Chromatography
Separation Techniques

Mottaleb et al., J Chromatogr Sep Tech 2015, 6:3
DOI: 10.4172/2157-7064.1000267

Research Article Open Access

Use of LC-MS and GC-MS Methods to Measure Emerging Contaminants Pharmaceutical and Personal Care Products (PPCPs) in Fish

M Abdul Mottaleb[1,2*], Michael K Bellamy[2], Musavvir Arafat Mottaleb[2] and M Rafiq Islam[2]

[1]*Center for Innovation and Entrepreneurship, 1402 North College Drive, Northwest Missouri State University, Maryville, Missouri 64468, USA*
[2]*Department of Natural Sciences, 800 University Drive, Northwest Missouri State University, Maryville, Missouri 64468, USA*

Abstract

Aquatic ecosystems are continuously contaminated by manufactured pharmaceutical and personal care products (PPCPs). Non-regulated, multi-purpose PPCP contaminants enter aquatic systems through sewage/wastewater treatment plants after consumption and use by humans and animals. These micro-pollutants receive increased attention worldwidesince significant levels of contamination have been found in various environmental compartments and organisms. Highly sophisticated equipment such as liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are reliable ways to determine PPCPs at sub-ppb levels from air, waters, sediments, effluents, aquatic organisms and human body fluids. Although the consequences of these pollutants are gradually becoming visible, their potential impacts on aquatic ecosystems and organisms are poorly known. Some studies have suggested that PPCPs are persistent and have bioaccumulation potential leading to ecological effects and abnormalities in fish. Other findings illustrate that PPCPs can impair swimming behavior in fathead minnow (*Pimephales promelas*), interfere with thyroid axis in the zebra fish (*Danio rerio*), or form adducts with hemoglobin and/or suitable protein breakdown PPCPs products. Thus, this review focuses on PPCPs emerging contaminants concern with regards to sources, occurrences, analytical methods, fate and biological transformation.

Keywords: PPCPs; Micro-pollutants; Emerging contaminants; Biomarkers; Aquatic organisms; LC-MS and GC-MS

Introduction

In modern life, one of the most important issues in the world is the exposure to man-made chemicals that cause interference of regular activities such as reproduction and development of different organisms in the environment [1,2]. Some of them are hazardous and present potential or actual threat to human health, wildlife, aquatic organisms and/or soundings [2]. Newer analytical techniques have made it possible to identify these compounds at extremely low level of the order of sub-ng/g. These are frequently detected in different environmental compartments including surface waters, wastewaters, air, wildlife and fish, and had not been recognized previously at such low levels. These compounds are often referred to as "emerging contaminants" (ECs) because adequate information associated with their presence, occurrence, fate, transport and mechanisms are not available to assess their risk to human health and the ecosystem [3]. ECs are used daily in homes, farms, businesses and industry as detergents, fragrances, prescription and non-prescription drugs, disinfectants, and pesticides etc. Some ECs have been commonly found in water resources around the world and across the USA [4-6].

PPCPs as Endocrine Disruptors

Emerging evidence from wildlife and laboratory studies indicates that some chemicals may interfere with the endocrine system. Compounds identified include pesticides, polychlorinated biphenyls, dioxins, furans, alkyl phenols, and steroid hormones. These chemicals are routed to ecosystems through wastewater treatment plants. Several studies reported that many ECs present in municipal wastewater effluent can act as endocrine disruptors at concentrations capable of inducing fish feminization [7,8]. The feminization has been linked to exposure to compounds that mimic estrogen activity. However, it has also been determined that thousands of the compounds have the potential to interact with components of the endocrine system, altering the natural action of hormones [9,10] in both freshwater and marine fish species [4,8,11-13]. The occurrence of some ECs correlates with ecological effects and sexual abnormalities in fish [14-16]. In other studies, complex mixtures of ECs at environmentally relevant concentrations were reported to inhibit the growth of human embryonic cells [17,18]. Other evidence suggests that some ECs are persistent in the environment and survive through conventional water treatment plant and ultimately reaching the aquatic organisms [18]. Overall, an important concern, posed by ECs, is the interference of reproduction and development of aquatic organisms and wildlife [1,2,19].

Release of PPCPs into the Environment

Pharmaceuticals and personal care products (PPCPs) are a major class of ECs commonly used in human and animal applications. PPCPs are many chemical compounds with a variety of chemical structures, conformations, functional groups, polarities and characteristics. PPCPs include prescription and non-prescription drugs together with fragrances, cosmetic ingredients, diagnostic agents, biopharmaceuticals, and growth enhancing constituents used in livestock operations. Tons of these chemicals are produced annually worldwide [20]. After consumption, PPCPs are released into ecosystems via urine, feces or residues as either parent compounds or their metabolites. PPCPs enter the environmental system through effluent discharge from wastewater/sewage treatment plants, inappropriate disposal of expired or unused

***Corresponding author:** M Abdul Mottaleb, Center for Innovation and Entrepreneurship, 1402 North College Drive, Northwest Missouri State University, Maryville, Missouri 64468, USA, Tel: +1 660 562 0820; Fax: +1 660 562 1188; E-mail: mmottaleb@yahoo. com and mmottal@nwmissouri. edu

Received February 22, 2015; **Accepted** March 05, 2015; **Published** March 15, 2015

Citation: Mottaleb MA, Bellamy MK, Mottaleb MA, Islam MR (2015) Use of LC-MS and GC-MS Methods to Measure Emerging Contaminants Pharmaceutical and Personal Care Products (PPCPs) in Fish. J Chromatogr Sep Tech 6: 267. doi:10.4172/2157-7064.1000267

drugs, shower drain, residues from drug manufacturing companies, nursing homes and hospital facilities.

Wastewater treatment plants are not generally designed to eliminate the PPCPs because they are non-regulated water pollutants [21]. Based on the process design of the treatment plants, the elimination rates of drugs range from <10% (e. g. atenolol and carbamazepine) to almost complete removal (e. g. propranolol) [22]. As these compounds are continuously released into aquatic systems, the effluents from the wastewater treatment plants are considered as the main routes of human pharmaceuticals into the environment [23], reaching concentrations of ng/L to µg/L [24]. Aquatic organisms are consistently exposed to these non-regulated PPCPs, as environmental contaminants.

Most PPCPs/ECs ultimately end up in the aquatic environment. Consequently, these compounds frequently appear in ecosystems and are frequently detected in different environmental compartments and organisms at different concentration levels [25]. PPCPs have been frequently detected in different environmental matrices such as air, waters, sediments, sewage sludge, humans and fish [26-45]. The high rate of occurrence of PPCPs is due to the fact that they are easily dissolved in aquatic environment and do not evaporate at normal temperature and pressures. Moreover some PPCPs appear to show low biodegradation rate and high lipophilicity, and have high bioaccumulation potential as environmental contaminants [21]. Several studies have been published that further discuss the sources of PPCPs and their transportation from personal usages to waters and aquatic organisms [3,18, 21,22-27].

Until recently, many around the world were unaware that a new environmental health concern had emerged. Now, regulatory authorities, health agencies, and professional organizations, all over the globe are informed of the growing PPCP problem which drives research on the presence, occurrence, fate of the PPCPs and metabolites [26,27]. Today, the U. S. Environmental Protection Agency (EPA) and other organizations are working together to improve its understanding of a number of ECs, particularly PPCPs.

Occurrences and Effects of PPCPs on Organisms

PPCPs and their metabolites have been detected in aquatic and terrestrial organisms [28,29], surface water [30], air [31], sewage effluent [32], lake Michigan water and sediments [33], industrial sewage sludge [34], municipal effluents [35], marine sediments [36], marine mammals [37], effluent-dominated river water fish [38,39], Pecan Creek fish [40] and German fish specimen bank [41], fish-eating birds and fish [42], receiving marine waters and marine bivalves [43]. PPCPs and metabolites have also been identified in human milk [44], and human blood [45]. More importantly, multiple studies have indicated that PPCPs are not only accumulated but also subsequently metabolized to reactive intermediates that form covalently-bound protein adducts in human [46] and aquatic organisms such as fish [47,48].

Studies indicate that many PPCPs are environmentally persistent, bioactive, and have bioaccumulation potential [49-52]. For example, the PPCPs known astriclosan (TCS), an antimicrobial agent, has been widely used in dental care products, disinfectants, hand soaps, footwear, skin care creams and textiles. TCS and its methyl metabolites were detected in surface waters [53], biosolids [54], fish [55], and algae [56]. Still, the fate and chemistry of TCS are not fully understood. TCS is quite stable to hydrolysis; however its photolysis was identified as one of the major pathways of degradation in surface waters [57]. Other research groups have shown that TCS in surface water may be toxic to certain algae species. Specifically, Orvos et al. [58] found no observer-effect concentration (72-h growth) at 500 ng/L for algae *Scenedesmus subspicatus* while Wilson [59] reported that TCS may cause significant increase in Synedra algae and a substantial reduction of the rare genus Chlamydomonas algae at 15 ng/L and 150 ng/L. Levy et al. [60] demonstrated that TCS can block bacterial lipid biosynthesis inhibiting the enzyme enoyl-acyl carrier protein reductase, which leads to a possible development of bacterial resistance to TCS. Recent studies have shown that TCS impaired swimming behavior and altered expression of excitation-contraction coupling proteins in fathead minnow (*Pimephales promelas*) [61] and interfered with thyroid axis in the zebra fish (*Danio rerio*) [62].

Analytical Methods

Modern equipment has made it made possible to detect PPCPs from different matrices at sub-ng/g levels. The main advances in PPCPs analysis have been made using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) techniques. Pharmaceuticals comprised of polar compounds are easily dissolved in water or polar solvents, which is special advantage for LC-MS analysis. Employing isocratic or gradient elution in LC method, complex composites/mixtures of sample can be separated using different polarities (polar or medium polar or mixed polar) mobile phases with an analytical column such as C18. The separated compounds are characterized with MS detection. LC - tandem mass spectrometry (LC-MS/MS) with positive- and or negative modes of operations using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are able to detect PPCPs up to sub-ppb level. The presence of unknown compounds are confirmed and identified by comparing the mass signals and retention times of unknown samples to known standards.

On the other hand, personal care products (PCPs) are relatively non-polar and are more easily dissolved and extracted in relatively non-polar organic solvents. After cleaned up, the samples are analyzed by GC- MS/MS or - selected ion monitoring (SIM) modes with election ionization (EI) or negative ion chemical ionization (NICI) based on sample nature. The GC-MS/MS or GC-SIM-MS methods are capable of detecting PPCPs down to sub-ng/g levels. The presence of unknown compounds are characterized establishing over 80% to 99% agreement with standard compounds of the respective samples [40,63]. Examples of some analyses performed by LC-MS and GC-MS are illustrated below.

Analysis of PPCPs by LC-MS and GC-MS

Many researchers have reported the identification and analysis of emerging PPCPs contaminants [39,42,64]. Our research group [39,64] developed LC-MS/MS methods for determination of pharmaceuticals and metabolites from environmental fish. Specifically, non-linear gradient elution of water and methanol solvents consisting of 0. 1% (v/v) formic acid in water and 100% methanol are passed through a C18 analytical column at a flow rate 350 µL/min to achieve the separation of the complex mixture of PPCPs. An auto-sampler is used to inject 10 µL sample solution. Column effluents are monitored by MS/MS equipped with an electrospray interface (ESI). Figure 1 displays chromatographic separation of 25 target pharmaceutical drugs and metabolites, 5 surrogates and 2 internal standards that were spiked to the clean fish tissues by LC-MS/MS using electrospray ionization (ESI) positive (+) and negative (-) modes.

Employing the extraction protocol and LC-MS/MS method [64] all target compounds were analyzed from environmental fish samples that were obtained from the Pecan Creek, Denton, Texas, downstream

from the effluent discharge. The presence and characterization of the target analytes were confirmed based on comparison of retention time and relative intensities of fragment ions observed from spiked and environmental fish specimens. Four pharmaceutical compounds were detected over method detection limits (MDLs). Figure 2 displays a typical LC-MS/MS ion chromatogram for identification and determined the concentration of dyphenhydramine (0. 66-1. 32 ng/g) ,diltiazem (0. 11-0. 27 ng/g), carbamazepine (0. 83-1. 44 ng/g), norfluoxetine (3. 49-5. 114 ng/g) drugs [64]. Method performance associated with method detection limits (MDLs), limit of detection (LOD) and limit of quantitation (LOQ) of 25 target compounds were compared and illustrated in Table 1. It was estimated that MDLs were approximately 3 to 10 times higher than LOD for a majority of the target analytes. In the LC-ESI-MS/MS analyses, matrix influences played a critical role that was essential to consider. These matrix effects are caused by co-extracted constituents that affect analyte ionization using either ESI positive or negative modes [42]. An approach, developed by our group [64] was used to measure the matrix influence for extraction solvents that promoted recoveries. The matrix effect in analyzing the samples that influence mass signal generation, matrix-match calibration curve was proven to be excellent method that minimized the matrix effect in quantitation of analytes from the environmental samples such as fish [64].

GC-MS is a highly efficient tool widely used to analyze semi-volatile and volatile organic personal care products at extremely low levels from environmental samples. Sample nature and complexity are critical considerations in choosing the GC-MS techniques. Extraction/pre-concentration and clean-up steps are required in preparation of samples for GC-MS examination. As fish samples are complex matrices containing lipids, fat etc. , a wide range of extraction and clean-up techniques are needed to handle the samples prior to analysis [40,63,65-68]. Extraction methodologies include Soxhlet extraction, microwave assisted extraction, ultrasound-solid liquid extraction, and pressurized liquid extraction (PLE) and clean-up approaches are silica gel, florisil, and/or gel permeation chromatography (GPC) [40,63,65-68]. Figure 3 shows a schematic diagram of fish sample extraction, pre-concentration and clean-up protocols for GC-MS analysis [63].

Our research group developed GC-SIM-MS and GC-MS/MS

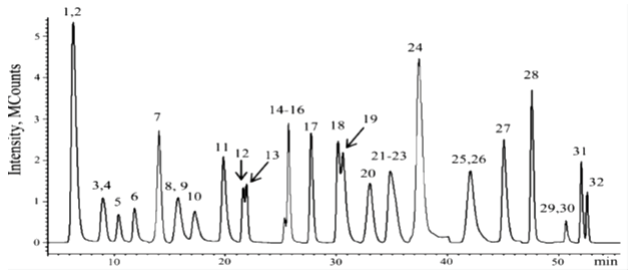

Figure 1: LC-MS/MS total ion chromatogram resulting from analysis of clean tissue spiked with a mixture of pharmaceutical standards. Peak identifications are as follows: (1) acetaminophen-d4, (2) acetaminophen, (3) atenolol, (4) cimetidine, (5) codeine, (6) 1,7-dimethylxanthine, (7) lincomycin, (8) trimethoprim, (9) thiabendazole, (10) caffeine, (11) sulfamethoxazole, (12) 7-aminoflunitrazepam-d7 (+IS), (13) metoprolol, (14) propranolol, (15) diphenhydramine-d3, (16) diphenhydramine, (17) diltiazem, (18) carbamazepine-d10, (19) carbamazepine, (20) tylosin, (21) fluoxetine-d6, (22) fluoxetine, (23) norfluoxetine, (24) sertraline, (25) erythromycin, (26) clofibric acid, (27) warfarin, (28) miconazole, (29) ibuprofen-13C3, (30) ibuprofen, (31) meclofenamic acid (-IS), and (32) gemfibrozil. (Reproduced with permission from (64), © American Chemical Society).

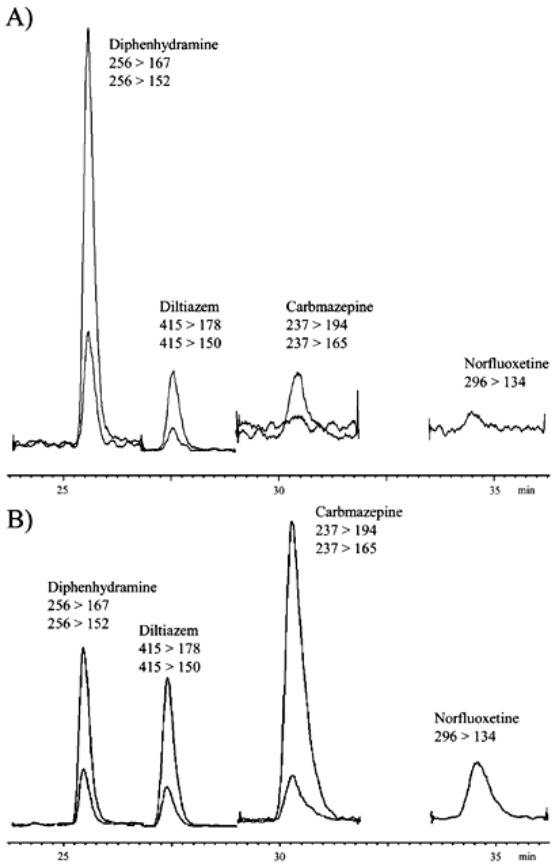

Figure 2: LC-MS/MS reconstituted ion chromatograms displaying analyte-specific quantitation and qualifier ions monitored for (A) a tissue extract from a fish (Lepomis sp.) collected in Pecan Creek and (B) an extract from clean' tissue spiked with known amounts of diphenhydramine (1.6 ng/g), diltiazem (2.4 ng/g), carbamazepine (16 ng/g), and norfluoxetine (80 ng/g). The higher m/z fragment is more intense in all cases. (Reproduced with permission from (64), © American Chemical Society).

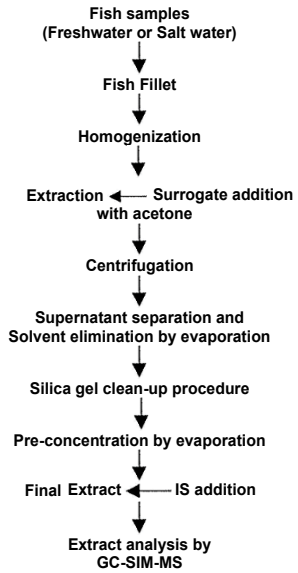

Figure3: A simplified schematic diagram of the experimental procedure used for extraction and analysis of nitromusks, antimicrobial agent and antihistamine from edible fish fillets. (*Reproduced with permission from (63), © Elsevier*).

Analyte	Linear range,[a] (ng/g)	LOD,[b](ng/g)	LOQ[c] (ng/g)	MDL[d] (ng/g)
Acetaminophen	3.12 - 400	0.30	0.99	4.40
Atenolol	1.25 - 160	0.48	1.62	1.48
Cimetidine	0.625 - 80	0.24	0.81	1.04
Codeine	4.60 - 600	1.07	3.55	6.11
1,7-dimethylxanthine	0.625 - 80	0.17	0.58	1.02
Lincomycin	3.12 - 400	0.63	2.09	5.53
Trimethoprim	1.25 - 160	0.79	2.63	2.15
Thiabendazole	1.25 - 160	0.14	0.47	2.63
Caffeine	3.12 - 400	0.34	1.15	3.93
Sulfamethoxazole	1.25 - 160	0.23	0.76	2.29
Metoprolol	1.25 - 160	0.25	0.85	2.50
Propranolol	0.625 - 80	0.01	0.03	1.07
Diphenhydramine	0.0625 - 8	0.01	0.03	0.05
Diltiazem	0.09 - 12	0.04	0.13	0.12
Carbamazepine	0.625 - 80	0.03	0.12	0.54
Tylosin	3.12 - 400	1.18	3.93	5.02
Fluoxetine	4.69 - 600	0.76	2.54	6.73
Norfluoxetine	3.12 - 400	0.32	1.08	2.90
Sertraline	3.12 - 400	0.21	0.71	3.57
Erythromycin	3.12 - 400	0.85	2.84	6.42
Clofabric acid	1.25 - 160	0.10	0.32	2.69
Warfarin	0.625 - 80	0.09	0.29	0.86
Miconazole	3.12 - 400	0.39	1.32	10.8
Ibuprofen	25 - 3200	3.14	10.4	45.9
Gemfibrozil	3.12 - 400	0.25	0.85	6.68

Table 1: Investigated linear range, LOD, LOQ, and MDL for target analytes in fish muscle tissue[a].

[a]clean tissues employed in the determination of these parameters were extracted using a 1:1 mixture of 0.1 M acetic acid (pH 4) and methanol. [b]LOD, calculated as3 times the standard deviation in the background signal observed for the replicate analysis of a tissue blank. [c]LOQ, calculated as 10 times the standard deviation in the background signal observed for the replicate analysis of a tissue blank, d MDL, determined by multiplying the one-sided Student's t–statistics at 99% confidence limit times the standard deviation observed for eight replicate analyses of a matrix spike (spiking level ≤ 10 x MDL). (*SOURCE Reproduced with permission from reference 64, Copyright© American Chemical Society*).

methods [40,41] for PPCPs analysis from fish and other environmental samples following U. S. EPA protocols. For example, Mottaleb et al. [40] detected the presences of PCPs in environmental fish collected from Pecan Creek and Clear Creek streams, Denton, TX, USA. Figure 4 represents a GC-SIM-MS total ion chromatogram for standard solution displaying the separation of compounds that were targeted in the environmental fish collected from the Pecan Creek, Denton, Texas.

Employing the extraction protocols illustrated (Figure 3) [63],we recently reported the concentration of four frequently observed PPCPs in edible fish fillets obtained from grocery stores by GC-SIM-MS [63]. In this investigation, the presence of the target compounds in fish extracts was confirmed based on similar mass spectral features and retention times compared to pure standards. Mass spectral features and retention times of the target compounds obtained from the fish extracts were used for characterization by comparing with the authentic standards. Figure 5 is a GC-SIM-MS ion chromatogram for (A) a standard solution containing, 100 pg/μL of triclosan, an antimicrobial agent and (B) a Whiting (genus *Merlangius*) fillet extract. Individual identities of the compounds extracted from the grocery store fish fillet were characterized based on comparison of the relative ion abundance ratios between the quantification and the qualifier ions mass signals. The presence of target compounds in the fish fillet extracts was confirmed when the difference of the relative abundance ratio was less than or equal to approximately ± 20%, or an agreement of the relative abundance ratio of 80% or over. Figure 5 compares typical mass spectra derived from the ion chromatogram. These spectra show an excellent agreement of the mass spectral features/mass signals with a variation of about ± 10%. When similar agreement of ion relative abundance ratio and retention features were observed, then the presence, characterization and quantification of other compounds in the different fish samples were established. Table 2 shows the concentrations of the compounds that were characterized and quantified in grocery stores fish species. The values of detected compounds from grocery store samples are approximately 1 to 3 orders of magnitude lower than the fish that were collected from the environmental sites [39-42,64].

Biological Transformation and Effects of PPCPs

Biotransformation of any chemicals is a critical consideration because the compounds get metabolized in biological system forming different species or breakdown products that may induce numerous issues over the period of time. The effects of PPCPs differ from those of conventional pollutants because drugs are intentionally designed to interact with cellular receptors at low concentrations and to cause specific biological effects. Unintended adverse effects can also occur from interaction with non-target receptors. Environmental toxicology focuses on acute effects of exposure rather than chronic effects. Effects on aquatic life are a major concern because aquatic organisms receive more exposure risks than do human, and are exposed with continual and multi-generational basis with higher concentrations of PPCPs in untreated water possible low dose effects. The risks posed to aquatic organisms by trace level concentration of the PPCPs are largely unknown. Some of the known potential impacts on organisms include delayed development in fish, delayed metamorphosis in frogs, and a variety of reactions including altered behavior and reproduction [1,2]. Overall, the behavior and fate of pharmaceuticals and their metabolites in the aquatic environment and organism are not well established. The low volatility and increase polarity of pharmaceuticals indicates that distribution in the environment occurs primarily through aqueous transport, but also via food chain dispersal. Recent studies have indicated that many pharmaceuticals and metabolites are environmentally persistent, bioactive, and have potential for bioaccumulation [49,50]. Acute aquatic toxicities of drugs and metabolites were examined on marine bacterium (*Vibrio fischeri*), a freshwater invertebrate (*Daphnia magna*), and the Japanese medaka fish (*Oryzia slatipes*) by Kim et al. [69]. They demonstrated that *Daphnia* was the most susceptible among the tested organisms. Correa and Hoffmann [70] studied the variation of magnitude of response effect of drugs d-amphetamine, sodium pentobarbital, diazepam, β-carboline, and saline before and after inducing into of knife-fish (*Gymnotus carapo*). They concluded a reduction of the degree of alertness by the barbiturate and a decrease in emotionality and/or stress by the benzodiazepine with the novelty response. Brandao et al. [71] evaluated the biochemical and behavioral effects of neuro-active anticonvulsant drugs (diazepam, carbamazepine, and phenytoin) on pumpkin-seed sunfish (*Lepomis gibbosus*) and showed behavioral changes of sunfish through oxidative stress parameters such as glutathione reductase, glutathione S-transferases, catalase and lipid peroxidation.

Mottaleb et al. [47,72,73] investigated the biotransformation and toxicokinetics of PPCPs known as nitro musks fragrance ingredients, musk xylene (MX), and musk ketone (MK) using trout fish as model. The fish were exposed to nitro musks compounds. Details regarding fish exposure, extraction, and analysis of breakdown product or metabolites have been reported [47,72,73]. Previously we demonstrated the formation of amine (cysteine - hemoglobin) adducts through enzymatic biotransformation reaction between MX/MK and

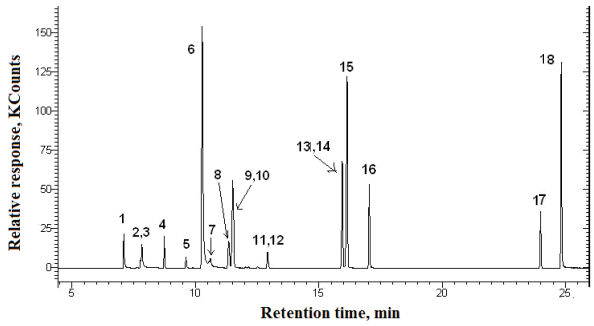

Figure 4: GC-SIM-MS representative total ion chromatogram for a calibration standard. Peak identifications are as follows: (1) *m*-toluamide, (2) benzophenone, (3) benzophenone-d_{10}, (4) celestolide, (5) pentachloronitro benzene, (6) phenanthrene-d_{10}, (7) *p-n*-octylphenol, (8) galaxolide, (9) tonalide, (10) musk xylene, (11) *p-n*-nonylphenol, (12) [$^{13}C_6$]*p-n*-nonylphenol, (13) 4-methylbenxylidine camphor, (14) 2,2'- dinitrobiphenyl, (15) musk ketone, (16) triclosan, (17) mirex, and (18) octocrylene. (*Reproduced with permission from (40), 2009, © Elsevier*).

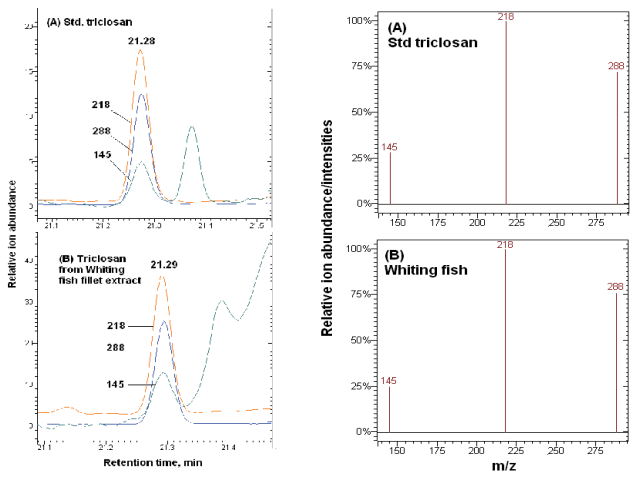

Figure 5: (i) Representative GC-SIM-MS ion chromatograms for (A) a standard solution containing, 100 pg/µl of triclosan and (B) a Whiting (genus *Merlangius*) fillet extract (left). (5 ii) Typical GC-SIM-MS mass spectra for (A) standard triclosanand (B) Whiting fillet extract solution derived by selecting the data file from Fig. 5 (i) (A), retention time 21.28 min and Fig. 5 (i) (B), retention time 21.29 min, respectively (right). (*Reproduced with permission from (63), © Elsevier*).

Name of fish	Concentration of analytes in edible fish fillets (ng g^{-1})				
	HHCB	**AHTN**	**DPH**	**MK**	**TCS**
Tilapia	0.876	0.813	0.679	nd	4.122
Catfish	0.276	0.429	0.939	nd	2.086
Swai	0.336	0.190	0.189	nd	1.782
Flounder	0.892	0.904	1.182	nd	7.472
Salmon	0.250	0.068	1.037	nd	0.942
Whiting	0.263	0.431	0.503	nd	3.699
Pollock	0.163	0.304	0.692	nd	1.011
Yellow fin Tuna	0.343	0.269	0.811	nd	2.292

Table 2: Concentration of target analytes in fish fillets received from local grocery stores, Maryville, Missouri, USAand their comparison with environmental fish samples.
nd – not detected. (*Source:Reproduced with permission from (63), © Elsevier*).

trout [47,74]. The reduction of a nitro-group in MX and MK led to the formation of amine adducts of hemoglobin that could be suitable as a biochemical endpoint useful for exposure monitoring and assessment of potential hazards resulting from MX and MK compounds [47,72-74].

Recently our group [48] also reported that the bound metabolites obtained from liver proteins may be used as indicators of internal exposure to chemical carcinogens. Table 3 illustrates the relationship between exposure time and the uptake of different dosages of MX and MK compounds over a period of 7 days. The metabolites of nitro musks or other related nitroarenes, bound to the cysteine sulfhydryl group (– SH) of proteins in liver as biomarkers of exposure, could potentially be used to assess continuous exposure over a longer time range, and thus, may be better suited for risk assessment than quantitation of urinary metabolites [75]. The biological transformation processes of MX and MK to their corresponding amine metabolites, with cysteine containing proteins in the liver results in adducts formation are shown in Figure 6. Nitroarenes are enzymatically reduced to nitroso reactive intermediates, nitrosoarenes, capable of covalently binding with the – SH group of cysteine amino acids in proteins to form an acid/base labile sulfonamide adducts that hydrolyzes to aromatic amines in the presence of aqueous base [76]. The aromatic amines were considered to be good dosimeters for the target tissue [77].

Spike recovery studies and limit of detection (LOD) measurements of 2-amino-MX, 4-amino-MX and 2-amino MK metabolites were accomplished as 95-114% with relative standard deviation <10% and 0. 91-3. 8 ng/g, respectively. Table 4 illustrated the concentration of metabolites observed in the trout liver [48]. The half-lives of 2-AMX, 2-AMK and 4-AMX metabolites were estimated to be 2 - 9 days in the trout liver based on the assumption of first-order kinetics. The individual values of the elimination rate constants and the half-lives of the 2-AMX, 2-AMK and 4-AMX metabolites in the fish liver suggested that the toxico kinetics are more complex than a simple first-order reaction because additional internal biological processes transpire in the living organisms. Nitromusks (MX and or MK) were identified as inducers of hepatic cytochrome P450 2B enzymes and P450 1A1 and 1A2 isoenzymes [78,79] and are non-genotoxic [80,81]. Although the non-genotoxic carcinogenesis is not fully understood, it is believed that a non-genotoxic mechanism, such as increased cell proliferation, might be responsible for the increase in the liver tumors [82]. In our investigation [48], the MX and or MK-cysteine-protein adducts in fish liver were used to monitor nitromusks hazards as biomarkers of exposure.

Conclusions

PPCPs are increasingly being used in human and animal applications for numerous purposes. The ultimate fates of these chemicals are in aquatic systems, where organisms get exposed over an extended period of time through wastewater treatment plants and other sources. Continuous loading of the parent compounds and metabolites of PPCPs will reach harmful concentrations that adversely affect the freedom of aquatic creatures. Thus, periodic measurements of exposure level of those compounds are very important. Modern analytical techniques such as LC-MS and GC-MS have made it possible to detect extremely low levels of those chemicals.

To address the challenge of PPCPs as emerging contaminants, regulatory authorities or agencies or health care professionals need to work collectively. An important and effective way aspect to reduce the load of PPCPs and their metabolites in wastewaters and surface

Exposure time, Day	MX exposure					MK exposure				
	MX conc. (mg/mL)	Fish wet weight, (g)	Liver wet weigh, (g)	MX dose / trout, (mg)	Average dosing level, (mg/g)	MK conc. (mg/mL)	Fish wet weigh, (g)	Liver wet weigh, (g)	MK dose / trout, (mg)	Average dosing level, (mg/g)
1- Day	10	202	0.75	2.0	0.01	10	257	0.88	2.6	0.01
		256	0.87	2.5			237	0.77	2.4	
		165	0.50	1.6			222†	NC	2.2	
	30	180	0.75	5.4	0.03	30	199	0.70	6.0	0.03
		256	0.92	7.5			230	0.76	6.9	
		280‡	1.41	8.4			212	0.69	6.3	
	100	236	0.76	24.0	0.10	100	272	1.26	27.0	0.10
		264	0.92	26.0			271	1.26	27.0	
		204	0.74	20.0			197	0.78	20.0	
	300	250	0.88	75.0	0.30	300	190	0.70	57.0	0.30
		310	1.52	90.0			270	1.18	81.0	
		227	0.71	69.0			250	1.00	75.0	
	Control	206	0.76	0.20 mL, exposed with salmon oil only						
		304	1.52	0.30 mL, exposed with salmon oil only						
		184	0.70	0.18 mL, exposed with salmon oil only						
3-Days	30	208	0.79	6.3	0.03	30	278	1.22	8.4	0.03
		244	0.81	7.2			156	0.46	4.5	
		193	0.71	6.0			196	0.68	6.0	
	Control	253	0.89	0.25 mL, exposed with salmon oil only						
		272	1.23	0.27 mL, exposed with salmon oil only						
		233	0.75	0.23 mL, exposed with salmon oil only						
7-Days	30	212‡	0.71	6.3	0.03	30	121	0.30	3.6	0.03
		230	0.76	6.9			241	0.88	7.2	
		204	0.74	6.0			167	0.53	5.1	
	Control	273	1.12	0.27 mL, exposed with salmon oil only						
		305	1.45	0.30 mL, exposed with salmon oil only						
		250	0.89	0.25 mL, exposed with salmon oil only						

†Trout was found dead and the liver sample was not collected (NC). ‡Trout was found sick (not equilibrium condition) and the collected liver specimen was not used in this study for composite preparation. All control liver samples were mixed together to make one control composite specimen. The collected MX and or MK exposed liver samples were composited mixing three liver for each dosing level with exception of sick or dead fish liver. (*Source: Reproduced with permission from (48), 2012, © Elsevier*).

Table 3: *In vivo* trout exposure dosing schedule with nitro musk compounds and salmon oil vehicle.

Exposure period (Day)	Exposure level MX or MK (mg/g)	Nitro musk metabolites (ng/g)		
		2-AMX	4-AMX	2-AMK
1-Day	0.01	94.0	2404.4	115.4
	0.03	492.0	12588.5	505.5
	0.10	444.1	10325.9	426.6
	0.30	259.1	5147.3	396.1
3-Days	0.03	213.6	6097.6	357.8
7-Days	0.03	113.5	2988.3	298.0
Controls	None	Not detected		

(*Source: Reproduced with permission from (48), © Elsevier*).

Table 4: Concentration of nitro musk metabolites in trout liver samples using hydrolyzed extraction.

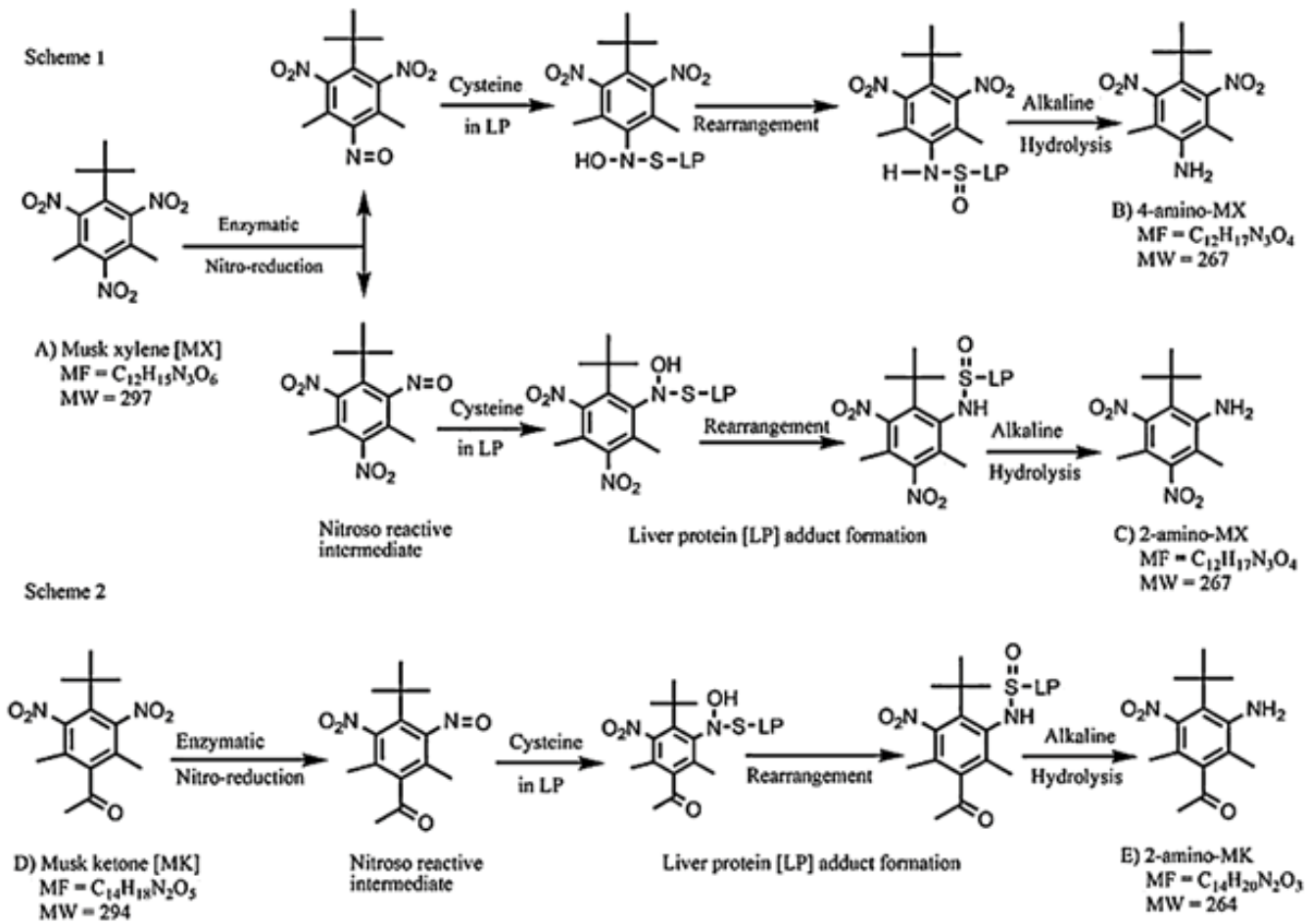

Figure 6: A possible biological transformation pathway of nitro musks (MX or MK) to corresponding metabolite amino compounds illustrating formation of nitroso adduct with cysteine containing proteins in the fish liver. Scheme 1: musk xylene (A) to 4-amino-musk xylene (B) and to 2-amino-musk xylene (C). Scheme 2: musk ketone (D) to 2-amino-musk ketone (E) (*Reproduced with permission from (48), © Elsevier*).

waters is to develop new sewage treatment processes. This requires understanding the fate of PPCPs during sewage treatment plants for implementation of better removal techniques. Consumers need to be aware of the consequences of PPCPs to aquatic organisms and ecosystems, and should follow the regulatory agencies disposal guidelines to make our environment friendly for all living organisms. At the same time, scientists and toxicologists should continue to investigate the transport, fate, toxicity and their potential physiological and psychological effects on humans and wildlife as well as relationship between bioaccumulation and diseases.

References

1. Stone R (1994) Environmental estrogens stir debate. Science 265: 308-310.
2. Ashby J, Houthoff E, Kennedy SJ, Stevens J, Bars R, et al. (1997) The challenge posed by endocrine-disrupting chemicals. Environ Health Perspect 105: 164-169.
3. Daughton CG, Ternes TA (1999) Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 107 Suppl 6: 907-938.
4. Hinck JE, Blazer VS, Schmitt CJ, Papoulias DM, Tillitt DE (2009) Widespread occurrence of intersex in black basses (Micropterus spp.) from U.S. rivers, 1995-2004. Aquat Toxicol 95: 60-70.
5. Loraine GA, Pettigrove ME (2006) Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in southern California. Environ Sci Technol 40: 687-695.
6. Barnes KK, Kolpin DW, Furlong ET, Zaugg SD, Meyer MT, et al. (2008) A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States - I) Groundwater. Sci Total Environ 402: 192-200.
7. Hahlbeck E, Griffiths R, Bengtsson BE (2004) The juvenile three-spined stickleback (Gasterosteus aculeatus L.) as a model organism for endocrine disruption. I. Sexual differentiation. Aquat Toxicol 70: 287-310.
8. Harris CA, Hamilton PB, Runnalls TJ, Vinciotti V, Henshaw A, et al. (2011) The consequences of feminization in breeding groups of wild fish. Environ Health Perspect 119: 306-311.
9. Xu P, Drewes JE, Kim T, Bellona C, Amy G (2006) Effect of membrane fouling on transport of emerging organic contaminants in NF/RO membrane applications. J Membrane Sci 279: 165-175.
10. Drewes JE, Hoppe C, Jennings T (2006) Fate and transport of N-nitrosamines under conditions simulating full-scale groundwater recharge operations. Water Environ Res 78: 2466-2473.
11. Jakimska A, SliwkaKaszynska M, Nagorski P, KotWasik A, Namiesnik J (2014) Environmental fate of two psychiatric drugs, diazepam and sertraline: Phototransformation and investigation of their photoproducts in natural waters. J Chromatogr Sep Tech 5: 253.
12. Blazer VS, Iwanowicz LR, Iwanowicz DD, Smith DR, Young JA, et al. (2007) Intersex (testicular oocytes) in smallmouth bass from the Potomac River and selected nearby drainages. J Aquat Anim Health 19: 242-253.

13. De Metrio G, Corriero A, Desantis S, Zubani D, Cirillo F, et al. (2003) Evidence of a high percentage of intersex in the Mediterranean swordfish (Xiphias gladius L.). Mar Pollut Bull 46: 358-361.

14. Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP (1998) Widespread sexual disruption in wild fish. Environ SciTechnol 32: 2498-2506.

15. Vajda AM, Barber LB, Gray JL, Lopez EM, Woodling JD, et al. (2008) Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environ Sci Technol 42: 3407-3414.

16. Vajda AM, Barber LB, Gray JL, Lopez EM, Bolden AM, et al. (2011) Demasculinization of male fish by wastewater treatment plant effluent. Aquat Toxicol 103: 213-221.

17. Pomati F, Orlandi C, Clerici M, Luciani F, Zuccato E (2008) Effects and interactions in an environmentally relevant mixture of pharmaceuticals. Toxicol Sci 102: 129-137.

18. Westerhoff P, Yoon Y, Snyder S, Wert E (2005) Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. Environ SciTechnol 39: 6649-6663.

19. Lange A, Katsu Y, Ichikawa R, Paull GC, Chidgey LL, Coe TS (2008) Altered sexual development in roach (Rutilusrutilus) exposed to environmental concentrations of the pharma¬ceutical 17a-ethinylestradiol and associated expression dynamics of aromatases and estrogen receptors. Toxicol Sci 106: 113–123.

20. Christen V, Hickmann S, Rechenberg B, Fent K (2010) Highly active human pharmaceuticals in aquatic systems: A concept for their identification based on their mode of action. Aquat Toxicol 96: 167-181.

21. Daughton CG (2004) Non-regulated water contaminants: emerging research. Environ Impact Assess Rev 24: 711-732.

22. Miege C, Choubert JM, Ribeiro L, Eusebe M, Coquery M (2009) Fate of pharmaceuticals and personal care products in wastewater treatment plants--conception of a database and first results. Environ Pollut 157: 1721-1726.

23. Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. Aquat Toxicol 76: 122-159.

24. Mompelat S, Le Bot B, Thomas O (2009) Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. Environ Int 35: 803-814.

25. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, et al. (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environ Sci Technol 36: 1202-1211.

26. Brodin T, Fick J, Jonsson M, Klaminder J (2013) Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science 339: 814-815.

27. Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FA (2007) Food web-specific biomagnification of persistent organic pollutants. Science 317: 236-239.

28. Snyder SA, Westerhoff P, Yoon Y, Sedlak DL (2003) Pharmaceuticals, personal care products and endocrine disruptors in water: implications for the water industry. Environ Eng Sci 20: 449–469.

29. van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13: 57-149.

30. Wu C, Huang X, Witter JD, Spongberg AL, Wang K, et al. (2014) Occurrence of pharmaceuticals and personal care products and associated environmental risks in the central and lower Yangtze river, China. Ecotoxicol Environ Saf 106: 19-26.

31. Kallenborn R, Gatermann R, Planting S, Rimkus GG, Lund M, et al. (1999) Gas chromatographic determination of synthetic musk com-pounds in Norwegian air samples. J Chromatogr A 846: 295-306.

32. Osemwengie LI, Steinberg S (2001) On-site solid-phase extraction and laboratory analysis of ultra-trace synthetic musks in municipal sewage effluent using gas chromatography-mass spectrometry in the full-scan mode. J Chromatogr A 932: 107-118.

33. Blair BD, Crago JP, Hedman CJ, Klaper RD (2013) Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern. Chemosphere 93: 2116-2123.

34. Berset JD, Bigler P, Herren D (2000) Analysis of nitro musk compounds and their amino metabolites in liquid sewage sludges using NMR and mass spectrometry. Anal Chem 72: 2124-2131.

35. Gagne F, Blaise C, Andre C (2006) Occurrence of pharmaceutical products in a municipal effluent and toxicity to rainbow trout (Oncorhynchusmykiss) hepatocytes. Ecotoxicol Environ Safety 64: 329-336.

36. Beretta M, Britto V, Tavares TM, Silva SMT, Pletsch AL (2014) Occurrence of Pharmaceutical and personal care products (PPCPs) in marine sediments in the Todosos Santos Bay and the north coast of Salvador, Bahia, Brazil. J Soils Sediments 14: 1278-1286.

37. Nakata H (2005) Occurrence of synthetic musk fragrances in marine mammals and sharks from Japanese coastal waters. Environ Sci Technol 39: 3430-3434.

38. Mottaleb MA, Brumley WC, Pyle SM, Sovocool GW (2004) Determination of a bound musk xylene metabolite in carp hemoglobin as a biomarker of exposure by gas chromatography-mass spectrometry using selected ion monitoring. J Anal Toxicol 28: 581-586.

39. Ramirez AJ, Brain RA, Usenko S, Mottaleb MA, O'Donnell JG, et al. (2009) Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. Environ Toxicol Chem 28: 2587-2597.

40. Mottaleb MA, Usenko S, O'Donnell JG, Ramirez AJ, Brooks BW, et al. (2009) Gas chromatography-mass spectrometry screening methods for select UV filters, synthetic musks, alkylphenols, an antimicrobial agent, and an insect repellent in fish. J Chromatogr A 1216: 815-823.

41. Subedi B, Mottaleb MA, Chambliss CK, Usenko S (2011) Simultaneous analysis of select pharmaceuticals and personal care products in fish tissue using pressurized liquid extraction combined with silica gel cleanup. J Chromatogr A 1218: 6278-6284.

42. Tanoue R, Nomiyama K2, Nakamura H3, Hayashi T4, Kim JW5, et al. (2014) Simultaneous determination of polar pharmaceuticals and personal care products in biological organs and tissues. J Chromatogr A 1355: 193-205.

43. McEneff G, Barron L, Kelleher B, Paull B, Quinn B (2014) A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves. Sci Tot Environ 476-477: 317-326.

44. Liebl B, Ehrenstofer S (1993) Nitro musk in human milk. Chemosphere27: 2253-2260.

45. Hu Z, Shi Y, Niu H, Cai Y, Jiang G, et al. (2010) Occurrence of synthetic musk fragrances in human blood from 11 cities in China. Environ Toxicol Chem 29: 1877-1882.

46. Riedel J, Dekant W (1999) Biotransformation and toxicokinetics of musk xylene in humans. Toxicol Appl Pharmacol 157: 145-155.

47. Mottaleb MA, Brumley WC, Curtis LR, Sovocool GW (2005) Nitro musk adducts of rainbow trout hemoglobin: dose-response and toxicokinetics determination by GC-NICI-MS for sentinel species. Am Biotech Lab 23: 26-29.

48. Mottaleb MA, Osemwengie LI, Islam MR, Sovocool GW (2012) Identification of bound intro musk-protein adducts in fish liver by gas chromatography-mass spectrometry: Biotransformation, dose-response and toxicokinetics of nitro musk metabolites protein adducts in trout liver as biomarkers of exposure. Aquat Toxicol 106-107: 164-172.

49. Valdes ME, Ames MV, Bistoni Mde L, Wunderlin DA (2014) Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the SuquÃa River basin (CÃ³rdoba, Argentina). Sci Total Environ 472: 389-396.

50. Gomez E, Bachelot M, Boillot C, Munaron D, Chiron S, et al. (2012) Bioconcentration of two pharmaceuticals (benzodiazepines) and two personal care products (UV filters) in marine mussels (Mytiluisgalloprovincialis) under controlled laboratory conditions. Environ Sci Pollut Res19: 2561-2569.

51. Peck AM (2006) Analytical methods for the determination of persistent ingredients of personal care products in environmental matrices. Anal Bioanal Chem 386: 907-939.

52. Mackay D, Barnthouse L (2010) Integrated risk assessment of household chemicals and consumer products: addressing concerns about triclosan. Integr Environ Assess Manag 6: 390-392.

53. Wu JL, Lam NP, Martens D, Kettrup A, Cai Z (2007) Triclosan determination in water related to wastewater treatment. Talanta 72: 1650-1654.

54. Behera SK, Oh SY, Park HS (2010) Sorption of triclosan onto activated carbon,

kaolinite and montmorillonite: effects of pH, ionic strength, and humic acid. J Hazard Mater 179: 684-691.

55. Rudel H, Bohmer W, Muller M, Fliedner A, Ricking M, et al. (2013) Retrospective study of triclosan and methyl-triclosan residues in fish and suspended particulate matter: results from the German Environmental Specimen Bank. Chemosphere 91: 1517-1524.

56. Coogan MA, Edziyie RE, La Point TW, Venables BJ (2007) Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in North Texas wastewater treatment plant receiving stream. Chemosphere 67: 1911-1918.

57. Aranami K, Readman JW (2007) Photolytic degradation of triclosan in freshwater and seawater. Chemosphere 66: 1052-1056.

58. Orvos DR, Versteeg DJ, Inauen J, Capdevielle M, Rothenstein A, et al. (2002) Aquatic toxicity of triclosan. Environ Toxicol Chem 21: 1338-1349.

59. Wilson BA, Smith VH, deNoyelles F Jr, Larive CK (2003) Effects of three pharmaceutical and personal care products on natural freshwater algal assemblages. Environ Sci Technol 37: 1713-1719.

60. Levy CW, Roujeinikova A, Sedelnikova S, Baker PJ, Stuitje AR, et al. (1999) Molecular basis of triclosan activity. Nature 398: 383-384.

61. Fritsch EB, Connon RE, Werner J, Davies RE, Beggel S, Feng W, Pessah IN (2013) Triclosan impairs swimming behavior and alters expression of excitation-contraction soupling proteins in Fathead Minnow (Pimephalespromelas). Environ SciTechnol 47: 2008-2017.

62. Pinto PIS, Guerreiro EM, Power DM (2013) Triclosan interferes with the thyroid axis in the zebrafish (Daniorerio). Toxicol Res 2: 60-69.

63. Foltz J, Mottaleb MA, Meziani MJ, Rafiq Islam M (2014) Simultaneous detection and quantification of select nitromusks, antimicrobial agent, and antihistamine in fish of grocery stores by gas chromatography-mass spectrometry. Chemosphere 107: 187-193.

64. Ramirez AJ, Mottaleb MA, Brooks BW, Chambliss CK (2007) Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry. Anal Chem 79: 3155-3163.

65. Balmer ME, Buser HR, Muller MD, Poiger T (2005) Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss Lakes. Environ Sci Technol 39: 953-962.

66. Rudel H, Bohmer W, Schroter-Kermani C (2006) Retrospective monitoring of synthetic musk compounds in aquatic biota from German rivers and coastal areas. J Environ Monit 8: 812-823.

67. Bohmer W, Muller J, Rudel H, Schroter-Kermani C (2004) Retrospective monitoring of alkylphenols and alkylphenol monoethoxylates in aquatic biota from 1985 to 2001: results from the German Environmental Specimen Bank. Environ Sci Technol 38: 1654-1661.

68. Navarro P, Bustamante J, Vallejo A, Prieto A, Usobiaga A, et al. (2010) Determination of alkylphenols and 17beta-estradiol in fish homogenate. Extraction and clean-up strategies. J Chromatogr A 1217: 5890-5895.

69. Kim Y, Choi K, Jung J, Park S, Kim PG, Park J (2007) Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. Environ Int 33: 370-375.

70. Correa L (1999) Effect of drugs that alter alertness and emotionality on the novelty response of a weak electric fish, Gymnotus carapo. Physiol Behav 65: 863-869.

71. Brandao FP, Rodrigues S, Castro BB, Goncalves F, Antunes SC, et al. (2013) Short-term effects of neuroactive pharmaceutical drugs on a fish species: biochemical and behavioural effects. Aquat Toxicol 144-145: 218-29.

72. Mottaleb MA, Zimmerman JD, Moy TW (2008) Biological transformation, kinetics and dose-response assessments of bound musk ketone hemoglobin adducts in rainbow trout as biomarkers of environmental exposure. J Environ Sci 20: 878-884.

73. Mottaleb MA, Moy TW, Zimmerman JH (2006) Biotransformation of musk xylene in trout haemoglobin: does-response and toxicokinetics of musk xylene metabolites haemoglobin adducts by gas chromatography-mass spectrometry. Int J Enviorn Anal Chem 86: 743-756.

74. Mottaleb MA, Zhao X, Curtis LR, Sovocool GW (2004) Formation of nitro musk adducts of rainbow trout hemoglobin for potential use as biomarkers of exposure. Aquat Toxicol 67: 315-324.

75. Farmer PB, Neumann HG, Henschler D (1987) Estimation of exposure of man to substances reacting covalently with macromolecules. Arch Toxicol 60: 251-260.

76. Sabbioni G (1994) Hemoglobin binding of nitroarenes and quantitative structure-activity relationships. Chem Res Toxicol 7: 267-274.

77. Skipper PL, Tannenbaum SR (1994) Molecular dosimetry of aromatic amines in human populations. Environ Health Perspect 102 Suppl 6: 17-21.

78. Minegishi K, Nambaru S, Fukuoka M, Tanaka A, Nishimaki-Mogami T (1991) Distribution, metabolism, and excretion of musk xylene in rats. Arch Toxicol 65: 273-282.

79. Mersch-Sundermann V, Reinhardt A, Emig M (1996) [Mutagenicity, genotoxicity and cogenotoxicity of environmentally relevant nitro musk compounds]. Zentralbl Hyg Umweltmed 198: 429-442.

80. Emig M, Reinhardt A, Mersch-Sundermann V (1996) A comparative study of five nitro musk compounds for genotoxicity in the SOS chromotest and Salmonella mutagenicity. Toxicol Lett 85: 151-156.

81. Api AM, Ford RA, San RH (1995) An evaluation of musk xylene in a battery of genotoxicity tests. Food Chem Toxicol 33: 1039-1045.

82. Lehman-McKeeman LD, Johnson DR, Caudill D, Stuard SB (1997) Mechanism-based inactivation of mouse hepatic cytochrome P4502B enzymes by amine metabolites of musk xylene. Drug Metab Dispos 25: 384-389.