Étude de l'état de santé des rivières karstiques en relation avec les pressions anthropiques sur leurs bassins versants.

VOLET
Evaluation des dangers et risques liés aux contaminants chimiques

4. Résidus médicamenteux

Pierre-Marie Badot, Eric Lucot, Audrey Bolard, François Degiorgi

1er septembre 2019

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – Place Leclerc - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

Avertissement

Ce document présente les résultats obtenus en matière de contaminations par les résidus médicamenteux. Ces données ont fait l'objet d'une publication scientifique (cf. annexes). Les éléments marquants sont brièvement résumés ici.

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

INTRODUCTION

Depuis plusieurs dizaines d'années, un faisceau de signes, mesures et observations montrent que les rivières de Franche-Comté subissent une érosion lente mais continue de leurs fonctions biologiques :

- des proliférations algales récurrentes ;
- des phénomènes de colmatages des fonds par des fines ou des feutrages organiques de plus en plus intenses ;
- des eaux en période de crue présentant fréquemment une teinte "chocolat" lorsque le débit dépasse le module ;
- une raréfaction voire une disparition d'espèces réputées sensibles (grands plécoptères, écrevisses pieds blancs, éphémères, trichoptères…) ;
- des captures de salmonidés par les pêcheurs montrant une nette tendance à la baisse ;
- une remontée des espèces médianes ou basales (comme l'ombre ou de nombreuses espèces d'insectes aquatiques) vers les secteurs apicaux ;
- …

Cette évolution négative semble s'être affirmée, sinon accélérée, depuis peu. Des mortalités massives de salmonidés sont survenues en 2010 et 2011, notamment au moment de leur période de reproduction.

De tels processus d'altération ont également été observés sur d'autres cours d'eau calcaires franc-comtois. Dans le cas de la Loue, ces phénomènes ont été d'autant plus spectaculaires que cette rivière était parmi les moins perturbées et présentait des stocks de salmonidés encore très importants jusqu'en 2008. La Loue et ses affluents constituent un observatoire représentatif pour rechercher les origines de l'appauvrissement général des ressources écologiques des rivières karstiques.

Depuis juillet 2012, le laboratoire Chronoenvironnement (UMR 6249, CNRS/UFC/UBFC) a entrepris avec le soutien financier de l'agence de l'eau Rhône Méditerranée Corse, puis du conseil régional de Bourgogne - Franche-Comté et du conseil départemental du Doubs, un programme de recherches centré sur ce réseau hydrographique pour atteindre les objectifs suivants :

1. caractériser de manière approfondie l'état de santé actuel de la Loue et ses évolutions avec des méthodes plus précises que celles employées dans les suivis réglementaires de la qualité des eaux réalisés dans le cadre de la directive cadre sur l'eau ;

2. appréhender les mécanismes de perturbations des fonctions biologiques du cours d'eau par l'analyse conjointe des compartiments fluviatiles et des principaux étages de l'édifice biologique ;
3. identifier les contaminants présents dans les différents compartiments de l'écosystème et leurs voies de transferts, hiérarchiser leurs impacts possibles, examiner leurs sources potentielles à l'échelle du bassin versant ;
4. explorer les relations existant entre l'évolution des activités socio-économiques du bassin versant de la Loue d'une part et la qualité des eaux et les capacités d'autoépuration de la rivière d'autre part.

La première tranche (tranche 1), réalisée entre juillet 2012 et fin 2014, s'est essentiellement attachée aux deux premiers objectifs. Elle a permis d'établir un diagnostic détaillé de l'état de la rivière et de sérier les hypothèses et scenarii visant à rendre compte des dégradations observées dans la rivière.

La deuxième tranche (tranche 2A) a été conduite de juillet 2012 à septembre 2015. Les investigations en matière de contaminants ont été effectuées dans différentes matrices environnementales (eaux, effluents de STEP, MES, sédiments, biote) et ont permis l'identification de multiples contaminants : pesticides chlorés, pyréthrinoïdes, hydrocarbures aromatiques polycycliques (HAP) ou bien encore résidus médicamenteux. Nous avons également établi que ces contaminations sont éminemment variables (i) en ce qui concerne leur nature chimique, (ii) leur occurrence temporelle et (iii) leur localisation spatiale, sans qu'il soit à ce stade possible d'identifier des *patterns* réguliers.

Les pesticides organochlorés et les pyréthrinoides, mais aussi les HAP sont les polluants les plus fréquemment mis en évidence dans les différentes matrices. Les HAP sont retrouvés de manière quasi systématique ou très fréquente dans les sédiments, les MES et le biote (algues). Les pesticides organochlorés, notamment l'hexachlorobenzène, le lindane, le DDT et ses métabolites sont souvent présents dans les poissons qui ont été analysés.

Ces contaminations atteignent des niveaux suspectés d'induire des effets toxiques avérés. Au cours de la troisième tranche (tranche 2B), nous avons donc entrepris de caractériser quantitativement la dangerosité de certains contaminants organiques persistants mis en évidence dans l'écosystème aquatique, afin d'être en mesure d'évaluer dans quelle mesure ces contaminants pourraient contribuer aux dysfonctionnements écologiques constatés seuls ou en conjonction avec d'autres facteurs stressants.

Au cours de la quatrième tranche (tranche 3), des analyses de pesticides, d'éléments en traces métalliques et de HAP ont été conduites sur les eaux lysimétriques, les eaux de surface, les MES et les sédiments en lien avec les suivis lysimétriques effectués sur les bassins versants du Grand Bief et de Plaisir Fontaine depuis 2016.

RESIDUS MEDICAMENTEUX ET CONTEXTE REGIONAL

Les médicaments humains et vétérinaires contiennent des substances chimiques douées d'activité biologique – les principes actifs – qui sont administrées pour restaurer ou une corriger une fonction biologique défaillante. Pour être efficace, une substance médicamenteuse doit pouvoir être assimilée par un organisme et elle doit être suffisamment persistante et stable pour que son effet puisse s'exercer.

En règle générale, les unités de traitement des stations d'épuration classiques ne sont pas équipées pour traiter ces molécules qui peuvent ainsi rejoindre les cours d'eau, les lacs ou les étendues océaniques, mais aussi les sols au travers des épandages de boues ou d'effluents d'élevage. En outre, les usines de potabilisation ne sont pas toutes dotées de moyen efficace pour éliminer ces substances avant distribution des eaux destinées à la consommation humaine.

Lorsque ces substances médicamenteuses sont libérées dans l'environnement, les propriétés mêmes, qui en font des médicaments efficaces – activité biologique, biodisponibilité, persistance – les rendent dangereuses pour les organismes non cible. A l'instar de la faune sauvage et de la flore, l'espèce humaine peut être exposée de manière non volontaire, notamment par les eaux de boisson et les denrées alimentaires.

La consommation de produits médicamenteux étant particulièrement importante dans les régions développées, les risques de contaminations environnementales y sont plus marqués.

L'occurrence de très nombreux médicaments et de leurs résidus dans les eaux douces est avérée dans de nombreux systèmes aquatiques (cf. références *in* Chiffre *et al.* 2016 en annexe) et constitue ainsi un sujet croissant de préoccupations pour les autorités et institutions en charge de l'environnement et de la santé publiqué.

Le massif jurassien constitue une région exempte de très grandes agglomérations, où la densité de population demeure faible par rapport à de nombreuses régions du monde. Le contexte géomorphologique – karst – de la région est connu pour faciliter rapidement les transferts au sein des bassins versants. Peu d'informations sont disponibles à l'échelle internationale concernant les contaminants médicamenteux dans ce type de milieu. L'objectif de notre étude visait d'une part à déterminer dans quelle mesure des résidus médicamenteux contaminent les eaux de la Loue et du Doubs et d'autre part à évaluer l'implication éventuelle de ces substances dans les dysfonctionnements observés dans les rivières karstiques du massif (Loue et Doubs). Pour cela, en partenariat avec l'Institut des Sciences Analytiques de l'Université de Lyon, nous avons quantifié 31 molécules pharmaceutiques dans les effluents de 2 STEP (Ornans et Pontarlier) et à l'amont et à l'aval des rejets dans les cours d'eau récepteurs (Loue et Doubs).

CONTAMINATIONS PAR LES RESIDUS MEDICAMENTEUX

Les résultats et leur analyse détaillée sont fournis dans Chiffre *et al.* 2016 (cf. annexe). Seuls les principaux résultats et conclusions sont brièvement résumés ici.

Tableau 1. Concentrations en résidus médicamenteux (µg L^{-1}) dans les eaux de la Loue.

Loue River (µg L^{-1})	Compound	PNEC[a]	MAC-EQS[b]	AA-EQS[c]	March 2014			May 2015		
Class					Spring	Upstream	Downstream	Spring	Upstream	Downstream
Antibiotic	Ciprofloxacin	938	0.363	0.089	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Ofloxacin	0.016			0.0007	0.0032	0.0042	*0.02* a	<LOD	<LOD
	Roxithromycin	4			<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Sulfamethoxazole	0.027	2.7	0.6	0.0015	<LOD	*0.0729* a	<LOD	<LOD	<LOD
	Trimethoprim	2.6	1100	60	0.0001	0.0003	0.0155	0.0015	0.0013	0.0013
Anti-convulsant	Carbamazepine	13.8	2550	0.5	0.0019	0.0022	*0.0568* c	<LOD	<LOD	<LOD
Anti-inflammatory	Diclofenac	0.1		0.05	<LOD	<LOD	*0.1665* c	<LOD	<LOD	<LOD
	Ibuprofen	1.65	23	0.3	<LOD	<LOD	0.0283	<LOD	<LOD	<LOD
	Ketoprofen	15.6			0.039	0.0462	0.0445	<LOD	<LOD	<LOD
	Naproxen	2.62	370	1.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Paracetamol	1			0.1317	0.158	0.1389	0.0021	0.0131	0.0196
Anti-parasitic	Metronidazole	2.5			<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Diuretic	Furosemide				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Fungicide	Econazole				<LOD	<LOD	<LOD	<LOD	0.0059	<LOD
Hormone	α-Estradiol			0.000037	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Androstenedione				<LOD	<LOD	<LOD	<LOD	<LOD	0.0056
	β-Estradiol			0.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Estriol				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Estrone			3.6	0.0021	0.0039	0.0057	<LOD	<LOD	<LOD
	Gestodene				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Levonorgestrel				<LOD	<<LOD	<LOD	<LOD	<LOD	<LOD
	Norethindrone				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Progesterone				<LOD	0.0015	<LOD	<LOD	<LOD	<LOD
	Testosterone				0.0001	<LOD	<LOD	0.0028	0.0028	0.0029
Lipid regulator	Bezafibrate		76	460	<LOD	<LOD	0.0112	<LOD	<LOD	<LOD
	Fenofibrate	0.1			<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Psychotropic	Fluvoxamine				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Lorazepam				<LOD	<LOD	0.0088	<LOD	<LOD	<LOD
	Oxazepam				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
β-Blocker	Atenolol	30	330	150	<LOD	0.0028	0.0196	<LOD	<LOD	<LOD
	Propanolol		12,000	160	<LOD	<LOD	<LOD	0.0001	<LOD	0.0002

Values in italics exceed threshold values (a, b, c)

PNEC predicted no-effect concentration, *MAC-EQS* environmental quality standard relative to the maximum allowable concentration, *AA-EQS* annual average concentration

[a] Data from Kümmerer and Henninger (2003); Sanderson et al. (2003); Ferrari et al. (2003); Lindqvist et al. (2005); Hoeger et al. (2005); Isidori et al. (2005); Ra et al. (2008); Quinn et al. (2008)

[b, c] http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards/?_ga=1.12802039.1593799960.1475153874

Les valeurs italisées et encadrées en rouge dans les Tableaux 1 et 2 indiquent des dépassements des valeurs seuils disponibles dans la littérature. Dans les eaux de la Loue, 4 molécules présentent des dépassements : l'ofloxacine (antibiotique), le sulfaméthoxazole (antibiotique), ainsi que la carbamazépine (anti-convulsant) et le diclofénac (anti-inflammatoire).

Tableau 2. Concentrations en résidus médicamenteux (µg L^{-1}) dans les eaux du Doubs.

River Doubs					April 2014		May 2015	
					Upstream	Downstream	Upstream	Downstream
Class	Compound	PNEC	MAC-EQS	AA-EQS				
Hormone	α-Estradiol			0.000037	<LOD	<LOD	<LOD	<LOD
Hormone	Androstenedione				<LOD	<LOD	<LOD	<LOD
β-Blocker	Atenolol	30	330	150	<LOD	0.1146	0.0204	<LOD
Hormone	β-Estradiol			0.4	<LOD	<LOD	<LOD	<LOD
Lipid regulator	Bezafibrat		76	460	<LOD	<LOD	<LOD	<LOD
Anti-convulsant	Carbamazepine	13.8	2550	0.5	0.0011	0.1488	0.0224	0.0002
Antibiotic	Ciprofloxacin	938	0.363	0.089	<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Diclofenac	0.1		0.05	<LOD	*0.3005* c	<LOD	<LOD
Fungicide	Econazole				<LOD	<LOD	<LOD	<LOD
Hormone	Estriol				<LOD	<LOD	<LOD	<LOD
Hormone	Estrone			3.6	0.0038	0.0292	0.0001	<LOD
Lipid regulator	Fenofibrate	0.1			<LOD	<LOD	<LOD	<LOD
Psychotropic	Fluvoxamine				<LOD	<LOD	<LOD	<LOD
Diuretic	Furosemide				<LOD	<LOD	<LOD	<LOD
Hormone	Gestodene				<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Ibuprofen	1.65	23	0.3	<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Ketoprofen	15.6			0.0394	0.0452	<LOD	0.0065
Hormone	Levonorgestrel				<LOD	<LOD	<LOD	<LOD
Psychotropic	Lorazepam				<LOD	0.0039	<LOD	<LOD
Anti-parasitic	Metronidazole	2.5			<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Naproxen	2.62	370	1.7	<LOD	<LOD	<LOD	<LOD
Hormone	Norethindrone				<LOD	<LOD	<LOD	<LOD
Antibiotic	Ofloxacin	0.016			0.0013	0.002	<LOD	<LOD
Psychotropic	Oxazepam				<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Paracetamol	1			0.1207	0.2736	0.0122	0.0196
Hormone	Progesterone				<LOD	0.0113	<LOD	<LOD
β-Blocker	Propanolol		12,000	160	0.0015	<LOD	<LOD	0.004
Antibiotic	Roxithromycin	4			<LOD	<LOD	<LOD	<LOD
Antibiotic	Sulfamethoxazole	0.027	2.7	0.6	<LOD	*0.1258* a	<LOD	<LOD
Hormone	Testosterone				<LOD	<LOD	0.003	0.0026
Antibiotic	Trimethoprim	2.6	1100	60	0.0616	0.0138	0.0059	0.0013

Values in italics exceed threshold values (a, b, c)

PNEC predicted no-effect concentration, *MAC-EQS* environmental quality standard relative to the maximum allowable concentration, *AA-EQS* annual average concentration

Dans les eaux du Doubs, le sulfaméthoxazole et le diclofénac dépassaient les valeurs seuils. D'autres résidus médicamenteux (paracétamol, kétoprofène, différents oestrogènes...) sont présents de manière très régulière dans les deux cours d'eaux.

Les effluents de STEP étant vraisemblablement une des sources majeures de résidus médicamenteux, ces matrices ont également été prélevées et analysées.

Tableau 3. Concentrations en résidus médicamenteux (ng L^{-1}) dans les effluents des STEP de Pontarlier (A) et d'Ornans (B).

Class	Compound	Range[a]	WWTP A effluent		WWTP B effluent	
			April	May	March	May
Antibiotic	Ciprofloxacin	72–96.3	<LOD	<LOD	<LOQ	<LOD
	Ofloxacine		18.3	53	47.7	303
	Roxithromycine		<LOQ	<LOD	<LOQ	<LOD
	Sulfamethoxazole	115–578	655.7	615	1380.4	640
	Trimethoprim	100–229	61.6	46.4	241.2	<LOQ
Anti-convulsant	Carbamazepine	157–832	442.5	566	811.4	1007
Anti-inflammatory	Diclofenac	50–680	965.7	467	2476.3	1055
	Ibuprofen	80.5–1960	<LOD	<LOD	526.7	126
	Ketoprofen	86–392	143	260.5	107.2	<LOD
	Naproxen	26–1890	254.6	<LOD	355.3	100.1
	Paracetamol	108–11,309	<LOD	<LOD	<LOD	<LOD
Anti-parasitic	Metronidazole		35.8	<LOQ	31.5	<LOQ
Diuretic	Furosemide		<LOQ	<LOQ	<LOQ	<LOQ
Fungicide	Econazole		<LOD	<LOD	<LOD	<LOD
Hormone	A estradiol	0.8–3	<LOD	<LOD	<LOD	<LOD
	B estradiol	2.8–3	<LOQ	<LOD	<LOQ	<LOD
	Androstenedione		<LOD	5.6	<LOD	9.1
	Estriol		<LOD	<LOD	<LOD	<LOD
	Estrone		10	2.4	44.2	2.6
	Gestodene		<LOD	<LOD	<LOD	<LOD
	Levonorgestrel		<LOD	<LOD	<LOD	<LOD
	Progesterone		<LOD	<LOD	<LOD	<LOD
	Testosterone		<LOD	<LOD	<LOD	<LOD
Lipid regulator	Bezafibrate	25–816	15.6	57.6	27	<LOD
	Fenofibrate		<LOD	<LOD	<LOD	<LOD
Psychotropic	Fluvoxamine		<LOD	<LOD	<LOD	<LOD
	Lorazepam		28.6	47.4	41.4	123.5
	Norethindrone		<LOD	<LOD	<LOD	<LOD
	Oxazepam		<LOQ	167.5	<LOQ	286.5
β-Blocker	Atenolol	154–843	324.1	381	324.5	169.5
	Propanolol		1.5	<LOQ	6.8	17.5

[a] Togola and Budzinski (2008); Miège et al. (2009); Rosal et al. (2010); Loos et al. (2013); Luo et al. (2014)

Les rejets des 2 STEP contiennent une grande variété de molécules médicamenteuses à des concentrations non négligeables. Le diclofénac (965 et 2476 ng L^{-1}), le sulfaméthoxazole (655 aet 1380 ng L^{-1}) et la carbamazépine (566

and 1007 ng L^{-1}) montrent les concentrations les plus élevées dans les rejets des deux stations.

Le Tableau 4 fournit pour chacune des substances analysées (i) les concentrations prédites sans effet (*Predicted No Effect Concentrations*, PNEC) lorsqu'elles existent dans la littérature scientifique, (ii) les quotients de risques (*Risk Quotients*, RQ), c'est-à-dire les ratio entre la concentration de chaque substance et la PNEC correspondante. Un quotient de risque supérieur à 1 indique l'existence d'un problème potentiel pour les organismes aquatiques alors qu'un ratio inférieur ou égal à 1 correspond à l'absence de risque significatif.

Tableau 4. Concentrations prédites sans effet (PNEC) et quotient de risques (RQ) de différents résidus médicamenteux (µg L^{-1}) présents dans les eaux de la Loue et du Doubs et les effluents des STEP de Pontarlier (A) et d'Ornans (B).

Class	Compound	PNEC	WWTP A March 2014 RQ	May 2015 RQ	WWTP B April 2014 RQ	May 2015 RQ
Hormone	A estradiol		–	–	–	–
Hormone	Androstenedione		–	–	–	–
β-Blocker	Atenolol	30	0.01	0.01	0.01	0.01
Hormone	B estradiol		–	–	–	–
Lipid regulator	Bezafibrat		–	–	–	–
Anti-convulsant	Carbamazepine	13.8	0.06	0.07	0.03	0.04
Antibiotic	Ciprofloxacin	938	–	–	–	–
Anti-inflammatory	Diclofenac	9.7	0.26	0.11	0.10	0.05
Fungicide	Econazole		–	–	–	–
Hormone	Estriol		–	–	–	–
Hormone	Estrone		–	–	–	–
	Fenofibrate	0.1	–	–	–	–
Psychotropic	Fluvoxamine		–	–	–	–
Diuretic	Furosemide		–	–	–	–
	Gestodene		–	–	–	–
	Ibuprofen	1.65	0.32	0.08	–	–
	Ketoprofen	15.6	0.01	–	0.01	0.02
	Levonorgestrel		–	–	–	–
	Lorazepam		–	–	–	–
	Metronidazole	2.5	0.01	–	0.01	–
	Naproxen	2.62	0.14	0.04	0.10	–
	Norethindrone		–	–	–	–
	Ofloxacin	0.016	2.98	18.94	1.14	3.31
	Oxazepam		–	–	–	–
	Paracetamol	1	–	–	–	–
	Progesterone		–	–	–	–
β-Blocker	Propanolol		–	–	–	–
	Roxithromycin	4	–	–	–	–
	Sulfamethoxazole	0.027	51.13	23.70	24.29	22.78
	Testosterone		–	–	–	–
	Trimethoprim	2.6	0.09	–	0.02	0.02

L'ofloxacine et le sulfaméthoxazole sont les substances les plus critiques en termes de risques environnementaux. A notre connaissance, les réglementations européennes et françaises n'ont jusqu'ici pas intégré de valeurs limites pour les

concentrations des résidus médicamenteux dans les rejets des STEP. Cependant, les mesures effectuées montrent que les concentrations de toute une série de résidus médicamenteux () dépassent les références de qualité proposée par le Swiss Ecotox Centre (www.centreecotox.ch), ce qui montre l'urgence de mettre en place une surveillance régulière et la nécessité de prendre des mesures visant à réduire les niveaux de contamination et les possibles effets néfastes de ces substances, notamment ceux liés aux perturbations endocriniennes.

CONCLUSIONS

Les résultats obtenus montrent que les deux rivières étudiées, la Loue et le Doubs, sont contaminées de manière sensible par plusieurs résidus médicamenteux, et plus particulièrement par la carbamazépine, le diclofénac, l'ofloxacine et le sulfaméthoxazole.

Nos résultats montrent que très vraisemblablement, les rejets des stations d'épuration constituent la source majeure de contamination des cours d'eaux.

En l'absence de cadre réglementaire clair, il faut insister sur le fait que la littérature scientifique sur cette problématique est de plus en fournie et abonde d'évidences montrant la dangerosité et les risques liés à la présence de ces substances dans les environnements aquatiques (voir par exemple Ebele *et al.*, 2017[1], Fent *et al.* 2006[2], Kaczala et Blum, 2016[3], Mohapatra et al. 2016[4] et références citées).

Nos résultats indiquent que plusieurs composés - carbamazépine, diclofénac, ofloxacine et sulfaméthoxazole - dépassent les valeurs seuils actuellement disponibles dans la littérature.

En l'état actuel des connaissances, il n'est pas possible d'évaluer la part prise par ces contaminants dans la dégradation de la qualité des eaux de la Loue et des rivières karstiques du massif jurassien, mais il est certain que ces molécules, dont certaines peuvent aussi être d'un usage répandu en médécine vétérinaire, participent à cette dégradation.

Toute mesure visant à limiter les usages non indispensables de ces molécules (tant en médecine humaine que vétérinaire) ou à mieux traiter les effluents (rejets de STEP, effluents d'élevage, aquaculture) contribuera à préserver les milieux récepteurs et à mieux préserver la santé publique.

[1] Anekwe Jennifer Ebele, Mohamed Abou-Elwafa Abdallah, Stuart Harrad, 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants* 3, 1-16
[2] K Fent, AA. Weston, D Caminada. 2006 Ecotoxicology of human pharmaceuticals, *Aquatic Toxicology* 76, 122–159.
[3] F Kaczala S Blum. 2016. The Occurrence of Veterinary Pharmaceuticals in theEnvironment: *A Review. Current Analytical Chemistry*, 2016, 12, 169-182
[4] D. P. Mohapatra & M. Cledón & S. K. Brar &R. Y. Surampalli. 2016. Application of Wastewater and Biosolids in Soil: Occurrence and Fate of Emerging Contaminants. *Water Air Soil Pollut* (2016) 227: 77

Annexe 71 - Analyse chimique des eaux et effluents - printemps 2014	Code station				PON2	PON4	EFFP	SOURCE	ORN2	ORN4	EFF0
	Nom des stations				Amont proche	Aval proche	STEP Pontarlier	Source	Amont	Aval proche	STEP Ornans
	Code échantillon				EAUX		EFFLUENT	EAUX			EFFLUENT
	LOD ng/L eaux de surface	LOQ ng/L eaux de surface	LOD ng/L effluents	LOQ ng/L effluents	avril 2014	CHRONO ENVIRONNEMENT		mars 2014		CHRONO ENVIRONNEMENT	
Substances											
A estradiol	0,2	0,6	0,2	0,6	nd	nd	nd	nd	nd	nd	nd
Androstènedione	0,1	0,2	0,1	0,2	nd	nd	nd	nd	<	nd	nd
Aténolol	0,03	0,1	0,03	0,1	nd	114,6	324,1	nd	2,8	19,6	324,5
B estradiol	0,1	0,4	0,1	0,4	<	<	<	<	<	<	<
Bézafibrate	0,8	2,6	0,8	2,6	nd	<	15,6	nd	<	11,2	27,0
Carbamazépine	0,1	0,2	0,1	0,2	1,1	148,3	442,5	1,9	2,2	56,8	811,4
Ciprofloxacine	1	3,3	1	3,3	nd	nd	nd	nd	nd	>	>
Diclofénac	0,5	1,8	0,5	1,8	nd	300,5	965,7	<	<	166,5	2476,3
Econazole	0,3	0,9	0,3	0,9	nd	nd	nd	nd	nd	nd	nd
Estriol	15	50	15	50	nd	nd	nd	nd	nd	nd	nd
Estrone	0,03	0,1	0,03	0,1	3,8	29,2	10,0	2,1	3,9	5,7	44,2
Fénofibrate	0,2	0,6	0,2	0,6	nd	nd	nd	nd	nd	nd	nd
Fluvoxamine	0,3	1	0,3	1	nd	nd	nd	nd	nd	nd	nd
Furosémide	30,4	101,2	30,4	101,2	nd	nd	<	nd	nd	nd	<
Gestodène	1,2	4	1,2	4	nd	nd	nd	nd	nd	nd	nd
Ibuprofène	0,2	0,6	0,2	0,6	<	<	nd	<	<	28,3	526,7
Ketoprofen	1,5	5	1,5	5	39,4	45,2	143,0	39,0	46,2	44,5	107,2
Levonorgestrel	4	13,3	4	13,3	nd	nd	nd	nd	nd	nd	nd
Lorazepam	0,7	2,2	0,7	2,2	nd	3,9	28,6	nd	nd	8,8	41,4
Métronidazole	0,4	1,4	0,4	1,4	nd	nd	35,8	nd	nd	<	31,5
Naproxène	4,6	15,3	4,6	15,3	nd	<	254,6	nd	nd	<	355,3
Norethindrone	1,9	6,2	1,9	6,2	nd	nd	nd	nd	nd	nd	nd
Ofloxacine	0,1	0,2	0,1	0,2	1,3	2,0	18,3	0,7	3,2	4,2	47,7
Oxazépam	0,2	0,5	0,2	0,5	>	>	>	>	>	>	>
Paracétamol	26	86,5	26	86,5	120,7	273,6	nd	131,7	158,0	138,9	nd
Progestérone	0,4	1,3	0,4	1,3	nd	11,3	nd	nd	1,5	nd	nd
Propanolol	0,9	3	0,9	3	<	<	1,5	<	<	<	6,8
Roxithromycine	0,03	0,1	0,03	0,1	nd	>	>	nd	nd	nd	>
Sulfaméthoxazole	0,1	0,2	0,1	0,2	nd	125,8	655,7	1,5	nd	72,9	1380,4
Testostérone	0,2	0,7	0,2	0,7	nd	nd	nd	<	<	nd	nd
Triméthoprime	0,03	0,1	0,03	0,1	0,3	13,8	61,6	0,1	0,3	15,5	241,2

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

Annexe 72 - Analyse chimique des eaux et effluents - juin 2015	Code station				PON2	PON4	EFFP	SOURCE	ORN2	ORN4	EFF0
	Nom des stations				Amont proche	Aval proche	STEP Pontarlier	Source	Amont	Aval proche	STEP Ornans
	Code échantillon				EAUX		EFFLUENT	EAUX			EFFLUENT
	LOD ng/L eaux de surface	LOQ ng/L eaux de surface	LOD ng/L effluents	LOQ ng/L effluents	juin 2015	CHRONO ENVIRONNEMENT		juin 2015		CHRONO ENVIRONNEMENT	
Substances											
A estradiol	0,05	0,2	2,2	7,1	nd	nd	nd	nd	nd	nd	nd
Androstènedione	0,1	0,3	3,8	4,8	nd	nd	5,6	nd	nd	nd	9,1
Aténolol	0,5	1,5	7	23	20,4	nd	381,0	nd	nd	nd	169,5
B estradiol	0,05	0,2	2,2	7,1	nd	nd	nd	nd	nd	nd	nd
Bézafibrate	0,5	1,7	31	38,4	nd	nd	57,6	nd	nd	nd	<
Carbamazépine	0,02	0,05	0,03	0,1	22,4	0,2	566,0	<	<	<	1007,0
Ciprofloxacine	2,1	7	24,5	28,2	nd	nd	nd	nd	nd	nd	nd
Diclofénac	0,8	2,7	130	171	nd	nd	467,0	nd	nd	nd	1055,0
Econazole	0,2	0,5	2,82	9,3	nd	nd	nd	nd	5,9	nd	nd
Estriol	71,1	234,6	233,3	700	nd	nd	nd	nd	nd	nd	nd
Estrone	0,03	0,1	0,4	1,2	0,1	<	2,4	nd	<	nd	2,6
Fénofibrate	0,04	0,1	1,9	6,3	nd	nd	nd	nd	<	nd	nd
Fluvoxamine	0,8	2,6	3,2	10,5	nd	nd	nd	nd	<	nd	nd
Furosémide	9,5	31,4	285	414	nd	nd	<	nd	nd	nd	<
Gestodène	0,5	1,5	2,4	8	nd	nd	nd	nd	nd	nd	nd
Ibuprofène	0,6	2,1	4,8	9,8	nd	nd	nd	nd	nd	nd	126,0
Ketoprofen	0,2	0,7	15	16	nd	6,5	260,5	nd	nd	nd	<
Levonorgestrel	3,9	12,9	4	13,3	nd	nd	nd	nd	nd	nd	nd
Lorazepam	0,04	0,1	0,2	0,5	<	nd	47,4	nd	nd	nd	123,5
Métronidazole	3,7	12,2	272,7	900	nd	<	<	nd	nd	nd	<
Naproxène	1,5	5,1	12,7	19,2	nd	nd	<	nd	nd	nd	100,1
Norethindrone	0,5	1,7	3,8	12,4	nd	nd	nd	nd	nd	nd	nd
Ofloxacine	0,06	0,2	1,5	4,6	<	<	53,0	20,0	nd	nd	303,0
Oxazépam	0,2	0,5	3,2	9,6	69,6	nd	167,5	nd	nd	nd	286,5
Paracétamol	2,3	7,7	75,9	130,3	12,2	22,6	nd	2,1	13,1	19,6	nd
Progestérone	0,5	1,6	3,8	8	nd	nd	nd	nd	nd	nd	nd
Propanolol	0,04	0,1	1,1	3,3	nd	4,0	<	0,1	<	0,2	17,5
Roxithromycine	0,6	1,9	9,4	10,6	nd	nd	nd	<	nd	nd	nd
Sulfaméthoxazole	0,04	0,1	0,4	1	<	<	615,0	<	<	<	640,0
Testostérone	0,2	0,6	6	15	3,0	2,6	nd	2,8	2,9	<	nd
Triméthoprime	0,03	0,1	6,8	8,2	5,9	1,3	46,4	1,4	1,3	1,3	<

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

Occurrence of pharmaceuticals in WWTP effluents and their impact in a karstic rural catchment of Eastern France

Axelle Chiffre, François Degiorgi, Audrey Buleté, Loïc Spinner & Pierre-Marie Badot

Environmental Science and Pollution Research

ISSN 0944-1344
Volume 23
Number 24

Environ Sci Pollut Res (2016)
23:25427-25441
DOI 10.1007/s11356-016-7751-5

Springer

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

Environ Sci Pollut Res (2016) 23:25427–25441
DOI 10.1007/s11356-016-7751-5

RESEARCH ARTICLE

Occurrence of pharmaceuticals in WWTP effluents and their impact in a karstic rural catchment of Eastern France

Axelle Chiffre[1] · François Degiorgi[1] · Audrey Buleté[2] · Loïc Spinner[2] · Pierre-Marie Badot[1]

Received: 12 April 2016 / Accepted: 18 September 2016 / Published online: 3 October 2016

Abstract The occurrence of pharmaceuticals in freshwater ecosystems provokes increasing concern due to their potential risk to non-target organisms and to human health. Pharmaceuticals are used in both human and veterinary medicine and are essentially released into the environment via wastewater treatment plants (WWTPs) and from livestock. In this study, 31 pharmaceuticals were analyzed in effluent and surface water upstream and downstream of two WWTPs in the Loue–Doubs rural karstic catchment in Eastern France. Diclofenac (965 and 2476 ng L^{-1}), sulfamethoxazole (655 and 1380 ng L^{-1}) and carbamazepine (566 and 1007 ng L^{-1}) displayed the highest levels in the effluents of both WWTPs. Diclofenac levels were also high in surface water samples 300 and 166 ng L^{-1} in the River Doubs and the River Loue, respectively, followed by paracetamol (273 and 158 ng L^{-1}) and sulfamethoxazole (126 and 73 ng L^{-1}). In both rivers, the most critical compounds were found to be the antibiotic sulfamethoxazole (risk quotient (RQ) from 23.7 to 51.1) and ofloxacine (RQ from 1.1 to 18.9), which reached levels inducing toxic effects in aquatic organisms. This study showed that WWTP effluents are the major sources of the pharmaceuticals, but raw discharges from human residences, pastures and livestock manure represent significant sources of contamination of surface water and groundwater. The aim of this study was to assist scientists and authorities in understanding occurrence and sources of pharmaceuticals in order to improve water quality management in chalk streams.

Keywords Pharmaceuticals · Chalk streams · WWTP · Veterinary use · Risk assessment

Responsible editor: Roland Kallenbom

Electronic supplementary material The online version of this article (doi:10.1007/s11356-016-7751-5) contains supplementary material, which is available to authorized users.

✉ Pierre-Marie Badot
pierre-marie.badot@univ-fcomte.fr

[1] University of Bourgogne Franche-Comté, Chrono-environnement, UMR 6249 UFC/CNRS usc INRA, 16 route de Gray, 25030 Besançon cedex, France

[2] University of Lyon, Institut des Sciences Analytiques, UMR 5280, CNRS, 5 rue de la Doua, 69100 Villeurbanne, France

Introduction

About 4000 pharmaceuticals including antibiotics, lipid regulators, beta-blockers and anti-depressants are currently consumed by humans in the European Union. France is the fourth largest consumer of human pharmaceuticals in the world with over €26.8 billion spent on prescription drugs in 2013 (ANSM 2014). It is also the largest consumer of veterinary compounds in the European Union with more than 300 veterinary pharmaceuticals like antibiotics, anti-parasitics and hormones (Kools et al. 2008). From the large number of molecules available, several compounds (antibiotics, anti-inflammatories) are used in both human and veterinary medicine.

Once administered, pharmaceuticals are only partially metabolized by the human body and are excreted as parent compounds or as a mixture with metabolites which find their way into the water cycle (Djemba 2006). Most municipal wastewater treatment plants (WWTPs) are unable to totally eliminate either parent or metabolized pharmaceuticals from wastewaters (Joss et al. 2006; Verlicchi et al. 2012). Removal efficiency depends on the treatment process used and on the chemical and physical properties of the various pharmaceuticals (Kasprzyk-Hordern et al. 2009 ; Luo et al. 2014; Patrolecco et al. 2014). Therefore, WWTPs are considered to be the

principal source of pharmaceuticals in aquatic ecosystems (Kolpin et al. 2002; Choi et al. 2008 ; Conley et al. 2008). However, besides human input via wastewater, livestock production has been recognized as a major source of contamination (Kemper 2008; Bártíková et al. 2016). In addition, a recent study conducted in Spain showed that average concentrations of pharmaceuticals were positively related to the population density and the livestock units (Osorio et al. 2016). Veterinary pharmaceuticals are released into the environment through the excretion of drugs and/or their metabolites in urine and faeces of livestock, the discharge of aquaculture products or the spreading of manure and slurry from treated animals (Boxall et al. 2003; Kümmerer 2009; Iglesias et al. 2013).

Due to the risks for aquatic life, attention is now being paid to the occurrence of pharmaceuticals in the environment worldwide. Monitoring of pharmaceuticals in aquatic ecosystems was recognized in European Union in the Water Framework Directive (European Union 2000) with the addition of three compounds (17α-ethinylestradiol, 17β-estradiol, diclofenac) to the watch list of substances. However, studies have reported the occurrence of more than a hundred pharmaceuticals at concentrations ranging from nanogram per litre to microgram per litre in municipal wastewater effluents (Al Aukidy et al. 2012; Patrolecco et al. 2014), surface water (Kasprzyk-Hordern et al. 2008; Kolpin et al. 2002; Conley et al. 2008) and groundwater (Fent et al. 2006).

Low levels of pharmaceuticals are known to exert short-term and long-term toxicities on non-target organisms in the aquatic environment depending on their biological effect and their bioavailability (Ferrari et al. 2003; Brooks et al. 2005; Boxall et al. 2004; Fent et al. 2006; Santos et al. 2010). For example, natural and synthetic hormones have been reported to cause adverse effects like feminization of fish in large rivers (Santos et al. 2010). Chronic toxicity trials performed on brown trout (*Salmo trutta fario*) showed cytological damage and a reduction of hematocrit values after 21 days of exposure to 0.5 g L^{-1} of diclofenac (Hoeger et al. 2005).

Despite increased knowledge about the occurrence of pharmaceuticals in France (Togola and Budzinski 2008; Vulliet and Cren-Olivé 2011), there are still little data available about the occurrence of pharmaceuticals in specific areas like karstic rural catchments. Indeed, karstic groundwater systems are known to be highly vulnerable to contamination and many micropollutants from agriculture, livestock farming and private households can be transported in conduits over long distances (Heinz et al. 2008 ; Einsiedl et al. 2010). In the study area (the French Jura mountains), the watershed is characterized by a thin layer of soil and a karstic geological formation with alternating large conduits and smaller fractures with two main chalk streams: the Rivers Doubs and Loue (Bichet and Campy 2008). These rivers are renowned for trout fishing and invertebrate diversity and density. Although the Loue River is still considered by national authorities (http://www.rhone-mediterranee.eaufrance.fr) as being in good ecological status, fish mortality and loss of macroinvertebrates have been occurring for several years (ONEMA et al. 2012). Furthermore, local authorities need to increase their knowledge about source and impact of pharmaceutical contamination in the catchment in order to improve water quality management.

The Loue–Doubs watershed is covered by agricultural and forest areas, and the population density is low. The agricultural areas are mainly composed of permanent grasslands used for dairy farming and production of protected designation of origin cheese. Considering the high rates of consumption of numerous pharmaceuticals in France and/or their potential ecotoxicological effects on aquatic communities, assessing risk requires additional data on the occurrence of pharmaceuticals in chalk streams situated in rural catchments and identifying the risk situation. The aims of this study were (1) to quantify 31 pharmaceuticals in the effluents of two WWTPs of the karstic Doubs–Loue catchment and in the receiving waters, (2) to evaluate upstream and downstream levels in order to discriminate pollution sources and (3) to assess the risk for aquatic organisms by calculating the risk quotient of the main pharmaceuticals.

Materials and methods

Study area

The study was conducted in the Haut-Doubs Haute-Loue catchment (Eastern France), which drains an area of about 2320 km^2. The substratum of the watershed is dominated by limestone with many karst structures and large conduits (Bichet and Campy 2008). According to the agricultural census, the land used for agriculture in 2010 represented 860 km^2 mainly composed of pastures and grassland (94 %). This corresponds to 42 % of the catchment. Livestock is dominated by dairy cows for cheese production. In addition, about 84 % of the agricultural land receives amendments in the form of manure (53 %), a mixture of manure and pig slurry (46 %) and sludge from WWTP (1 %).

Two conventional activated sludge plants (WWTP A and WWTP B) discharge their effluent into the Rivers Doubs and the Loue, respectively, and were monitored in this study (Fig. 1). Water of the River Doubs was sampled upstream and downstream of WWTP A, which treats domestic and industrial wastewater of the town of Pontarlier and its agglomeration of 55,000 people equivalent (PE). In the Loue, river water was sampled upstream and downstream of the WWTP B, which collects wastewater from the town of Ornans (5000 PE). The characteristics of each WWTP are summarized in Table 1. We also sampled the karst

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

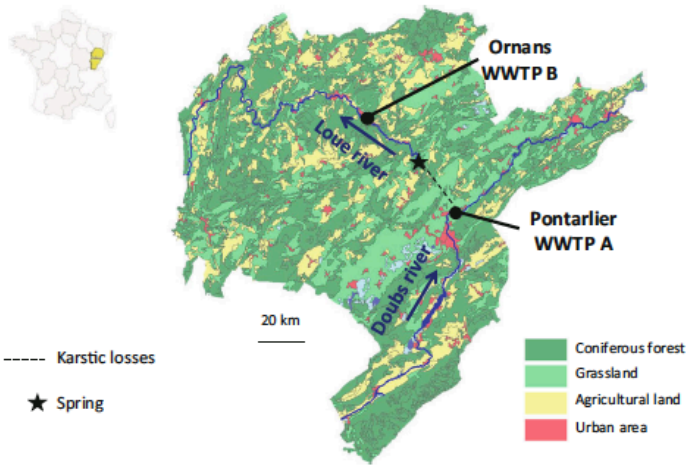

Fig. 1 Location of sampling site indicating land use

spring of the Loue River, which mainly corresponds to losses from the Doubs River downstream of the WWTP A (Jacquemin 1984). Sampling was carried out in April 2014 and in May 2015 in the River Doubs and in March 2014 and May 2015 in the River Loue. Effluent and river discharges were relatively constant during both sampling campaigns. The daily average flow velocities of WWTP A and WWTP B discharge were, respectively, 14,900 and 505 $m^3\ d^{-1}$. The daily mean flow rates were 2.38 and 3.06 $m^3\ s^{-1}$ in the River Doubs and 11.5 and 11.2 $m^3\ s^{-1}$ in the River Loue for the first and the second campaigns, respectively. In each campaign, grab surface water subsamples were collected upstream ($n = 6$) and downstream ($n = 6$) of both WWTP discharges and in the River Loue spring ($n = 6$). Precisely, in each site, two replicates of 1-l water samples were taken at three points along the channel transects in order to reduce spatial variability. Subsamples of river water and effluent were mixed and then put in three 2-l amber glass flasks. For effluents, we collected several grab samples ($n = 6$) in 1-l glass bottle, which were mixed and put in three 2-l amber glass flasks. All bottles were previously cleaned with hydrochloric acid and ultra-pure water. All the samples were immediately refrigerated and kept at 4 °C until analysis. Well mixing of effluents in surface water was verified by

Table 1 Characteristics of both WWTPs

	WWTP A	WWTP B
People equivalent	55,000	5000
Effluent flow rate ($m^3\ J^{-1}$)	20,800	430
Sewer	Combined sewer system	Combined sewer system
Treatment	Activated sludge: - Primary treatment - Secondary aerobic treatment - Dephosphatation	Activated sludge - Primary treatment -Secondary aerobic treatment - Dephosphatation
Biological reactor (m^3)	6550	900
HRT (h)	181	40
SRT (days)	19	14
Type of sewage	Domestic/industrial	Domestic/industrial
Effluent daily discharge flow rate ($m^3\ d^{-1}$) for the first campaign	7618	702
Effluent daily discharge flow rate ($m^3\ d^{-1}$) for the second campaign	7640	505
Dilution rate in river (%) for the first campaign	3	0.1
Dilution rate in river (%) for the second campaign	2	0.1

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

measuring electric conductivity upstream and downstream of the WWTP (Supporting information).

Compounds

The compounds selected for study belong to different drug families: antibiotics, anti-convulsants, anti-inflammatories, fungicides, hormones, lipid regulators, psychotropics and β-blockers (Table 2). The choice of these compounds was based upon the French pharmaceutical consumption and predicted environmental concentrations as well as ecotoxicological, pharmacological and physicochemical data (Besse and Garric 2008) and upon their occurrence in previous environmental surveys in French surface water and groundwater (Vulliet and Cren-Olivé 2011).

Analysis

Analytical methods were adapted from Vulliet and Cren-Olivé (2011) for pharmaceuticals and hormones. These analytical

Table 2 List of the analyzed pharmaceuticals classified by their therapeutic activity, identification number (CAS), annual consumption in France, veterinary use and approved or not approved in France

Class	Compound	CAS number	Annual consumption (kg) in France (ANSM 2014)	Use or origin
Antibiotic	Ciprofloxacin	85721-33-1	12,186	H/V[c]
	Ofloxacin	82419-36-1	4137	H/V[b]
	Roxithromycin	80214-83-1	3404	H/V[b]
	Sulfamethoxazole	723-46-6	16,730	H/V[a]
	Trimethoprim	738-70-5	3346	H/V[a]
Anti-convulsant	Carbamazepine	298-46-4	33,514	H
Anti-inflammatory	Diclofenac	15307-86-5	39,000	H
	Ibuprofen	15687-27-1	240,024	H
	Ketoprofen	22071-15-4	21,697	H/V[a]
	Naproxen	22204-53-1	37,332	H
	Paracetamol	103-90-2	3,303,077	H/V[a]
Anti-parasitic	Metronidazole	443-48-1	36,545	H/V[a]
Diuretic	Furosemide	54-31-9	21,288	H/V[a]
Fungicide	Econazole	27220-47-9		H
Hormone	α-Estradiol	57-63-6		H
	Androstenedione	63-05-8		H
	β-Estradiol	50-28-2		H
	Estriol	50-27-1		H
	Estrone	53-16-7		H/V
	Gestodene	60282-87-3		H
	Levonorgestrel	17489-40-6		H
	Norethindrone	68-22-4		H
	Progesterone	57-83-0		H/V
	Testosterone	58-22-0		H/V
Lipid regulator	Fenofibrate	49562-28-9	86,000	H
	Bezafibrate	41859-67-0	20,852	H
Psychotropic	Fluvoxamine	54739-18-3		H
	Lorazepam	846-49-1		H
	Oxazepam	604-75-1	6195	H
β-Blocker	Atenolol	29122-68-7	18,337	H
	Propanolol	4199-09-1		H

All human pharmaceutical uses are approved in France

[a] Veterinary pharmaceuticals approved in France (http://www.ircp.anmv.anses.fr/results.aspx)

[b] Veterinary pharmaceuticals not approved in France (Bártíková et al. 2016)

[c] Metabolite of enrofloxacin

Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

methods were evaluated with the appropriate matrix (e.g. effluent and surface water), resulting in an updated limit of detection (LOD) and limit of quantification (LOQ). Recoveries were between 60 and 120 %. Regarding the pollutants analyzed, two different analytical procedures were used for the different pollutant groups: α-estradiol, β-estradiol, estriol, estrone, ibuprofen and furosemide compounds were analyzed in electrospray ionization positive mode and other compounds in negative mode. Depending on the physicochemical properties of the compounds, in order to get suitable results in terms of sensitivity and recovery, extraction process had to be divided into several extractions with different extraction solvents. River water and effluent samples were filtered in fibreglass (142-mm diameter, pore 0.7 μm). They were similar overall for the solid-phase extraction (SPE). The water samples were extracted by SPE using the AutoTrace SPE workstation (Supplementary data). Finally, samples were analyzed by ultra-high-performance liquid chromatography (UHPLC, Agilent®) coupled with a tandem mass spectrometer (QqQ ABSciex®) in multiple reaction monitoring (MRM) mode. The injection volume was 40 μL at a flow rate of 400 μL min^{-1}. The source temperature was 550 °C. Each compound was characterized by its retention time, two MRM transitions and the ratio between the areas of MRM 1 and MRM 2. In a batch, each sample was injected twice. Instrumental performance was checked with the injection of quality control samples (pharmaceuticals dissolved in methanol). Extraction performance was checked following the signal areas of internal standards. Matrix-matched calibration was used for quantification. All compounds were obtained from Sigma-Aldrich with purity higher than or equal to 97 % (St. Quentin Fallavier, France). LC-MS-grade acetonitrile and methanol, ammonium formate, formic acid and citric acid were obtained from Fluka (Sigma-Aldrich). The water used was purified by a Milli-Q water system (Millipore, France).

Environmental risk assessment

The levels of each compound were compared to acute and chronic quality standards proposed by the Swiss Ecotox Centre (www.centreecotox.ch). The levels of pharmaceuticals in surface water and effluent were compared to the maximum allowable concentration (MAC), which corresponded to acute quality standards, and to annual average concentration (AA), which corresponded to chronic quality standards. The chronic quality standard is particularly relevant for the assessment of the impact of long-term pollution on aquatic organisms. Furthermore, the potential risk of each pharmaceutical was assessed by calculating its risk quotient (RQ) defined as the ratio between the maximum measured environmental concentration and the predicted no-effect concentration (PNEC). PNEC is the concentration below which no adverse effects of exposure in an ecosystem are measured and is calculated by applying an assessment factor (10 or 1000) on the lowest ecotoxicological values reported in the literature such as the no-observed-effect concentration (NOEC) or the concentration that causes adverse effects in 50 % of the test organisms (EC_{50}), respectively, for the most sensitive species assayed.

Results and discussion

Occurrence of selected pharmaceuticals in the effluents

WWTP A effluent

In the effluent from WWTP A, 13 and 12 pharmaceuticals of the selected compounds were quantified, respectively, in April 2014 and May 2015 (Table 3). For both campaigns, diclofenac (965 and 467 ng L^{-1}), sulfamethoxazole (655 and 615 ng L^{-1}) and carbamazepine (442 and 566 ng L^{-1}) displayed the highest concentrations. Concentrations of anti-inflammatories (diclofenac, naproxen), antibiotics (sulfamethoxazole, trimethoprim, metronidazole), oestrogen (estrone) and beta-blocker (propanolol) decreased in May 2015 compared to April 2014. The anti-inflammatory naproxen was among the most concentrated (254 ng L^{-1}) pharmaceuticals in April 2014 but was not detected in May 2015. Conversely, the psychiatric drug oxazepam was not quantified in April 2014 but was measured in May 2015 (167 ng L^{-1}).

WWTP B effluent

In April 2014, 14 pharmaceuticals among the 31 compounds selected were quantified and 5 were detected but not quantified (Table 3). In the second campaign of May 2015, 12 pharmaceuticals were quantified and 3 detected but not quantified among the 31 pharmaceuticals selected. For both campaigns, the three compounds at the highest concentrations were the analgesic diclofenac (2476 and 1055 ng L^{-1}), the antibiotic sulfamethoxazole (1380 and 640 ng L^{-1}) and the anti-epileptic carbamazepine (811 and 1007 ng L^{-1}).

Comparison between the two effluents

Among the selected pharmaceuticals, 20 of the 31 were detected and quantified at least once in both WWTP effluents. For both sampling campaigns and effluents, seven compounds were always detected (atenolol, carbamazepine, diclofenac, estrone, lorazepam, ofloxacine, sulfamethoxazole). Maximum concentrations found in this study for diclofenac, sulfamethoxazole and carbamazepine were in accordance with previously published data on the occurrence of these compounds (Verlicchi et al. 2012; Patrolecco et al. 2014).

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

Table 3 Concentrations (ng L^{-1}) of pharmaceuticals in effluents of WWTP A and WWTP B range in the literature

Class	Compound	Range[a]	WWTP A effluent		WWTP B effluent	
			April	May	March	May
Antibiotic	Ciprofloxacin	72–96.3	<LOD	<LOD	<LOQ	<LOD
	Ofloxacine		18.3	53	47.7	303
	Roxithromycine		<LOQ	<LOD	<LOQ	<LOD
	Sulfamethoxazole	115–578	655.7	615	1380.4	640
	Trimethoprim	100–229	61.6	46.4	241.2	<LOQ
Anti-convulsant	Carbamazepine	157–832	442.5	566	811.4	1007
Anti-inflammatory	Diclofenac	50–680	965.7	467	2476.3	1055
	Ibuprofen	80.5–1960	<LOD	<LOD	526.7	126
	Ketoprofen	86–392	143	260.5	107.2	<LOD
	Naproxen	26–1890	254.6	<LOD	355.3	100.1
	Paracetamol	108–11,309	<LOD	<LOD	<LOD	<LOD
Anti-parasitic	Metronidazole		35.8	<LOQ	31.5	<LOQ
Diuretic	Furosemide		<LOQ	<LOQ	<LOQ	<LOQ
Fungicide	Econazole		<LOD	<LOD	<LOD	<LOD
Hormone	A estradiol	0.8–3	<LOD	<LOD	<LOD	<LOD
	B estradiol	2.8–3	<LOQ	<LOD	<LOQ	<LOD
	Androstenedione		<LOD	5.6	<LOD	9.1
	Estriol		<LOD	<LOD	<LOD	<LOD
	Estrone		10	2.4	44.2	2.6
	Gestodene		<LOD	<LOD	<LOD	<LOD
	Levonorgestrel		<LOD	<LOD	<LOD	<LOD
	Progesterone		<LOD	<LOD	<LOD	<LOD
	Testosterone		<LOD	<LOD	<LOD	<LOD
Lipid regulator	Bezafibrate	25–816	15.6	57.6	27	<LOD
	Fenofibrate		<LOD	<LOD	<LOD	<LOD
Psychotropic	Fluvoxamine		<LOD	<LOD	<LOD	<LOD
	Lorazepam		28.6	47.4	41.4	123.5
	Norethindrone		<LOD	<LOD	<LOD	<LOD
	Oxazepam		<LOQ	167.5	<LOQ	286.5
β-Blocker	Atenolol	154–843	324.1	381	324.5	169.5
	Propanolol		1.5	<LOQ	6.8	17.5

[a] Togola and Budzinski (2008); Miège et al. (2009); Rosal et al. (2010); Loos et al. (2013); Luo et al. (2014)

Concentrations of diclofenac (2476 ng L^{-1}) and sulfamethoxazole (1380 ng L^{-1}) in both effluents were higher than the range of average concentrations found in other studies: from 50 to 680 ng L^{-1} for diclofenac and from 115 to 578 ng L^{-1} for sulfamethoxazole (Miège et al. 2009; De la Cruz et al. 2012; Loos et al. 2013). Although these compounds had low excretion rates by humans, we found them at high concentrations (Luo et al. 2014). Such levels suggest that these pharmaceuticals were frequently consumed. In addition, levels of naproxen, trimethoprim, carbamazepine, atenolol and estrone measured in this study were in accordance with those reported in other reports (Miège et al. 2009; Rosal et al. 2010; De la Cruz et al. 2012; Loos et al. 2013) dealing with the occurrence of pharmaceuticals in WWTP effluents from different countries. Even though ibuprofen was among the most frequently detected compounds according to the review of Verlicchi et al. (2012), it was not quantified in the effluent of WWTP A. However, ibuprofen concentrations in WWTP B effluent were in agreement with average concentrations (from 80.5 to 1960 ng L^{-1}) reported in the literature (Miège et al. 2009; Loos et al. 2013). Ciprofloxacin, roxithromycine, α-estradiol, β-estradiol, estriol and bezafibrate were not measured in the effluents although they were found in samples of other surveys (Miège et al. 2009; Loos et al. 2013; Gabet-Giraud et al. 2014). Differences of concentrations between the two effluents may be due to local variations in pharmaceutical

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

consumption, which likely determine the amounts of compounds in WWTP influent. Moreover, higher raw wastewater volume from industries and surface runoff during rainfall may have had a dilution effect on pharmaceutical concentrations in WWTP A (Kasprzyk-Hordern et al. 2009). Other factors like the type of wastewater treatment, temperature, hydraulic retention time (HRT) and sedimentation retention time (SRT) may affect the removal efficiency of the plant and, finally, the concentration of pharmaceuticals and may explain seasonal variations (Vieno et al. 2005; Patrolecco et al. 2014). Indeed, the largest biological reactor and highest HRT and SRT of WWTP A may improve removal efficiency of pharmaceuticals compared to WWTP B (Table 3). Furthermore, the chemical properties (log K_{ow}, pKa) of each compound determined their fate in the aqueous or particulate phase, so total concentrations of pharmaceuticals may be underestimated if taking into account only aqueous phase. Indeed, da Silva et al. (2013) showed that among 43 pharmaceuticals analyzed in WWTP effluent, 30 % were predominantly bound to suspended particulate matter.

Occurrence of selected pharmaceuticals in river water

Occurrence of pharmaceuticals in the Doubs River

Among the pharmaceuticals selected, six compounds (paracetamol, ketoprofen, estrone, ofloxacine, carbamazepine and trimethoprim) displayed lower concentrations downstream of WWTP A (from 0.3 to 120.7 ng L^{-1}), while 11 compounds (diclofenac, paracetamol, carbamazepine, sulfamethoxazole, atenolol, ketoprofen, estrone, trimethoprim, progesterone, lorazepam and ofloxacine) occurred at higher concentrations (from 2.0 to 300.5 ng L^{-1}, Fig. 2). Estrone and progesterone were measured in higher concentrations downstream of the WWTP (29.2 and 11.3 ng L^{-1}) than in the effluent (10 ng L^{-1} and not detected). Furthermore, paracetamol was not quantified in the effluent, while its concentration was higher downstream (273.6 ng L^{-1}) than upstream (120.7 ng L^{-1}) of the WWTP A. In May 2015, concentrations ranged from 0.1 to 69.6 ng L^{-1} (estrone and oxazepam, respectively) in upstream site and from 0.2 to 22.6 ng L^{-1} (carbamazepine and paracetamol, respectively) downstream of WWTP A. Paracetamol and testosterone were quantified upstream and downstream of WWTP A but not in the effluent. Oxazepam, carbamazepine, atenolol, trimethoprim and estrone were measured in higher concentrations upstream than downstream of the WWTP A.

Occurrence of pharmaceuticals in the Loue River

In March 2014, among the 31 pharmaceuticals selected, eight were quantified upstream of WWTP B (paracetamol, carbamazepine, atenolol, trimethoprim, ketoprofen, ofloxacine, estrone and progesterone), while 12 (diclofenac, paracetamol, sulfamethoxazole, carbamazepine, ketoprofen, ibuprofen, atenolol, trimethoprim, bezafibrate, lorazepam, estrone, ofloxacine) were found downstream of the discharge (Fig. 2). Except for paracetamol and progesterone, concentrations of the compounds were higher downstream of WWTP B discharge than upstream. The highest concentrations were found for diclofenac (166 ng L^{-1}), paracetamol (158 ng L^{-1}) and sulfamethoxazole (73 ng L^{-1}). In May 2015, only four pharmaceuticals (paracetamol, econazole, testosterone and trimethoprim) were quantified upstream and five (paracetamol, androstenedione, testosterone, trimethoprim and propanolol) downstream of the discharge of WWTP B. Three pharmaceuticals (trimethoprim, paracetamol and testosterone) were quantified downstream of the WWTP B but not in the effluent. Concentrations of compounds were much lower than in March 2014, the highest being for paracetamol (20 ng L^{-1}).

In both rivers, occurrence of carbamazepine, diclofenac, sulfamethoxazole and ketoprofen is consistent with previous reports dealing with the analysis of pharmaceutical residues in surface water (Gros et al. 2007; Vulliet and Cren-Olivé 2011). Estrone occurred upstream and downstream of the WWTP in the first campaign and ranged from 3.9 to 29.2 ng L^{-1}. It is regularly detected in surface water at concentrations from 0.1 to few ng L^{-1} because of its natural excretion by women and since it is a by-product of estradiol biodegradation (Daughton and Ternes 1999, Vulliet et al. 2009). Concentrations of estrone found in the Rivers Doubs and Loue were slightly higher than those reported in other rivers. In Germany, Zuehlke et al. (2005) found concentrations of estrone from 0.16 to 0.86 ng L^{-1}, Kuch and Ballschmiter (2001) found an average concentration of 0.7 ng L^{-1}, and in Italy, Laganà et al. (2004) reported 8 ng L^{-1}. In this study, testosterone was also quantified in several samples at concentrations of 2.6 to 3 ng L^{-1} but was not detected in the effluent of either WWTP. In China, Chang et al. (2011) reported very low concentrations of testosterone in WWTP effluent (0.2 to 1.2 ng L^{-1}). Furthermore, livestock excretion may represent another source of hormone release. Indeed, animal manure has been identified as a major source of natural steroids reaching the environment (Shore and Shemesh 2009). Bartelt-Hunt et al. (2011) reported the co-occurrence of pharmaceuticals and steroid hormones like estrone and testosterone in wastewater from cattle and swine facilities and in groundwater. Other compounds found in river water or WWTP effluents in this study are also used as veterinary drugs (Table 2). In France, according to the allowed list of veterinary medicines (https://www.anses.fr), eight compounds are used in both human and veterinary medicine: estriol, furosemide, ketoprofen, metronidazole, paracetamol, progesterone, sulfamethoxazole and trimethoprim. Trimethoprim and sulfamethoxazole, frequently observed in our samples, are usually employed in veterinary and human medicine due to their broad spectrum of

Tel. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

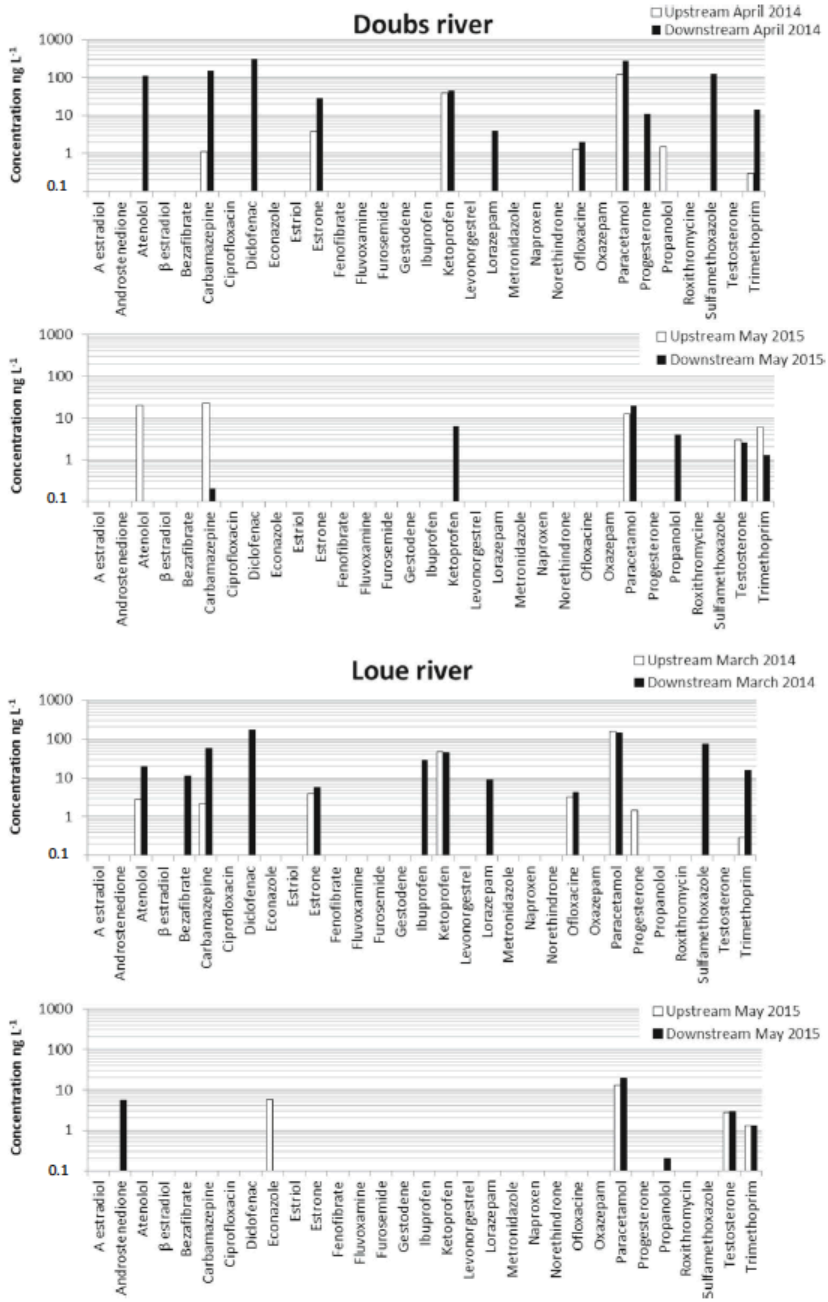

Fig. 2 Concentration (*Y* axis log scale) for the selected pharmaceuticals in the water of the River Doubs and the River Loue for the two sampling campaigns

activity and are among the most common pharmaceuticals used to prevent or treat bacterial infection (Pastor-Navarro et al. 2009). In addition, the degradation of some pharmaceuticals used in veterinary medicine produces metabolites, which correspond to human medicine. For example, ciprofloxacin is a major metabolite of enrofloxacine (EMEA 1998). Therefore, it was not possible to quantify the contribution of pharmaceuticals from agricultural input in the Rivers

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

Doubs and Loue. Nevertheless, due to the large numbers of pasture animals in the catchment, the veterinary origin of some pharmaceuticals (e.g. anti-microbials, anti-parasitics) cannot be excluded and will be investigated in further research. In Spain, Iglesias et al. (2013) suggested that the presence of anti-parasitic drugs only employed in veterinary medicine attested that veterinary residue inputs occur in the environment even though farming activities like dairy and meat production result in less drug residue pollution than human activity.

In the present study, estriol and α- and β-estradiols were not found in river water or effluent samples. Due to seasonal variation of flow rate, concentration of steroid oestrogen may vary and showed cyclical pattern (Johnson 2010). So samples collected during these field surveys may reflect a low seasonal concentration period. Furthermore, this may be due to their hydrophobic properties enhancing adsorption onto suspended particulate matter and sediment (Nie et al. 2015). For example, estradiol has been quantified in river sediments from the River Ouse (UK) at concentrations from <0.3 to 1.2 ng g^{-1} and from 0.4 to 3.3 ng g^{-1} dry weight for estrone (Labadie et al. 2007). Numerous studies have identified WWTPs as the main source of pharmaceuticals in surface water, suspended matter and sediment (Silva et al. 2013; Patrolecco et al. 2014). Moreover, da Silva et al. (2013) highlighted that analysis limited to the water phase resulted in underestimation of the concentrations of pharmaceuticals and that suspended particulate matter (SPM) had to be considered. Indeed, Yang et al. (2015) found that carbamazepine and trimethoprim were the most frequently detected compounds in an essentially urban catchment. In Finland, Lahti and Oikari (2011) found that SPMs downstream of WWTP were frequently contaminated with pharmaceuticals; however, they also quantified ibuprofen and ofloxacin in SPM located in a rural area. The authors assumed that the pharmaceuticals came from independent households but did not consider agricultural sources. Nevertheless, the highest concentrations of most of the compounds tested occurred downstream of the WWTPs, confirming that WWTP effluents represent the main source of ecosystem contamination. The dilution factor (Table 1) in rivers (from 0.1 to 3 %) and sorption onto suspended solids may explain why surface water concentrations of most of the pharmaceuticals investigated remain quite low after effluent discharge (Gómez et al. 2012). Therefore, surface water concentrations will partially depend on the stream flow rate and on the flow rate of the effluent. The occurrence of compounds in upstream sites may be explained by input from buildings not yet connected to the sewage network and/or by sewage overflow.

Furthermore, the presence of pharmaceuticals in the River Loue spring may be linked to groundwater contamination (Fig. 3). In March 2014 and May 2015, respectively, seven and five pharmaceuticals were quantified among 31 selected compounds. Two antibiotics (ofloxacine and trimethropim) and one anti-inflammatory (paracetamol) were found in both campaigns. Carbamazepine, estrone, ketoprofen and sulfamethoxazole were quantified in the March 2014 survey but not in May 2015. During the first campaign, the highest concentrations were found for paracetamol (132 ng L^{-1}) and ketoprofen (39 ng L^{-1}), while, in May 2015, the highest concentration was for ofloxacine (20 ng L^{-1}). Propanolol and testosterone were quantified only in the second campaign (0.1 and 2.8 ng L^{-1}). These results are in accordance with a study in a karst system of the Swiss Jura which found that ketoprofen, diclofenac and sulfamethoxazole occurred frequently in the springs in the nanogram-per-litre range (Morasch 2013). They also showed that the swallow hole draining an agricultural plain was the main entry path for micropollutants into the karst system and the connected springs and suggested domestic sewers as additional sources of contamination. Contamination of groundwater mainly results from landfill leachate, groundwater–surface interaction

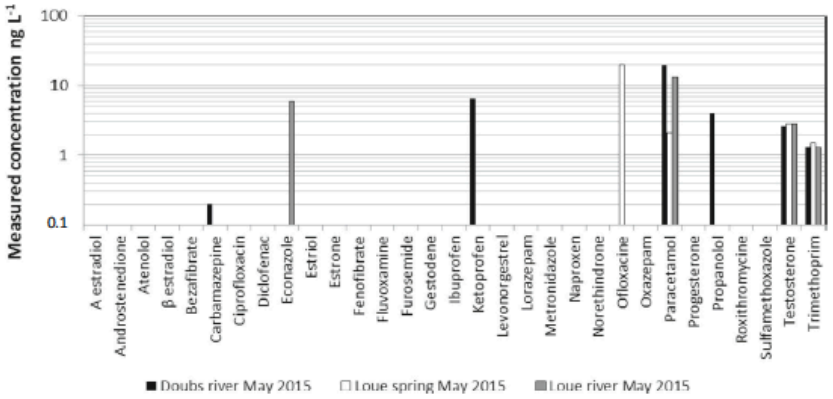

Fig. 3 Concentration of pharmaceuticals downstream of WWTP A in the River Doubs near the karstic losses, in the karst spring of the Loue River and upstream of WWTP B in the River Loue

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

and infiltration of contaminated water from agricultural land and/or sewer systems. Thin soil coverage with a low field capacity and highly permeable local infiltration pathways leads to groundwater recharging very rapidly after precipitation events. Transfer of compounds in the karst system led to decreased concentrations of pharmaceuticals due to different processes such as dilution, adsorption, degradation and travel time. Consequently, our results are fully consistent with the fact that karst springs are highly vulnerable and show strong variations in spring discharge and water quality (Heinz et al. 2008).

Toxicity

Many studies have demonstrated acute toxicities of pharmaceuticals on aquatic organisms from microgram per litre to milligram per litre (Cleuvers 2003; Ferrari et al. 2003).

Table 4 Concentrations (μg L^{-1}) of pharmaceuticals in the River Loue

Loue River (μg L^{-1})	Compound	PNEC[a]	MAC-EQS[b]	AA-EQS[c]	March 2014			May 2015		
Class					Spring	Upstream	Downstream	Spring	Upstream	Downstream
Antibiotic	Ciprofloxacin	938	0.363	0.089	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Ofloxacin	0.016			0.0007	0.0032	0.0042	*0.02* a	<LOD	<LOD
	Roxithromycin	4			<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Sulfamethoxazole	0.027	2.7	0.6	0.0015	<LOD	*0.0729* a	<LOD	<LOD	<LOD
	Trimethoprim	2.6	1100	60	0.0001	0.0003	0.0155	0.0015	0.0013	0.0013
Anti-convulsant	Carbamazepine	13.8	2550	0.5	0.0019	0.0022	*0.0568* c	<LOD	<LOD	<LOD
Anti-inflammatory	Diclofenac	0.1		0.05	<LOD	<LOD	*0.1665* c	<LOD	<LOD	<LOD
	Ibuprofen	1.65	23	0.3	<LOD	<LOD	0.0283	<LOD	<LOD	<LOD
	Ketoprofen	15.6			0.039	0.0462	0.0445	<LOD	<LOD	<LOD
	Naproxen	2.62	370	1.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Paracetamol	1			0.1317	0.158	0.1389	0.0021	0.0131	0.0196
Anti-parasitic	Metronidazole	2.5			<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Diuretic	Furosemide				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Fungicide	Econazole				<LOD	<LOD	<LOD	<LOD	0.0059	<LOD
Hormone	α-Estradiol			0.000037	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Androstenedione				<LOD	<LOD	<LOD	<LOD	<LOD	0.0056
	β-Estradiol			0.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Estriol				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Estrone			3.6	0.0021	0.0039	0.0057	<LOD	<LOD	<LOD
	Gestodene				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Levonorgestrel				<LOD	<<LOD	<LOD	<LOD	<LOD	<LOD
	Norethindrone				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Progesterone				<LOD	0.0015	<LOD	<LOD	<LOD	<LOD
	Testosterone				0.0001	<LOD	<LOD	0.0028	0.0028	0.0029
Lipid regulator	Bezafibrate		76	460	<LOD	<LOD	0.0112	<LOD	<LOD	<LOD
	Fenofibrate	0.1			<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Psychotropic	Fluvoxamine				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Lorazepam				<LOD	<LOD	0.0088	<LOD	<LOD	<LOD
	Oxazepam				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
β-Blocker	Atenolol	30	330	150	<LOD	0.0028	0.0196	<LOD	<LOD	<LOD
	Propanolol		12,000	160	<LOD	<LOD	<LOD	0.0001	<LOD	0.0002

Values in italics exceed threshold values (a, b, c)

PNEC predicted no-effect concentration, *MAC-EQS* environmental quality standard relative to the maximum allowable concentration, *AA-EQS* annual average concentration

[a] Data from Kümmerer and Henninger (2003); Sanderson et al. (2003); Ferrari et al. (2003); Lindqvist et al. (2005); Hoeger et al. (2005); Isidori et al. (2005); Ra et al. (2008); Quinn et al. (2008)

[b, c] http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards/?_ga=1.12802039.1593799960.1475153874

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

For example, the EC_{50}s for the crustacean *Daphnia magna* were reported to be >13,800 μg L^{-1}, 22,430 μg L^{-1}, 25.20 mg L^{-1} and 31.75 mg L^{-1} for carbamazepine, diclofenac, sulfamethoxazole and ofloxacin, respectively (Ferrari et al. 2003; Isidori et al. 2005). However, as concentrations in surface water are in the nanogram-per-litre or microgram-per-litre ranges, most pharmaceuticals did not cause lethal toxicity. Nevertheless, chronic exposure to low concentrations of pollutants may lead to sublethal effects, including changes in behaviour for example. De Lange et al. (2006) demonstrated a change of behaviour in *Gammarus pulex* exposed to 10 ng L^{-1} ibuprofen, which corresponded to concentrations 10^4 to 10^7 times lower than the previously reported lowest observable effect concentration (LOEC) and in the range of environmentally occurring concentrations. Schwaiger et al. (2004) showed that long-term exposure to diclofenac in environmentally relevant concentrations led to an impairment of the general health of fishes. However, pharmaceuticals in mixtures may trigger chronic and synergetic effects. Moreover, the toxic

Table 5 Concentrations of pharmaceuticals (μg L^{-1}) in the River Doubs

River Doubs					April 2014		May 2015	
Class	Compound	PNEC	MAC-EQS	AA-EQS	Upstream	Downstream	Upstream	Downstream
Hormone	α-Estradiol			0.000037	<LOD	<LOD	<LOD	<LOD
Hormone	Androstenedione				<LOD	<LOD	<LOD	<LOD
β-Blocker	Atenolol	30	330	150	<LOD	0.1146	0.0204	<LOD
Hormone	β-Estradiol			0.4	<LOD	<LOD	<LOD	<LOD
Lipid regulator	Bezafibrat		76	460	<LOD	<LOD	<LOD	<LOD
Anti-convulsant	Carbamazepine	13.8	2550	0.5	0.0011	0.1488	0.0224	0.0002
Antibiotic	Ciprofloxacin	938	0.363	0.089	<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Diclofenac	0.1		0.05	<LOD	*0.3005* c	<LOD	<LOD
Fungicide	Econazole				<LOD	<LOD	<LOD	<LOD
Hormone	Estriol				<LOD	<LOD	<LOD	<LOD
Hormone	Estrone			3.6	0.0038	0.0292	0.0001	<LOD
Lipid regulator	Fenofibrate	0.1			<LOD	<LOD	<LOD	<LOD
Psychotropic	Fluvoxamine				<LOD	<LOD	<LOD	<LOD
Diuretic	Furosemide				<LOD	<LOD	<LOD	<LOD
Hormone	Gestodene				<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Ibuprofen	1.65	23	0.3	<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Ketoprofen	15.6			0.0394	0.0452	<LOD	0.0065
Hormone	Levonorgestrel				<LOD	<LOD	<LOD	<LOD
Psychotropic	Lorazepam				<LOD	0.0039	<LOD	<LOD
Anti-parasitic	Metronidazole	2.5			<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Naproxen	2.62	370	1.7	<LOD	<LOD	<LOD	<LOD
Hormone	Norethindrone				<LOD	<LOD	<LOD	<LOD
Antibiotic	Ofloxacin	0.016			0.0013	0.002	<LOD	<LOD
Psychotropic	Oxazepam				<LOD	<LOD	<LOD	<LOD
Anti-inflammatory	Paracetamol	1			0.1207	0.2736	0.0122	0.0196
Hormone	Progesterone				<LOD	0.0113	<LOD	<LOD
β-Blocker	Propanolol		12,000	160	0.0015	<LOD	<LOD	0.004
Antibiotic	Roxithromycin	4			<LOD	<LOD	<LOD	<LOD
Antibiotic	Sulfamethoxazole	0.027	2.7	0.6	<LOD	*0.1258* a	<LOD	<LOD
Hormone	Testosterone				<LOD	<LOD	0.003	0.0026
Antibiotic	Trimethoprim	2.6	1100	60	0.0616	0.0138	0.0059	0.0013

Values in italics exceed threshold values (a, b, c)

PNEC predicted no-effect concentration, *MAC-EQS* environmental quality standard relative to the maximum allowable concentration, *AA-EQS* annual average concentration

UMR 6249 CNRS-UFC ast INRA Chrono-Environnement – Univ. de Franche-Comte – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

effect of multiple compounds at concentrations below the LOEC may produce significant effects in the following generations (Parrott and Bennie 2009). Melvin et al. (2014) demonstrated an increase of toxicity in amphibians exposed to a mixture of compounds (naproxen, carbamazepine and sulfamethoxazole) commonly occurring in wastewater, compared to exposures to the single compounds. Chronic exposure to a mixture of antibiotic with sulfamethoxazole and trimethoprim at 50 μg L^{-1} led to a significant decrease in the sex ratio of the first brood of *D. magna* (Flaherty and Dodson 2005). This indicated that mixtures of antibiotics potentially found in aquatic ecosystems may affect the developmental and reproductive processes of non-target aquatic organisms and trigger responses in other aquatic populations.

The RQs, i.e. the ratio between the measured concentration of each compound and their corresponding PNEC, were calculated for river water (Tables 4 and 5) and effluent samples (Table 6). RQ in river water showed that ofloxacin and sulfamethoxazole were the most critical compounds and posed a medium and high environmental risk (RQ >0.1 and RQ >1), respectively. Up to now, European and French legislation has not provided limits for the concentrations of pharmaceuticals in WWTP effluent. However, comparison of concentrations of pharmaceuticals in surface water showed that several compounds (carbamazepine, diclofenac, ofloxacine and sulfamethoxazole) exceeded chronic quality standard proposed by the Swiss Ecotox Centre (www.centreecotox.ch). Furthermore, we evaluated the toxicity level of effluents

Table 6 Predicted no-effect concentration (PNEC μg L^{-1}) and corresponding risk quotients (RQs) defined as the ratio between the maximum measured environmental concentration and the predicted no-effect concentration (PNEC) for the selected compounds in both WWTP effluents

Class	Compound	PNEC	WWTP A March 2014 RQ	WWTP A May 2015 RQ	WWTP B April 2014 RQ	WWTP B May 2015 RQ
Hormone	A estradiol		–	–	–	–
Hormone	Androstenedione		–	–	–	–
β-Blocker	Atenolol	30	0.01	0.01	0.01	0.01
Hormone	B estradiol		–	–	–	–
Lipid regulator	Bezafibrat		–	–	–	–
Anti-convulsant	Carbamazepine	13.8	0.06	0.07	0.03	0.04
Antibiotic	Ciprofloxacin	938	–	–	–	–
Anti-inflammatory	Diclofenac	9.7	0.26	0.11	0.10	0.05
Fungicide	Econazole		–	–	–	–
Hormone	Estriol		–	–	–	–
Hormone	Estrone		–	–	–	–
	Fenofibrate	0.1	–	–	–	–
Psychotropic	Fluvoxamine		–	–	–	–
Diuretic	Furosemide		–	–	–	–
	Gestodene		–	–	–	–
	Ibuprofen	1.65	0.32	0.08	–	–
	Ketoprofen	15.6	0.01	–	0.01	0.02
	Levonorgestrel		–	–	–	–
	Lorazepam		–	–	–	–
	Metronidazole	2.5	0.01	–	0.01	–
	Naproxen	2.62	0.14	0.04	0.10	–
	Norethindrone		–	–	–	–
	Ofloxacin	0.016	2.98	18.94	1.14	3.31
	Oxazepam		–	–	–	–
	Paracetamol	1	–	–	–	–
	Progesterone		–	–	–	–
β-Blocker	Propanolol		–	–	–	–
	Roxithromycin	4	–	–	–	–
	Sulfamethoxazole	0.027	51.13	23.70	24.29	22.78
	Testosterone		–	–	–	–
	Trimethoprim	2.6	0.09	–	0.02	0.02

by calculating RQ values for each compound separately. Table 6 shows that, for both WWTPs, ofloxacin and sulfamethoxazole pose high environmental risk (RQ >1). Moreover, diclofenac, ibuprofen and naproxen may cause medium risk in effluent of WWTP A with RQ >0.1. These results indicate that both WWTP discharges constituted a significant risk for the aquatic environment in the Rivers Doubs and Loue. Even though most of the compounds tested are discharged at low concentrations, the daily continuous discharge into rivers may contribute to adverse effects on aquatic biota due to chronic and mixture toxicities. Little is known about the possible long-term effects and low-level exposure to pharmaceuticals (Santos et al. 2010). While knowledge regarding the environmental occurrence of veterinary pharmaceuticals is increasing, information in the literature on their fate and effects is limited (Bártíková et al. 2016). In addition, veterinary medicines are often introduced in the same area in combination with other pollutants like pesticides, which may increase adverse effects on non-target organisms. Therefore, aquatic organisms may be exposed to mixture of human and veterinary pharmaceuticals and pesticides that could have synergetic adverse effects. There is a definite need for studies addressing long-term effects of low doses of veterinary medicines and their degradation products in the environment, the behaviour of mixtures of veterinary drugs and the interactions of veterinary drugs with other pharmaceuticals or chemicals in the ecosystem.

Conclusion

The occurrence of 31 pharmaceuticals in two WWTPs located in a rural and karstic catchment has been established together for the first time with their concentrations in the receiving waters, the Rivers Doubs and Loue. In the WWTP effluents and surface water, diclofenac and sulfamethoxazole were detected in relatively high levels showing that WWTPs are not able to efficiently remove pharmaceuticals. Furthermore, this study showed that the levels of ofloxacin and sulfamethoxazole in surface water can pose a risk, highlighting their potential hazard for the health of the aquatic ecosystem. In addition, the occurrence of some pharmaceuticals upstream of the WWTP and in the karst spring of the Loue River indicated other domestic and agricultural releases in the catchment and transport of the contaminants through the karst system. In order to improve water quality management and to reduce the impact on the aquatic ecosystem, authorities have to consider WWTPs as hotspots of contamination and take into account diffuse sources in the catchment due to the high risk of transfers in the karst system.

Acknowledgments We thank A. Bolard and E. Chanez for sampling assistance. This study was financially supported by the Agence de l'eau Rhône-Méditerranée-Corse, La Région Franche-Comté and the Doubs département. The support of Peter Winterton with English editing is also greatly acknowledged.

References

Al Aukidy M, Verlicchi P, Jelic A et al (2012) Monitoring release of pharmaceutical compounds: occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. Sci Total Environ 438:15–25. doi:10.1016/j.scitotenv.2012.08.061

ANSM (2014) Analyse des ventes de médicaments en France en 2013.

Bartelt-Hunt S, Snow DD, Damon-Powell T, Miesbach D (2011) Occurrence of steroid hormones and antibiotics in shallow groundwater impacted by livestock waste control facilities. J Contam Hydrol 123:94–103. doi:10.1016/j.jconhyd.2010.12.010

Bártíková H, Podlipná R, Skálová L (2016) Veterinary drugs in the environment and their toxicity to plants. Chemosphere 144:2290–2301. doi:10.1016/j.chemosphere.2015.10.137

Besse J-P, Garric J (2008) Human pharmaceuticals in surface waters: implementation of a prioritization methodology and application to the French situation. Toxicol Lett 176:104–123. doi:10.1016/j.toxlet.2007.10.012

Bichet V, Campy M (2008) Montagnes du Jura—Géologie et paysages, NEO.

Boxall ABA, Fogg LA, Blackwell PA et al (2004) Veterinary medicines in the environment. Rev Environ Contam Toxicol 180:1–91

Boxall ABA, Kolpin DW, Halling-Sørensen B, Tolls J (2003) Are veterinary medicines causing environmental risks? Environ Sci Technol 37:286A–294A

Brooks BW, Chambliss CK, Stanley JK et al (2005) Determination of select antidepressants in fish from an effluent-dominated stream. Environ Toxicol Chem 24. doi:10.1897/04-081R.1

Chang H, Wan Y, Wu S et al (2011) Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: comparison to estrogens. Water Res 45:732–740. doi:10.1016/j.watres.2010.08.046

Choi K, Kim Y, Park J et al (2008) Seasonal variations of several pharmaceutical residues in surface water and sewage treatment plants of Han River, Korea. Sci Total Environ 405:120–128. doi:10.1016/j.scitotenv.2008.06.038

Cleuvers M (2003) Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol Lett 142:185–194. doi:10.1016/S0378-4274(03)00068-7

Conley JM, Symes SJ, Schorr MS, Richards SM (2008) Spatial and temporal analysis of pharmaceutical concentrations in the upper Tennessee River basin. Chemosphere 73:1178–1187. doi:10.1016/j.chemosphere.2008.07.062

da Silva AK, Amador J, Cherchi C et al (2013) Emerging pollutants—part I: occurrence, fate and transport. Water Environ Res 85:1978–2021. doi:10.2175/106143013X13698672323065

Daughton CG, Ternes TA (1999) Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 107:907–938

De la Cruz N, Giménez J, Esplugas S et al (2012) Degradation of 32 emergent contaminants by UV and neutral photo-Fenton in domestic wastewater effluent previously treated by activated sludge. Water Res 46:1947–1957. doi:10.1016/j.watres.2012.01.014

De Lange HJ, Noordoven W, Murk AJ et al (2006) Behavioural responses of *Gammarus pulex* (Crustacea, Amphipoda) to low

UMR 6249 CNRS-UFC usc INRA Chrono-Environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

concentrations of pharmaceuticals. Aquat Toxicol 78:209–216. doi:10.1016/j.aquatox.2006.03.002

Einsiedl F, Radke M, Maloszewski P (2010) Occurrence and transport of pharmaceuticals in a karst groundwater system affected by domestic wastewater treatment plants. J Contam Hydrol 117:26–36. doi:10.1016/j.jconhyd.2010.05.008

EMEA (1998) Committee for Veterinary Medicinal Products. Enrofloxacin. The European Agency for the Evaluation of Medicinal Products

European Union (2000) Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy.

Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. Aquat Toxicol 76:122–159. doi:10.1016/j.aquatox.2005.09.009

Ferrari B, Paxéus N, Giudice RL et al (2003) Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicol Environ Saf 55:359–370. doi:10.1016/S0147-6513(02)00082-9

Flaherty CM, Dodson SI (2005) Effects of pharmaceuticals on Daphnia survival, growth, and reproduction. Chemosphere 61:200–207. doi:10.1016/j.chemosphere.2005.02.016

Gabet-Giraud V, Miège C, Jacquet R, Coquery M (2014) Impact of wastewater treatment plants on receiving surface waters and a tentative risk evaluation: the case of estrogens and beta blockers. Environ Sci Pollut Res Int 21:1708–1722. doi:10.1007/s11356-013-2037-7

Gómez MJ, Herrera S, Solé D et al (2012) Spatio-temporal evaluation of organic contaminants and their transformation products along a river basin affected by urban, agricultural and industrial pollution. Sci Total Environ 420:134–145. doi:10.1016/j.scitotenv.2012.01.029

Gros M, Petrović M, Barceló D (2007) Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (Northeast Spain. Environ Toxicol Chem 26:1553–1562. doi:10.1897/06-495R.1

Heinz B, Birk S, Liedl R et al (2008) Water quality deterioration at a karst spring (Gallusquelle, Germany) due to combined sewer overflow: evidence of bacterial and micro-pollutant contamination. Environ Geol 57:797–808. doi:10.1007/s00254-008-1359-0

Hoeger B, Köllner B, Dietrich DR, Hitzfeld B (2005) Water-borne diclofenac affects kidney and gill integrity and selected immune parameters in brown trout (*Salmo trutta* f. fario. Aquat Toxicol 75: 53–64. doi:10.1016/j.aquatox.2005.07.006

Iglesias A, Nebot C, Vázquez BI et al (2013) Detection of veterinary drug residues in surface waters collected nearby farming areas in Galicia, north of Spain. Environ Sci Pollut Res 21:2367–2377. doi:10.1007/s11356-013-2142-7

Isidori M, Lavorgna M, Nardelli A et al (2005) Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Sci Total Environ 346:87–98. doi:10.1016/j.scitotenv.2004.11.017

Jacquemin P (1984) Réponses hydrodynamiques des hauts bassins du Doubs et de la Loue. Université de Franche-comté

Jjemba PK (2006) Excretion and ecotoxicity of pharmaceutical and personal care products in the environment. Ecotoxicol Environ Saf 63: 113–130. doi:10.1016/j.ecoenv.2004.11.011

Johnson AC (2010) Natural variations in flow are critical in determining concentrations of point source contaminants in rivers: an estrogen example. Environ Sci Technol 44:7865–7870. doi:10.1021/es101799j

Joss A, Zabczynski S, Göbel A et al (2006) Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. Water Res 40:1686–1696. doi:10.1016/j.watres.2006.02.014

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res 42: 3498–3518. doi:10.1016/j.watres.2008.04.026

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res 43:363–380. doi:10.1016/j.watres.2008.10.047

Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. Ecol Indic 8:1–13. doi:10.1016/j.ecolind.2007.06.002

Kolpin DW, Furlong ET, Meyer MT 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. doi:10.1021/es011055j

Kools SAE, Moltmann JF, Knacker T (2008) Estimating the use of veterinary medicines in the European union. Regul Toxicol Pharmacol 50:59–65. doi:10.1016/j.yrtph.2007.06.003

Kuch HM, Ballschmiter K (2001) Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. Environ Sci Technol 35:3201–3206

Kümmerer K (2009) The presence of pharmaceuticals in the environment due to human use—present knowledge and future challenges. J Environ Manag 90:2354–2366. doi:10.1016/j.jenvman.2009.01.023

Kümmerer K, Henninger A (2003) Original article promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin. Microbiol Infect 9(12):1203–1214 doi:10.1111/j.1469-0691.2003.00739.x

Labadie P, Cundy AB, Stone K et al (2007) Evidence for the migration of steroidal estrogens through river bed sediments. Environ Sci Technol 41:4299–4304. doi:10.1021/es063062j

Laganà A, Bacaloni A, De Leva I et al (2004) Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. Anal Chim Acta 501: 79–88. doi:10.1016/j.aca.2003.09.020

Lahti M, Oikari A (2011) Pharmaceuticals in settleable particulate material in urban and non-urban waters. Chemosphere 85:826–831. doi:10.1016/j.chemosphere.2011.06.084

Lindqvist N, Tuhkanen T, Kronberg L (2005) Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. Water Res 39:2219–2228. doi:10.1016/j.watres.2005.04.003

Loos R, Carvalho R, António DC et al (2013) EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res 47:6475–6487. doi:10.1016/j.watres.2013.08.024

Luo Y, Guo W, Ngo HH et al (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ 473–474:619–641. doi:10.1016/j.scitotenv.2013.12.065

Melvin SD, Cameron MC, Lanctôt CM (2014) Individual and mixture toxicity of pharmaceuticals naproxen, carbamazepine, and sulfamethoxazole to Australian striped marsh frog tadpoles (*Limnodynastes peronii*. J Toxicol Environ Health A 77:337–345. doi:10.1080/15287394.2013.865107

Miège C, Choubert JM, Ribeiro L et al (2009) Fate of pharmaceuticals and personal care products in wastewater treatment plants—conception of a database and first results. Environ Pollut 157:1721–1726. doi:10.1016/j.envpol.2008.11.045

Morasch B (2013) Occurrence and dynamics of micropollutants in a karst aquifer. Environ Pollut 173:133–137. doi:10.1016/j.envpol.2012.10.014

Nie M, Yan C, Dong W et al (2015) Occurrence, distribution and risk assessment of estrogens in surface water, suspended particulate matter, and sediments of the Yangtze Estuary. Chemosphere 127:109–116. doi:10.1016/j.chemosphere.2015.01.021

UMR 6249 CNRS-UFC use INRA Chrono-Environnement – Univ. de Franche-Comté – 16, route de Gray – F 25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr

ONEMA, Villeneuve A, Humbert, JF, et al. (2012) Rapport d'expertise sur les mortalités de poissons et les efflorescences de cyanobactéries de la Loue. ONEMA

Osorio V, Larrañaga A, Aceña J et al (2016) Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers. Sci Total Environ 540:267–277. doi:10.1016/j.scitotenv.2015.06.143

Parrott JL, Bennie DT (2009) Life-cycle exposure of fathead minnows to a mixture of six common pharmaceuticals and triclosan. J Toxicol Environ Health A 72:633–641. doi:10.1080/15287390902769428

Pastor-Navarro N, Maquieira A, Puchades R (2009) Review on immunoanalytical determination of tetracycline and sulfonamide residues in edible products. Anal Bioanal Chem 395:907–920. doi:10.1007/s00216-009-2901-y

Patrolecco L, Capri S, Ademollo N (2014) Occurrence of selected pharmaceuticals in the principal sewage treatment plants in Rome (Italy) and in the receiving surface waters. Environ Sci Pollut Res:1–13. doi:10.1007/s11356-014-3765-z

Quinn B, Gagné F, Blaise C (2008) An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, Hydra attenuata. Sci Total Environ 389:306–314. doi:10.1016/j.scitotenv.2007.08.038

Ra JS, Lee BC, Chang NI, Kim SD (2008) Comparative whole effluent toxicity assessment of wastewater treatment plant effluents using *Daphnia magna*. Bull Environ Contam Toxicol 80:196–200. doi:10.1007/s00128-007-9344-y

Rosal R, Rodríguez A, Perdigón-Melón JA et al (2010) Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Res 44:578–588. doi:10.1016/j.watres.2009.07.004

Sanderson H, Johnson DJ, Wilson CJ et al (2003) Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. Toxicol Lett 144:383–395

Santos LHMLM, Araújo AN, Fachini A et al (2010) Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J Hazard Mater 175:45–95. doi:10.1016/j.jhazmat.2009.10.100

Schwaiger J, Ferling H, Mallow U et al (2004) Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: part I: histopathological alterations and bioaccumulation in rainbow trout. Aquat Toxicol 68:141–150. doi:10.1016/j.aquatox.2004.03.014

Shore LS, Shemesh M (2009) Naturally produced steroid hormones and their release into the environment. Pure Appl Chem 75:1859–1871. doi:10.1351/pac200375111859

Silva C, Oliveira C, Gravato C, Almeida JR (2013) Behaviour and biomarkers as tools to assess the acute toxicity of benzo(a)pyrene in the common prawn *Palaemon serratus*. Mar Environ Res 90:39–46. doi:10.1016/j.marenvres.2013.05.010

Togola A, Budzinski H (2008) Multi-residue analysis of pharmaceutical compounds in aqueous samples. J Chromatogr A 1177:150–158. doi:10.1016/j.chroma.2007.10.105

Verlicchi P, Al Aukidy M, Zambello E (2012) Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment—a review. Sci Total Environ 429:123–155. doi:10.1016/j.scitotenv.2012.04.028

Vieno NM, Tuhkanen T, Kronberg L (2005) Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environ Sci Technol 39:8220–8226. doi:10.1021/es051124k

Vulliet E, Cren-Olivé C (2011) Screening of pharmaceuticals and hormones at the regional scale, in surface and groundwaters intended to human consumption. Environ Pollut 159:2929–2934. doi:10.1016/j.envpol.2011.04.033

Vulliet E, Cren-Olivé C, Grenier-Loustalot M-F (2009) Occurrence of pharmaceuticals and hormones in drinking water treated from surface waters. Environ Chem Lett 9:103–114. doi:10.1007/s10311-009-0253-7

Yang Y-Y, Toor GS, Williams CF (2015) Pharmaceuticals and organochlorine pesticides in sediments of an urban river in Florida, USA. J Soils Sediments 15:993–1004. doi:10.1007/s11368-015-1077-7

Zuehlke S, Duennbier U, Heberer T (2005) Determination of estrogenic steroids in surface water and wastewater by liquid chromatography–electrospray tandem mass spectrometry. J Sep Sci 28:52–58. doi:10.1002/jssc.200301727

UMR 6249 CNRS-UFC usc INRA Chrono-environnement – Univ. de Franche-Comté – 16, route de Gray - F-25030 BESANÇON CEDEX
Tél. 03 81 66 57 09 – Fax 03 81 66 57 97 – pierre-marie.badot@univ-fcomte.fr, francois.degiorgi@univ-fcomte.fr