TRENDS IN THE ANALYSIS OF POLLUTANT TRANSFORMATION PRODUCTS IN THE MARINE ENVIRONMENT

ANDREAS PETSAS, MARIA VAGI, ANASTASIA NIKOLAOU* and MARIA KOSTOPOULOU

Department of Marine Sciences, University of Aegean University Hill, GR-81100, Mytilene, Greece, corresponding author: nnikol@aegean.gr

ABSTRACT

During the past decades, the occurrence of several categories of pollutants in the marine environment has been well documented. Polycyclic aromatic hydrocarbons, pesticides and herbicides, pharmaceuticals and personal care products, plasticizers, organotin compounds have been detected in seawater, marine sediments and marine organisms. Many of these compounds exhibited toxic effects and were classified and regulated as priority pollutants, emerging pollutants and/or endocrine disruptors, posing risks to the quality of the marine environment, the balance of marine ecosystems, and human health.

The advanced analytical instrumentation and sample preparation techniques available in the recent years resulted in extended research for the above categories of compounds in the marine environment, with a vast number of scientific publications globally regarding the levels and toxic effects of such compounds in water, sediment and biota. The resulting information is of critical importance for the protection of the marine environment, especially in the framework of Marine Strategy Directive of the European Union.

However, the occurrence of marine pollutants can create further problems, in regard to the transformation products formed by their decomposition, hydrolysis, photolysis, biodegradation in the marine environment. As the evolution in the field of analytical methods for the determination of pollutants in increasingly low levels and with improved accuracy is continuing, particular emphasis on research towards these new compounds is necessary in order to estimate the actual risk involved for the marine environment quality. Recently, a significant number of relevant publications have dealt with the subject, identifying new pollutant transformation products, pathways of formation, fate and possible adverse environmental and health effects.

In this framework, the present study aims to examine and present the current trends in the analysis of pollutant transformation products in the marine environment, as well as to identify the relevant topics with emerging needs for investigation. In particular, through the study of recent relevant papers, the following trends were identified, collectively presented and discussed: (a) TPs formation pathways and related mechanisms, (b) analytical aspects, (c) concentration levels detected in samples from marine environment, (d) toxicity of these compounds. According to the findings, as some transformation products are equally or even more toxic than the parent compounds, the importance of further investigation of the subject is highlighted, in order to have a clearer picture of their speciation and role in the marine environment, while many more compounds of this category remain to be discovered.

KEYWORDS: transformation products, pollutants, analysis, toxicity, marine environment

1. INTRODUCTION

The occurrence of several categories of pollutants in the marine environment has been well documented by many researchers during the last decades. Polycyclic aromatic
hydrocarbons, pesticides and herbicides, pharmaceuticals and personal care products, plasticizers, organotin compounds are the major pollutant categories that have been detected in seawater, marine sediments and marine organisms (Nikolaou et al., 2007, 2013, Kostopoulou et al., 2008). Many of these compounds exhibited toxic effects and were classified and regulated as priority pollutants, emerging pollutants and/or endocrine disruptors, posing risks to the quality of the marine environment, the balance of marine ecosystems, and human health (Kostopoulou et al., 2013).

After their release into the environment, pollutants are subject to transformation processes such as chemical and photochemical degradation. These processes can take place during wastewater treatment, during transport of the pollutants via rivers and runoff or in the marine environment which is their final receiver (Nikolaou et al., 2013). Different transformations can occur in all these environmental compartments, resulting in the formation of a variety of transformation products (TPs), many of which have still not been identified. There is a large gap of knowledge about their formation pathways, their levels in environmental samples and their ecotoxicity, especially in the case of marine environment, where their analysis in trace levels still presents a challenge, due to the complexity of the samples and the presence of large amounts of organic compounds. TPs have been studied mainly in wastewater samples, where recent relevent research has shown that some of them can be more toxic than the parent compounds, thus raising concern for their effects on ecosystems, organisms and eventually human health (Nikolaou et al., 2009, Fatta-Kassinos et al., 2011).

The advanced analytical instrumentation and sample preparation techniques available in the recent years resulted in extended research for the above categories of compounds in the marine environment, with a vast number of scientific publications globally regarding the levels and toxic effects of such compounds in water, sediment and biota (Kostopoulou et al., 2008, Devier et al., 2011). The resulting information is of critical importance for the protection of the marine environment, especially in the framework of Marine Strategy Directive of the European Union. As the evolution in the field of analytical methods for the determination of pollutants in increasingly low levels and with improved accuracy is continuing, particular emphasis on research towards these new compounds is necessary in order to estimate the actual risk involved for the marine environment quality. Recently, several relevant publications have identified new pollutant TPs, pathways of formation, fate and possible adverse environmental and health effects (Farre et al., 2008, Voogt and Laane, 2009, Nikolaou et al., 2009b, Sirtori et al., 2012, Salvado et al., 2012).

In this framework, the present study aims to examine and present the current trends in the analysis of pollutant TPs in the marine environment, as well as to identify the relevant topics with emerging needs for investigation. In particular, through the study of recent relevant papers, the following trends were identified, collectively presented and discussed: (a) TPs formation pathways and related mechanisms, (b) analytical aspects, (c) concentration levels detected in samples from marine environment, (d) toxicity of these compounds. According to the findings, as some TPs are equally or even more toxic than the parent compounds, the importance of further investigation of the subject is highlighted, in order to have a clearer picture of their speciation and role in the marine environment, while many more compounds of this category remain to be discovered.

2. FORMATION PATHWAYS

The formation pathways of TPs in the marine environment include various processes that induce chemical transformations of the molecules of pollutants. These processes can be abiotic or biotic. Examples of abiotic transformation processes, occurring particularly at the upper layers of the water column, are the hydrolysis, the direct photochemical degradation as well as indirect photochemical pathways, such as reactions with singlet...
oxygen (1O2), hydroxyl radicals (dOH), peroxy radicals (dOOR), photo-excited organic matter, and other reactive species. These reactions are often complex and lead to multiple TPs, some of which have been found to be more toxic than the parent compounds, or retain the properties of the parent compounds e.g. some dehydrated products of tetracyclines and photodegradation products of ofloxacin retain antibiotic activity (Farre et al., 2008).

Among the biotic processes that result in the formation of TPs, the most prominent is biodegradation. Biodegradation can transform many organic compounds that are being used by microorganisms as an energy source. The cometabolism process, in which an organic compound is modified but not utilized for growth also results in biodegradation of pollutants. Biodegradation processes become more important at lower levels of the water column, where sunlight cannot affect the pollutant molecules, or in cases of pollutants are adsorbed onto suspended particles (Farre et al., 2008, Nikolaou et al., 2009b). Further biotic processes that can lead to TPs formation involve the activity of plants and animals that can detoxify or excrete contaminants after uptake, without complete elimination of their molecules. This can take place because of accumulation of the pollutants in adipose tissue or the absence of appropriate enzyme systems necessary for biotransformation. Biotic processes result in the formation of TPs with increased polarity and different physico-chemical properties than the parent compounds, resulting in distinct environmental behavior. Such processes are commonly initiated in the wastewater treatment plants by bacteria and protozoa, but they are continued in the marine environment by the contribution of various organisms (Fatta-Kassinos et al., 2011).

3. ANALYTICAL ASPECTS
Among the analytical techniques most commonly applied for the investigation of pollutant TPs in environmental samples, the most preferable today is Liquid Chromatography combined with Mass Spectrometry (LC-MS) and related techniques, such as ultraperformance LC-MS (UPLC-MS). By the use of such analytical techniques, sensitive analytical methods have been developed and used to determine TPs of pollutants at ultra-trace levels in environmental samples. The use of advanced instrumentation such as LC coupled to ion-trap (IT)-MS or time-of-flight (ToF)-MS, has enabled the identification and determination of more TPs in environmental samples, providing complementary data for the structural elucidation of unknown compounds. For confirmation purposes, hybrid triple-quadrupole ToF-MS (QqToF-MS) are very useful (Farre et al., 2008, Kostopoulou et al., 2008, Nikolaou et al., 2009).

Further than the advanced detection and idetification technology, a very important analytical aspect regards the sample preparation and cleanup aiming to minimize the matrix effects, because the levels of pollutants and especially the levels of TPs in the marine environment are very low. An analyte-preconcentration procedure is usually applied in order to increase analytical sensitivity, often requiring high enrichment factors (100–10,000), usually achieved by solid-phase extraction (SPE). Much progress has been made in various SPE techniques both for sample preparation and extract cleanup to minimize matrix effects during the last years (Farre et al., 2008, Kostopoulou et al., 2008).

4. OCCURRENCE AND TOXICITY
The occurrence, fate and toxicity of pollutant TPs in the aquatic environment is largely unknown. There has been a small number of studies reporting the toxicity of metabolites and degradation products of pollutants, as well as their occurrence mainly in wastewater influents and effluents (Nikolaou et al., 2007, 2009, 2009b, Fatta-Kassinos et al., 2011), while similar research in the marine environment is very limited. Characteristic examples of such recent research, outlining the need to obtain more knowledge on the subject, are summarized below.
4.1 TPs of pharmaceuticals
Clofibric acid, a metabolite of lipid-lowering agents clofibrate, etofibrate and etofyllin clofibrate, was detected in effluents, freshwater (rivers and lakes) and even in seawater. Fluoxetine and sertraline and metabolites norfluoxetine and desmethylsertraline were detected in fish sampled from the wild in the U.S.A., therefore reflecting potential bioaccumulation. The accumulated levels of fluoxetine and sertraline found in brain were 1.6 ng/g and 4.3 ng/g respectively (Farre et al., 2008).

Farre et al. (2008) studied the ecotoxicological hazard potential of 42 pharmaceuticals from 22 therapeutic groups, including metabolites formed in humans, and reported that, on average, 50% of a parent drug was metabolized, and 70% was excreted with urine, albeit with large variations among drugs. The same researchers concluded that metabolism reduced the toxic potential of all but eight drugs, and that ibuprofen and its metabolites in a mixture could pose an ecotoxicological risk.

The ecotoxicity of triclosan and its metabolite methyl-triclosan has been studied on the marine bacteria V. fischeri by Farre et al. (2008), who reported a toxic synergism between triclosan and linear alkylbenzene sulfonates. The acute aqueous toxicity values (96-h LC50) reported for triclosan and metabolite were 305 lg/L for adult shrimp, 154 lg/L for larvae, and 651 lg/L for embryos. The phytoplankton species was the most sensitive species tested, with a 96-h EC50 value of 3.55 lg/L. Moreover, adult grass shrimp were found to accumulate methyl-triclosan after 14-day exposure to 100 lg/L triclosan, indicating formation of this metabolite in seawater and its potential to bioaccumulate in higher organisms (Farre et al., 2008).

4.2 TPs of Flumequine
Sirtori et al. (2012) investigated the direct photolysis of flumequine (FLU) in demineralised water (DW) and in synthetic seawater (SW), and identified the major phototransformation products. FLU was susceptible to transformation when subjected to direct solar radiation. Slower degradation kinetics were observed in SW. 14 Photolytic TPs were identified by (LC–TOF–MS) (Figure 1).

Figure 1. TPs of FLU (Sirtori et al., 2012)
The transformation mechanism of FLU begins with the opening of the heterocyclic ring by oxidation of the double bond, and continued with loss of the fluorine atom and hydroxylation of the aromatic ring. The authors investigated the acute toxicity to *Vibrio fischeri* and *Daphnia magna* bioassays for the first and last irradiated solutions and found that in the SW the most persistent PTPs are highly toxic to *D. magna* but less so to *V. fischeri*. The results with *D. magna* showed toxicity 65–80%, higher after illumination in sea water (Sirtori et al., 2012).

4.3 TPs of PDBEs
Salvado et al. (2012) studied the TPs of polybromodiphenyl ethers (PBDEs) in superficial sediments from the Gulf of Lion. The levels of PPBDEs (sum of all PBDE congeners except BDE-209) varied from non-detectable to 2.6 ng/g with a mean value of 0.43 ng/g. BDE 209 predominated, originating from the commercial mixture deca-BDE, in all sediment samples at concentrations ranging between 0.06 and 140 ng/g. This compound and the less brominated BDE exhibited a southwestward decreasing concentration gradient following the dominant marine currents and bottom relief. The results indicated a progressive transformation of the dumped BDE 209 with a depletion paralleled by increases of the less brominated BDEs (from 8.6% to 22%). These less brominated compounds were accumulated at about 100-140 km away from the Rhone prodelta, e.g. at the end of the submarine canyons, evidencing that these transformation compounds can be accumulated at long distances from the dumping sites in the marine system (Salvado et al., 2012).

4.4 TPs pf 2,6-DNT and picric acid
Nipper et al. (2005) determined the toxicity of TPs of 2,6-dinitrotoluene (2,6-DNT) and 2,4,6-trinitrophenol (picric acid) in spiked sandy and fine-grained marine sediments and in seawater. The major identified biotransformation product of 2,6-DNT was 2-amino-6-nitrotoluene (2-A-6-NT), followed by 2-nitrotoluene (2-NT) and other minor components, and some of the main breakdown products of picric acid were 2,4-dinitrophenol and amino dinitrophenols, including picramic acid (Nipper et al., 2004). The researchers found that the toxicity of pore water from sediments spiked with 2,6-DNT decreased for the macro-alga, *Ulva fasciata*, zoospores as biotransformation proceeded, but increased for the copepod, *Schizopera knabeni*, nauplii. According to their findings, the primary biotransformation product of 2,6-DNT, 2-amino-6-nitrotoluene, was also more toxic than the parent compound to copepod *nauplii*, but not to alga zoospores, in spiked seawater tests. Two biotransformation products of picric acid, picramic acid and 2,4-DNP, were more toxic than their parent compound. After six-months incubation, the porewater toxicity from picric acid-spiked sediments decreased significantly, and fine-grained sediment spiked with either ordnance compound had lower toxicity than its sandy counterpart, suggesting faster microbial transformation in the former and production of less toxic products. Photo-transformation of 2,6-DNT in seawater resulted in a reduction in toxicity. The results of the study showed that neither 2,6-DNT nor its main biotransformation product, 2-A-6-NT, were highly toxic to copepod, *S. knabeni*, adult female survival. (LC50 values of 357 and >277 lmol/L respectively). The copepod *nauplii* hatching and survival endpoint, however, indicated significantly higher toxicity of the biotransformation product, with LC50 values of 50 and 303 lmol/L for 2-A-6-NT and 2,6-DNT, respectively. However, the opposite happened in *U. fasciata* zoospore germination tests, where the toxicity of the parent compound, 2,6-DNT, was markedly higher than that of 2-A-6-NT. Regarding the TPs of picric acid, 2,4-DNP and picramic acid, they proved to be more toxic to copepods and macro-alga zoospores than their parent compound (Nipper et al., 2005).
4.5 TPs of APEO surfactants
Zoller (2006) investigated the occurrence and toxicity of the nonionic biodegradation-resistant alkylphenol ethoxylate (APEO) surfactants and their TPs in Israel’s estuarine and coastal zone seawater and reported concentrations in sewage-containing rivers, estuaries and 50–60-m offshore sea (Mediterranean) water 12.5–75.1, 4.2–25.0 and 0.9–2.6 μg/L, respectively. Their toxicity was assessed by egg production by zebrafish, exposed to 75, 25 and 10 μg/L of a typical industrial APEOs that was reduced up to 89.6%, 84.7% and 76.9%, respectively, between the 8th and 28th days of exposure, indicating a possible APEOs-related ecotoxicological health risk problem. In a follow-up study (Zoller and Plaut, in press), the same authors found that exposure of zebrafish to concentrations of 10 and 25 Ag/L of commercial NP3EO and NP15EO (both found to be mixtures of homologic APEOs, picking at 3 and 15 EO units, respectively), resulted in significant reduction in egg production in the case of the first (NP3EO; 25 Ag/L), but not in the second (NP15EO), showing a higher toxicity especially for the short-chained compared to the longer-chained APEOs (Zoller, 2006).

4.6 TPs of azaarenes
Voogt and Laane (2009) investigated the occurrence and toxicity of azaarones, the TPs (oxidized derivatives) of azaarenes, a group of newly emerging chemical compounds. Azaarenes are polycyclic aromatic heterocyclic compounds containing one nitrogen atom in one of the aromatic rings. Azaarones, the oxidized TPs of the azaarenes (Figure 2) are often more toxic than the parent compound. These researchers determined for the first time the concentration of 7 azaarenes and their TPs in the surface sediments (fraction < 63 lm) of the Dutch coastal zone of the North Sea. The concentrations of acridine, benz[a]acridine, benz[c]acridine and 5,6-benzoquinoline varied between 10–63, 3.9–25, 3.3–11 and 3.98–10.84 ng/g, respectively. Concentrations of 7,8-benzoquinoline and phenanthridine were below the limit of detection. 2-Hydroxyquinoline and 5-hydroxyquinoline, probably metabolites of quinoline, were present in relatively high concentrations: 7.4–949 and 11–188 ng/g. The range in the concentrations of 6-hydroxyquinoline was 4–16 ng/g. The concentrations of acridine in the sediments varied between 9.97 and 63.5 ng/g, while its transformation product 9(10H)-acridone was detected only at one sampling station (11.2 ng/g). The concentrations of 6(5H)-phenanthridinone varied between 4.2–28 ng/g (Voogt and Laane, 2009).

The highest concentrations of the sum of azaarenes and the sum of the concentration of their metabolites were detected close to the coast and lower concentrations further offshore. The analysis in sediments showed that the cumulative concentrations of TPs were about four times the cumulative concentrations of the azaarenes. The concentrations of azaarenes and their metabolites measured were in the same order of magnitude as those found to induce phototoxicity to algae. The acute and chronic toxicity of azaarenes to several fish species, a few aquatic invertebrates, and marine algae had been published previously (Gissel-Nielsen and Nielsen, 1996; Wiegman et al., 2001). According to the study of Wiegman et al. (2001), some of the oxidized derivatives of azaarenes are easily produced photochemically in the aquatic environment and they are more toxic to algae than the parent compounds (Voogt and Laane, 2009).
5. CONCLUSIONS AND OUTLOOK

The occurrence of various categories of toxic pollutants has been well documented in the aquatic environment and raises concern for the ecosystems quality and human health. The evolution of analytical techniques and the optimization of procedures has enabled the analysis of pollutants at lower levels in environmental samples and has also revealed several TPs formed during biotic and abiotic processes. Such research is still very limited in the marine environment, however several studies have indicated that some of these TPs are present in considerable amounts in marine water and sediments, and can have more toxic effects than the parent compounds. Moreover, many of these compounds and their levels of formation, as well as other categories of TPs still remain unknown. One of the key challenges in environmental chemistry and ecotoxicology is to identify such TPs and their toxic effects in order to safeguard the quality of the marine environment.
REFERENCES