4$), Suwannee River Natural Organic Matter (SRNOM), its fractions Suwannee River Fulvic Acids (SRFA) and Humic Acids (SRHA) and pore water from a swamp were individually added to ultrapure water and spiked with a PQ-mix standard. Analysis of these samples demonstrated that predominantly SRHAs and the porewater result in a significant reduction in PQ signal intensities (Figure 9). Fulvic acids, which are generally of lower molecular weight and stronger acidity than humic acids (Weng et al., 2006), seem to interact less strongly with PQs while humic acids showed a stronger interaction. We hypothesize that the higher molecular weight of humic acids may form insoluble complexes with PQs, making them unavailable to be detected and quantified by the used method. However, humic acids are much less common in surface water than fulvic acids (fulvic acids make up 81-95% of humic substances, (Linnik et al., 2013)) and thus, such effects are expected to a lesser extend in this matrix. As a proof of principle, the water from the Elsterbecken in Leipzig (Germany) was analysed, showing signals for PQ-2 (Figure 10), which demonstrated that a) analysis in surface water is generally feasible and b) concentrations in surface water may be sufficient to be detected with this method. Consequently, the analysis of wastewater treatment plant effluent was deemed less promising and the focus of the sampling campaign was shifted towards surface water after consultation with the UBA.

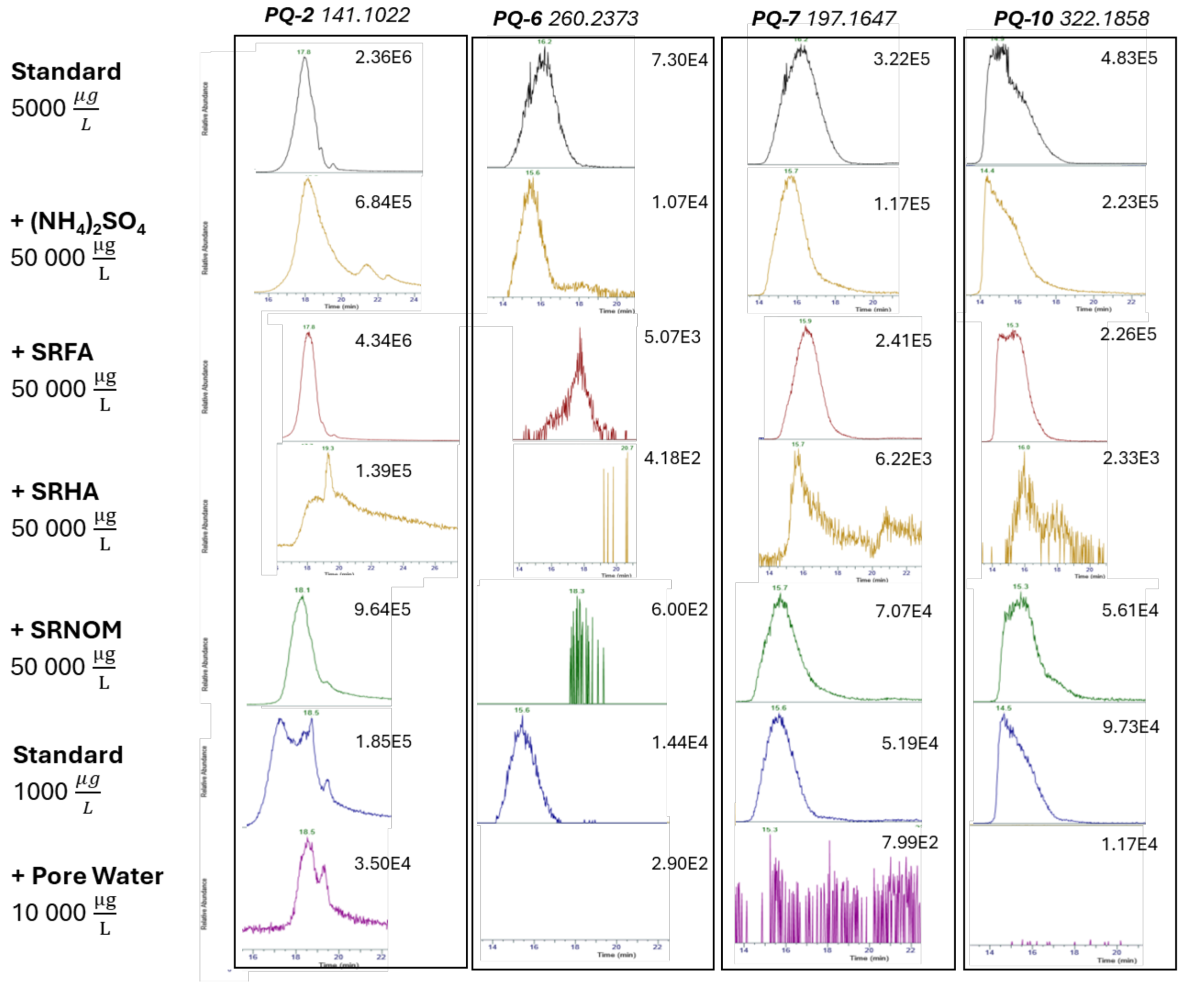
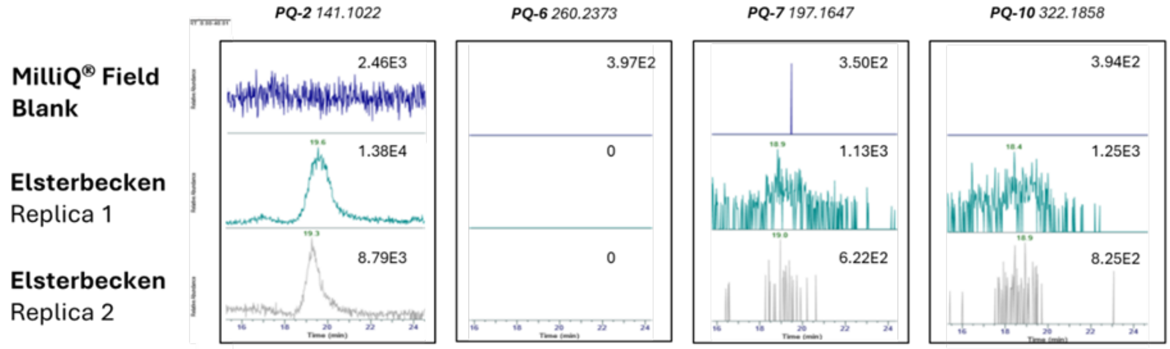


Figure 9: Influence of sulfate and DOM on PQ signal intensities

Source: own illustration, Helmholtz-Centre for Environmental Research

Figure 10: Elugram of PQs in a field blank and surface water of the Elsterbecken in Leipzig



Source: own illustration, Helmholtz-Centre for Environmental Research

5 Surface water monitoring

5.1 Samples

Samples for the monitoring campaign were taken from surface water bodies in Saxony and Saxony-Anhalt in Germany at the end of September and beginning of October 2024 (Table 4). Grab samples were taken in high density polyethylene (HDPE) bottles, stored at 4-degree Celsius and analysed within 2 weeks of sampling. Before the sampling took place in September, there were heavy rainfall events (130L/m^2 in Saxony and 80 L/m^2 in Saxony-Anhalt, which is each twice the long term means), even resulting in flooding of Elbe and its tributaries. Further rainfall events also occurred in between and during the sampling in October. Thus, the samples might be influenced by additional dilution due to heavy rain fall events or be contaminated through run-off. Sampled streams varied in size from 0.09 m^3/s (Parthe) to 1360 m^3/s (Elbe) and were influenced by a variety of potential contamination sources.

Table 4: Sampling locations.

Surface water name	Sampling date	GPS coordinates	Potential contamination sources
Pleiße	23.09.2024	51.30055 °N 12.37391 °O	Historically influenced by mining and rehabilitation of mining areas
Floßgraben	23.09.2024	51.30243 °N 12.35583 °O	WWTP Markleeberg
Paußnitz	23.09.2024	51.30016 °N 12.34679 °O	
Weiße Elster	23.09.2024	51.30429 °N 12.34054 °O	WWTP Knautnaundorf
Parthe	24.09.2024	51.37981 °N 12.42360 °O	
Mulde	24.09.2024	51.36229 °N 12.72021 °O	
Elbe Magdeburg	24.09.2024	51.12301 °N 11.63643 °O	
Alte Elbe Magdeburg	24.09.2024	52.13263 °N 11.65932 °O	
Tanger Tangerhütte	25.09.2024	52.43406 °N 11.81694 °O	Ironworks Water Association Stendal-Osterburg
Tanger Tangermünde	25.09.2024	52.54182 °N 11.97806 °O	WWTP Lüderitz
Elbe Tangermünde	25.09.2024	52.54335 °N 11.97954 °O	
Alte Elbe Bertingen	25.09.2024	52.35290 °N 11.81632 °O	WWTP Rogätz

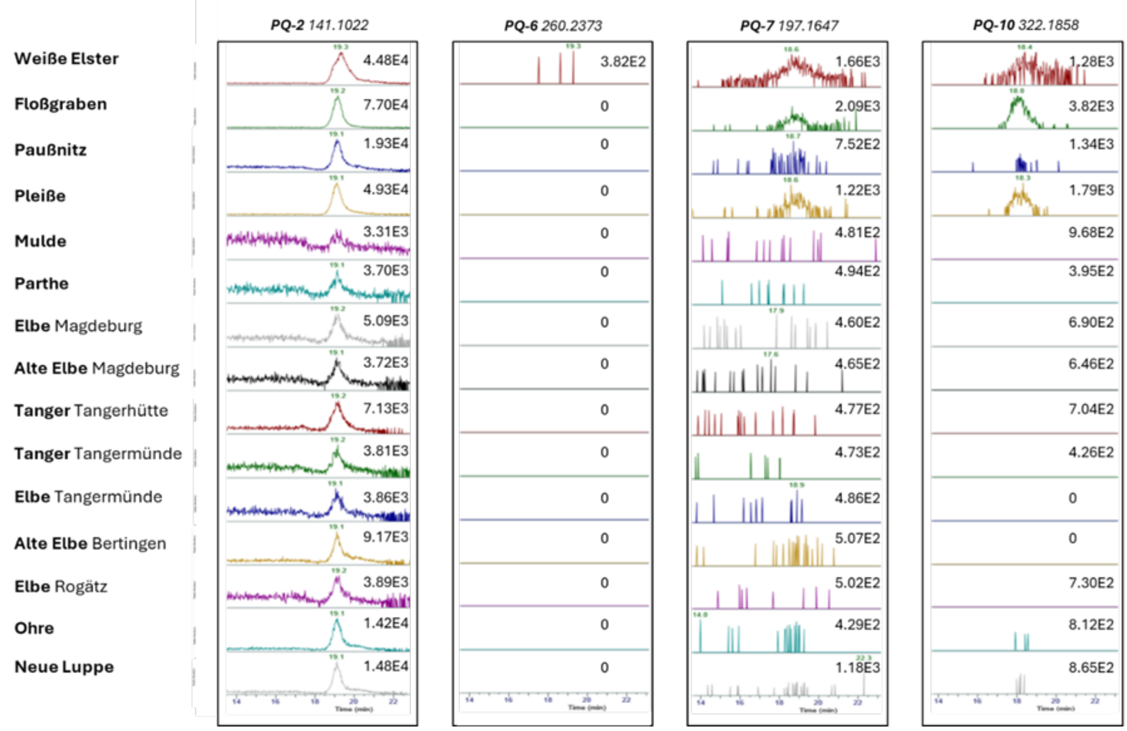
Surface water name	Sampling date	GPS coordinates	Potential contamination sources
Elbe Rogätz	25.09.2024	52.31436 °N 11.76921 °O	
Ohre	25.09.2024	52.28968 °N 11.69993 °O	WWTP Wolmirstedt
Neue Luppe	30.09.2024	51.36767 °N 12.31392 °O	WWTP Rosenthal

5.2 Monitoring

5.2.1 Occurrence in surface waters

The 15 selected surface water samples were analysed via direct injection (Figure 11), which led to the detection of PQ-2 in in most surface water samples. The largest peak areas were detected in the surface waters Weiße Elster, Floßgraben, Paußnitz, Pleiße, Ohre and Neue Luppe. The surface waters Floßgraben and Pleiße also showed signals for PQ-10 that where, however, of too low intensity for a reliable detection. Consequently, all surface waters were enriched via freeze drying (samples were frozen and sublimated at low pressure; enrichment factor of 20) and reanalysed. Analysis of the enriched samples (Figure 12) led to the detection of PQ-7 in the surface waters Pleiße, Floßgraben and Weiße Elster and PQ-10 in the surface waters Pleiße, Floßgraben, Paußnitz, and Weiße Elster. While elution times in standards and samples may be different this is to be expected with SEC since the elution time reflects the molecular weight distribution and is not indicative for a certain analyte.

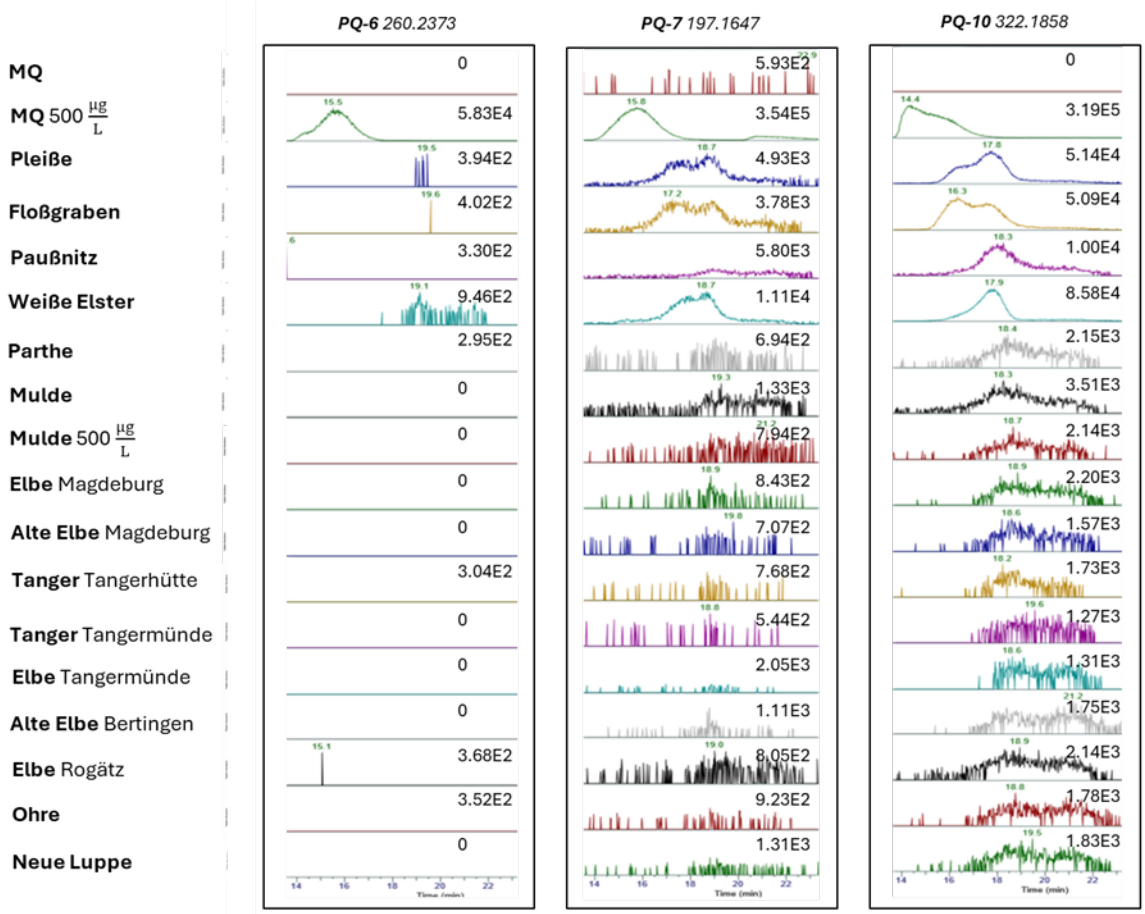
Figure 11: Elugrammes of PQ-2, 6, 7, and 10 in monitored surface waters



Source: own illustration, Helmholtz-Centre for Environmental Research

Generally, the most impacted samples are those with strong influence of household wastewater through wastewater treatment plants. Exceptions here are the Paußnitz and Pleiße in which wastewater treatment plants do not directly discharge. The Pleiße is influenced by mining and rehabilitation mining activities sometimes leading to a brown colouring of the water through iron compounds. Since PQs are known to be used in the mining industry (Carmona-Ribeiro & De Melo Carrasco, 2013) this could be a potential source of this contamination. Consequently, these results indicate a widespread presence of PQ-substances especially in surface waters impacted by urban wastewater, which needs to be confirmed by further investigations with larger sample sizes.

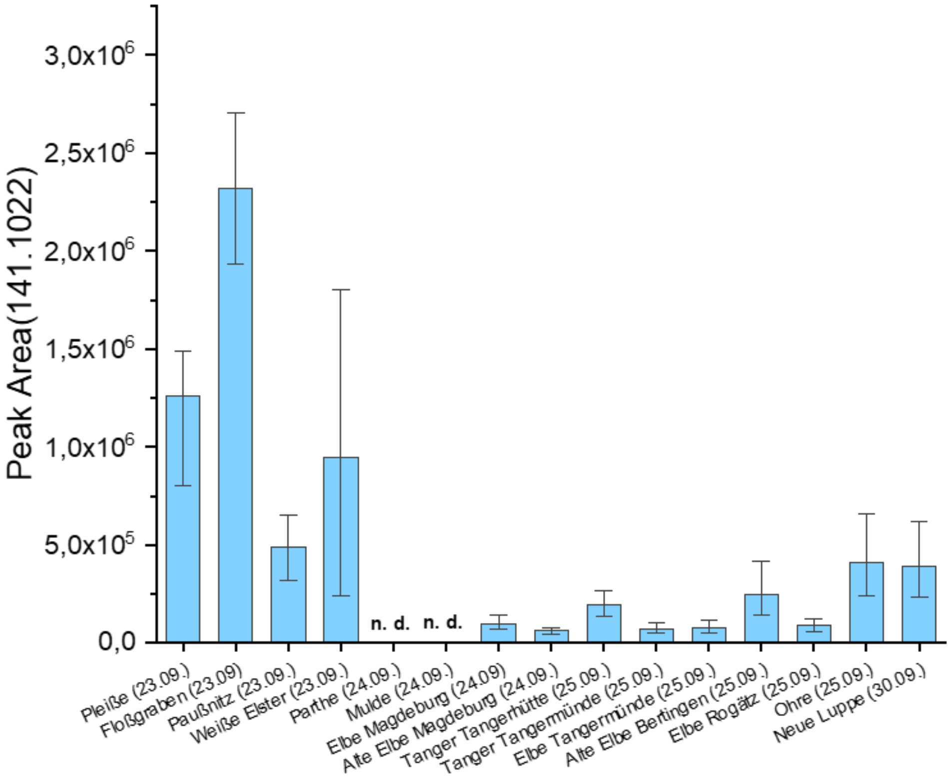
Figure 12: Elugrammes of PQ-6, 7, and 10 in monitored surface waters after freeze drying



Source: own illustration, Helmholtz-Centre for Environmental Research

Due to the wide variety of uses for PQ-substances (e.g. in personal care products (Wang et al., 2023)), the absence of isotope labelled internal standards, and the inability to detect the intact molecule false positives were the main concern to obtain valid information from the monitoring. Thus, the 15 surface water samples selected for the monitoring campaign were sampled and analysed in triplicate. All bottles were rinsed before the sampling and the final wash step was collected and analysed to exclude contamination of the bottles. Additionally, field blanks (sampling bottles were filled with ultra-pure water at the sampling site) were taken at the sampling locations. Neither the field blanks nor the water of the final wash step of the sampling bottles showed signals at the relevant elution time of any of the PQs analysed. Areas of triplicate samplings vary strongly for some samples respectively (e.g. Weiße Elster), further highlighting that exact quantification is not possible with the current iteration of the method (Figure 13).

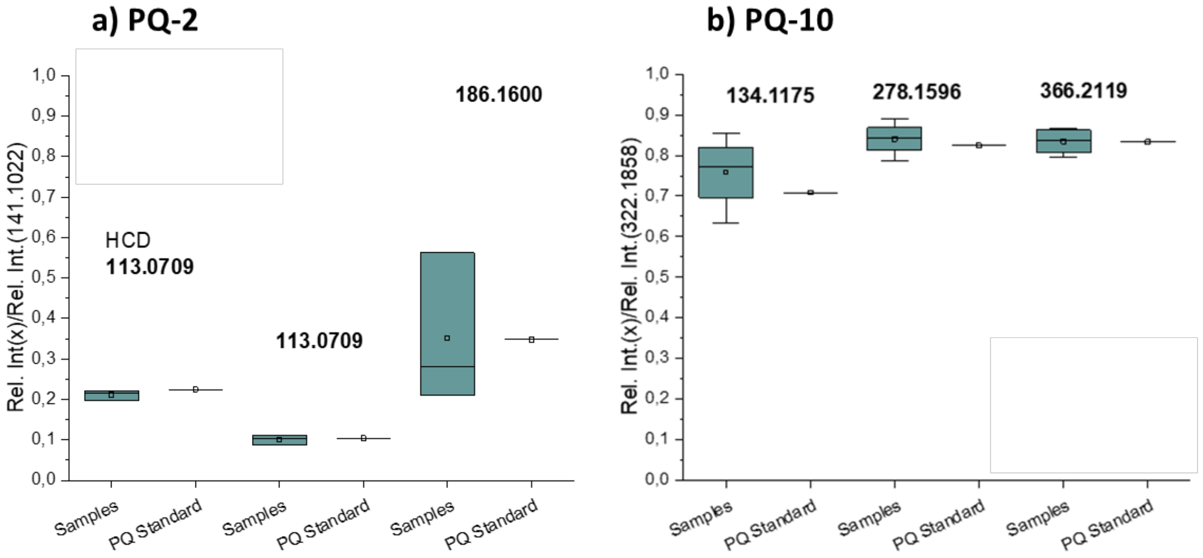
Figure 13: Signal intensities of PQ-2 (m/z 141.1022) in surface water samples



Source: own illustration, Helmholtz-Centre for Environmental Research

Another potential source of false positive results is the presence of other high molecular mass substances that form isobaric fragments during in-source fragmentation. The risk of false positives caused by this can be significantly reduced if several fragments and the ratio between fragments are considered. For PQ-2 three in-source fragments (m/z 141.1022, m/z 113. 0709, and m/z 186.1600) and one MS^3 fragment formed upon HCD fragmentation of m/z 141.1022 (m/z 113.0709) were evaluated in the directly analysed samples. For PQ-10 four in-source fragments (m/z 322.1858, m/z 134.1175, m/z 278.1596, and m/z 366.2119) were analysed in the samples enriched with freeze drying. The intensity of the most abundant fragment (m/z 141.1022 for PQ-2 and m/z 322.1858 for PQ-10, respectively) were set to 1 in each sample and the relative intensities for the other fragments were calculated and compared to the standard (Figure 14). For both analytes the fragment ratios of the standards were within the interquartile range of the samples, significantly reducing the possibility of false positives.

Figure 14: **Relative intensities of diagnostic fragments of (a) PQ-2 and (b) PQ-10 (both after freeze drying) compared to the quantifier in contaminated samples and standards.**



Source: own illustration, Helmholtz-Centre for Environmental Research

5.2.2 Concentration estimation

In light of the widespread detection of PQ-2 concentrations need to be known to assess the risk these chemicals may pose to the aquatic environment. However, several key limitations exacerbate accurate quantification:

- **Limited standard availability:** Standards of PQ-compounds are often of technical quality and not well-defined, containing low molecular weight contaminations and are of unknown molecular weight. This may be especially relevant since response factors may be molecular weight dependent as it has been observed for PEO (Pauelsen et al., 2023).
- **Complex and incompletely understood interactions with NOM:** PQs appear to interact with compounds of the NOM in a complex and incompletely understood way.
- **Poor reproducibility of enrichment:** attempts to enrich PQs from surface water with either freeze drying or solid phase extraction suffer from poor reproducibility for yet unknown reasons. Thus, enriched samples may only be used for qualitative assessment until this can be improved.

For these reasons, exact quantification of PQs in surface water is not feasible with the current iteration of the method and even concentration estimation is limited to PQ-2, which was detected after direct injection. Concentrations of PQ-2 in surface waters were calculated based on the highest intensity fragment and compared to the second highest intensity fragment (qualifier), which was mostly in good agreement. However, given the current limitations of the method concentrations can only be seen as an estimation of the order of magnitude instead of exact concentrations (Table 5).

Table 5: Estimated concentrations of PQ-2 in surface water samples

Surface water name	Estimated concentration[a] (in µg/L)	Standard deviation (in µg/L), n = 3	Deviation of qualifier[b] (in %)
Pleiße	$8.08 \cdot 10^2$	$2.09 \cdot 10^2$	-7.57
Floßgraben	$1.49 \cdot 10^3$	$2.02 \cdot 10^2$	-32.4
Paußnitz	$3.16 \cdot 10^2$	$8.69 \cdot 10^1$	-11.0
Weiße Elster	$6.07 \cdot 10^2$	$4.14 \cdot 10^2$	27.8
Parthe	n. d.	n. d.	n. d.
Mulde	n. d.	n. d.	n. d.
Elbe Magdeburg	$6.40 \cdot 10^1$	$2.00 \cdot 10^1$	0.96
Alte Elbe Magdeburg	$4.24 \cdot 10^1$	8.74	-7.58
Tanger Tangerhütte	$1.26 \cdot 10^2$	$3.48 \cdot 10^1$	-21.6
Tanger Tangermünde	$4.61 \cdot 10^1$	$1.38 \cdot 10^1$	16.2
Elbe Tangermünde	$5.03 \cdot 10^1$	$1.68 \cdot 10^1$	2.24
Alte Elbe Bertingen	$1.61 \cdot 10^2$	$7.65 \cdot 10^1$	0.61
Elbe Rogätz	$5.81 \cdot 10^1$	$1.76 \cdot 10^1$	-3.05
Ohre	$2.63 \cdot 10^2$	$1.14 \cdot 10^2$	-19.3
Neue Luppe	$2.50 \cdot 10^2$	$1.05 \cdot 10^2$	-24.0

a) based on quantifier (m/z 141.1022)
b) based on qualifier (m/z 186.1600)

Estimated concentrations for PQ-2 range from 10^1 to the 10^3 µg/L. Environmental effects of PQ-compounds are only scarcely studied, with a clear focus on PQ-6 as most studied compound (Table 6). In the absence of effect data for PQ-2 estimated concentrations were compared to effect studies for PQ-6 instead. The upper limit of the estimated concentrations for PQ-2 are in the low mg/L range and thus exceed the 72h ErC_{50} (concentration at which a 50 percent reduction in growth rate is observed) for growth inhibition for the freshwater algae *Raphidocelis subcapitata* (0.12-0.26 mg/L for PQ-6) (Hansen et al., 2023) and the LC_{50} (concentration at which a mortality rate of 50 percent is reached) for the water flea species *Ceriodaphnia dubia* (0.32 mg/L) (Steinmetz et al., 2024). Consequently, environmental PQ-2 concentrations may be in the range where adverse effects on aquatic species might occur. However, since concentrations can only be estimated roughly, it is still unclear if the chosen analytical approach only detects bioavailable PQs or also PQs bound in soluble complexes, and toxicity data for PQ-2 is missing, more sophisticated data is required for a detailed risk assessment.

Table 6: **Overview of effect data for PQ-compounds.**

Tested PQ-compound	Organisms and End point	LC50/EC50	Study
PQ-6	freshwater green algae Raphidocelis subcapitata growth inhibition	72 h ErC_50=0.12-0.26 mg/L	(Hansen et al., 2023)
PQ-6	Daphnia immobilisation	48 h-EC50 20.2 g/L	(Gomes et al., 2014)
PQ-6	zebra fish Danio rerio and the cladoceran D. magna	LC50 and immobilisation EC50 values higher than 10 mg/L	(Gomes et al., 2014)
PQ-6	Ceriodaphnia dubia	$48\ h\ LC_{50}$ 320 $\frac{\mu g}{L}$	(Steinmetz et al., 2024)

6 Conclusions

This study demonstrated that PQ-compounds cannot be analyzed for with conventional non-target screening workflows due to the absence of high-intensity diagnostic ions, and thus a dedicated workflow had to be developed. Consequently, an analytical workflow established for the environmental analysis of PEO that utilizes SEC for separation and in-source fragmentation to form diagnostic fragments was adopted for PQ-2, 6, 7, and 10. While analytical limitations still remain and render exact quantification infeasible qualitative analysis in surface water was shown to be possible. A monitoring in surface water with the developed workflow led to two key observations:

- **PQ-compounds seem to be widely present in surface waters:** In this study PQ-2 was detected in 13 of the 15 analyzed surface waters from Saxony-Anhalt and Saxony (Germany) with direct injection while PQ-7 and 10 were detected in 3, respectively 4 surface waters after freeze drying. In light of the high limits of detection this is remarkable.
- **PQ-compounds were estimated to be partially present in concentrations were adverse effects may occur:** Concentrations for PQ-2 were estimated up to the low mg/L range, which seems exceptionally high and requires confirmation. If these concentrations are realistic, they exceed the toxicity thresholds that have been reported for specific aquatic species for other PQ-compounds.

Based on these observations the following actions seem necessary to accurately assess the risk that PQ-compounds pose to the environment and devise regulatory action if required:

- **Analytical methods for PQs need to be further developed and optimized:** The developed method for PQ-2, 6, 7, and 10 allows for the qualitative assessment of these 4 PQs in surface water at environmentally relevant concentrations for the first time, but is still hampered by high detection limits and inaccurate quantification. Thus, it seems mandatory to expand the method to include more PQ-compounds, reduce detection limits and improve accuracy of quantification. This will not only demand methodological optimizations but also requires high-quality and well-described analytical standards. If more such standards with well-defined molecular weight are available this will also enable molecular weight determination in environmental samples which might improve the understanding of sources.
- **Widespread environmental monitoring of PQs is required to establish realistic concentrations, assess current exposure status and identify sources and hotspots:** Initial data implies that PQs are widely present and partially occur in high concentrations. Once well-defined standards and a more sophisticated method is available a larger scale monitoring is required to confirm estimated concentration ranges and identify entrance pathways and hotspots. As emission and exposure data are currently lacking, such data are key to decide on follow up regulatory measures.
- **The environmental fate of PQs and especially their interaction with NOM needs to be better understood:** If the estimated environmental concentrations for PQs can be confirmed it becomes essential to understand their environmental fate and behavior. This includes biotransformation, bioaccumulation, indirect photolysis as well as the seemingly high relevant interaction with natural organic matter.
- **Toxicity tests for PQs need to consider the most environmentally prevalent PQs and realistic effective concentrations:** So far toxicity studies for PQ-compounds are scarce and predominantly focused on PQ-6. In light of these initial results toxicity studies for the most

environmentally prevalent PQs seem necessary. Improvements in analytical methods and available standards may also significantly improve toxicity studies. In light of the scarcity of high-quality standards, so far mostly technical standards or technical products are used for such studies, which may contain low molecular weight contaminants as a leftover of the production process. Thus, it may be unclear if the PQ-polymers themselves or these contaminations are mainly responsible for the observed effects. Additionally, the lack of analytical methods renders it impossible to consider effective concentrations instead of added concentrations in toxicity studies, which can lead to an underestimation of effects, especially for substances with are prone to sorption.

7 List of references

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