OPUR : Observatoire d'hydrologie urbaine en Île de France
Thème de recherche R2: Diagnostic et optimisation des systèmes d'assainissement vis-à-vis des polluants et des micropolluants
Action de recherche R2.6

ÉVALUATION ECOTOXICOLOGIQUE D'UN POLLUANT PHARMACEUTIQUE D'INTERET EMERGENT (LE FUROSEMIDE) ET DE SES PRODUITS DE DEGRADATION

Rapport final
Thèse de doctorat de Fidji Sandré
Juin 2024

- *Thèse réalisée au Laboratoire Eau Environnement et Systèmes Urbains, sous la direction de Laure Garrigue-Antar et Christophe Morin.*

laboratoire eau environnement systemes urbains

Ecole doctorale Sciences, Ingénierie et Environnement (SIE) - ED n°531
Laboratoire Eau, Environnement et systèmes Urbaines (LEESU)

Thèse de doctorat

Bourse de thèse du Ministère de l'Education Supérieur, de la Recherche et de l'Innovation
Financé par le programme de recherche OPUR
En vue de l'obtention du grade de Docteur en Ecotoxicologie
Délivré par l'Université Paris Est Créteil

Présentée le 8 juin 2023 par :

Fidji SANDRÉ

Évaluation écotoxicologique d'un polluant pharmaceutique d'intérêt émergent (le furosémide) et de ses produits de dégradation

---

Composition du jury :

Olivier GEFFARD — rapporteur
Directeur de recherche à l'INRAe Lyon

Jérôme CACHOT — rapporteur
Professeur à l'Université de Bordeaux

Séverine LE FAUCHEUR — Examinateur
Enseignant chercheur à l'Université de Pau et des pays de l'Adour

Sandrine PAIN-DEVIN — Examinateur
Maître de conférences à l'Université de Lorraine

Laure GARRIGUE-ANTAR — Directrice de thèse
Professeure à l'Université Paris Est Créteil

Christophe MORIN — Co-directeur de thèse
Professeur à l'Université Paris Est Créteil

Ecole doctorale Sciences, Ingénierie et Environnement (SIE) - ED n°531
Laboratoire Eau, Environnement et systèmes Urbaines (LEESU)

Thèse de doctorat

En vue de l'obtention du grade de Docteur en Ecotoxicologie
Délivré par l'Université Paris Est Créteil

Présentée le 8 juin 2023 par :

Fidji SANDRÉ

Évaluation écotoxicologique d'un polluant pharmaceutique d'intérêt émergent (le furosémide) et de ses produits de dégradation

"It's a long way to the top if you wanna Rock & Roll" - AC/DC

Remerciements

Je tiens à remercier en premier lieu **Régis Moilleron**, directeur du Leesu, pour m'avoir accueillie au laboratoire. Merci de m'avoir soutenue à l'école doctorale, de t'être rendu disponible pour répondre à mes (nombreuses) requêtes et pour tes avis toujours très constructifs. Tu as rendu la vie au laboratoire plus sympathique pour moi et tous les doctorants (le canapé, les soirées jeux, tes super blagues bien-sûr,..).

Je souhaite également exprimer ma reconnaissance aux membres de mon jury de thèse, **Olivier Geffard**, **Jérôme Cachot**, **Sandrine Pain-Devin** et **Séverine Le Faucheur**, pour avoir accepté d'évaluer ce travail. Merci pour votre temps et votre engagement.

Merci également au programme **OPUR** pour le soutien financier.

J'adresse ensuite mes sincères remerciements à mes encadrants, **Laure Garrigue-Antar** et **Christophe Morin**, pour m'avoir donné l'opportunité de travailler sur cette thèse. Merci de m'avoir entraînée avec vous dans ce projet passionnant! Christophe, merci pour ta gentillesse, ton soutien et tes encouragements. Tes conseils et tes commentaires m'ont beaucoup aidée. Merci également d'avoir toujours répondu aux S.O.S. aquariums! Laure, tu m'as laissée libre d'aborder ce sujet comme je le souhaitais tout en étant très (très) présente. Merci pour la confiance que tu m'as accordée et ton soutien sans faille. Nous avons partagé de nombreuses galères (fichus poissons!), mais nous avons aussi bien rigolé. J'ai eu la chance d'avoir une directrice de thèse passionnée par la recherche et toujours souriante. Merci pour toutes les portes que tu m'as ouvertes.

Je tiens à remercier chaleureusement les membres de mon comité de suivi de thèse, **Stéphanie Simon** et **Chahrazade El-Amri** pour leur écoute lors des réunions, leurs conseils bienveillants et leurs encouragements.

Cette thèse à été le fruit de collaboration et d'échanges avec de nombreuses personnes. Je tiens alors à remercier tous mes collègues et collaborateurs. En particulier, merci **Emilie Caupos** pour tes explications précieuses et ton aide tout au long de la partie analytique de mon projet. Merci **Julien Le Roux** pour tes conseils et tes commentaires, c'est un plaisir de travailler avec toi. Merci à **Christophe Pommier** d'avoir débloqué une situation désespérée. Ton enthousiasme sur le projet nous a fait chaud au cœur. Merci **Michaël Rivard** pour ton implication sur le projet et la synthèse du pyridinium. Merci **Benoit Vallée** pour m'avoir formé à la culture cellulaire, pour ta grande aide sur cette partie du projet et pour ton incontestable humour. Merci **Sandrine Bourgoin**, pour ton aide sur la partie protéomique de ma thèse, pour ta disponibilité et ta gentillesse ainsi qu'à **Damien Haber** et **José Courty** pour votre aide précieuse. Je remercie également **Sonia Decker** pour toute l'aide et les ressources apportées. Merci à tous ceux qui ont, de près ou de loin, contribué à cette thèse.

Je tiens à remercier chaleureusement tous mes super stagiaires pour leur travail, leur bonne humeur, leur grande implication et, bien-sûr, leurs gâteaux. Merci **Marion**, ma première stagiaire, d'avoir partagé les galères de Roulépur avec moi. Merci **Antoine**, qui fait des script R pour le plaisir le dimanche soir et **Luna** qui laisse des commentaires sur les documents à minuit, pour votre sérieux (parfois trop). Merci **Aliénor** pour toute ton aide avec les poissons (et ton amour pour les daphnies) et **Alexis** pour tout le travail fourni. Vous êtes au top!

Merci à tous les résidents (actuels et anciens) de la MSE et ses environs, pour votre bonne humeur, les gâteaux, les soirées jeux, les Secret-Santas, les gâteaux, le sport, les Inktobers, les gâteaux, les pauses Sutom du midi, et bien sûr, les gâteaux. Merci **Nadia**, **Pierre**, **Nadège**, **Julien**, **Julia**, **Antoine**, **Manel**, **Lamyae**, **Rachid**, **Max** et les anciens **Bertille**, **Claudia** et **Robin**, pour tous les bons moments, **Chandirane**, pour toute l'aide que tu m'as apportée, **Angélique** pour les suggestions de thé les plus pointues, **My-dung** pour tes encouragements, la motivation pour le sport et, bien-sûr, la soirée nems, **Christelle** pour les bonnes rigolades, et merci **Claire** pour toute ton aide, tes conseils, ton écoute, les photos de toutous et pour les longs moments à râler dans le labo de bio. Un grand merci aussi aux deux autres membres du "trio infernal". **Nina**, tes heures passées sur le canapé du Leesu sont une source d'inspiration pour moi. Merci d'avoir partagé ton amour pour le projet Roulepur, pour toute ton aide, les activités de rempotage et de création d'étiquettes et d'affiches et les bonnes excuses pour procrastiner. **Maxime** mon guide parisien, merci pour toutes les sorties et les bons moments ensemble, les randos, cinéma, canoë, aprèm jeux, l'organisation de la journée à Koezio et pour le support émotionnel au labo le weekend. J'espère que l'on fera pleins d'autres "détours touristiques".

Bien évidemment, merci à ma famille, **Katy**, **Bruno**, **Enzo**, **Annette** et **Inox** pour votre soutien et les incessants "quand est-ce qu'on te voit ?". Je n'ai plus l'excuse "je ne peux pas, je rédige ma thèse" maintenant.

Merci mes petits bourdons, **Alexia**, **Ludovic**, **Louis**, **Gaëlle** et **Laura** pour votre soutien et vos encouragements. Merci **Nico** pour le réconfort, le gras, le gras, encore le gras, tes SUPERBES pizzas, le refourgage de potion magique pour les algues, les petites plantes, plus de petites plantes, les tioups, et tous les bons moments que l'on a partagé malgré les galères de nos thèses respectives. Merci **Vincent** pour ta frivolité, le ravitaillement en fromage de survie et les MAGNIFIQUES cartes sur R. Tu étais le souffle imprévisible de cette fin de thèse. Merci **Océane** d'avoir partagé mes galères depuis les cours de chimie organique jusqu'à la fin de cette thèse et pour les séances de psychanalyses à distances. On reprend très vite en face à face. Merci mes petits pâtés, **Myriam**, **Noémie** et **Linh** de m'avoir écoutée et encouragée. Enfin, à mes Charentais préférés, **Benoit**, **Morgane**, **Pauline**, merci d'être là depuis le début ♥.

Liste des publications et participation à des conférences

Articles & revue

- **Publié en 2023** dans Chemosphere

Sandre F., Huynh N., Caupos E., El-Mrabet L., Partibane C., Lachaise I., Pommier C., Rivard M., Morin C., Moilleron R., Le Roux J., Garrigue-Antar L. Occurrence and fate of an emerging drug pollutant and its by-products during conventional and advanced wastewater treatment: case study of furosemide. Chemosphere 2023, 322, 138212, https://doi.org/10.1016/j.chemosphere.2023.138212.

- **Publié en 2022** dans Water (travaux de master)

Sandré F., Huynh N., Gromaire M.-C., Varrault G., Morin C., Moilleron R., Le Roux J., Garrigue-Antar L. Road Runoff Characterization: Ecotoxicological Assessment Combined with (Non-)Target Screenings of Micropollutants for the Identification of Relevant Toxicants in the Dissolved Phase. Water 2022, 14, 511. DOI: 10.18785/gcr.3001.14

- **Publié en 2019** dans Gulf and caribbean research (travaux de master)

Sandre F., Dromard C. R., Le Menach K., Bouchon-Navaro Y., Cordonnier S., Tapie N., Budzinski H., Bouchon C. 2019. Detection of Adsorbed Chlordecone on Microplastics in Marine Sediments in Guadeloupe: A Preliminary Study. Gulf and Caribbean Research 30 (1): GCFI 8-GCFI 14. DOI: 10.3390/w14040511

- **En préparation pour soumission** dans Environmental Science and Technology

Sandre F., Morin C., Garrigue-Antar L. Furosemide, a pharmaceutical pollutant of emerging interest: Origin and fate in the aquatic environment. (Revue)

- **En préparation pour soumission** dans Environmental Science and Pollution Research

Sandre F., Duval A., Golven A., Morin C., Garrigue-Antar L. First approach for the ecotoxicological assessment of a ubiquitous pharmaceutical compound and its transformation products.

Communications par affiche

- **Internationale, 2018** (travaux de master)

Sandre F., Dromard C. R., Le Menach K., Bouchon-Navaro Y., Tapie N., Budzinski H., Bouchon C. 2018. Contamination of marine sediment by microplastics and absorbed organochlorine pollution (chlordecone) in coral reefs of Guadeloupe (Lesser Antilles). 71th conference of the Gulf and Caribbean Fisheries Institute (GCFI): 5-9 November 2018, San Andres, Colombia.

- **Nationale, 2019** (travaux de master)

Sandré F., Flanagan K., Guttmann Y., Rabate M., Moilleron R., Gromaire M-C., Garrigue-Antar L., Morin C. Approche écotoxicologique de l'efficacité du traitement d'eaux de voirie par des ouvrages de dépollution par un modèle de larve de poisson zèbre. Colloque de la Société d'Écotoxicologie Fondamentale et Appliquée (SEFA), Juin 2019, Lyon, France.

- **Nationale, 2020**

Sandre F., Garrigue-Antar L., Morin C. Évaluation écotoxicologique d'un polluant pharmaceutique émergent : le Pyridinium du Furosémide (PoF). Colloque de la Société Française d'Ecotoxicologie Fondamentale et Appliquée (SEFA), Juillet 2020, Virtuel, France.

Communications Orales

- **Internationale, 2020**

Sandre F., Morin C., Garrigue-Antar L. Ecotoxicological Study of an Emerging Pharmaceutical Pollutant: The Pyridinium of Furosemide (PoF). SETAC North America 41st Annual Meeting, November 2020, Virtual, United States

- **Internationale, 2020**

Huynh N., Sandre F., Garrigue-Antar L., Morin C., Caupos E. Gromaire M-C., Moilleron R., Le Roux J. Evaluation of road runoff treatment systems performance by non-target screening combined with ecotoxicological studies. 16th Annual Workshop On Emerging High-Resolution Mass Spectrometry And LC-MS/MS Applications In Environmental Analyses And Food Safety, October 2020, Online meeting, Spain

- **Internationale, 2022**

Sandre F., Morin C., Garrigue-Antar L. Ecotoxicological evaluation of a pharmaceutical pollutant (furosemide) and its degradation products. 12th Micropol & Ecohazard conference, 6-10 June 2022, Santiago de Compostella, Spain

- **Internationale, 2022 - Prix du public pour la meilleure communication orale**

Sandre F., Garrigue-Antar L. Étude écotoxicologique d'un polluant pharmaceutique (le furosémide) et de ses produits de dégradation. Colloque joint de la Société Francophone de Santé et Environnement (SFSE) et de la Fondation Rovaltain, 23-25 novembre 2022 Valence, France

- **Nationale, 2020**

Sandre F., Garrigue-Antar L., Morin C. Présentation de la zebrabox et application à l'écotoxicologie (étude de cas). Webinaire de la Plateforme Régionale d'Analyse Multi-milieux des Micros-Contaminants (PRAMMICS) de l'OSU-EFLUVE. Novembre 2020. pod.u-pec.fr/video/0905-matinales-prammics-17-novembre-2020-pole-biologique/

- **Nationale, 2022**

Sandre F., Morin C., Garrigue-Antar L. Évaluation écotoxicologique d'un polluant pharmaceutique (le furosémide) et de ses produits de dégradation et étude de leur transfert vers l'environnement. Journée scientifique du programme OPUR, phase 5, 06 janvier 2022

- **Nationale, 2022 - Prix de la meilleure communication orale**

Sandre F., Duval A., Golven A., Morin C., Garrigue-Antar L. Évaluation écotoxicologique d'un polluant pharmaceutique et de ses produits de dégradation par une approche multi-modèle. Colloque de la Société Française d'Ecotoxicologie Fondamentale et Appliquée (SEFA), 30 juin-1 juillet 2022 Metz, France

- **Nationale, 2023**

Sandre F., Interview pour les 10 ans de la Fondation Rovaltain pour la recherche en environnement. https://www.youtube.com/watch?v=ZVhZYpUDWjI&ab_channel=FondationRovaltain

- **Locale, 2021**

Sandre F., Garrigue-Antar L., Morin C. Évaluation écotoxicologique d'un polluant pharmaceutique (le furosémide) et de ses produits de dégradation. Journée scientifique de l'Ecole Doctorale Sciences Ingénierie, Environnement (ED SIE), 25 juin 2021, virtuel

- **Locale, 2021**

Sandre F., Garrigue-Antar L., Morin C. Évaluation écotoxicologique d'un polluant pharmaceutique (le furosémide) et de ses produits de dégradation et étude de leur transfert vers l'environnement. LEESURIALES, Séminaire interne au laboratoire, 15-16 juin 2021, Créteil, France

- **Locale, 2021**

Sandre F., Garrigue-Antar L., Morin C. Évaluation écotoxicologique d'un polluant pharmaceutique et de ses produits de dégradation par une approche multi-modèle. LEESURIALES, Séminaire interne au laboratoire, 11-12 juillet 2022, Créteil, France

Résumé

De nombreux médicaments sont consommés quotidiennement et sont ensuite évacués dans les eaux usées. Cependant, les Stations de Traitement des Eaux Usées (STEU) ne sont pas conçues pour éliminer efficacement ces composés, qui sont alors rejetés dans l'environnement aquatique et représentent un danger pour les écosystèmes. Parmi ces polluants pharmaceutiques, certains sont très fréquemment retrouvés dans les eaux de surface: c'est notamment le cas du furosémide. C'est l'un des médicaments les plus utilisés dans le monde. Considéré comme un médicament essentiel par l'Organisation Mondiale de la Santé, c'est un puissant diurétique de l'anse largement prescrit pour traiter l'insuffisance cardiaque et rénale ou l'hypertension. Fortement consommé, persistant et peu éliminé par les STEU, le furosémide s'inscrit peu à peu comme un composé d'intérêt émergent.

De plus, au cours de son transit vers l'environnement, le furosémide peut être dégradé en plusieurs sous-produits, qui sont encore très mal caractérisés. La première partie de ce travail a pour but de quantifier dans l'environnement deux d'entre eux, également connus comme métabolites humains : La saluamine, connue depuis longtemps, et le pyridinium du furosémide, récemment découvert, possible inducteur de maladies neurodégénératives sont en effet particulièrement préoccupants. Une méthode de chromatographie liquide couplée à de la spectrométrie de masse a été développée pour les quantifier. L'analyse de différents échantillons (EHPAD, STEU, rivière) a montré pour la première fois leur présence dans le milieu aquatique. En parallèle, l'efficacité de dégradation du furosémide par des procédés de traitement avancé (UV/H_2O_2, Chloration, Ozonation) a été évaluée en plus de la formation de nouveaux produits de dégradation. La chloration et l'ozonation se sont révélées très efficaces pour éliminer le furosémide mais produisent en revanche de la saluamine.

Le furosémide, la saluamine et le pyridinium, peuvent donc présenter un risque important pour les organismes non cibles. La seconde partie de cette thèse a donc pour objectif d'évaluer leur toxicité, à des concentrations fortes et environnementales, sur des modèles représentatifs d'un écosystème aquatique (poisson, daphnie, algue). Plusieurs bioessais ont été développés afin d'évaluer la toxicité aiguë, la modification des traits fonctionnels, le stress oxydant, ou encore leur impact sur le comportement. Nos résultats montrent un effet, non seulement des produits de dégradation, mais également du furosémide dès les concentrations environnementales. De plus, une première approche sur les effets cocktails a été menée sur les daphnies et montre un effet synergique de ces molécules.

La saluamine et le pyridinium étant des métabolites humains, la troisième partie de cette étude s'intéresse donc à leur impact sur les cellules humaines de foie, de rein et de neuroblastome. Des tests de toxicité aiguë montrent également un effet plus important des sous-produits et du mélange par rapport au furosémide. Des analyses protéomiques ont aussi été réalisées afin d'identifier certains mécanismes d'action au travers de l'expression de protéines dérégulées.

Finalement, ces travaux soulignent l'importance de mieux caractériser les produits de dégradation lors de l'évaluation du risque lié à un micropolluant, car ils peuvent se révéler plus toxiques que leur molécule parent. L'approche multi-modèle est également pertinente du fait de la sensibilité différente des organismes aux micropolluants. Ces résultats mettent en lumière l'intérêt d'étudier les effets cocktails, à ce jour encore peu documentés dans les conditions environnementales. De nombreux composés pharmaceutiques comme le furosémide peuvent avoir des effets délétères sur l'écosystème même à des concentrations trace. Il serait alors intéressant d'intégrer ces composés aux listes de polluants suivis qui ne sont pour l'instant pas du tout pris en compte dans les réglementations.

Abstract

Many drugs are consumed daily and then discharged into wastewater. However, wastewater treatment plants (WWTPs) are not designed to effectively eliminate these compounds, which are then released into the aquatic environment, thus representing a hazard for ecosystems. Among these pharmaceutical pollutants, some are very frequently found in surface water, as in the case of furosemide. It is one of the most widely used drugs in the world. Considered as an essential drug by the World Health Organization, it is a powerful loop diuretic widely prescribed to treat heart and kidney failure or hypertension. Highly consumed, persistent and poorly eliminated by WWTPs, furosemide is increasingly becoming a compound of emerging concern.

Moreover, during its transfer to the environment, furosemide can be degraded into several by-products, which remain poorly characterized to date. The first part of this work aims to quantify two of them, also known as human metabolites, in the environment: Saluamine, known for a long time, and the recently discovered pyridinium of furosemide, possible inducer of neurodegenerative diseases, are indeed particularly concerning. A liquid chromatography-mass spectrometry method has been developed to quantify them. The analysis of different samples (EHPAD, STEU, river) showed for the first time their presence in the aquatic environment. In parallel, the degradation efficiency of furosemide by advanced treatment processes (UV/H_2O_2, chlorination, ozonation) has been evaluated in addition to the formation of new degradation products. Chlorination and ozonation were found to be very effective in removing furosemide but produce saluamine.

Furosemide, saluamine and pyridinium, may therefore present a significant risk to non-target organisms. The second part of this thesis aims at evaluating their toxicity, at high and environmental concentrations, on representative models of an aquatic ecosystem (fish, daphnia, algae). Several bioassays were developed to evaluate acute toxicity, modification of functional traits, oxidative stress, or their impact on behavior. Our results show an effect, not only of degradation products, but also of furosemide at environmental concentrations. Moreover, a first approach on the cocktail effects was carried out on daphnids and shows a synergistic effect of these molecules.

Saluamine and pyridinium being human metabolites, the third part of this study focuses on their impact on human liver, kidney and neuroblastoma cells. Acute toxicity tests also showed a greater effect of the by-products and the mixture compared to furosemide. Proteomic analyses were also performed to identify certain mechanisms of action through the expression of deregulated proteins.

Finally, this work underlines the importance of better characterizing the degradation products during the risk assessment of a micropollutant, because they can be more toxic than their parent molecule. The multi-model approach is also relevant because of the different sensitivity of organisms to micropollutants. These results highlight the interest of studying cocktail effects, which are still poorly documented under environmental conditions. Many pharmaceutical compounds such as furosemide can have adverse effects on the ecosystem even at trace concentrations. It would then be interesting to include these compounds in the lists of monitored pollutants that are currently not taken into account at all in the regulations.

Sommaire

Liste des abréviations

DCE _ Directive Cadre sur l'Eau
ECHA _ European CHemical Agency
EFESE _ Évaluation française des écosystèmes et des services
EHPAD _ Établissement d'Hébergement pour Personnes Agées Dépendantes
ERE _ Evaluation du Risque Environnemental
FUR _ Furosemide
ISO _ International Organization for Standardization
LEESU _ Laboratoire Eau, Environnement et Systèmes Urbains
MDMA _ 3,4-Methylenedioxymethamphetamine
NQE _ Norme Qualité Environnement
OCDE _ Organisation de Coopération et de Développement Économiques
OMS _ Organisation Mondiale de la Santé
PCB _ PolyChloroBiphenyls
PEC _ Predicted Environmental Concentration
PNEC _ Predicted No Effect Concentration
PYR _ Pyridinium du furosémide
QSAR _ Quantitative Structure Activity Relationship
REACH _ Registration, Evaluation, Authorisation and Restriction of Chemicals
SAL _ Saluamine
SDAGES _ Schéma Directeur d'Aménagement et de Gestion des Eaux
SNDS _ Système National des Données de Santé
STEU _ Station de Traitement des Eaux Usées
UPLC-MS-MS _ Ultra-Performance Liquid Chromatography with tandem Mass Spectrometry
UPLC-IMS-QTOF _ Ultra-Performance Liquid Chromatography Ion Mobility Separation Quadruple Time-of-Flight

Projets & programmes
DOREMIPHARM _ Développement d'Outils Robustes d'Évaluation pour les MIlieux aquatiques du danger des substances PHARMaceutiques
MEIDSEINE _ Étude de la contamination par les médicaments de l'estuaire de la Seine
OCAPI _ Organisation des cycles Carbone, Azote, Phosphore dans les territoires
OPUR _ Observatoire des Polluants Urbains
POSEIDON _ Assessment of technologies for the removal of pharmaceuticals and personal care products in sewage and drinking water facilities to improve the indirect potable water reuse
REMPAR _ Présence et devenir de micropolluants d'intérêt dans le réseau d'assainissement du Bassin d'Arcachon
REMPHARMAWATER _ Ecotoxicological assessments and removal technologies for pharmaceuticals in wastewaters

Liste des Tableaux & Figures

Avant propos

Ce travail de recherche a été mené au Laboratoire Eau, Environnement, et Systèmes Urbains (LEESU, UMR MA 102) à l'Université Paris Est Créteil.

Cette thèse s'inscrit dans le cadre du programme de recherche OPUR phase 5 (Observatoire des Polluants Urbains) qui a pour but d'améliorer les connaissances sur la production et le transfert des flux d'eau et de contaminants dans les eaux urbaines. L'axe de recherche R2.6, dont ce travail dépend, s'intéresse aux nouvelles méthodes de caractérisation des polluants et micropolluants au travers de l'analyse par screening qualitatif et l'écotoxicologie.

Certaines expériences ont été menées en collaboration avec l'IMRB (Institut Mondor de Recherche Biomédicale (IMRB, U955 Inserm, équipe I-Biot), l'ICMPE (Institut de Chimie et des Matériaux Paris-Est, UMR CNRS 7182, équipe ECCO) et la plateforme de protéomique de l'Université Paris-Est Créteil.

Introduction générale

Introduction générale

En Europe, une quantité croissante de nouvelles molécules chimiques est mise sur le marché chaque année. En 2022, plus de 22 000 substances sont enregistrées par l'Agence Européenne des produits chimiques (ECHA)[1]. Ces dernières peuvent se retrouver dans les eaux usées en étant rejetées directement par des industries ou transportées par les eaux de ruissellement des sols et des routes, qui peuvent être contaminées par des produits biocides ou des hydrocarbures (Paijens et al. 2019, Sandré et al. 2022). Ils peuvent également être issus de l'utilisation quotidienne de médicaments et de produits cosmétiques qui sont éliminés dans les eaux usées lorsqu'ils sont utilisés et métabolisés par l'organisme (Godoy et al. 2015, Cizmas et al. 2015).

Les eaux usées, alors chargées de ces composés, sont conduites dans les Stations de Traitement des Eaux Usées (STEU). Ces STEU, de superficie et de fonctionnement variable selon le volume d'eau à traiter, sont équipées de différents systèmes de traitement chimiques, physiques ou biologiques conçus pour purifier l'eau avant de la rejeter dans l'environnement (voir Chapitre II). Ces procédés sont conçus pour éliminer les macrodéchets, retirer les graisses, décanter les boues et éliminer des polluants organiques comme l'azote. Malheureusement, ces systèmes ne permettent pas d'éliminer les micropolluants (polluants présents en faible concentrations) tels que les produits cosmétiques, les biocides, les hormones et bien-sûr, les médicaments (**Figure 1**)(Chiffre et al. 2016, Cizmas et al. 2015).

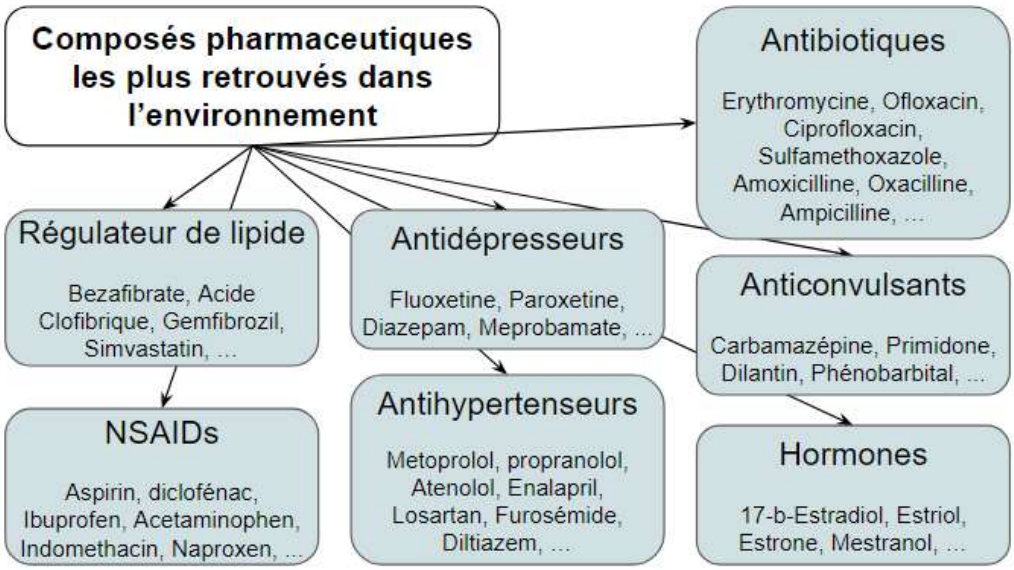


Figure 1. Les composés pharmaceutiques les plus fréquemment retrouvés dans l'environnement, adapté de Cizmas et al. 2015.

La qualité des eaux rejetées dans le milieu s'en trouve donc fortement détériorée et la présence de ces composés dans l'environnement déstabilise les écosystèmes (Cizmas et al. 2015). Afin de pallier cette dégradation progressive du milieu aquatique, un cadre réglementaire s'instaure progressivement autour des masses d'eau.

[1] https://echa.europa.eu/fr/registration-statistics consulté le 27/12/2022

En France, depuis 1964, la gestion des masses d'eau est organisée en fonction des bassins versants (surface géographique pour laquelle toutes les eaux sont drainées vers un cours d'eau principal et ses affluents). Des agences de l'eau sont créées pour gérer ces 12 bassins (**Figure 2**).

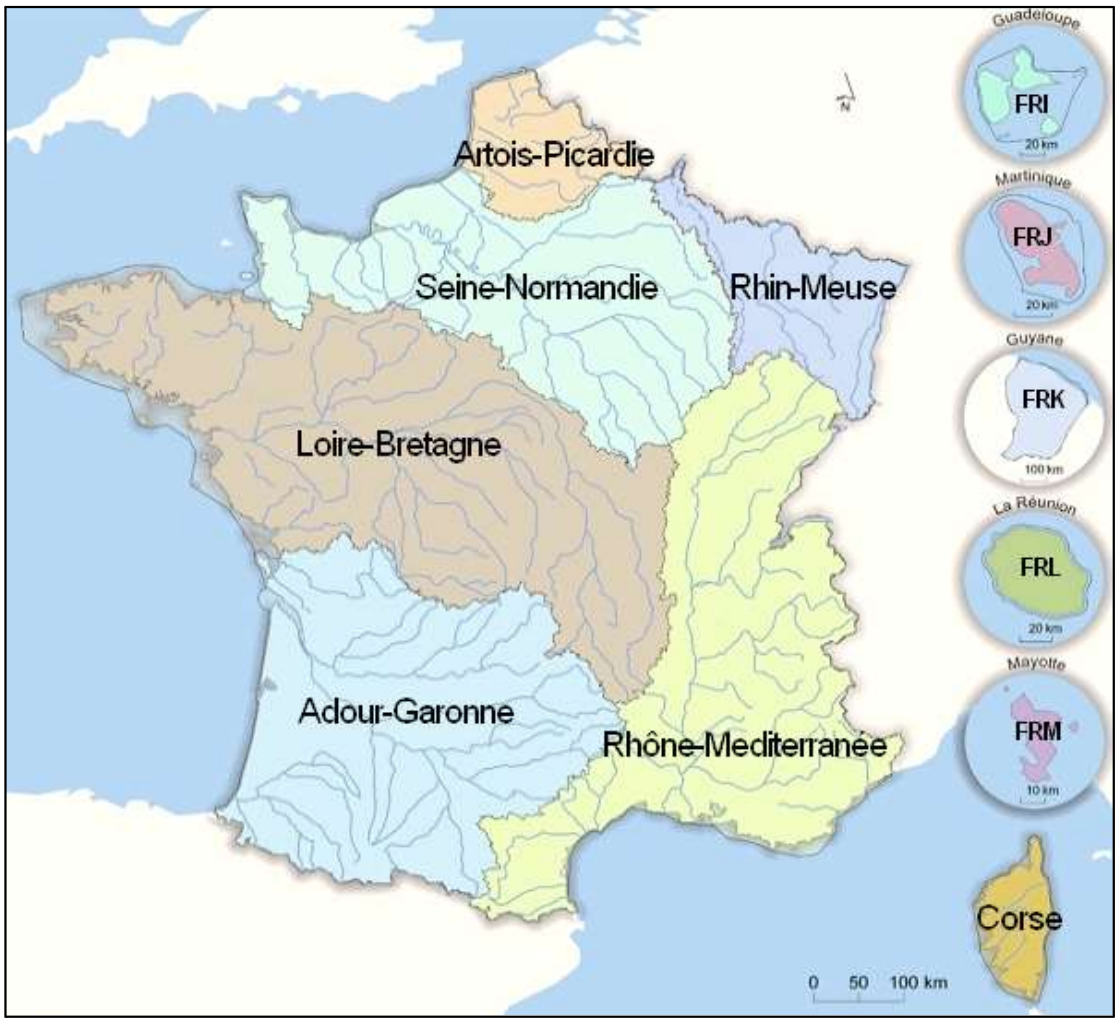


Figure 2. Les 12 bassins hydrographiques français. Schéma adapté de oieau.fr. (Bassin Adour-Garonne, Artois-Picardie, Loire-Bretagne, Rhin-Meuse, Rhône-Méditerranée, Corse, Seine-Normandie, Guadeloupe (FRI), Guyane (FRK), Martinique (FRJ), La Réunion (FRL), Mayotte(FRM)).

Depuis 1992, un Schéma Directeur d'Aménagement et de Gestion des Eaux (SDAGE) est déterminé pour chacun de ces bassins hydrographiques pour cadrer les activités des différents usagers de l'eau (industriels, agriculteurs, consommateurs)[2]. Enfin, en 2000, une Directive Cadre sur l'Eau (DCE)[3] est mise en place au niveau Européen dont l'un des objectifs est d'obtenir un bon état des masses d'eau pour les 27 pays membres de l'Union Européenne. La DCE impose à la fois un bon état écologique, défini par une bonne santé des écosystèmes et mesuré par des indicateurs biologiques, et un bon état chimique, déterminé en fonction de valeurs seuils pour 41 substances : 8 substances dites dangereuses (annexe IX de la DCE) et 33 substances prioritaires (annexe X de la DCE), présentées dans le **tableau 1**.

La liste des substances prioritaires recense des métaux, des hydrocarbures et des biocides, choisis en raison de leur toxicité, persistance, bioaccumulation, potentiel cancérigène, occurrence dans le milieu

[2] https://www.ecologie.gouv.fr/gestion-leau-en-france
[3] Directive 2000/60/CE du Parlement européen et du Conseil du 23 octobre 2000

aquatique, production et usage. Ces polluants dits “historiques”, ont été largement étudiés et des réglementations ont été mises en place pour limiter leur impact sur l'environnement. A l’inverse, de nouveaux polluants récemment identifiés, dits “émergents” ou des des composés présents depuis longtemps qui sont considérés comme préoccupant depuis peu, dits "d'intérêt émergent", ne sont pas encore pris en compte dans ces réglementations. C’est notamment le cas des composés pharmaceutiques qui sont de plus en plus reconnus comme un enjeu de santé publique et environnementale.

Tableau 1. Liste des substances prioritaires et dangereuses de la DCE.

Substances prioritaires figurant à l'annexe X de la DCE, Directive 2008/105/CE					
1	Alachlore	14	Endosulfan	27	Pentachlorophénol
2	**Anthracène**	15	**Fluoranthène**	28	**Hydrocarbures Aromatiques Polycycliques (HAP)**
3	Atrazine	16	Hexachlorobenzène		**Benzo-[a]-pyrène**
4	Benzène	17	Hexachlorobutadiène		**Benzo-[b]-fluoranthène**
5	**Pentabromodiphényléther (PBDE)**	18	Hexaclorocyclohexane		**Benzo-[k]-fluoranthène**
6	**Cadmium et ses composés**	19	Isoproturon		**Benzo-[g,h,i]-perylène**
7	C10-13-chloroalcanes	20	**Plomb et ses composés**		Indeno-[1,2,3-cd]-pyrène
8	Chlorfenvinphos	21	**Mercure et ses composés**	29	Simazine
9	Chlorpyrifos	22	**Naphtalène**	30	Composés du tributylétain
10	1,2-Dichloroéthane	23	**Nickel et ses composés**	31	Trichlorobenzènes (tous isomères)
11	Dichlorométhane	24	**Nonylphénols**	32	Trichlorométhane
12	**Di(2-éthylhexyl)phtalate (DEHP)**	25	**Octylphénols**	33	Trifluraline
13	Diuron	26	Pentachlorobenzène		

Substances de la directive 76/464	
1	DDT total
2	Aldrine
3	Dieldrine
4	Endrine
5	Isodrine
6	Tétrachlorure de carbone
7	Tétrachloroéthylène
8	Trichloroéthylène

Ces derniers ont pourtant un usage croissant et de nombreuses molécules actives sont commercialisées chaque année. En France, pas moins de 3,1 milliards de boîtes de médicaments ont été consommées représentant plus de 2800 substances actives en 2013[4]. Cependant, la conception et la fabrication des médicaments visent essentiellement la protection de la santé humaine (Marchand-Pilard 2021). Une autorisation de mise sur le marché est obligatoire pour tous les médicaments, mentionnant l’identité, les caractéristiques, la qualité, la sécurité et l’efficacité du médicament accompagné d'études pharmacologiques, pharmacocinétiques et toxicologiques respectives (Directive 2001/83/CE). Depuis 2005, une évaluation du Risque Environnementale (ERE) est également demandée (Directive 2004/27/CE), basé sur les concentrations environnementales prédites (PEC) et les concentrations sans effets observées (PNEC) pour l’environnement. Cependant, l’ERE n’a pas d’incidence sur l’autorisation du composé. Si un risque est avéré à la suite de cette évaluation, l’entreprise doit seulement indiquer le risque environnemental ainsi que les conditions de stockage et d’élimination recommandées (Directive 2001/83/CE). Cette mesure de prévention vise à sensibiliser les professionnels de santé mais n’empêche pas la commercialisation et donc, finalement, la présence de ces composés dans le milieu aquatique.

Le furosémide est un exemple édifiant de cette pollution (**figure 3.a**). Ce diurétique de l’anse de la famille des sulfonamides, commercialisé depuis 1964 (Stokes and nunn 1964) est très rapidement devenu un médicament essentiel selon l’Organisation Mondiale de la Santé en raison de son efficacité, de sa sûreté et de son importance pour la santé publique. Il est donc largement consommé dans le monde et, de ce fait, omniprésent dans le milieu aquatique. Il est fréquemment retrouvé dans les effluents de STEU, dans les eaux de surface, mais aussi parfois dans les eaux souterraines. Cependant, malgré sa présence abondante, peu de données existent concernant son impact sur le milieu. Quelques évidences de génotoxicité et

[4] Analyse des ventes de médicaments en France en 2013, Rapport de l’ANSM, juin 2014.

cytotoxicité ont été relevées chez le poisson zèbre (Rocco et al. 2010), d'hépatotoxicité chez les rongeurs (Peterson 2013) et une activité estrogénique sur un modèle in vitro mais peu d'études se focalisent sur son écotoxicité. De plus, le furosémide peut être dégradé au cours de sa consommation par l'Homme (métabolisation), son transit dans les réseaux d'eaux usées et dans les STEU (hydrolyse, biodégradation, oxydations chimiques) et sa résidence dans les cours d'eau (photodégradation), formant ainsi plusieurs produits de dégradation (Bindschedler et al. 1997, Andreasen et al. 1982, Laurencé et al. 2014, Gros et al. 2020, Zoumpouli et al. 2021, Starling et al. 2019).

Certains de ces sous-produits sont connus depuis quelques années. Le furfural (**figure 3.d**), par exemple, est bien étudié du fait de son utilisation dans l'industrie chimique (solvant pour les colorants, inhibiteur de corrosion, réactif pour la synthèse de médicaments, …)(Grosse et al. 2017). Des données sur sa toxicité existent mais il semble représenter un risque moindre car il est rapidement biodégradé dans l'environnement (Mandalika et al. 2014). La saluamine (**figure 3.c**) est en revanche plus stable. Bien que ce produit de dégradation soit connu depuis les années 80, une seule étude, à notre connaissance, s'intéresse à sa toxicité et montre un impact sur le foie et les reins chez le rat (Al-Omar et al. 2009). Enfin, découvert en 2011, un nouveau composé nommé Pyridinium du furosémide (**figure 3.b**) est identifié (Laurencé et al. 2011). Une étude récente montre alors qu'il engendrerait des marqueurs précoces caractéristiques de maladies neurodégénératives chez la souris (Laurencé et al. 2019).

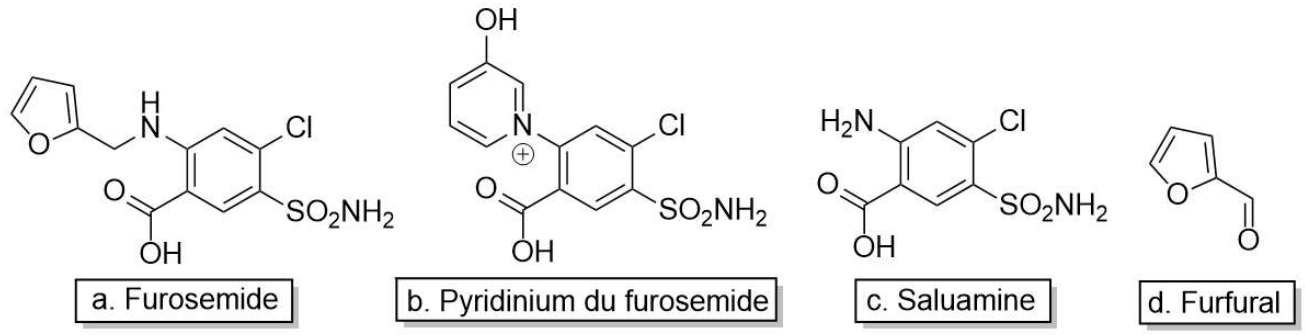


Figure 3. Structure du furosémide (a) et de ses produits de dégradation; le pyridinium du furosémide (b), la saluamine (c) et le furfural (d).

Finalement, ces molécules sont assez mal caractérisées et bien qu'il existe quelques rares études sur leur toxicité (à forte concentrations), leur écotoxicité n'a jamais été étudiée. Une première question se pose alors:

Est-ce que le furosémide et ses produits de dégradation présentent un risque pour l'écosystème aquatique, à des concentrations pertinentes dans l'environnement ?

L'objectif de cette thèse est donc d'évaluer la potentielle écotoxicité du furosémide et de ses produits de dégradation sur l'environnement, et dans une autre mesure leurs impacts toxiques possibles sur les cellules humaines. Pour cela, ce travail de thèse est séparé en quatre chapitres.

Chapitre I. Pour aborder ce travail, ce premier chapitre dresse un état de l'art sur le furosémide. Au travers d'un article de revue, différents aspects du furosémide sont documentés ; sa consommation, son occurrence dans différentes matrices, sa persistance, ses produits de dégradations connus ainsi que leur toxicité. L'objectif de ce chapitre est de bien identifier l'ampleur de la contamination au furosémide et de mieux appréhender les informations manquantes à son sujet.

Revue - Pharmaceutical pollutants of emerging concern. Origin and fate of furosemide and its degradation products in the aquatic environment, a review

Chapitre II. Le seconde chapitre discute du devenir du furosémide et de ses produits de dégradation de leur origine jusqu'au milieu aquatique. Dans une première partie, les concentrations dans différentes matrices, l'efficacité des STEU et les concentrations trouvées au niveau d'institutions médicalisées (EHPAD), sont détaillées dans un article de recherche accompagné d'une étude comparative de traitements oxydatifs sur le furosémide.

Article - Occurrence and fate of an emerging drug pollutant and its by-products in wastewater: case study of furosemide.

Dans une seconde partie, nous nous intéresserons plus particulièrement à la contamination par le furosémide du territoire français. La consommation, les flux, ainsi que la présence du furosémide dans le bassin versant Seine-Normandie seront estimés. La discussion portera également sur la présence du furosémide dans l'urine, problématique pour la réutilisation des urines comme engrais. L'objectif de ce chapitre est de dresser un portrait "chimique" du furosémide et de mieux définir l'origine des produits de dégradation ainsi que leurs concentrations environnementales.

Chapitre III. Ce chapitre porte sur l'évaluation écotoxicologique du furosémide et de ses produits de dégradation. Cette partie vise à mieux caractériser la toxicité des produits de dégradation pour lesquels peu d'informations sont disponibles. Un première partie porte sur le développement d'une méthodologie employée pour évaluer la toxicité du furosémide et ses produits de dégradation. Les approches basées sur la toxicité aiguë présentent de nombreuses limites lors de l'évaluation de risque dans l'environnement. Une approche plus intégrative pour l'étude de ces composés et de leur mélange a été développée. A l'aide d'approches multimodèles, la toxicité et l'écotoxicité de ces composés sont évaluées dans un deuxième article de recherche.

Article - First approach for the ecotoxicological assessment of a ubiquitous pharmaceutical compound (furosemide) and its transformation products.

L'objectif de ce chapitre est d'estimer le risque que représentent les trois composés pour les organismes aquatiques en utilisant des approches sensibles.

Chapitre IV. Le dernier chapitre aborde la problématique de l'impact des produits de dégradation sur les cellules humaines. La saluamine et le pyridinium étant également des métabolites humains, dans cette partie ont été réalisés des tests de toxicité aiguë sur des lignées cellulaires humaines en culture (foie, rein, neuroblastome) et des analyses protéomiques. L'objectif de ce chapitre est de mieux comprendre le mode d'action de ces métabolites, d'identifier les voies impliquées et certaines cibles biologiques au travers de l'expression de protéines dérégulées.

Chapitre I.

Le furosémide, un polluant d'intérêt émergent

Chapitre I.
Le furosémide, un polluant d'intérêt émergent

I. Etat de l'art sur le furosémide

La pollution de l'eau par les micropolluants est un sujet d'actualité récurrent et alarmant. Ces molécules sont camouflées tout autour de nous : des retardateurs de flammes dans nos peintures et textiles, des parabènes dans nos produits cosmétiques, des biocides dans nos produits ménagers; etc. Elles se retrouvent alors dans les eaux de ruissellement, les déchets ménagers et les eaux usées qui vont au final se déverser dans les milieux récepteurs.

Les micropolluants sont issus, au moins en partie, de l'activité humaine et, bien qu'ils soient présents en faible concentration, ils ont des conséquences délétères sur la biosphère. Des plans d'action nationaux ont alors été mis en place pour lutter contre cette pollution et préserver la qualité des eaux et la biodiversité. Le plan national de lutte contre les micropolluants (2016-2021)[5] succède aux plans nationaux de lutte contre les polychlorobiphényles (PCB), le plan national sur les micropolluants, et le plan national sur les résidus de médicaments. Ce nouveau plan a pour objectif de réduire les émissions de toutes les molécules susceptibles de polluer les ressources en eau en accord avec les objectifs fixés par la DCE.

En revanche, les composés pharmaceutiques sont des micropolluants à part, il est très difficile de réguler leur émission "à la source" à cause de leur usage thérapeutique, indispensable pour la santé humaine. Quelques mesures ont été instaurées dans le cadre de l'ancien plan national sur les résidus de médicaments, comme la mise en place d'un dispositif de récupération des déchets (industriels, officinaux et grossistes) ou de campagnes de sensibilisation des professionnels de première ligne[6]. Ces dispositifs dits de "gestion des risques" sont bien insuffisants compte tenu de l'ampleur de la contamination des résidus pharmaceutiques. D'autres objectifs en matière d'évaluation des risques ou de recherche fondamentale ont été suivis (état des lieux de la contamination des eaux et des sols, développement d'outils, campagnes de mesures). Cependant, ces molécules sont nombreuses, il est difficile d'appliquer un suivi sur chacune d'entre elles. Il est alors nécessaire de prioriser les molécules d'intérêt en fonction notamment de leur toxicité, de leur persistance et de leur bioaccumulation.

Ce chapitre détaille le choix du furosémide comme molécule "modèle" dans notre étude ainsi que les raisons pour lesquelles il devrait être considéré comme un composé d'intérêt émergent. Une étude bibliographique sur le furosémide a été réalisée et présentée dans un article de revue. Divers aspects sont traités ; ses caractéristiques et son utilisation, sa consommation, son occurrence dans les eaux usées, eaux de surface, eaux souterraines, boues de STEU, sédiments, et autres matrices, sa dégradation dans les stations de traitement des eaux usées et au travers de différents processus de dégradation avancée, ses produits de dégradations connus, sa toxicité ainsi que celle de ces produits de dégradation. Cette analyse de la bibliographie recoupe plus de 300 références et vise à dresser un portrait global de la molécule.

[5] Plan micropolluants 2016-2021 pour préserver la qualité des eaux et la biodiversité
[6] Plan national sur les résidus de médicaments dans les eaux (PNRM) 2010-2015

Résumé de l'article : Pharmaceutical pollutants of emerging concern. Origin and fate of furosemide and its degradation products in the aquatic environment, a review.

De nombreux composés pharmaceutiques se retrouvent dans l'environnement en raison de leur élimination incomplète par les stations d'épuration des eaux usées (STEU). Certains composés sont parfois présents en concentrations importantes et représentent donc un risque pour l'environnement aquatique.

Le furosémide est l'un des médicaments les plus utilisés dans le monde. Considéré comme un médicament essentiel par l'Organisation Mondiale de la Santé, ce puissant diurétique de l'anse est largement utilisé pour traiter l'hypertension, l'insuffisance cardiaque et rénale et de nombreuses autres applications. Cependant, cette forte consommation entraîne également une émission importante de furosémide dans les eaux usées et dans le milieu récepteur où des concentrations de quelques centaines de ng/L à plusieurs milliers ont été trouvées dans la littérature, faisant du furosémide un composé très préoccupant.

De plus, au cours de son parcours dans les systèmes d'eaux usées et les STEU, le furosémide peut être dégradé par divers processus entraînant la production de plus de 40 sous-produits. Le furosémide peut donc présenter un risque important pour la santé des écosystèmes, non seulement en raison de ses effets cytotoxiques, génotoxiques et hépatotoxiques directs chez les animaux, mais aussi indirectement par le biais de ses produits de dégradation, qui sont mal caractérisés.

De nombreux articles classent le furosémide comme un polluant prioritaire en fonction de sa présence dans l'environnement, de sa persistance, de son élimination par les STEU, de sa toxicité et de son écotoxicité. Nous présentons ici une revue de l'état de l'art de ce polluant d'intérêt émergent, en le suivant, de sa consommation à son devenir dans l'environnement aquatique. Les points de discussion incluent la présence du furosémide dans diverses matrices, l'efficacité de nombreux processus de dégradation du furosémide, la production ultérieure de produits de dégradation suite à ces traitements, ainsi que leur toxicité.

Pharmaceutical pollutants of emerging concern. Origin and fate of furosemide and its degradation products in the aquatic environment, a review

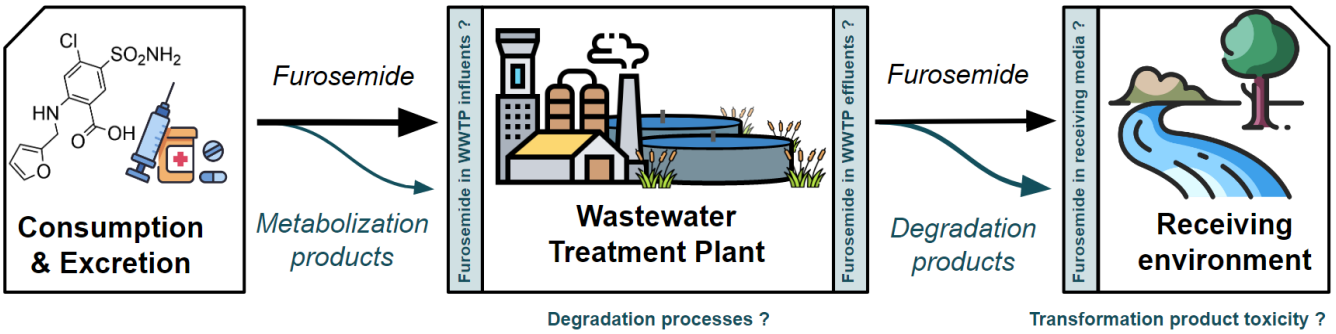
Fidji Sandre [1], Régis Moilleron [1], Christophe Morin [1, 2], Laure Garrigue-Antar [1]

[1] Leesu, Univ Paris Est Creteil, Ecole des Ponts, Creteil, F-94010, France.

[2] IUT - Sénart Fontainebleau, 36 Rue Georges Charpak, 77567 Lieusaint

Contact: sandrefidji@gmail.com ; laure.garrigue-antar@u-pec.fr

Graphical abstract



Abstract

Many pharmaceutical compounds end up in the environment due to incomplete removal by wastewater treatment plants (WWTPs). Some compounds are sometimes present in significant concentrations and therefore represent a risk to the aquatic environment. Furosemide is one of the most widely used drugs in the world. Considered as an essential drug by the World Health Organization, this powerful loop diuretic is used extensively to treat hypertension, heart and kidney failure and many other purposes. However, this important consumption also results in a significant release of furosemide in wastewater and in the receiving environment where concentrations of a few hundred ng/L to several thousand have been found in the literature, making furosemide a compound of great concern. Also, during its transport in wastewater systems and WWTPs, furosemide can be degraded by various processes resulting in the production of more than 40 by-products. Furosemide may therefore present a significant risk to ecosystem health due not only to its direct cytotoxic, genotoxic and hepatotoxic effects in animals, but also indirectly through its transformation products, which are poorly characterized. Many articles classify furosemide as a priority pollutant according to its occurrence in the environment, its persistence, its elimination by WWTPs, its toxicity and ecotoxicity. Here, we present a state-of-the-art review of this emerging pollutant of interest, tracking it, from its consumption to its fate in the aquatic environment. Discussion points include the occurrence of furosemide in various matrices, the efficiency of many processes for the degradation of furosemide, the subsequent production of degradation products following these treatments, as well as their toxicity.

Keywords

Furosemide, Occurrence, Degradation Process, Transformation Products, Ecotoxicity

Introduction

The presence of pharmaceutical compounds in water has been investigated since the 1970s (Hignite & Azarnoff 1977; Aherne & Briggs 1989; Ternes 1998; Stumpf et al. 1999). Since the discovery of clofibric acid, a drug metabolite, in wastewater treatment plants (WWTPs) (Stan et al. 1994), the presence of pharmaceutical residues in the aquatic environment has become a significant issue in the field of environmental sciences and is becoming increasingly important due to the growing consumption of drugs worldwide (Corvaisier 2000). These substances cover a very wide range of human and veterinary uses, therefore, there are hundreds of active substances that can be found in different environments (Halling-Sørensen et al. 1997; Heberer 2002; Kümmerer 2001; Godoy et al. 2015). Pharmaceutical compounds are created to be biologically active, although they were created for human or domestic animals, their action also extend to non-targeted populations which is why their occurrence and their persistence in the environment poses a risk to organisms and ecosystems (Richardson & Bowron 1985; Aherne et al. 1990; Kümmer 2001; Isidori et al. 2005).

Pharmaceutical residues may be released into the aquatic environment after their use due to inadequate removal by WWTPs (Boxall et al. 2014) but also during their manufacture by pharmaceutical companies (Kleywegt et al. 2015; Wolf et al. 2012). Moreover, despite some guidelines such as controls on the marketing of pharmaceutical products by institutions as the European CHemical Agency (ECHA) or the U.S. Food and Drug Administration, there are no global regulations limiting their concentrations in effluents (Kuster & Adler 2014). There is no monitoring of the life cycle of these molecules and the presence of degradation products is not taken into account either. Some of those molecules, sometimes hardly metabolized by humans and excreted in unchanged form, are introduced into the environment through wastewater systems (Verlicchi 2016; Daughton

& Ruhoy 2009). Part of these compounds is degraded and is therefore not (or barely) found in the environment, but their degradation products can be identified there and their effects on ecosystems are very poorly documented (Grandcoin et al. 2017, Maculewicz et al. 2022). These compounds are sometimes more toxic than their parent molecules (Świacka et al. 2020). Related chemical structures and physicochemical properties of the original molecules, raises many concerns about their adverse environmental and human health impacts. Given the hundreds of thousands of anthropogenic substances and their degradation products, including those generated during the elimination processes in WWTPs (Corvaisier, 2000), it is clear that the research and the analysis of these compounds are extremely complex and require powerful methods of detection and identification, such as non-targeted screening techniques. These methods allow the detection of a large number of compounds which are for the most part unknown. Studying all these compounds seems to be an overly large task, therefore, there is a need to prioritize them. Some well-known medicines are frequently found in prioritization lists such as Ibuprofen, Diclofenac or Paracetamol (Zuccato 2004; Riva et al. 2015). Of all these frequently occurring pollutants, furosemide, which is considerably less documented in aquatic media, will be the subject of this review.

Furosemide (4-chloro-N-furfuryl-5-sulphamoylanthranillic acid), has been marketed since 1964 (Stokes and Nunn 1964) under several brand names, of which the most common are Lasilix, Lasix, Frusemide, Edemid and Furix (DrugBank DB00695). Its physico-chemical properties are presented in **Table 1**.

Table 1. Informations and physico-chemical properties of furosemide

Property	Value	Reference
n° CAS	54-31-9	PubChem
n° ECHA	100.000.185	PubChem
ATC code	C03CA01	PubChem
Molecular Weight (g/mol)	330,744	PubChem
Formula	$C_{12}H_{11}ClN_2O_5S$	PubChem
Water solubility 25°C (mg/L)	0.25	Beyer et al. 2000
Water solubility 30°C (mg/L)	73.1	Yalkowsky et al. 2010
Log Kow	2.03	PubChem
Melting point (°C)	206	O'Neil, 2013
Pka 1 (Carboxyl)	3.8	PubChem
Pka 2	7.5	PubChem
Hydrogen bond acceptors	7	PubChem
Hydrogen bond donors	3	PubChem

This drug is a powerful diuretic derived from sulphonamides, acting on the kidneys by inhibiting the reabsorption of electrolytes to ultimately increase water loss from the body (DrugBank DB00695, RXLIST, HumanMetabolomeDataBase, Abbott & Kovacic 2018). Considered as cardiovascular agent or antihypertensive agent (Li et al, 2019; Bjorklund et al. 2016) furosemide is used in humans in cases of cardiac, hepatic or renal insufficiency, high blood pressure and oedema (Abbott & Kovacic 2008; ESSEC Report, 2012; Rodriguez-Cillero et al. 2017; Van Kraaij et al. 1997; Cheng & Nayar 2009) and it is also used in children, for diuretic and anti-inflammatory properties (Prandota 2001). It has anti-epileptic (Haglund & Hochman 2005) and antioxidant

(Lahet et al. 2003) properties but is not used primarily for this purpose. In addition, it is used in the veterinary field to limit the consequences of volume overload in renal and cardiac diseases in dogs, horses and ponies (Hinchcliff & Muir 1991; Chetboul et al. 2017; abbott & Kovacic 2008). Furosemide has also been reported in diverted uses, for example it has been found undeclared in food supplements for weight loss (Ivaova & Ivanov 2019) and also used in sport to help athletes lose weight quickly. It is also used to mask doping products diluting drug concentrations by increasing the volume of water in the urine but also by lowering the pH, thus modifying the excretion of other doping agents making them less detectable (Espinosa Bosch et al., 2008; Luiz et al. 2013; Cadwallader et al. 2010; Ventura & Segura 1996, Hinchcliff & Muir 1991). It is classified, as well as other diuretics, as a masking agent on the WADA Prohibited List (2009). Furosemide is the 2nd most frequently detected diuretic in drug screenings (23%) (Cadwallader et al. 2010). It could be used to mix recreational drugs such as MDMA to counteract the secretion of antidiuretic hormones (Schroder et al. 2010). Furosemide has many applications and is widely distributed across the world. Therefore, it quickly became a pollutant of interest in many countries such as Spain, France, Italy, India, Switzerland, Denmark, Greece, USA, or China (Chinnaiyan et al. 2018; Li et al. 2019; Zuccato 2004; US RX list; Besse et al. 2008; Kostich et al. 2014; Christensen et al. 2009). Therefore, many studies classify it as a priority pollutant based on several parameters (***Figure 1***).

Figure 1. Criteria based on which furosemide is considered a priority pollutant

	zuccato 2004	Zuccato et. al 2005	Besse & Garric 2008	Munoz et al. 2008	De Voogt et al. 2009	Christensen et al. 2009	Roos et al. 2012	Dong et al. 2013	Gillard et al. 2014	Kostich et al. 2014	Riva et al. 2014	Daouk et al. 2015	Kleywegt et al. 2015	Guo et al. 2016	Mansour et al. 2016	Machado et al. 2017	Chinnaiyan et al. 2018	Li et al. 2019	Ahmed 2020	Khan et al. 2020
Production, sale or consumption					x			x			x			x	x	x	x		x	x
Occurence in environment	x				x		x		x	x		x					x			x
Persistance/ stability in freshwater	x	x		x	x		x					x								x
Removal in WWTP or input in wastewater								x			x		x				x			
Excretion rate											x					x				
PEC			x							x										
Toxicity				x	x				x			x			x					x
Ecotoxicity, risk assessement				x		x	x	x	x			x		x	x					

The most common criteria are production or consumption sales data. These are easily accessible data which provide direct information on the extent of use of the compound. This information can be used to assess Predicted Environmental Concentrations (PEC). The persistence or stability of the compound is also frequently mentioned. Indeed, a stable compound will not be degraded in the wastewater treatment plants or in the environment and will therefore tend to accumulate in the receiving environment and then determine its

occurrence. Furosemide is effectively found in many countries and a large number of studies around Europe, Asia or North America in WWTPs effluents, surface water and even in sludge or sediments (see the "occurence in the environment" section). This ubiquity of furosemide leads many researchers to question its toxicity and its ecotoxicity, which is the second most cited priority criterion. In order to assess the toxicity of furosemide, several studies use tools or indicators combining several parameters. Methods such as the Occurrence, Persistence, Bioaccumulation, Toxicity (OPBT) approach, based on several relevant indicators for the evaluation of the environmental impact, allows to prioritize the molecules (Daouk et al. 2015). The Measured or Predicted Environmental Concentration/Predicted no Effect Concentration (MEC/PNEC or PEC/PNEC) ratio is the most commonly used in ecotoxicology to determine a risk because it is based both on the concentration of a pollutant in the environment and also the sensitivity of the species studied (Chinnaiyan et al. 2018; Roos et al. 2012, Besse et al. 2008). The EC5/MC95 (EC5 ¼ 5th percentile effect concentration; MC95 ¼ 95th percentile measured concentration) (Christensen et al. 2009), is a probabilistic approach quite similar to PEC/PNEC ratio.

Finally, there are many reasons to monitor the environmental fate of furosemide. Many pharmacological studies have been carried out on this compound (Abbot & Kovacic 2008, Huang et al. 2016, Ahmed 2020), but quite few on its presence, degradation and impact on the environment. Here, we present a state-of-the-art review of this emerging pollutant of interest, tracking it, from its consumption to its fate in the aquatic environment.

Consumption of furosemide

Diuretic compounds are commonly used to treat cardiovascular problems which are one of the main causes of hospitalization in the world (Roger et al. 2011). More than 80% of patients receive a loop diuretic for heart failure but it is also used for oedema disorders or high blood pressure (Buttard, 2016). The incidence of heart failure is growing (Savarese & Lund 2017) and makes diuretics a highly represented class of drugs in many countries (**Table 2**).

Table 2. Diuretic consumption by country in Defined daily dosage per 1 000 inhabitants per day (DDD) in 2017.

Denmark	Germany	Sweden	Iceland	Portugal	Finland	United Kingdom	Nether-lands	Czech Republic	Canada	Spain	Slovak Republic	Hungary	Italy
83,2	69,6	61,9	54	51,8	51,7	50,3	48,9	48,1	44,1	43,3	42,9	40,6	38,7

Belgium	Chile	Slovenia	Norway	Luxem-bourg	Greece	Australia	Estonia	Lithuania	Austria	Latvia	Israel	Korea	Turkey
38,6	35,7	35,2	32	31,3	28,3	25,1	24,7	22,6	19,1	18,5	18,4	18,3	8,1

Consumption in Defined daily dosage of the ATC class C03, named "Diuretics" by the WHO. Data from OCDE. Stats 2017.

In some countries, a significant portion of the population consumes diuretics (Arrubla et al. 2016). Diuretics are extensively used in elderly people and some studies even mention their overuse (Rodriguez-Cillero et al. 2017; Van Kraaij et al. 1997). In France, Buttard (2016) mentions that more than one third of people over 75 years of age are administered diuretics and 70% of the prescriptions are considered inappropriate by this author.

The class of diuretics (C03) is separated into several categories defined by the World Health Organization (WHO). Categories C03A and C03B are the low-ceiling diuretics regrouping thiazides, plain or in combination

with potassium-sparing agents such as Hydrochlorothiazide (Petrovic & Verlicchi 2014, Treadgold et al. 2012, Schuster et al. 2008, Cheng & Nayar 2009). The C03C category includes the high-ceiling diuretics also known as loop diuretics such as furosemide, torsemide, bumetanide, piretanide, ethacrynic acid and tienilic acid. The C03D category is for aldosterone antagonists and other potassium-sparing agents and the C03E category is for diuretics and potassium-sparing agents in combination. The most commonly prescribed categories for hypertension are thiazide diuretics and loop diuretics (3A, B and C) (Rimoy et al. 2009, Shalavadi et al. 2018) whereas loop diuretics (C03C) prevail over the other categories for the treatment of cardiac congestion (Boulestreau et al. 2018; Buttard 2016; Cheng & Nayar 2009).

Furosemide is then one of the most widely prescribed diuretics (Murray & Hall 1997; ANSM 2013; Ahmed 2020; Thapa & Singh 2019; Osunmakinde et al. 2013; ClinCalc DrugStats Database 2018; Papageorgiou et al. 2016) and it is also used in the formulation of several products such as co-amilofruse or co-amilozide (Treadgold et al. 2012) even if it is not necessarily the best choice over bumetanide and torsemide based on price, bioavailability and hospitalization rates of patients treated (Wargo & Banta 2009; DiNicolantonio 2012). Wargo & Banta (2009) hypothesized that furosemide is the most widely used because it was the first loop diuretic approved by the U.S. Food and Drug Administration in 1966. It was also considered to be the least toxic for humans (Prandota & Witkowska 1976) which is probably why it was the most prescribed diuretic in the late 1990s (Murray & Hall 1997). Since 1977, it is considered as an essential medicine by the World Health Organization based on its efficiency, safety and public health importance, which means that it must be available at all times in health systems. The mass of furosemide prescribed or consumed after the 2000s is noteworthy in many European countries (**table 3**).

Table 3. Sale, prescription or consumption of furosemide in kg per year in several European countries.

Country	Year	Population	Mass sold (kg)	Mass prescribed (kg)	Mass consumed (kg)	**Mass per inhabitant (mg)**
Poland	2013	38 040 000	6 604 [a]	-	-	**173.6**
Poland	2014	38 010 000	6 915 [a]	-	-	**181.9**
Hungary	2014 - 2018*	9 817 400*	1 013 [b]	-	-	**110.3**
Denmark	2000	5 349 217	3 812[L]			**712.6**
Sweden	2002	8 925 000	6 960 [c]	-	-	**526.9**
Sweden	2011	9 449 000	-	4 979 [d]	-	**779.8**
Germany	1999	82 100 000	-	25 334 [e]	-	**308.6**
Germany	2000	82 210 000	-	26 098 [e]	-	**317.5**
United-Kingdom (Wales)	2006	2 977 000	-	1 298 [f]	-	**436.0**
Italy	2001	56 970 000	-	6 400 [e]	-	**112.3**
Italy	2010	59 280 000	-	-	18 606 [h]	**313.9**
Italy	2013	60 230 000	-	-	20 000 [i]	**332.1**
United-Kingdom	2009	62 280 000	-	-	20 872 [j]	**335.1**
France	2014	66 310 000	-	-	21 288 [k]	**321.0**

*Average sales and average population between 2014 and 2018 [a] Giebułtowicz et al. 2016, [b] Krakko et al. 2019, [c] Carlsson et al. 2006, [d] Lindim et al. 2016, [e] Huschek et al. 2004, [f] Kasprzyk-Hordern et al. 2008b, [g] Calamari et al. 2003, [h] Al Aukidy et al. 2012, [i] Riva et al. 2015, [j] Boxall et al. 2014, [k] Chiffre et al. 2016, [L] Kjølholt et al. 2003. Data for population size comes from Eurostats.eu. Mass per inhabitant is the mass sold, prescribed or consumed divided by the population.

According to **table 3**, furosemide prescription contributes 1 to 26 tons per year in European countries and corresponds to a few hundred mg per inhabitant. In Germany and Poland, data from two consecutive years show an increase in furosemide prescriptions year over year and an increase is also observed for consumption in Italy between 2010 and 2013. The consumption per inhabitant does not reflect the proportion of the population that actually consumes furosemide. As furosemide is used for cardiovascular diseases and especially by elderly people, the growing consumption of furosemide is likely linked to the aging of the German, Polish and Italian populations (Vancea & Sole-Casals 2016) as it is the case for other drugs such as antidepressants (Fratiglioni et al. 1999). Indeed, studies focusing on hospitals, households or care homes have shown that furosemide is among the 20 most consumed drugs in terms of mass in the United-Kingdom and that care homes contribute 67.5% of total mass (Treadgold 2012). In France, a significant consumption of furosemide (595 to 1779 mg/day) has been noted in several nursing homes for elderly people (Lacorte et al. 2017). However, the estimated concentrations in the environment given by these authors were relatively low, which was attributed to the fact that part of the furosemide was absorbed in the disposable diapers of the incontinent patients in the establishments studied. In addition, Schuster et al. (2007) showed that consumption of furosemide in Germany is much higher by the general public than in hospitals mostly because patients purchase their medication at the pharmacy and consume it in their private residence.

Thus, in the general context of a growing consumption of medicines, both in high income and emerging countries, and population aging, the use of diuretics and in particular furosemide, is set to follow this trend and to increase over time. The high concentrations observed in the sewers and wastewater networks can be linked to the important consumption of furosemide in residential care homes, hospitals and mostly by the general public.

Occurrence in the environment

Furosemide has been detected and quantified on many occasions in the aquatic environment; very widely and often at high concentrations in wastewater (influents and urban effluents from WWTPs, hospitals, industries...), in surface waters (rivers, lakes, seas, oceans, estuaries, and deltas) at concentrations ranging from the ng/L level to a few hundred of ng/L and sometimes in groundwater and drinking water (wells, catchments, tap water). Most studies focus on water phase concentrations, however, considering its physico-chemical characteristics (e.g., organic carbon/water partition coefficient (Log Koc = 24.92), octanol/water partition coefficient (Log Kow= 2,03)), it is also found in suspended matter, WWTP sludge and sediments. These matrices are often not considered, therefore furosemide concentrations may be underestimated. It is also investigated in other matrices such as food in order to estimate the sanitary risk in the case of treated wastewater reuse for crop irrigation.

1. Occurrence in medicalised institutions wastewaters

Medicalized institutions gather a larger number of patients, then, high concentrations of furosemide are expected in their effluents. For example, according to Ort et al. 2010, hospital discharges account for 5.9% of furosemide in total wastewater discharges but are more spatially condensed. Depending on the users of the different institutions (veterinary or human hospital, maternity, oncological institution...), significant concentrations of furosemide can be found directly at the outlet and even after treatment by the wastewater treatment plants (**Figure 2**).

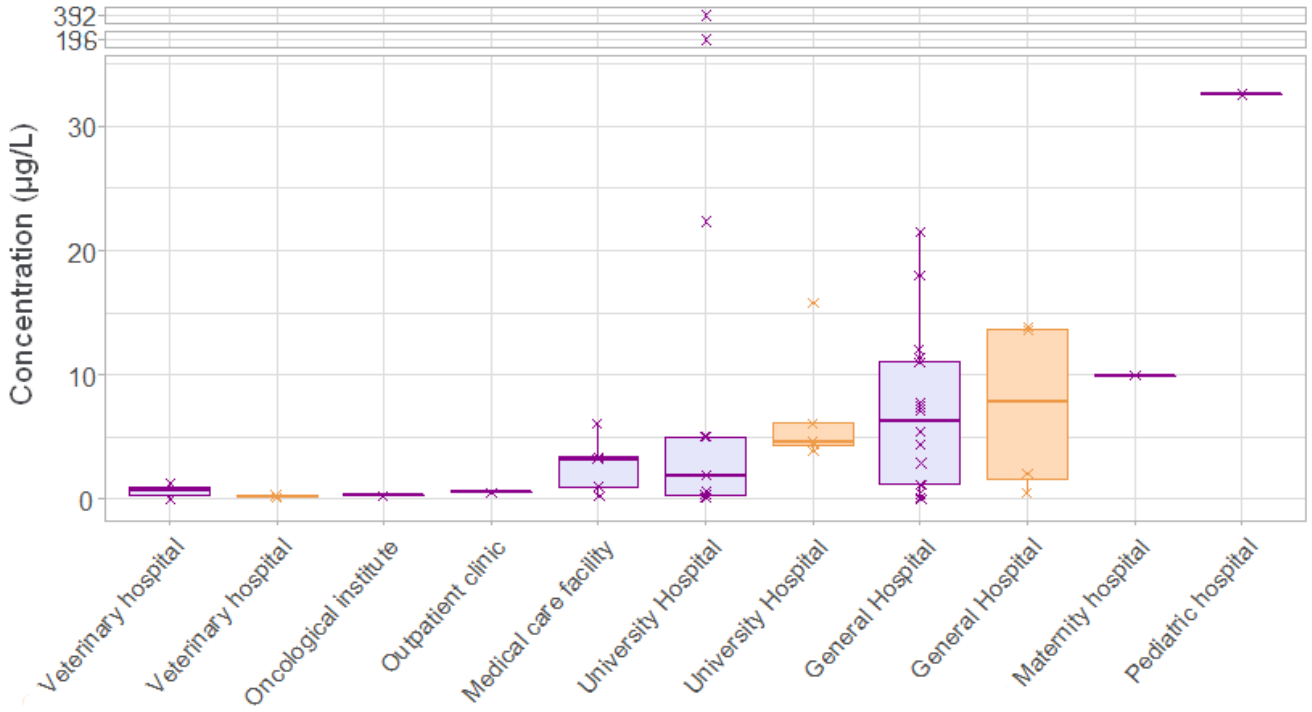


Figure 2. Furosemide concentration in WWTP effluents and influents (µg/L) of various types of medical institutions. Boxplot is plotted using R software with the ggplot2 function. Each x represents a value of furosemide in medical institutions. The yellow boxplot shows the concentration in the raw water, the purple boxplot shows the concentration in the wastewater after treatment. Afsa et al. 2020; Ajo et al. 2018; Fernandez 2018; Gillard et al. 2014; Gomez-Canela et al. 2019; Huber et al. 2016; Kleywegt et al. 2016; Kovalova et al. 2012; Lee et al. 2014; Mackuľak et al. 2019; Mir-Tutusaus et al. 2017; Nagarnaik et al. 2010; Nelson et al. 2011; Nielsen et al. 2013; Oliveira et al. 2015; Santos et al. 2013; Thomas & Langford 2009; Verlicchi et al. 2012a; Wahlberg et al. 2011; Yilmaz et al. 2017.

Studies on raw water from medical institutions are less common than on treated wastewater. Overall, the concentrations found in raw water are slightly higher than their equivalent in treated water except for veterinary hospitals. Furosemide concentrations are around a few hundred ng/L in both sample types, which is relatively low but can be explained by a less important use of furosemide in veterinary medicine. The median concentrations in general hospitals influent (7818 ng/L) are a little higher than in the effluents (6295 ng/L) which seems coherent because the effluents are treated. The same applies to university hospitals; median concentration in inlet waters are 4600 ng/L and median concentrations in outlet are 3450 ng/L but mean concentrations are much higher due to very high values of 196 000 and 392 000 ng/L found in a Norwegian hospital effluent (Thomas & Langford 2009). There is a visible difference between general hospitals and university hospitals. In nursing homes, the median concentration is around 3.2 µg/L and the values found by the different authors are quite close. Two relatively high concentrations were found in a maternity hospital and a pediatric hospital (Santos et al. 2013) which confirms that the use of furosemide in children is also important. Indeed, Prandota (2001) reports its use in premature infants with chronic lung disease, children with nephrotic syndrome, acute or chronic renal failure, congestive heart failure, progressive hydrocephalus. Finally the median concentration of health facilities for human patients is 3915 ng/L. Gillard et al. (2014) found a close value (3207 ng/L) for eight institutions in Belgium (hospitals, neuro-psychiatric units or rest homes). The authors also found important furosemide concentrations up to 57900 ng/L and hypothesized that these concentrations may be related to its heavy use in psychiatric units to counteract the side effects of several psychotropic drugs which have a known anti-diuretic activity (Spigset & Hedenmalm 1995). While healthcare facilities constitute a major source of high furosemide contamination locally, discharges from individual patients' homes may be less concentrated, but probably more prevalent.

2. Occurrence in global wastewater treatment plants

The largest number of studies found on the presence of furosemide focus on WWTPs and most of them are conducted in Europe. The highest concentrations of furosemide are found in wastewater influents (**Figure 3**).

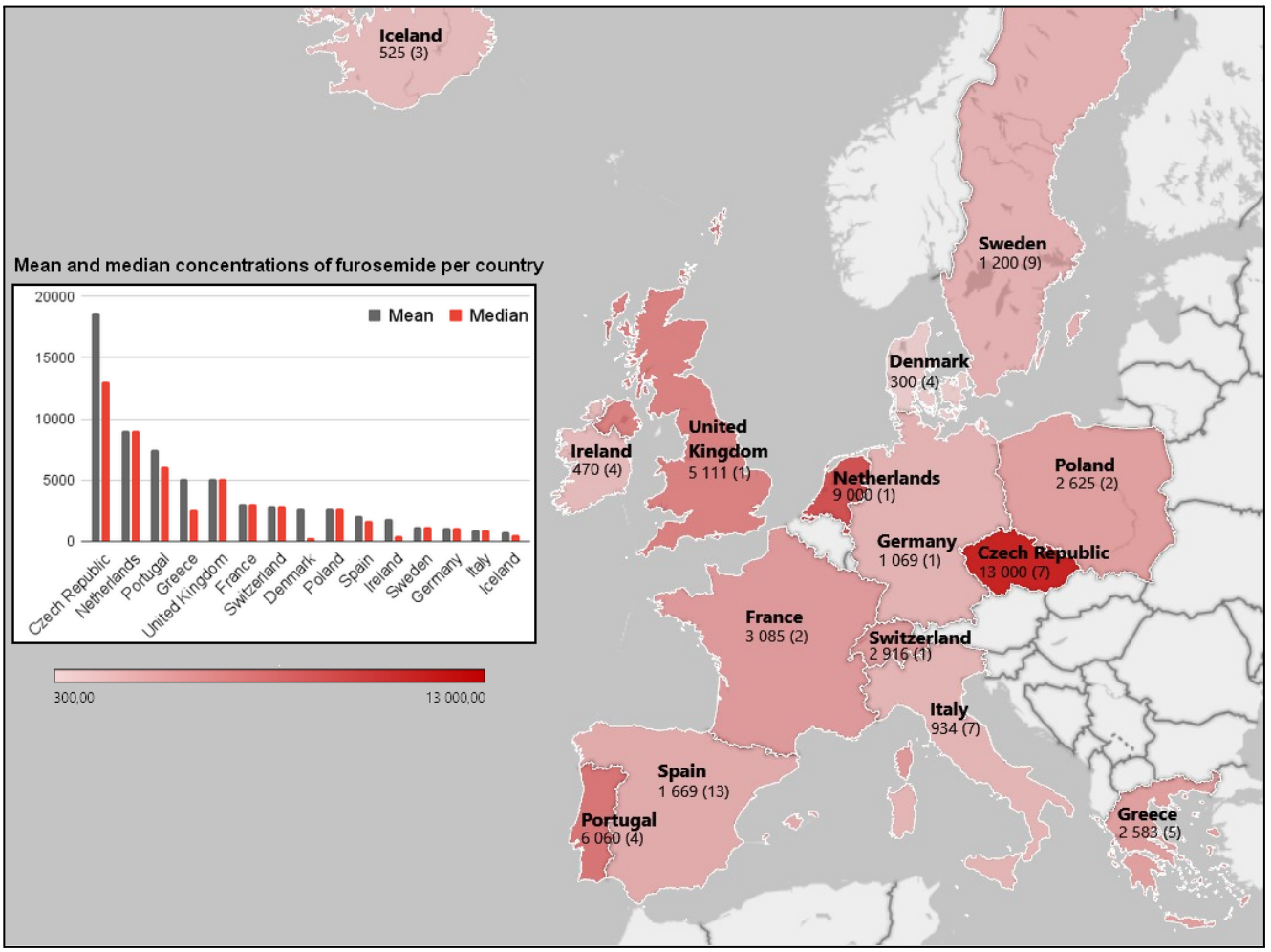


Figure 3. Furosemide median concentration in municipal, urban, industrial, agricultural or unspecified WWTP influents (ng/L). The figure has been produced by Excel Map. The red gradient on the map represents the range of median concentrations, which also appear under the name of each country. The number of studies per country is indicated in parentheses. Czech Republic: Rozman et al. 2017; Chen et al. 2016; Vymazal et al. 2016; Denmark: Huber et al. 2016; Jacobsen et al. 2004; Mogensen et al. 2008; Kjølholt et al. 2003; France: Sandre et al. 2023; Norway: Møskeland et al. 2006; Germany: Schröder et al. 2010; Greece: Papageorgiou et al. 2019; Dasenaki & Thomaidis 2015; Papageorgiou et al. 2019; Papageorgiou et al. 2016; Iceland: Huber et al. 2016; Ireland: Lacey 2008; Lacey et al. 2012; Lacey et al. 2008; Italy: Verlicchi et al. 2013; Verlicchi et al. 2012a; Castiglioni et al. 2018; Feo et al. 2020; Netherlands: Jie 2012; Poland: Kot-Wasik et al. 2016; Portugal: Sousa et al. 2011; Santos et al. 2013; Salgado et al. 2010; Spain: Ibanez et al. 2013; Gros et al. 2012; Gros et al. 2009; Rosal et al. 2010; Ginebreda et al. 2011; Fernandez 2018; Perez et al. 2010; Fernandez 2010; Collado et al. 2014; Teijon et al. 2010; Urtiaga et al. 2013; Čelić et al. 2019; Klamerth et al. 2013; Sweden: Gros et al. 2016; Kim 2018; Falås et al. 2012; Stockholm Vatten 2010; Baresel et al. 2019; Wahlberg et al. 2011; Switzerland: Lee et al. 2014; United-Kingdom: Kasprzyk-Hordern et al. 2009.

In Europe, the median concentration of furosemide is around 2600 ng/L in WWTP influent with a range of variation between 300 ng/L for Denmark to 13000 ng/L for the Czech Republic. The average concentration of furosemide is around 4 300 ng/L and average concentrations for all these countries are quite close to the medians, which shows that there are not too many extreme concentrations except in the Czech Republic, where Rozman et al. (2017) found a maximum concentration reaching 71 500 ng/L in the municipality of Onšov. This result is puzzling given the very low number of inhabitants (230 in 2022), and average age of the population (45.6 years). In this village, furosemide was the pollutant with the highest concentration, ahead of the other commonly-found compounds paracetamol, caffeine and ibuprofen. A much greater number of

studies on furosemide is carried out in Spain than other european countries, but the concentrations are below the averages and medians. Compared with the furosemide consumption data shown in **table 2**, interestingly, a high concentration of furosemide in the raw water is not necessarily linked to a high consumption, as it is the case for Sweden, for example, but this could be biased by the low number of consumption data.

Outside Europe, a few studies have quantified furosemide in wastewater treatment plant inlets. In North America, concentrations of 1830 ng/L in Canada (Kleywegt et al. 2016), median concentrations of 3565 ng/L in the United States (Lara-Martin et al. 2014; Oliveira et al. 2015) and 436 ng/L in Mexico (Lesser et al. 2018; Estrada-Arriaga et al. 2016) have been quantified, thus in the same orders of magnitude as in Europe. In Asia and Africa, the concentrations of furosemide are globally lower than in Europe, with 170 ng/L, 330 ng/L in Vietnam and Korea respectively (Kuroda et al. 2015; Lee 2014) and 200 ng/L in Tunisia (Afsa et al. 2019). However, due to the small amount of data available, it is difficult to conclude on furosemide contamination in these locations.

Globally, several hundred to several thousand nanograms per liter of furosemide were found in untreated wastewater. It has also been found in landfill leaching ponds up to 3840 ng/L (Rodriguez-Navas et al. 2013) and fitness center discharges up to 102 ng/L (Schröder et al. 2010). Fortunately, wastewater treatment processes are sometimes effective in removing furosemide, so it is expected to find lower concentrations after treatment. Although its concentration is reduced, furosemide has been found across Europe in municipal, urban, industrial and agricultural treated wastewater effluents (**Figure 4**).

Many more studies have been carried out on wastewater treatment plant outlets than on inlets. These studies allow the evaluation of the treatment efficiency but also the quantification of pollutants transfer/discharge from WWTPs to the environment. In WWTP outlets, the median concentration in Europe is around 940 ng/L and the average concentration is 1 260 ng/L. The range of variation of the medians is from 44 ng/L in Slovakia (Alygizakis et al. 2019) to 2 900 ng/L in Denmark (Huber et al. 2016; Jacobsen et al. 2004; Matamoros et al. 2009; UNESCO, 2017). Several concentrations far above 1000 ng/L were found in Denmark (4500 ng/L in Jacobsen et al. (2004); 7200 ng/L in Matamoros et al. (2009); 1300 ng/L in UNESCO (2017)) and the highest average concentration reported is in the Czech Republic with 4130 ng/L due to two very high concentrations in Rozman et al. (2017) and Vymazal et al. (2016) (11000 and 26000 ng/L). The Czech Republic is the country in which the highest concentrations of furosemide are found both before and after WWTPs.

In North America, a value of 80 ng/L was found by Estrada-Arriaga et al. (2016) in Mexico and a median value of 640 ng/L has been obtained (Batt et al. 2008; Lara-Martin et al. 2014; Oliveira et al. 2015; Meador et al. 2016) in U.S.A. In Asia, median concentrations of furosemide of 562, 497 and 3601 ng/L have been found in Japan (Hanamoto et al. 2018; Nakada et al. 2007), Malaysia (Al-Odaini et al. 2010; Al-Odaini et al. 2013) and Korea (Lee 2014; Kim 2018), respectively. This last value is surprisingly high compared to those usually found in treated water. Finally, in Africa, concentrations of 67 ng/L and 1300 ng/L have been found in Tunisia (Afsa et al. 2019) and Uganda (Dalahmeh et al. 2019). In these cases, there are less data available than in European countries, even concerning WWTP outputs to our knowledge, no data at all have been published from South America or Oceania to date. In five of the countries mentioned above, the medium concentrations of furosemide were higher at the outlet of the WWTP than at the inlet (Canada, Denmark, Ireland, Korea, and

Sweden). Keeping in mind that the small number of samples available may not allow to draw firm conclusions, this difference might be due to the fact that inlet/outlet samples were not necessarily paired and outlet data outnumbered those from inlet. Inlet data of samples with higher concentrations may have been missed, as temporal variations are frequently observed (cf below §6).

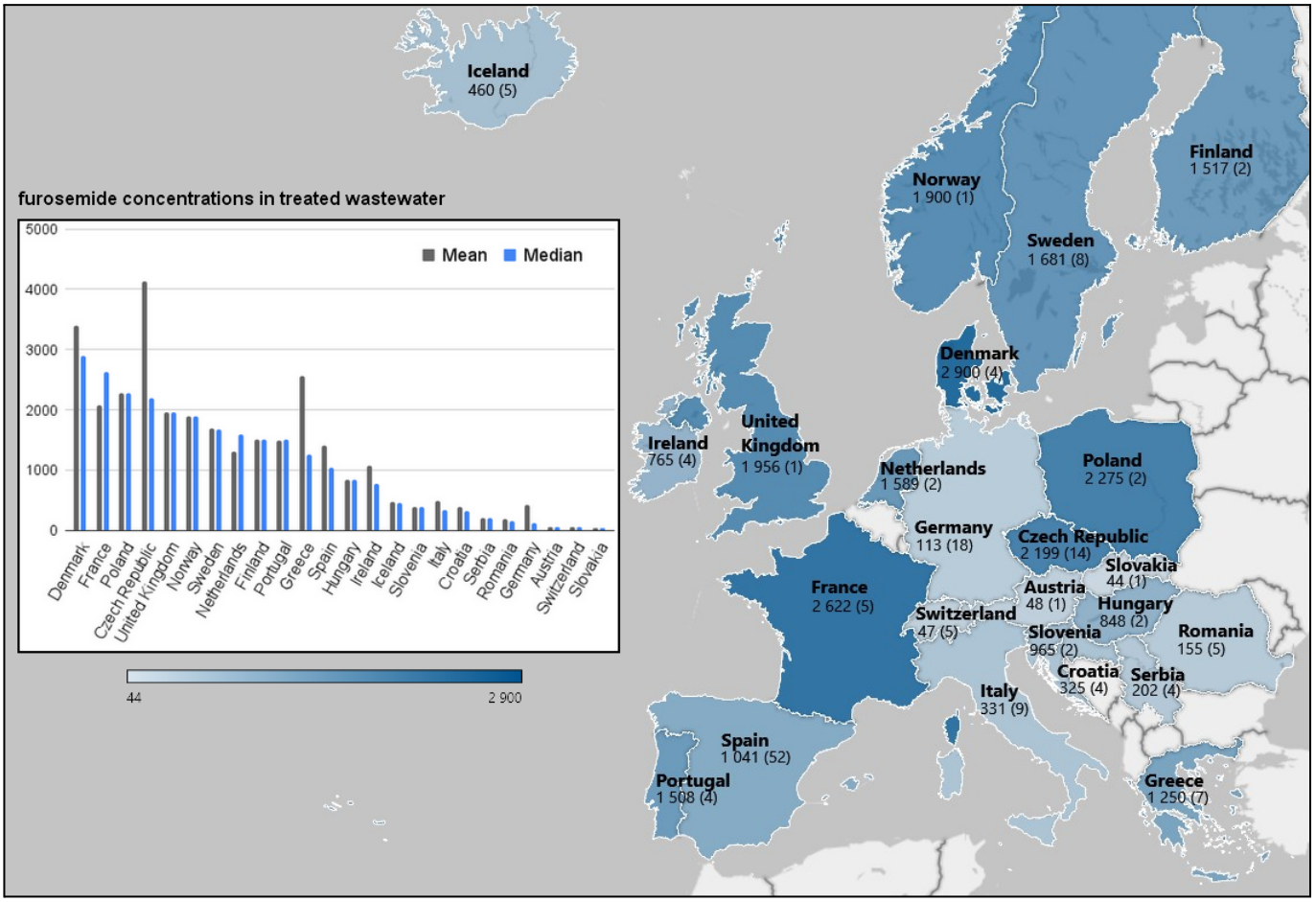


Figure 4. Furosemide median concentration in municipal, urban, industrial, agricultural or unspecified WWTP effluents (ng/L). The figure has been produced by Excel Map. The blue gradient on the map represents the median concentrations. The number of studies per country is indicated in parentheses. Greece: Papageorgiou et al. 2019; Dasenaki & Thomaidis 2015; Papageorgiou et al. 2016; Finckh et al. 2022; Hungary: Alygizakis et al. 2019; Finckh et al. 2022; Iceland: Huber et al. 2016; Ireland: Lacey 2008; Lacey et al. 2012; Italy: Al Aukidy et al. 2012;Verlicchi et al. 2013; Riva et al. 2014; Castiglioni et al. 2018; Verlicchi et al. 2012; Zuccato et al. 2005; Netherland: Finckh et al. 2022; Jie 2012; Norway: Møskeland et al. 2006; Poland: Kot-Wasik et al. 2016; Giebułtowicz et al. 2016; Portugal: Sousa et al. 2012; Salgado et al. 2011; Santos et al. 2013; Sousa et al. 2011; Romania: Petre et al. 2016; Burcea et al. 2021; Alygizakis et al. 2019; Finckh et al. 2022; Serbia: Petrovic et al. 2014; Alygizakis et al. 2019; Finckh et al. 2022; Slovakia: Alygizakis et al. 2019; Slovenia: Alygizakis et al. 2019; Finckh et al. 2022; Spain: Ibanez et al. 2013; Prieto-Rodriguez et al. 2012; Fernandez 2018; Campos-Manas et al. 2017; Valcarcel et al. 2011; Rodriguez-Navas et al. 2013; Collado et al. 2014; Prieto-Rodriguez et al. 2013; Diaz-Garduno et al. 2017; Gomez et al. 2008; Gros et al. 2009; Rosal et al. 2010; Ginebreda et al. 2011; Jelic et al. 2011; Gracia-Lor et al. 2012; Bueno et al. 2012; Perez et al. 2010; Lopez-Serna et al. 2010; Teijon et al. 2010; Acuña et al. 2015; Čelić et al. 2019; Bueno et al. 2012; Collado et al. 2014; Biel-Maeso et al. 2018; Campos-Manas et al. 2017; Gros et al. 2012; Urtiaga et al. 2013; Martinez-Bueno et al. 2007; Lopez-Serna et al. 2012; Finckh et al. 2022; Sweden: Gros et al. 2016; Kim 2018; Falås et al. 2012; Wahlberg et al. 2011; Gros et al. 2016; Finckh et al. 2022; Switzerland: Finckh et al. 2022; Denmark: Huber et al. 2016; Jacobsen et al. 2004; Matamoros et al. 2009; Mogensen et al. 2008; Kjølholt et al. 2003; United-Kingdom: Kasprzyk-Hordern et al. 2009.

The highest concentrations of furosemide in the aquatic environment were found by Kleywegt et al. (2019) in effluents from a pharmaceutical facility in Canada (1 200 000 ng/L), quite close to the concentrations found in Denmark by UNESCO (2017) in WWTP effluents (1 300 000 ng/L), thus suggesting the proximity of a pharmaceutical facility to the sampling point. Indeed, the river downstream of the pharmaceutical facility in

Canada shows very significant furosemide concentrations (3650 ng/L), underlining that this type of factories contributes significantly to the contamination of the rivers, raising serious environmental concerns.

Finally, the median concentrations at the outlets of WWTPs are almost three times less concentrated than the concentrations at the inlet of WWTPs, which highlights that there is a substantial overall reduction of furosemide in the aqueous phase by the treatment plants. However, another possibility would be that part of it could be transferred to solid phases such as sewage treatment plant sludge (**figure 5**). For instance, Petrovic & Verlicchi (2014) have shown that 2% of the furosemide present in the wastewater is transferred to the sludge, thus making it likely to find significant contents in the sludge of furosemide-loaded waters, as observed in WWTPs.

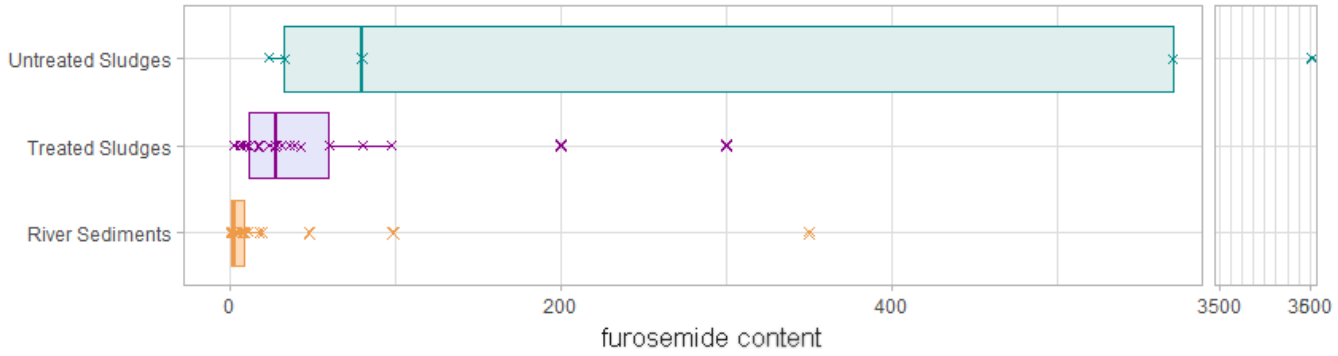


Figure 5. Furosemide contents in river sediments and WWTP sludge (ng/g dw). Boxplot is plotted using R software with the ggplot2 function. Each x represents a value of furosemide inThe cyan boxplots represent furosemide concentrations in untreated sludge (no digestion or biological treatments). The magenta boxplot represents the concentrations in the sludge after treatment and the yellow boxplot represents the concentrations in the sediment. Björklund et al. 2016; Ferrari et al. 2011; Ferreira Da Silva et al. 2011; Huber et al. 2016; Sadutto et al. 2021; Ginebreda et al. 2011; Gros et al. 2019; Jelic et al. 2009; Narumiya et al. 2013; Riva et al.2021; Rodriguez-Rodriguez 2012; Salgado et al. 2010; Wahlberg et al. 2011. Concentrations are given in ng/g of dry content except for Ferrari et al. 2011 which is expressed as ng/g of wet content.

According to **figure 5**, the median furosemide content in raw sludge is 80 ng/g.dw and the median content of furosemide in the treated sludge is 28.0 ng/g.dw. In treated sludge, furosemide is 3 times less concentrated than in raw sludge, meaning that the different sludge treatments might be relatively efficient for the removal of furosemide. In comparison, Riva et al. (2021) found concentrations around 11.9 ng/g.dw for atenolol and 21.7 ng/g.dw for carbamazepine in treated sludge, which are a little lower. Hydrochlorothiazide on the other hand, another diuretic, was found in slightly higher content (36.7 ng/g.dw). Different parameters can influence the affinity of furosemide to sludge but the partition coefficient of furosemide (**table 4**) between the dissolved and particulate phase (Kd) suggests that it has a rather good affinity with these matrices.

Table 4. Kd value for Furosemide in different matrices.

Matrix	Kd (L/kg)	references
Suspended Matter from WWTP influent	43	Jelic et al. 2012
Suspended Matter from residential care home sewage	6.5	Sandre et al. 2022
Sludge after MBR	2.1	Park et al. 2017
Sludge after A2O	2.1	Park et al. 2017
Digested sludge	110	Jelic et al. 2012
Thickness sludge	127	Jelic et al. 2012

Stuer-Laurisden et al. (2000) further showed that the affinity of furosemide with sludge is several orders of magnitude more important than suggested by the partition coefficients; indeed Svahn & Bjorklund 2015 underlined that the sorption mechanisms are effectively too complicated to be estimated from the Log p (also log Kow, octanol/water partition coefficient) or log d (pH-dependent partitioning coefficient) of the molecules. The Kd value depends on several parameters, including nature and concentration of organic carbon and pH which varies during wastewater treatment (between 5 and 9) which could explain why the Kd of digested sludge is lower than that of thickened sludge (Jelic et al. 2012).

It is important to analyze the solid phases as well as the aqueous phase when evaluating the extent of furosemide contamination, as significant concentrations of this substance are present in both. By doing so, a more comprehensive understanding of the contamination can be obtained.

Moreover, with increasing water stress in some countries, water reuse is becoming an important issue but could be a source of micropollutant contamination. Sludge spreading and the use of urine as fertilizer could also generate significant contamination. A few studies were then interested in the transfer of furosemide in food products and shows that it can accumulate (weakly) in food crops irrigated by wastewater: Compagni et al. (2019) showed, through a complex model, a possible accumulation of furosemide especially in the roots of maize, rice and ryegrass, but it can be transferred to the rest of the plant by the phloem resulting in a possible accumulation in leaves and seeds. However, not all plants seem to accumulate. Indeed, Martinez-piernas et al. (2019) did not detect furosemide in tomatoes while tomato plants were irrigated with reused water containing 1700 ng/L of furosemide. Reuse of wastewater could then present a risk but more studies on the transfer of pharmaceutical compounds in food products would be necessary to give a clear answer to that question.

3. Occurrence in surface waters

A lot of studies quantified furosemide in surface water but only a few focus on lakes, seas, oceans, estuaries and deltas. River waters are the most studied for the presence of pharmaceutical compounds and furosemide has been quantified in many locations (***Figure 6***).

Considering the data in **figure 6**, the median concentration of furosemide in European rivers is 93.0 ng/L, which is quite close to the median concentration found for other pharmaceuticals in river (sulfamethoxazole (83 ng/L), primidone (70.9 ng/L) or naproxen (98.0 ng/L)) (Hughes et al. 2013). The average concentration is 265.1 ng/L and the maximum concentration found is 6894 ng/L in Portugal (Palma et al. 2020) which is almost 30 times above average. Maximum concentrations in rivers in several countries exceed the predicted environmental concentration (PEC) of furosemide of 100 ng/L. In non-European countries, the maximum quantified concentration (1234.8 ng/L) is found in the Hudson River in the United States (Cantwell et al. 2018). This relatively high concentration has been explained by the proximity of a sewage treatment plant discharge which contributes strongly to the volume of water present especially during low tides.

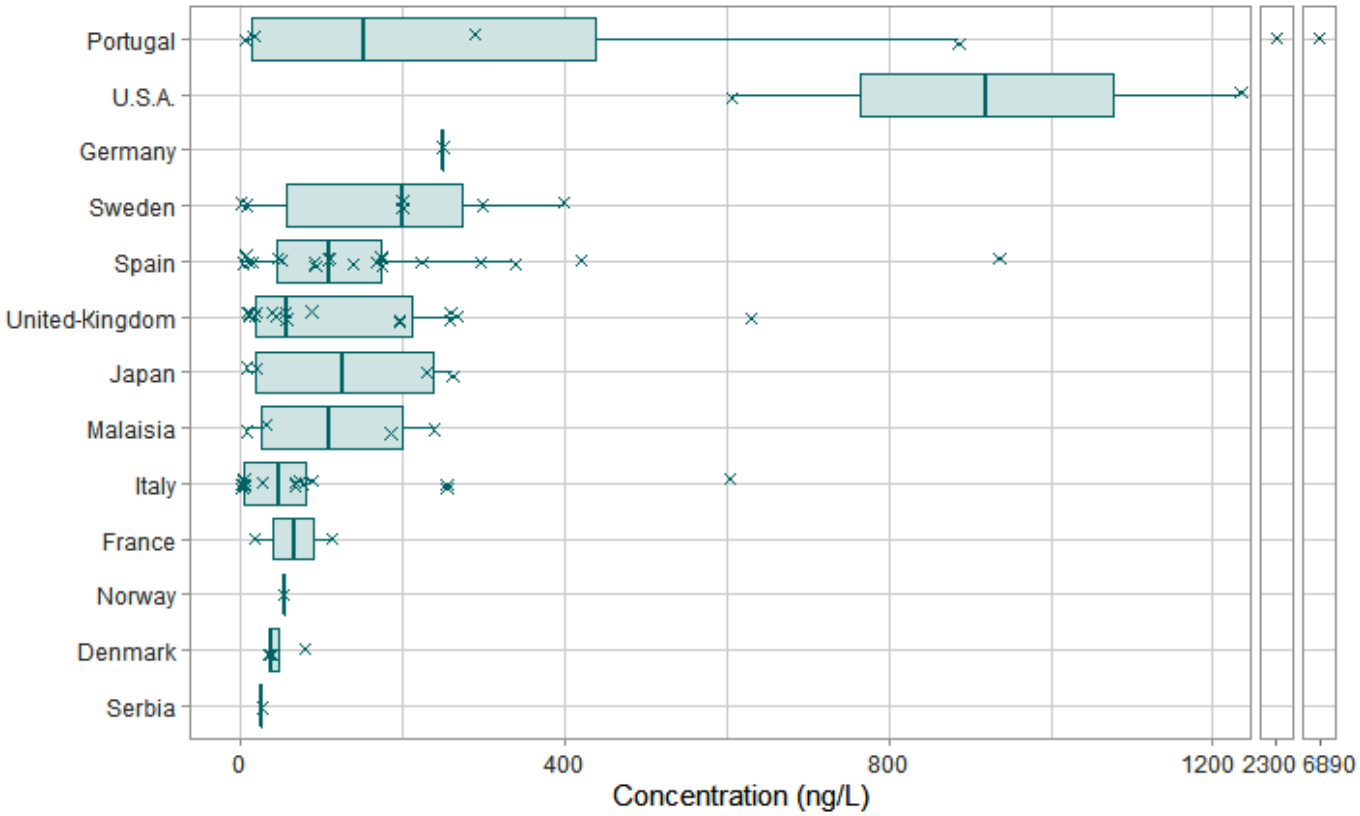


Figure 6. concentrations of furosemide (ng/L) in surface water in several countries. Boxplot is plotted using R software with the ggplot2 function. Each x represents a value of furosemide in surface water. U.S.A.: Srinivasan 2012; Cantwell et al. 2018; Portugal: Almeida et al. 2017; Palma et al. 2020; Germany: Bakken et al. 2018; Sweden: Wahlberg et al. 2011; Spain: Osorio et al. 2012; Osorio et al. 2014; Huerta-Fontela et al. 2011; Lopez-Serna et al. 2010; Banjac et al. 2015; Köck-Schulmeyer et al. 2011; Ferreira Da Silva et al. 2011; Lopez-Serna et al. 2012; Lopez-Serna et al. 2011; Acuña et al. 2014; Gros et al. 2009; Gracia-Lor et al. 2012; Matamoros et al. 2010; Valcarcel et al. 2013; Huerta et al. 2016; United-kingdom: Kasprzyk-Hordern et al. 2009; Kasprzyk-Hordern et al. 2008; Kasprzyk-Hordern et al. 2008 (bis); White et al. 2019; Boxall et al. 2014; Munro et al. 2019; Japan: Nakada et al. 2007; Komori et al. 2013; Tamura et al. 2017; Malaisia: Al-Odaini et al. 2010; Al-Odaini et al. 2012; Al-Odaini et al. 2013; Italy: Zuccato et al. 2000; Zuccato et al. 2004; Zuccato et al. 2005; Castiglioni et al. 2018; Ferrari et al. 2011; Riva et al. 2014; Calamari et al. 2003; France: Celle-jeanton et al. 2014; Idder et al. 2013; Norway : Møskeland et al. 2006; Denmark: Matamoros et al. 2012; Björklund et al. 2016; Khalaf et al. 2009; Mogensen et al. 2008; Serbia: Petrovic et al. 2014.

In other compartments, concentrations are lower in most cases. Furosemide was detected in Lake Mälaren in Sweden (Wahlberg et al. 2011) and quantified at 12 ng/L in Lake Brabrand in Denmark (Matamoros et al. 2012) which receives relatively little water from urban effluents. It has been quantified in Estuaire du Grouet in France up to 30 ng/L (Togola et al. 2008), then in coastal waters in Spain at 47 ng/L (Rodriguez-Navas et al. 2013) and in Portugal at 2300 ng/L (Almeida et al. 2017). No explanation was provided by Almeida et al. (2017) to explain this latter concentration, which is extremely high for coastal waters. Indeed, although the waters of the Tejo estuary discharge in the vicinity of the sampling point in the Almeida et al. (2017) study before passing through Lisbon, a more recent study did not detect furosemide in the estuary. Generally, furosemide is rarely detected in coastal or oceanic waters (Biel-Maeso et al. 2018; Afsa et al. 2019; Feo et al. 2020), probably because of the importance of the water volumes involved (dilution effect). Finally, furosemide is actively searched and widely found in surface waters. Concentrations are around a few hundred ng/L in rivers but extreme concentrations can be encountered occasionally, due to the probable presence of sources nearby (WWTPs, pharmaceutical industries, combined sewer overflows…). Furosemide is also found in lakes, but much less frequently because of rare contamination sources.It is also rather weakly detected in estuaries and coastal waters because of the important dilution. Studying the surface water content constitutes the first step toward risk assessment, providing an idea of exposure concentrations of non-target organisms in these environments. In addition, as in the case of sludge,

some of the furosemide may be found in the sediment (**figure 5**). The median furosemide content in sediment is 2.7 ng/g.dw, which is higher than that for carbamazepine (0.203 ng/g) but lower than that for atenolol (17.5 ng/g.dw) found in Ferrari et al. (2011). As furosemide has a low polar to apolar surface ratio, it is expected to form more hydrogen interactions with sediments, while carbamazepine and atenolol which are less polar, will sorb with apolar (van der Waals and π−π) interactions (Bäuerlein et al. 2012). In sediments, furosemide is 10 times less concentrated when in treated sludge. This disparity may be due to a significant dilution effect in river water. On the other hand, Björklund et al. (2016) calculated a very high Kd in sediment for furosemide (2517 L/kg). However, the furosemide content in sediment found by these authors is very high (350 ng/g.dw) compared to other values found in the literature (**figure 7**) and could explain this high Kd, which would therefore not be representative of the affinity of furosemide with sediments.

4. Occurrence in ground and drinking waters

Pharmaceutical residues in groundwater are quite well studied in the literature to investigate the magnitude of the contamination, while analysis of drinking water aims at assessing the risk that residues represent for the population or ecosystems. These environments are rather protected but not totally devoid of contamination, as shown in **table 5** for furosemide.

Table 5. Concentration of furosemide in groundwater in ng/L

Country	Location	Concentration (ng/L)
Spain	Delta Besos	284 [1]
	Poble Sec District	22.8 [1]
	Llobregat delta	138 [2]
	Barcelona	218 [3]
	Llobregat delta	8 [4]
	Llobregat delta	17 [4]
France	Normandie	14 [5]
	Aubepierre	189 [6]
	Basse-normandie	29 [6]
	Tarzy	12 [6]
	Aubergenville	8 [6]
Sweden	Åre municipality	26 [7]
	Stockholm	detected [8]

[1] Lopez-Serna et al. 2013, [2] Teijon et al. 2010, [3] Candela et al. 2016, [4] Cabeza et al. 2012, [5] Tracol & Duchemin 2007, [6] Blum et al. 2011, [7] Rostvall 2017, [8] Wahlberg et al. 2011

Pharmaceutical compounds are generally less concentrated in groundwater than pesticides. There are still several sources that contribute to the pharmaceutical compounds contamination to groundwater such as loss from sewage systems, urban runoff by Sustainable Urban Drainage systems which promote the infiltration of runoff water locally (Erikson et al. 2007), wastewater discharges, industrial discharges or infiltration of river water to the aquifer (Jurado et al. 2012; Sui et al. 2015). Furosemide is quantified occasionally in groundwater. Whereas concentrations of a dozen to a few hundred nanograms per liter have been found in some cases in France and Spain, most of the time furosemide is not even detected (Gros et al. 2012; Meffe & Bustamante 2014; Vulliet & Cren-Olivé 2011; Benotti et al. 2006; Valcarcel et al. 2011; Stackelberg et al. 2014; Mogensen, et al. 2008). It has not been found either in natural river biofilm (Huerta et al. 2016). Furosemide is also rarely detected in drinking

water (Gracia-Lor et al. 2012; Anderson et al. 2010; Marube et al. 2017; Zuccato et al. 2000; Lopez-Serna et al. 2010). To our best knowledge, it has been detected once in drinking water in Sweden (Wahlberg et al. 2011) and measured once in tap water in Poland at a concentration of 29 ng/L (Giebułtowicz et al. 2016). It is rarely detected because drinking water treatment processes are relatively effective for furosemide (Baken et al. 2018). Indeed, chlorination, ozonation and UV radiation are usual treatments for the potabilization of water and the secondary amine or the furan ring in furosemide reacts quickly with chlorine or ozone during advanced treatments and it is therefore quickly photodegraded by UV lights (Sandre et al. 2023). However, the degradation of furosemide can lead to the synthesis of degradation by-products (see section "Degradation products of furosemide"), for example, De Jongh et al. (2012) found 1-acetyl-1-methyl-2-phenylhydrazide, a phenazone degradation product in drinking water. Globally, groundwater and drinking water are rather spared from pharmaceuticals; most studies do not even detect furosemide. Sources contributing to furosemide in groundwater are limited and drinking water undergoes sufficient treatments to degrade it completely.

5. Temporal variation

The presence of furosemide in the receiving environment depends both on its use by humans and on the efficiency of its elimination by the WWTPs, which depends on several parameters. Several authors have then observed temporal variations of furosemide concentrations. Compagni et al. (2019) observed lower concentrations of furosemide in summer (≈70 ng/L) than in winter (≈180 ng/L). As furosemide is well photodegraded, especially in rivers (Hanamoto et al. 2014), the authors hypothesized that the decrease in concentration in summer is due to increased radiation favoring its photolysis. In addition, a more important degradation of furosemide in wastewater treatment plants is also observed in summer (54%) over winter (8%) by Castiglioni et al. (2006). Riva et al. (2021) observes similar variations in WWTP sludges with 8 ng/g.dw in summer and 31 ng/g.dw in winter. This degradation could be then due to a more important biological activity with a warmer temperature. However, Kot-wasik et al. (2016) did not find significant variations in furosemide concentrations between seasons. On the other hand, Moreno-gonzales et al. (2014) and Feo et al. (2020) observed a higher contribution of furosemide by river to coastal waters (in the Mar menor lagoon and the Bay Augusta) in spring. Similarly, effluent of a WWTP in Canada, Singh et al. (2015) also found more important concentrations of furosemide in spring than in fall and they did not detect it in summer.

In Mexico, variations of furosemide concentrations are observed between the dry and wet seasons. Estrada-arriaga et al. (2016) reported two to three times higher concentrations of furosemide in municipal WWTP influent in the dry season (514 ng/L) compared to the wet season (173 ng/L), which could be due to dilution of furosemide during the rainy season. They also noted a better performance of elimination of WWTPs during the dry season because the residence time of wastewater is longer in the WWTP and there is less water to be treated. Weekly variations could also potentially exist. However, Salgado et al. (2011) who measured micropollutants the same two days on two consecutive weeks and Còmez-Canela et al. (2019) who analyzed two residential care homes over 5 consecutive days, did not observe any particular pattern between the different days of monitoring. Fries et al. (2016) further observed within-day concentration variations with repeated tendencies at 12 am, 2 pm and 4 pm between two consecutive days which indicates that the primary source of contamination is human excretion.

6. Prediction of the furosemide load into the environment

Another way to determine the furosemide load in the environment is to predict it using models based on several inputs. Sales, consumption or production data allow the estimation of the concentrations emitted, while

calculations of the treatment plants performance allow to evaluate the charges released into the environment (Gomez-Canela et al. 2019; Moreno-González et al. 2014; Zuccato et al. 2004; Zuccato et al. 2005; Wahlberg et al. 2011; Dong et al. 2013; Castiglioni et al. 2006; Kleywegt et al. 2019; Riva et al. 2015; Boxall et al. 2014; Samoilenko & Yermakovych 2014). However, discrepancies are often observed between predictions and actual environmental concentrations (Verlicchi 2016; Stuer-Lauridsen et al. 2000). Some authors consider the percentage of excretion of furosemide or/and removal rate in WWTPs (Lindim et al. 2016; Riva et al. 2014; Sedlak et al. 2005; Zuccato et al. 2005), while others calculate the potential loadings according to physico-chemical parameters such as partition coefficients (Kd, Log Kow) or pKa (Stuer-Lauridsen et al. 2000). In fact, several complex variables such as the biotransformation and degradation of furosemide in water or in water treatment processes, as well as its seasonal variations, must be taken into account.

Finally, furosemide is widely found in the receiving water. The sources of contamination are multiple and furosemide can be found in very high concentrations in the effluents of medical institutions and urban effluents. The median concentration in the raw wastewaters is about 3 times higher than in the treated ones, suggesting that furosemide is partly eliminated by WWTPs. Groundwater and drinking water catchments are relatively uncontaminated, but furosemide is widely found in rivers throughout the world at a median concentration of 45 ng/L, with many more studies focused on Europe. The environmental concentrations are generally lower because of the important dilution in the environment. Furosemide also has a good affinity with the solid phase and has been detected in numerous studies in river sediments, but also in sludge from wastewater treatment plants. Variations in concentrations, especially seasonal, are observed. Overall, this section reveals a significant furosemide contamination that could potentially be problematic for human health and ecosystems, as there is limited knowledge about the fate of this compound. Moreover, the degradation of furosemide leads to the production of numerous by-products which can be equally problematic both in terms of identification and toxicity.

Fate of furosemide from its consumption to receiving water

After consumption, furosemide will pass through the human body before being excreted in urine. It will be then transferred to the sewer network and the wastewater treatment plants, where it will be eliminated with various efficiency.

1. Metabolization

In adults, most of furosemide (between 69 and 99%) is excreted within the first 4 hours after intake regardless of the mode of administration, mainly through urine and marginally through feces (Calesnick et al. 1966; Aranda et al. 1982, Stankiewicz et al. 2015). Furosemide can be metabolized in the liver and the guts by uridine diphosphate glucuronyltransferase but mostly (85%) in the kidneys (Phakdeekitcharoen & Boonyawat 2012). It is highly bonded to plasma albumin (>90%) which leads to a low-efficiency filtration by the glomerulus (Prandota & Witkowska 1976; Phakdeekitcharoen & Boonyawat 2012; Andreasen et al. 1974). Finally, between 11% and 23% of furosemide is excreted in its glucuronide form (Zuccato et al. 2005; Bindschedler et al. 1997; Riva et al. 2015; Andreasen et al. 1981; Aranda et al. 1982; Boles Ponto & Schoenwald 1990). In newborns, glucuronide form is almost non-existent because of their immature glucuronidation capacity (Hammarlund-udenaes & Benet 1989). However, furosemide remains mostly untransformed and an average of 66% is excreted in unchanged form in adults (42.3% in Bindschedler et al. 1997; 90% in Zuccato 2005; 65.8% in Andreasen et al. 1981) and 84.5% in children (Aranda et al. 1982). It is therefore mostly unchanged furosemide that is received in the wastewater system.

2. Degradation in WWTPs and advanced process

The degradation of furosemide in water treatment plants is quite variable. Effective degradation rates around 70% have been found in Matamoros et al. (2009), Sandré et al. (2023) and Kasprzyk-Hordern et al. (2009). These rates have been explained by more efficient wastewater treatment plants processes and/or time of sampling: due to the higher temperature in summer, the biodegradation activity is indeed stronger and furosemide is better degraded. Medium elimination rates 25-40-50-54% were found in Park et al. (2016), kot-wasik et al. (2016), Gros et al. (2010) and Castiglioni et al. (2006) respectively, in conventional mechanical-biological treatment. These percentages of removal can be much increased by adding tertiary or quaternary treatments in the treatment plants, as shown in **figure 7**.

The degradation of furosemide by innovative processes has been widely studied in more than 35 papers. The effectiveness of these processes appears very variable, ranging from complete elimination of furosemide to no elimination, or even higher furosemide concentrations following Membrane Bio Reactor (MBR) processes (Nielsen et al. 2013). This phenomenon has been explained by the deconjugation of glucuroconjugated forms of furosemide, increasing the concentration of quantifiable "native" furosemide (Kovalova et al. 2012, Kosma et al. 2014). Due to their highly variable efficiency, it is difficult to conclude on the performance of MBR (from -14 to 95% elimination of furosemide) and absorption on xylite (from 15 to 100% elimination). The effectiveness of xylite depends on the residence time of the water within the process, a longer time resulting in a more effective absorption (Rosvall 2017). The efficiency of the MBR relies on the upstream processes and the type of membrane used. The lowest removal rates reported for furosemide are obtained with ceramic membranes (Nielsen et al. 2013, Joannis-Cassan et al. 2021). On average, biological treatments (Activated sludge, Anaerobic-Anoxic-Aerobic system (A2O), Peroxidase, Plant, Fugus, Constructed wetland, Chitosan film, MBR) are not much more efficient than physical-chemical treatments (i.e., all the rest): Average efficiency of 74% versus 70%, respectively. Filtration techniques such as sand filtration and ultrafiltration are not the most efficient (<50%) although ultrafiltration coupled with Gas-phase Pulsed corona discharge (PCD) shows better performances. On the other hand, Urtiaga et al. (2013) shows that the reverse osmosis can completely eliminate furosemide due to the membranes that retain even small organic molecules. The majority of the processes found in the literature are oxidation processes. Their efficiency is very variable, and depends on the reactivity of the oxidants involved with furosemide (Sandre et al. 2022). Ozonation processes are among the most efficient due to the quick reaction of ozone with the furan ring and the aniline group of furosemide (Zoumpouli et al. 2021). Photo-Fenton, chlorination, electro-oxidation and peroxydation are close to complete elimination of furosemide. Peroxidases have also been shown to be very effective (Almaqdi et al. 2019), but the process has not been tested at the scale of a WWTP and may be difficult to implement because it requires the addition of enzymes in high concentrations (in proportion to the micropollutants) and the buffering of the PH. On the other hand, Ultra-Violet (UV) radiation appears rather ineffective. Despite its high sensitivity to photodegradation (Bundgaard et al. 1988), the wastewater matrix must be too complex to allow optimal removal of furosemide. UV-H_2O_2 process on the other hand shows good efficiency because the addition of H_2O_2 results in the formation of reactive oxygen species which will react strongly with furosemide (Miklos et al. 2018). The elimination of furosemide by absorption is also interesting. Absorption on Xylite, lignite or activated charcoal (in powder or granulated form) shows an efficiency of more than 60%. These techniques have the advantage of trapping the molecules and therefore potentially release less by-products than oxidation methods (Cuthbertson et al. 2019).

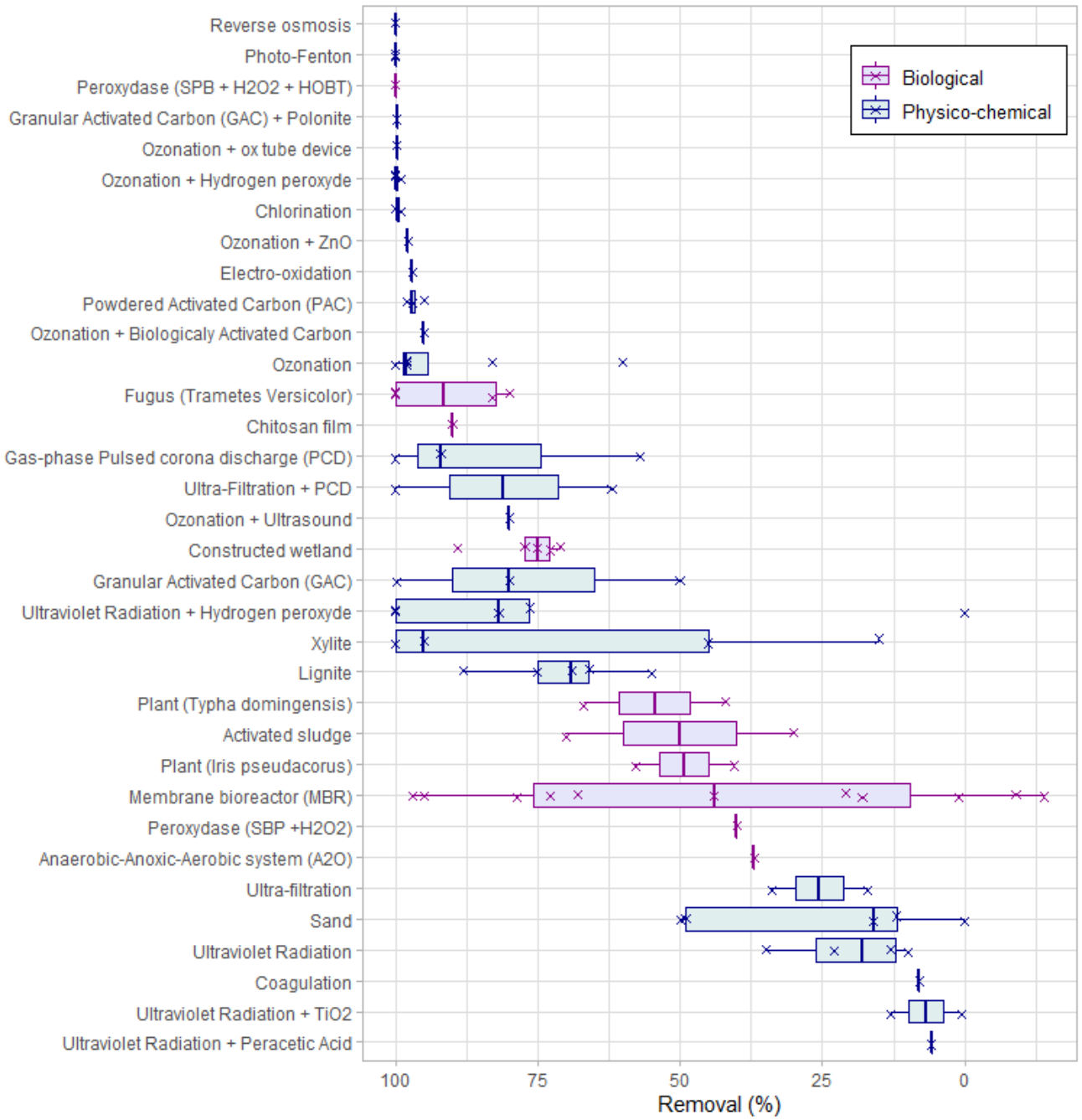


Figure 7. Removal efficacy of furosemide in percentage by different advanced processes. Boxplot is plotted using R software with the ggplot2 function. Each x represents a value of furosemide removal. The purple boxes represent the techniques for which the removal of furosemide is done by a biological process and the blue boxes represent those for which the process is physicochemical. Ahmed et al. 2016; Ajo et al. 2018; Almaqdi et al. 2019; Arola et al. 2017; Badia-Fabregat et al. 2015; Badia-Fabregat et al. 2016; Cruz-Morato et al. 2014; Cuervo-Lumbaque 2020; Gomez et al. 2008; Heidari et al. 2020; Huerta fontela 2011; Ibanez et al. 2013; Ikonen et al. 2021; Jie 2012; Joannis-Cassan et al. 2020; kim et al. 2014; Klamerth et al. 2013; Kovalova et al. 2012; Kovalova et al. 2013; Llorens-Blanch et al. 2015; Machado et al. 2017; Machado et al. 2020; Munoz et al. 2009; Nielsen et al. 2013; Park et al. 2017; Reungoat et al. 2012; Rizzi et al. 2020; Rosal et al. 2010; Rostvall 2017; Sandre et al. 2022; Singh et al. 2015; Urtiaga et al. 2013; Verlicchi et al. 2015; Vymazal et al. 2017; Kjølholt et al. 2003

3. Natural degradation

Under environmental conditions, furosemide has several degradation pathways. First, furosemide has been known to be photodegradable for a long time: it is sensitive to visible light with fluorescent lamp (Katsura et al. 2015), sunlight (Starling et al. 2019) and UV radiation (Bundgaard et al. 1988; Moore & Burt 1981) which will react with the chlorine, furfuryl or sulfamoyl groups (Sandre et al. 2022). Although its photodegradation is unlikely in the wastewater system, it is expected once discharged into the aquatic environment. It has been reported to be

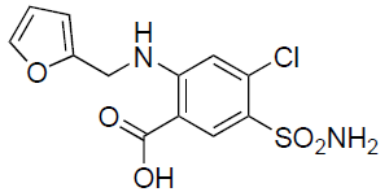
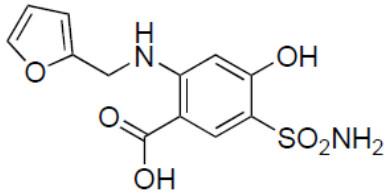
biodegraded by environmental bacteria such as *Agrobacterium tumefaciens* and *Arthrobacter ureafaciens* (Laurencé et al. 2014), fungus like *Aspergillus candidus*, *Cunninghamella echinulata* and *Trametes versicolor* (Laurencé et al. 2014, Olvera-Vargas et al. 2016, Badia-Fabregat et al. 2015, 2016) and anaerobic microorganisms (Narumya et al. 2013, Gros et al. 2020), forming several by-products. Furosemide can also be hydrolyzed to a lesser extent in the human stomach (Andreasen et al. 1982) and in aqueous solutions (Bundgaard et al. 1988). At constant temperature and pH, Bundgaard et al. (1998) describes a first order kinetic, and an improvement of the hydrolysis rate when the pH decreases. The thermal decomposition of furosemide was studied in the early 2000s by Beyers et al. (2000). However, furosemide only degrades at 218.1°C which is far from the environmental conditions.

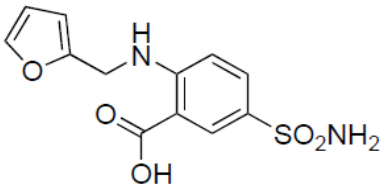
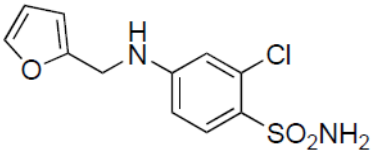
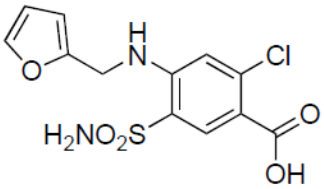
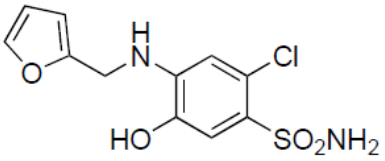
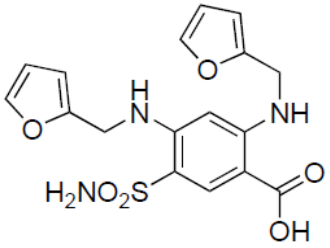
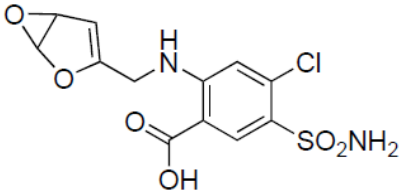
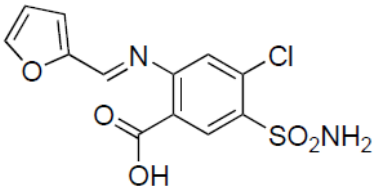
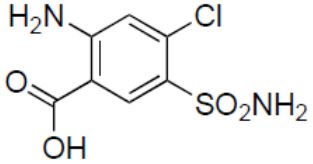
In summary, furosemide is rather well degraded in water treatment plants and many additional advanced processes allow further reduction of its concentration. Among the most efficient processes (> 90%) are absorption processes on chitosan and on activated carbon, followed by oxidation processes such as ozonation, chlorination, electro-oxidation, photo-Fenton and reverse osmosis. The elimination of furosemide by fungi (*Trametes versicolor*) also seems promising. However, furosemide, like many other anthropogenic compounds including pharmaceuticals, still remains in WWTP treated effluent and significant concentrations can be discharged in the receiving water.

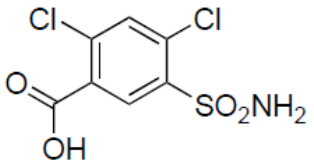
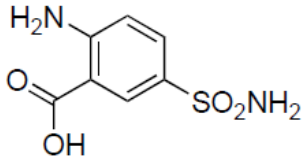
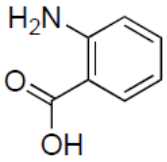
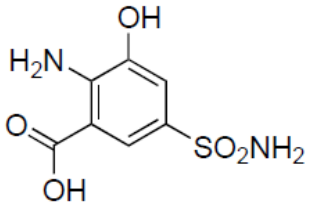
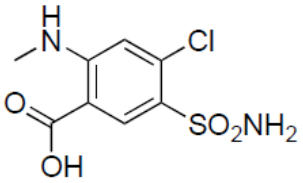
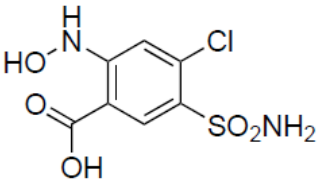
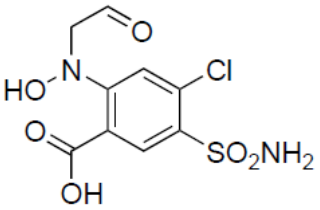
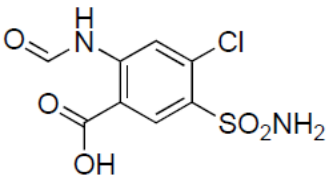
Degradation products of furosemide

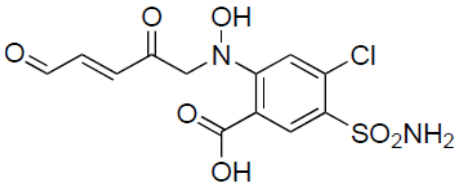
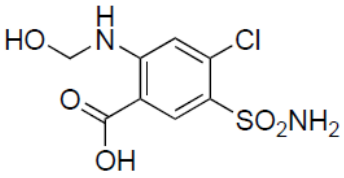
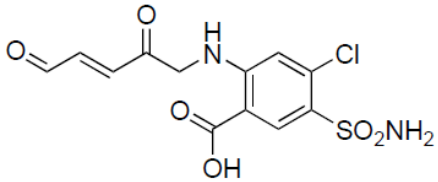
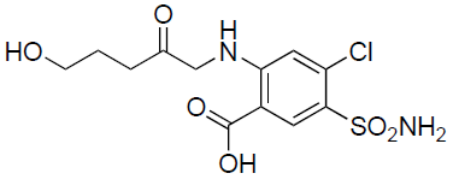
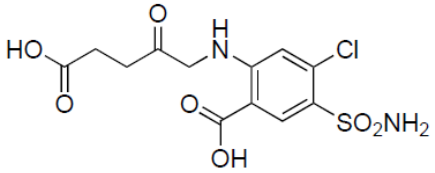
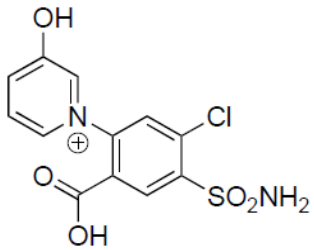
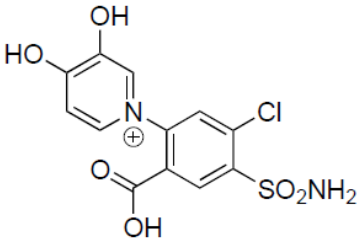
During its passage in the human body, in wastewater systems, in treatment plants or in the environment, furosemide undergoes several degradation pathways, leading sometimes to its real elimination, but more frequently to the concomitant production of by-products, more or less stable, thus resulting in an apparent loss when assessing removal in the WWTPs. These by-products can be formed by various processes, and may remain unidentified if global and sensitive analytical methods such as LC-MS/MS or HRMS are not applied. Furthermore, they may have a significant toxicity, which is currently understudied (poorly taken in account). The structure of several degradation products has been identified in the literature (**Table 6**). Some of these have been known for a long time and others have been identified only recently with the improvement of screening techniques.

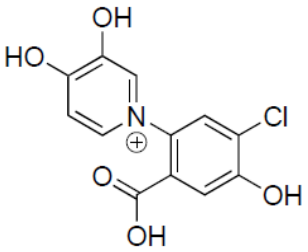
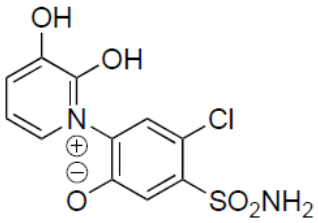
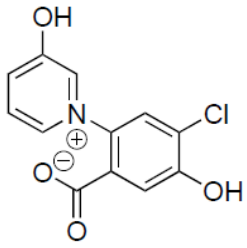
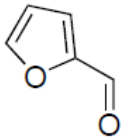
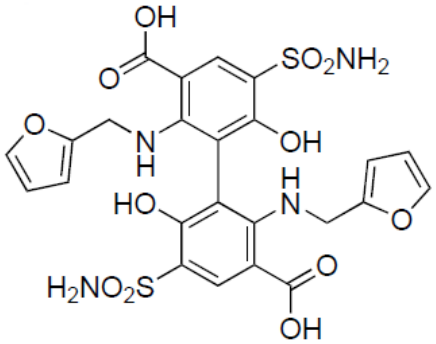
Table 6. Degradation products of furosemide.

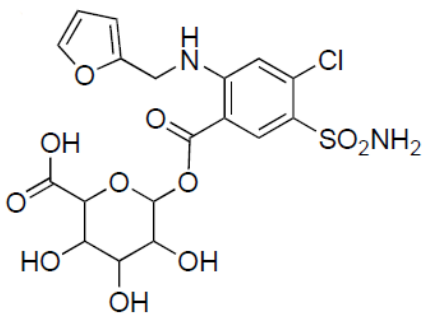
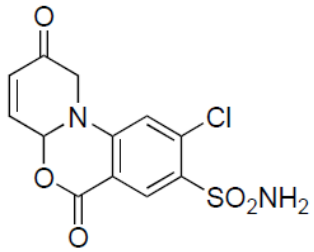
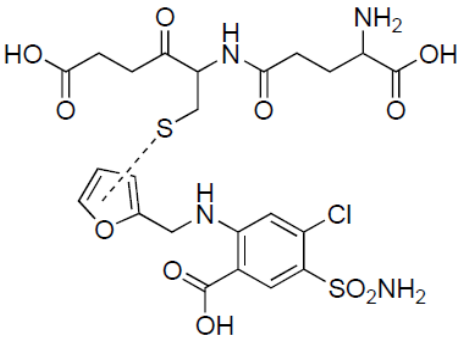
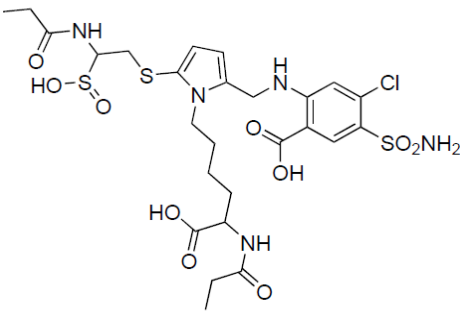
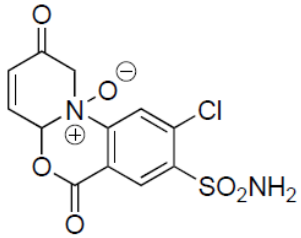
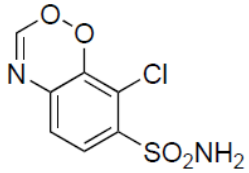
N°	Structure	Mass	Name and formula	Reference
Furosemide and furosemide-like structures				
1		**330.7**	**Furosemide** $C_{12}H_{11}ClN_2O_5S$	
2		**312.3**	**Photodegradation product** $C_{12}H_{12}N_2O_6S$	Yagi et al. 1991 Mizuma et al. 1998 Jakimska et al. 2014 Katsura et al. 2015 Sandre et al. 2023

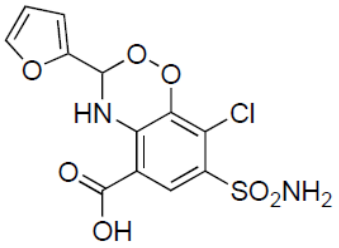
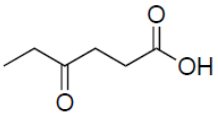
3		**296.3**	**Photodegradation product** $C_{12}H_{12}N_2O_5S$	Jakimska et al. 2014
4		**286.7**	**Photodegradation product** $C_{11}H_{11}ClN_2O_3S$	Vargas et al. 1998
5		**330.7**	**Photodegradation product** $C_{12}H_{11}ClN_2O_5S$	Carda-Broch et al. 2000
6		**302.7**	**Photodegradation product** $C_{11}H_{11}ClN_2O_4S$	Vargas et al. 1998
7		**391.4**	**Photodegradation product** $C_{17}H_{17}N_3O_6S$	Carda-Broch et al. 2000
8		**346.7**	**Metabolization or anodic oxidation product** $C_{12}H_{11}ClN_2O_6S$	Mitchell et al. 1976 Laurencé et al. 2011
9		**328.7**	**Anodic oxidation product** $C_{12}H_9ClN_2O_5S$	Laurencé et al. 2011 Bukkitgar & Shetti 2016
Saluamine and saluamine-like structures				
10		**250.7**	**Saluamine** **Acid catalyzed hydrolysis, Electro-Fenton, metabolization, photodegradation product** $C_7H_7ClN_2O_4S$	Beyers et al. 2000 Carda-Broch et al. 2000 Williams et al. 2007 Baranowska et al. 2010 Laurencé et al. 2011 Peterson 2013 Laurencé et al. 2014 Jakimska et al. 2014 Li et al. 2014 Katsura et al. 2015 Almaqdi et al. 2019

11		**270.1**	**Photodegradation product** $C_7H_5Cl_2NO_4S$	Carda-Broch et al. 2000
12		**216.2**	**Photodegradation product** $C_7H_8N_2O_4S$	Jakimska et al. 2014
13		**137.1**	**Photodegradation product** $C_7H_7NO_2$	Carda-Broch et al. 2000
14		**232.2**	**Photodegradation product** (The position of the hydroxyl group can vary on the ring) $C_7H_8N_2O_5S$	Jakimska et al. 2014
15		**264.7**	**Ozonation product** $C_8H_9ClN_2O_4S$	Tsilikilis et al. 2016 Zoumpouli et al. 2021
16		**266.7**	**Ozonation product** $C_7H_7ClN_2O_5S$	Zoumpouli et al. 2021
17		**308.7**	**Ozonation product** $C_9H_9ClN_2O_6S$	Zoumpouli et al. 2021
18		**278.7**	**Ozonation product** $C_8H_7ClN_2O_5S$	Tsilikilis et al. 2016 Zoumpouli et al. 2021

19		**362.7**	**Ozonation product** $C_{12}H_{11}ClN_2O_7S$	Zoumpouli et al. 2021
20		**280.7**	**Chlorination product** $C_8H_9ClN_2O_5S$	Sandre et al. 2023
21		**346.7**	**Anodic oxidation product** $C_{12}H_{11}ClN_2O_6S$	Laurencé et al. 2011
22		**346.7**	**Anodic oxidation, Metabolization, Biotransformation, Ozonation product** $C_{12}H_{11}ClN_2O_6S$	Williams et al. 2007 Olvera-Vargas et al. 2016 Peterson 2013 Zoumpouli et al. 2021
23		**364.8**	**Metabolization product** $C_{12}H_{13}ClN_2O_7S$	Bukkitgar & Shetti 2016 Williams et al. 2007 Peterson 2013
Pyridinium and pyridinium-like structures				
24		**329.7**	**Pyridinium of furosemide** **Chemical, enzymatic oxidation, bioconversion, photodegradation product** $C_{12}H_{10}ClN_2O_5S$	Chen & Burka 2007 Laurencé et al. 2011 Peterson 2013 Laurencé et al. 2014 Laurencé et al. 2019
25		**345.7**	**Bioconversion product** $C_{12}H_{10}ClN_2O_6S$	Laurencé et al. 2014

26		**282.7**	**Bioconversion product** (The position of the hydroxyl group can vary on the pyridin ring) $C_{12}H_9ClNO_5$	Laurencé et al. 2014
27		**316.7**	**Bioconversion product** (The position of the hydroxyl group can vary on the pyridin ring) $C_{11}H_9ClN_2O_5S$	Laurencé et al. 2014
28		**328.7**	**Ozonation product** $C_{12}H_9ClN_2O_5S$	Zoumpouli et al. 2021
Dimer, glucuronide form and other structures				
29		**96.1**	**Furfural** **Anodic oxidation, photodegradation product** $C_5H_4O_2$	Carda-Broch et al. 2000 Laurencé et al. 2014
30		**622.6**	**Photodegradation product** $C_{24}H_{22}N_4O_{12}S_2$	Dellagreca et al. 2004 Isidori et al. 2006
31	-	**352**	**Photodegradation product** $C_{14}H_{14}N_3O_6S$	Katsura et al. 2015
32	-	**555**	**Photodegradation product** $C_{20}H_{19}N_4O_{11}S_2$	Katsura et al. 2015

33		**506.9**	**Furosemide glucuronide** **Metabolization product** $C_{18}H_{19}ClN_2O_{11}S$	Mizuma et al. 1998 Williams et al. 2007 Peterson 2013
34		**328.7**	**Metabolization product** $C_{12}H_9ClN_2O_5S$	Peterson 2013
35		**636.1**	**Metabolization product** $C_{23}H_{28}ClN_4O_{11}S_2$	Williams et al. 2007 Peterson 2013
36		**682.2**	**Metabolization product** $C_{24}H_{32}ClN_5O_{10}S_3$	Williams et al. 2007 Peterson 2013
37		**344.7**	**Chemical and enzymatic oxidation** $C_{12}H_9ClN_2O_6S$	Chen & Burka 2007
38		**248.7**	**Ozonation product** $C_7H_5ClN_2O_4S$	Tsilikilis et al. 2016

39		**360.7**	**Ozonation product** $C_{12}H_9ClN_2O_7S$	Tsilikilis et al. 2016
40	-	**276**	**Ozonation product**	Aalizadeh et al. 2018
41	-	**288**	**Ozonation product**	Aalizadeh et al. 2018
42	-	**198**	**Ozonation product**	Tsilikilis et al. 2016
43		**116.1**	**Thermal decomposition** $C_5H_8O_3$	Carda-Broch et al. 2000
44	-	**205**	**Peroxidase product**	Almaqdi et al. 2019
45	-	**118**	**Peroxidase product**	Almaqdi et al. 2019

As mentioned earlier, the glucuronide form (product **33**) is the second most common form of furosemide. It is quite well referenced in the early literature (Bindschedler et al. 1997, Andreasen et al. 1981, Aranda et al. 1982, Mizuma et al. 1998). pH has a strong influence on its stability, it is more stable at pH<6, while more basic pH leads to a rearrangement into a β-glucuronide. However, both forms ultimately hydrolyze into furosemide (Hammarlund-udenaes & Benet 1989). The half-life of furosemide glucuronide in aqueous medium at physiological pH is 5.3 hours (Yang et al. 2006). Like furosemide, its glucuronide form is photodegraded but 20 times faster (sekikawa et al. 1995). The chlorine atom can be substituted by an hydroxyl group by photodegradation leading to the product **36**. Peterson (2013) lists the three major pathways for the formation of saluamine (**10**), the glucuronide form (**33**) and the Glutathione S-conjugate form (**35**). Two other degradation products of furosemide have been long known: furfural (product **29**) and saluamine (product **10**). Furfural is a small, highly soluble molecule that is also produced naturally by plants such as corn (Yue et al. 2022) and is rapidly biodegraded (Mandalika et al. 2014). Saluamine is the most referenced degradation product of furosemide and has been identified as a metabolite in humans (Hammarlund-udenaes & Benet 1989, Andreasen et al. 1981). It can be obtained by several processes : Electro-Fenton (Laurencé et al. 2011), acid catalyzed hydrolysis (Beyers et al. 2000), photodegradation (Della Greca et al. 2004; Katsura et al. 2015, Jakimska et al. 2014) and biodegradation (Laurencé et al. 2014, Olvera-Vargas et al. 2016). It was also found after incubation in sediments spiked with furosemide (Li et al. 2014).

The pyridinium of furosemide (**24**) has been identified as a degradation product of furosemide much more recently. Initially produced by electrochemistry (Laurencé et al. 2011), it was then obtained by furosemide bioconversion by two fungi, *Aspergillus candidus* and *Cunninghamella echinulata* (Laurencé et al. 2014, Olvera-Vargas et al. 2016) and finally found in the urine of patients treated with furosemide (Laurencé et al. 2019), therefore qualifying it as a human metabolite. Moreover, Chen & Burka (2007) detect a pyridinium ion in microsomal incubations of furosemide which corresponds to pyridinium of furosemide.

The gamma-ketocarboxylic acid (product **23**) has been identified on several occasions as well (Bukkitgar & Shetti 2016; Williams et al. 2007; Peterson 2013). According to the reaction pathway proposed by Olvera-Vargas et al.

2016, this product would be an intermediate preceding the formation of the pyridinium of furosemide (**24**) or the formation of a hydroxy ketone product (**22**), which can be hydrolyzed to saluamine (**10**). The hydroxyketone (**24**) has also been repeatedly identified as an anodic oxidation, metabolization or biotransformation product (Olvera-Vargas et al. 2016; Williams et al. 2007; Peterson 2013, respectively).

Most of the degradation products structures are proposed following NMR or mass spectrometry analysis but the absence of analytical standards makes it challenging to ascertain their authenticity. Some products have been obtained by the same processes in several other studies, which reinforces their probability to be genuine. Thus, ozonation products ***4*** and ***18*** were found in two different studies (Zoumpouli et al. (2021);Tsilikidis et al. (2016)) and product ***30***, a dimer from photodegradation of furosemide was observed both by Isidori et al. (2006) and Dellagrace et al. (2004). Product ***2*** seems to be the most frequently found both as a photodegradation product (Jakimska et al. 2014, Sandre et al. 2023, Katsura et al. 2015, Yagi et al. 1991) and as a degradation product of furosemide glucuronide (Mizuma et al. 1998). On the other hand, several products were described only once in the literature. It may be due to their very low concentration, or because they are intermediates of transformation and disappear quickly (Tsilikidis et al. 2016, Sandre et al. 2023, Aalizadeh et al. 2018). The lack of analytical standards makes the analysis of these compounds more complicated (Chen and Burka 2007). These structures are mostly similar to furosemide, saluamine or pyridinium, with variations in hydroxyl groups, chlorine substitutions or rearrangements. The prevalence of a degradation product depends on the media conditions. For example, Jakimska et al. (2014) detected saluamine and product ***2*** in several matrices, whereas product ***12*** was only found in treated wastewater and could therefore be a result of water treatment processes. Saluamine is one of the least well documented degradation products. Saluamine has rarely been searched for, and, to our knowledge, we were the first to quantify it in effluents of wastewater treatment plants, with concentrations up to 470 ng/L (Sandre et al. 2023). We also recently quantified pyridinium of furosemide in WWTP effluent over 200 ng/L. The presence of these two compounds has been verified unambiguously using analytical standards, which is not the case for the remaining by-products.

In conclusion, even if furosemide is apparently well eliminated by wastewater treatment plants, a lot of degradation by-products are produced and progressively identified thanks to the progress in analytical chemistry, in particular non targeted/suspect screening. However, they remain understudied, in the absence of analytical standards. The glucuronide form, the most well-known, degrades rapidly but some historical degradation products (saluamine, pyridinium of furosemide) have been identified on many occasions and could eventually be problematic if found in the environment.

Toxicity and ecotoxicity of furosemide and its degradation products

1. Health hazard of furosemide

As a pharmaceutical substance, the toxicity of furosemide on humans has been widely reviewed in various medical journals. Numerous adverse effects have been reported such as dehydration in the most common case (23%), hydrolytic disturbance (21%), orthostatic hypotension (10%), osteoporosis (7%), urinary disorders (4%), gastroenterological disorders (3%), rhythm disorders (3%) and gout attacks (2%) (Buttard 2016) and sometimes associated with jaundice (Peterson 2013). In rodents, it has shown evidence of toxicity (hepatotoxicity with depletion of GSH (reduced form of glutathion) and protein thiols, liver necrosis) at high concentrations (Peterson 2013).

2. Environmental toxicity of furosemide

The impact of furosemide in the environment and aquatic fauna has been less investigated than in humans, but a few studies aim to assess the risk of pharmaceutical compounds. Risk calculations are based on ratios between Measured Environmental Concentrations (MEC) or Predicted Environmental Concentrations (PEC) and Predicted No-Effect Concentrations (PNEC). PNEC are determined from the Effect Concentrations (ECs) obtained experimentally on different classes of organisms or estimated using different models such as ECOSAR (**Table 7**).

Table 7. ECs (Lethality, Immobilization or growth inhibition) value for several organisms exposed to furosemide.

	Species	Duration	EC (mg/L)	Type of data	Reference
Bacteria	*Aliivibrio fischeri*	EC10 15 min	**7.5**	Experimental	Di Nica et al. 2016
		EC20 15 min	**72.3**	Experimental	Olvera-Vargas et al. 2016
		EC50 15 min	**33.2**	Experimental	Di Nica et al. 2016
		EC50 30 min	**> 200**	Experimental	Isidori et al. 2006
Green algae	*Desmodesmus subspicatus*	EC50 72H	**322.2**	Experimental	Guo 2015
	Pseudokirchneriella subcapitata	EC50 72H	**142**	Experimental	Christensen et al. 2009
		EC50 -	**19.8**	ECOSAR	Kuzmanovic et al. 2015
Zooplankton	*Brachionus calyciflorus*	EC50 24H	**> 100**	Experimental	Isidori et al. 2006
Crustacean	*Artemia salina*	EC50 24H	**273.0**	Experimental	Diaz-sosa et al. 2020
		EC50 48H	**225.1**	Experimental	Diaz-sosa et al. 2020
	Daphnia magna	EC50 -	**560**	ECOSAR	Kuzmanovic et al. 2015
		EC50 24H	**60.6**	Experimental	Isidori et al. 2006
		EC50 48H	**239.0**	Experimental	Christensen et al. 2009
	Thamnocephalus Platyurus	EC50 24H	**70.6**	Experimental	Isidori et al. 2006
	Ceriodaphnia dubia	EC50 48H	**84.1**	Experimental	Isidori et al. 2006
Cnidarian	*Hydra vulgaris*	Acute tox.	**> 1**	Experimental	Pascoe et al., 2003
Teleosts	*Pimephales promelas*	EC50 -	**521**	ECOSAR	Kuzmanovic et al. 2015
	Cyprinodon variegatus	EC50 96H	**497**	Experimental	Christensen et al. 2009
	Cyprinus orfus	EC50	**> 500**	Experimental	Hanisch et al. 2002
Cell line	*Oncorhynchus mykiss*	EC50 24H	**1 131**	Experimental	Christensen et al. 2009
	Poeciliopsis lucida hepatoma	EC50 24H	**2 576**	Experimental	Christensen et al. 2009

EC = Effect Concentration

The bacterium *Aliivibrio fischeri* seems to be the most sensitive to furosemide followed by the green algae *Selenastrum capricornutum*. Among invertebrates, the crustacean *Daphnia magna* appears as the most sensitive organism. In comparison, the EC50s obtained in the fish cell lines appear to be quite high. However, these results cannot be extrapolated to toxicological relevance in whole organisms and ecosystems because these *in vitro* tests

do not take into account systemic effects of the molecule, neither organ/tissue specific effects nor possible detoxification processes.

It should be noted that the data summarized in **Table 7** for lethality, immobilization or growth inhibition are obtained in acute exposure (i.e., short duration exposure). Chronic exposures (i.e., long term exposure) would be more representative of environmental conditions. For chronic exposures, Isidori et al. (2006) indeed obtained much lower EC50s for *Brachionus calyciflorus* (2.5 mg/L) and *Ceriodaphnia dubia* (2.3 mg/L) versus 100 mg/L and 84.1 mg/L in acute exposure, respectively. Riva et al. (2019) and Mendoza et al. (2015) set a PNEC of 45.15 µg/L and 1,56 µg/L based on the ECs of algal, crustacean and fish toxicity tests. These PNEC are then used to determine the Risk Quotient (RQ) or Hazard Quotient (HQ) by calculating the ratio MEC/PNEC. When this ratio is greater than 1, it is considered that there is a risk for the environment. According to its risk quotient, furosemide presents a high toxicological risk for invertebrates and moderate risk for fish (Papageorgiou et al. 2016). Furosemide is often detected in rivers at concentrations higher than the Predicted Environmental Concentrations (Besse & Garric, 2008). As a result, risk assessments may underestimate its impact. Moreover, most of the time, ECs are based on the lethality of compounds to organisms, which is not a very sensitive parameter. For example, based on those lethality values, Lacey (2008) and Carlsson et al. (2006) considered furosemide as not problematic for aquatic environments. However, an increasing number of studies reported that analysis of organism behavior, which integrates many physiological processes, is environmentally more relevant to anticipate impacts on survival, as behavioral toxicity can be observed at concentrations 10 to 100 times lower than lethal concentrations (Legradi et al. 2018, Sandre et al. 2022). Thus, furosemide, at the concentrations mentioned above, may well have a negative impact on aquatic organism survival.

Other markers may be also relevant to study. For example, genotoxic and cytotoxic effects of furosemide were shown by Rocco et al. (2010) on *Danio rerio* and by Mondal et al. (2012) in mice hepatocytes and cytotoxicity in mouse and rat along with an irreversible binding to hepatocyte were found by Williams et al. (2007). Peterson (2013) reported several evidences of hepatotoxicity in rodents and Fent et al. (2006) noted an estrogenic activity in *in vitro* experiments. This endocrine disruption potential could have negative impacts not only at the individual level but also at the population level, endangering its survival.

3. Toxicity of furosemide degradation products

The toxicity of furfural (product **29**) has been well studied because it is used to produce furfuryl alcohol which is extensively used for metal casting industry, as a solvent for dyes, as a corrosion inhibitor, in flavors and fragrances and also as a reagent for drug synthesis (Grosse et al. 2017). Furfural exposure in humans occurs through the lungs or skin and provokes irritations (Flek and Sedivec 1978). In rats, it can induce lung hemorrhage and edema after oral or dermal exposure and the Lethal Dose (LD50) is 102 mg/kg which is quite high (Reed and Kwok 2014). Furfural is rapidly metabolized by enteric bacteria under both aerobic and anaerobic conditions and 83 to 88% is excreted in urine in 72 h (Boopathy et al. 1993). In the environment, furfural presents a higher toxicity than furosemide with an LC50 of 29 mg/L for 24 h exposure and 13 mg/L for 72 h exposure in *Daphnia magna*. For fish, LC50 after 96 h exposure is 10.5 mg/L for *Poecilia reticulata* and 32 mg/L for *Pimephales promelas* which is also lower than furosemide for different fish species. Chronic exposure of fishes to furfural leads to growth retardation, morphological abnormalities, and lethargy (Reed and Kwok 2014). There is little data concerning the other degradation products of furosemide. Saluamine (product **10**) is only referenced as a degradation product of furosemide and is therefore much less studied. Only one study investigated its toxicity (Al-Omar et al. (2009),

which showed that it induces changes in some body parameters in mouse models such as increased alanine and aspartate aminotransferase, increased creatinine, reduced blood glucose, liver and kidney congestion. We recently showed that pyridinium of furosemide (product **24**) leads to the development of characteristic biomarkers of Parkinson's disease in mice and generates oxidative stress and inhibition of the mitochondrial respiratory chain (Laurencé et al. 2019). Furthermore, Olvera-Vargas et al. (2016) noted an EC50 of 34.4 mg/L and an EC20 of 18.9 mg/L in *Aliivibrio fischeri* for 15 min exposure, which is lower than the EC20 of 72.3 mg/L they obtained for furosemide in the same conditions. Taken together, these results suggest that pyridinium of furosemide is also more toxic than furosemide. Isidori et al. (2006) conducted a study on photodegradation product of furosemide (Product ***30***) and showed that EC50 values were lower for the degradation product than for furosemide for *Brachionus calyciflorus* (1.04 mg/L) and *Ceriodaphnia dubia* (0.57 mg/L) reflecting a higher toxicity. Moreover, an Ames test showed mutagenic activity of this by-product at concentrations between 6.25 and 100 mg/L. Olvera-Vargas et al. (2016) also noted a more important toxicity for the hydroxyketone product (product **22**) with an EC20 of 37.42 mg/L in *Aliivibrio fischeri* for 15 min exposure. Mitchell et al. (1976) mentioned a metabolite corresponding to product **8** which would be more toxic than furosemide and causes hepatic necrosis. For a more complete evaluation of the environmental impact, mixtures should also be taken into account. Indeed, interactions between molecules can lead to synergistic or antagonistic effects (cocktail effects) and are quite complex. Because furosemide is a medication, it is frequently in interaction with other compounds such as benzalkonium chloride or Spironolactone (commercialized in mixture by Sanofi Aventis since 1981). However, there is very few data about the ecotoxicity of the mixture or about the interactions between furosemide and its degradation products. To the best of our knowledge, only the study of Pomati et al. (2006), which showed a 30% decrease in liver cell (HEK293) proliferation in the presence of a mixture of 30 compounds including furosemide, presents toxicity data in mixtures.

Whereas the acute toxicity of furosemide is overall quite low, its chronic toxicity has been shown to be more important. Furosemide has hepatotoxic effects in rodents, cytotoxic and genotoxic effects in fish and an estrogenic activity has also been demonstrated in vitro. In addition, three of its degradation products are rather preoccupying, as the limited information available (Olvera-Vargas et al. 2016; Laurencé et al. 2019) suggests that they have a more important acute toxicity than their parent molecule, affecting physiological parameters, growth and mitochondrial respiration. Furthermore, this could be also the case for some of the other identified degradation products, on which standards do not even exist and for those remaining to be discovered to date.

Conclusion

Given the wide consumption of medicines, increasing demographical urban pressure and population aging, the chemical and ecotoxicological assessment of pharmaceuticals and their metabolites constitutes major challenges. This situation is expected to worsen in the frame of climate change and modification of water availability and uses, requiring the implementation of suitable management measures based on scientific/technological progress. Improving our understanding of drugs follow-up to and in the environment in the framework of water resources protection against these new pollutants is crucial. Furosemide is a good example of contaminants of emerging concern. It is a widely prescribed loop diuretic for the elderly, but also for younger adults and children, and in veterinary medicine, for almost 60 years. It is one of the most sold drugs in the world and considered essential by the WHO. Many studies classify it as a priority pollutant based on its production, sale or consumption, its occurrence in the environment, its persistence in freshwater, its removal in WWTP or input in wastewater, its excretion rate and its toxicity or ecotoxicity. Indeed, a significant consumption of furosemide, in particular in

European countries, has been noted. Important concentrations are prescribed and consumed in medical institutions or by individuals. After its consumption, furosemide can be metabolized or degraded in WWTPs with an average removal of over 50%. However, new processes or processes used for other purposes are being studied to obtain a better degradation of furosemide. The oxidation processes are the most frequent and also seem to be the most efficient. However, furosemide will be degraded into several by-products. The best known is the glucuronide conjugate, but it is not of great concern because it is rapidly degraded. On the other hand, saluamine, pyridinium of furosemide, furfural, and certain products resulting from metabolization or photodegradation present a higher toxicity than the parent molecule. There are also new or simply little studied degradation products for which information on their toxicity is needed.

Furosemide is highly concentrated at sources such as hospitals and WWTP influents but also after treatment of these effluents for which most studies are conducted in Europe. Furosemide is also found in WWTP sludges. As a consequence, furosemide and its degradation products end up in the receiving environment. In river water, furosemide is found at a few hundred nanograms per liter and has been observed in groundwater. It has also been reported in sediment. Therefore, furosemide is widely present in the environment and could be a problem due to its occurrence and persistence in water. Although its acute toxicity is moderate, other endpoints are impacted and its degradation products are more toxic for those of which we have information. Unfortunately, little is known about their fate in the environment. It is then absolutely necessary to continue the chemical, toxicological and ecotoxicological characterization of these pollutants, and in a wider scope, those of pharmaceuticals and their degradation products. Non-targeted analytical chemistry approaches are a powerful tool to find new degradation products. Adverse outcome pathway (AOP) studies could be interesting approaches to understand the mechanisms of action of these new pollutants and multi-model approaches in ecotoxicology could lead to a better understanding of their off-target effects and the environmental risk for ecosystems.

Bibliography

Aalizadeh, R., Nika, M.-C., Thomaidis, N.S., 2019. Development and application of retention time prediction models in the suspect and non-target screening of emerging contaminants. Journal of Hazardous Materials 363, 277–285. https://doi.org/10.1016/j.jhazmat.2018.09.047

Abbott, L.M., Kovacic, J., 2008. The pharmacologic spectrum of furosemide. J Veter Emer Crit 18, 26–39. https://doi.org/10.1111/j.1476-4431.2007.00267.x

Acuña, V., von Schiller, D., García-Galán, M.J., Rodríguez-Mozaz, S., Corominas, L., Petrovic, M., Poch, M., Barceló, D., Sabater, S., 2015. Occurrence and in-stream attenuation of wastewater-derived pharmaceuticals in Iberian rivers. Science of The Total Environment 503–504, 133–141. https://doi.org/10.1016/j.scitotenv.2014.05.067

Afsa, S., Hamden, K., Lara Martin, P.A., Mansour, H.B., 2020. Occurrence of 40 pharmaceutically active compounds in hospital and urban wastewaters and their contribution to Mahdia coastal seawater contamination. Environ Sci Pollut Res 27, 1941–1955. https://doi.org/10.1007/s11356-019-06866-5

Agence nationale de Sécurité du médicament et des produits de santé, 2013. Analyse des ventes de médicaments en France en 2013 36.

Aherne, G.W., Briggs, R., 1989. The relevance of the presence of certain synthetic steroids in the aquatic environment. Journal of Pharmacy and Pharmacology 41, 735–736. https://doi.org/10.1111/j.2042-7158.1989.tb06355.x

Aherne, G.W., Hardcastle, A., Nield, A.H., 1990. Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples. Journal of Pharmacy and Pharmacology 42, 741–742. https://doi.org/10.1111/j.2042-7158.1990.tb06574.x

Ahmed, N.J., 2020. The Outpatient Use of Furosemide in a Public Hospital. JPRI 79–83. https://doi.org/10.9734/jpri/2020/v32i730463

Ajo, P., Preis, S., Vornamo, T., Mänttäri, M., Kallioinen, M., Louhi-Kultanen, M., 2018. Hospital wastewater treatment with pilot-scale pulsed corona discharge for removal of pharmaceutical residues. Journal of Environmental Chemical Engineering 6, 1569–1577. https://doi.org/10.1016/j.jece.2018.02.007

Al Aukidy, M., Verlicchi, P., Jelic, A., Petrovic, M., Barcelò, D., 2012. Monitoring release of pharmaceutical compounds: Occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. Science of The Total Environment 438, 15–25. https://doi.org/10.1016/j.scitotenv.2012.08.061

Almaqdi, K.A., Morsi, R., Alhayuti, B., Alharthi, F., Ashraf, S.S., 2019. LC-MSMS based screening of emerging pollutant degradation by different peroxidases. BMC Biotechnol 19, 83. https://doi.org/10.1186/s12896-019-0574-y

Almeida, C., Ahmad, S.M., Nogueira, J.M.F., 2017. Bar adsorptive microextraction technique - application for the determination of pharmaceuticals in real matrices. Anal Bioanal Chem 409, 2093–2106. https://doi.org/10.1007/s00216-016-0156-y

Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I., Surif, S., 2010. Multi-residue analytical method for human pharmaceuticals and synthetic hormones in river water and sewage effluents by solid-phase extraction and liquid chromatography–tandem mass spectrometry. Journal of Chromatography A 1217, 6791–6806. https://doi.org/10.1016/j.chroma.2010.08.033

Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I., Surif, S., Abdulghani, M., 2013. The occurrence of human pharmaceuticals in wastewater effluents and surface water of Langat River and its tributaries, Malaysia. International Journal of Environmental Analytical Chemistry 93, 245–264. https://doi.org/10.1080/03067319.2011.592949

Al-Odaini, N.A., Zakaria, M.P., Zali, M.A., Juahir, H., Yaziz, M.I., Surif, S., 2012. Application of chemometrics in understanding the spatial distribution of human pharmaceuticals in surface water. Environ Monit Assess 184, 6735–6748. https://doi.org/10.1007/s10661-011-2454-3

Al-Omar, I.A., Al-Ashban, R.M., Shah, A.H., 2009. Toxicity Studies on 4-Chloro-5-Sulfamoylanthranilic Acid the Degradation Product of a Loop Diuretic Furosemide. Research Journal of Pharmacology 63–77, 3.

Alygizakis, N.A., Besselink, H., Paulus, G.K., Oswald, P., Hornstra, L.M., Oswaldova, M., Medema, G., Thomaidis, N.S., Behnisch, P.A., Slobodnik, J., 2019. Characterization of wastewater effluents in the Danube River Basin with chemical screening, in vitro bioassays and antibiotic resistant genes analysis. Environment International 127, 420–429. https://doi.org/10.1016/j.envint.2019.03.060

Anderson, P., Denslow, N., Drewes, J.E., Olivieri, A., Schlenk, D., Snyder, S., 2010. Monitoring Strategies for Chemicals of Emerging Concern (CECs) in Recycled Water. State Water Resources Control Board Repport.

Andreasen, F., Botker, H., Lorentzen, K., 1982. In vitro studies on the hydrolysis of frusemide in gastrointestinal juices. British Journal of Clinical Pharmacology 14, 306–309. https://doi.org/10.1111/j.1365-2125.1982.tb01984.x

Andreasen, F., Christensen, C.K., Jakobsen, F.K., Mogensen, C.E., 2009. The Use of HPLC to Elucidate the Metabolism and Urinary Excretion of Furosemide and its Metabolic Products. Acta Pharmacologica et Toxicologica 49, 223–229. https://doi.org/10.1111/j.1600-0773.1981.tb00897.x

Andreasen, F., Jakobsen, P., 2009. Determination of Furosemide in Blood Plasma and its Binding to Proteins in Normal Plasma and in Plasma from Patients with Acute Renal Failure. Acta Pharmacologica et Toxicologica 35, 49–57. https://doi.org/10.1111/j.1600-0773.1974.tb00724.x

Aranda, J.V., Lambert, C., Perez, J., Turmen, T., Sitar, D.S., 1982. Metabolism and renal elimination of furosemide in the newborn infant. The Journal of Pediatrics 101, 777–781. https://doi.org/10.1016/S0022-3476(82)80319-3

Arola, K., Kallioinen, M., Reinikainen, S.-P., Hatakka, H., Mänttäri, M., 2018. Advanced treatment of membrane concentrate with pulsed corona discharge. Separation and Purification Technology 198, 121–127. https://doi.org/10.1016/j.seppur.2017.07.011

Arrubla, J.P., Cubillos Vargas, J.A., Ramirez Vargas, C.A., Arredondo Gonzalez, J.A., Arias Isaza, C.A., Paredes Cuervo, D., 2016. Pharmaceutical and personal care products in domestic wastewater and their removal in anaerobic treatment systems: Septic tank - up flow anaerobic filter. ing.inv. 36, 70–78. https://doi.org/10.15446/ing.investig.v36n1.53076

Badia-Fabregat, M., Lucas, D., Gros, M., Rodríguez-Mozaz, S., Barceló, D., Caminal, G., Vicent, T., 2015. Identification of some factors affecting pharmaceutical active compounds (PhACs) removal in real wastewater. Case study of fungal treatment of reverse osmosis concentrate. Journal of Hazardous Materials 283, 663–671. https://doi.org/10.1016/j.jhazmat.2014.10.007

Badia-Fabregat, M., Lucas, D., Pereira, M.A., Alves, M., Pennanen, T., Fritze, H., Rodríguez-Mozaz, S., Barceló, D., Vicent, T., Caminal, G., 2016. Continuous fungal treatment of non-sterile veterinary hospital effluent: pharmaceuticals removal and microbial community assessment. Appl Microbiol Biotechnol 100, 2401–2415. https://doi.org/10.1007/s00253-015-7105-0

Baken, K.A., Sjerps, R.M.A., Schriks, M., van Wezel, A.P., 2018. Toxicological risk assessment and prioritization of drinking water relevant contaminants of emerging concern. Environment International 118, 293–303. https://doi.org/10.1016/j.envint.2018.05.006

Baranowska, I., Wilczek, A., Baranowski, J., 2010. Rapid UHPLC Method for Simultaneous Determination of Vancomycin, Terbinafine, Spironolactone, Furosemide and Their Metabolites: Application to Human Plasma and Urine. Anal. Sci. 26, 755–759. https://doi.org/10.2116/analsci.26.755

Baresel, C., Harding, M., Junestedt, C., 2019. Removal of pharmaceutical residues from municipal wastewater using UV/H2O2 (No. B 2354).

Batt, A.L., Kostich, M.S., Lazorchak, J.M., 2008. Analysis of Ecologically Relevant Pharmaceuticals in Wastewater and Surface Water Using Selective Solid-Phase Extraction and UPLC−MS/MS. Anal. Chem. 80, 5021–5030. https://doi.org/10.1021/ac800066n

Bäuerlein, P.S., Mansell, J.E., ter Laak, T.L., de Voogt, P., 2012. Sorption Behavior of Charged and Neutral Polar Organic Compounds on Solid Phase Extraction Materials: Which Functional Group Governs Sorption? Environ. Sci. Technol. 46, 954–961. https://doi.org/10.1021/es203404x

Benotti, M.J., Fisher, S.C., Terracciano, S.A., 2006. Occurrence of Pharmaceuticals in Shallow Ground Water of Suffolk County, New York, 2002–2005 (U.S. Geological Survey Open-File Report 2006–1297), Open-File Report.

Besse, J.-P., Garric, J., 2008. Human pharmaceuticals in surface waters. Toxicology Letters 176, 104–123. https://doi.org/10.1016/j.toxlet.2007.10.012

Beyers, H., Malan, S.F., van der Watt, J.G., de Villiers, M.M., 2000. Structure-Solubility Relationship and Thermal Decomposition of Furosemide. Drug Development and Industrial Pharmacy 26, 1077–1083. https://doi.org/10.1081/DDC-100100271

Biel-Maeso, M., Baena-Nogueras, R.M., Corada-Fernández, C., Lara-Martín, P.A., 2018. Occurrence, distribution and environmental risk of pharmaceutically active compounds (PhACs) in coastal and ocean waters from the Gulf of Cadiz (SW Spain). Science of The Total Environment 612, 649–659. https://doi.org/10.1016/j.scitotenv.2017.08.279

Bindschedler, M., Degen, P., Flesch, G., de Gasparo, M., Preiswerk, G., 1997. Pharmacokinetic and pharmacodynamic interaction of single oral doses of valsartan and furosemide. European Journal of Clinical Pharmacology 52, 371–378. https://doi.org/10.1007/s002280050303

Björklund, E., Svahn, O., Bak, S., Bekoe, S.O., Hansen, M., 2016. Pharmaceutical Residues Affecting the UNESCO Biosphere Reserve Kristianstads Vattenrike Wetlands: Sources and Sinks. Arch Environ Contam Toxicol 71, 423–436. https://doi.org/10.1007/s00244-016-0303-7

Blum, A., Allier, D., Ghestem, J.P., Lopez, B., Moly, F., 2011. BRGM/RP- 59135-FR- Campagne exceptionnelle d'analyse des substances présentes dans les eaux souterraines (No. BRGM/RP-59135-FR).

Boles Ponto, L.L., Schoenwald, R.D., 1990. Furosemide (Frusemide) A Pharmacokinetic/Pharmacodynamic Review (Part 1)1 5, 381–408.

Boopathy, R., Bokang, H., Daniels, L., 1993. Biotransformation of furfural and 5-hydroxymethyl furfural by enteric bacteria. Journal of Industrial Microbiology 11, 147–150. https://doi.org/10.1007/BF01583715

Boulestreau, R., Lalanne, P., Durieux, W., Delarche, N., 2018. Prescription de furosémide en médecine générale en Aquitaine. Enquête de pratique auprès des médecins généralistes maîtres de stages universitaires. Annales de Cardiologie et d'Angéiologie 67, 327–333. https://doi.org/10.1016/j.ancard.2018.08.012

Boxall, A.B.A., Keller, V.D.J., Straub, J.O., Monteiro, S.C., Fussell, R., Williams, R.J., 2014. Exploiting monitoring data in environmental exposure modelling and risk assessment of pharmaceuticals. Environment International 73, 176–185. https://doi.org/10.1016/j.envint.2014.07.018

Bueno, M.J.M., Gomez, M.J., Herrera, S., Hernando, M.D., Agüera, A., Fernández-Alba, A.R., 2012. Occurrence and persistence of organic emerging contaminants and priority pollutants in five sewage treatment plants of Spain: Two years pilot survey monitoring. Environmental Pollution 164, 267–273. https://doi.org/10.1016/j.envpol.2012.01.038

Bukkitgar, S.D., Shetti, N.P., 2016. Electrochemical oxidation of loop diuretic furosemide in aqueous acid medium and its analytical application. Cogent Chemistry 2, 1152784. https://doi.org/10.1080/23312009.2016.1152784

Bundgaard, H., Nørgaard, T., Nielsen, N.M., 1988. Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions. International Journal of Pharmaceutics 42, 217–224. https://doi.org/10.1016/0378-5173(88)90178-0

Burcea, A., Boeraş, I., Mihuţ, C.-M., Bănăduc, D., Matei, C., Curtean-Bănăduc, A., 2020. Adding the Mureş River Basin (Transylvania, Romania) to the List of Hotspots with High Contamination with Pharmaceuticals. Sustainability 12, 10197. https://doi.org/10.3390/su122310197

Buttard, M., 2016. Évaluation de la prescription du furosémide chez la personne âgée de 75 ans et plus : étude observationnelle, prospective réalisée dans le service de Médecine Interne Gériatrie du Centre Hospitalier Universitaire de Dijon-Bourgogne. Université de Bourgogne, UFR des Sciences de Santé de Dijon.

Cabeza, Y., Candela, L., Ronen, D., Teijon, G., 2012. Monitoring the occurrence of emerging contaminants in treated wastewater and groundwater between 2008 and 2010. The Baix Llobregat (Barcelona, Spain). Journal of Hazardous Materials 239–240, 32–39. https://doi.org/10.1016/j.jhazmat.2012.07.032

Cadwallader, A.B., De La Torre, X., Tieri, A., Botrè, F., 2010. The abuse of diuretics as performance-enhancing drugs and masking agents in sport doping: pharmacology, toxicology and analysis: Diuretics in sport doping. British Journal of Pharmacology 161, 1–16. https://doi.org/10.1111/j.1476-5381.2010.00789.x

Calamari, D., Zuccato, E., Castiglioni, S., Bagnati, R., Fanelli, R., 2003. Strategic Survey of Therapeutic Drugs in the Rivers Po and Lambro in Northern Italy. Environ. Sci. Technol. 37, 1241–1248. https://doi.org/10.1021/es020158e

Calesnick, B., Christensen, J.A., Richter, M., 1966. Absorption and Excretion of Furosernide-S35 in Human Subjects. PSEBM, Section of Human Pharmacology 123.

Campos-Mañas, M.C., Plaza-Bolaños, P., Sánchez-Pérez, J.A., Malato, S., Agüera, A., 2017. Fast determination of pesticides and other contaminants of emerging concern in treated wastewater using direct injection coupled to highly sensitive ultra-high performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 1507, 84–94. https://doi.org/10.1016/j.chroma.2017.05.053

Candela, L., Tamoh, K., Vadillo, I., Valdes-Abellan, J., 2016. Monitoring of selected pharmaceuticals over 3 years in a detrital aquifer during artificial groundwater recharge. Environ Earth Sci 75, 244. https://doi.org/10.1007/s12665-015-4956-8

Cantwell, M.G., Katz, D.R., Sullivan, J.C., Shapley, D., Lipscomb, J., Epstein, J., Juhl, A.R., Knudson, C., O'Mullan, G.D., 2018. Spatial patterns of pharmaceuticals and wastewater tracers in the Hudson River Estuary. Water Research 137, 335–343. https://doi.org/10.1016/j.watres.2017.12.044

Carda-Broch, S., Esteve-Romero, J., García-Alvarez-Coque, M.C., 2000. Furosemide assay in pharmaceuticals by Micellar liquid chromatography: study of the stability of the drug. Journal of Pharmaceutical and Biomedical Analysis 23, 803–817. https://doi.org/10.1016/S0731-7085(00)00378-2

Carlsson, C., Johansson, A.-K., Alvan, G., Bergman, K., Kühler, T., 2006. Are pharmaceuticals potent environmental pollutants? Science of The Total Environment 364, 67–87. https://doi.org/10.1016/j.scitotenv.2005.06.035

Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D., Zuccato, E., 2006. Removal of Pharmaceuticals in Sewage Treatment Plants in Italy. Environ. Sci. Technol. 40, 357–363. https://doi.org/10.1021/es050991m

Castiglioni, S., Davoli, E., Riva, F., Palmiotto, M., Camporini, P., Manenti, A., Zuccato, E., 2018. Data on occurrence and fate of emerging contaminants in a urbanised area. Data in Brief 17, 533–543. https://doi.org/10.1016/j.dib.2018.01.029

Čelić, M., Gros, M., Farré, M., Barceló, D., Petrović, M., 2019. Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). Science of The Total Environment 652, 952–963. https://doi.org/10.1016/j.scitotenv.2018.10.290

Celle-Jeanton, H., Schemberg, D., Mohammed, N., Huneau, F., Bertrand, G., Lavastre, V., Le Coustumer, P., 2014. Evaluation of pharmaceuticals in surface water: Reliability of PECs compared to MECs. Environment International 73, 10–21. https://doi.org/10.1016/j.envint.2014.06.015

Chahwakilian, P., Ferrier-Battner, A., 2012. Synthèse de l'étude européenne sur la consommation de médicaments. ESSEC, LIR.

Chen, L.-J., Burka, L.T., 2007. Chemical and Enzymatic Oxidation of Furosemide: Formation of Pyridinium Salts. Chem. Res. Toxicol. 20, 1741–1744. https://doi.org/10.1021/tx700262z

Chen, Y., Vymazal, J., Březinová, T., Koželuh, M., Kule, L., Huang, J., Chen, Z., 2016. Occurrence, removal and environmental risk assessment of pharmaceuticals and personal care products in rural wastewater treatment wetlands. Science of The Total Environment 566–567, 1660–1669. https://doi.org/10.1016/j.scitotenv.2016.06.069

Cheng, J.W.M., Nayar, M., 2009. A review of heart failure management in the elderly population. The American Journal of Geriatric Pharmacotherapy 7, 233–249. https://doi.org/10.1016/j.amjopharm.2009.10.001

Chetboul, V., Pouchelon, J.-L., Menard, J., Blanc, J., Desquilbet, L., Petit, A., Rougier, S., Lucats, L., Woehrle, F., the TEST study investigators, 2017. Short-Term Efficacy and Safety of Torasemide and Furosemide in 366 Dogs with Degenerative Mitral Valve Disease: The TEST Study. J Vet Intern Med 31, 1629–1642. https://doi.org/10.1111/jvim.14841

Chiffre, A., Degiorgi, F., Buleté, A., Spinner, L., Badot, P.-M., 2016. Occurrence of pharmaceuticals in WWTP effluents and their impact in a karstic rural catchment of Eastern France. Environ Sci Pollut Res 23, 25427–25441. https://doi.org/10.1007/s11356-016-7751-5

Chinnaiyan, P., Thampi, S.G., Kumar, M., Mini, K.M., 2018. Pharmaceutical products as emerging contaminant in water: relevance for developing nations and identification of critical compounds for Indian environment. Environ Monit Assess 190, 288. https://doi.org/10.1007/s10661-018-6672-9

Christensen, A.M., Markussen, B., Baun, A., Halling-Sørensen, B., 2009. Probabilistic environmental risk characterization of pharmaceuticals in sewage treatment plant discharges. Chemosphere 77, 351–358. https://doi.org/10.1016/j.chemosphere.2009.07.018

Collado, N., Rodriguez-Mozaz, S., Gros, M., Rubirola, A., Barceló, D., Comas, J., Rodriguez-Roda, I., Buttiglieri, G., 2014. Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. Environmental Pollution 185, 202–212. https://doi.org/10.1016/j.envpol.2013.10.040

Corvaisier, N., 2000. Les substances médicamenteuses rejetées dans les eaux usées urbaines. OFFICE INTERNATIONAL DE L'EAU, ENGREF.

Cruz-Morató, C., Lucas, D., Llorca, M., Rodriguez-Mozaz, S., Gorga, M., Petrovic, M., Barceló, D., Vicent, T., Sarrà, M., Marco-Urrea, E., 2014. Hospital wastewater treatment by fungal bioreactor: Removal efficiency for pharmaceuticals and endocrine disruptor compounds. Science of The Total Environment 493, 365–376. https://doi.org/10.1016/j.scitotenv.2014.05.117

Cuthbertson, A.A., Kimura, S.Y., Liberatore, H.K., Summers, R.S., Knappe, D.R.U., Stanford, B.D., Maness, J.C., Mulhern, R.E., Selbes, M., Richardson, S.D., 2019. Does Granular Activated Carbon with Chlorination Produce Safer Drinking Water? From Disinfection Byproducts and Total Organic Halogen to Calculated Toxicity. Environ. Sci. Technol. 53, 5987–5999. https://doi.org/10.1021/acs.est.9b00023

Dalahmeh, S., Björnberg, E., Elenström, A.-K., Niwagaba, C.B., Komakech, A.J., 2020. Pharmaceutical pollution of water resources in Nakivubo wetlands and Lake Victoria, Kampala, Uganda. Science of The Total Environment 710, 136347. https://doi.org/10.1016/j.scitotenv.2019.136347

Daouk, S., Chèvre, N., Vernaz, N., Bonnabry, P., Dayer, P., Daali, Y., Fleury-Souverain, S., 2015. Prioritization methodology for the monitoring of active pharmaceutical ingredients in hospital effluents. Journal of Environmental Management 160, 324–332. https://doi.org/10.1016/j.jenvman.2015.06.037

Dasenaki, M.E., Thomaidis, N.S., 2015. Multianalyte method for the determination of pharmaceuticals in wastewater samples using solid-phase extraction and liquid chromatography–tandem mass spectrometry. Anal Bioanal Chem 407, 4229–4245. https://doi.org/10.1007/s00216-015-8654-x

Data Bridge Market research, 2021. Frusemide Market – Global Industry Trends and Forecast to 2028 | URL https://www.databridgemarketresearch.com/reports/global-frusemide-market

Daughton, C.G., Ruhoy, I.S., 2009. Environmental footprint of pharmaceuticals: the significance of factors beyond direct excretion to sewers. Environ Toxicol Chem 28, 2495. https://doi.org/10.1897/08-382.1

de Jongh, C.M., Kooij, P.J.F., de Voogt, P., ter Laak, T.L., 2012. Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water. Science of The Total Environment 427–428, 70–77. https://doi.org/10.1016/j.scitotenv.2012.04.010

de Voogt, P., Janex-Habibi, M.-L., Sacher, F., Puijker, L., Mons, M., 2009. Development of a common priority list of pharmaceuticals relevant for the water cycle. Water Science and Technology 59, 39–46. https://doi.org/10.2166/wst.2009.764

Della Greca, M., Iesce, M.R., Previtera, L., Rubino, M., Temussi, F., 2004. A new photoproduct of the drug furosemide in aqueous media. Environ Chem Lett 2, 155–158. https://doi.org/10.1007/s10311-004-0080-9

Delli Compagni, R., Gabrielli, M., Polesel, F., Turolla, A., Trapp, S., Vezzaro, L., Antonelli, M., 2020. Risk assessment of contaminants of emerging concern in the context of wastewater reuse for irrigation: An integrated modelling approach. Chemosphere 242, 125185. https://doi.org/10.1016/j.chemosphere.2019.125185

Di Nica, V., Villa, S., Finizio, A., 2017. Toxicity of individual pharmaceuticals and their mixtures to *Aliivibrio fischeri* : Experimental results for single compounds and considerations of their mechanisms of action and potential acute effects on aquatic organisms: Aquatic toxicity of pharmaceuticals. Environ Toxicol Chem 36, 807–814. https://doi.org/10.1002/etc.3568

Díaz-Garduño, B., Pintado-Herrera, M.G., Biel-Maeso, M., Rueda-Márquez, J.J., Lara-Martín, P.A., Perales, J.A., Manzano, M.A., Garrido-Pérez, C., Martín-Díaz, M.L., 2017. Environmental risk assessment of effluents as a whole emerging contaminant: Efficiency of alternative tertiary treatments for wastewater depuration. Water Research 119, 136–149. https://doi.org/10.1016/j.watres.2017.04.021

Diaz-Sosa, V.R., Tapia-Salazar, M., Wanner, J., Cardenas-Chavez, D.L., 2020. Monitoring and Ecotoxicity Assessment of Emerging Contaminants in Wastewater Discharge in the City of Prague (Czech Republic). Water 12, 1079. https://doi.org/10.3390/w12041079

DiNicolantonio, J.J., 2012. Should torsemide be the loop diuretic of choice in systolic heart failure? Future Cardiology 8, 707–728. https://doi.org/10.2217/fca.12.54

Dong, Z., Senn, D.B., Moran, R.E., Shine, J.P., 2013. Prioritizing environmental risk of prescription pharmaceuticals. Regulatory Toxicology and Pharmacology 65, 60–67. https://doi.org/10.1016/j.yrtph.2012.07.003

Ekowati, Y., Buttiglieri, G., Ferrero, G., Valle-Sistac, J., Diaz-Cruz, M.S., Barceló, D., Petrovic, M., Villagrasa, M., Kennedy, M.D., Rodríguez-Roda, I., 2016. Occurrence of pharmaceuticals and UV filters in swimming pools and spas. Environ Sci Pollut Res 23, 14431–14441. https://doi.org/10.1007/s11356-016-6560-1

Eriksson, E., Baun, A., Scholes, L., Ledin, A., Ahlman, S., Revitt, M., Noutsopoulos, C., Mikkelsen, P.S., 2007. Selected stormwater priority pollutants — a European perspective. Science of The Total Environment 383, 41–51. https://doi.org/10.1016/j.scitotenv.2007.05.028

Espinosa Bosch, M., Ruiz Sánchez, A.J., Sánchez Rojas, F., Bosch Ojeda, C., 2008. Recent developments in analytical determination of furosemide. Journal of Pharmaceutical and Biomedical Analysis 48, 519–532. https://doi.org/10.1016/j.jpba.2008.07.003

Estrada-Arriaga, E.B., Cortés-Muñoz, J.E., González-Herrera, A., Calderón-Mólgora, C.G., de Lourdes Rivera-Huerta, Ma., Ramírez-Camperos, E., Montellano-Palacios, L., Gelover-Santiago, S.L., Pérez-Castrejón, S., Cardoso-Vigueros, L., Martín-Domínguez, A., García-Sánchez, L., 2016. Assessment of full-scale biological nutrient removal systems upgraded with physico-chemical processes for the removal of emerging pollutants present in wastewaters from Mexico. Science of The Total Environment 571, 1172–1182. https://doi.org/10.1016/j.scitotenv.2016.07.118

Falås, P., Andersen, H.R., Ledin, A., Jansen, J. la C., 2012. Occurrence and reduction of pharmaceuticals in the water phase at Swedish wastewater treatment plants. Water Science and Technology 66, 783–791. https://doi.org/10.2166/wst.2012.243

Fantuzzi, G., Aggazzotti, G., Righi, E., Predieri, G., Castiglioni, S., Riva, F., Zuccato, E., 2018. Illicit drugs and pharmaceuticals in swimming pool waters. Science of The Total Environment 635, 956–963. https://doi.org/10.1016/j.scitotenv.2018.04.155

Fent, K., Escher, C., Caminada, D., 2006. Estrogenic activity of pharmaceuticals and pharmaceutical mixtures in a yeast reporter gene system. Reproductive Toxicology 22, 175–185. https://doi.org/10.1016/j.reprotox.2006.04.010

Feo, M.L., Bagnati, R., Passoni, A., Riva, F., Salvagio Manta, D., Sprovieri, M., Traina, A., Zuccato, E., Castiglioni, S., 2020. Pharmaceuticals and other contaminants in waters and sediments from Augusta Bay (southern Italy). Science of The Total Environment 739, 139827. https://doi.org/10.1016/j.scitotenv.2020.139827

Fernandez, D.L., 2018. Fungal treatment for the elimination of pharmaceutical compounds from wastewater: chemical, microbiological and ecotoxicological evaluation. Barcelona, Bellaterra.

Ferrari, F., Gallipoli, A., Balderacchi, M., Ulaszewska, M.M., Capri, E., Trevisan, M., 2011. Exposure of the Main Italian River Basin to Pharmaceuticals. Journal of Toxicology 2011, 1–11. https://doi.org/10.1155/2011/989270

Finckh, S., Beckers, L.-M., Busch, W., Carmona, E., Dulio, V., Kramer, L., Krauss, M., Posthuma, L., Schulze, T., Slootweg, J., Von der Ohe, P.C., Brack, W., 2022. A risk based assessment approach for chemical mixtures from wastewater treatment plant effluents. Environment International 164, 107234. https://doi.org/10.1016/j.envint.2022.107234

Flek, J., Sedivěc, V., 1978. The Absorption, metabolism and excretion of furfural in man. Int. Arch Occup Environ Heath 41, 159–168. https://doi.org/10.1007/BF00572888

Fratiglioni, L., De Ronchi, D., Agüero Torres, H., 1999. Worldwide Prevalence and Incidence of Dementia. Drugs & Aging 15, 365–375. https://doi.org/10.2165/00002512-199915050-00004

Fries, E., Mahjoub, O., Mahjoub, B., Berrehouc, A., Lions, J., Bahadir, M., n.d. OCCURRENCE OF CONTAMINANTS OF EMERGING CONCERN (CEC) IN CONVENTIONAL AND NON- CONVENTIONAL WATER RESOURCES IN TUNISIA. Fresenius Environmental Bulletin 25.

Giebułtowicz, J., Stankiewicz, A., Wroczyński, P., Nałęcz-Jawecki, G., 2016. Occurrence of cardiovascular drugs in the sewage-impacted Vistula River and in tap water in the Warsaw region (Poland). Environ Sci Pollut Res 24337–24349. https://doi.org/DOI 10.1007/s11356-016-7668-z

Gillard, N., Detry, B., Robert, C., Nonet, S., Samou, Y., Moise, E., Bauwens, F., Delahaut, P., 2014. Detection of pharmaceutical products in untreated hospital wastewater. Quality Assurance and Safety of Crops & Foods 6, 459–467. https://doi.org/10.3920/QAS2012.0177

Ginebreda, A., Jelić, A., Petrović, M., López de Alda, M., Barceló, D., 2012. New indexes for compound prioritization and complexity quantification on environmental monitoring inventories. Environ Sci Pollut Res 19, 958–970. https://doi.org/10.1007/s11356-011-0557-6

Godoy, A.A., Kummrow, F., Pamplin, P.A.Z., 2015. Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment - A review. Chemosphere 138, 281–291. https://doi.org/10.1016/j.chemosphere.2015.06.024

Gómez, M.J., Martínez Bueno, M.J., Agüera, A., Hernando, M.D., Fernández-Alba, A.R., Mezcua, M., 2008. Evaluation of ozone-based treatment processes for wastewater containing microcontaminants using LC-QTRAP-MS and LC-TOF/MS. Water Science and Technology 57, 41–48. https://doi.org/10.2166/wst.2008.809

Gómez-Canela, C., Pueyo, V., Barata, C., Lacorte, S., Marcé, R.M., 2019. Development of predicted environmental concentrations to prioritize the occurrence of pharmaceuticals in rivers from Catalonia. Science of The Total Environment 666, 57–67. https://doi.org/10.1016/j.scitotenv.2019.02.078

Gracia-Lor, E., Martínez, M., Sancho, J.V., Peñuela, G., Hernández, F., 2012. Multi-class determination of personal care products and pharmaceuticals in environmental and wastewater samples by ultra-high performance liquid-chromatography-tandem mass spectrometry. Talanta 99, 1011–1023. https://doi.org/10.1016/j.talanta.2012.07.091

Grandcoin, A., Piel, S., Baurès, E., 2017. AminoMethylPhosphonic acid (AMPA) in natural waters: Its sources, behavior and environmental fate. Water Research 117, 187–197. https://doi.org/10.1016/j.watres.2017.03.055

Gros, M., Ahrens, L., Levén, L., Koch, A., Dalahmeh, S., Ljung, E., Lundin, G., Jönsson, H., Eveborn, D., Wiberg, K., 2020. Pharmaceuticals in source separated sanitation systems: Fecal sludge and blackwater treatment. Science of The Total Environment 703, 135530. https://doi.org/10.1016/j.scitotenv.2019.135530

Gros, M., Blum, K.M., Jernstedt, H., Renman, G., Rodríguez-Mozaz, S., Haglund, P., Andersson, P.L., Wiberg, K., Ahrens, L., 2017. Screening and prioritization of micropollutants in wastewaters from on-site sewage treatment facilities. Journal of Hazardous Materials 328, 37–45. https://doi.org/10.1016/j.jhazmat.2016.12.055

Gros, M., Petrović, M., Barceló, D., 2009. Tracing Pharmaceutical Residues of Different Therapeutic Classes in Environmental Waters by Using Liquid Chromatography/Quadrupole-Linear Ion Trap Mass Spectrometry and Automated Library Searching. Anal. Chem. 81, 898–912. https://doi.org/10.1021/ac801358e

Gros, M., Petrović, M., Ginebreda, A., Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environment International 36, 15–26. https://doi.org/10.1016/j.envint.2009.09.002

Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. Journal of Chromatography A 1248, 104–121. https://doi.org/10.1016/j.chroma.2012.05.084

Grosse, Y., Loomis, D., Guyton, K.Z., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Mattock, H., Straif, K., 2017. Some chemicals that cause tumours of the urinary tract in rodents. The Lancet Oncology 18, 1003–1004. https://doi.org/10.1016/S1470-2045(17)30505-3

Guo, J., 2015. Impact of pharmaceuticals on algal species. University of York.

Guo, J., Boxall, A., Selby, K., 2015. Do Pharmaceuticals Pose a Threat to Primary Producers? Critical Reviews in Environmental Science and Technology 45, 2565–2610. https://doi.org/10.1080/10643389.2015.1061873

Haglund, M.M., Hochman, D.W., 2005. Furosemide and Mannitol Suppression of Epileptic Activity in the Human Brain. Journal of Neurophysiology 94, 907–918. https://doi.org/10.1152/jn.00944.2004

Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E., 1998. Occurrence, fate and effects of pharmaceutical substances in the environment- A review. Chemosphere 36, 357–393. https://doi.org/10.1016/S0045-6535(97)00354-8

Hammarlund-Udenaes, M., Benet, L.Z., 1989. Furosemide pharmacokinetics and pharmacodynamics in health and disease—An update. Journal of Pharmacokinetics and Biopharmaceutics 17, 1–46. https://doi.org/10.1007/BF01059086

Hanamoto, S., Kawakami, T., Nakada, N., Yamashita, N., Tanaka, H., 2014. Evaluation of the photolysis of pharmaceuticals within a river by 2 year field observations and toxicity changes by sunlight. Environ. Sci.: Processes Impacts 16, 2796–2803. https://doi.org/10.1039/C4EM00448E

Hanamoto, S., Nakada, N., Yamashita, N., Tanaka, H., 2018. Source estimation of pharmaceuticals based on catchment population and in-stream attenuation in Yodo River watershed, Japan. Science of The Total Environment 615, 964–971. https://doi.org/10.1016/j.scitotenv.2017.10.013

Hanisch, B., Abbas, B., Kratz, W., 2002. Ökotoxikologische Bewertung von Humanarzneimitteln in aquatischen Ökosystemen, Landesumweltamt Brandenburg, Potsdam, Germany (No. 39). landesumweltamt brandenburg.

Heberer, T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicology Letters 131, 5–17. https://doi.org/10.1016/S0378-4274(02)00041-3

Heidari, Z., Alizadeh, R., Ebadi, A., Oturan, N., Oturan, M.A., 2020. Efficient photocatalytic degradation of furosemide by a novel sonoprecipited ZnO over ion exchanged clinoptilolite nanorods. Separation and Purification Technology 242, 116800. https://doi.org/10.1016/j.seppur.2020.116800

Hignite, C., Azarnoff, D.L., 1977. Drugs and drug metabolites as environmental contaminants : chlorophenoxyisobutyrate and salicylic acid in sewage water effluent. Life Sciences 20, 337–342.

Hinchcliff, K.W., Muir, W.W., 1991. Pharmacology of Furosemide in the Horse: A Review. Journal of Veterinary Internal Medicine 5, 211–218. https://doi.org/10.1111/j.1939-1676.1991.tb00951.x

Huber, S., Remberger, M., Kaj, L., Schlabach, M., Jörundsdóttir, H.Ó., Vester, J., Arnórsson, M., Mortensen, I., Schwartson, R., Dam, M., 2016. A first screening and risk assessment of pharmaceuticals and additives in personal care products in waste water, sludge, recipient water and sediment from Faroe Islands, Iceland and Greenland. Science of The Total Environment 562, 13–25. https://doi.org/10.1016/j.scitotenv.2016.03.063

Huerta, B., Rodriguez-Mozaz, S., Nannou, C., Nakis, L., Ruhí, A., Acuña, V., Sabater, S., Barcelo, D., 2016. Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river. Science of The Total Environment 540, 241–249. https://doi.org/10.1016/j.scitotenv.2015.05.049

Huerta-Fontela, M., Galceran, M.T., Ventura, F., 2011. Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. Water Research 45, 1432–1442. https://doi.org/10.1016/j.watres.2010.10.036

Hughes, S.R., Kay, P., Brown, L.E., 2013. Global Synthesis and Critical Evaluation of Pharmaceutical Data Sets Collected from River Systems. Environ. Sci. Technol. 47, 661–677. https://doi.org/10.1021/es3030148

Huschek, G., Hansen, P.D., Maurer, H.H., Krengel, D., Kayser, A., 2004. Environmental risk assessment of medicinal products for human use according to European Commission recommendations. Environ. Toxicol. 19, 226–240. https://doi.org/10.1002/tox.20015

Ibáñez, M., Gracia-Lor, E., Bijlsma, L., Morales, E., Pastor, L., Hernández, F., 2013. Removal of emerging contaminants in sewage water subjected to advanced oxidation with ozone. Journal of Hazardous Materials 260, 389–398. https://doi.org/10.1016/j.jhazmat.2013.05.023

Idder, S., Ley, L., Mazellier, P., Budzinski, H., 2013. Quantitative on-line preconcentration-liquid chromatography coupled with tandem mass spectrometry method for the determination of pharmaceutical compounds in water. Analytica Chimica Acta 805, 107–115. https://doi.org/10.1016/j.aca.2013.10.041

Ikonen, J., Nuutinen, I., Niittynen, M., Hokajärvi, A.-M., Pitkänen, T., Antikainen, E., Miettinen, I.T., 2021. Presence and Reduction of Anthropogenic Substances with UV Light and Oxidizing Disinfectants in Wastewater—A Case Study at Kuopio, Finland. Water 13, 360. https://doi.org/10.3390/w13030360

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L., Parrella, A., 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Science of The Total Environment 346, 87–98. https://doi.org/10.1016/j.scitotenv.2004.11.017

Isidori, M., Nardelli, A., Parrella, A., Pascarella, L., Previtera, L., 2006. A multispecies study to assess the toxic and genotoxic effect of pharmaceuticals: Furosemide and its photoproduct. Chemosphere 63, 785–793. https://doi.org/10.1016/j.chemosphere.2005.07.078

Ivanova, S., Ivanov, K., 2019. Undeclared furosemide in food supplements. Biomedical Research 30, 733–737. https://doi.org/10.35841/biomedicalresearch.30-19-314

Jacobsen, B.N., Kjersgaard, D., Winther-Nielsen, M., Gustavson, K., 2004. Combined chemical analyses and biomonitoring at Avedoere wastewater treatment plant in 2002. Water Science and Technology 50, 37–43. https://doi.org/10.2166/wst.2004.0306

Jakimska, A., Śliwka-Kaszyńska, M., Reszczyńska, J., Namieśnik, J., Kot-Wasik, A., 2014. Elucidation of transformation pathway of ketoprofen, ibuprofen, and furosemide in surface water and their occurrence in the aqueous environment using UHPLC-QTOF-MS. Anal Bioanal Chem 406, 3667–3680. https://doi.org/10.1007/s00216-014-7614-1

Jelic, A., Fatone, F., Di Fabio, S., Petrovic, M., Cecchi, F., Barcelo, D., 2012. Tracing pharmaceuticals in a municipal plant for integrated wastewater and organic solid waste treatment. Science of The Total Environment 433, 352–361. https://doi.org/10.1016/j.scitotenv.2012.06.059

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., Barcelo, D., 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Research 45, 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010

Jelić, A., Petrović, M., Barceló, D., 2009. Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. Talanta 80, 363–371. https://doi.org/10.1016/j.talanta.2009.06.077

Jie, L., 2012. Potential of advanced wastewater treatment processes to remove pharmaceutical compounds under PILLS/SLIK Project.

Joannis-Cassan, C., Rodriguez Castillo, A.S., Dezani, C., Gómez-Canela, C., Reoyo-Prats, B., Calas-Blanchard, C., Barata, C., Lacorte, S., Plantard, G., 2021. Towards an innovative combined process coupling biodegradation and photo-oxidation for the removal of pharmaceutical residues. J Chem Technol Biotechnol 96, 755–763. https://doi.org/10.1002/jctb.6589

Junwon, P., 2016. Removal Characteristics and Predictive Model of Pharmaceutical and Personal Care Products (PPCPs) in Membrane Bioreactor (MBR) Process. https://doi.org/10.14989/doctor.k19984

Jurado, A., Vàzquez-Suñé, E., Carrera, J., López de Alda, M., Pujades, E., Barceló, D., 2012. Emerging organic contaminants in groundwater in Spain: A review of sources, recent occurrence and fate in a European context. Science of The Total Environment 440, 82–94. https://doi.org/10.1016/j.scitotenv.2012.08.029

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 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 Research 43, 363–380. https://doi.org/10.1016/j.watres.2008.10.047

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008a. Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography–electrospray tandem mass spectrometry. Anal Bioanal Chem 391, 1293–1308. https://doi.org/10.1007/s00216-008-1854-x

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008b. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Research 42, 3498–3518. https://doi.org/10.1016/j.watres.2008.04.026

Katsura, S., Yamada, N., Nakashima, A., Shiraishi, S., Furuishi, T., Ueda, H., 2015. Identification of Furosemide Photodegradation Products in Water–Acetonitrile Mixture. Chem. Pharm. Bull. 63, 617–627. https://doi.org/10.1248/cpb.c15-00122

Khalaf, H., Salste, L., Karlsson, P., Ivarsson, P., Jass, J., Olsson, P.-E., 2009. In vitro analysis of inflammatory responses following environmental exposure to pharmaceuticals and inland waters. Science of The Total Environment 407, 1452–1460. https://doi.org/10.1016/j.scitotenv.2008.10.016

Khan, A.H., Khan, N.A., Ahmed, S., Dhingra, A., Singh, C.P., Khan, S.U., Mohammadi, A.A., Changani, F., Yousefi, M., Alam, S., Vambol, S., Vambol, V., Khursheed, A., Ali, I., 2020. Application of advanced oxidation processes followed by different treatment technologies for hospital wastewater treatment. Journal of Cleaner Production 269, 122411. https://doi.org/10.1016/j.jclepro.2020.122411

Kim, I., 2018. Removal of Residual Pharmaceuticals in a Secondary Effluent from a Sewage Treatment Plant by Ozonation. J Korean Soc Environ Eng 40, 487–494. https://doi.org/10.4491/KSEE.2018.40.12.487

Kim, M., Guerra, P., Shah, A., Parsa, M., Alaee, M., Smyth, S.A., 2014. Removal of pharmaceuticals and personal care products in a membrane bioreactor wastewater treatment plant. Water Science and Technology 69, 2221–2229. https://doi.org/10.2166/wst.2014.145

Kjølholt, J., Nielsen, P., Stuer-Lauridsen, F., 2003. Hormonforstyrrende stoffer og lægemidler i spildevand (No. Miljøprojekt n°799).

Klamerth, N., Malato, S., Agüera, A., Fernández-Alba, A., 2013. Photo-Fenton and modified photo-Fenton at neutral pH for the treatment of emerging contaminants in wastewater treatment plant effluents: A comparison. Water Research 47, 833–840. https://doi.org/10.1016/j.watres.2012.11.008

Klamerth, N., Malato, S., Agüera, A., Fernández-Alba, A., Mailhot, G., 2012. Treatment of Municipal Wastewater Treatment Plant Effluents with Modified Photo-Fenton As a Tertiary Treatment for the Degradation of Micro Pollutants and Disinfection. Environ. Sci. Technol. 46, 2885–2892. https://doi.org/10.1021/es204112d

Kleywegt, S., Payne, M., Ng, F., Fletcher, T., 2019. Environmental loadings of Active Pharmaceutical Ingredients from manufacturing facilities in Canada. Science of The Total Environment 646, 257–264. https://doi.org/10.1016/j.scitotenv.2018.07.240

Kleywegt, S., Pileggi, V., Lam, Y.M., Elises, A., Puddicomb, A., Purba, G., Di Caro, J., Fletcher, T., 2016. The contribution of pharmaceutically active compounds from healthcare facilities to a receiving sewage treatment plant in Canada: Contribution of pharmaceutically active compounds to STP effluent. Environ Toxicol Chem 35, 850–862. https://doi.org/10.1002/etc.3124

Komori, K., Suzuki, Y., Minamiyama, M., Harada, A., 2013. Occurrence of selected pharmaceuticals in river water in Japan and assessment of their environmental risk. Environ Monit Assess 185, 4529–4536. https://doi.org/10.1007/s10661-012-2886-4

Kosma, C.I., Lambropoulou, D.A., Albanis, T.A., 2014. Investigation of PPCPs in wastewater treatment plants in Greece: Occurrence, removal and environmental risk assessment. Science of The Total Environment 466–467, 421–438. https://doi.org/10.1016/j.scitotenv.2013.07.044

Kostich, M.S., Batt, A.L., Lazorchak, J.M., 2014. Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. Environmental Pollution 184, 354–359. https://doi.org/10.1016/j.envpol.2013.09.013

Kot-Wasik, A., Jakimska, A., Śliwka-Kaszyńska, M., 2016. Occurrence and seasonal variations of 25 pharmaceutical residues in wastewater and drinking water treatment plants. Environ Monit Assess 188, 661. https://doi.org/10.1007/s10661-016-5637-0

Kovalova, L., Siegrist, H., Singer, H., Wittmer, A., McArdell, C.S., 2012. Hospital Wastewater Treatment by Membrane Bioreactor: Performance and Efficiency for Organic Micropollutant Elimination. Environ. Sci. Technol. 46, 1536–1545. https://doi.org/10.1021/es203495d

Kovalova, L., Siegrist, H., von Gunten, U., Eugster, J., Hagenbuch, M., Wittmer, A., Moser, R., McArdell, C.S., 2013. Elimination of Micropollutants during Post-Treatment of Hospital Wastewater with Powdered Activated Carbon, Ozone, and UV. Environ. Sci. Technol. 47, 7899–7908. https://doi.org/10.1021/es400708w

Krakkó, D., Licul-Kucera, V., Záray, G., Mihucz, V.G., 2019. Single-run ultra-high performance liquid chromatography for quantitative determination of ultra-traces of ten popular active pharmaceutical ingredients by quadrupole time-of-flight mass spectrometry after offline preconcentration by solid phase extraction from drinking and river waters as well as treated wastewater. Microchemical Journal 148, 108–119. https://doi.org/10.1016/j.microc.2019.04.047

Kümmerer, K., 2001. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. Chemosphere 45, 957–969. https://doi.org/10.1016/S0045-6535(01)00144-8

Kuroda, K., Nakada, N., Hanamoto, S., Inaba, M., Katayama, H., Do, A.T., Nga, T.T.V., Oguma, K., Hayashi, T., Takizawa, S., 2015. Pepper mild mottle virus as an indicator and a tracer of fecal pollution in water environments: Comparative evaluation with wastewater-tracer pharmaceuticals in Hanoi, Vietnam. Science of The Total Environment 506–507, 287–298. https://doi.org/10.1016/j.scitotenv.2014.11.021

Küster, A., Adler, N., 2014. Pharmaceuticals in the environment: scientific evidence of risks and its regulation. Phil. Trans. R. Soc. B 369, 20130587. https://doi.org/10.1098/rstb.2013.0587

Kuzmanović, M., Ginebreda, A., Petrović, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. Science of The Total Environment 503–504, 289–299. https://doi.org/10.1016/j.scitotenv.2014.06.056

Lacey, 2008. Assessment of pharmaceutical residue levels in three Irish sewage treatment plants. Dublin City University, Ireland.

Lacey, C., Basha, S., Morrissey, A., Tobin, J.M., 2012. Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland. Environ Monit Assess 184, 1049–1062. https://doi.org/10.1007/s10661-011-2020-z

Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A., Tobin, J.M., 2008. An LC–MS method for the determination of pharmaceutical compounds in wastewater treatment plant influent and effluent samples. Talanta 75, 1089–1097. https://doi.org/10.1016/j.talanta.2008.01.011

Lacorte, S., Luis, S., Gómez-Canela, C., Sala-Comorera, T., Courtier, A., Roig, B., Oliveira-Brett, A.M., Joannis-Cassan, C., Aragonés, J.I., Poggio, L., Noguer, T., Lima, L., Barata, C., Calas-Blanchard, C., 2018.

Pharmaceuticals released from senior residences: occurrence and risk evaluation. Environ Sci Pollut Res 25, 6095–6106. https://doi.org/10.1007/s11356-017-9755-1

Lahet, J.J., Lenfant, F., Courderot-Masuyer, C., Ecarnot-Laubriet, E., Vergely, C., Durnet-Archeray, M.J., Freysz, M., Rochette, L., 2003. In vivo and in vitro antioxidant properties of furosemide. Life Sciences 73, 1075–1082. https://doi.org/10.1016/S0024-3205(03)00382-5

Läkemedelsrester I Stockholms vattenmiljö-Förekomst, förebyggande atgärder och rening av avloppsvatten, 2010. Stockholm Vatten Repport.

Langford, K.H., Thomas, K.V., 2009. Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works. Environment International 35, 766–770. https://doi.org/10.1016/j.envint.2009.02.007

Lara-Martín, P.A., González-Mazo, E., Petrovic, M., Barceló, D., Brownawell, B.J., 2014. Occurrence, distribution and partitioning of nonionic surfactants and pharmaceuticals in the urbanized Long Island Sound Estuary (NY). Marine Pollution Bulletin 85, 710–719. https://doi.org/10.1016/j.marpolbul.2014.01.022

Laurencé, C., Rivard, M., Lachaise, I., Bensemhoun, J., Martens, T., 2011. Preparative access to transformation products (TPs) of furosemide: a versatile application of anodic oxidation. Tetrahedron 67, 9518–9521. https://doi.org/10.1016/j.tet.2011.10.006

Laurencé, C., Rivard, M., Martens, T., Morin, C., Buisson, D., Bourcier, S., Sablier, M., Oturan, M.A., 2014. Anticipating the fate and impact of organic environmental contaminants: A new approach applied to the pharmaceutical furosemide 7. http://dx.doi.org/10.1016/j.chemosphere.2014.05.036

Laurencé, C., Zeghbib, N., Rivard, M., Lehri-Boufala, S., Lachaise, I., Barau, C., Le Corvoisier, P., Martens, T., Garrigue-Antar, L., Morin, C., 2019. A new human pyridinium metabolite of furosemide, inhibitor of mitochondrial complex I, is a candidate inducer of neurodegeneration. Biochemical Pharmacology 160, 14–23. https://doi.org/10.1016/j.bcp.2018.12.007

Lee, S., 2014. Source, occurrence and modeling of pharmaceuticals, personal care products and estrogens on the Geongan river basin in Korea. Kyoto University, Japan.

Lee, Y., Kovalova, L., McArdell, C.S., von Gunten, U., 2014. Prediction of micropollutant elimination during ozonation of a hospital wastewater effluent. Water Research 64, 134–148. https://doi.org/10.1016/j.watres.2014.06.027

Legradi, J.B., Di Paolo, C., Kraak, M.H.S., van der Geest, H.G., Schymanski, E.L., Williams, A.J., Dingemans, M.M.L., Massei, R., Brack, W., Cousin, X., Begout, M.-L., van der Oost, R., Carion, A., Suarez-Ulloa, V., Silvestre, F., Escher, B.I., Engwall, M., Nilén, G., Keiter, S.H., Pollet, D., Waldmann, P., Kienle, C., Werner, I., Haigis, A.-C., Knapen, D., Vergauwen, L., Spehr, M., Schulz, W., Busch, W., Leuthold, D., Scholz, S., vom Berg, C.M., Basu, N., Murphy, C.A., Lampert, A., Kuckelkorn, J., Grummt, T., Hollert, H., 2018. An ecotoxicological view on neurotoxicity assessment. Environ Sci Eur 30, 46. https://doi.org/10.1186/s12302-018-0173-x

Lesser, L.E., Mora, A., Moreau, C., Mahlknecht, J., Hernández-Antonio, A., Ramírez, A.I., Barrios-Piña, H., 2018. Survey of 218 organic contaminants in groundwater derived from the world's largest untreated wastewater irrigation system: Mezquital Valley, Mexico. Chemosphere 198, 510–521. https://doi.org/10.1016/j.chemosphere.2018.01.154

Li, Y., Zhang, L., Liu, X., Ding, J., 2019. Ranking and prioritizing pharmaceuticals in the aquatic environment of China. Science of The Total Environment 658, 333–342. https://doi.org/10.1016/j.scitotenv.2018.12.048

Li, Z., Maier, M.P., Radke, M., 2014. Screening for pharmaceutical transformation products formed in river sediment by combining ultrahigh performance liquid chromatography/high resolution mass spectrometry with a rapid data-processing method. Analytica Chimica Acta 810, 61–70. https://doi.org/10.1016/j.aca.2013.12.012

Lindim, C., van Gils, J., Georgieva, D., Mekenyan, O., Cousins, I.T., 2016. Evaluation of human pharmaceutical emissions and concentrations in Swedish river basins. Science of The Total Environment 572, 508–519. https://doi.org/10.1016/j.scitotenv.2016.08.074

Llorens-Blanch, G., Badia-Fabregat, M., Lucas, D., Rodriguez-Mozaz, S., Barceló, D., Pennanen, T., Caminal, G., Blánquez, P., 2015. Degradation of pharmaceuticals from membrane biological reactor sludge with Trametes versicolor. Environ. Sci.: Processes Impacts 17, 429–440. https://doi.org/10.1039/C4EM00579A

López-Serna, R., Jurado, A., Vázquez-Suñé, E., Carrera, J., Petrović, M., Barceló, D., 2013. Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metropolis of Barcelona, Spain. Environmental Pollution 174, 305–315. https://doi.org/10.1016/j.envpol.2012.11.022

López-Serna, R., Pérez, S., Ginebreda, A., Petrović, M., Barceló, D., 2010. Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography-electrospray–tandem mass spectrometry. Talanta 83, 410–424. https://doi.org/10.1016/j.talanta.2010.09.046

López-Serna, R., Petrović, M., Barceló, D., 2011. Development of a fast instrumental method for the analysis of pharmaceuticals in environmental and wastewaters based on ultra high performance liquid chromatography (UHPLC)–tandem mass spectrometry (MS/MS). Chemosphere 85, 1390–1399. https://doi.org/10.1016/j.chemosphere.2011.07.071

Luiz, V.H.M., Pezza, L., Pezza, H.R., 2013. Rapid determination of furosemide by combined spot test/diffuse reflectance spectroscopy to detect doping in sport. Microchemical Journal 109, 68–72. https://doi.org/10.1016/j.microc.2012.04.002

Lumbaque, E.C., 2020. Degradation of pharmaceuticals in hospital wastewater by solar photo-Fenton processes. UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL.

Machado, A.I., Dordio, A., Fragoso, R., Leitão, A.E., Duarte, E., 2017. Furosemide removal in constructed wetlands: Comparative efficiency of LECA and Cork granulates as support matrix. Journal of Environmental Management 203, 422–428. https://doi.org/10.1016/j.jenvman.2017.08.002

Machado, A.I., Fragoso, R., Dordio, A.V., Duarte, E., 2020. Performance of *Iris pseudacorus* and *Typha domingensis* for furosemide removal in a hydroponic system. International Journal of Phytoremediation 22, 863–871. https://doi.org/10.1080/15226514.2020.1717431

Maculewicz, J., Kowalska, D., Świacka, K., Toński, M., Stepnowski, P., Białk-Bielińska, A., Dołżonek, J., 2022. Transformation products of pharmaceuticals in the environment: Their fate, (eco)toxicity and bioaccumulation potential. Science of The Total Environment 802, 149916. https://doi.org/10.1016/j.scitotenv.2021.149916

Mackuľak, T., Grabic, R., Špalková, S., Belišová, N., Škulcová, A., Slavík, O., Horký, P., Gál, M., Filip, J., Híveš, J., Vojs Staňová, M., Medveďová, A., Marton, M., Birošová, L., 2019. Hospital wastewaters treatment: Fenton reaction vs. BDDE vs. ferrate(VI). Environmental Science and Pollution Research 31812–31821. https://doi.org/10.1007/s11356-019-06290-9

Mandalika, A., Qin, L., Sato, T.K., Runge, T., 2014. Integrated biorefinery model based on production of furans using open-ended high yield processes. Green Chem. 16, 2480–2489. https://doi.org/10.1039/C3GC42424C

Mansour, F., Al-Hindi, M., Saad, W., Salam, D., 2016. Environmental risk analysis and prioritization of pharmaceuticals in a developing world context. Science of The Total Environment 557–558, 31–43. https://doi.org/10.1016/j.scitotenv.2016.03.023

Martínez Bueno, M.J., Agüera, A., Gómez, M.J., Hernando, M.D., García-Reyes, J.F., Fernández-Alba, A.R., 2007. Application of Liquid Chromatography/Quadrupole-Linear Ion Trap Mass Spectrometry and Time-of-Flight Mass Spectrometry to the Determination of Pharmaceuticals and Related Contaminants in Wastewater. Anal. Chem. 79, 9372–9384. https://doi.org/10.1021/ac0715672

Martínez-Piernas, A.B., Plaza-Bolaños, P., Fernández-Ibáñez, P., Agüera, A., 2019. Organic Microcontaminants in Tomato Crops Irrigated with Reclaimed Water Grown under Field Conditions: Occurrence, Uptake, and Health Risk Assessment. J. Agric. Food Chem. 67, 6930–6939. https://doi.org/10.1021/acs.jafc.9b01656

Marube, L., Caldas, S., dos Santos, E., Michaelsen, A., Primel, E., 2017. Multi-Residue Method for Determination of Thirty-Five Pesticides, Pharmaceuticals and Personal Care Products in Water Using Ionic Liquid-Dispersive Liquid-Liquid Microextraction Combined with Liquid Chromatography-Tandem Mass Spectrometry. J. Braz. Chem. Soc. https://doi.org/10.21577/0103-5053.20170234

Matamoros, V., Arias, C.A., Nguyen, L.X., Salvadó, V., Brix, H., 2012. Occurrence and behavior of emerging contaminants in surface water and a restored wetland. Chemosphere 88, 1083–1089. https://doi.org/10.1016/j.chemosphere.2012.04.048

Matamoros, V., Jover, E., Bayona, J.M., 2010. Part-per-Trillion Determination of Pharmaceuticals, Pesticides, and Related Organic Contaminants in River Water by Solid-Phase Extraction Followed by Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry. Anal. Chem. 82, 699–706. https://doi.org/10.1021/ac902340e

Meador, J.P., Yeh, A., Young, G., Gallagher, E.P., 2016. Contaminants of emerging concern in a large temperate estuary. Environmental Pollution 213, 254–267. https://doi.org/10.1016/j.envpol.2016.01.088

Meffe, R., de Bustamante, I., 2014. Emerging organic contaminants in surface water and groundwater: A first overview of the situation in Italy. Science of The Total Environment 481, 280–295. https://doi.org/10.1016/j.scitotenv.2014.02.053

Miklos, D.B., Remy, C., Jekel, M., Linden, K.G., Drewes, J.E., Hübner, U., 2018. Evaluation of advanced oxidation processes for water and wastewater treatment – A critical review. Water Research 139, 118–131. https://doi.org/10.1016/j.watres.2018.03.042

Mir-Tutusaus, J.A., Parladé, E., Llorca, M., Villagrasa, M., Barceló, D., Rodriguez-Mozaz, S., Martinez-Alonso, M., Gaju, N., Caminal, G., Sarrà, M., 2017. Pharmaceuticals removal and microbial community assessment in a continuous fungal treatment of non-sterile real hospital wastewater after a coagulation-flocculation pretreatment. Water Research 116, 65–75. https://doi.org/10.1016/j.watres.2017.03.005

Mitchell, J.R., Nelson, W.L., Potter, W.Z., Sasame, H.A., Jollow, J.D., 1976. Metabolic activation of furosemide to a chemically reactive, hepatotoxic metabolite. The journal of pharmacology and experimental therapeutics 199, 41–52.

Mizuma, T., Benet, L.Z., Lin, E.T., 1998. High-performance liquid chromatographic determination and identification of acyl migration and photodegradation products of furosemide 1-O-acyl glucuronide. Journal of Chromatography B: Biomedical Sciences and Applications 718, 153–162. https://doi.org/10.1016/S0378-4347(98)00357-0

Mogensen, B., Bossi, B., Kjær, J., Juhler, R., Boutrup, S., 2008. NOVANA-Screeningsundersøgelse af det akvatiske miljø, Lægemidler og triclosan i punktkilder og vandmiljøet (No. Faglig rapport fra DMU nr. 638, 2008).

Mondal, S.C., Tripathi, D.N., Vikram, A., Ramarao, P., Jena, G.B., 2012. Furosemide-induced genotoxicity and cytotoxicity in the hepatocytes, but weak genotoxicity in the bone marrow cells of mice: Furosemide-induced genotoxicity and cytotoxicity in mice. Fundamental & Clinical Pharmacology 26, 383–392. https://doi.org/10.1111/j.1472-8206.2011.00927.x

Moreno-González, R., Rodríguez-Mozaz, S., Gros, M., Pérez-Cánovas, E., Barceló, D., León, V.M., 2014. Input of pharmaceuticals through coastal surface watercourses into a Mediterranean lagoon (Mar Menor, SE Spain): Sources and seasonal variations. Science of The Total Environment 490, 59–72. https://doi.org/10.1016/j.scitotenv.2014.04.097

Møskeland, T., Kelley, A., Sollie, O., Hansen, N., Køppen, B., Heier, L., 2006. Mapping of selected substances in pharmaceuticals and cosmetics. Deliveries and current state. (No. Rapport 949/2006).

Muñoz, I., Rodríguez, A., Rosal, R., Fernández-Alba, A.R., 2009. Life Cycle Assessment of urban wastewater reuse with ozonation as tertiary treatment. Science of The Total Environment 407, 1245–1256. https://doi.org/10.1016/j.scitotenv.2008.09.029

Munro, K., Martins, C.P.B., Loewenthal, M., Comber, S., Cowan, D.A., Pereira, L., Barron, L.P., 2019. Evaluation of combined sewer overflow impacts on short-term pharmaceutical and illicit drug occurrence in a heavily urbanised tidal river catchment (London, UK). Science of The Total Environment 657, 1099–1111. https://doi.org/10.1016/j.scitotenv.2018.12.108

Murray, M.D., Haag, K.D., Black, P.D., Hall, S.D., Brater, C., 1997. Variable Furosemide Absorption and Poor Predictability of Response in Elderly Patients. General clinical research center at an academic medical center.

Nagarnaik, P., Batt, A., Boulanger, B., 2010. Concentrations and mass loadings of cardiovascular pharmaceuticals in healthcare facility wastewaters. J. Environ. Monit. 12, 2112. https://doi.org/10.1039/c0em00216j

Nakada, N., Komori, K., Suzuki, Y., Konishi, C., Houwa, I., Tanaka, H., 2007. Occurrence of 70 pharmaceutical and personal care products in Tone River basin in Japan. Water Science and Technology 56, 133–140. https://doi.org/10.2166/wst.2007.801

Narumiya, M., Nakada, N., Yamashita, N., Tanaka, H., 2013. Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. Journal of Hazardous Materials 260, 305–312. https://doi.org/10.1016/j.jhazmat.2013.05.032

Nelson, E.D., Do, H., Lewis, R.S., Carr, S.A., 2011. Diurnal Variability of Pharmaceutical, Personal Care Product, Estrogen and Alkylphenol Concentrations in Effluent from a Tertiary Wastewater Treatment Facility. Environ. Sci. Technol. 45, 1228–1234. https://doi.org/10.1021/es102452f

Nielsen, U., Hastrup, C., Klausen, M.M., Pedersen, B.M., Kristensen, G.H., Jansen, J.L.C., Bak, S.N., Tuerk, J., 2013. Removal of APIs and bacteria from hospital wastewater by MBR plus O3, O3 + H2O2, PAC or ClO2. Water Science and Technology 67, 854–862. https://doi.org/10.2166/wst.2012.645

Nordic Council of Ministers, 2012. PPCP monitoring in the Nordic Countries – Status Report. Nordic Council of Ministers. https://doi.org/10.6027/TN2012-519

Oliveira, T.S., Murphy, M., Mendola, N., Wong, V., Carlson, D., Waring, L., 2015. Characterization of Pharmaceuticals and Personal Care products in hospital effluent and waste water influent/effluent by direct-injection LC-MS-MS. Science of The Total Environment 518–519, 459–478. https://doi.org/10.1016/j.scitotenv.2015.02.104

Olvera-Vargas, H., Leroy, S., Rivard, M., Oturan, N., Oturan, M., Buisson, D., 2016. Microbial biotransformation of furosemide for environmental risk assessment: identification of metabolites and toxicological evaluation. Environ Sci Pollut Res 23, 22691–22700. https://doi.org/10.1007/s11356-016-7398-2

O'Neil, M., 2013. The Merck index : an encyclopedia of chemicals, drugs, and biologicals., 15th ed. Cambridge, UK : Royal Society of Chemistry.

Ort, C., Lawrence, M.G., Reungoat, J., Eaglesham, G., Carter, S., Keller, J., 2010. Determining the fraction of pharmaceutical residues in wastewater originating from a hospital. Water Research 44, 605–615. https://doi.org/10.1016/j.watres.2009.08.002

Osorio, V., Marcé, R., Pérez, S., Ginebreda, A., Cortina, J.L., Barceló, D., 2012. Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions. Science of The Total Environment 440, 3–13. https://doi.org/10.1016/j.scitotenv.2012.08.040

Osorio, V., Proia, L., Ricart, M., Pérez, S., Ginebreda, A., Cortina, J.L., Sabater, S., Barceló, D., 2014. Hydrological variation modulates pharmaceutical levels and biofilm responses in a Mediterranean river. Science of The Total Environment 472, 1052–1061. https://doi.org/10.1016/j.scitotenv.2013.11.069

Osunmakinde, C.S., South Africa, Water Research Commission, 2013. Verification and validation of analytical methods for testing the levels of PPHCPs (pharmaceutical & personal health care products) in treated drinking water and sewage: report to the Water Research Commission.

Palma, P., Fialho, S., Lima, A., Novais, M.H., Costa, M.J., Montemurro, N., Pérez, S., de Alda, M.L., 2020. Pharmaceuticals in a Mediterranean Basin: The influence of temporal and hydrological patterns in environmental risk assessment. Science of The Total Environment 709, 136205. https://doi.org/10.1016/j.scitotenv.2019.136205

Papageorgiou, M., Kosma, C., Lambropoulou, D., 2016. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. Science of The Total Environment 543, 547–569. https://doi.org/10.1016/j.scitotenv.2015.11.047

Papageorgiou, M., Zioris, I., Danis, T., Bikiaris, D., Lambropoulou, D., 2019. Comprehensive investigation of a wide range of pharmaceuticals and personal care products in urban and hospital wastewaters in Greece. Science of The Total Environment 694, 133565. https://doi.org/10.1016/j.scitotenv.2019.07.371

Park, J., Yamashita, N., Park, C., Shimono, T., Takeuchi, D.M., Tanaka, H., 2017. Removal characteristics of pharmaceuticals and personal care products: Comparison between membrane bioreactor and various biological treatment processes. Chemosphere 179, 347–358. https://doi.org/10.1016/j.chemosphere.2017.03.135

Pascoe, D., Karntanut, W., Müller, C.T., 2003. Do pharmaceuticals affect freshwater invertebrates? A study with the cnidarian Hydra vulgaris. Chemosphere 51, 521–528. https://doi.org/10.1016/S0045-6535(02)00860-3

Pérez, G., Fernández-Alba, A.R., Urtiaga, A.M., Ortiz, I., 2010a. Electro-oxidation of reverse osmosis concentrates generated in tertiary water treatment. Water Research 44, 2763–2772. https://doi.org/10.1016/j.watres.2010.02.017

Peterson, L.A., 2013. Reactive Metabolites in the Biotransformation of Molecules Containing a Furan Ring. Chem. Res. Toxicol. 26, 6–25. https://doi.org/10.1021/tx3003824

Petre, J., Galaon, T., Iancu, V.I., Cruceru, L., Niculescu, M., 2016. Simultaneous liquid chromatography- tandem mass spectrometry determination of some pharmaceuticals and antimicrobial disinfectant agents in surface water and in urban wastewater. Journal of Environmental Protection and Ecology 17, 119–126.

Petrović, M., Škrbić, B., Živančev, J., Ferrando-Climent, L., Barcelo, D., 2014. Determination of 81 pharmaceutical drugs by high performance liquid chromatography coupled to mass spectrometry with hybrid triple quadrupole–linear ion trap in different types of water in Serbia. Science of The Total Environment 468–469, 415–428. https://doi.org/10.1016/j.scitotenv.2013.08.079

Petrovic, M., Verlicchi, P., 2014. Water treatment plants and pharmaceutical residues in Catalonia and Italy. Contributions to Science 135–150. https://doi.org/10.2436/20.7010.01.198

Phakdeekitcharoen, B., Boonyawat, K., 2012. The added-up albumin enhances the diuretic effect of furosemide in patients with hypoalbuminemic chronic kidney disease: a randomized controlled study. BMC Nephrol 13, 92. https://doi.org/10.1186/1471-2369-13-92

Prandota, J., 2001. Clinical Pharmacology of Furosemide in Children: A Supplement: American Journal of Therapeutics 8, 275–289. https://doi.org/10.1097/00045391-200107000-00010

Prandota, J., Witkowska, M., 1976. Pharmacokinetics and metabolism of furosemide in man. European Journal of Drug Metabolism and Pharmacokinetics 1, 177–181. https://doi.org/10.1007/BF03189275

Prieto-Rodriguez, L., Miralles-Cuevas, S., Oller, I., Agüera, A., Puma, G.L., Malato, S., 2012. Treatment of emerging contaminants in wastewater treatment plants (WWTP) effluents by solar photocatalysis using low TiO2 concentrations. Journal of Hazardous Materials 211–212, 131–137. https://doi.org/10.1016/j.jhazmat.2011.09.008

Prieto-Rodríguez, L., Oller, I., Klamerth, N., Agüera, A., Rodríguez, E.M., Malato, S., 2013. Application of solar AOPs and ozonation for elimination of micropollutants in municipal wastewater treatment plant effluents. Water Research 47, 1521–1528. https://doi.org/10.1016/j.watres.2012.11.002

Reed, N.R., Kwok, E.S.C., 2014. Furfural, in: Encyclopedia of Toxicology. Elsevier, pp. 685–688. https://doi.org/10.1016/B978-0-12-386454-3.00147-0

Reungoat, J., Escher, B.I., Macova, M., Argaud, F.X., Gernjak, W., Keller, J., 2012. Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. Water Research 46, 863–872. https://doi.org/10.1016/j.watres.2011.11.064

Richardson, M.L., Bowron, J.M., 2011. The fate of pharmaceutical chemicals in the aquatic environment. Journal of Pharmacy and Pharmacology 37, 1–12. https://doi.org/10.1111/j.2042-7158.1985.tb04922.x

Rimoy, G., Justin-Temu, M., Nilay, C., 2009. Prescribing Patterns and Cost of Antihypertensive Drugs in Private Hospitals in Dar es Salaam, Tanzania. East and Central African Journal of Pharmaceutical Sciences 11, 69–73. https://doi.org/10.4314/ecajps.v11i3.46285

Riva, F., Zuccato, E., Castiglioni, S., 2015. Prioritization and analysis of pharmaceuticals for human use contaminating the aquatic ecosystem in Italy. Journal of Pharmaceutical and Biomedical Analysis 106, 71–78. https://doi.org/10.1016/j.jpba.2014.10.003

Riva, F., Zuccato, E., Pacciani, C., Colombo, A., Castiglioni, S., 2021. A multi-residue analytical method for extraction and analysis of pharmaceuticals and other selected emerging contaminants in sewage sludge. Anal. Methods 13, 526–535. https://doi.org/10.1039/D0AY02027C

Rizzi, V., Gubitosa, J., Fini, P., Romita, R., Nuzzo, S., Gabaldón, J.A., Gorbe, M.I.F., Gómez-Morte, T., Cosma, P., 2020. Chitosan film as recyclable adsorbent membrane to remove/recover hazardous pharmaceutical pollutants from water: the case of the emerging pollutant Furosemide. Journal of Environmental Science and Health, Part A 1–12. https://doi.org/10.1080/10934529.2020.1853985

Rocco, L., Frenzilli, G., Fusco, D., Peluso, C., Stingo, V., 2010. Evaluation of zebrafish DNA integrity after exposure to pharmacological agents present in aquatic environments. Ecotoxicology and Environmental Safety 73, 1530–1536. https://doi.org/10.1016/j.ecoenv.2010.07.032

Rodriguez-Cillero, C., Menu, D., d'Athis, P., Perrin, S., Dipanda, M., Asgassou, S., Guepet, H., Mazen, E., Manckoundia, P., Putot, A., 2017. Potentially inappropriate use of furosemide in a very elderly population: An observational study. Int J Clin Pract 71, e12975. https://doi.org/10.1111/ijcp.12975

Rodríguez-Navas, C., Björklund, E., Bak, S.A., Hansen, M., Krogh, K.A., Maya, F., Forteza, R., Cerdà, V., 2013. Pollution Pathways of Pharmaceutical Residues in the Aquatic Environment on the Island of Mallorca, Spain. Arch Environ Contam Toxicol 65, 56–66. https://doi.org/10.1007/s00244-013-9880-x

Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelić, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrović, M., Caminal, G., Barceló, D., Vicent, T., 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus Trametes versicolor in bioslurry reactor. Journal of Hazardous Materials 233–234, 235–243. https://doi.org/10.1016/j.jhazmat.2012.07.024

Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Adams, R.J., Berry, J.D., Brown, T.M., Carnethon, M.R., Dai, S., de Simone, G., Ford, E.S., Fox, C.S., Fullerton, H.J., Gillespie, C., Greenlund, K.J., Hailpern, S.M., Heit, J.A., Ho, P.M., Howard, V.J., Kissela, B.M., Kittner, S.J., Lackland, D.T., Lichtman, J.H., Lisabeth, L.D., Makuc, D.M., Marcus, G.M., Marelli, A., Matchar, D.B., McDermott, M.M., Meigs, J.B., Moy, C.S., Mozaffarian, D., Mussolino, M.E., Nichol, G., Paynter, N.P., Rosamond, W.D., Sorlie, P.D., Stafford, R.S., Turan, T.N., Turner, M.B., Wong, N.D., Wylie-Rosett, J., 2011. Heart Disease and Stroke Statistics—2011 Update: A Report From the American Heart Association. Circulation 123. https://doi.org/10.1161/CIR.0b013e3182009701

Roos, V., Gunnarsson, L., Fick, J., Larsson, D.G.J., Rudén, C., 2012. Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Science of The Total Environment 421–422, 102–110. https://doi.org/10.1016/j.scitotenv.2012.01.039

Rosal, R., Rodríguez, A., Perdigón-Melón, J.A., Petre, A., García-Calvo, E., Gómez, M.J., Agüera, A., Fernández-Alba, A.R., 2010. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Research 44, 578–588. https://doi.org/10.1016/j.watres.2009.07.004

Rostvall, A., 2017. Evaluation of sorption materials for the removal of organic micropollutants in domestic wastewater and their potential infiltration in groundwater. Uppsala University.

Rozman, D., Hrkal, Z., Váňa, M., Vymazal, J., Boukalová, Z., 2017. Occurrence of Pharmaceuticals in Wastewater and Their Interaction with Shallow Aquifers: A Case Study of Horní Beřkovice, Czech Republic. Water 9, 218. https://doi.org/10.3390/w9030218

Sadutto, D., Andreu, V., Ilo, T., Akkanen, J., Picó, Y., 2021. Pharmaceuticals and personal care products in a Mediterranean coastal wetland: Impact of anthropogenic and spatial factors and environmental risk assessment. Environmental Pollution 271, 116353. https://doi.org/10.1016/j.envpol.2020.116353

Salgado, R., Marques, R., Noronha, J.P., Mexia, J.T., Carvalho, G., Oehmen, A., Reis, M.A.M., 2011. Assessing the diurnal variability of pharmaceutical and personal care products in a full-scale activated sludge plant. Environmental Pollution 159, 2359–2367. https://doi.org/10.1016/j.envpol.2011.07.004

Salgado, R., Noronha, J.P., Oehmen, A., Carvalho, G., Reis, M.A.M., 2010. Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. Water Science and Technology 62, 2862–2871. https://doi.org/10.2166/wst.2010.985

Samoilenko, N., Yermakovych, I., 2014. Analysis of studies in the field of wastewater pollution by pharmaceutical contaminants. National Technical University «Kharkiv Polytechnic institute».

Sandre, F., Huynh, N., Caupos, E., El-Mrabet, L., Partibane, C., Lachaise, I., Pommier, C., Rivard, M., Morin, C., Moilleron, R., Le Roux, J., Garrigue-Antar, L., 2023. Occurrence and fate of an emerging drug pollutant and its by-products during conventional and advanced wastewater treatment: Case study of furosemide. Chemosphere 322, 138212. https://doi.org/10.1016/j.chemosphere.2023.138212

Sandré, F., Huynh, N., Gromaire, M.-C., Varrault, G., Morin, C., Moilleron, R., Le Roux, J., Garrigue-Antar, L., 2022. Road Runoff Characterization: Ecotoxicological Assessment Combined with (Non-)Target Screenings of Micropollutants for the Identification of Relevant Toxicants in the Dissolved Phase. Water 14, 511. https://doi.org/10.3390/w14040511

Santos, L.H.M.L.M., Gros, M., Rodriguez-Mozaz, S., Delerue-Matos, C., Pena, A., Barceló, D., Montenegro, M.C.B.S.M., 2013. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. Science of The Total Environment 461–462, 302–316. https://doi.org/10.1016/j.scitotenv.2013.04.077

Savarese, G., Lund, L.H., 2017. Global Public Health Burden of Heart Failure. Cardiac Failure Review 03, 7. https://doi.org/10.15420/cfr.2016:25:2

Schröder, H., Gebhardt, W., Thevis, M., 2010. Anabolic, doping, and lifestyle drugs, and selected metabolites in wastewater—detection, quantification, and behaviour monitored by high-resolution MS and MS n before and after sewage treatment. Anal Bioanal Chem 398, 1207–1229. https://doi.org/10.1007/s00216-010-3958-3

Schuster, A., Hädrich, C., Kümmerer, K., 2008. Flows of Active Pharmaceutical Ingredients Originating from Health Care Practices on a Local, Regional, and Nationwide Level in Germany—Is Hospital Effluent Treatment an Effective Approach for Risk Reduction? Water Air Soil Pollut: Focus 8, 457–471. https://doi.org/10.1007/s11267-008-9183-9

Sedlak, D.L., Pinkston, K., Huang, C.-H., 2005. Occurrence Survey of Pharmaceutically Active Compounds. American Water Works Association.

Sekikawa, H., Yagi, N., Lin, E.T., Benet, L.Z., 1995. Apparent intramolecular acyl migration and hydrolysis of furosemide glucuronide in aqueous solution. Biol pharm bull 18, 134–139.

Shalavadi, M., Chandrashekhar, V., Manohar, K., Nihar, K., Srikanth, D., 2018. A Prospective Study on Prescription Pattern of Antihypertensive Drugs and Drug Interactions at a Tertiary Care Teaching Hospital. Am J Pharmacol 1, 7.

Silva, B.F. da, Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85, 1331–1339. https://doi.org/10.1016/j.chemosphere.2011.07.051

Singh, A., Havixbeck, J.J., Smith, M.K., Shu, Z., Tierney, K.B., Barreda, D.R., El-Din, M.G., Belosevic, M., 2015. UV and hydrogen peroxide treatment restores changes in innate immunity caused by exposure of fish to reuse water. Water Research 71, 257–273. https://doi.org/10.1016/j.watres.2015.01.003

Sousa, M.A., Gonçalves, C., Cunha, E., Hajšlová, J., Alpendurada, M.F., 2011. Cleanup strategies and advantages in the determination of several therapeutic classes of pharmaceuticals in wastewater samples by SPE–LC–MS/MS. Anal Bioanal Chem 399, 807–822. https://doi.org/10.1007/s00216-010-4297-0

Sousa, M.A., Gonçalves, C., Vilar, V.J.P., Boaventura, R.A.R., Alpendurada, M.F., 2012. Suspended TiO2-assisted photocatalytic degradation of emerging contaminants in a municipal WWTP effluent using a solar pilot plant with CPCs. Chemical Engineering Journal 198–199, 301–309. https://doi.org/10.1016/j.cej.2012.05.060

Srinivasan, V.N., 2012. Developing a Watershed-Level Protocol for Choosing Indicator Compounds for EDCs/PPCPs Using Analytical Methods and Chemometrics. https://doi.org/10.7275/XVYM-NJ73

Stackelberg, P.E., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Henderson, A.K., Reissman, D.B., 2004. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. Science of The Total Environment 329, 99–113. https://doi.org/10.1016/j.scitotenv.2004.03.015

Stan, H.-J., Heberer, T., Linkerhägner, M., 1992. Occurrence of chlorofibric acid in the aquatic system: is their therapeutic use responsible for the loads found in surface, ground- and drinking water? Vom Wasser 83, 57–68.

Stankiewicz, A., Giebułtowicz, J., Stankiewicz, U., Wroczyński, P., Nałęcz-Jawecki, G., 2015. Determination of selected cardiovascular active compounds in environmental aquatic samples – Methods and results, a review of global publications from the last 10 years. Chemosphere 138, 642–656. https://doi.org/10.1016/j.chemosphere.2015.07.056

Starling, M.C.V.M., Souza, P.P., Le Person, A., Amorim, C.C., Criquet, J., 2019. Intensification of UV-C treatment to remove emerging contaminants by UV-C/H2O2 and UV-C/S2O82−: Susceptibility to photolysis and investigation of acute toxicity. Chemical Engineering Journal 376, 120856. https://doi.org/10.1016/j.cej.2019.01.135

Stokes, W., Nunn, L., 1964. A New Effective Diuretic -Lasix. British Medical Journal 2, 910–914.

Stuer-Lauridsen, F., Birkved, M., Hansen, L.P., Holten Lützhøft, H.-C., Halling-Sørensen, B., 2000. Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. Chemosphere 40, 783–793. https://doi.org/10.1016/S0045-6535(99)00453-1

Stumpf, M., Ternes, T.A., Wilken, R.-D., Silvana Vianna Rodrigues, Baumann, W., 1999. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. Science of The Total Environment 225, 135–141. https://doi.org/10.1016/S0048-9697(98)00339-8

Sui, Q., Cao, X., Lu, S., Zhao, W., Qiu, Z., Yu, G., 2015. Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: A review. Emerging Contaminants 1, 14–24. https://doi.org/10.1016/j.emcon.2015.07.001

Svahn, O., Björklund, E., 2015. Describing sorption of pharmaceuticals to lake and river sediments, and sewage sludge from UNESCO Biosphere Reserve Kristianstads Vattenrike by chromatographic asymmetry factors and recovery measurements. Journal of Chromatography A 1415, 73–82. https://doi.org/10.1016/j.chroma.2015.08.061

Świacka, K., Michnowska, A., Maculewicz, J., Caban, M., Smolarz, K., 2021. Toxic effects of NSAIDs in non-target species: A review from the perspective of the aquatic environment. Environmental Pollution 273, 115891. https://doi.org/10.1016/j.envpol.2020.115891

Tamura, I., Yasuda, Y., Kagota, K., Yoneda, S., Nakada, N., Kumar, V., Kameda, Y., Kimura, K., Tatarazako, N., Yamamoto, H., 2017. Contribution of pharmaceuticals and personal care products (PPCPs) to whole toxicity of water samples collected in effluent-dominated urban streams. Ecotoxicology and Environmental Safety 144, 338–350. https://doi.org/10.1016/j.ecoenv.2017.06.032

Teijon, G., Candela, L., Tamoh, K., Molina-Díaz, A., Fernández-Alba, A.R., 2010. Occurrence of emerging contaminants, priority substances (2008/105/CE) and heavy metals in treated wastewater and groundwater at Depurbaix facility (Barcelona, Spain). Science of The Total Environment 408, 3584–3595. https://doi.org/10.1016/j.scitotenv.2010.04.041

Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Research 32, 3245–3260. https://doi.org/10.1016/S0043-1354(98)00099-2

Thapa, R., Singh, S., 2019. A Study on Medicine Utilization Pattern in Outpatient Departments of Tertiary Care Centre in Kathmandu. Nep. Med. Coll. J. 21, 319–326. https://doi.org/10.3126/nmcj.v21i4.27630

Thomas, K.V., Langford, K.H., 2010. Point Sources of Human Pharmaceuticals into the Aquatic Environment, in: Kümmerer, K., Hempel, M. (Eds.), Green and Sustainable Pharmacy. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 211–223. https://doi.org/10.1007/978-3-642-05199-9_14

Togola, A., Amalric, L., Bristeau, S., 2008. Les substances pharmaceutiques dans les eaux superficielles et souterraines du bassin Loire-Bretagne (No. BRGM/RP-55578-FR).

Tracol, R., Duchemin, J., 2007. Evaluation de l'occurrence des résidus de médicaments dans un échantillon de nappes souterraines vulnérables du bassin seine-normandie utilisées pour la production d'eau destinée à la consommation humaine (ddass - drass de basse normandie). (DDASS - DRASS DE BASSE NORMANDIE).

Treadgold, J.W., 2012. The sources and environmental fate of pharmaceuticals and personal care products in lowland river catchments. Imperial College London.

Tsilikidis, I., Nika, M.-C., Christophoridis, C., 2015. Identification of ozonation transformation products of furosemide using LC-HR-MS/MS. Proceedings of the 14th International Conference on Environmental Science and Technology Rhodes, Greece, 3-5 September 2015 1.

Urtiaga, A.M., Pérez, G., Ibáñez, R., Ortiz, I., 2013. Removal of pharmaceuticals from a WWTP secondary effluent by ultrafiltration/reverse osmosis followed by electrochemical oxidation of the RO concentrate. Desalination 331, 26–34. https://doi.org/10.1016/j.desal.2013.10.010

U.S. Department of Health and Human Services, 1998. Environmental Assessment of Human Drug and Biologics Applications.

Valcárcel, Y., Alonso, S.G., Rodríguez-Gil, J.L., Castaño, A., Montero, J.C., Criado-Alvarez, J.J., Mirón, I.J., Catalá, M., 2013. Seasonal variation of pharmaceutically active compounds in surface (Tagus River) and tap water (Central Spain). Environ Sci Pollut Res 20, 1396–1412. https://doi.org/10.1007/s11356-012-1099-2

Valcárcel, Y., Alonso, S.G., Rodríguez-Gil, J.L., Maroto, R.R., Gil, A., Catalá, M., 2011. Analysis of the presence of cardiovascular and analgesic/anti-inflammatory/antipyretic pharmaceuticals in river- and drinking-water of the Madrid Region in Spain. Chemosphere 82, 1062–1071. https://doi.org/10.1016/j.chemosphere.2010.10.041

van Kraaij, D.J.W., Jansen, R.W.M.M., Bruijns, E., Gribnau, F.W.J., Hoefnagels, W.H.L., 1997. Diuretic Usage and Withdrawal Patterns in a Dutch Geriatric Patient Population. Journal of the American Geriatrics Society 45, 918–922. https://doi.org/10.1111/j.1532-5415.1997.tb02959.x

Vancea, M., Solé-Casals, J., 2016. Population Aging in the European Information Societies: Towards a Comprehensive Research Agenda in eHealth Innovations for Elderly. Aging and disease 7, 526. https://doi.org/10.14336/AD.2015.1214

Vargas, F., Martinez Volkmar, J., Sequera, J., Mendez, H., Rojas, J., Fraile, G., Velasquez, M., Medina, R., 1998. Photodegradation and phototoxicity studies of furosemide. Involvement of singlet oxygen in the photoinduced hemolysis and lipid peroxidation. Journal of Photochemistry and Photobiology B: Biology 42, 219–235.

Ventura, R., Segura, J., 1996. Detection of diuretic agents in doping control. Journal of Chromatography B: Biomedical Sciences and Applications 687, 127–144. https://doi.org/10.1016/S0378-4347(96)00279-4

Verlicchi, P., 2016. Pharmaceutical Concentrations and Loads in Hospital Effluents: Is a Predictive Model or Direct Measurement the Most Accurate Approach?, in: Verlicchi, P. (Ed.), Hospital Wastewaters, The Handbook of Environmental Chemistry. Springer International Publishing, Cham, pp. 101–133. https://doi.org/10.1007/698_2016_24

Verlicchi, P., Al Aukidy, M., Galletti, A., Petrovic, M., Barceló, D., 2012a. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. Science of The Total Environment 430, 109–118. https://doi.org/10.1016/j.scitotenv.2012.04.055

Verlicchi, P., Al Aukidy, M., Zambello, E., 2015. What have we learned from worldwide experiences on the management and treatment of hospital effluent? — An overview and a discussion on perspectives. Science of The Total Environment 514, 467–491. https://doi.org/10.1016/j.scitotenv.2015.02.020

Verlicchi, P., Al Aukidy, M., Zambello, E., 2012b. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. Science of The Total Environment 429, 123–155. https://doi.org/10.1016/j.scitotenv.2012.04.028

Verlicchi, P., Galletti, A., Petrovic, M., Barceló, D., Al Aukidy, M., Zambello, E., 2013. Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed — Analysis of their respective contributions. Science of The Total Environment 454–455, 411–425. https://doi.org/10.1016/j.scitotenv.2013.03.044

UNESCO, 2017. Pharmaceuticals in the aquatic environment of the Baltic Sea region – A status report. UNESCO Emerging Pollutants in Water Series – No, 1. UNESCO Publishing, Paris.

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

Vymazal, J., Dvořáková Březinová, T., Koželuh, M., Kule, L., 2017. Occurrence and removal of pharmaceuticals in four full-scale constructed wetlands in the Czech Republic – the first year of monitoring. Ecological Engineering 98, 354–364. https://doi.org/10.1016/j.ecoleng.2016.08.010

WADA, 2009. The 2009 prohibited list international standard - The World Anti-Doping Code.

Wahlberg, C., Björlenius, B., Paxéus, N., 2011. Fluxes of 13 selected pharmaceuticals in the water cycle of Stockholm, Sweden. Water Science and Technology 63, 1772–1780. https://doi.org/10.2166/wst.2011.124

Wargo, K.A., Banta, W.M., 2009. A Comprehensive Review of the Loop Diuretics: Should Furosemide Be First Line? Ann Pharmacother 43, 1836–1847. https://doi.org/10.1345/aph.1M177

White, D., Lapworth, D.J., Civil, W., Williams, P., 2019. Tracking changes in the occurrence and source of pharmaceuticals within the River Thames, UK; from source to sea. Environmental Pollution 249, 257–266. https://doi.org/10.1016/j.envpol.2019.03.015

Williams, D.P., Antoine, D.J., Butler, P.J., Jones, R., Randle, L., Payne, A., Howard, M., Gardner, I., Blagg, J., Park, B.K., 2007. The Metabolism and Toxicity of Furosemide in the Wistar Rat and CD-1 Mouse: a Chemical and Biochemical Definition of the Toxicophore. J Pharmacol Exp Ther 322, 1208–1220. https://doi.org/10.1124/jpet.107.125302

Wolf, L., Zwiener, C., Zemann, M., 2012. Tracking artificial sweeteners and pharmaceuticals introduced into urban groundwater by leaking sewer networks. Science of The Total Environment 430, 8–19. https://doi.org/10.1016/j.scitotenv.2012.04.059

Yagi, N., Kemotsu, H., Sekikawa, H., Takada, M., 1991. Study on the photolysis and hydrolysis of furosemide in aqueous solution. Biol pharm bull 39, 454–457.

Yalkowsky, S.H., He, Y., Jain, P., 2010. Handbook of Aqueous Solubility Data, 2nd ed. CRC Press, Boca Raton. https://doi.org/10.1201/EBK1439802458

Yang, X.-X., Hu, Z.-P., Alex Boelsterli, U., Zhou, S.-F., 2006. Drug Acyl Glucuronides: Reactivity and Analytical Implication. CPA 2, 259–277. https://doi.org/10.2174/157341206777934608

Yilmaz, G., Kaya, Y., Vergili, I., Beril Gönder, Z., Özhan, G., Ozbek Celik, B., Altinkum, S.M., Bagdatli, Y., Boergers, A., Tuerk, J., 2017. Characterization and toxicity of hospital wastewaters in Turkey. Environ Monit Assess 189, 55. https://doi.org/10.1007/s10661-016-5732-2

Yue, Z., Sun, L.-L., Sun, S.-N., Cao, X.-F., Wen, J.-L., Zhu, M.-Q., 2022. Structure of corn bran hemicelluloses isolated with aqueous ethanol solutions and their potential to produce furfural. Carbohydrate Polymers 288, 119420. https://doi.org/10.1016/j.carbpol.2022.119420

Zoumpouli, G.A., Zhang, Z., Wenk, J., Prasse, C., 2021. Aqueous ozonation of furans: Kinetics and transformation mechanisms leading to the formation of α,β-unsaturated dicarbonyl compounds. Water Research 203, 117487. https://doi.org/10.1016/j.watres.2021.117487

Zuccato, E., 2004. Prediction of the environmental load of pharmaceuticals contaminating the marine environment. A case for the Adriatic Sea. Novel contaminants and pathogens in coastal waters. 4.

Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R., 2000. Presence of therapeutic drugs in the environment. The Lancet 355, 1789–1790. https://doi.org/10.1016/S0140-6736(00)02270-4

Zuccato, E., Castiglioni, S., Fanelli, R., 2005. Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. Journal of Hazardous Materials 122, 205–209. https://doi.org/10.1016/j.jhazmat.2005.03.001

Databases :

ClinCalc DrugStats Database, 2018.

DrugBank. Furosemide - https://go.drugbank.com/drugs/DB00695

HMDB, 2006. Furosemide HMDB0001933 - Human Metabolome Database.

OCDE. OCDE.Stat - Diuretic.

PubChem. Furosemide - https://pubchem.ncbi.nlm.nih.gov/compound/3440

RxList, 2020. Lasix - RxList.

Il est crucial d'améliorer notre compréhension du suivi des médicaments vers et dans l'environnement dans le cadre de la protection des ressources en eau contre ces nouveaux polluants.

Cette revue dresse un portrait global du furosémide et souligne son omniprésence. C'est l'un des médicaments les plus vendus dans le monde, largement prescrit aux personnes âgées, mais aussi aux jeunes adultes et aux enfants, ainsi qu'en médecine vétérinaire, depuis près de 60 ans. Au niveau de sources telles que les hôpitaux et les influents de STEU, des fortes concentrations de furosémides sont retrouvées. Le traitement de ces eaux étant insuffisant, on en retrouve en concentrations importantes en sortie de STEU et, de ce fait, on en quantifie également dans les rivières autour de quelques centaines de nanogrammes par litre, et aussi parfois dans les eaux souterraines.

De nouveaux procédés sont étudiés pour une meilleure élimination des micropolluants dans les STEU. Les procédés d'oxydation se sont montrés particulièrement efficaces pour obtenir une meilleure dégradation du furosémide. Cependant, ce dernier est dégradé en plusieurs sous-produits dont la saluamine, le pyridinium du furosémide, le furfural, et certains produits issus de la métabolisation ou de la photodégradation. Ces composés présentent parfois une toxicité plus importante que celle du furosémide et nécessitent d'être mieux caractérisés. Il existe également des produits de dégradation pour lesquels il n'existe aucune information de toxicité et qui pourraient être préoccupants.

Largement consommé, persistant, très présent dans l'environnement, potentiellement toxique, pour toutes ces raisons, de nombreuses études classent le furosémide comme polluant prioritaire. Malheureusement, on sait peu de choses sur son devenir dans l'environnement. Il est nécessaire de développer des outils pour étudier l'impact de cette molécule et de mieux caractériser ses produits de dégradation.

Chapitre II.

Origine et devenir du furosémide et de ses produits de dégradation dans le milieu aquatique

Chapitre II.
Origine et devenir du furosémide et de ses produits de dégradation dans le milieu aquatique

I. Devenir du furosémide dans les eaux usées : état des lieux en France

Pour protéger le milieu aquatique, les eaux usées domestiques (toilettes, douche, vaisselle, lave-linge, ménage, cuisine,...), les eaux usées industrielles (usines, installations agricoles, laboratoires, imprimeries,..) et parfois les eaux pluviales (ruissellement de route, toiture, revêtements imperméables) nécessitent d'être traitées avant d'être rejetées[7]. C'est le rôle des Stations de Traitement des Eaux Usées (STEU). Ces eaux sont chargées de microorganismes potentiellement pathogènes et d'un cocktail complexe de molécules en phase dissoute ou particulaire, qu'elles soient organiques ou inorganiques (détergents, huiles, hydrocarbures, médicaments,...). Elles sont acheminées vers les STEU où elles subissent différents traitements d'assainissement (**Figure 4**).

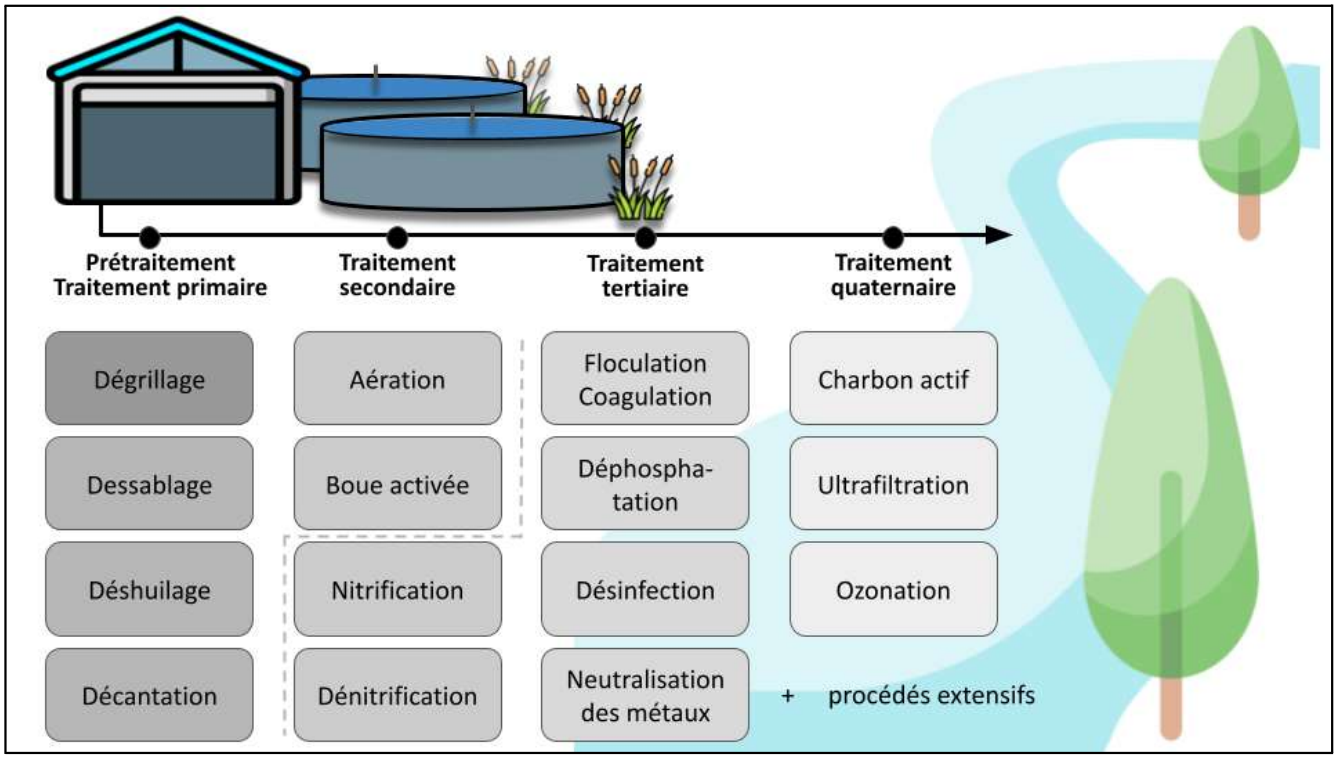


Figure 4. Différents processus pouvant être appliqués pour le traitement des eaux usées. Les informations utilisées pour produire ce schéma proviennent de l'Office International de l'Eau et des sites eaufrance.fr et ademe.fr. Certaines illustrations sont issues du site flaticon.com.

Un prétraitement (dégrillage, dessablage, déshuilage) et traitement primaire (décantation) enlèvent les grosses particules, les matières flottantes et les graisses. Ensuite, un traitement biologique (aération, traitement en lit biologique, boues activées) dégrade les matières organiques comme les nitrates, l'ammoniac ou certains polluants biodégradables présents dans les eaux usées, grâce à des filtres

[7] Service publique de l'assainissement francilien - Définitions - consulté le 13/01/2023

biologiques contenant des bactéries qui les digèrent. Un traitement chimique (neutralisation, adsorption, oxydation) ou physique (charbon actif, ultrafiltration, microfiltration) est parfois ajouté pour neutraliser certains contaminants ou pour favoriser leur élimination. Un traitement tertiaire pour éliminer les composés azotés ou phosphorées restants, ou une étape de désinfection pour limiter les risques de contamination bactériologique peuvent également être ajoutés[8]. En Europe, le traitement des eaux usées est obligatoire[9]. Cependant, les STEU ne comportent pas nécessairement toutes les étapes de traitement évoquées ci-dessus et présentés dans la figure 2 (seul le dégrillage est généralisé). Elles sont adaptées selon les contraintes en amont (taille de l'agglomération, charges de polluants à l'entrée, dispositions touristique, artisanale et/ou agricole de la commune, variation de débit), les contraintes en aval (performance épuratoire à atteindre, capacité de dilution du milieu) et les contraintes financières[10]. Dans certains cas, les STEU les plus optimisées disposent même d'un traitement quaternaire. Ce traitement a spécifiquement pour but de réduire la charge de micropolluants. Ces traitements sont rares en France puisque la politique appliquée par le plan national micropolluant est plutôt celle de la réduction à la source. En revanche, en Suisse, ces traitements sont déjà imposés pour les STEU d'une capacité de plus de 100 000 EH (Équivalent Habitant)[11]. Ces traitements peuvent comprendre par exemple des procédés d'oxydation comme l'ozonation, l'ajout de charbon actif ou encore des filtrations par osmose inverse.

Dans le chapitre précédent, nous avons comparé la capacité d'élimination du furosémide de plusieurs procédés cités dans la littérature scientifique. Dans ce chapitre, nous nous sommes intéressés à ces traitements quaternaires. Les procédés avancés les plus fréquents, soit l'ozonation, la chloration et l'irradiation UV avec ajout d'H_2O_2 utilisés initialement pour la désinfection, montrent une bonne élimination du furosémide. Nous nous sommes alors posé deux questions: En quoi le furosémide est-t-il transformé ? Est-ce que ces procédés favorisent la production de saluamine et de pyridinium du furosémide, considérés comme potentiellement problématiques ? La présence de ces deux sous-produits dans différentes matrices a également été examinée. En premier lieu, des échantillons potentiellement chargés en furosémide ont été analysés pour établir une première évidence de la saluamine et du pyridinium dans les eaux usées. Le furosémide étant majoritairement consommé par des personnes âgées, des rejets d'EHPAD se sont révélés être très intéressants. Les composés ont ensuite été recherchés en amont et en aval d'une STEU de l'agglomération parisienne pour sonder leur élimination ou, à l'inverse, leur production à l'issue des différents traitements. Les résultats de cette étude comparative de traitements oxydatifs sur le furosémide et des analyses de produits de dégradations sont présentés dans un article de recherche publié dans le journal Chemosphere.

[8] Le service public d'information sur l'eau - L'assainissement des eaux usées domestiques - consulté le 13/01/2023
[9] Directive européenne 91/271/CEE relative au traitement des eaux résiduaires urbaines (DERU)
[10] Circulaire N°97-31 du 17 février 1997
[11] Ordonnance du 28 octobre 1998 sur la protection des eaux (OEaux)

Résumé de l'article : Occurrence and fate of an emerging drug pollutant and its by-products during conventional and advanced wastewater treatment: Case study of furosemide

Les systèmes conventionnels de traitement des eaux usées ne sont pas conçus pour éliminer les composés pharmaceutiques des eaux usées. Ces composés peuvent être partiellement ou totalement dégradés et conduire éventuellement à des sous-produits plus préoccupants qui sont peu, voire pas du tout, étudiés. Dans ce contexte, nous avons étudié la dégradation du furosémide, un diurétique très fréquemment détecté dans les eaux usées, afin d'observer la formation de ses produits de dégradation. Les effluents de la station d'épuration Seine-Centre (Paris, France) ainsi que ceux de maisons de retraite (Dordogne, France) ont été analysés par UPLC-MS/MS afin de quantifier le furosémide et ses produits de dégradation connus, la saluamine et le pyridinium du furosémide. Des expériences d'oxydation (chloration, ozonation et photolyse UV avec du peroxyde d'hydrogène) ont également été réalisées sur des solutions de furosémide et sur de l'eau provenant d'établissements de soins résidentiels afin d'étudier la dégradation du furosémide et d'identifier ses produits d'oxydation par UPLC-IMS-QTOF.

Le furosémide a été bien dégradé dans les STEU de Seine-Centre (>75%) mais n'a pas produit un surcroît pour ses principaux produits de dégradation. La saluamine et le pyridinium du furosémide étaient déjà présents à des concentrations similaires à celles du furosémide dans les eaux usées brutes (~2,5-3,5 µg.L-1), et leur élimination dans les STEU était très élevée (>80%). Malgré les traitements, les trois composés sont restés présents à des concentrations de centaines de nanogrammes par litre. La chloration a dégradé le furosémide sans production de pyridinium, contrairement aux deux autres procédés. La chloration et l'ozonation ont également été efficaces pour l'élimination du furosémide et du pyridinium dans l'eau des maisons de soins résidentielles, mais elles ont entraîné la production de saluamine. À notre connaissance, il s'agit de la première preuve de la présence de saluamine et de pyridinium de furosémide dans des échantillons d'eau réels, que ce soit dans la phase particulaire ou dissoute.

Chemosphere 322 (2023) 138212

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Occurrence and fate of an emerging drug pollutant and its by-products during conventional and advanced wastewater treatment: Case study of furosemide

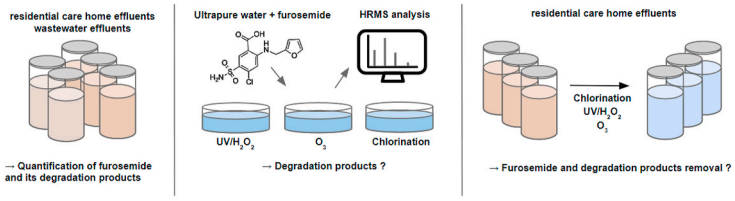
Fidji Sandre [a], Nina Huynh [a], Emilie Caupos [a,b], Lamyae El-Mrabet [a], Chandirane Partibane [a], Isabelle Lachaise [c], Christophe Pommier [d], Michael Rivard [c], Christophe Morin [e], Régis Moilleron [a], Julien Le Roux [a], Laure Garrigue-Antar [a,*]

[a] Leesu - Univ Paris Est Creteil, Ecole des Ponts, Creteil, F-94010, France
[b] Univ Paris Est Creteil, CNRS, OSU-EFLUVE, F-94010, Creteil, France
[c] ICMPE - Univ Paris Est Creteil, CNRS, UMR, 7182, 2 Rue Henri Dunant, F-94320, Thiais, France
[d] LDAR 24 - Laboratoire Départemental D'analyses et de Recherche, Coulounieix Chamiers, France
[e] IUT - Sénart Fontainebleau, 36 Rue Georges Charpak, 77567, Lieusaint, France

HIGHLIGHTS

- Pyridinium of furosemide and saluamine were quantified for the first time in wastewater samples.
- Seine-Centre WWTP shows a good removal of furosemide and its degradation products.
- Ozonation and chlorination completely degrade furosemide but may produce saluamine.
- New chlorination and UV/H_2O_2 degradation products of furosemide were identified.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Y Liu

Keywords:
Pharmaceutical
Wastewater
Residential care home
Degradation products
Oxidation

ABSTRACT

Conventional wastewater treatment systems are not designed to remove pharmaceutical compounds from wastewater. These compounds can be degraded into many other transformation products which are hardly, if at all, studied. In this context, we studied the occurrence and degradation of furosemide, a very frequently detected diuretic, along with its known degradation products in several types of wastewater. Influent and effluent from the Seine-Centre Wastewater Treatment Plant (WWTP) (Paris, France) as well as outlet of residential care homes (Dordogne, France) were analyzed by Ultra-Performance Liquid Chromatography-tandem Mass Spectrometry (UPLC-MS/MS) to quantify furosemide and its known degradation products, saluamine and pyridinium of furosemide. Oxidation experiments (chlorination, ozonation and UV photolysis with hydrogen peroxide) were then performed on furosemide solutions and on water from residential care facilities to study the degradation of furosemide by potential advanced processes, and also to identify unknown oxidation products by high-resolution mass spectrometry. Furosemide was well degraded in Seine-Centre WWTP (>75%) but did not increase the concentrations of its main degradation products. Saluamine and pyridinium of furosemide were already present at similar concentrations to furosemide in the raw wastewater (~2.5–3.5 $\mu g.L^{-1}$), and their removal in the WWTPs were very high (>80%). Despite their removal, the three compounds remained present in treated

* Corresponding author.
E-mail address: laure.garrigue-antar@u-pec.fr (L. Garrigue-Antar).

https://doi.org/10.1016/j.chemosphere.2023.138212
Received 30 November 2022; Received in revised form 14 February 2023; Accepted 20 February 2023
Available online 21 February 2023

wastewater effluents at concentrations of hundreds of nanograms per liter. Chlorination degraded furosemide without pyridinium production unlike the other two processes. Chlorination and ozonation were also effective for the removal of furosemide and pyridinium in residential care home water, but they resulted in the production of saluamine. To our knowledge this is the first evidence of saluamine and pyridinium of furosemide in real water samples in either the particulate or dissolved phase.

1. Introduction

In the context of a wide consumption of medicines in high income and emerging countries, increasing demographical urban pressure and population aging, hundreds of active substances used for therapeutic purposes and marketed worldwide end up in the wastewater system. Conventional wastewater treatment plant (WWTP) processes based on filtration, sand and grease removal, decantation and biological or physico-chemical treatments are not designed for the elimination of pharmaceutical compounds, so their removal can vary drastically depending on the molecule (e.g., its hydrophobicity) (Chiffre et al., 2016). The active substances of these drugs are discharged with WWTP effluents at concentrations ranging from $ng.L^{-1}$ to $\mu g.L^{-1}$ and end up in the aquatic environment, representing a risk for organisms (Cizmas et al., 2015, Okoye et al., 2022). To overcome this problem, the implementation of tertiary or quaternary treatments after conventional treatments is increasingly studied, and several countries (e.g., Switzerland, Germany) already regulate the removal of organic micropollutants from wastewater. The optimization of biological treatments, for example with the addition of fungus (*Trametes versicolor*) in bioreactors (Cruz-Morató et al., 2014; Badia-Fabregat et al., 2015), can be effective on potentially biodegradable compounds. However, the most frequent processes used to achieve a satisfactory removal of micropollutants include adsorption on activated carbon (Guillossou et al., 2019), membrane processes and oxidation processes such as ozonation (Guillossou et al., 2020).

Ozone has been used for a long time as both an oxidant and a disinfectant for drinking water production, and is increasingly applied for the treatment of wastewater effluents (Lim et al., 2022). It is a very powerful oxidant that can react directly with organic molecules, mainly by electrophilic reaction, and also indirectly by the production of radicals initiated by hydroxyl or hydroperoxide ions in the aqueous medium (Guo et al., 2012). Ozonation has been shown to be effective in the degradation of hormones, antibiotics, antivirals and pesticides (Lim et al., 2022). Advanced oxidation processes (AOPs) are also very efficient for the degradation of organic molecules (Miklos et al., 2018). These processes are based on the production of highly reactive hydroxyl radicals from one or more primary oxidants (e.g., chlorine, ozone, hydrogen peroxide, etc.) (Sarathy and Mohseni, 2006) or by photocatalysis (Prieto-Rodriguez et al., 2012). Advanced oxidation processes in which Ultraviolet (UV) radiation is combined with ozone, chlorine, peroxodisulfate or hydrogen peroxide are efficient to reach extensive degradation. The most common combination is with H_2O_2 because it is much less energy-consuming and expensive than with O_3, and it is also less sensitive to matrix changes and dissolved organic matter than sulfates. While advanced treatment processes for organic micropollutants are not widely used, many WWTP use a disinfection step before discharging effluents into the environment (e.g., in bathing areas). Another process commonly used for disinfection and inactivation of pathogenic microorganisms is based on UV irradiation (Hijnen et al., 2006). Direct UV photolysis with UV-C (100–280 nm) shows good efficiency on pesticides (Sanches et al., 2010) but some pharmaceutical compounds like carbamazepine are more resistant to it (Pereira et al., 2007).

Although oxidation treatments can be quite efficient for the degradation of micropollutants, they often form oxidation by-products. Transformation products (e.g., metabolites) of pharmaceuticals can also be already present in the incoming water or be formed during conventional biological processes. The formation of oxidation by-products depends on the mode of oxidation, the medium (especially the presence of nitrogen, halogens, organic matter) and the type of radical produced in the case of advanced oxidation (Miklos et al., 2018). These degradation by-products present a hazard for health (Stalter et al., 2016) and for the environment (Wang et al., 2018). Apart from the regulated disinfection by-products (e.g., trihalomethanes and haloacetic acids), hundreds of chlorination by-products have been identified (Zhang et al., 2012) from the reaction of chlorine with organic matter and their potential toxicity can be a threat to human health (Stefán et al., 2019; Mazhar et al., 2020). Many products are also generated during UV-H_2O_2 oxidations depending on the dose of oxidant, the UV fluence and the pH of the medium. Oxidation by-products are often more toxic than the parent compounds on algae, daphnia, and medaka fish (Wang et al., 2018), it is thus of critical importance to assess the toxicity of degradation products to fully establish the potential impacts of organic micropollutants on the environment.

To address the questions related to the presence of pharmaceuticals in the environment, we selected furosemide from a list of high-risk emerging pollutants (Besse and Garric, 2008) and subjected this drug to a predictive study aiming to anticipate the fate of organic contaminants (Laurencé et al., 2011; 2014; Olvera Vargas et al., 2016). Classified by the World Health Organization as an essential medicine, furosemide is a loop diuretic prescribed all over the world for hypertension and heart, liver, and kidney failure (Abbot and Kovacic 2008). Mostly eliminated unmodified by humans and moderately degraded in conventional treatment plants (between 8 and 54% in Castiglioni et al., 2006, >65% in Matamoros et al., 2009, 74% in Kasprzyk-Hordern et al., 2009, or between 30 and 80% according to the WWTP considered in Jelic et al., 2011), furosemide is also widely found in the environment from several tens to few thousand of $ng.L^{-1}$ in rivers (Banjac et al., 2015; Baken et al., 2018; Munro et al., 2019; White et al., 2019; Cantwell et al., 2018). Applied to furosemide, our predictive study confirmed that saluamine and furfural are two transformation products (TPs) of furosemide (Laurencé et al., 2014). More importantly, it also identified the pyridinium of furosemide as a TP and a human metabolite of this drug (Laurencé et al., 2019). Whereas furfural presents moderate acute toxicity in freshwater invertebrates ($LC50 = 13\ mg.L^{-1}$ for 72 h exposure in Reed and Kwok, 2014), saluamine shows acute toxicity in rats (Al-Omar et al., 2009). We also previously demonstrated that pyridinium of furosemide exhibits neurodegenerative properties in mice (Laurencé et al., 2019). Therefore, the fate of furosemide and its TPs in wastewater and in the environment needs to be assessed to fully characterize the potential impacts of this pharmaceutical on the aquatic ecosystems. Furosemide is also an interesting and highly relevant model to study the fate of pharmaceuticals along with their TPs. Especially, the production of saluamine and pyridinium of furosemide as well as their potential degradation during conventional and advanced wastewater treatment remains unknown to date. The purpose of this work was thus i) to assess the occurrence and removal of furosemide and its known TPs in a large WWTP and in wastewater from an elderly care facility, ii) to study their removal by several advanced oxidation processes (ozonation, UV/H_2O_2) and iii) to identify the subsequent formation of potential new TPs following these treatments.

2. Material & methods

2.1. Reagents

The furosemide-d5 standard was purchased from Cluzeau Info Labo. Internal standards (atenolol-d7 and sulfamethoxazole-d4) and saluamine (SAL) were purchased from Sigma-Aldrich. For the extraction and analysis, MS grade methanol was purchased from Fisher. Ultrapure water was produced by a Milli-Q® IQ 7000 ultrapure water system. Furosemide (FUR) was purchased from Sigma. Pyridinium of furosemide (PYR) was synthesized at ICMPE as previously described (Laurencé et al., 2011), and its structure has been confirmed by NMR with over 96% purity. Hydrogen peroxide 50% was purchased from CarlRoth.

2.2. Sampling sites

To assess the WWP efficiency on furosemide degradation, raw water (RW) and treated effluent water (TW) were collected on 2021-12-07 and 2021-12-08 from the Seine-Centre WWTP (Colombes, France), operated by the Paris public sanitation service (SIAAP) and treating wastewater of about 1 000 000 people equivalent with a flow of 240 000 m^3/day. The Seine-Centre WWTP is mostly based on biofiltration stages ensuring the treatment of organic carbon as well as nitrification and denitrification processes. For the oxidation tests on raw sewerage water samples, wastewater from residential care homes (RCH) was chosen because of its presumably high concentration of furosemide, thus allowing a better identification of the possible degradation products. Three wastewater outlets of health care institutions were collected in Dordogne, France. The first sample (RCH1) was collected on 2021-05-26 in a RCH of 10 000 m^2 located in Bergerac, in an urban area. It has a capacity of 90 beds plus 15 beds for Alzheimer's patients, 10 places in daycare centers and 5 emergency beds. The sample was taken from a lift station which only collects water from the facility. The second sample (RCH2) was collected on 2021-06-09 from a reeducation center located in a national forest at Antonne-et-Trigonant with 120 places and 40 beds for senior residents. The third sample (RCH3) was collected on 2021-06-08 from a RCH at Lolme with 90 beds and 5 daycare places. Several physico-chemical parameters were recorded: pH, Dissolved Organic Carbon (DOC), Chemical Oxygen Demand (COD) and Biological Oxygen Demand at 5 days (DBO5), Suspended Matter (SM), Total Kjeldahl Nitrogen (TKN), ammoniacal nitrogen and organic nitrogen (Table 1). The RCH outlet water samples were filtered on GF/D filters (2.7 μm pore size) and then on GF/F filters (0.7 μm pore size). pH and DOC were measured after filtration (Table 1). Particulate phase samples (i.e., particles collected on filters) were stored at −20 °C and freeze-dried until treatment for analysis. For RCH samples, it is interesting to note the large gap between the Kjeldahl nitrogen and ammonia nitrogen values, which implies a high proportion of organic nitrogen, reflecting the short residence time of the effluent and confirming the relatively fresh effluent sampling.

Table 1
Physico-chemical parameters of samples from WWTP raw wastewater (RW1 = 2021-12-07, RW2 = 2021-12-08), WWTP treated wastewater (TW1 = 2021-12-07, TW2 = 2021-12-08) and wastewater outlets of residential care homes (RCH). NA = Not available (not measured).

	RW1	RW2	TW1	TW2	RCH1	RCH2	RCH3
DOC ($mg_C.L^{-1}$)	NA	NA	<8	<8	113.0	429.0	135.8
COD ($mg_{O2}.L^{-1}$)	485	500	26	26	749	1844	640
BOD5 ($mg_{O2}.L^{-1}$)	189	189	5	5	416	894	329
SM ($mg.L^{-1}$)	231	246	4	5	320	400	150
TKN ($mg_N.L^{-1}$)	54.6	57.8	1.9	1.9	37	50	24
ammoniacal N ($mg_N.L^{-1}$)	43.8	45.5	0.3	0.3	6	16	7
organic N ($mg_N.L^{-1}$)	10.8	12.3	1.6	1.6	31	38	2

2.3. Oxidation assays

Furosemide degradation kinetics experiments were performed in ultrapure water spiked with 1 $mg.L^{-1}$ of furosemide. UV/H_2O_2 and ozonation were applied at high doses or exposure as described below, and chlorination was also conducted at high dose (35 $mgCl_2.L^{-1}$) to maximize the formation of degradation products (see Text S1). Oxidation processes were also applied on 200 mL filtered wastewater samples from RCH facilities.

Photodegradation under UV light with H_2O_2. The sample was placed in a beaker, under a UV lamp at 254 nm (UV–C; 0.34 mW/cm^2) with stirring. To establish degradation kinetics of furosemide in ultrapure water, 1 $mg.L^{-1}$ of H_2O_2 was added and aliquots of 1 mL were taken at 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 210 and 600 min. For the oxidation of wastewater from RCH, the H_2O_2 dose was determined according to the DOC value by using 0.375 mg of H_2O_2 per mg of DOC, and the total reaction time under UV irradiation was 5 h.

Ozonation. Ozone was produced by a generator with an external source (O_2 gas cylinder). The bottle containing the sample to be oxidized was immersed in an ice bath throughout the experiment. For the ozonation of furosemide solutions in ultrapure water, a concentrated ozone solution was first produced (33 mg/L O_3) and diluted in the furosemide samples at the required ozone concentrations, determined according to the DOC concentration of the sample (Equation (1)).

$$[O_3]\left(mgO_3.L^{-1}\right) = 2\ x\ DOC\ \left(mgC.L^{-1}\right) \quad (1)$$

Aliquots of 1 mL were collected at 1, 2, 4, 5, 7, 10, 15, 20, 30, 45 and 60 min. For the oxidation of RCH wastewater, ozone was bubbled directly into the samples for 1.5 h with an O_2 flow to the ozonator of 2 L h^{-1}. The residual O_3 was measured by colorimetry with indigo carmine using a double beam spectrometer (UV6300 PC, VWR) at 600 nm.

2.4. Analytical procedures for quantification

After filtration, the residential care home samples are split into several aliquots of which 5 are spiked. Furosemide and its TPs present in dissolved phases of wastewater samples were extracted through an automated extraction system (Thermo AutoTrace 280 SPE Instrument) on OASIS HLB cartridges (200 mg, 6 cc). After conditioning with methanol and ultrapure water, samples (200 mL) were loaded onto cartridges and eluted by 10 mL of methanol. The particulate phases of RCH1 and RCH2 samples were also analyzed. The filters (GF/D and GF/F) were frozen and freeze-dried to remove water and determine their dry mass. Furosemide and its TPs were extracted by assisted microwave extraction (Antoon Paar, Multiwave 3000). Two cycles of extractions of 30 min in a mixture of 60% methanol-40% dichloromethane (v/v) were performed (100 °C, 800 W). The extracts were filtered on folded filters washed with dichloromethane, evaporated to 1 mL under rotary evaporator and diluted in 100 mL ultrapure water to carry out a purification step on OASIS HLB cartridges. Cartridges were eluted with 10 mL of methanol, internal standards (Atenolol-d7 and Sulfamethoxazole-d4) were added to the purified extracts and the final volume was adjusted to 1 mL by evaporation. Extract concentrations were evaluated using internal calibration. Internal standards were chosen based on their similar retention time to the one of the targeted molecule (Table S2). An evaluation of matrix effects revealed their low variability for all molecules across 16 urine samples. Spiked samples were also extracted, leading to the evaluation of extraction yields of furosemide, saluamine and pyridinium. These extraction yields were used for the correction of concentrations in the samples. The detection and quantification limit of the instrument for each compound is presented in Table S3. Analyses were performed using Ultra Performance Liquid Chromatography coupled with a triple quadrupole detector (Acquity-TQD, Waters). Separation was carried out on an ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm) with a 15 min gradient elution from 90:10 ultra-pure

water (A) and methanol (B) both acidified with 0.1% formic acid, to 0:100 (A:B) maintained for 5 min, before reequilibration of the column (0.4 mL min^{-1}). For the detection, furosemide was ionized in negative electrospray mode, and saluamine and pyridinium of furosemide were ionized in positive mode. MS/MS acquisition was used (Table S2).

The phase distribution (K_d value) was calculated according to equation (2) (Park et al., 2017), where K_d is the solid-water distribution coefficient in $L.kg^{-1}$, Cs is the concentration of furosemide, saluamine or pyridinium of furosemide adsorbed onto MES $ng.L^{-1}$ and Cw is the concentration on furosemide, saluamine or pyridinium of furosemide in the liquid phase in $ng.L^{-1}$, and SS is that of suspended matter in the mixture in $mg.L^{-1}$.

$$K_d = \frac{Cs}{SS\ x\ Cw} \times 10^6 \quad (2)$$

2.5. Analytical procedures for the identification of TPs

Degradation kinetics experiments were analyzed by ultra-performance liquid-chromatography coupled to ion-mobility time-of-flight mass spectrometry (UPLC-IMS-QTOF, Vion, Waters) to enable the tentative identification of degradation products. The separation was carried out on an ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm) with a 25 min gradient from 98:2 ultra-pure water (A) and acetonitrile (B) both acidified with 0.1% formic acid, to 2:98 (A:B) maintained for 5 min, before reequilibration of the column. Ionization was performed by an electrospray source in both positive and negative mode, in low energy (6 V) and high energy ramp (20–56 V). Data was acquired and analyzed with the UNIFI software (Waters). Briefly, after 4D peak detection, the chromatograms at successive kinetics points were compared to spot potential TPs. Molecular formula attribution was performed with a restricted list of atoms (C, H, N, O, P, S and Cl) and several online libraries, as a part of Chemspider in UNIFI, were interrogated (Sigma-Aldrich, Drugbank, NIST, MassBank and LGC Standard) to tentatively identify the detected products. In order to ensure mass precision lockspray infusion was used during all the injections.

3. Results & discussion

3.1. Occurrence and removal in wastewater treatment plants

First, the concentrations of furosemide were evaluated in the Seine-Centre WWTP, along with the possible presence of its known degradation products saluamine and pyridinium of furosemide. This WWTP was chosen because it is located directly downstream of the city of Paris, thus receiving most of Parisian wastewaters.

The two collected raw water samples (2021-12-07 and 2021-12-08 samples) exhibited substantial concentrations of furosemide of 3351 and 2819 $ng.L^{-1}$ respectively (Table 2). These concentrations were consistent with those found in recent literature for WWTPs raw water in other European countries. Concentrations between 1 and 5 $\mu g.L^{-1}$ were found: 1491 $ng.L^{-1}$ in Sweden (Baresel et al., 2019), 2625 $ng.L^{-1}$ in Poland (Kot-Wasik et al., 2016), 2601 $ng.L^{-1}$ in Greece (Papageorgiou et al., 2019), 1901 $ng.L^{-1}$ and 3410 $ng.L^{-1}$ in Spain (Collado et al., 2014; Celic et al. 2019), 4577 $ng.L^{-1}$ in Portugal (Santos et al., 2013), 1652 ng.L^{-1} in Italy (Feo et al., 2020), 2916 $ng.L^{-1}$ and in Switzerland (Lee et al., 2014).

Table 2
Concentrations ($ng.L^{-1}$) of Furosemide, Pyridinium of Furosemide and Saluamine in WWTP water samples (RW = raw wastewater, TW = treated wastewater).

	2021-12-07		2021-12-08	
	RW1	TW1	RW2	TW2
Furosemide	3351	890	2819	727
Pyridinium of Furosemide	2612	255	2506	219
Saluamine	3018	272	3617	674

Our samples concentrations at 24 h interval were of the same order of magnitude, indicating little variation over this period. This result is in contrast to Gómez-Canela et al. (2019), who observed significant variations in furosemide concentrations over 5 consecutive days, but on nursing home effluents. However, for furosemide, other studies usually reported seasonal rather than weekly variations with lower concentrations in summer (Kot-Wasik et al., 2016; Delli Compagni et al., 2020).

The known degradation products of furosemide, saluamine, furfural and pyridinium of furosemide, have been searched for in wastewater. Furfural could not be analyzed with our method, the compound being possibly too small and/or too polar to be extracted and analyzed with the same method as the other three. Although described as a furosemide transformation product since long ago (Andreasen et al., 1982), reports on saluamine toxicity and occurrence in the environment are scarce. Nonetheless, we found saluamine and pyridinium of furosemide in WWTP raw water both at concentrations of a few thousands of $ng.L^{-1}$ (Table 2).

Interestingly, the distribution between the three compounds in raw water was about one third for each, which means that the degradation products are formed upstream of the WWTP, probably during the journey through the water system as commonly observed. Indeed, other drug TPs have already been found in WWTP influents such as N4-Acetylsulfamethoxazole, 2-hydroxycarbamazepine, O-desmethylvenlafaxine, 4-hydroxydiclofenac which are TPs of sulfamethoxazole, carbamazepine, venlafaxine and diclofenac, respectively (Aymerich et al., 2016). Furosemide could have been hydrolyzed or biodegraded in the wastewater transport system. The hydrolysis of furosemide has been known since the 70's and leads to the formation of saluamine in an acidic environment (Bundgaard et al., 1988; Andreasen et al., 1982) and some studies showed that saluamine and pyridinium of furosemide can be produced by biotransformation of furosemide by several microorganisms (Hezari and Davis, 1992; Laurencé et al., 2014; Olvera-vargas et al., 2016). These three compounds are still present in the two WWTP effluents samples at several hundred nanograms per liter (Table 2). Saluamine can be formed in water sediments (Li et al., 2014) and by photolysis (Bundgaard et al., 1988) but to our knowledge, this is the first evidence of saluamine and pyridinium of furosemide presence in wastewater.

All three compounds exhibited some removal in the WWTP. Yields of removal for each compound were very similar between the two sampling dates (Fig. 1). For furosemide, a reduction of over 70% was observed for both samples, which ranked in the higher range of furosemide removal by comparison with data from the literature. Matamoros et al. (2009) and Kasprzyk-Hordern et al. (2009) found similar furosemide removal rates (65% and 74%, respectively), but other studies found variable removals, with the lowest around 25% (Park, 2016), or 40–50% removal for WWTP with conventional mechanical-biological treatment (Kot-Wasik et al., 2016; Gros et al., 2010). In some cases, the sampling period could explain the differences in removal; Matamoros et al. (2009) and Kasprzyk-Hordern et al. (2009) samples were collected in spring and summer while Kot-Wasik et al. (2016) collected theirs in winter. Castiglioni et al. (2006) showed that the elimination rate of furosemide was much lower in winter than in summer (8% versus 54%). These differences could be related to the lower temperatures in winter, which possibly attenuated the biological activity. However, in our case, the samples were collected in winter. Thus, the differences in efficiency of the treatment plants for furosemide removal could be explained by the different processes used in the plants. For example, the plant studied in Matamoros et al. (2009) included several types of wetland constructs that were shown to be relatively efficient for the elimination of furosemide in recent literature (Ahmed et al., 2017; Machado et al., 2017). In the case of Seine-Centre, the purification process, which includes biofiltration, nitrification and denitrification processes, also promoted an efficient elimination of

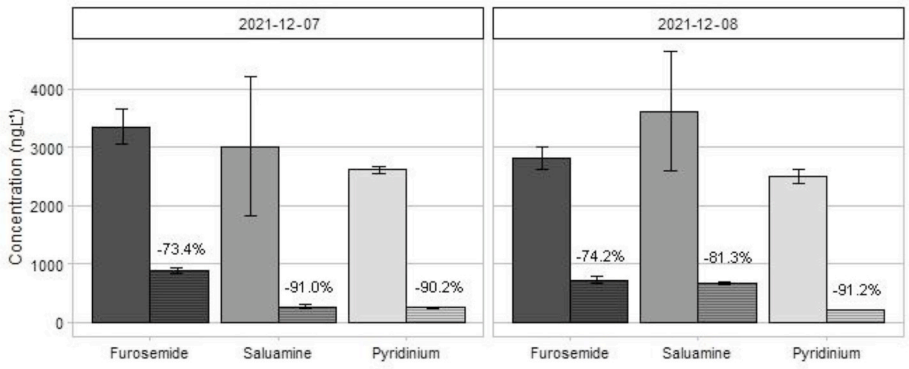


Fig. 1. Removal percentage of furosemide, saluamine and pyridinium of furosemide in Seine-Centre WWTP. Solid bars represent concentrations in raw samples and hatched bars represent concentrations in samples after treatment in WWTP. The percentages shown on the hatched bars indicate the percent removal for each compound. Error bars represent standard error.

furosemide.

In spite of a good elimination rate of furosemide and its TPs saluamine and pyridinium of furosemide (70–90% removal), several hundreds of nanograms per liter still remained in the wastewater effluent. Many authors reported significant concentrations of furosemide at the outlet of wastewater treatment plants (Table 3). Concentrations exceeding 10 $\mu g.L^{-1}$ were even found in a few studies (22 300 $ng.L^{-1}$ in UNESCO (2017), 26 000 $ng.L^{-1}$ in Vymazal et al. (2017), and 11 000 $ng.L^{-1}$ in Rozman et al. (2017)).

WWTP effluents concentrations not only depend on the influent concentrations, but also on the treatment processes used within each WWTP. In some cases, furosemide concentrations can be higher at the WWTP outlet than at the inlet (Kleywegt et al., 2016). Due to the fact that an important fraction of furosemide is excreted as a glucuro-conjugated form (Yang et al., 2006), and thus not taken into account during the analysis, this difference could be assigned to the deconjugation occurring during the WWTP treatment, leading to an increased concentration of the native, unchanged form of furosemide at the outlet. It cannot be excluded that in our raw samples, furosemide concentrations could be underestimated for similar reasons, and consequently, the removal percentage as well. Overall, the WWTP appeared to be relatively effective in removing furosemide as well as its TPs, thus showing that there was no additional production of the targeted TPs during the biological process.

3.2. Occurrence in residential care home wastewater

The concentration of furosemide and its TPs was then evaluated in RCH wastewater samples (Table 4). We anticipated this type of sample to be highly-charged in furosemide, as this medication is frequently prescribed at high doses to elderly people affected by chronic and age-related diseases such as hypertension and heart failure. Effluents from three different RCH were analyzed. In all three samples, significant amounts of furosemide were found, and to a lesser extent, of pyridinium of furosemide and saluamine, but their concentrations and proportions relative to each other were variable.

Furosemide concentration variation across the three sites could be directly related to their hosting capacity. The highest furosemide concentration was found in RCH2 which also had the highest hosting capacity and on the contrary, the lowest concentration was observed in RCH3 which also has the lowest number of beds. On the other hand, Gómez-Canela et al. (2019) showed that there were quite large furosemide concentration variations (of a few thousand nanograms per liter) within the same institution over several samples, thus making it more relevant in this case to discuss the orders of magnitude. The concentrations of furosemide in the RCH1 and RCH2 samples were quite close, but the concentration of saluamine was 2-fold higher in RCH2. The higher proportion of saluamine in RCH2 could be explained by a greater biological activity which could lead to a more important biodegradation of furosemide (Laurencé et al., 2014; Olvera-Vargas et al., 2016). The proportion of pyridinium of furosemide was also much lower in RCH2, implying a preferential transformation of furosemide to saluamine in this case. This difference could be explained by the neutral pH (7.10 at 20 °C) indeed the formation of saluamine is promoted in acidic conditions (Bundgaard et al., 1988; Andreasen et al., 1982).

Few studies have investigated the presence of furosemide in non-

Table 3
Concentrations (in $ng.L^{-1}$) of furosemide in WWTP effluents.

France[a]	Spain[b]	Portugal[c]	Italy[d]	Poland[e]	Denmark[f]	Sweden[g]	Czech Republic[h]	Greece[i]	Canada[j]	Mexico[k]	Tunisia[l]	Japan[m]	Malaysia[n]
2622	1604	2214	903	2670	612	410	1250	9965	1940	80	67	184	819

[a] Tracol and Duchemin, 2016.
[b] Celic et al. 2019
[c] Santos et al., (2013).
[d] Castiglioni et al., (2018).
[e] Giebułtowicz et al., (2016).
[f] Huber et al., (2016).
[g] Frieberg, 2018.
[h] Diaz-Sosa et al., (2020).
[i] Papageorgiou et al., (2016).
[j] Kleywegt et al., 2016.
[k] Estrada-Arriaga et al., (2016).
[l] Afsa et al., 2020.
[m] Hanamoto et al., (2018).
[n] Al-Odaini et al., (2013).

Table 4
Concentration of furosemide, saluamine and pyridinium of furosemide in residential care homes wastewater in dissolved and particulate phases.

	RCH1			RCH2			RCH3
	Dissolved phase ($ng.L^{-1}$)	Particulate phase ($ng.g^{-1}$)	particulate phase related to SM ($ng.L^{-1}$)	Dissolved phase ($ng.L^{-1}$)	Particulate phase ($ng.g^{-1}$)	particulate phase related to SM ($ng.L^{-1}$)	Dissolved phase ($ng.L^{-1}$)
Furosemide	65 108	160	17.8	69 762	150	26.6	37 038
Saluamine	4947	2160	244.8	8841	510	91.1	5498
Pyridinium of Furosemide	4070	260	29.5	274	10	2.1	1280

treated effluents of RCH. Gómez-Canela et al. (2019) reported concentrations up to 3200 $ng.L^{-1}$ and 3400 $ng.L^{-1}$ in two different senior residence effluents in Spain with a capacity of 103 and 96 beds, respectively, and Nagarnaik et al. (2010) found 1031 $ng.L^{-1}$ in nursing care facility effluents which were considerably lower than the concentrations in this study for an equivalent hosting capacity. Slightly higher concentrations (median 6120 $ng.L^{-1}$ and maximum 25 100 $ng.L^{-1}$) were found by Kleywegt et al. (2016) in a long care center for senior residents and other clients in Canada but with a much larger capacity (289 beds).

Furosemide and its major degradation products were also analyzed in the solid fraction of RCH1 and RCH2 samples (Table 4). Although the three compounds were difficult to analyze because of an important matrix effect, significant concentrations (several hundreds of nanograms per gram) were found.

Saluamine has only been detected once in natural sediments doped with furosemide (Li et al., 2014) but our present work uncovers for the first time the presence of pyridinium of furosemide in the solid fraction. In sediments, furosemide concentrations of 7, 98, and 350 ng g^{-1} were reported (Ferreira da Silva et al. (2011), Ferrari et al. (2011), and Björklund et al. (2016) respectively). The concentrations obtained in our study are closer to those obtained in WWTP sludge, where the concentrations reached up to 686 ng g^{-1} (Huber et al., 2016) and 3602 ng g^{-1} (Salgado et al., 2011). These values indicate that a significant fraction of furosemide is adsorbed onto the particulate phase. The partition coefficients between the liquid and solid phase (Kd) are good indicators of the compounds affinity with the solid phase. The Kd values of the suspended matter of the samples RCH1 and RCH2 were 7.7 and 5.4 L kg^{-1} respectively. Jelic et al. (2012) found a Kd of 43 L kg^{-1} for furosemide in the solid phase of WWTP effluent which is 5–8 fold higher than the values found in this study. The Kd value is very dependent on the matrix considered. For example, in sediments, Björklund et al. (2016) found a Kd of 2517 L kg^{-1} which shows a very good affinity of furosemide for sediments whereas Jelic et al. (2012) found a lower Kd in thickened sludge (127 L kg^{-1}) and digested sludge (110 L kg^{-1}). In a Membrane BioReactor (MBR) or Anaerobic-Anoxic-Aerobic system (A2O) -treated sludge, Park et al. (2017) obtained a Kd of 2.1, which is quite low in comparison. Narumiya et al. (2013) showed a decrease of the Kd for furosemide before and after sludge digestion. In sample RCH1, the Kd value of pyridinium of furosemide (12.5 L kg^{-1}) was quite close to the one of furosemide, but was much lower in RCH2 (0.36 L kg^{-1}), which shows a much better affinity of furosemide for the solid phase in sample 1. Kd values for saluamine were more important (103.7 and 18.3 L kg^{-1}), meaning that a significant fraction of saluamine was adsorbed on the solid phase. Thus, total saluamine load in wastewater could be largely underestimated when only performing the conventional analysis of the dissolved phase.

3.3. Advanced oxidation in pure water and in residential care houses wastewater

3.3.1. Oxidation of furosemide in ultrapure water

The partial resistance of hundreds of compounds to conventional wastewater treatments highlights the need to go further in the degradation processes. We chose two AOPs which have the potential to upgrade WWTPs worldwide and are already used in some countries like Japan or Switzerland (Prasse et al., 2015). Chlorination oxidation experiments were also performed (Figs. S3 and S4 and Table S1). Both oxidation processes have been used for a long time for disinfection and the oxidants produced are likely to react with furosemide. The photodegradation of furosemide has been known for several decades. UV irradiation leads to the substitution of chlorine by a hydroxyl group, to the hydrolysis of the furfuryl group, that results in the production of furfural and saluamine, or the oxidation of the sulfamoyl group (Bundgaard et al., 1988; Moore and Burt, 1981). Furosemide is also degraded in sunlight or artificial laboratory light which makes it a good candidate for UV degradation (Starling et al., 2019). The use of ozonation has increased in recent years for the treatment of wastewaters due to its effectiveness in removing organic compounds. As an electrophilic agent, the presence of electron-rich moieties such as aromatic compounds determines the reactivity with ozone (Lim et al., 2022). According to Zoumpouli et al. (2021), furosemide is expected to present an important reactivity with ozone due to the presence of the furan ring and the aniline group. However, the potential formation of furosemide TPs, especially pyridinium of furosemide, remains unknown for AOPs. AOPs are known to generate a variety of TPs that are potentially hazardous to ecosystems, and whose identification is a challenge due to the lack of analytical standards. Furosemide degradation was thus first investigated in ultrapure water spiked with furosemide. Pyridinium of furosemide was quantified after the oxidation, and aliquots were also taken to monitor the formation of unknown TPs by high-resolution mass spectrometry.

As anticipated, the two AOPs were effective in degrading furosemide under our experimental conditions. Ozonation was the most efficient (see Supplementary Information Figs. S1 and S2).

The pyridinium of furosemide was produced during both oxidation experiments (Table 5). During the UV/H_2O_2 oxidation, the aliquot taken at 210 min already presented a few micrograms per liter of pyridinium with 1.5% conversion rate of furosemide. This rate reached 5.8% after 300 min. However, during this 5 h-time lapse, less than 10% of furosemide was degraded, which means that pyridinium formation is slow, probably because it involves reaction intermediates (Olvera-vargas et al., 2016; Zoumpouli et al., 2021). Pyridinium of furosemide was also produced by ozonation within a few minutes. Its production started immediately after ozone addition, and its concentration increased gradually until 4 min, with a furosemide conversion rate of 3.2% which remained unchanged after 10 min, in conjunction with a total consumption of ozone and furosemide concentration reaching a plateau. As in the UV/H_2O_2 experiment, pyridinium of furosemide did not seem to be degraded by the ozone treatment. Pyridinium of furosemide was also

Table 5
Pyridinium of furosemide concentration and furosemide percentage of conversion during oxidation experiments.

	Time (min)	Concentration ($\mu g.L^{-1}$)	Conversion (%)
UV/H_2O_2	210	15.05	1.5
	300	58.43	5.8
Ozonation (3 $mg.L^{-1}$)	1	35.84	1.2
	2	58.17	1.9
	4	95.61	3.2
	10	93.29	3.1

detected after chlorination (35 $mgCl_2.L^{-1}$) of furosemide (3 $mg.L^{-1}$), with a maximum conversion rate of 1.4% after only 2 min of reaction time, but contrary to the other oxidation processes, its concentration quickly decreased after 5 min, probably because of the formation of chlorinated derivatives (Table S1). This result might be of importance for the quality of drinking water produced from resources impacted by furosemide, even though the fast degradation of pyridinium of furosemide seems to indicate a low risk of exposure.

3.3.2. *Oxidation of residential care houses wastewater*

In order to get insights on the AOPs efficiencies with furosemide heavily-loaded real samples and on the formation of TPs in the presence of wastewater matrix components, we next subjected the effluents from the three RCH samples to the two AOPs (Fig. 2). The overall abatement was quite variable from one sample to another and for the different compounds, depending on the water parameters and the oxidation process used. Ozonation was very effective at removing the three pollutants, with an important reduction (below quantification limit) of furosemide and pyridinium of furosemide for the three tested samples (Fig. 2). As for chlorination (supplementary data, Figs. S4 and SI), abatement rates of almost 100% were observed for furosemide and pyridinium. These results were thus consistent with the experiments conducted with ultrapure water. Although the lifetime of ozone in WWTP effluent is shorter because of background absorptions (Lim et al., 2022), ozone was injected continuously in the samples and therefore furosemide reached higher removals than in the ultra-pure water experiment (Figs. S2 and SI). This could also explain why pyridinium of furosemide was not observed in the ozonated samples, a higher ozone dose probably leading to its decomposition.

Our results are in accordance with those reported in the litterature. Muñoz et al. (2009) showed a 100% removal of furosemide and Gómez et al. (2008) a removal of 99% for both O_3 and O_3 with H_2O_2 treatments. Ikonen et al. (2021) obtained a reduction of 99.7% of furosemide in wastewater effluents and Lee et al. (2012) observed a reduction to below their detection limits with a dose of 2 mg $O_3.L^{-1}$. Experiments combining ozonation with other treatments such as biological activated carbon (Reungoat et al., 2012) or ultrasonication (Ibáñez et al., 2013) did not significantly improve the removal efficiency and showed that the ozone treatment is responsible for most of the elimination of furosemide.

Contrary to furosemide and pyridinium, saluamine was less removed and its removal rate was variable depending on the sample. Saluamine was well degraded in the RCH3 sample, but higher concentrations of saluamine were observed from RCH1 and RCH2 samples after ozonation. This production of saluamine could be explained by the conversion of both furosemide and pyridinium to saluamine in the conditions of these samples (e.g., different initial concentrations, competition reactions of ozone with wastewater constituents such as nitrites or organic matter leading to a decrease in saluamine decomposition). Contrary to experiments in ultrapure water, no additional formation of pyridinium was observed, and all pyridinium already present in the RCH samples was degraded by ozone.

Furosemide removal by UV/H_2O_2 was between 70 and 80% for all

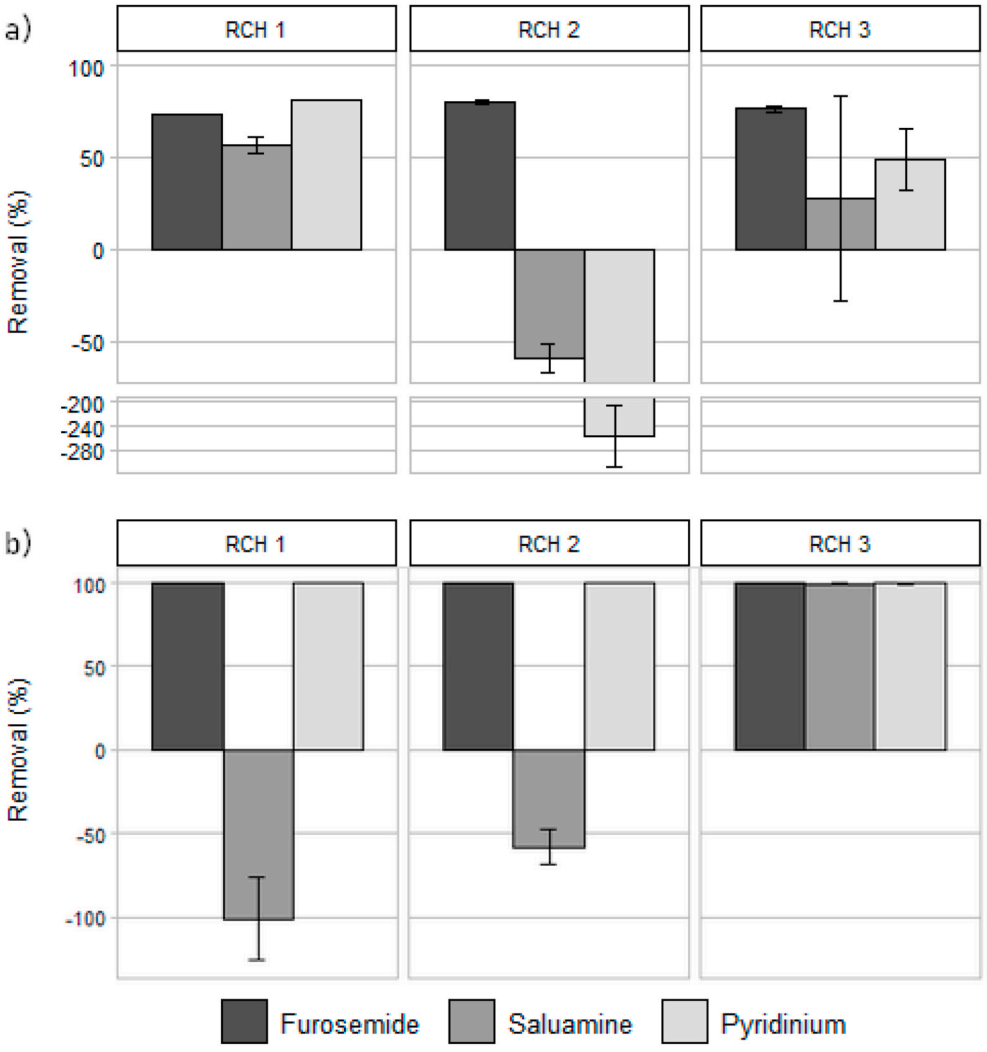


Fig. 2. Effectiveness of a) UV/H_2O_2 and b) ozonation on residential care home (RCH) discharge water. The bars represent removal efficiency in percentage calculated from initial concentration for each compound (dark grey = furosemide, medium grey = saluamine, light grey = Pyridinium of furosemide). Error bars represent standard error. For Ozonation, the samples were exposed for 1.5 h with 2 $L.h^{-1}$ O_3. For UV/H_2O_2, the samples were exposed for 5H to (0.375xCOD value)$mg.L^{-1}$ H_2O_2.

the samples, which is very close to the 82% removal found by Jie (2012) in hospital wastewater. However, the effectiveness of UV/H_2O_2 on furosemide elimination is still uncertain. Park et al. (2017) described the use of UV irradiation alone as not very effective on pharmaceuticals and personal care products, and only achieved 23% of removal of furosemide in WWTP post-treatment. However, as the treatment was performed during a purification process in a WWTP, the suspended particles can interfere and reduce the effectiveness of UV as compared to laboratory experiments performed with filtered samples. With UV/H_2O_2, Ikonen et al. (2021) found no removal of furosemide while Singh et al. (2015) observed a complete degradation, but with a lamp 8 times more powerful (Joule/cm^2). The efficiency of furosemide degradation by UV/H_2O_2 thus seems to be strongly dependent on the UV irradiation intensity. Similarly to ozonation and chlorination, large differences of removal were observed between the three samples for the two degradation products.

3.4. Generation of furosemide new transformation by-products

As these advanced oxidation processes have been reported to generate numerous by-products by reaction with organic contaminants, many of them being often potentially more toxic than their parent molecules (reviewed in Prasse et al., 2015), the formation of other transformation products of furosemide was investigated after the oxidation experiments by high-resolution mass spectrometry (HRMS). Noteworthy, two other by-products of furosemide were identified: one during chlorination (Fig. 2 e.) and one during UV/H_2O_2 treatment (Fig. 3**f.**).

To our knowledge, the product 3.**e.** was not clearly mentioned in the literature. However, Aalizadeh et al. (2019) found a by-product of furosemide of similar mass (*m/z* 276.99) after ozonation but they did not identify the structure. On the other hand, the compound 3.**f.** from UV/H_2O_2 oxidation has already been observed as a photodegradation product of furosemide after exposure to fluorescent lamps, and its structure was identified with ^{1}H NMR (Katsura et al., 2015). Its structure resembles the one of furosemide with *m/z* 311.03, except that the chlorine has been replaced by a hydroxyl group. In Jakimska et al. (2014), this product has been found after photodegradation experiments carried out on river water samples and was considered as one of the most persistent transformation products. These authors then found it in WWTP influents and effluents. In our study, the 3.**f** product formation kinetics was studied (see supplementary data, Fig. S4), showing that its degradation occurred after 1 h.

Jakimska et al. (2014) and Katsura et al. (2015) also identified saluamine and other degradation products (*m/z* 352, *m/z* 555, *m/z* 231, *m/z* 295, *m/z* 215) which not only corresponded to losses or substitution of Cl groups on furosemide or saluamine but also sometimes to recombination of fragments as in the study of Della-Greca et al. (2004), which identified a dimer (*m/z* 623) after photodegradation of furosemide. These by-products were not found in our oxidation experiments. The Della-Greca et al. (2004) dimer is larger than the other molecules and very polar: it is possible that our extraction method did not retain it. Moreover, these degradation products were observed after UV irradiation alone, thus it is conceivable that by combining UV with H_2O_2 treatment, they were further degraded as well, as described by (Starling et al., 2019). Indeed, these authors found similar degradation products of furosemide after UV and UV/H_2O_2 treatment, but they did not remain stable after H_2O_2 addition. If these compounds were present in our experiments, they may be below the limit of detection.

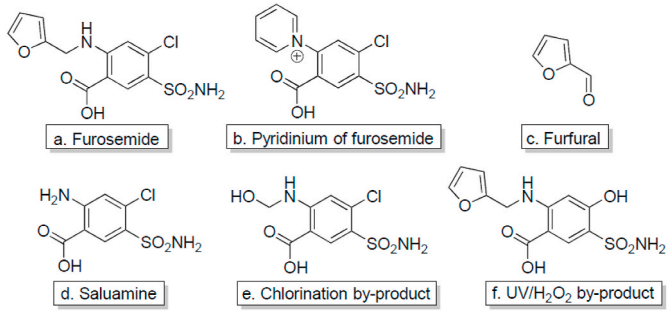


Fig. 3. Molecular Structure of a. furosemide (*m/z* 329.74) and its transformation products. b. Pyridinium of furosemide (*m/z* 328.73), c. Furfural (*m/z* 96.08), d. Saluamine (*m/z* 250.66). Transformation products identified by high-resolution mass spectrometry analysis: e. Chlorination by product *m/z* 276.99; f. UV/H_2O_2 by product *m/z* 311.03.

The *m/z* 276 and *m/z* 288 transformation products obtained after ozonation have also been mentioned in the literature but their structure could not be elucidated (Aalizadeh et al., 2019). In Zoumpouli et al. (2021), a molecule with a pyridinium structure (*m/z* 328) and a saluamine-like molecule (*m/z* 265) were also obtained by ozonation of the furan ring.

Taken together, our data show that ozonation, UV/H_2O_2 and chlorination degrade furosemide, generating several different by-products. High oxidant doses were chosen to maximize the formation of transformation products in order to enhance their analytical detection and identification, so their presence should still be investigated at realistic doses (e.g., at a specific ozone dose <1 mgO_3/mgDOC) and at the industrial scale (Guillossou et al., 2020). Besides saluamine production, the pyridinium of furosemide was formed under all three conditions but was only degraded by chlorination. Furthermore, chlorination also generated a product (*m/z* 276) never described before, but which was degraded after a few hours. Another product (*m/z* 311), resulting from photodegradation and previously reported (Katsura et al., 2015), was also identified.

4. Conclusion

This study provides new findings on the occurrence and fate of furosemide from its origin to wastewater treatment plants. The known degradation products of furosemide, saluamine and pyridinium of furosemide were searched and quantified for the first time in real samples (WWTP inlets and outlets and RCH wastewaters). The Seine-Centre WWTP showed a good removal of furosemide (>70%), saluamine (>80%) and pyridinium (>90%). However, concentrations of several hundred ng.L^{-1} were found after treatment and are released into the aquatic environment. These concentrations sometimes exceed 10 µg.L^{-1} in the literature for furosemide and may pose a risk to ecosystems.

To limit the release of these pharmaceutical compounds in the environment, we investigated AOPs and the possible production of TPs. UV/H_2O_2, chlorination and ozonation were first shown to be effective in degrading furosemide in ultrapure water. In RCH wastewater, ozonation and chlorination showed complete degradation of furosemide and pyridinium, but saluamine was still present in the samples after treatment due to a possible production. UV/H_2O_2 showed variable removal rates depending on the compounds. Although these treatments appeared quite effective, new TPs were identified following the chlorination (*m/z* 276) and ozonation (*m/z* 311) processes. The elimination of pollutants is used as a criterion for the evaluation of AOPs, but the presence of TPs should also be considered. Before concluding on the effectiveness of these advanced treatments, the toxicity and persistence of the end-products should be investigated. Thus, our study underlines the necessity and the relevance to use approaches such as non-targeted analysis for the detection of emerging pollutants, to unveil new TPs, combined with toxicity assessments in order to improve the characterization and design of wastewater treatment processes. Keeping in mind that these data alone do not provide information on their toxic risk and cocktail effects, our study highlights the need to develop an integrative strategy coupling state-of-the art chemical analysis techniques with ecotoxicological tests with defined end-points.

Credit author statement

Fidji Sandre: Investigation, Visualization, Writing- original draft & Editing; Nina Huynh: Methodology, Investigation, Review & Editing; Emilie Caupos: Methodology, Investigation, Review & Editing; Lamyae El-Mrabet: Investigation, Review & Editing; Chandirane Partibane: Investigation, Review & Editing; Isabelle Lachaise: Investigation, Review & Editing; Christophe Pommier: Investigation, Review & Editing; Michael Rivard: Supervision, Review & Editing; Christophe Morin: Review & Editing; Régis Moilleron: Review & Editing; Julien Le Roux: Supervision, Conceptualization, Methodology, Review & Editing; Laure Garrigue-Antar: Supervision, Conceptualization, Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Thanks to SIAAP for providing the WWTP inlet and outlet samples and to PRAMMICS platform (OSU-EFLUVE UMS 3563) for the analytical equipment (UPLC-IMS-QTOF and AutoTrace SPE). The funding was provided by the OPUR program and WaterOmics project (ANR-17-CE34-0009-01). Thanks to the LDAR sampling service and specifically Benoît CHIRON and Jérôme PRADES for the residential care homes samples. Thanks to Université Paris-Est Créteil and to the French ministry of higher education research and innovation for F. Sandre PhD fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.138212.

References

Aalizadeh, R., Nika, M.-C., Thomaidis, N.S., 2019. Development and application of retention time prediction models in the suspect and non-target screening of emerging contaminants. J. Hazard Mater. 363, 277–285. https://doi.org/10.1016/j.jhazmat.2018.09.047.

Abbott, L.M., Kovacic, J., 2008. The pharmacologic spectrum of furosemide. J. Vet. Emerg. Crit. Care 18, 26–39. https://doi.org/10.1111/j.1476-4431.2007.00267.x.

Afsa, S., Hamden, K., Lara Martin, P.A., Mansour, H.B., 2020. Occurrence of 40 pharmaceutically active compounds in hospital and urban wastewaters and their contribution to Mahdia coastal seawater contamination. Environ. Sci. Pollut. Res. 27, 1941–1955. https://doi.org/10.1007/s11356-019-06866-5.

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Thomaidis, N.S., Xu, J., 2017. Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review. J. Hazard Mater. 323, 274–298. https://doi.org/10.1016/j.jhazmat.2016.04.045.

Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I., Surif, S., Abdulghani, M., 2013. The occurrence of human pharmaceuticals in wastewater effluents and surface water of Langat River and its tributaries, Malaysia. Int. J. Environ. Anal. Chem. 93, 245–264. https://doi.org/10.1080/03067319.2011.592949.

Al-Omar, I.A., Al-Ashban, R.M., Shah, A.H., 2009. Toxicity studies on 4-chloro-5-sulfamoylanthranilic acid the degradation product of a loop diuretic furosemide. Res. J. Pharmacol. 63–77, 4.

Andreasen, F., Botker, H., Lorentzen, K., 1982. In vitro studies on the hydrolysis of frusemide in gastrointestinal juices. Br. J. Clin. Pharmacol. 14, 306–309. https://doi.org/10.1111/j.1365-2125.1982.tb01984.x.

Aymerich, I., Acuña, V., Barceló, D., García, M.J., Petrovic, M., Poch, M., Rodriguez-Mozaz, S., Rodríguez-Roda, I., Sabater, S., von Schiller, D., Corominas, Ll, 2016. Attenuation of pharmaceuticals and their transformation products in a wastewater treatment plant and its receiving river ecosystem. Water Res. 100, 126–136. https://doi.org/10.1016/j.watres.2016.04.022.

Badia-Fabregat, M., Lucas, D., Gros, M., Rodríguez-Mozaz, S., Barceló, D., Caminal, G., Vicent, T., 2015. Identification of some factors affecting pharmaceutical active compounds (PhACs) removal in real wastewater. Case study of fungal treatment of reverse osmosis concentrate. J. Hazard Mater. 283, 663–671. https://doi.org/10.1016/j.jhazmat.2014.10.007.

Baken, K.A., Sjerps, R.M.A., Schriks, M., van Wezel, A.P., 2018. Toxicological risk assessment and prioritization of drinking water relevant contaminants of emerging concern. Environ. Int. 118, 293–303. https://doi.org/10.1016/j.envint.2018.05.006.

Banjac, Z., Ginebreda, A., Kuzmanovic, M., Marcé, R., Nadal, M., Riera, J.M., Barceló, D., 2015. Emission factor estimation of ca. 160 emerging organic microcontaminants by inverse modeling in a Mediterranean river basin (Llobregat, NE Spain). Sci. Total Environ. 520, 241–252. https://doi.org/10.1016/j.scitotenv.2015.03.055.

Baresel, C., Harding, M., Junestedt, C., 2019. Removal of Pharmaceutical Residues from Municipal Wastewater Using UV/H2O2, vol. 28. Repport from Swedish Environmental Research Institute, 2019.

Besse, J.-P., Garric, J., 2008. Human pharmaceuticals in surface waters. Toxicol. Lett. 176, 104–123. https://doi.org/10.1016/j.toxlet.2007.10.012.

Björklund, E., Svahn, O., Bak, S., Bekoe, S.O., Hansen, M., 2016. Pharmaceutical residues affecting the UNESCO biosphere reserve kristianstads vattenrike wetlands: sources and sinks. Arch. Environ. Contam. Toxicol. 71, 423–436. https://doi.org/10.1007/s00244-016-0303-7.

Bundgaard, H., Nørgaard, T., Nielsen, N.M., 1988. Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions. Int. J. Pharm. 42, 217–224. https://doi.org/10.1016/0378-5173(88)90178-0.

Cantwell, M.G., Katz, D.R., Sullivan, J.C., Shapley, D., Lipscomb, J., Epstein, J., Juhl, A. R., Knudson, C., O'Mullan, G.D., 2018. Spatial patterns of pharmaceuticals and wastewater tracers in the Hudson River Estuary. Water Res. 137, 335–343. https://doi.org/10.1016/j.watres.2017.12.044.

Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D., Zuccato, E., 2006. Removal of pharmaceuticals in sewage treatment plants in Italy. Environ. Sci. Technol. 40, 357–363. https://doi.org/10.1021/es050991m.

Castiglioni, S., Davoli, E., Riva, F., Palmiotto, M., Camporini, P., Manenti, A., Zuccato, E., 2018. Mass balance of emerging contaminants in the water cycle of a highly urbanized and industrialized area of Italy. Water Res. 131, 287–298. https://doi.org/10.1016/j.watres.2017.12.047.

Čelić, M., Gros, M., Farré, M., Barceló, D., Petrović, M., 2019. Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). Sci. Total Environ. 652, 952–963. https://doi.org/10.1016/j.scitotenv.2018.10.290.

Chiffre, A., Degiorgi, F., Buleté, A., Spinner, L., Badot, P.-M., 2016. Occurrence of pharmaceuticals in WWTP effluents and their impact in a karstic rural catchment of Eastern France. Environ. Sci. Pollut. Control Ser. 23, 25427–25441. https://doi.org/10.1007/s11356-016-7751-5.

Cizmas, L., Sharma, V.K., Gray, C.M., McDonald, T.J., 2015. Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. Environ. Chem. Lett. 13, 381–394. https://doi.org/10.1007/s10311-015-0524-4.

Collado, N., Rodriguez-Mozaz, S., Gros, M., Rubirola, A., Barceló, D., Comas, J., Rodriguez-Roda, I., Buttiglieri, G., 2014. Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. Environ. Pollut. 185, 202–212. https://doi.org/10.1016/j.envpol.2013.10.040.

Cruz-Morató, C., Lucas, D., Llorca, M., Rodriguez-Mozaz, S., Gorga, M., Petrovic, M., Barceló, D., Vicent, T., Sarrà, M., Marco-Urrea, E., 2014. Hospital wastewater treatment by fungal bioreactor: removal efficiency for pharmaceuticals and endocrine disruptor compounds. Sci. Total Environ. 493, 365–376. https://doi.org/10.1016/j.scitotenv.2014.05.117.

Della Greca, M., Iesce, M.R., Previtera, L., Rubino, M., Temussi, F., 2004. A new photoproduct of the drug furosemide in aqueous media. Environ. Chem. Lett. 2, 155–158. https://doi.org/10.1007/s10311-004-0080-9.

Delli Compagni, R., Gabrielli, M., Polesel, F., Turolla, A., Trapp, S., Vezzaro, L., Antonelli, M., 2020. Risk assessment of contaminants of emerging concern in the context of wastewater reuse for irrigation: an integrated modelling approach. Chemosphere 242, 125185. https://doi.org/10.1016/j.chemosphere.2019.125185.

Diaz-Sosa, V.R., Tapia-Salazar, M., Wanner, J., Cardenas-Chavez, D.L., 2020. Monitoring and ecotoxicity assessment of emerging contaminants in wastewater discharge in the city of prague (Czech republic). Water 12, 1079. https://doi.org/10.3390/w12041079.

Estrada-Arriaga, E.B., Cortés-Muñoz, J.E., González-Herrera, A., Calderón-Mólgora, C.G., de Lourdes Rivera-Huerta, Ma, Ramírez-Camperos, E., Montellano-Palacios, L., Gelover-Santiago, S.L., Pérez-Castrejón, S., Cardoso-Vigueros, L., Martín-Domínguez, A., García-Sánchez, L., 2016. Assessment of full-scale biological nutrient removal systems upgraded with physico-chemical processes for the removal of emerging pollutants present in wastewaters from Mexico. Sci. Total Environ. 571, 1172–1182. https://doi.org/10.1016/j.scitotenv.2016.07.118.

Feo, M.L., Bagnati, R., Passoni, A., Riva, F., Salvagio Manta, D., Sprovieri, M., Traina, A., Zuccato, E., Castiglioni, S., 2020. Pharmaceuticals and other contaminants in waters and sediments from Augusta Bay (southern Italy). Sci. Total Environ. 739, 139827 https://doi.org/10.1016/j.scitotenv.2020.139827.

Ferrari, F., Gallipoli, A., Balderacchi, M., Ulaszewska, M.M., Capri, E., Trevisan, M., 2011. Exposure of the main Italian river basin to pharmaceuticals. J. Toxicol. 1–11. https://doi.org/10.1155/2011/989270, 2011.

Ferreira da Silva, B., Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85, 1331–1339. https://doi.org/10.1016/j.chemosphere.2011.07.051.

Frieberg, K., 2018. Effects of Effluent Wastewater in Developing Zebrafish (Danio rerio). Report from Uppsala University.

Giebułtowicz, J., Stankiewicz, A., Wroczyński, P., 2016. Occurrence of cardiovascular drugs in the sewage-impacted Vistula River and in tap water in the Warsaw region

(Poland). Environ. Sci. Pollut. Control Ser. 23, 24337–24349. https://doi.org/10.1007/s11356-016-7668-z.
Gómez, M.J., Martínez Bueno, M.J., Agüera, A., Hernando, M.D., Fernández-Alba, A.R., Mezcua, M., 2008. Evaluation of ozone-based treatment processes for wastewater containing microcontaminants using LC-QTRAP-MS and LC-TOF/MS. Water Sci. Technol. 57, 41–48. https://doi.org/10.2166/wst.2008.809.
Gómez-Canela, C., Sala-Comorera, T., Pueyo, V., Barata, C., Lacorte, S., 2019. Analysis of 44 pharmaceuticals consumed by elderly using liquid chromatography coupled to tandem mass spectrometry. J. Pharmaceut. Biomed. Anal. 168, 55–63. https://doi.org/10.1016/j.jpba.2019.02.016.
Gros, M., Petrović, M., Ginebreda, A., Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 36, 15–26. https://doi.org/10.1016/j.envint.2009.09.002.
Guillossou, R., Le Roux, J., Brosillon, S., Mailler, R., Vulliet, E., Morlay, C., Nauleau, F., Rocher, V., Gaspéri, J., 2020. Benefits of ozonation before activated carbon adsorption for the removal of organic micropollutants from wastewater effluents. Chemosphere 245, 125530. https://doi.org/10.1016/j.chemosphere.2019.125530.
Guillossou, R., Le Roux, J., Mailler, R., Vulliet, E., Morlay, C., Nauleau, F., Gasperi, J., Rocher, V., 2019. Organic micropollutants in a large wastewater treatment plant: what are the benefits of an advanced treatment by activated carbon adsorption in comparison to conventional treatment? Chemosphere 218, 1050–1060. https://doi.org/10.1016/j.chemosphere.2018.11.182.
Guo, Y., Yang, L., Wang, X., 2012. The application and reaction mechanism of catalytic ozonation in water treatment. Journal of Environmental & oJ Analytical Toxicology 2. https://doi.org/10.4172/2161-0525.1000150.
Hanamoto, S., Nakada, N., Yamashita, N., Tanaka, H., 2018. Source estimation of pharmaceuticals based on catchment population and in-stream attenuation in Yodo River watershed, Japan. Sci. Total Environ. 615, 964–971. https://doi.org/10.1016/j.scitotenv.2017.10.013.
Hezari, M., Davis, P.J., 1992. N-dealkylation of Furosemide to yield the mammalian metabolite CSA using Cunninghamella elegans. Drug Metabol. Dispos. 20, 92.
Hijnen, W.A.M., Beerendonk, E.F., Medema, G.J., 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. Water Res. 40, 3–22. https://doi.org/10.1016/j.watres.2005.10.030.
Huber, S., Remberger, M., Kaj, L., Schlabach, M., Jörundsdóttir, H.Ó., Vester, J., Arnórsson, M., Mortensen, I., Schwartson, R., Dam, M., 2016. A first screening and risk assessment of pharmaceuticals and additives in personal care products in waste water, sludge, recipient water and sediment from Faroe Islands, Iceland and Greenland. Sci. Total Environ. 562, 13–25. https://doi.org/10.1016/j.scitotenv.2016.03.063.
Ibáñez, M., Gracia-Lor, E., Bijlsma, L., Morales, E., Pastor, L., Hernández, F., 2013. Removal of emerging contaminants in sewage water subjected to advanced oxidation with ozone. J. Hazard Mater. 260, 389–398. https://doi.org/10.1016/j.jhazmat.2013.05.023.
Ikonen, J., Nuutinen, I., Niittynen, M., Hokajärvi, A.-M., Pitkänen, T., Antikainen, E., Miettinen, I.T., 2021. Presence and reduction of anthropogenic substances with UV light and oxidizing disinfectants in wastewater—a case study at kuopio. Finland. Water 13, 360. https://doi.org/10.3390/w13030360.
Jakimska, A., Śliwka-Kaszyńska, M., Reszczyńska, J., Namieśnik, J., Kot-Wasik, A., 2014. Elucidation of transformation pathway of ketoprofen, ibuprofen, and furosemide in surface water and their occurrence in the aqueous environment using UHPLC-QTOF-MS. Anal. Bioanal. Chem. 406, 3667–3680. https://doi.org/10.1007/s00216-014-7614-1.
Jelic, A., Fatone, F., Di Fabio, S., Petrovic, M., Cecchi, F., Barcelo, D., 2012. Tracing pharmaceuticals in a municipal plant for integrated wastewater and organic solid waste treatment. Sci. Total Environ. 433, 352–361. https://doi.org/10.1016/j.scitotenv.2012.06.059.
Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., Barcelo, D., 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res. 45, 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010.
Jie, L., 2012. Potential of Advanced Wastewater Treatment Processes to Remove Pharmaceutical Compounds under PILLS/SLIK Project.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 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. https://doi.org/10.1016/j.watres.2008.10.047.
Katsura, S., Yamada, N., Nakashima, A., Shiraishi, S., Furuishi, T., Ueda, H., 2015. Identification of furosemide photodegradation products in water–acetonitrile mixture. Chem. Pharm. Bull. 63, 617–627. https://doi.org/10.1248/cpb.c15-00122.
Kleywegt, S., Pileggi, V., Lam, Y.M., Elises, A., Puddicomb, A., Purba, G., Di Caro, J., Fletcher, T., 2016. The contribution of pharmaceutically active compounds from healthcare facilities to a receiving sewage treatment plant in Canada: contribution of pharmaceutically active compounds to STP effluent. Environ. Toxicol. Chem. 35, 850–862. https://doi.org/10.1002/etc.3124.
Kot-Wasik, A., Jakimska, A., Śliwka-Kaszyńska, M., 2016. Occurrence and seasonal variations of 25 pharmaceutical residues in wastewater and drinking water treatment plants. Environ. Monit. Assess. 188, 661. https://doi.org/10.1007/s10661-016-5637-0.
Laurencé, C., Rivard, M., Lachaise, I., Bensemhoun, J., Martens, T., 2011. Preparative access to transformation products (TPs) of furosemide: a versatile application of anodic oxidation. Tetrahedron 67, 9518–9521. https://doi.org/10.1016/j.tet.2011.10.006.
Laurencé, C., Rivard, M., Martens, T., Morin, C., Buisson, D., Bourcier, S., Sablier, M., Oturan, M.A., 2014. Anticipating the fate and impact of organic environmental contaminants: a new approach applied to the pharmaceutical furosemide. Chemosphere 113, 193–199. https://doi.org/10.1016/j.chemosphere.2014.05.036.
Laurencé, C., Zeghbib, N., Rivard, M., Lehri-Boufala, S., Lachaise, I., Barau, C., Le Corvoisier, P., Martens, T., Garrigue-Antar, L., Morin, C., 2019. A new human pyridinium metabolite of furosemide, inhibitor of mitochondrial complex I, is a candidate inducer of neurodegeneration. Biochem. Pharmacol. 160, 14–23. https://doi.org/10.1016/j.bcp.2018.12.007.
Lee, C.O., Howe, K.J., Thomson, B.M., 2012. Ozone and biofiltration as an alternative to reverse osmosis for removing PPCPs and micropollutants from treated wastewater. Water Res. 46, 1005–1014. https://doi.org/10.1016/j.watres.2011.11.069.
Lee, Y., Kovalova, L., McArdell, C.S., von Gunten, U., 2014. Prediction of micropollutant elimination during ozonation of a hospital wastewater effluent. Water Res. 64, 134–148. https://doi.org/10.1016/j.watres.2014.06.027.
Li, Z., Maier, M.P., Radke, M., 2014. Screening for pharmaceutical transformation products formed in river sediment by combining ultrahigh performance liquid chromatography/high resolution mass spectrometry with a rapid data-processing method. Anal. Chim. Acta 810, 61–70. https://doi.org/10.1016/j.aca.2013.12.012.
Lim, S., Shi, J.L., von Gunten, U., McCurry, D.L., 2022. Ozonation of organic compounds in water and wastewater: a critical review. Water Res. 213, 118053 https://doi.org/10.1016/j.watres.2022.118053.
Machado, A.I., Dordio, A., Fragoso, R., Leitão, A.E., Duarte, E., 2017. Furosemide removal in constructed wetlands: comparative efficiency of LECA and Cork granulates as support matrix. J. Environ. Manag. 203, 422–428. https://doi.org/10.1016/j.jenvman.2017.08.002.
Matamoros, V., Arias, C., Brix, H., Bayona, J.M., 2009. Preliminary screening of small-scale domestic wastewater treatment systems for removal of pharmaceutical and personal care products. Water Res. 43, 55–62. https://doi.org/10.1016/j.watres.2008.10.005.
Mazhar, M.A., Khan, N.A., Ahmed, S., Khan, A.H., Hussain, A., Rahisuddin, Changani F., Yousefi, M., Ahmadi, S., Vambol, V., 2020. Chlorination disinfection by-products in municipal drinking water – a review. J. Clean. Prod. 273, 123159 https://doi.org/10.1016/j.jclepro.2020.123159.
Miklos, D.B., Remy, C., Jekel, M., Linden, K.G., Drewes, J.E., Hübner, U., 2018. Evaluation of advanced oxidation processes for water and wastewater treatment – a critical review. Water Res. 139, 118–131. https://doi.org/10.1016/j.watres.2018.03.042.
Moore, D., Burt, C., 1981. Photosensitization by drugs in surfactant solutions. Photochem. Photobiol. 34, 431–439.
Muñoz, I., Rodríguez, A., Rosal, R., Fernández-Alba, A.R., 2009. Life Cycle Assessment of urban wastewater reuse with ozonation as tertiary treatment. Sci. Total Environ. 407, 1245–1256. https://doi.org/10.1016/j.scitotenv.2008.09.029.
Munro, K., Martins, C.P.B., Loewenthal, M., Comber, S., Cowan, D.A., Pereira, L., Barron, L.P., 2019. Evaluation of combined sewer overflow impacts on short-term pharmaceutical and illicit drug occurrence in a heavily urbanised tidal river catchment (London, UK). Sci. Total Environ. 657, 1099–1111. https://doi.org/10.1016/j.scitotenv.2018.12.108.
Nagarnaik, P., Batt, A., Boulanger, B., 2010. Concentrations and mass loadings of cardiovascular pharmaceuticals in healthcare facility wastewaters. J. Environ. Monit. 12, 2112. https://doi.org/10.1039/c0em00216j.
Narumiya, M., Nakada, N., Yamashita, N., Tanaka, H., 2013. Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. J. Hazard Mater. 260, 305–312. https://doi.org/10.1016/j.jhazmat.2013.05.032.
Okoye, C.O., Okeke, E.S., Okoye, K.C., Echude, D., Andong, F.A., Chukwudozie, K.I., Okoye, H.U., Ezeonyejiaku, C.D., 2022. Occurrence and fate of pharmaceuticals, personal care products (PPCPs) and pesticides in African water systems: a need for timely intervention. Heliyon 8, e09143. https://doi.org/10.1016/j.heliyon.2022.e09143.
Olvera-Vargas, H., Leroy, S., Rivard, M., Oturan, N., Oturan, M., Buisson, D., 2016. Microbial biotransformation of furosemide for environmental risk assessment: identification of metabolites and toxicological evaluation. Environ. Sci. Pollut. Control Ser. 23, 22691–22700. https://doi.org/10.1007/s11356-016-7398-2.
Papageorgiou, M., Kosma, C., Lambropoulou, D., 2016. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. Sci. Total Environ. 543, 547–569. https://doi.org/10.1016/j.scitotenv.2015.11.047.
Papageorgiou, M., Zioris, I., Danis, T., Bikiaris, D., Lambropoulou, D., 2019. Comprehensive investigation of a wide range of pharmaceuticals and personal care products in urban and hospital wastewaters in Greece. Sci. Total Environ. 694, 133565 https://doi.org/10.1016/j.scitotenv.2019.07.371.
Park, J., 2016. Removal Characteristics and Predictive Model of Pharmaceutical and Personal Care Products (PPCPs) in Membrane Bioreactor (MBR) Process. Thesis from Kyoto University. https://doi.org/10.14989/doctor.k19984.
Park, J., Yamashita, N., Park, C., Shimono, T., Takeuchi, D.M., Tanaka, H., 2017. Removal characteristics of pharmaceuticals and personal care products: comparison between membrane bioreactor and various biological treatment processes. Chemosphere 179, 347–358. https://doi.org/10.1016/j.chemosphere.2017.03.135.
Pereira, V.J., Linden, K.G., Weinberg, H.S., 2007. Evaluation of UV irradiation for photolytic and oxidative degradation of pharmaceutical compounds in water. Water Res. 41, 4413–4423. https://doi.org/10.1016/j.watres.2007.05.056.
Prasse, C., Stalter, D., Schulte-Oehlmann, U., Oehlmann, J., Ternes, T.A., 2015. Spoilt for choice: a critical review on the chemical and biological assessment of current wastewater treatment technologies. Water Res. 87, 237–270. https://doi.org/10.1016/j.watres.2015.09.023.
Prieto-Rodriguez, L., Miralles-Cuevas, S., Oller, I., Agüera, A., Puma, G.L., Malato, S., 2012. Treatment of emerging contaminants in wastewater treatment plants (WWTP)

effluents by solar photocatalysis using low TiO2 concentrations. J. Hazard Mater. 211 (212), 131–137. https://doi.org/10.1016/j.jhazmat.2011.09.008.
Reed, N.R., Kwok, E.S.C., 2014. Furfural. In: Encyclopedia of Toxicology. Elsevier, pp. 685–688. https://doi.org/10.1016/B978-0-12-386454-3.00147-0.
Reungoat, J., Escher, B.I., Macova, M., Argaud, F.X., Gernjak, W., Keller, J., 2012. Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. Water Res. 46, 863–872. https://doi.org/10.1016/j.watres.2011.11.064.
Rozman, D., Hrkal, Z., Váňa, M., Vymazal, J., Boukalová, Z., 2017. Occurrence of pharmaceuticals in wastewater and their interaction with shallow aquifers: a case study of horní beřkovice, Czech republic. Water 9, 218. https://doi.org/10.3390/w9030218.
Salgado, R., Marques, R., Noronha, J.P., Mexia, J.T., Carvalho, G., Oehmen, A., Reis, M. A.M., 2011. Assessing the diurnal variability of pharmaceutical and personal care products in a full-scale activated sludge plant. Environ. Pollut. 159, 2359–2367. https://doi.org/10.1016/j.envpol.2011.07.004.
Sanches, S., Barreto Crespo, M.T., Pereira, V.J., 2010. Drinking water treatment of priority pesticides using low pressure UV photolysis and advanced oxidation processes. Water Res. 44, 1809–1818. https://doi.org/10.1016/j.watres.2009.12.001.
Santos, L.H.M.L.M., Gros, M., Rodriguez-Mozaz, S., Delerue-Matos, C., Pena, A., Barceló, D., Montenegro, M.C.B.S.M., 2013. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: identification of ecologically relevant pharmaceuticals. Sci. Total Environ. 461, 302–316. https://doi.org/10.1016/j.scitotenv.2013.04.077. –462.
Sarathy, S.R., Mohseni, M., 2006. An overview of UV-based advanced oxidation processes for drinking water treatment. IUVA News 7, 16–27.
Singh, A., Havixbeck, J.J., Smith, M.K., Shu, Z., Tierney, K.B., Barreda, D.R., El-Din, M. G., Belosevic, M., 2015. UV and hydrogen peroxide treatment restores changes in innate immunity caused by exposure of fish to reuse water. Water Res. 71, 257–273. https://doi.org/10.1016/j.watres.2015.01.003.
Stalter, D., O'Malley, E., von Gunten, U., Escher, B.I., 2016. Fingerprinting the reactive toxicity pathways of 50 drinking water disinfection by-products. Water Res. 91, 19–30. https://doi.org/10.1016/j.watres.2015.12.047.
Starling, M.C.V.M., Souza, P.P., Le Person, A., Amorim, C.C., Criquet, J., 2019. Intensification of UV-C treatment to remove emerging contaminants by UV-C/H2O2 and UV-C/S2O82−: susceptibility to photolysis and investigation of acute toxicity. Chem. Eng. J. 376, 120856 https://doi.org/10.1016/j.cej.2019.01.135.
Stefán, D., Erdélyi, N., Izsák, B., Záray, G., Vargha, M., 2019. Formation of chlorination by-products in drinking water treatment plants using breakpoint chlorination. Microchem. J. 149, 104008 https://doi.org/10.1016/j.microc.2019.104008.
Tracol, R., Duchemin, J., 2016. Evaluation de l'occurrence des residus de medicaments dans un echantillon de nappes souterraines vulnerables du bassin seine-normandie utilisees pour la production d'eau destinee a la consommation humaine. Repport DDASS Basse Normandie.
UNESCO, 2017. Pharmaceuticals in the aquatic environment of the Baltic Sea region – A status report. UNESCO Emerging Pollutants in Water Series – No, 1. UNESCO Publishing, Paris.
Vymazal, J., Dvořáková Březinová, T., Koželuh, M., Kule, L., 2017. Occurrence and removal of pharmaceuticals in four full-scale constructed wetlands in the Czech Republic – the first year of monitoring. Ecol. Eng. 98, 354–364. https://doi.org/10.1016/j.ecoleng.2016.08.010.
Wang, W.-L., Wu, Q.-Y., Huang, N., Xu, Z.-B., Lee, M.-Y., Hu, H.-Y., 2018. Potential risks from UV/H2O2 oxidation and UV photocatalysis: a review of toxic, assimilable, and sensory-unpleasant transformation products. Water Res. 141, 109–125. https://doi.org/10.1016/j.watres.2018.05.005.
White, D., Lapworth, D.J., Civil, W., Williams, P., 2019. Tracking changes in the occurrence and source of pharmaceuticals within the River Thames, UK; from source to sea. Environ. Pollut. 249, 257–266. https://doi.org/10.1016/j.envpol.2019.03.015.
Yang, X.-X., Hu, Z.-P., Alex Boelsterli, U., Zhou, S.-F., 2006. Drug acyl glucuronides: reactivity and analytical implication. Curr. Pharmaceut. Anal. 2, 259–277. https://doi.org/10.2174/157341206777934608.
Zhang, H., Zhang, Y., Shi, Q., Hu, J., Chu, M., Yu, J., Yang, M., 2012. Study on transformation of natural organic matter in source water during chlorination and its chlorinated products using ultrahigh resolution mass spectrometry. Environ. Sci. Technol. 46, 4396–4402. https://doi.org/10.1021/es203587q.
Zoumpouli, G.A., Zhang, Z., Wenk, J., Prasse, C., 2021. Aqueous ozonation of furans: kinetics and transformation mechanisms leading to the formation of α,β-unsaturated dicarbonyl compounds. Water Res. 203, 117487 https://doi.org/10.1016/j.watres.2021.117487.

Supplementary informations:

Text S1. Methodology for chlorination experiment. The Cl_2 concentration of a commercial solution was measured with a spectrophotometer at a wavelength of λ = 292 nm. The sample is agitated for 30 minutes and then the required amount of chlorine is added directly to the sample. For the furosemide degradation kinetics experiments, ultrapure water was spiked with 3 $mg.L^{-1}$ furosemide and chlorine was added at a concentration of 35 $mgCl_2.L^{-1}$. Aliquots were taken in 1 mL vials at 0, 2, 5, 60 minutes and 24 h. For the experiments on outlet water from residential care homes, chlorine was added directly to the beaker with the sample at a concentration equal to three times the DOC concentration. The samples were placed under stirring for 4 h. A measurement of the residual chlorine was performed after 4 h by taking 3 mL of the sample with 75 µL of 1.7 M acetic acid and 750 µL of 1 M potassium

$$[Cl_2](mM) = \frac{A \times (3+0.75+0.075) \times 1000}{3 \times \varepsilon \times d}$$

iodide.

A is the absorbance measured at λ = 351 nm, ε is the molar extinction coefficient (ε = 26 900 $M^{-1}.cm^{-1}$) and d is the dilution factor of the reaction medium to make the measurement. Residual chlorine was quenched by adding sodium thiosulfate in excess.

Figure S1. Furosemide degradation under UV-Light with hydrogen peroxide

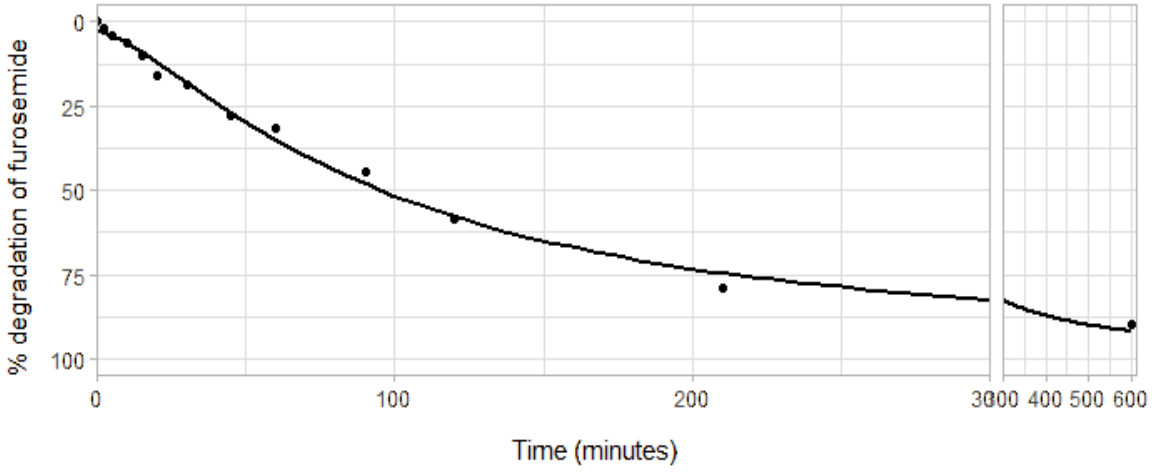


Figure S2. Furosemide degradation by ozonation and ozone consumption. (COLOR FIGURE)

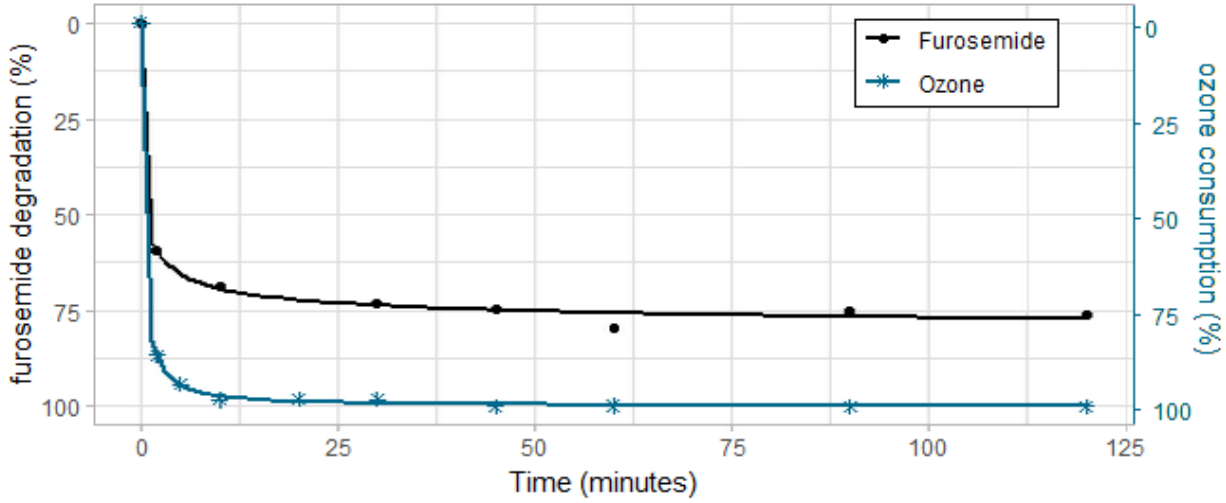
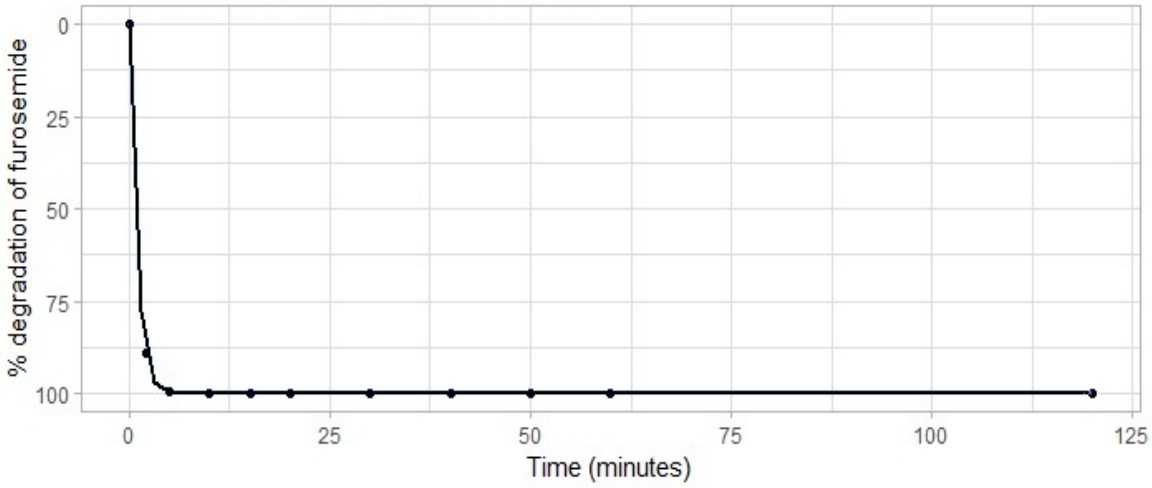


Figure S3. Furosemide degradation by chlorination



Chlorination is primarily used as a disinfectant, but is also an oxidizing agent capable of reacting with sulfur, primary and secondary amines or phenols groups of organic contaminants, thus eliminating them with variable efficiencies. Due to the presence of secondary amine, chlorine should react strongly with furosemide (Huerta-Fontela et al. 2011).

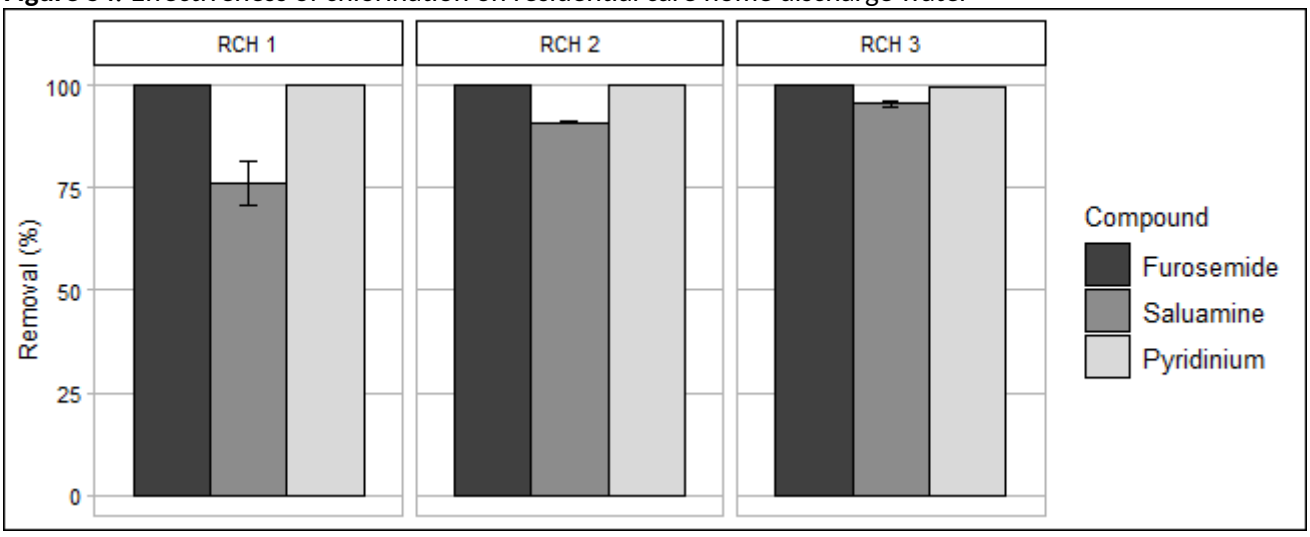
Huerta-Fontela, M., Galceran, M.T., Ventura, F., 2011. Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. Water Research 45, 1432–1442. https://doi.org/10.1016/j.watres.2010.10.036

Table S1. Pyridinium of furosemide concentration and percentage of conversion during chlorination

	Time (min)	Concentration (µg.L^{-1})	conversion (%)
Chlorination	2	41.20	1.4
	5	8.73	0.3

During chlorination, the pyridinium production seemed faster than with UV/H_2O_2, with a 1.4% furosemide conversion rate and a concentration of 41.2 µg.L^{-1} after only 2 minutes, which is consistent with the rapid degradation of furosemide in this condition (Fig. S1, SI). On the other hand, 3 minutes later, the pyridinium concentration was much lower. Thus, although it was formed rapidly, it also appeared to be removed rapidly by chlorination.

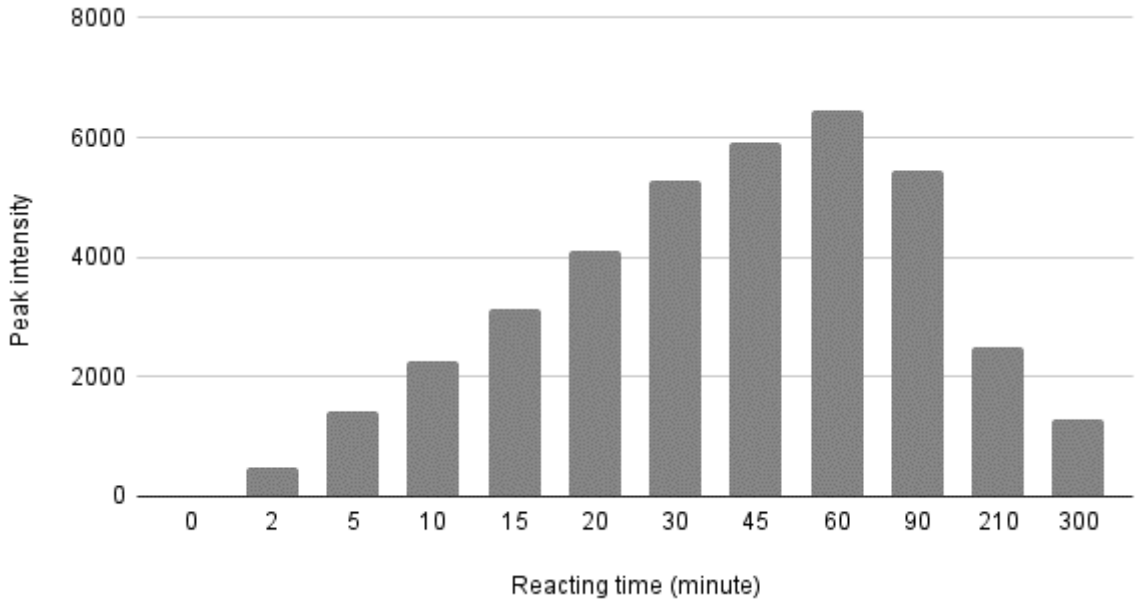
Figure S4. Effectiveness of chlorination on residential care home discharge water



The bars represent removal efficiency in percentage calculated from initial concentration for each compound (dark grey = furosemide, grey = saluamine, light grey = Pyridinium of furosemide). Error bars represent standard error. The samples were exposed for 4H to 1.3g.L^{-1} (3xCOD value).

Furosemide removal efficiency by chlorination was quite close to that found in the scientific literature. Huerta-Fontela et al. (2011) obtained an almost complete degradation of furosemide (>99%). As mentioned in part 2, the presence of primary and/or secondary amines on the three molecules which are very reactive with chlorine could explain their good degradation. The lower efficiency of chlorination observed for saluamine removal could be linked to the ability of both pyridinium and furosemide to degrade into saluamine, as shown in our earlier study (Laurencé et al. 2011). Thus, these transformations would result in an increase of this compound, that would attenuate the net effect of its removal by chlorination. Nevertheless, the efficiency of chlorination was similar to that observed in the reconstituted water experiments (S3). The complexity of the sample did not seem to impact the degradation process of furosemide.

Figure S5. Detection and monitoring of a transformation product after UV/H_2O_2 (product f.)



Monitoring of 2-(2-furylmethylamino)-4-hydroxy-5-sulfamoyl-benzoic acid (product f.) in negative mode. Mass= 311.0345 detected in NTS.

Table S2. *Retention time of each molecule and the corresponding internal standard, and the estimation of matrix effects for each internal standards: ME = 100 x (area in matrix / area in standard)*

	Electrospray	RT	ME (%)	ME SD
Pyridinium of Furosemide	+	1.0		
Atenolol-d7	+	2.1	85.6	36.8
Saluamine	+	2.5		
Sulfamethoxazole-d4	-	3.9	85.5	18.0
Furosemide	-	5.8		
Furosemide-d5	-	5.8	82.5	9.5

Table S3. *Acquisition parameters*

	Molecular mass (g.mol^{-1})	Mass of the analyzed ionic fragment (m/z)	Electrospray	Cone tension (V)	Collision energy (eV)	RT (min)	LOD instr (µg.L^{-1})	LOQ instr (µg.L^{-1})
Furosemide	330,74	204,84	-	32	20	5.70	0.890	2.966
Saluamine	250,66	233,10	+	20	16	2.4	7.941	26.470
Pyridinium of Furosemide	328,72	249,02	+	50	40	0.9	0.270	0.899

Cette étude fournit de nouveaux résultats sur l'occurrence et le devenir du furosémide depuis son origine jusqu'aux stations d'épuration des eaux usées. Dans les rejets d'EHPAD, le furosémide a été trouvé en forte concentration, allant de 37 000 ng/L à 70 000 ng/L, confirmant que l'échantillonnage de résidences médicalisées était un choix pertinent pour l'étude du furosémide. Les analyses en LC-MS/MS ont également montré, pour la première fois dans des échantillons d'eaux usées, la présence de pyridinium du furosémide, entre 300 et 4 000 ng/L, et de saluamine, entre 5 000 et 9 000 ng/L. Ces sous-produits ont également été quantifiés dans les eaux usées urbaines autour de 3 000 ng/L chacun, montrant une proportion plus importante des produits de transformations que dans les rejets d'EHPAD. Cette différence pourrait être expliquée par un temps de résidence plus long dans les réseaux d'eau usées par rapport aux échantillons issus des résidences médicalisées directement prélevés à la source, donnant ainsi plus de temps au furosémide de se dégrader en pyridinium ou saluamine (biodégradation, hydrolyse,..). Enfin, les trois composés ont également été quantifiés dans les rejets de STEU. Malgré une bonne élimination du furosémide (>70%), de la saluamine (>80%) et du pyridinium (>90%) dans la STEU de Seine-Centre (Colombes), des concentrations de plusieurs centaines de nanogrammes par litre sont rejetées dans le milieu naturel. Afin de limiter le rejet de ces composés dans l'environnement, il est nécessaire d'optimiser les procédés de traitement.

Par ailleurs, les procédés de traitement quaternaires étudiés dans cet article ont montré une efficacité variable. Les traitements par ozonation et chloration ont un taux d'élimination proche de 100% ce qui est pertinent avec les résultats obtenus dans la littérature scientifique (Cf chapitre I). Le traitement par UV/H_2O_2 en revanche s'est montré moins efficace (entre 70% et 80% d'abattement du furosémide) et très dépendant de la composition des échantillons. Dans la littérature, le taux d'élimination du furosémide par UV/H_2O_2 varie de 0 à 100% selon le type d'échantillon également, mais aussi en fonction des procédés en amont. La dégradation du furosémide est relativement bien documentée. En revanche, aucune donnée n'existait sur le pyridinium du furosémide et la saluamine. Globalement, les mêmes tendances ont été observées pour la dégradation du pyridinium que pour le furosémide, avec une dégradation proche de 100% par chloration et ozonation. Cette similitude pourrait être expliquée par le fait que les deux composés ont une structure proche et beaucoup de groupements fonctionnels en commun. La saluamine, en revanche, est systématiquement moins bien dégradée, voire même, produite, en particulier par l'ozonation. De plus, de nouveaux sous-produits ont été identifiés suite aux processus de chloration (276 m/z) et d'ozonation (311 m/z).

L'élimination des polluants est utilisée comme critère d'évaluation des ces procédés avancés, mais cette étude souligne que la présence des produits de dégradation doit également être considérée. Ceci souligne également la nécessité et la pertinence d'utiliser des approches telles que l'analyse non ciblée pour la détection de polluants émergents, combinées à des évaluations de toxicité afin d'améliorer la caractérisation et la conception des processus de traitement des eaux usées. Des procédés d'adsorption, comme le charbon actif, pourraient être des solutions prometteuses pour empêcher le relargage de nouveaux sous-produits, non caractérisés, et potentiellement délétères pour l'écosystème aquatique. L'absence de standards analytiques rend très difficile leur quantification, mais aussi leurs études toxicologiques. Empêcher leur formation semble donc être la meilleure alternative.

II. Mesure du furosémide dans le bassin Seine-Normandie

En France, la gestion des masses d'eau s'organise en fonction des bassins versants. Une agence de l'eau est associée à chacun de ces bassins, et fixe des objectifs environnementaux à atteindre via des Schémas Directeurs d'Aménagement et de Gestion des Eaux (SDAGE). Afin d'orienter les actions de ces SDAGE, des programmes de surveillance sont nécessaires. Un réseau de surveillance est alors mis en place pour chaque bassin hydrographique équipé de diverses stations de prélèvements pour effectuer un suivi de la qualité des eaux et évaluer la présence de polluants d'intérêt.

Les 41 substances listées par l'article 16 de la DCE sont systématiquement suivies. En fonction des bassins versants, et donc des SDAGES, d'autres polluants d'intérêt peuvent être ajoutés. Au niveau du bassin versant Seine-Normandie, par exemple, 286 paramètres sont suivis systématiquement issus des listes obligatoires et d'une liste spécifique comprenant notamment 32 résidus médicamenteux (comprenants quelques sous-produits) (Ethynyl estradiol, Carbamazépine, Diclofénac, Ibuprofène, Kétoprofène, Paracétamol, Sulfaméthoxazole, Acide fénofibrique, Oxazépam, Estrone, Norethindrone, Sotalol, Erythromycine, Ofloxacine, Clarithromycine, Ciprofloxacine, Acide diatrizoïque, Amiodarone, Tramadol, Carbamazépine époxide, Métronidazole, Trimétazidine, Cyclophosphamide, Metformine, O-Demethyltramadol, Carboxy Ibuprofen, Acide niflumique, Triclocarban, 1-Hydroxy Ibuprofen, 2-Hydroxy Ibuprofen, Azithromycine).

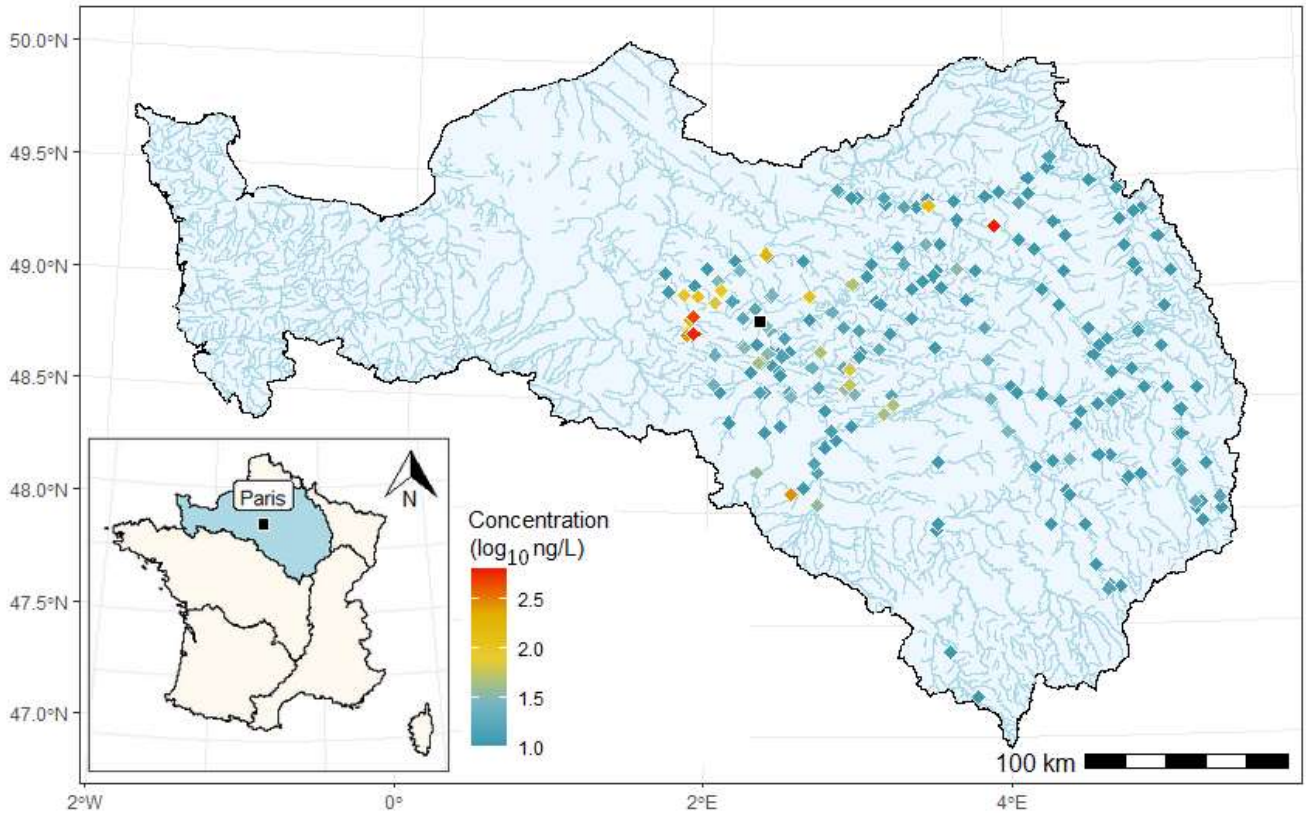


Figure 5. Concentrations de furosémide dans le bassin Seine-Normandie en 2019. La quantification du furosémide (code SANDRE 5364) est réalisée par HPLC-MS/MS par le laboratoire EUROFINS IPL EST EST avec une limite de détection de 0,007 µg/L et une limite de quantification de 0,02 µg/L. Les échantillons proviennent des préleveurs d'EUROFINS IPL EST EST et du département de Seine et Marne, prélevés en 2019. Les données brutes ont été fournies par l'agence de l'eau Seine-Normandie. La figure a été réalisée sous Rstudio et les fonds de cartes ont été téléchargés depuis geo.data.gouv.fr.

Ces polluants sont parfois également suivis dans le cadre de programmes de recherches pérennes associés à ce bassin et financés en partie par l'agence de l'eau Seine-Normandie comme le programme OPUR (auquel est rattachée cette thèse), le programme Piren-Seine, ou encore le GIP Seine aval. Ainsi, en 2019, le furosémide a également été suivi sur l'est du bassin de manière ponctuelle, et échantillonné sur 205 stations. Les résultats des mesures réalisées sur les prélèvements de 2019 sont présentés dans la **figure 5**.

Sur le bassin versant Seine-Normandie, près de 82% des sites échantillonnés en Ile-de-France et en amont présentent des concentrations inférieures à la limite de quantification de 20 ng/L, révélant des cours d'eau relativement épargnés par la contamination au furosémide et des concentrations environnementales assez faibles. Près de 17% des cours d'eau présentent des concentrations de furosémide comprises entre 20 et 100 ng/L, soit du même ordre de grandeur que celles relevées dans les rivières européennes (Cf. Revue, Chapitre I, concentrations médianes 78,5 ng/L).

Une dizaine de sites (1%) présentent cependant des valeurs supérieures à 100 ng/L. Une concentration maximale de 623 ng/L est observée au nord-est de Paris. Ce site est positionné sur la Vesle, juste en aval de Reims. Cette petite rivière est un affluent de l'Aisne, elle-même un affluent de l'Oise, qui se jette dans la Seine. En 2019, la ville comprenait 181 194 habitants, avec une proportion de de plus de 65 ans dans la moyenne nationale (21%) et fait partie des quelques villes du bassin versant avec une population supérieure à 2 000 habitants (<90%)[12]. La présence de plusieurs STEU rejetant dans la Vesle, en amont de la station de prélèvement, présentées en **figure 6**, pourrait expliquer la concentration importante de furosémide.

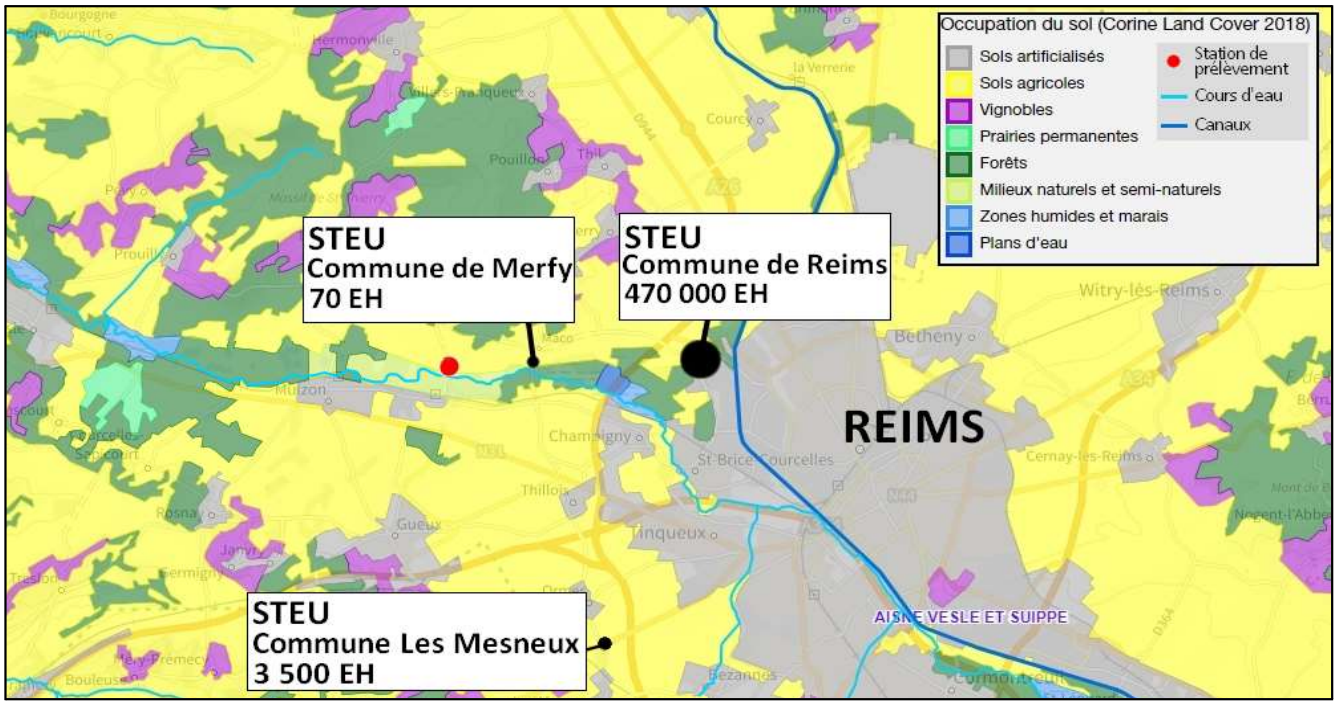


Figure 6. Potentielles sources de contamination en furosémide de la Vesle en région rémoise. Le fond de carte est issu du portail geo.eau-seine-normandie.fr et la position et capacité des STEU sont issues du portail assainissement.developpement-durable.gouv.fr. EH = Equivalent habitant.

[12] https://www.eau-seine-normandie.fr/

Des concentrations importantes sont retrouvées en Ile-de-France, notamment sur la Seine. La forte densité de population de l'agglomération parisienne pourrait expliquer des rejets plus importants en micropolluants par les stations de traitement des eaux usées, bien qu'elles aient des capacités de traitement importantes (Equivalent Habitant, EH) (voir **figure 7**).

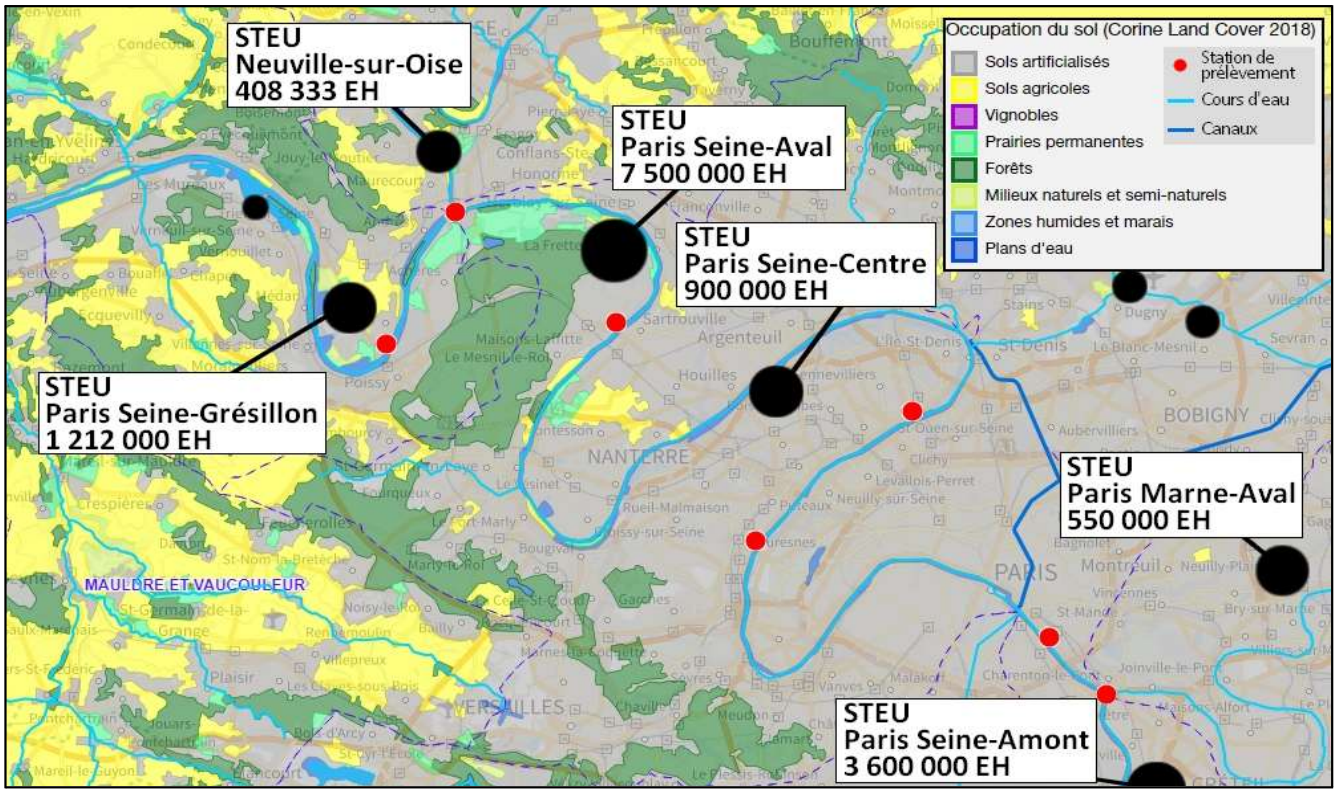


Figure 7. Potentielles sources de la contamination en furosémide de la Seine au niveau de l'agglomération parisienne. *Le fond de carte est issu du portail geo.eau-seine-normandie.fr et la position et capacité des STEU sont issues du portail assainissement.developpement-durable.gouv.fr. EH = Equivalent habitant.*

Les concentrations observées dans la Seine sont cependant moins importantes que sur la Vesle en aval de Reims, pour une densité de population bien supérieure. Le débit moyen de la Seine étant bien plus important que celui de la Vesle (393 m^3/s à Poissy contre 7,52 m^3/s sur la Vesle), la dilution des rejets de STEU est également plus importante. Il se peut également que les STEU de l'agglomération parisienne, traitant des volumes bien plus importants, soient plus performantes.

Des concentrations fortes sont également retrouvées autour du bassin Mauldre et Vaucouleur. Ce bassin reçoit des eaux issues de la ville de Versailles (84 808 habitants en 2019), et la présence de plusieurs hôpitaux pourrait contribuer à l'augmentation des concentrations de furosémide dans les cours d'eau. Les stations de traitement des eaux usées sur ce bassin ont des capacités (EH) inférieures à celles des stations traitant les eaux de l'agglomération parisienne, mais sont en revanche bien plus nombreuses (voir **figure 8**). De plus, Nebot et al. (2015) ont montré que les stations de traitement des eaux usées rurales pouvaient contribuer de façon importante au rejet de micropolluants dans l'eau. De plus, l'assainissement non collectif est très étendu sur le réseau et peut contribuer largement à la contamination et la présence abondante de micropolluants sur ce bassin a par ailleurs déjà été relevée par l'agence de l'eau Seine-Normandie (SDAGE AESN 2022).

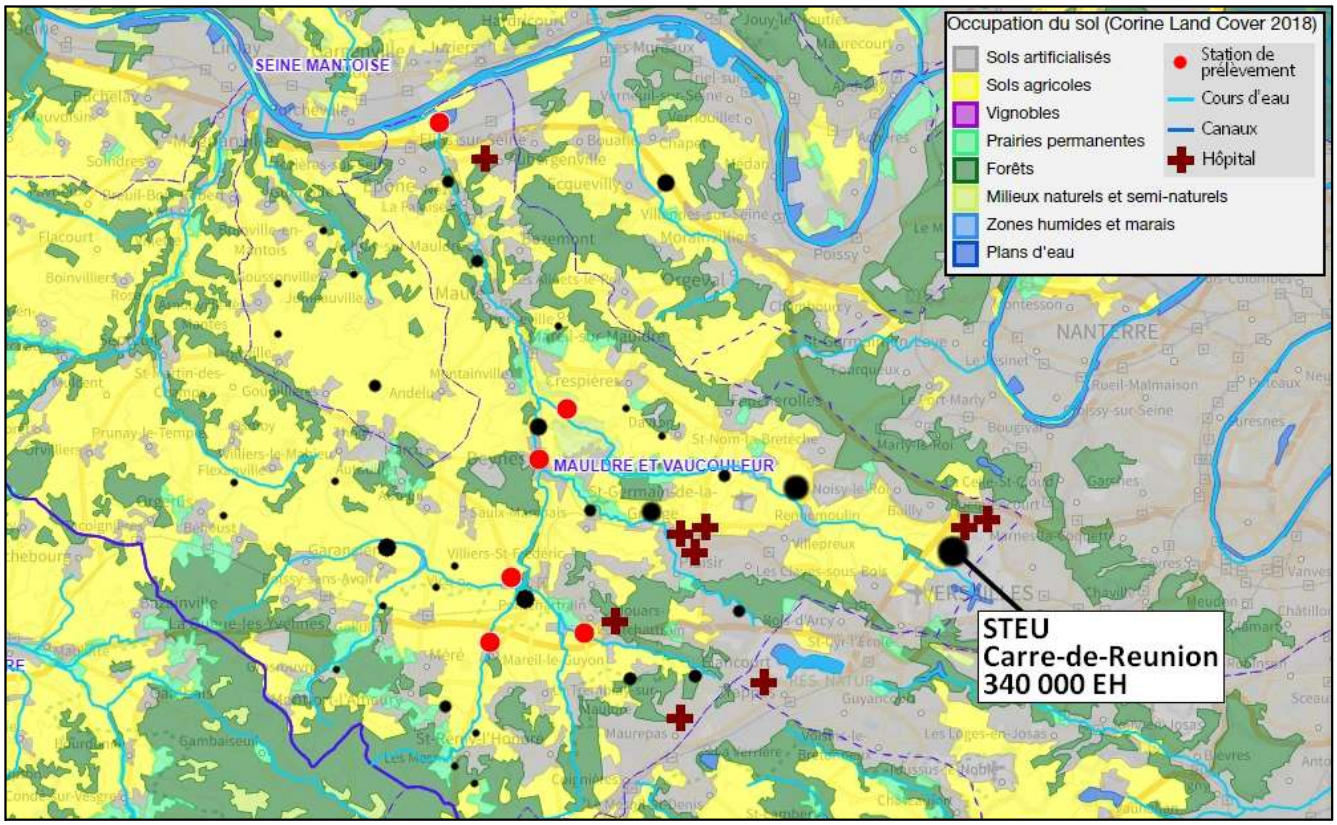


Figure 8. sources de la contamination en furosémide du bassin Mauldre et Vaucouleurs de l'agglomération de Versailles. Le fond de carte est issu du portail geo.eau-seine-normandie.fr et la position et capacité des STEU sont issues du portail assainissement.developpement-durable.gouv.fr. EH = Equivalent habitant.

Un autre site au sud de Paris, vers Corbeilles, présente une concentration de 274 ng/L (**figure 9**).

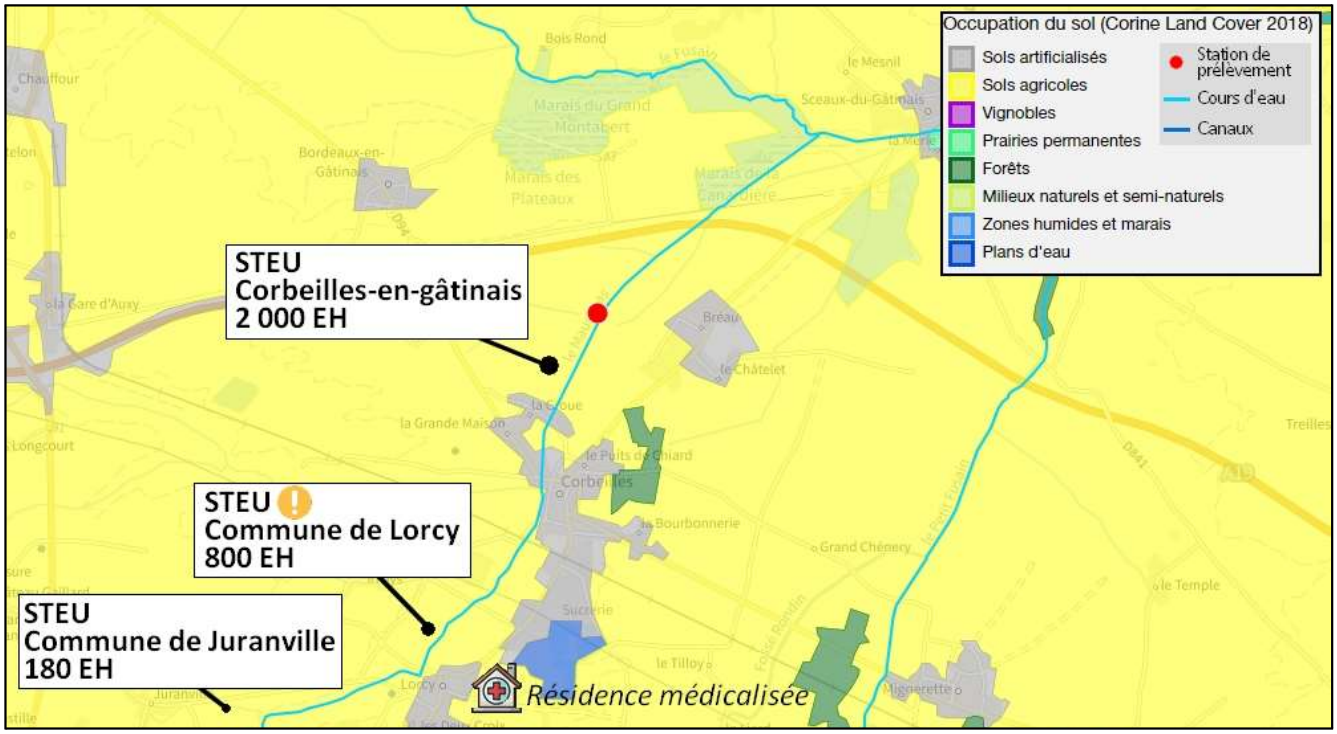


Figure 9. Carte d'occupation du territoire autour de la commune de Lorcy. EH = Equivalent habitant. Le fond de carte est issu du portail geo.eau-seine-normandie.fr et la position et capacité des STEU sont issues du portail assainissement.developpement-durable.gouv.fr.

C'est une petite ville (1 551 habitants en 2019) mais la présence d'une résidence médicalisée[13] juste en amont, dans la commune de Lorcy (579 habitants en 2019) pourrait expliquer cette contamination importante. De plus, la STEU traitant les eaux de cette commune est répertoriée comme "conforme" en termes d'équipement, bien que "non conforme" en termes de performance[14], ce qui pourrait également contribuer à la contamination mesurée.

En conclusion, même si des concentrations importantes sont retrouvées ponctuellement à proximité de sites particuliers (EHPAD, ville à forte densité de population,..), la contamination des rivières par le furosémide est globalement plutôt diffuse. Finalement, plus de 2 500 stations de traitement des eaux usées traitent les eaux usées de 18 millions d'habitants sur le bassin versant Seine-Normandie, mais ces eaux doivent être suffisamment traitées, et/ou les polluants dilués.

III. Calculs des flux sur la station de Poissy

Le rejet de micropolluants dans le milieu n'étant généralement pas un apport ponctuel mais permanent, le calcul du flux permet de suivre la progression de la pollution dans le temps et dans l'espace, ce qui est crucial pour la planification et la mise en œuvre de mesures de réduction de la pollution. En France, plusieurs projets de recherche ont bien pris en compte ce paramètre pour étudier le devenir des polluants de leur origine vers les milieux. Par exemple, le projet Cosmeteau[15] (2015 - 2018, porté par le LEESU) qui s'intéresse aux micropolluants issus des produits cosmétiques comme les parabènes et leurs produits de substitution, décrit les changements de composition de l'eau depuis la source jusqu'à la Seine, en passant par toutes les étapes du système d'épuration. Le projet REMPAR[16], lui, étudie la contamination en micropolluants organiques (médicaments, hormones, pesticides, parabènes et biocides, filtres UV) et métalliques du bassin d'Arcachon en étudiant les apports du bassin versant jusqu'aux exutoires en mer. Le projet MEDSEINE[17] également, a pour but d'étudier la contamination par les composés pharmaceutiques (SP) de l'estuaire de la Seine. Il est alors intéressant de calculer un flux de polluants (la quantité de polluants qui passe à travers une zone donnée à un moment donné) plutôt qu'une concentration ponctuelle car cela permet de mieux comprendre la façon dont la pollution se déplace et se propage. Dans notre cas, la station de Poissy est particulièrement intéressante puisqu'elle se situe juste en aval de Paris et récupère les eaux de la Seine après la confluence de ses trois principaux affluents, l'Yonne, la Marne et l'Oise. Ce site permet notamment d'étudier le transit du furosémide vers l'ouest du bassin versant. L'agence de l'eau Seine-Normandie s'est ainsi appliquée à calculer les flux des résidus médicamenteux surveillés par l'agence de l'eau Seine-Normandie en 2019, en se basant sur le site de Poissy. Le calcul des flux est basé sur l'**équation 1**:

$$\textbf{Flux} = k\,(\textstyle\sum \mathrm{CiQi} / \sum \mathrm{Qi}) * Q_{moyen} \qquad \textbf{(Equation 1)}$$

[13] https://www.hostellerieduchateaudelorcy.com/
[14] assainissement.developpement-durable.gouv.fr
[15] https://www.leesu.fr/Presentation-de-Cosmet-eau
[16] https://www.siba-bassin-arcachon.fr/qualite-de-l-eau/rempar
[17] https://www.seine-aval.fr/projet/medseine/

Avec Ci, la concentration en polluant en µg/L (Lorsque le résultat est inférieur à la limite de quantification), Qi, le débit journalier en L/s issus de la banque HYDRO[18] et Q_{moyen} calculé à partir des Qi d'une année. Les débits de la station de Poissy (03125000) ont été reconstitués à partir de 7 stations hydrométriques (H7611010; H7853010; H7833540; H7843010; H7742020; H4340020; H5841070) du fait de l'absence d'une station hydrologique proche de la station de surveillance qualité.

Au niveau de la station de Poissy, les composés pharmaceutiques suivis ont formé un flux annuel de 24,05 T/an. Parmi ceux-ci, le flux de furosémide a été d'environ 650 kg/an, soit 2,7% du flux total.

IV. Vente de furosémide en France

Pour expliquer une partie des apports de furosémide dans les cours d'eaux, les données de vente de médicaments pour les années 2019, 2020 et 2021 ont été extraites de la base de données nationale Open Medic[19] qui se base sur le Système National des Données de Santé (SNDS) et présentées dans le **tableau 2**.

Tableau 2. Masse de furosémide vendue en France et en Seine-Normandie en 2019, 2020 et 2021.

Vente 2019 (Tonne)	Vente 2020 (Tonne)	Vente 2021 (Tonne)	Coefficient population	Zone géographique
3,113	3,131	3,114	1	Ile-de-France
0,236	0,240	0,244	0,2	Centre Val de loire
0,228	0,230	0,226	0,15	Bourgogne Franche-comté
1,495	1,545	1,542	0,95	Normandie
0,524	0,542	0,546	0,2	Haut de France
0,702	0,698	0,685	0,21	Grand Est
6,297	6,386	6,357	-	Bassin Seine-Normandie
29,472	**30,016**	**29,862**	**-**	**France**

Les coefficients population sont calculés en fonction de la portion de la population de la région incluse dans la zone géographique du bassin versant, à partir du recensement communal. Pour la région Île-de-France, entièrement incluse dans le bassin versant, le coefficient vaut 1.

La masse moyenne de furosémide vendue sur le bassin Seine-Normandie pour les trois années est de 6,3 tonnes, soit 21% de la consommation française. La population du bassin Seine-Normandie comprend environ 25% de la population française, ce qui est cohérent avec les valeurs de consommation. En France, 29,8 tonnes de furosémide ont été vendues en moyenne, ce qui représente une masse importante mais pertinente du fait qu'il soit l'une des molécules les plus vendues en France (**tableau 3**).

[18] https://www.hydro.eaufrance.fr/
[19] https://www.data.gouv.fr/fr/datasets/open-medic-base-complete-sur-les-depenses-de-medicaments-interregimes/

Tableau 3. Top 20 des molécules les plus vendues en 2021 en nombre de boîtes.

Rang	Molécule	Nombre de boîtes vendues
1	Paracetamol	138649526
2	Acetylsalicylique Acide	41119529
3	Metformine	34316600
4	Codéine	32689052
5	Colecalciferol	32063844
6	Amoxicilline	30939677
7	Esomeprazole	29326718
8	Bisoprolol	23308020
9	Atorvastatine	21097457
10	Alprazolam	20434659
11	Omeprazole	18806500
12	**Furosemide**	**18391996**
13	Pantoprazole	18138760
14	Oxazepam	17372350
15	Phloroglucinol	17085177

Les données utilisées sont issues de la base de données Open Medic pour l'année 2021. Les lignes en vert correspondent aux molécules suivies par l'agence de l'eau Seine-Normandie.

D'autre part, la masse vendue entre 2019 et 2020 a augmenté de plus de 500 kg. Cette augmentation coïncide avec le début de l'épidémie de Sars-CoV 2. En effet, pendant les périodes de confinement, les prescriptions de médicaments liés au diabète et aux maladies cardiovasculaires ont largement augmenté (**figure 10**), dont celles de furosémide (+ 19% après la 11ème semaine de 2019), ce qui correspond aux dates du premier confinement en France (Weill et al. 2020).

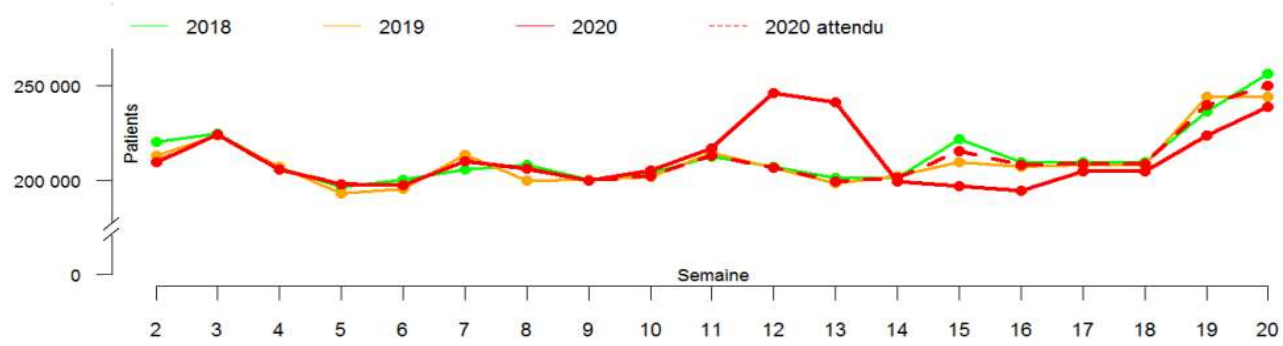


Figure 10. Évolution de la délivrance de furosémide au cours des 20 premières semaines de 2018, 2019 et 2020. Graphique extrait du rapport EPIPHARE, 2020.

En revanche, l'augmentation des prescriptions ne dure que quelques jours et est probablement dûe à un stockage lors du début du confinement. En outre, durant la pandémie, le furosémide a aussi été proposé comme médicament pour traiter certains symptômes de la COVID-19 (Kevorkian et al. 2021). Il a récemment été montré que le coronavirus du syndrome respiratoire aigu sévère (SarS-CoV 2) dérégule la production des angiotensines I et II (protéines du système hormonal localisé dans le rein) dont le rôle est de réguler la pression artérielle dans l'organisme, provoquant des oedèmes pulmonaires (Francisco Santos et al. 2021). L'infection virale initiale est suivie d'une réponse inflammatoire de l'hôte qui engendre une sécrétion importante de cytokines inflammatoires (par exemple, IL-6 et TNFα) provoquant des lésions des tissus pulmonaires. Le furosémide est capable d'inhiber la production de ces cytokines et grâce à cette

propriété anti-inflammatoire, présente de bons résultats pour traiter ces symptômes (Brennecke et al. 2020). Ces auteurs ont également rapporté que le furosémide possède en plus des activités antivirales, qui bloquent les perturbations des concentrations des cations intracellulaires provoquées par le virus en inhibant le transporteur Na+/K+/2Cl-.

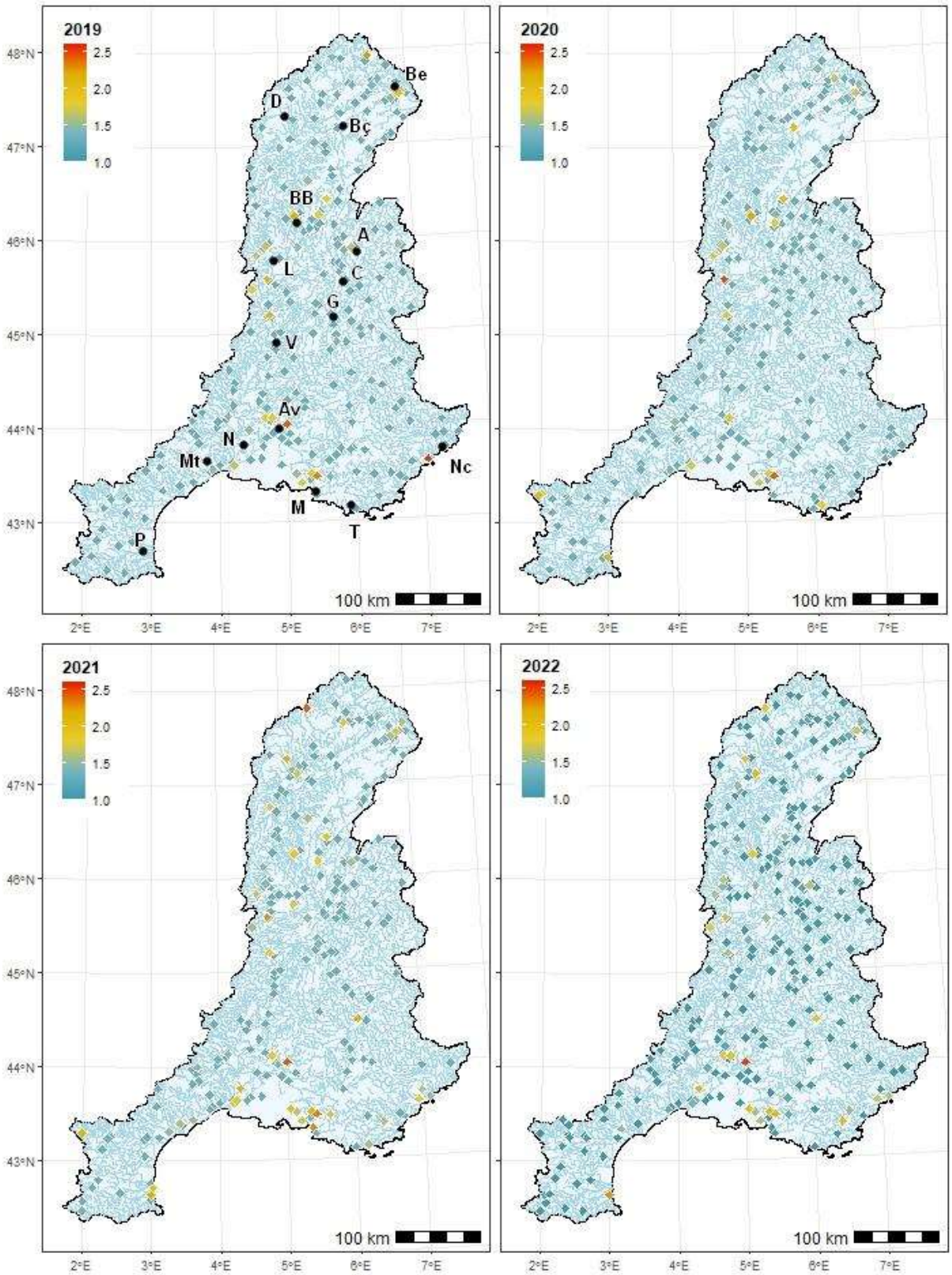


Figure 11. Concentrations de furosémide sur le bassin Rhône-Méditerranée. *Les données brutes ont été extraites de la base de données Naïade. La figure a été réalisée sous Rstudio et les fonds de cartes ont été téléchargés depuis geo.data.gouv.fr. A=Annecy; Av=Avignon; BB=Bourg en Bresse; Be=Belfort; Bç=Besançon; C=Chambord; D=Dijon; G=Grenoble; L=Lyon; M=Marseille; Mt=Montpellier; N=Nîme; Nc=Nice; P=Perpignan; T=Toulon*

Cependant, les données issues de la base de données d'Open Medic indiquent seulement les médicaments prescrits et vendus en officine. Le furosémide utilisé dans les établissements de soins ne sont alors pas pris en compte et les augmentations liées à l'épidémie pourraient ne pas se voir ici. En revanche, les concentrations analysées dans les rejets traduisent les variations de la consommation globale. Le furosémide étant un polluant suivi dans le bassin Rhône-Méditerranée depuis plusieurs années, l'évolution des concentrations sont alors comparées pour les années 2019, 2020, 2021 et 2022 dans la **figure 11**. Globalement, les concentrations de furosémide ne varient pas significativement d'une année à l'autre. La pandémie de COVID-19 ne semble pas avoir eu d'impact sur les rejets de furosémide sur le bassin Rhône-Méditerranée. Cependant, la **figure 11** permet d'identifier des sites où le furosémide est une problématique récurrente. Les stations les plus impactées au sud du bassin correspondent à des zones géographiques marquées par des agglomérations importantes. La zone au sud correspond aux villes de Marseille, Avignon, Nimes et Montpellier, la zone au sud-ouest correspond à la ville de Perpignan et la zone au sud, à Cannes et Nice. Aussi, comme sur le bassin Seine-Normandie en 2019, la contamination de furosémide est globalement plutôt diffuse.

V. Réutilisation des déchets humains et contamination au furosémide

Jusqu'alors nous avons discuté des procédés de traitement des eaux usées, cependant, ces eaux contiennent également des matières organiques qui, après séparation dans les STEU, vont former les boues. On distingue alors les boues primaires (après décantation), physico-chimiques (agglomération par un réactif) et biologiques (bactéries nourries de matières organiques)[20]. Comme pour le traitement de l'eau, plusieurs procédés sont appliqués pour assainir, et potentiellement valoriser ces boues: centrifugation, épaississement par gravité ou séchage pour réduire leur teneur en eau, stabilisation de la matière organique, désinfection, etc. Comme pour le traitement des eaux, les procédés pour le traitement des boues sont coûteux et énergivores. Des projets s'intéressent donc à la séparation des matières à la source, avant même leur entrée dans le réseau d'assainissement. Le programme de recherche OCAPI (porté par le LEESU)[21] s'intéresse justement à cette problématique. Lancé en 2014, le programme étudie les cycles biogéochimiques et en particulier la gestion des excrétions humaines. Ces matières, très riches, pourraient être une source importante de nutriments et l'urine en particulier est une source importante d'azote, de phosphore et potassium qui pourraient être un atout pour l'agriculture. Le projet Agrocapi (rapport Agrocapi, 2022), lié au programme OCAPI, s'est intéressé en particulier à la valorisation de l'urine humaine en agriculture, sous forme de matières fertilisantes (urinofertilisants). Cependant, la présence de micropolluants dans ces urines peut représenter un risque de contamination pour les systèmes biologiques dans lesquels elles sont épandues.

Dans ce contexte, nous avons souhaité déterminer les concentrations de certains composés pharmaceutiques, dont le furosémide, la saluamine et le pyridinium du furosémide. Pour cela, une méthode d'analyse par chromatographie liquide à haute performance-spectrométrie de masse en tandem (UPLC-MS/MS) a été développée au cours du stage de Thomas Flahou (Rapport de stage T. Flahou, 2022).

[20] https://www.siaap.fr/former-transmettre/mieux-comprendre-lassainissement/ consulté le 18/01/23
[21] https://www.leesu.fr/ocapi/

L'étude est basée sur 21 composés pharmaceutiques consommés en France, comprenant 4 antipsychotiques, 3 antibiotiques, 2 antiarythmiques, 2 anti-inflammatoires non stéroïdiens, méthylxanthines (caféine), un diurétique, un antiépileptique, un antihypertenseur, 2 antalgiques, un anti histaminique, une hormone de synthèse et un agent de contraste. Des échantillons d'urine issus d'un festival de musique électronique à Poitiers et du festival électro Nantes, ont été récupérés en 2020-2021. Les résultats de quantification de ces composés sont présentés dans le **tableau 4**.

Tableau 4. Quantification de 21 composés pharmaceutiques dans les urines de deux festivals.

	Concentration en µg/L		
Composé	**Urine Poitiers**	**Urine Nantes**	**Moyenne**
Paracétamol*	52,04	19241,99	9647,015
Caféine*	782,71	886,08	834,395
Naproxène*	37,23	145	91,115
Furosemide	**54,83**	**0,34**	**27,585**
Saluamine*	**11,72**	**24,65**	**18,185**
Clarithromycine*	9,44	6,24	7,84
Tramadol	3,9	0,94	2,42
Ethinylestradiol	1,89	2,22	2,055
Lidocaïne	2,2	0,8	1,5
Diclofénac	0,75	0,88	0,815
Gabapentine	0,81	0,69	0,75
Sulfamethoxazole	1,3	0	0,65
Sulfadiazine	0,74	0,13	0,435
Carbamazépine	0,01	0,82	0,415
Amoxicilline	0,14	0,45	0,295
Aténolol	0,11	0,21	0,16
Ranitidine	0,1	0,15	0,125
Iopromide	0,03	0,18	0,105
Amisulpride	0,17	0	0,085
Pyridinium du furosémide	**0,11**	**0,04**	**0,075**
Irbesartan	0	0,02	0,01
Trimethoprime	0	0	0

* Le rendement n'était pas applicable. Le rendement est calculé en fonction du dopage, celui-ci étant faible par rapport à la concentration obtenues de certaines molécules, la précision est réduite. Dans le cas des molécules marquées * , les concentrations peuvent être sous estimées.

Parmi les composés quantifiés, le paracétamol est sans surprise le médicament le plus concentré dans les urines. Utilisé comme anti-douleur (antalgique) et anti-fièvre, c'est le composé le plus utilisé en France par les particuliers (hors hôpital) vendues sous différentes marques commerciales comme Efferalgan, Dafalgan, Doliprane,.. (Rapport ANSM, 2013). La caféine est un psycho-analeptique présent dans la composition de certains médicaments, mais il est surtout consommé sous forme de boissons comme le café ou le thé. On suppose que c'est cette consommation qui place la caféine en deuxième sur notre liste. Le naproxène est

la troisième molécule la plus retrouvée. C'est un anti-inflammatoire non stéroïdien souvent utilisé pour traiter les douleurs, fièvres et inflammations. Le furosémide arrive en 4ème position avec des concentrations de plusieurs dizaines de microgrammes, ce qui est surprenant à première vue pour un médicament majoritairement prescrit pour les personnes âgées. Il semblerait que le furosémide soit utilisé pour contrebalancer l'effet secondaire de la MDMA (3,4-Methylenedioxymethamphetamine) aussi connue sous le nom d'ecstasy (Bora et al. 2016), souvent associée au milieu festif (techno, rave parties et musique électronique). De plus, dans le cadre d'une recherche de produits dopants dans des eaux municipales, le furosémide a été retrouvé en concentration plus importante que la moyenne (d'un ordre de grandeur) alors que la présence de MDMA a été avérée dans ces eaux (Schroder et al. 2010). La MDMA est une substance psychostimulante dérivée de la méthamphétamine, considérée en France comme stupéfiant[22]. La prise de MDMA serait accompagnée d'une augmentation de la température corporelle associée à une sudation importante entraînant une déshydratation. Pour pallier cet effet, les consommateurs ont tendance à boire beaucoup d'eau conduisant à de l'hyponatrémie (diminution trop importante de sodium dans le plasma) et l'hypokaliémie (diminution de potassium sérique), ainsi que la sécrétion d'hormones antidiurétiques (Houdou, 2004). L'usage d'un diurétique comme le furosémide paraît alors intuitif, mais peut en fait s'avérer très dangereux car il amplifie la déplétion de sodium et de potassium[23] pouvant évoluer vers la formation d'oedèmes.

La saluamine est également présente en fortes concentrations. Les échantillons ont été stockés dans des bidons soumis à l'air extérieur, la chaleur et le soleil, rendant possible une dégradation du furosémide dans ces conditions de stockage. En particulier, l'hydrolyse du furosémide en saluamine serait favorisée en milieu acide (Bundgaard et al. 1988; Andreasen et al. 1982). Le pH de l'urine étant en moyenne plutôt acide (4,5-7,5), cette voie de dégradation pourrait être privilégiée dans les conditions de stockage et expliquerait également que la saluamine soit le métabolite majoritaire par rapport au pyridinium du furosémide. Il est également possible que le pyridinium ait été formé, mais soit ensuite dégradé en saluamine (Sandré et al. 2023). La température de stockage ne devrait pas impacter directement le furosémide, celui-ci étant stable jusqu'à 218,1 °C (Beyers et al. 2000), cependant les températures estivales, plus chaudes, pourraient favoriser le développement de microorganismes. Ces derniers pourraient potentiellement dégrader le furosémide (Hezari & Davis 1993; Laurencé et al. 2014; Olvera-Vargas et al. 2016), bien que dans ces conditions particulières, cela n'ait pas été montré.

Au final, des concentrations importantes de résidus pharmaceutiques ont été retrouvées dans les urines de festivaliers, ce qui pourrait poser problème pour la réutilisation à des fins agricoles. Ces urines devraient être traitées ou très fortement diluées pour être utilisées sans risque sur le biote mais aussi sur l'Homme. En effet, une partie des composés pharmaceutiques peut être transférée dans les plantes (Delli Compagni et al. 2020) qui doivent, dans ce contexte, être consommées. Les urines de festivaliers ne semblent, dans ce cas, pas les plus adaptées pour être récupérées directement à la source pour réutilisation. L'urine d'individus n'ayant pris aucun médicament pourrait être intéressante en revanche, mais difficile à récupérer et plus rare (excluant par exemple toutes les femmes sous contraception). Un traitement des urines avant réutilisation pourrait réduire ce problème mais nécessiterait des installations

[22] Convention de 1971 sur les substances psychotropes - OMS
[23] https://www.worldsbest.rehab/furosemide-and-mdma/

supplémentaires et une dépense d'énergie. La récupération de l'azote et du phosphore des urines semble quand même intéressante au regard des traitements conventionnels des STEU. Il serait également intéressant d'analyser d'autres types d'urines pour comparer la charge en micropolluants. Dans le cadre du projet Med-Urinagri[24], qui prend la suite du projet Agrocapi, les urines issues de tout le territoire du plateau de Saclay, sont traitées par passage sur un filtre à charbon actif avant les analyses de micropolluants. Il serait pertinent d'inclure certains produits stupéfiants, comme la MDMA par exemple, à la liste des polluants analysés.

VI. Conclusion sur l'origine et le devenir du furosémide et de ses produits de dégradation dans le milieu aquatique

Dans ce chapitre, nous avons pu constater que le furosémide, mais aussi ces produits de dégradation étaient très présents, notamment en France. Bien que le furosémide soit correctement dégradé par les STEU (70%), il reste encore très présent et se retrouve transformé en pyridinium du furosémide, saluamine, et quelques autres sous produits. Les procédés d'oxydation avancés semblent mieux éliminer le furosémide, mais ils sont encore très rares en France, et surtout, ils engendrent la formation de plusieurs sous-produits, dont en particulier la saluamine. Le furosémide, la saluamine et le pyridinium sont alors largement retrouvés depuis leurs sources (EHPAD, entrées de STEU) vers le milieu récepteur (sortie de STEU, rivières).

En France, et en particulier au niveau du bassin hydrographique Seine-Normandie ou Rhône-Méditerranée, la contamination du furosémide est plutôt diffuse. Le furosémide est suffisamment bien traité ou suffisamment dilué pour se trouver en dessous des seuils de quantification (20 ng/L). On retrouve cependant certains points d'intérêt où les concentrations sont plus fortes (jusqu'à quelques centaines de nanogrammes par litre) à proximité d'EHPAD, sans surprise, ou de grosses agglomérations. Des flux importants de micropolluants sont retrouvés dans la Seine après confluence de ses trois principaux affluents. Le furosémide représente une part non négligeable de ce flux. Cet apport peut être expliqué par le fait que le furosémide soit une des molécules les plus vendues en France. Aussi, bien que le furosémide ait été testé comme traitement pour soulager certains symptômes du COVID-19, les concentrations dans les rivières (en particulier du bassin Rhône-Méditerranée) ne semblent pas varier significativement.

Enfin, le furosémide, mais aussi la saluamine ont été quantifiés en abondance dans les urines de festivaliers. Ce résultat surprenant vis-à-vis du type de population attendue dans ce genre d'événement (très rarement au-dessus de 70 ans), pourrait être expliqué par son utilisation couplée à la consommation récréative de certaines drogues psychotropes. Finalement, quelle que soit la matrice analysée, le furosémide est toujours présent ainsi que la saluamine. Le pyridinium est également quantifié dans toutes ces matrices, souvent en concentrations un peu inférieures aux deux autres composés. Ce chapitre met en lumière la nécessité de développer une méthodologie pour l'étude de la toxicité du furosémide et de ces produits de dégradation, au sujet desquels trop peu d'informations sont disponibles.

[24] https://www.leesu.fr/ocapi/les-projets/med-urinagri/

Chapitre III.

Evaluation de l'écotoxicité par une approche multi-modèle

Chapitre III.
Evaluation de l'écotoxicité par une approche multi-modèle

Dans le chapitre précédent, nous avons confirmé et discuté l'omniprésence du furosémide dans le milieu aquatique, dans lequel nous avons aussi mis en évidence pour la première fois ses deux métabolites la saluamine et le pyridinium du furosémide. Cependant, les analyses chimiques ne permettent pas de connaître la toxicité des molécules ou encore leurs interactions (effets cocktails). Dans ce chapitre, nous nous intéressons alors à l'effet de ces molécules sur les organismes aquatiques. Les substances polluantes peuvent engendrer des effets variés sur la croissance, la santé, la capacité de reproduction, le comportement, ou encore la survie des organismes. Ces impacts peuvent se répercuter sur différents niveaux d'intégration allant de l'individu jusqu'à un déséquilibre des populations, des communautés ou bien même de l'écosystème. Dans ce contexte, il est alors intéressant de prendre en compte les effets toxiques, le plus largement possible, en étudiant plusieurs organismes représentatifs du milieu aquatique. Dans notre cas, les composés étudiés sont présents à la fois dans la colonne d'eau et dans les sédiments. Cependant, la quantification dans les sédiments s'est révélée plus difficile à cause d'effets matrices importants (cf Chapitre II, article) et le furosémide dans les matrices solides est bien moins documenté que dans les milieux aqueux. Dans ce contexte, nous avons choisi d'étudier dans un premier temps, uniquement l'impact sur des espèces présentes dans la colonne d'eau.

I. Approches basées sur la toxicité aigüe

Les études pour évaluer les potentiels effets d'une substance chimique sur les organismes vivants dans l'environnement sont en général basées sur des tests de toxicité aiguë. Ces études consistent généralement à exposer des organismes (végétaux, invertébrés, vertébrés,..) à des concentrations croissantes d'une substance chimique pendant une courte période de temps (24, 48, 72, 96 heures) et de mesurer les effets immédiats sur la survie, la croissance et les paramètres physiologiques. Elles ont l'avantage d'être basées sur des normes définies par les organismes gouvernementaux et les organismes internationaux en charge de la réglementation des produits chimiques, comme l'Organisation de Coopération et de Développement Économiques (OCDE) ou l'International Organization for Standardization (ISO). Cette standardisation permet d'obtenir des valeurs de références homogènes d'un laboratoire à l'autre à partir de protocoles vérifiés et optimisés. Les résultats de ces études de toxicité dite "aigüe" permettent de déterminer la concentration la plus faible d'une substance chimique qui cause des effets nocifs, soit la PNEC (Predicted No Effect Concentration). Les PNEC les plus basses permettent d'identifier les organismes les plus sensibles à la substance: ces approches sont nécessaires lors des études d'évaluation de risque environnemental défini par le ratio PEC/PNEC (où PEC est la Predicted Environmental Concentration), appelé Quotient de Risque. Les valeurs seuil définies pour les polluants suivis par la DCE se basent sur des Normes de Qualité Environnementales (NQE)[25] elles-mêmes définies à

[25] Rapport de l'Ineris "Méthodologie utilisée pour la détermination de normes de qualité environnementale (NQE)", 2011

partir de ces PNEC. Ces NQE représentent "la concentration d'un polluant ou d'un groupe de polluants dans l'eau, les sédiments ou le biote qui ne doit pas être dépassée afin de protéger la santé humaine et l'environnement" et sont établies à partir d'une méthodologie standardisée (**figure 12**)[26].

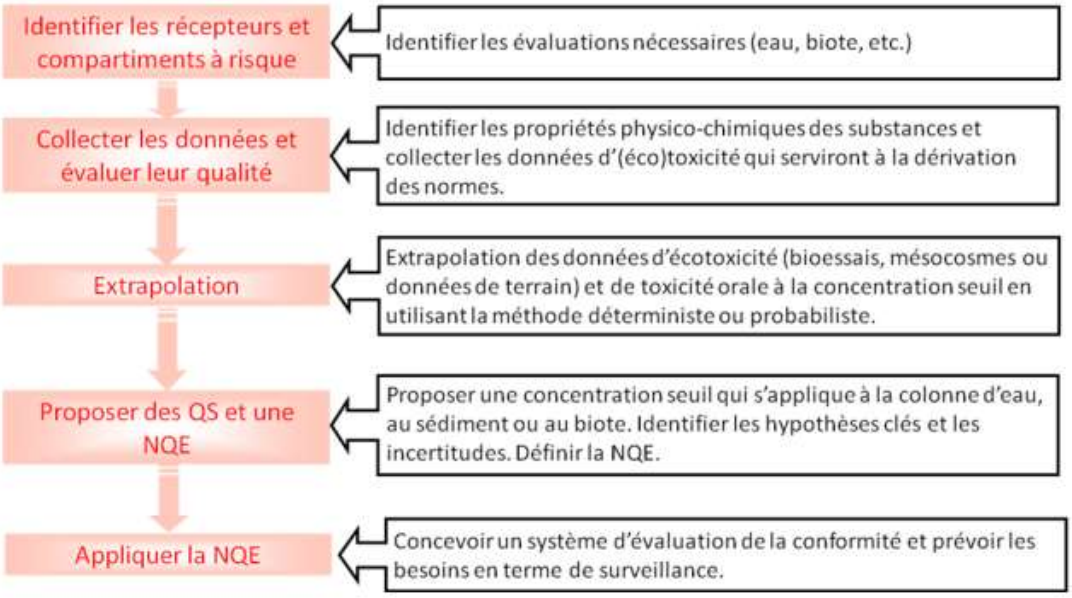


Figure 12. Méthodologie pour la mise en place d'une NQE. D'après le "Draft Technical Guidance for Deriving Environmental Quality Standards" (2010).

Les NQE peuvent varier en fonction de la substance ou du paramètre en question (par exemple, la concentration de certaines substances chimiques dans l'eau, la température de l'air, etc.) et doivent être définies pour différents groupes d'organismes. Les modèles de toxicité aiguë présentent cependant une limite. Les tests se basent en général sur des paramètres assez peu sensibles comme la mortalité ou l'immobilisation, et, bien qu'ils soient faciles à observer, les concentrations d'effet obtenues sont alors relativement élevées (en général autour du mg/L) et sont peu représentatives des concentrations environnementales.

Certaines études comme celles menées dans le projet DOREMIPHARM (2012-2015, porté par l'Ineris)[27], développent des approches plus sensibles pour la détermination des PNEC. Le projet vise à fournir des données écotoxicologiques sur des médicaments prioritaires, notamment le diclofénac et la carbamazépine, en utilisant dans un premier temps les test normés classiques, puis des biomarqueurs, et enfin une étude en mésocosme avec différents niveaux biologiques. Dans le même esprit, nous avons cherché à développer des tests plus subtils pour établir des PNEC, basées sur des paramètres plus sensibles sur des organismes modèles, pertinents pour l'étude du milieu aquatique.

[26] Directive 2000/60/CE du Parlement européen et du Conseil du 23 octobre 2000
[27] https://umr-sebio.fr/index.php/programmes-recherche/doremipharm-2012-2015

II. Choix de modèles pour l'étude de la toxicité sur le milieu aquatique

Différents critères s'appliquent pour le choix des organismes modèles ; il faut connaître la biologie de l'espèce (ex: publications sur le sujet), maîtriser la zootechnie et l'environnement (reproduction, maintenance,..), maîtriser les outils expérimentaux disponibles (génétique, bases de données, …), connaître la législation liée à l'expérimentation animale pour les modèles vertébrés, et estimer la faisabilité des expériences (œufs, embryons, matériel biologique suffisant,...). De plus, pour étudier l'impact de micropolluant dans l'environnement, il faut sélectionner des espèces représentatives du milieu et suffisamment sensibles.

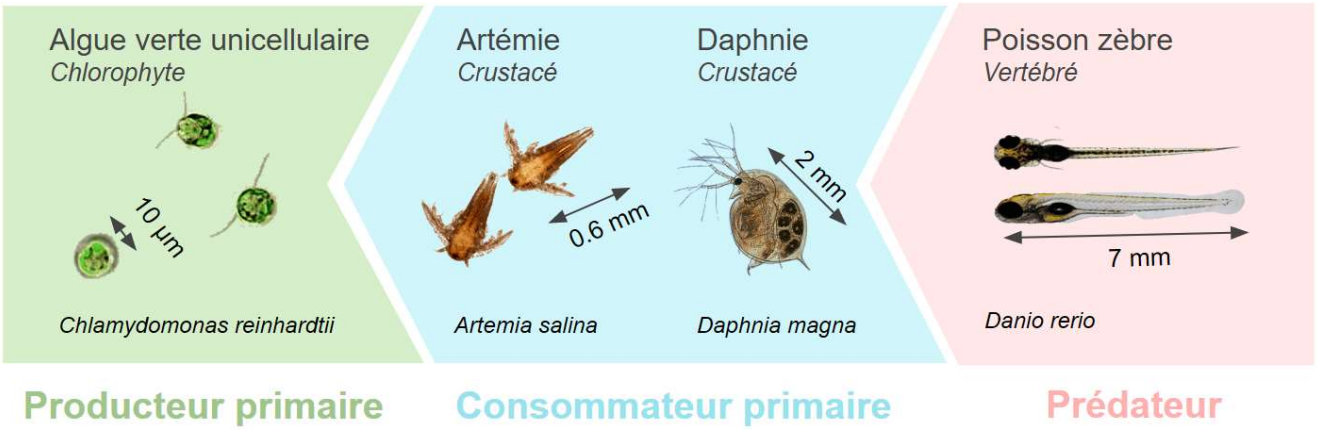


Figure 13. Représentativité des organismes modèles utilisés au cours de la thèse.

Notre choix s'est porté sur des organismes de niveau trophique différents et ayant donc des rôles écosystémiques divers (**figure 13**). Dans cette partie, différents modèles biologiques utilisés pour cette étude seront décrits: Le poisson zèbre (*Danio rerio*), la daphnie (*Daphnia magna*), l'artémie (*Artemia salina*) et une algue verte (*Chlamydomonas reinhardtii*), qui sont bien caractérisés et largement employés pour la recherche scientifique.

A. Le poisson zèbre (Danio rerio)

Le poisson est un modèle de choix pour l'étude d'impacts de contaminants dans le milieu aquatique sur les vertébrés: c'est en effet le groupe de vertébré le plus représenté avec plus de 22000 espèces connues de poissons "osseux" (ostéichthyens) et "cartilagineux" (chondrichtyens)[28]. En France, les poissons représentaient 7,3% des modèles animaux utilisés pour la recherche scientifique en 2020[29] (les plus fréquents étant les rats, les souris et les lapins). L'un des plus connus est le poisson-zèbre ou *Danio rerio*, un poisson d'aquarium commun appartenant à la famille des *Cyprinidae*. Il est originaire de l'Asie du Sud-Est et mesure généralement entre 3,5 et 5 cm de long. Il est caractérisé par des bandes verticales noires et blanches qui lui donnent son nom, visible sur la **figure 14.A**.

[28] https://www.universalis.fr/encyclopedie/vertebres/2-classification/
[29] Ministère de l'Enseignement supérieur, de la recherche et de l'innovation, Enquête statistique sur l'utilisation des animaux à des fins scientifiques

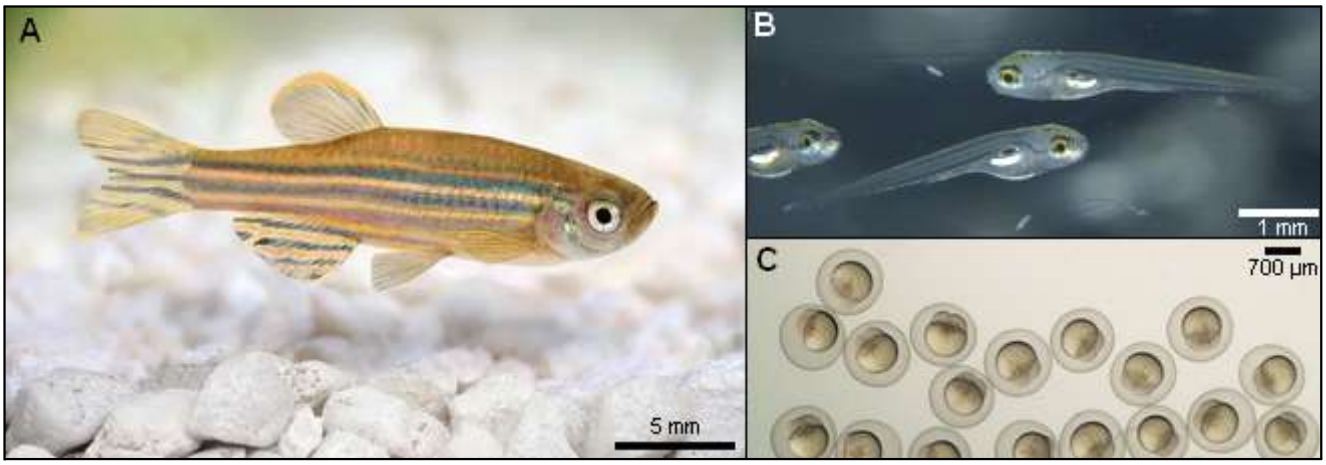


Figure 14. Photographie d'un poisson zèbre adulte (A), à l'état larvaire (B) et à l'état embryonnaire (œuf en développement)(C). L'image (A) issue du site aquablog.fr, l'image (B) sur ens.psl.eu et l'image (C) imp.ac.at.

Pacifique et sociable, il est considéré comme l'une des rares espèces de poisson domestique, au même titre que la carpe koï (*Cyprinus carpio*), le guppy (*Poecilia reticulata*), le poisson combattant (*Betta splendens*), et le poisson rouge et japonais (*Carassius auratus*)[30], ne nécessitant donc pas de certificat de capacité pour être maintenu en aquarium. Il est alors souvent utilisé pour les aquariums communautaires mais aussi largement pour la recherche scientifique en raison de sa robustesse, sa petite taille, sa reproduction facile, rapide et toute l'année, produisant de nombreux œufs (Spence et al. 2007).

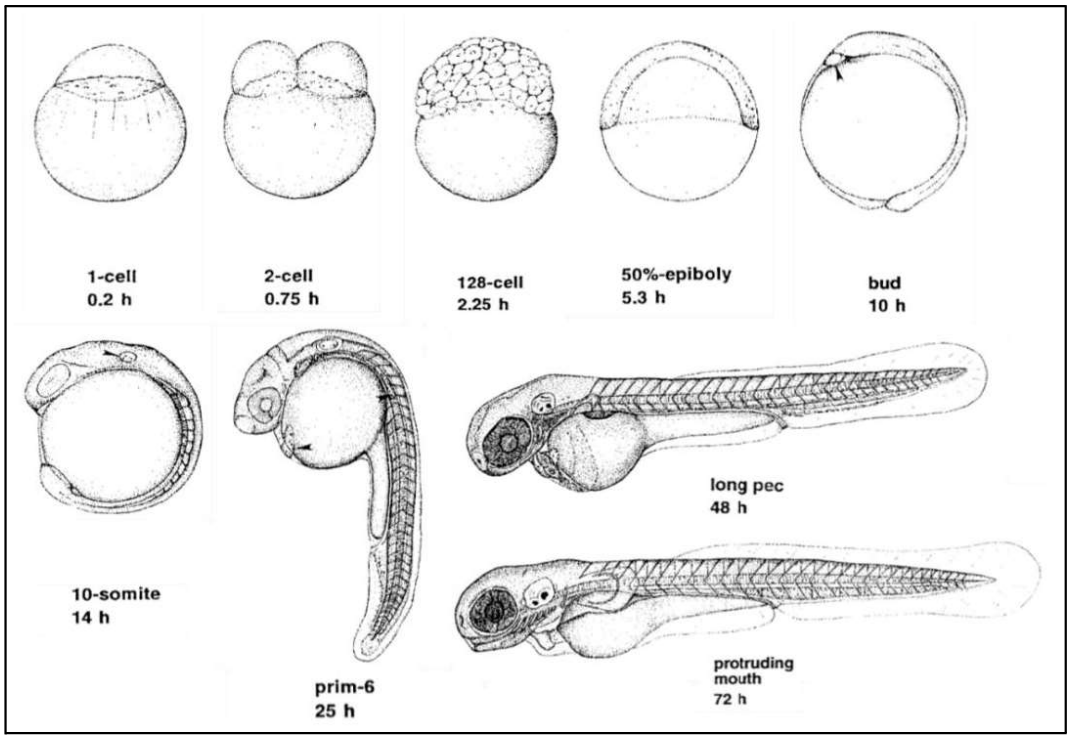


Figure 15. Principaux stades de développement du poisson zèbre. Schéma simplifié du développement du poisson zèbre issu de Kimmel et al. (1995). Durant les 24 heures qui suivent l'éclosion, cinq stades sont observés : zygote, clivage, blastula, gastrula et segmentation. Quarante huit heures après la fécondation, la larve éclot.

Son développement embryonnaire rapide avec une organogenèse quasiment complète à 72 h (**figure 15**), et ses œufs transparents facilement observables et manipulables en font un modèle animal

[30] Arrêté du 11 août 2006 Fixant la liste des espèces, races ou variétés d'animaux domestiques

particulièrement adapté pour les études de développement, de formation de l'embryon et de la segmentation (Strähle et al. 2011, Kimmel et al. 1995). Quatre grands stades se distinguent dans la vie d'un poisson zèbre : embryon, larve, juvénile et adulte (Belanger et al. 2010). A partir du stade de larve autonome (larves se nourrissant elles-mêmes), la directive 2010/63 concernant la protection des animaux utilisés à des fins scientifiques[31] s'applique et le dépôt d'une saisine est obligatoire.

Son génome est intégralement séquencé depuis 2009 et il existe de nombreux orthologues à des gènes humains avec plus de 70% d'homologie (avec souvent 2 gènes orthologues pour chaque gène humain à cause d'une duplication générale du génome chez les Téléostéens). De plus, son ADN facilement modifiable permet la création de nombreux mutants[32]. C'est donc un modèle d'étude pertinent en biomédecine ; il est utilisé pour étudier les voies moléculaires impliquées dans certains cancers (Völkel et al. 2018), pour comprendre le rôle de certaines protéines impliquées dans la maladie d'Alzheimer (Newman et al. 2014; Saleem & Kannan 2018), pour étudier le métabolisme des lipides et de l'adipose liées au diabète (Zang et al. 2018), pour étudier l'insuffisance rénale (Poureetezadi & Wingert 2016), pour le développement des nouvelles pistes pour la régénération des tissus cardiaques (Beffagna 2019), etc. De plus, le comportement du poisson zèbre a été extensivement étudié (Kalueff et al. 2013) et il est largement utilisé en écotoxicologie, notamment pour l'évaluation des polluants, tels que les métaux lourds toxiques, les perturbateurs endocriniens et les polluants organiques (Magyary 2018). Les larves sont également utilisées pour des tests de toxicité aiguë normés (OCDE 326).

B. La daphnie (Daphnia magna)

La Daphnie est un crustacé d'eau douce appartenant à la famille des *Daphniidae*. Les crustacés sont abondamment présents dans les océans et dans les eaux douces et font partie des arthropodes, qui rassemblent les quatre cinquièmes des espèces animales actuelles connues[33]. La structure fondamentale des crustacés est composée d'une tête avec deux paires d'antennes et une paire de mandibules, suivie de segments similaires avec des appendices biramés (divisés en deux branches) (**figure 16**). Chaque segment a un squelette externe en chitine. Elle est commune dans les lacs, les étangs et les rivières à travers l'Europe et l'Amérique du Nord. De plus, les daphnies sont des organismes de base dans les écosystèmes aquatiques: elles se nourrissent d'algues unicellulaires, de bactéries et de détritus et sont des proies privilégiées pour de nombreux poissons planctivores[34], lui conférant un rôle écologique majeur.

Il existe plusieurs sous-genres de daphnies, mais les plus connus et les plus utilisés en recherche sont les *Daphnia magna* et les *Daphnia pulex* en raison de leur cycle de vie court et de leur facilité de maintenance en culture. *Daphnia magna* est particulièrement utilisée en recherche pour sa grande taille: également connue sous le nom de "grande daphnie", la femelle mesure environ 5 mm de long et est de couleur vert pâle à marron foncé, visible sur le **figure 16.A**. Le mâle, plus petit, mesure jusqu'à 2 mm.

---

[31] Directive 2010/63/UE du parlement européen et du conseil du 22 septembre 2010 relative à la protection des animaux utilisés à des fins scientifiques
[32] http://zfin.org/action/fish/search
[33] https://www.universalis.fr/encyclopedie/crustaces/
[34] https://pure.mpg.de/pubman/faces/ViewItemOverviewPage.jsp?itemId=item_1506950

Elle se distingue de *Daphnia pulex* par la présence de peignes sous la griffe abdominale (Toyota et al. 2016) visible sur la **figure 16.C**.

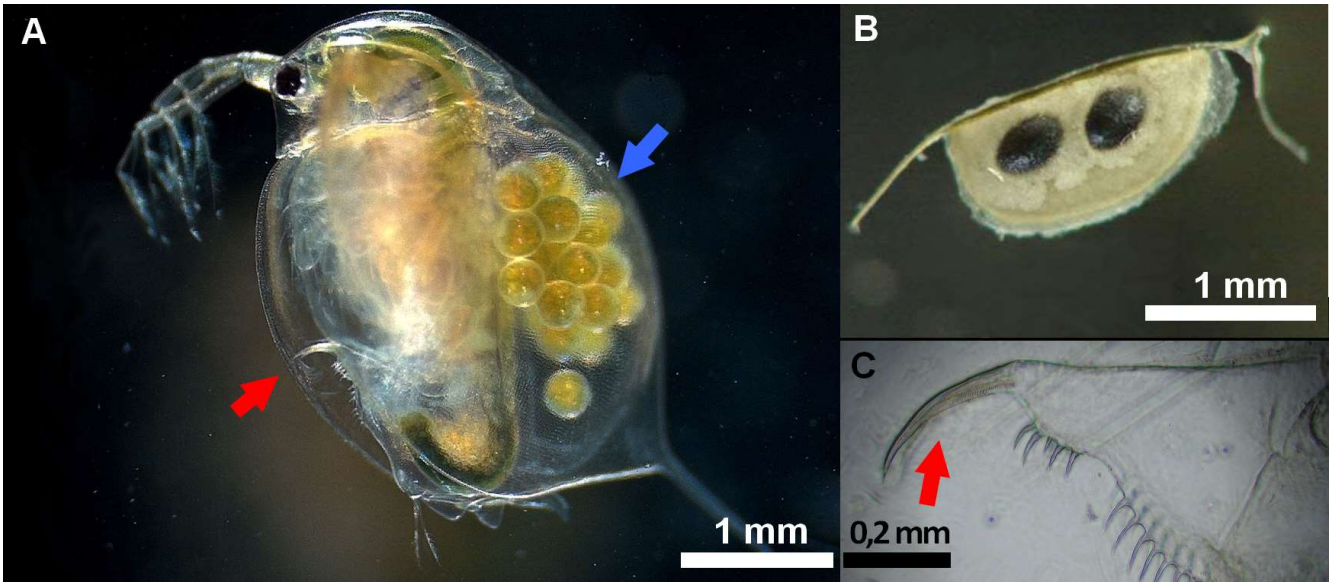


Figure 16. Photographie d'une daphnie (*Daphnia magna*) femelle (A), d'un éphippium (B) et d'une griffe abdominale (C). Les images (A) et (C) sont issues de wikipédia.org, l'image (B) provient du site aqualiment.com. La flèche rouge sur l'image (A) indique la position de la griffe abdominale représentée sur l'image (C), la flèche bleue indique le sac gestationnel.

Avant l'éclosion, 12 grands stades de développement peuvent être identifiés chez la daphnie, présentés sur la **figure 17** (Mittmann et al. 2014). Un dimorphisme sexuel ne peut être observé qu'après le stade 11. Le développement embryonnaire de la daphnie s'achève au bout de seulement 46 h (Obreshkove & Fraser 1940).

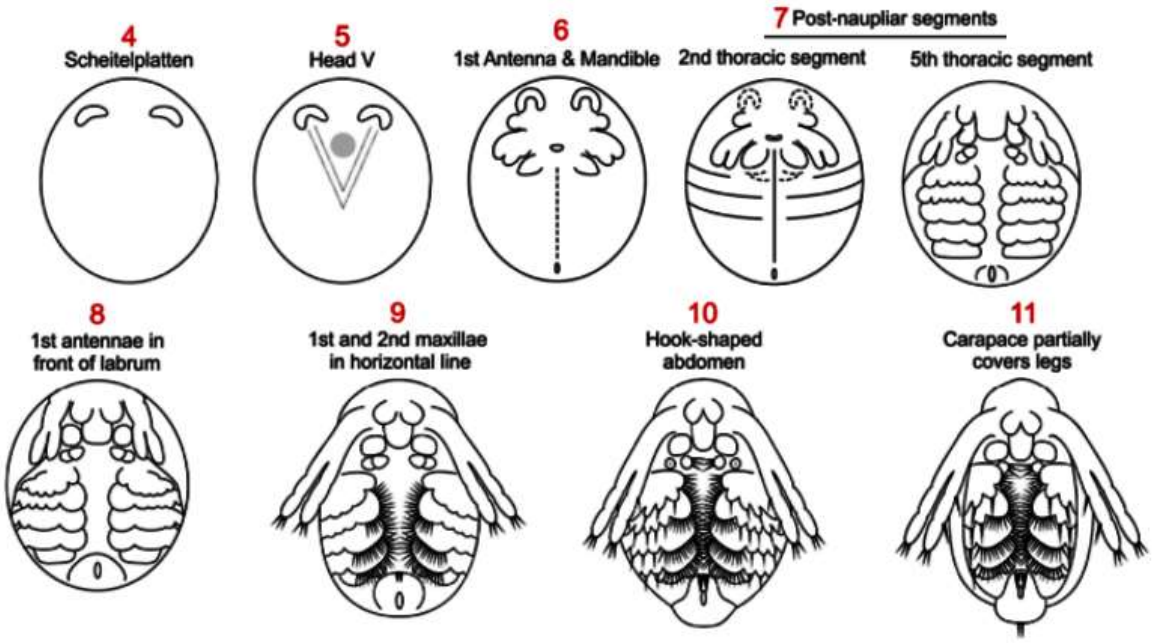


Figure 17. Principales étapes du développement de *Daphnia magna (vue ventrale)*. Le schéma ci-dessus est issu de Mittmann et al. 2014. Brièvement, les œufs sont libérés dans le sac gestationnel (figure 15.A) et se clivent pour former un blastoderme, puis migrent pour former une épibolie. La "Scheitelplatte" apparaît (stade 4) et détermine les axes antérieur-postérieur et dorso-ventral. Le système nerveux antérieur est délimité par une zone en forme de V (stade 5). Les appendices apparaissent aux stades 6 à 9. L'abdomen prend une forme de crochet et les maxilles développent des soies (stade 10) et la carapace se développe (stade 11). Les antennes et la colonne continuent de s'allonger jusqu'à l'éclosion (stade 12).

Les daphnies se reproduisent préférentiellement par parthénogénèse (reproduction assexuée), et produisent uniquement des femelles. Cependant, en conditions d'environnement défavorable, elles produisent des mâles. Une reproduction sexuée permet ensuite de produire des paires d'œufs de survie ou éphippium (**figure 16. B**), conçus pour résister aux conditions difficiles jusqu'aux périodes plus favorables où le cycle de reproduction peut avoir de nouveau lieu. Ses modes de reproduction sont explicités sur la **figure 18**.

Son temps de génération court rend la daphnie adaptée pour les études sur la reproduction, notamment l'impact de perturbateurs endocriniens (Abe et al. 2015). Un test normé sur la reproduction a été établi (OCDE 211). Son mode de reproduction par parthénogenèse permet également de générer des clones, intéressant pour la reproductibilité entre les différents réplicats lors de manipulations. De plus, les invertébrés ne sont, à ce jour, pas soumis à des réglementations sur l'expérimentation animale. Seule la vente d'invertébrés d'eau douce nécessite un certificat de capacité[35].

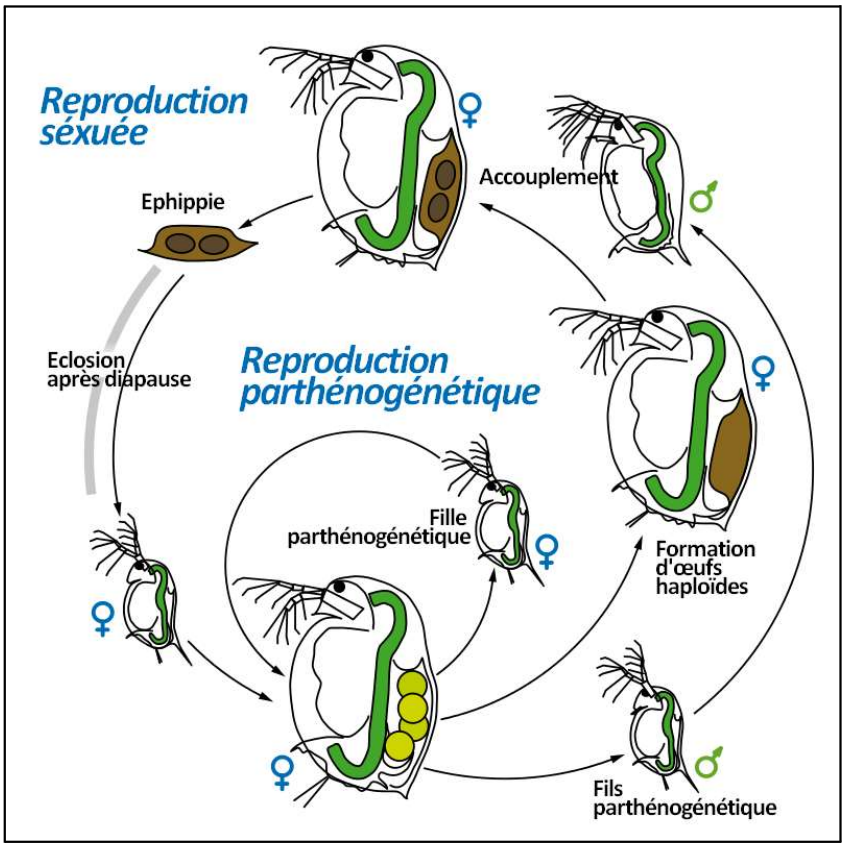


Figure 18. Reproduction parthénogénétique et sexuée des daphnies. *Image modifiée issue du site wikipédia.org*

La daphnie est utilisée en écotoxicologie comme organisme modèle pour évaluer les effets des perturbations environnementales sur les écosystèmes aquatiques (Ebert 2022) mais également en écologie et en biologie évolutive. Elle est considérée comme un organisme de test idéal pour cette application en raison de sa reproduction rapide, de sa sensibilité aux substances chimiques et de sa disponibilité en grande quantité. Elle est souvent utilisée pour évaluer la qualité de l'eau et pour la mise en place des normes réglementaires pour les polluants (OCDE 202, ISO 6341:2012). Comme pour le poisson zèbre, son développement est facilement observable sous microscope grâce à sa transparence. Par

[35] certificat de capacité N° 67-084 autorisant la vente et le transit de tous les poissons et invertébrés d'eau douce.

ailleurs, la daphnie est le premier crustacé dont le génome a été séquencé. Un total de 15 721 gènes ont été identifiés chez *Daphnia magna* (Lee et al. 2019).

C. L'artémie (Artemia salina)

L'artémie est une espèce de crustacé d'eau salée appartenant à la famille des *Artemiidae*, elle est également connue sous le nom de "cypris" ou "artémia". Elle est commune dans les lacs et les étangs salins à travers le monde, notamment en Amérique du Nord, en Europe et en Asie. L'espèce la plus connue est *Artemia salina*, qui peut mesurer jusqu'à une quinzaine de millimètres une fois adulte, visible sur la **figure 19.A**.

Figure 19. Photographie d'*Artemia salina* adulte (A), de nauplii (B) et d'œufs (C). L'image (A) est issue du site aqua-store.fr, l'image (B) du site istockphoto.com, et l'image (C) du site sciencephoto.com

Elle dispose de trois yeux et de 11 paires de pattes. Les mâles se distinguent des femelles par le fait que leurs secondes antennes sont nettement plus grosses car utilisées pour l'accouplement. Leur sang contient le pigment hémoglobine, que l'on trouve également chez les vertébrés.

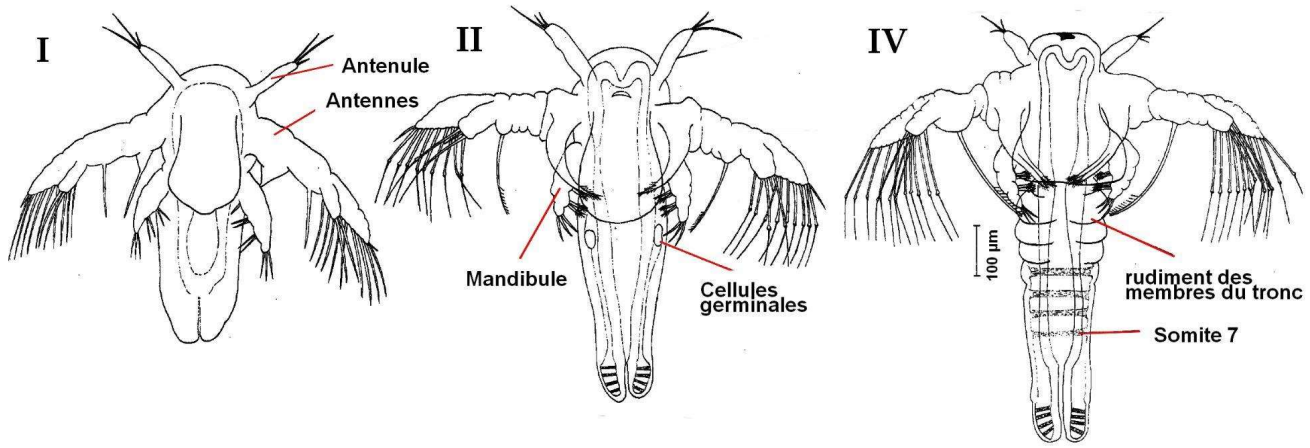


Figure 20. Les quatre principaux stades de développement d'*Artemia salina* (vue ventrale). Schéma adapté de Anderson 1967. A 20°C, le stade I dure environ 20 h, ici la forme d'un nauplius d'Artemia salina est illustré juste après l'éclosion. Le stade II dure environ 10 h, ici le nauplius est représenté immédiatement avant la deuxième mue. Le stade 3 dure 40 h environ. A partir du stade IV les artémies commencent à se nourrir.

Au cours du développement des artémies, il existe quatre stades de développement distincts (**figure 20**). Les nauplii (jeunes artémies) de stade I ont éclos entre 22-27 heures après l'immersion des œufs et peuvent nager directement. Ensuite, les antennes se développent et des segments sont ajoutés progressivement au tronc à partir d'une zone de croissance située immédiatement devant le telson (dernier segment de l'abdomen).

Artemia salina est utilisée dans la recherche scientifique dans de nombreux domaines de la recherche, notamment la biologie de l'environnement, l'écotoxicologie, la biotechnologie, la génétique, la biologie des systèmes et la biologie marine, en raison de sa simplicité de culture, de sa croissance rapide et de sa résistance aux conditions environnementales extrêmes. Comme la daphnie, elle a la capacité de se reproduire de manière asexuée et de produire des œufs parthénogénétiques. On peut ainsi étudier les effets des perturbations environnementales telles que la salinité, la température, la qualité de l'eau et la pollution par les métaux lourds, évaluer l'impact de contaminants sur la reproduction et leur accumulation (Persoone & Wells 1987). Elle est également employée pour évaluer la qualité de l'eau et pour la mise en place des normes réglementaires pour les polluants (FD ISO 14669) et des tests de toxicité aiguë (Sarah et al. 2021).

D. Les algues vertes (Chlamydomonas reinhardtii)

Les algues vertes unicellulaires sont communes dans les milieux aquatiques et peuvent être trouvées dans les lacs, les rivières, les étangs et les marais. Elles font partie de la classe des Chlorophycées et sont caractérisées par leur capacité à produire de l'oxygène grâce à la photosynthèse (photoautotrophe). Les organismes autotrophes ou primaires, constituent généralement le premier maillon d'une chaîne alimentaire, et sont à l'origine de quasiment toute la matière organique dans un écosystème.

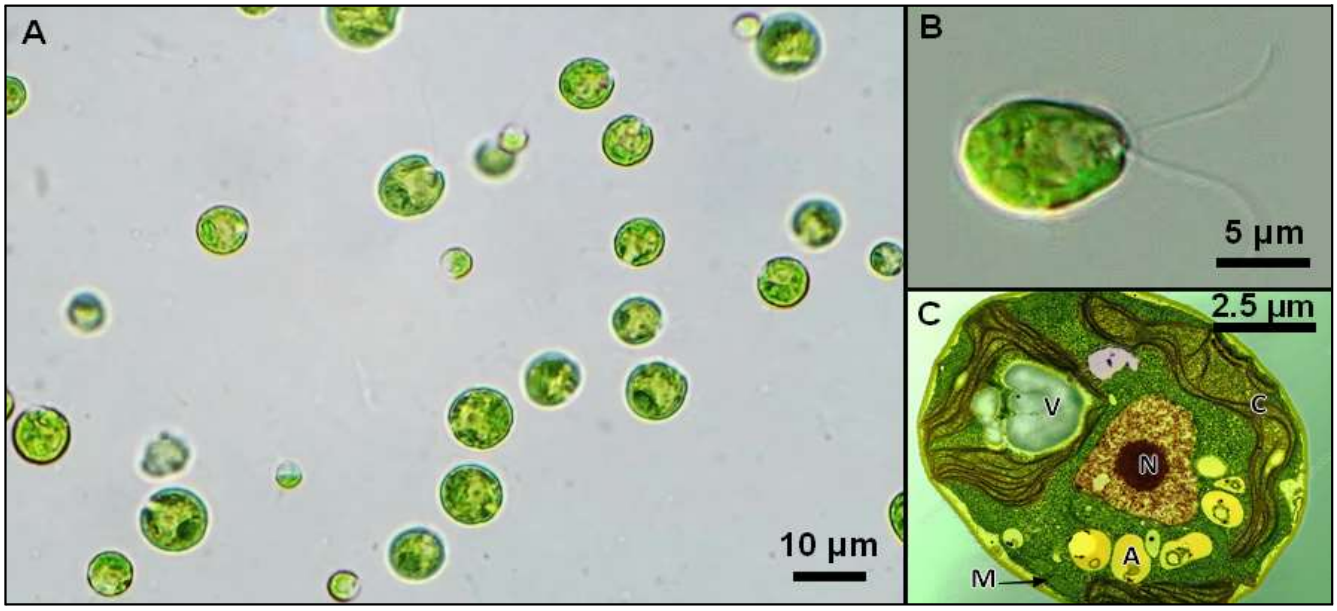


Figure 21. Photographies (A, B) et coupe (C) de *Chlamydomonas reinhardtii*. L'image (A) est issue de algalramblings.wordpress.com (B) de Wang, 2018 et (C) de sciencephoto.com. L'image (C) montre une section de C. reinhardtii vue en Microscopie Électronique à Transmission montrant le noyau (N), le chloroplaste (C), les grains d'amidon (A), vacuoles (V), mitochondries (M).

Il existe des centaines d'espèces d'algues vertes unicellulaires, mais *Chlamydomonas reinhardtii* est la plus couramment utilisée en laboratoire. Cette microalgue verte unicellulaire de 6-8 µm possède un chloroplaste, un stigma (tache oculaire photosensible) et deux flagelles extérieurs à la cellule, visibles sur la **figure 21**.

En conditions favorables, *C. reinhardtii* se reproduit de manière asexuée (**figure 22.A et B**). Pendant la reproduction asexuée, les cellules se développent et subissent deux ou plusieurs cycles de mitose et de cytokinèse (division) avant que les cellules filles éclosent de l'ancienne paroi cellulaire. En conditions défavorables, *C. reinhardtii* peut se reproduire de manière sexuée (**figure 22.C**) (De Carpentier et al. 2019). Le cycle cellulaire peut être synchronisé en fonction de la photopériode (Lemaire et al. 1999).

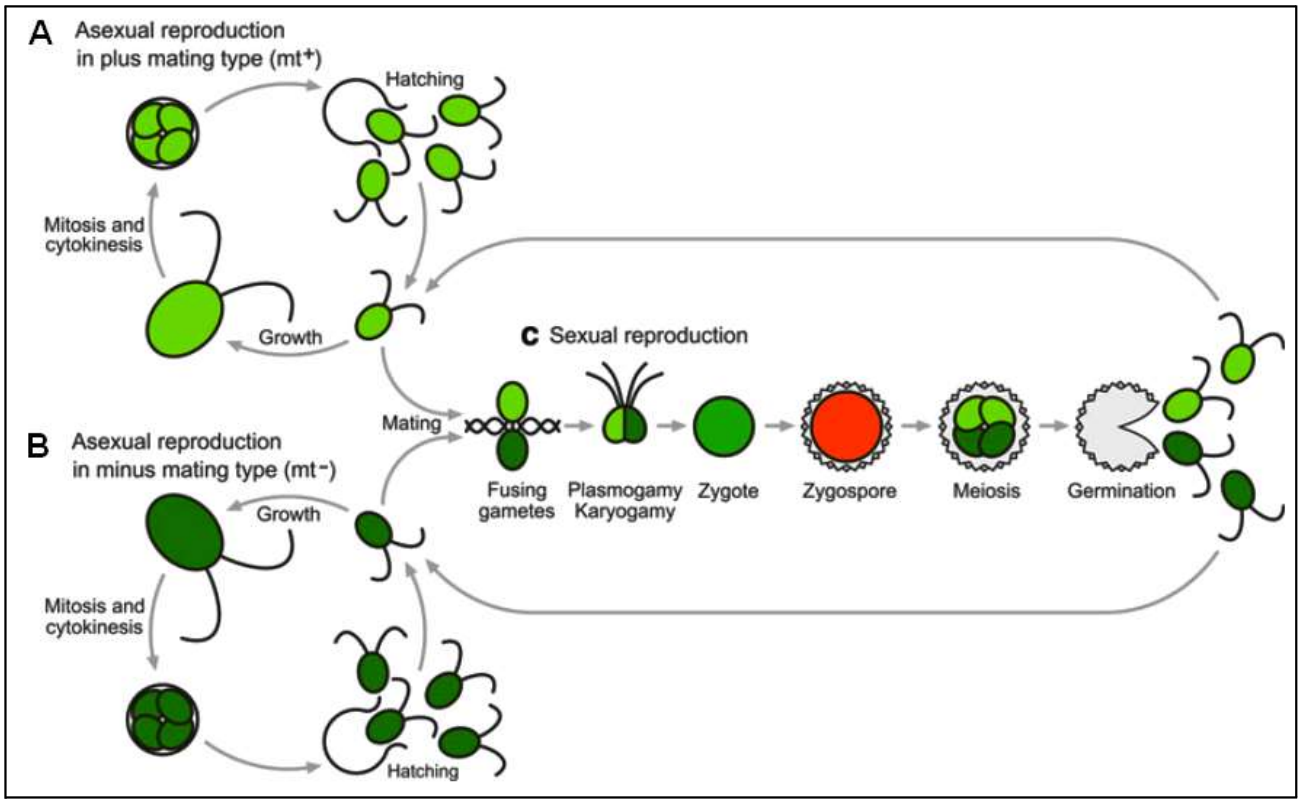


Figure 22. Reproduction asexuée (A, B) et sexuée (C) de *Chlamydomonas reinhardtii*. La figure est issue de Hallmann 2010. Les schémas A et B montrent la reproduction asexuée par division cellulaire. Le schéma C montre la reproduction sexuée. Les cellules asexuées des deux types d'accouplement se développent en gamètes et les gamètes de types d'accouplement opposés vont former des agrégats par agglutination flagellaire. Après la libération des parois cellulaires, les structures d'accouplement se forment, et les gamètes fusionnent pour former un zygote qui mûrit ensuite en une zygospore diploïde. Quatre cellules haploïdes se forment par méiose (deux de chaque type d'accouplement).

Le génome de cette algue est entièrement séquencé depuis 2007 (Merchant et al. 2007) et de nombreux gènes et protéines sont déjà caractérisés. Il existe une grande communauté de chercheurs travaillant sur cette algue, ce qui facilite l'accès à des outils de recherche tels que des lignées génétiquement modifiées, des anticorps et des outils de biologie moléculaire[36]. Elle est fréquemment utilisée en raison de sa simplicité de culture, de sa croissance rapide, de son cycle de vie court et de sa capacité à se reproduire de manière asexuée. Elle obtient son énergie par la photosynthèse mais elle peut vivre sans lumière si elle a accès à une autre source de carbone (mixotrophe).

[36] efor.fr/chlamydomonas-reinhardtii/

C. reinhardtii est utilisée dans de nombreux domaines de la recherche, notamment la biologie cellulaire, la génétique, la biologie des systèmes, la biotechnologie et la biologie de l'environnement. Elle est utilisée pour étudier les processus fondamentaux de la photosynthèse, les mécanismes de réponse face aux perturbations environnementales, la régulation de la croissance, la régulation génétique et la biologie de l'énergie (Miguez et al. 2021, De Carpentier et al. 2019).

III. Evaluation écotoxicologique du furosémide et de ces produits de dégradation

Cette section traite de la toxicité des trois composés étudiés. Des bioessais ont été effectués sur des organismes modèles mentionnés précédemment. Bien que la toxicité aiguë ait été initialement évaluée, elle présente des limites quant à sa pertinence écologique. Pour cette raison, des échantillons d'eau de rivière ont été prélevés en amont et en aval de l'agglomération de Paris pour déterminer les concentrations environnementales des composés, puis des tests spécifiques ont été réalisés à ces concentrations. Les résultats de ces tests sont présentés dans l'article ci-dessous.

Résumé de l'article : First approach for the ecotoxicological assessment of a ubiquitous pharmaceutical compound (furosemide) and its transformation products.

De nombreux médicaments sont consommés quotidiennement et rejetés dans les eaux usées. Or, les stations d'épuration des eaux usées (STEU) ne sont pas conçues pour éliminer efficacement ces composés, qui sont ensuite rejetés dans l'environnement aquatique et représentent un danger pour les écosystèmes. Certains produits chimiques, comme le furosémide (FUR), l'un des médicaments les plus utilisés au monde, sont omniprésents dans les rivières. De plus, le FUR peut être dégradé en plusieurs sous-produits encore mal caractérisés tels que la saluamine (SAL) et le pyridinium du furosémide (PYR).

Cette étude vise à évaluer le risque environnemental de ces composés par le biais de bioessais sur différents modèles représentatifs de l'environnement aquatique (poissons, crustacés, algues). Les concentrations environnementales des trois composés ont été déterminées par LC-MS/MS dans la Seine (France). Ensuite, une étude sur le comportement, la cardiotoxicité, la production de stress oxydatif, les modifications physiologiques et la létalité a été réalisée. Les concentrations d'effet et les scores de toxicité ont été déterminés pour FUR, PYR et SAL.

Des concentrations comprises entre 30 et 250 ng/L ont été trouvées et montrent pour la première fois la présence de PYR et de SAL dans l'environnement. Les tests de toxicité aiguë et les scores de toxicité établis ont montré que le SAL et le PYR sont plus toxiques que leur molécule mère (FUR) et pourraient avoir un effet délétère sur l'écosystème aquatique en impactant la survie de différents organismes, et en particulier sur la daphnie, qui s'est révélée être particulièrement sensible.

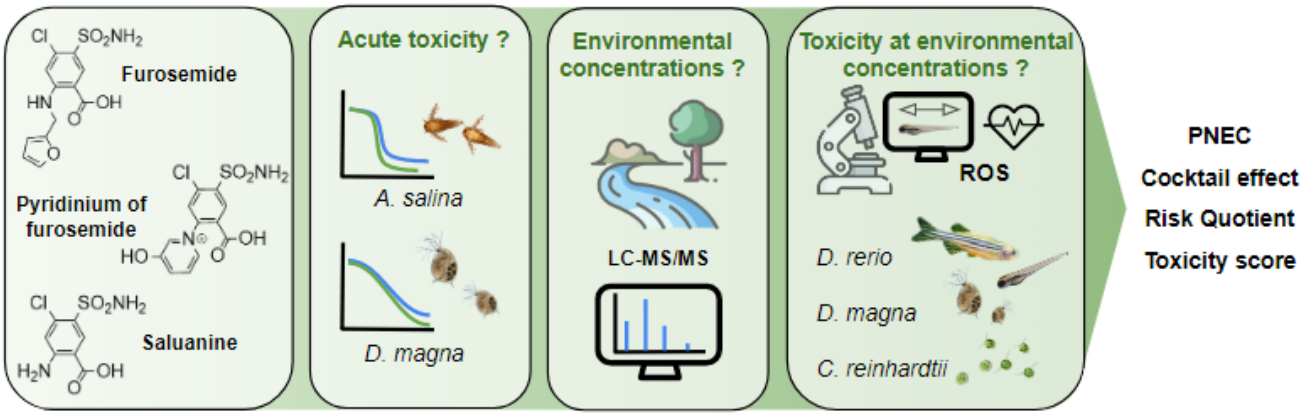
First approach for the ecotoxicological assessment of a ubiquitous pharmaceutical compound (furosemide) and its transformation products.

Fidji Sandré [1], Aliénor Duval [1], Luna Grimault [1], Alexis Golven [1], Christophe Morin [1, 2], Laure Garrigue-Antar [1]

[1] Leesu - Univ Paris Est Creteil, Ecole des Ponts, Creteil, F-94010, France

[2] IUT - Sénart Fontainebleau, 36 Rue Georges Charpak, 77567 Lieusaint

Graphical abstract



Abstract

Many drugs are consumed daily and then discharged into wastewater. However, wastewater treatment plants (WWTPs) are not designed to effectively eliminate these compounds, which are then released into the aquatic environment and represent a danger for ecosystems. Some chemicals, such as furosemide (FUR), one of the most used drugs in the world, are omnipresent in rivers. Moreover, the FUR can be degraded into several by-products that are still poorly characterized such as saluamine (SAL) and pyridinium of furosemide (PYR). This study aimed to assess the environmental risk of these compounds through bioassays on different models representative of the aquatic environment (fish, crustacean, algae). The concentrations of the three compounds in the Seine river (France) determined by LC-MS/MS were found between 30 and 250 ng/L and highlighted for the first time the presence of PYR and SAL in the environment. Then, all three compounds have induced deleterious effects either on mobility, heart rate and oxidative stress of *D. rerio* and *D. magna* and on mobility, growth rate, mobility and size of *C. reinhardtii*. The acute toxicity tests and the calculated toxicity scores showed that SAL and PYR were more toxic than their parent molecule (FUR) and could have a deleterious effects on the aquatic ecosystem by impacting the survival of different organisms, in particular daphnia, which has been shown to be particularly sensitive. All three compounds can impact aquatic organisms and thus cause deleterious effects on ecosystems. This study highlights the pressing necessity to consider degradation products in risk studies

Introduction

Pharmaceutical compounds presence in water media has been investigated for nearly fifty years (Aherne & Briggs 1989; Hughes et al. 2013) and in a context of high demographic growth and aging population, are increasingly consumed. These anthropogenic pollutants transit via wastewater systems before ending up in receiving environments following incomplete elimination by water treatment plants (Chiffre et al. 2016). Due to the ubiquitous presence of these drug residues, their transformation products are emerging as a new threat to human health and the environment.

These products, resulting from various processes, such as metabolism (phase I, phase II), biotic transformation (aerobic or anaerobic bioconversion), or abiotic transformation (hydrolysis, photodegradation, chemical oxidation, thermal decomposition) also end up in the aquatic environment (Maculewicz et al. 2022). There is a wide variety of these compounds and while recent non-targeted analysis techniques have been able to identify a large number of them (Nihemaiti et al. 2022), characterizing them all is a daunting task. In some cases, these compounds may be present in higher concentrations than their parent molecules, even when those are not detected (Battaglin et al. 2014, Battaglin et al. 2003, Kołecka et al. 2019, Langford and Thomas 2011). They can be more toxic than their parent compounds (Isidori et al. 2006), and thus can have many impacts on non-target organisms and be potentially deleterious to ecosystem well-being (Świacka et al. 2020). Pharmaceuticals are not properly regulated in the environment and it is very difficult to reduce their sources because of their therapeutic use.

In this context, this study focuses on a pharmaceutical pollutant of emerging interest: furosemide (FUR). Ranked as a priority pollutant by various prioritization methodologies (Besse and Garric, 2008 Chinnaiyan et al 2018), FUR is a powerful diuretic of the sulfonamide family acting on the loop of Henle. It is frequently prescribed for hypertension, heart or kidney failure, and cirrhosis (Abbott et al. 2018) in humans and it also has anti-inflammatory, anti-epileptic and antioxidant properties (Sandré et al. 2023b). FUR is ubiquitous in the aquatic environment. In Europe, it is found at concentrations of around hundred ng/L in rivers and groundwater, and several thousands of ng/L in wastewater (Sandré et al. 2023a). It is also captured by sludge and sediments due to a high partition coefficient (Kd) between the dissolved and particulate phase (Jelic et al. 2012).

From its passage through the body to its release in the environment, *via* sewage network and wastewater treatment plants, FUR undergoes degradation into several by-products. Saluamine (SAL) has been identified as a metabolite of FUR for a long time (Hammarlund-udenaes & Benet 1989, Andreasen et al. 1981, Laurencé et al. 2014) but surprisingly remains poorly documented to date. To our knowledge, only Al-Omar et al. (2009) reported on the toxicity of SAL, demonstrating its negative impact on rat liver and kidneys. We then obtained a new transformation product of furosemide, the pyridinium of furosemide (PYR), by electro-oxidation and bioconversion by fungi and yeasts (Laurencé et al. 2011, Laurencé et al. 2014, Olvera-Vargas et al. 2016), that we later identified in urine of furosemide-treated patients as a genuine human metabolite. We further demonstrated its neurotoxic properties in rodents and cytotoxicity on human neuroblastoma cells (Laurencé et al. 2019). However, there is currently no toxicological data on aquatic species, even though this pharmaceutical and its by-products ultimately end up in aquatic media.

The increasing incidence of cardiovascular diseases and the aging of the population are leading to a notable augmentation of furosemide prescription numbers over the years (Sandré et al. 2023b). Although a substantial amount of toxicological studies exist on furosemide (Abbot et al. 2018), its toxicity in the environment has been very little studied. Moreover, the ecotoxicological risks associated with its degradation products are still unknown.

The aim of this work is then to provide a better understanding of the impact of these compounds through a multi-model approach taking into account organisms of different trophic levels with various ecosystem roles. Thus, a vertebrate (zebrafish, *Danio rerio*), two crustaceans (*Daphnia magna* and *Artemia salina*) and a unicellular green algae (*Chlamydomonas reinhardtii*) were selected for their representativeness in the aquatic environment and their sensitivity. These models are widely used in research in various fields and are consequently very well characterized (Spence et al. 2007, De Carpentier et al. 2019, Persoone & Wells 1987, Tkaczyk et al. 2020). Although multi-organism approaches (population, community, food web) are not uncommon, they are still rarely taken into account in risk studies and associated regulations. The acceptance and validation of these models are complex but they allow a better evaluation of the risks due to a better representativeness (Leenhardt et al. 2023).

In the present study, the concentrations of FUR, PYR and SAL were determined in the Seine upstream and downstream of the Paris metropolitan area (France) to assess the environmental contamination by these compounds. Acute toxicity tests were first conducted on crustaceans to compare the toxicity of furosemide to that of its byproducts. Then, more sensitive tests were performed at environmental concentrations. Cardiotoxicity and oxidative stress were evaluated on daphnia and zebrafish, taking into account furosemide's use in heart disease and its antioxidant properties (Abbott et al. 2008, Lahet et al. 2003), as well as behavioral tests and measurements of physiological parameters on algae. The Lowest Observed Effect Concentrations (LOECs) of these compounds were assessed for these different parameters, and a toxicity score was determined for FUR, PYR and SAL. Their interaction in a mixture was also monitored.

Material & methods

I. Reagents & instruments

The compounds tested in this study, furosemide (FUR) and saluamine (SAL) were purchased from Sigma-Aldrich. The pyridinium of furosemide (PYR) was synthesized at ICMPE (UMR CNRS 7182, Thiais, France), as described previously (Laurencé et al. 2011) and its structure was confirmed by NMR (purity >96%). The stock solutions of FUR, SAL and PYR were prepared in the respective media of each organism with 5% dimethylsulfoxide (DMSO), except for the algae experiments, where DMSO was replaced by methanol due to its lower toxicity on algae (Pino et al. 2016). The DMSO and methanol used to prepare the stock solutions (purity >99.9%) were purchased from Sigma-Aldrich. For chemical analyses, the furosemide-d5 standard was purchased from Cluzeau Info Labo. Internal standards (atenolol-d7 and sulfamethoxazole-d4) were from Sigma-Aldrich. For extraction and analysis, MS grade methanol was from Fisher. The ultrapure water was produced by a Milli-Q® IQ 7000 ultrapure water system.

Compounds used in the preparation of embryonic medium (E3) for zebrafish larvae (14.6 g NaCl; 0.63 g KCl; 2.43 g $CaCl_2$; 4.07 g $MgSO_4$, for 1L), reconstituted water for daphnia (11.76 g $CaCl_2$; 4.93 g $MgSO_4$; 2.59 g

$NaHCO_3$; 0.23 g KCl) and artemia (+NaCl for 3. 5%) and TAP medium for green algae (54 mg K_2HPO_4; 28 mg KH_2PO_4; 200 mg NH_4Cl; 50 mg $MgSO_4$; 1.21 mL Tris base, 0.5 mL glacial acetic acid, 0.5 mL Hutner's Trace Elements) were purchased from Sigma-Aldrich, with the exception of the Hutner solution which was purchased from the Chlamydomonas Resource Center.

For oxidative stress measurements, the dichlorofluorescein diacetate (DCFDA) probe was purchased from Sigma-Aldrich. To measure gene expression of biomarkers in zebrafish larvae, MP Biomedical's Lysing Matrix A kit (compatible with the FastPrep-24 homogenizer), Qiagen's RNeasy MiniKit, Qiagen's RNAse-Free DNAse, kit Promega Quantifluor RNA System, Agilent's Affinity Multiple Temperature cDNA Synthesis and Agilent's Brilliant III Ultra-Fast SYBR Green qPCR Master Mix were used.

For low doses, referred to as "environmental", concentrations were set according to the concentration values found in rivers during this study and the concentrations at the outlet of WWTPs in our previous work (Sandré et al. 2023a).

II. Animals

The zebrafish larvae (*Danio rerio*) and daphnids (*Daphnia magna*) used were obtained from the laboratory rearing facilities. Artemia (*Artemia salina*) were purchased as dehydrated cysts from the supplier JBL. The wild-type algae (*Chlamydomonas reinhardtii*) strain CC-503 was provided by the LCQB laboratory (Paris, France).

Adult zebrafish of the AB tübingen strain were maintained in a controlled environment in 70 L tanks with a photoperiod of 14 hours day and 10 hours night. The temperature was 28.0°C with weekly variations lower than 1°C and the pH between 7.1 and 7.6. Water parameters were regularly monitored (nitrates, nitrites, chlorine, ammonium, dissolved CO2,...). Three days before breeding, males and females were separated by a grid in the same tank. For reproduction, two females were placed in the evening with 4 males in a reproduction tank equipped with a grid at the bottom allowing the passage of eggs. The eggs were collected the next morning at the beginning of the diurnal cycle, sorted and washed three times in Embryo medium (E3). All experiments were carried out at 26°C between the first and the 6th day post-fertilization (dpf) before the larvae are able to feed independently (Directive 2010/63/EU).

Adult daphnids were maintained in culture in the laboratory in 5 L glass containers with a photoperiod of 16 h day 8 h night. They were fed daily with a fresh culture of green microalgae (*Chlamydomonas reinhardtii*) and the medium was renewed weekly. For ecotoxicological tests, daphnids of at least 3 generations were used. Then, adult daphnids were isolated in a separate container by filtration on a 0.5 cm mesh and filtered again 24 h later to recover the neonates (<24h) used for the experiments (see experimental section).

Artemia cysts were grown in an appropriate environment to allow hatching. 1 g of dehydrated artemia cysts were placed in an Erlenmeyer flask of 500 mL of water with 3.5% salinity. They were maintained in a water bath at 25°C and under constant bubbling for 24 h. The hatched artemia nauplii were then collected for the toxicological tests (see experimental section).

Chlamydomonas reinhardtii strains were kept in sterile 2 mL vials containing gelled TAP medium. Before starting the experiments, the algae were transferred to liquid TAP medium for an acclimatization period (two weeks). Liquid algal cultures were maintained at 21°C with constant agitation (100 rpm), in Erlenmeyer flasks with membrane caps to maintain sterile conditions but allow gas passage. The algae were transplanted regularly to maintain them in the growth phase at the time of the experiments. The presence of undesirable microorganisms in the cultures was regularly controlled under the microscope.

III. Sampling and chemical analysis

In order to determine the environmental concentrations of FUR, PYR and SAL, river water samples were collected from the Seine River upstream (Juvisy-sur-Orge, Essonne) and downstream (Triel-sur-Seine, Yvelines) of the Paris metropolitan area, France, on 12/21/2021. The samples were processed and analyzed by a high-performance Liquid Chromatography-Tandem Mass Spectrometry method as described previously (Sandré et al. 2023a). Briefly, the samples were filtered at 0.7 µm on GF/F filters and were then separated into seven 500 mL aliquots of which 5 were spiked. Compounds in the dissolved phase were extracted by an automatic extraction system (Thermo AutoTrace 280 SPE Instrument) onto OASIS HLB cartridges (200 mg, 6cc), conditioned with methanol and ultrapure water. After sample passage, the cartridges were eluted with 10 mL of methanol. Internal standards (Atenolol-d7 and sulfamethoxazole-d4) were added and the samples were concentrated to 1 mL by evaporation. The analyses were performed with a triple quadrupole UPLC (Acquity-TQD, waters) and the compounds were separated by an ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 x 100 mm) with a 15 min elution gradient in a 90:10 mixture of ultrapure water and methanol acidified with 0.1% formic acid. The concentration of the extracts was evaluated by internal calibration.

IV. Toxicological and ecotoxicological tests

A. Acute toxicity

The acute toxicity of the compounds on *Artemia salina* was evaluated based on the immobilization criterion after 24 h of exposure (10-450 mg/L FUR, 10-450 mg/L PYR or 5-350 mg/L SAL). Five 24 h old-nauplii were captured in 20µL of water reconstituted at 3.5% salinity and placed in the wells of 96-well plates. The volume of the wells was completed to 200 µL by the exposure solutions (prepared from stock solutions) in the reconstituted saline water. 12 wells were exposed for each condition and 5 replicates were performed for each experiment. The plates were maintained at 21°C for 24 h and the number of immobile artemia (no movement for at least 10 s) was counted under a binocular magnifier (OPTIKA SFX-91).

Acute toxicity on *Daphnia magna* was also evaluated on immobilization parameters after 48 h exposure (20-750 mg/L FUR, 20-750 mg/L PYR or 20-250 mg/L SAL). Neonate daphnids were collected randomly in 20 µL of reconstituted water and placed in a 96-well plate (one neonate/well). Twelve daphnids were exposed for each condition and 3 replicates were performed for each experiment. The volume of the wells was completed to 200µL with exposure solutions.

Acute toxicity on *Danio rerio* was evaluated on lethality parameters (no heart beat) after 96 h exposure. The EC50s could not be obtained at the concentrations tested, as more concentrated solutions required higher percentages of DMSO which could have significant effects above 5 %. However, physiological effects have been observed between 50 and 700 mg/L exposure of FUR.

The tests systematically included a control and a DMSO vehicle control. In the controls, less than 10% of the individuals had to be immobilized for the test to be considered valid.

B. Cardiotoxicity

Neonate daphnids were exposed for 24 h to 50-500 ng/L FUR, PYR or SAL and 2 replicates were performed for a total of 14 individuals per condition. The heart of *D. magna* was visible by transparency at the dorsal level and heartbeats were counted using a hand-held counter under an inverted optical microscope. Heartbeats were recorded over 15 s because of the high mobility of *D. magna* and reported in Beats Per Minute (BPM).

D. rerio eggs were exposed from 1 dpf to 6 dpf. Heartbeats were counted on the animals used for the behavioral tests exposed at 50-500 ng/L FUR or SAL, 50-500-2000 ng/L for PYR. The experiment was performed in triplicate for a total of 16 individuals per condition. The heart of *D. rerio* was visible in the ventral side of the heart chamber. Beats were counted over a period of one minute.

C. Behavioral assay

D. rerio and *D. magna* were subjected to a stress (periods of darkness) and their mobility was evaluated using a Zebrabox (Viewpoint) which recorded individual movement of the organisms in real time. The light-dark transition test on *D. rerio* larvae has been described previously (Sandré et al. 2022). Briefly, *D. rerio* eggs were exposed from 1 to 6 dpf in 96-well plates at concentration ranges between 50 and 2000 ng/L except for FUR for which higher concentrations were found in the literature (50000 ng/L). Plates were then analyzed using the Zebrabox with a program involving a 60 min acclimatization period followed by three 5 min dark periods spaced by 10 min light periods. The movement of the larvae was integrated every minute. The experiment was performed in triplicate for a total of 36 exposed individuals.

A similar light-dark transition assay was adapted for *D. magna*. Neonate daphnids were maintained in reconstituted water for 12 days and then exposed with the exposure solutions for 24 h in 96-well plates at concentrations between 50 and 2000 ng/L of FUR, PYR or SAL. The plates were then analyzed with the Zebrabox with a protocol similar to that applied for *D. rerio* but with a shorter acclimatization period (10 min). Daphnia movement was integrated every 30 seconds. The experiment was performed in duplicate for a total of 24 exposed individuals.

D. Measurement of oxidative stress

Reactive Oxygen Species (ROS) generated by exposure of *D. rerio* larvae to the three molecules (1-10-100-500-1000 ng/L) were quantified using the 2',7'-dichlorofluorescein diacetate probe (H_2DCFDA, Sigma-Aldrich) which enters the cells and, in the presence of ROS or esterases, is oxidized to 2',7'-dichlorofluorescein (DCF) which then emits a fluorescent signal whose intensity is proportional to the amount of ROS present. For this purpose, 24 h eggs were placed in black 96-well plates (COSTAR 3915) with one egg per well and 8 wells per condition. Excess water was removed with a pipette, taking care not to damage the eggs, and the wells were filled with 200 µL of exposure solution, DMSO at the concentrations present in the wells, or E3 medium to be used as negative control, or positive control after addition of H_2O_2. Plates were placed in the incubator at 26°C for 5 days. In the control wells, 100 µL of medium was removed and replaced with 100µL of H_2O_2 to a final concentration of 1.5 g/L, and the plates were further incubated 15 min. After incubation, 20 µL of a 10 µg/mL DCFDA solution was added to each well. Then, the plates were

briefly shaken and returned to the incubator in the dark. After 30 min, fluorescence measurements were performed using a plate reader (Fluoroskan Ascent, Thermo Electron Corporation) and Ascent Software for Fluoroskan with λexcitation = 485 nm, λemission = 538 nm, and 100 ms exposure time.

E. Algal growth, size and mobility

C. reinhardtii cultures were acclimatized in the dark for two weeks. Sterile Erlenmeyer flasks filled with 50 mL of TAP control or 50-200-700 ng/L or 1 mg/L FUR, PYR or SAL medium were inoculated with 250000 cells per mL in an exponential growth phase. The number of algae was counted by manual counting on a malassez cell using a microscope (IM-3F OPTIKA) after 24 h and at 120 h of exposure. At 120 h, two aliquots of algae per replicate were photographed and analyzed under a microscope on calibration slides, and 4 replicates were taken per condition. The areas were calculated with the Danioscope software on about 20 algae per photograph. In parallel, on the calibration grid, the percentage of immobile algae (during at least 10 s) or having lost their flagella was recorded. For each condition, two counts were performed for two different replicates.

V. Expression of oxidative stress-related genes

The expression of 3 genes encoding antioxidant enzymes (*sod1*: superoxide dismutase, *gpx*: glutathione peroxidase and *cat*: catalase) was measured by RT-qPCR on *D. rerio*. Twenty-four hours post fertilization, eggs were collected and placed in a 12-well plate (10 eggs/well). Excess fluid was removed by pipetting. Wells were then filled with 3 mL of FUR, PYR, SAL, E3 or DMSO solution. Larvae were exposed for 5 days during which the plates were maintained in an oven at 26°C.

The larvae were ground using the MP Biomedicals FastPrep-24 Kit. Total RNA was then extracted and purified with the Qiagen RNeasy 2 kit. A DNAse treatment step was added with the Qiagen RNAse-Free Kit DNAse in order to remove any trace of DNA contamination. At this stage, a fluorimetric quantification was performed to determine the quantity of extracted RNA (Promega Quantifluor RNA System kit). The purity of the extracted RNAs was also verified using the Biowave DNA WP spectrophotometer.

Next, the mRNAs were reverse transcribed using the Affinity Multiple Temperature cDNA Synthesis kit. Once the cDNA template was obtained, qPCR was performed using the Brilliant III Ultra-Fast SYBR Green qPCR Master Mix kit (see qPCR and Melting-Curve in SI.SI) The primers used are listed in **table 1**. The specificity of each sequence was verified on NCBI. actb1 (actin beta) was used as a housekeeping gene.

Table 1. List of primers used for the RT-qPCR step.

	F 5'-3'	R 5'-3'	Reference
actb1	AAGTGCGACGTGGACA	GTTTAGGTTGGTCGTCGTTTGA	Gonzalez et al. 2006
sod1	GGCCAACCGATAGTGTTAGA	CCAGCGTTGCCAGTTTTTAG	Liu et al. 2020
gpx	GCCCGCATTCAGATTCCTCA	AACGCACCACTTGGCCCTC	Liu et al. 2020
cat	CCAGCCACGCTTCCTTGAGT	TCTCTCGGCTTCATTTAGCACCT	Liu et al. 2020

Finally, the results were processed with the ΔΔCt method that quantifies the expression of one or more genes of interest based on one or more reference genes.

VI. Calculation of toxicity scores

Toxicity scores were calculated taking into account the percent effect (difference from the respective test controls) and the effect concentration. These scores were weighted by an impact coefficient according to the following formula:

$$Toxicity\ score = \frac{\%\ measured\ effect}{Log(Effect\ concentration)} \times Impact\ coefficient$$

The impact coefficient was set to 1 if the measured parameter directly impacted the survival of the organism (lethality), 0.8 if the parameter indirectly measured the survival of the population (growth, reproduction, ...), 0.6 if the parameter measured an indirect impact on the survival of organisms (mobility, stress response, oxidative stress, ...) and 0.4 if the parameter describes another physiological change in the organism (algae size, heart rate, ...).

VII. Assessment of cocktail effects

The synergistic effects of FUR, PYR and SAL at high (concentration ranges underneath the EC50) and low (ie environmental) (50, 500 and 2000 ng/L) concentrations were evaluated on *D. magna*. Compounds were tested in pairs (FUR-PYR, FUR-SAL, PYR-SAL), and three concentrations were tested per compound within each mixture. Each experiment was performed in triplicate.

For high concentrations, the parameter measured was immobility. The exposures were carried out in the same way as in the acute toxicity tests; neonate daphnids were exposed and the immobility was measured after 48 hours of exposure. For environmental doses, the parameter measured was the mobility of daphnia. The exposures were performed in the same way as in the behavioral tests. Twelve-day-old daphnia were exposed in 96-well plates for 24 hours before being analyzed using the Zebrabox with the light-dark program. The antagonistic, additive or synergistic effects of the mixtures were modeled using the Combenefit software (Di Veroli et al. 2016). BLISS and LOEWE models were compared.

Results & discussion

I. Acute toxicity

Acute toxicity tests are classically used to determine the toxicity of a substance. These studies generally consist in exposing organisms (plants, invertebrates, vertebrates,...) to increasing concentrations of a chemical substance during a short period of time. Numerous standardized tests exist for the study of different parameters (lethality, immobilization, growth,...) and these tests allow the determination of no-effect concentrations, necessary for environmental risk assessment studies.

Furosemide acute toxicity is well documented and has been determined on various organisms such as the bacterium *Aliivibrio fischeri* (Olvera-Vargas et al. 2016, Di Nica et al. 2016, Isidori et al. 2006), different algal species (Kuzmanovic et al. 2015, Guo 2015, Christensen et al. 2009), zooplankton (Isidori et al. 2006), different crustacean species (Diaz-Sosa et al. 2020, Kuzmanovic et al. 2015, Isidori et al. 2006, Christensen et al. 2009), cnidarians (Pascoe et al, 2003) and several fish species (Kuzmanovic et al. 2015, Christensen et al. 2009). There

are also acute toxicity values on fish cell lines (Christensen et al. 2009). In contrast, very few studies exist on the toxicity of SAL and PYR. Only one study investigated the toxicity of SAL and showed that it induces changes in some biological parameters in mouse models, such as increased alanine and aspartate aminotransferase, increased creatinine, reduced blood glucose, liver and kidney congestion (Al-Omar et al. 2009). Two of our previous work investigated PYR toxicity *in vitro* and *in vivo*. We indeed showed that PYR induces a dose-dependent decrease in cell survival (SH-SY5Y) with an EC50 at 973 ± 46 µM after 96h of exposure (MTT test) leading to a 175% increase in caspase-3 activity (linked to cell apoptosis), generates oxidative stress and causes respiratory chain complex I inhibition. After in vivo per-os exposure at high dose in mice, PYR induces the development of characteristic markers of neurodegenerative diseases (α-synuclein accumulation, dopaminergic neurons death, Tau alterations in hippocampus)(Laurencé et al. 2014, 2019). However, no studies have been conducted on aquatic organisms. In this context, in order to compare the toxicity of PYR and SAL degradation products with their parent molecule, the acute toxicity was determined on *D. magna* after 48 h and *A. salina* after 24 h exposure. The results are presented in **figure 1**.

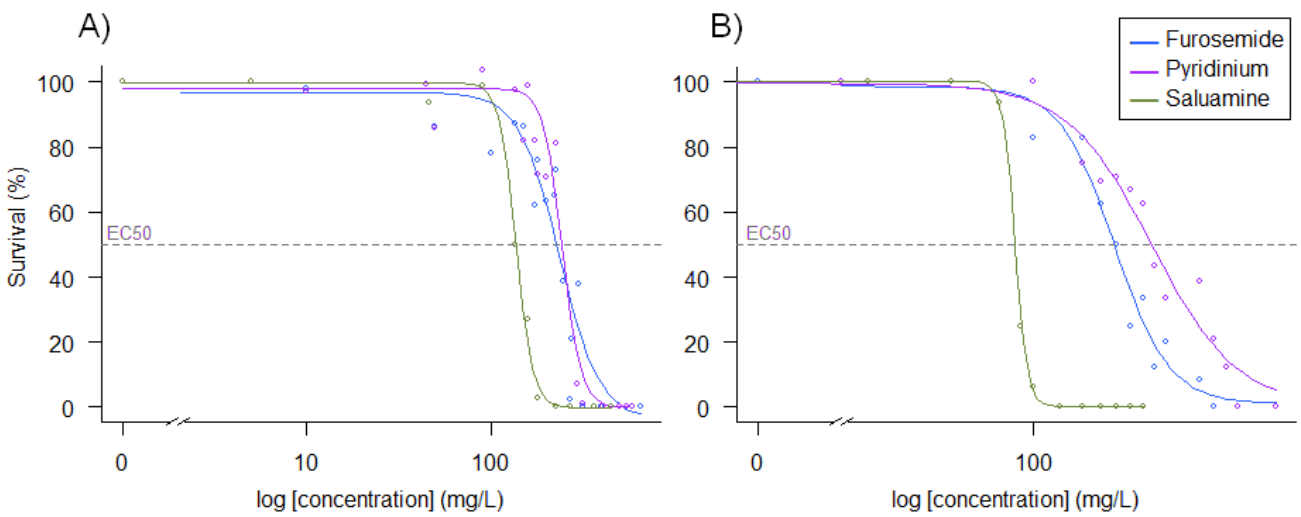


Figure 1. Dose-response curves after 24 h of exposure to furosemide, pyridinium of furosemide and saluamine on *A. salina* (A) and after 48 h on *D. magna* (B).The dose-response curves were drawn using the R software with the drm function. The quality of the models was verified according to the definition of the slope, the high limit and the low limit. The EC50 were determined from the dose response curves. Each point represented the average value of 5 replicates for A. salina and 3 for D. magna. Standard errors are not shown for clarity.

For FUR, the EC50 values obtained are 236.3 ± 15.6 mg/L for *A. salina* and 195.5 ± 18.5 mg/L for *D. magna* (figure 1). Diaz-Sosa et al. (2020) reported an EC50 after 24 h of exposure of 273.0 mg/L for *A. salina*, which is quite close to the value obtained in this study. Christensen et al. (2009) reported a 48 h EC50 for *D. magna* of 239.0 mg/L which is 18% higher than the value obtained in the present study. In contrast, greater variations in EC50 values for *D. magna* have been observed in the literature, for example, Isidori et al. (2006) obtained an abnormally lower 24 h EC50 (60.6 mg/L) than the 48 h EC50 obtained by Christensen et al. (2009). These variations could possibly be attributed to the different methodologies used (standardized OECD 202 test for the median 48 h toxicity; standardized ISO 6341 test for the 24 h toxicity). For PYR, the EC50 values obtained are 244.9 ± 7.0 mg/L for *A. salina* and 268.6 ± 39.7 mg/L for *D. magna*. PYR and FUR toxicity are quite close and the similar structure of the two compounds could explain this proximity. Our previous results also show a higher toxicity of PYR on the SH-SY5Y cell line (MTT test) (Laurencé et al. 2014). However, Olvera-Vargas et al. (2016) estimated an EC20 of 18.9 mg/L in *Aliivibrio fischeri* for a 15-min exposure for PYR and 72.3 mg/L for

FUR suggesting a higher toxicity of PYR in this model. For SAL, the EC50 values obtained are 137.7 ± 1.0 mg/L for *A. salina* and 85.4 ± 0.7 mg/L for *D. magna*. In both cases, the 50% effect concentration of SAL is well above that of its parent molecule, indicating a higher toxicity.

Acute toxicity tests performed on fish models are scarcer; only one study reported an EC50 at 96 h for SAL in *Cyprinodon variegatus* (497.0 mg/L, OECD 203). Tests on vertebrates are more constrained (ethics and regulations) and furosemide precipitates at high concentrations, making it necessary to add large amounts of organic solvent, which can bias the EC50 value or significantly increase the exposure time over 96 h. Tests were performed on *D. rerio* at 96 h but the concentrations tested were insufficient to reach the EC50 (result in SI.II). On the other hand, hemorrhages appeared around 350 mg/L at 72 h of exposure to furosemide (**figure 2A**).

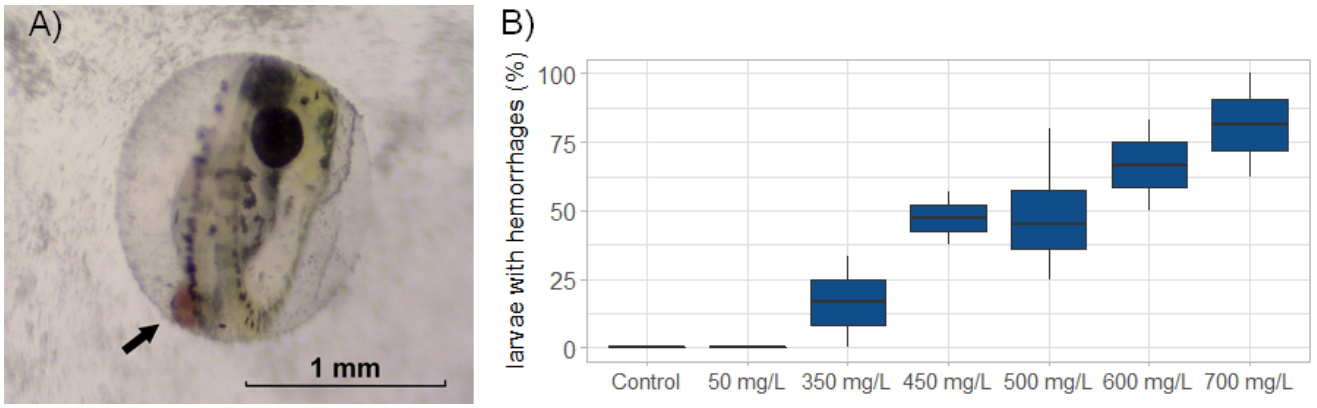


Figure 2. Presence of hemorrhages in furosemide-exposed larvae. (A) Photograph of a 96 dpf zebrafish larvae exhibiting a hemorrhage (black arrow). (B) Percentages of larvae showing hemorrhages. Control = E3 medium; number of individuals = 20 per condition.

The presence of hemorrhage has been linked to abnormal development in *D. rerio* embryo (Chen et al. 2020, McCormick et al. 2010). Although observed here at high concentrations, the presence of hemorrhages after 72h of exposure in *D. rerio* showed that FUR can permeate the chorion and damage the organism before hatching, leading to exposure at very early stages of development. The occurrence of hemorrhage was directly dependent on the concentration of FUR used (**figure 2.B**). No hemorrhages were observed after PYR or SAL exposures, but tremors were observed in larvae exposed to PYR at high concentrations (50-700 mg/L), but could not be quantified because the phenomenon was not continuously visible. Furthermore, the presence of oedemas, trunk malformations (light to severe torsion) or flotation problems (probably related to malformation or growth retardation of the swim bladder) were sometimes observed after exposure to either 3 compounds.

Overall, the acute toxicity of FUR was relatively low, with EC50s around 200 mg/L in both crustaceans tested, in agreement with the scientific literature. The acute toxicity of PYR was in the same order of magnitude. On the other hand, the effect concentration of SAL was almost twice as low as the other two compounds, thus showing its higher toxicity. However, the concentrations tested were far from representative of environmental concentrations, making it difficult to anticipate their effects in the natural environment. It was then necessary to evaluate the toxicity at environmental concentrations, requiring the use of more sensitive tests and endpoints (see below). This is especially important considering that furosemide has been shown to be toxic on

other endpoints. Indeed, Rocco et al. (2010) and Isidori et al. (2006) have shown genotoxic and cytotoxic properties of furosemide on aquatic organisms.

II. Environmental concentrations

FUR has been well documented in the scientific literature and has been frequently detected and quantified in various surface waters (Sandré et al. 2023b). Its degradation products, on the other hand, are poorly documented. SAL has been detected in FUR-spiked sediments (Li et al. 2014, 2015). PYR and SAL have been obtained by biodegradation in controlled environments (Laurencé et al. 2014, Olvera-Vargas et al. 2016, Badia-Fabregat et al. 2015, 2016, Narumiya et al. 2013, Gros et al. 2020). However, no study to our knowledge has identified the presence of PYR or SAL in natural surface waters to date. In this context, in order to obtain an estimate of environmental concentrations, FUR, PYR and SAL were quantified in the Seine upstream and downstream of the Paris area (**table 2**) by LCMS-MS.

Table 2. Concentration of furosemide, pyridinium of furosemide and saluamine in the Seine river upstream (Juvisy-sur-Orge) and downstream (Triel-sur-Seine) of Paris and in Seine-Centre wastewater treatment plant outlet samples (ng/L)

	Upstream (Juvisy-sur-Orge)	Downstream (Triel-sur-Seine)	WWTP (Seine-centre) [1]
Furosemide	29.3 ± 8.6	186.8 ± 4.6	808.5
Pyridinium of furosemide	243.7 ± 2.7	62.3 ± 3.9	237.0
Saluamine	65.0 ± 27.2	92.8 ± 7.2	473.0

[1] Average value of the two samples at the outlet of Seine-centre from Sandre et al. (2023a). WWTP = Wastewater Treatment Plant.

The median concentration of FUR in European rivers is around 80 ng/L, the same order of magnitude as in our study (Sandre et al. 2023) and SAL concentrations are quite close to those of FUR. On the other hand, the concentrations of PYR are higher upstream.

Downstream concentrations of FUR and SAL were found higher than the upstream ones, most probably due to wastewater discharges from the Paris metropolitan area loaded with high concentrations of these compounds. The opposite was observed for PYR, in higher concentrations upstream than downstream. It is possible that pyridinium is discharged in lower concentrations by the WWTPs (better elimination) in the basin, as is the case for the Seine-Centre station. In addition, PYR could be degraded into SAL, as shown previously (Sandré et al 2023a), thus contributing to the increased concentrations of SAL observed. Moreover, the relatively low concentrations of SAL could be explained by the fact that it has a very good affinity with the particulate phase with a partition coefficient between the dissolved and particulate phase (Kd value) up to 10 times higher than FUR and PYR (Sandré et al. 2023). An important part of the saluamine contamination could then be missed because our results only reflected the concentrations in the dissolved phase.

III. Oxidative stress and cardiotoxicity

The above results underlined that other characteristics, more sensitive than the parameters classically observed in standard tests (lethality, immobilization, growth, reproduction,...), can be interesting to estimate

the toxicity of compounds. For example, another effect potentially induced on target organisms would be an increase in oxidative stress. Oxidative stress is a commonly studied parameter in ecotoxicology because it can be representative of the damage caused to organisms exposed to one or more pollutants. We previously showed that exposure to furosemide pyridinium leads to an increase in the amount of ROS produced mitochondria isolated from human cells (SH-SY5Y) (Laurencé et al. 2019). Thus, it was of interest to study the evolution of oxidative stress in non-target organisms. Measurements of oxidative stress produced by *D. rerio* larvae after exposure to FUR, PYR and SAL were measured by fluorescence using an H_2DCFDA probe (**figure 3**).

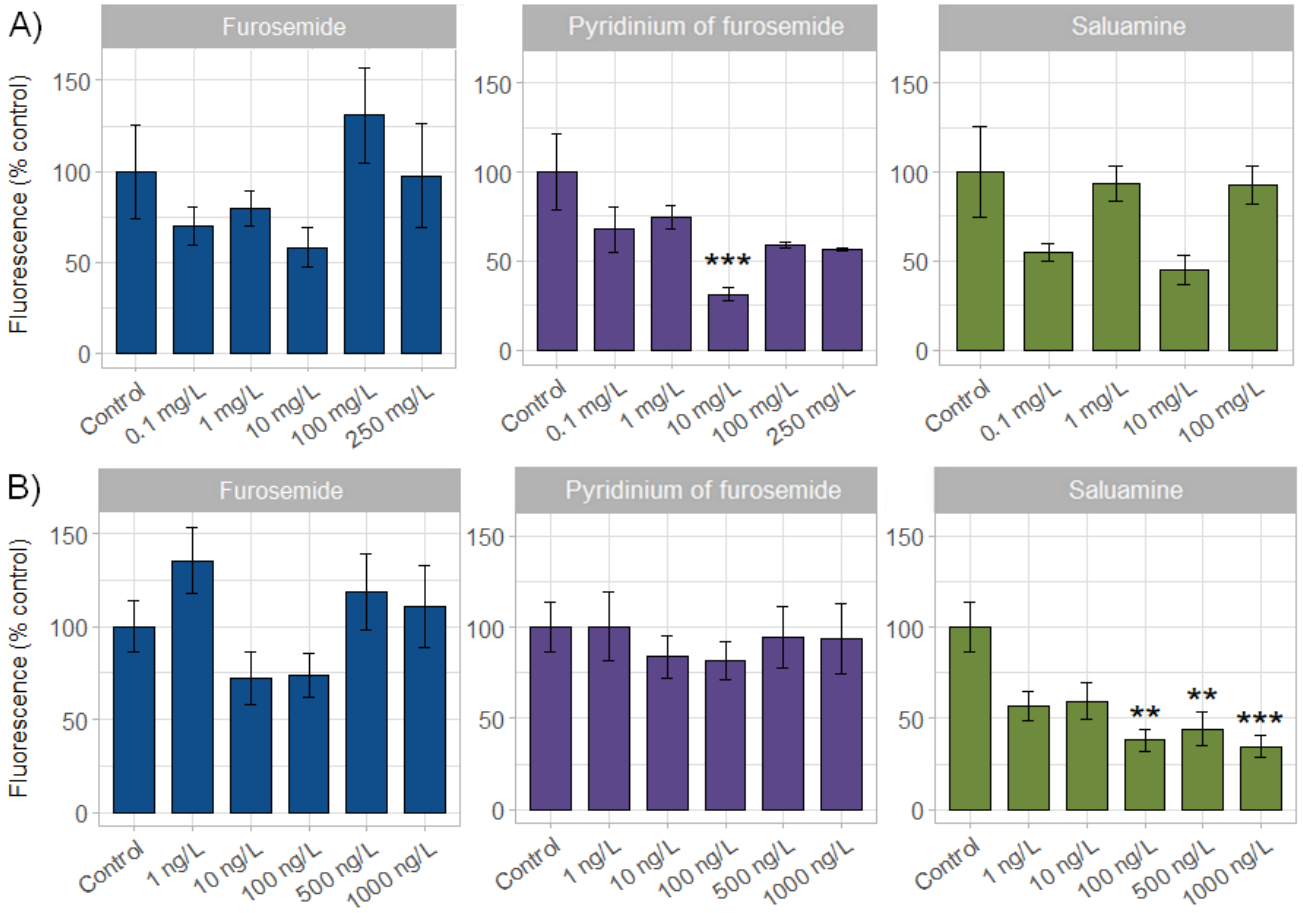


Figure 3. Measurement of reactive oxygen species by fluorescence in *D. rerio* larvae chronically exposed (5 dpf) to high and environmental concentrations of furosemide, pyridinium of furosemide and saluamine *(A) at high concentrations and (B) at environmental concentrations.* Control = E3 medium; number of individuals = 16 per condition; * = p-value < 0.05; ** = p-value < 0.01; *** = 0.001 (non-parametric Kruskal-Wallis test). A positive control for H_2O_2 and exposures to DMSO were also performed (results in SI.III). As a significant effect of DMSO was found for the highest concentrations, the values in the graph were then normalized by DMSO. At environmental concentrations, the percentage of DMSO is reduced to 5.10^{-6}% in the most concentrated solutions, no effect of DMSO was observed at these concentrations. The value at 250 mg/L SAL is not shown because this concentration resulted in 100 % larval mortality.

The H_2DCFDA probe provides a fluorescent signal proportional to the amount of ROS produced by the larvae. When the eggs were chronically exposed to high concentrations (0.1 to 250 mg/L) of FUR, PYR and SAL, the fluorescent signal was generally lower in exposed larvae compared to controls. Despite this trend, the differences were not statistically significant because of a very large variability in the fluorescence values. Only one significant effect was observed after exposure to 10 mg/L of PYR resulting in a 63% decrease in fluorescence. At environmental concentrations (1 to 1000 ng/L), a significant decrease in fluorescence was observed after exposure to SAL between 100 and 1000 ng/L. In both cases, these decreases suggest an antioxidant effect of the molecules. These results differed from our previous study (Laurencé et al. 2019), who

demonstrated a 57% increase in the amount of ROS in mitochondria isolated from SH-SY5Y cells accompanied by a 52% increase in the amount of superoxide anion (O_2^-) as well as a 68% decrease in the enzyme aconitase (inhibited in the presence of ROS) upon exposure to 1 µM of PYR. Thus, whereas PYR is able to induce ROS production at the cellular level, this response clearly depends on the scale (organelle, cell or whole organism) and the model used. Nonetheless, taken together, the results highlighted that PYR has the capacity to alter the redox balance. Acute exposures have also been performed (results in SI.IV) but the same concentrations were not sufficient to induce a similar response. Lahet et al. (2003) demonstrated an antioxidant effect of furosemide on human erythrocytes in an in vitro study using the ORAC assay (an assay to determine the antioxidant capacity of plasma). As early as 1 µM (329 µg/L) of FUR, a decrease in the fluorescence emitted by allophycocyanin was observed: the hypothesis put forward is that of a ROS scavenging capacity by furosemide. This result is in agreement with the study of Kang et al. (1998). However, this decrease was not observed *in vivo* in rats because FUR has a plasma protein binding capacity of 95%. However, if FUR binds plasma proteins, it can no longer scavenge ROS, which explains this *in vitro*/*in vivo* difference.

The impact of these three molecules at the transcriptomic level was then evaluated through the expression of three genes belonging to the triad of antioxidant system genes most studied in ecotoxicology (sod1 : superoxide dismutase, gpx : glutathione peroxidase and cat : catalase). In vivo, their expression allows to take in charge the ROS generated naturally during biological reactions (notably O_2^- by sod1 and H_2O_2 by gpx and cat). When exposed to stress, these genes can be deregulated. The expression of these three genes was quantified following chronic exposure to 50 ng/L, 500 ng/L and 1 mg/L of FUR, PYR and SAL (**figure 4**).

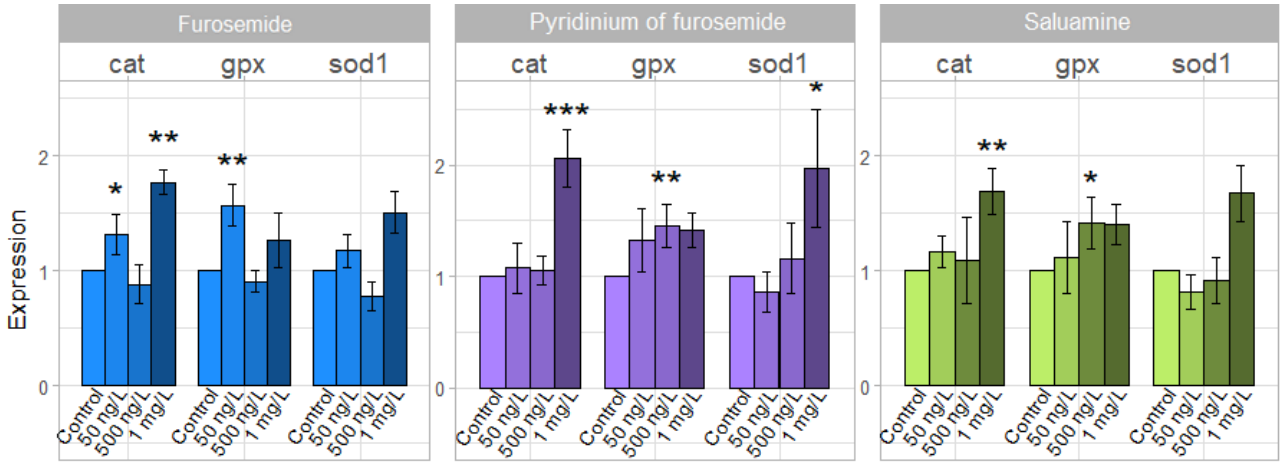


Figure 4. Expression of three enzymes involved in oxidative stress, superoxide dismutase (sod1), glutathione peroxidase (gpx) and catalase (cat), measured by RT-qPCR on *D. rerio* larvae exposed chronically to FUR, PYR or SAL. Zebrafish larvae were exposed for 5 days to 50 ng/L, 500 ng/L, or 1 mg/L FUR, PYR or SAL; Error bars represent standard deviations; Significances result from a 1-factor ANOVA: * = p-value < 0.05; ** = p-value < 0.01; *** = 0.001.

The results obtained by RT-qPCR indicated that exposure to a high concentration (1 mg/L) of the three compounds caused a significant increase in the expression of catalase, an enzyme that takes up H_2O_2 to reduce it to H_2O and O_2. For PYR, the antioxidant effect observed with H_2DCFDA also appeared to be related to the significant increase in the expression of sod1, an essential component of the free radical scavenging cell system. At environmental concentrations (50 and 500 ng/L), an over-expression of gpx was observed for the three compounds. Thus, the antioxidant effect observed may originate at the transcriptional level. Furthermore, the three molecules were able to induce an antioxidant response in aquatic vertebrate models

even at very low concentrations. The antioxidant effect of SAL is all the more worrying since this molecule is well present in the environment and has been shown to be the final metabolite of FUR: indeed, FUR can degrade directly to PYR or SAL, and PYR can itself be oxidized to SAL (Sandré et al. 2023a).

Overall, we thus observed that our three molecules of interest can have a significant antioxidant effect and that this effect is related to the increase in the expression of enzymes involved in the cellular antioxidant defense system, in particular hydrogen peroxide decomposition. At first glance, a decrease in the amount of ROS could be beneficial to organisms, as they are known to cause DNA damage and are therefore mutagenic and potentially carcinogenic agents. Overproduction of ROS can also cause inflammation and activate DNA damage repair mechanisms, or apoptosis (Finaud et al. 2006), while low levels of ROS can lead to cell growth and survival (Sies & Jones 2020). ROS hold many roles within cells including keeping the stability of the cell cycle by inhibiting the action of certain mitosis repressors (Son et al. 2011) or by stimulating mitosis activators (Ogrunc et al. 2014). They also have an important role in the immune response as they are involved in the removal of antigens by phagocytes. Thus, redox homeostasis is crucial for living organisms (Chio et al. 2017), and exposure to our compounds, through affecting cellular redox balance, may strongly disrupt many essential processes and signaling pathways.

Another relevant parameter to measure is the impact on the heart. As FUR is prescribed to fight diseases affecting the cardiovascular system, a potential impact on the heart of non-target organisms may be conceivable. In addition, we have previously shown the occurrence of hemorrhages after exposure to high doses of FUR and the presence of hemorrhages is often accompanied by cardiac malformations and yolk sac malformation (Duan et al. 2021). Moreover, heart rate measurement has proven to be more sensitive than immobilization measurement for the study of micropollutant toxicity (Fekete-Kertész et al. 2016). The cardiotoxic potential of FUR and its degradation products at environmental concentrations was measured in *D. rerio* and *D. magna* (**figure 5**).

On *D. rerio (***figure 5A***)*, FUR had no effect on the heart rate at realistic environmental concentrations. On the other hand, a significant increase in BPM was revealed at 2000 ng/L for PYR, which corresponds rather to concentrations of WWTP effluents (Sandre et al. 2023a) and as early as 50 ng/L for SAL. The increase in heart rate of *D. rerio* could be explained by a need to accelerate the metabolism to allow the elimination of the compound from the body as hypothesized by Osterauer & Köhler (2008). In addition, Carlsson et al. (2013) also noted a change in heart rate in *D. rerio* after 48 h exposure to flumethrin and toltrazuril associated with greater sensitivity to acute toxicity tests. Experiments on *D. magna* showed no significant effect of the three compounds on the heartbeat. Furthermore, the average BPM found for the control group (280 BPM) is consistent with the scientific literature (Liang et al. 2017). However, a decreasing trend of the heart rate could be noted. At high concentration on the other hand, a significant heart rate decrease was observed after exposure to FUR from 225 mg/L and to PYR from 300 mg/L. A different behavior was observed for SAL; at 90 mg/L and 70 mg/L an increase in BPM was observed (see results in SI.V).

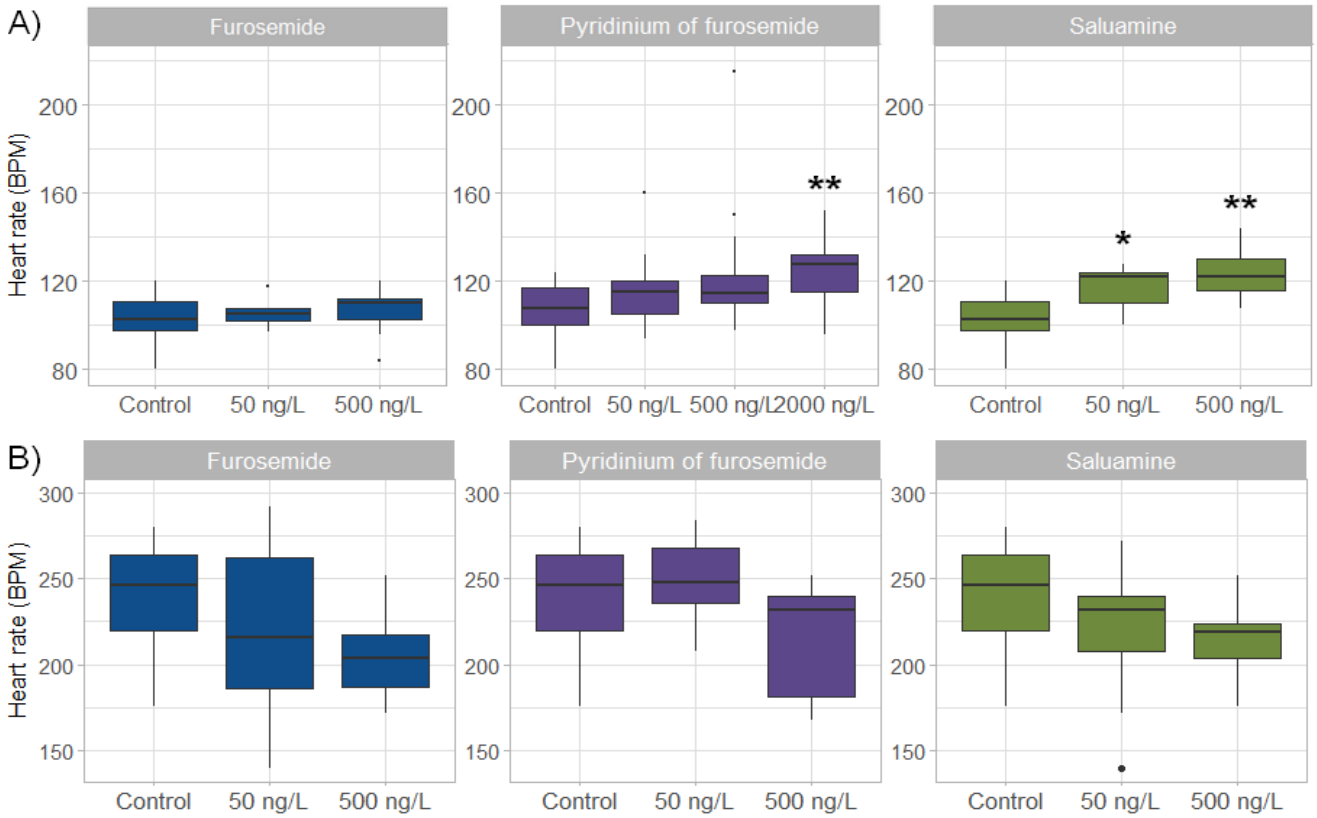


Figure 5. Evaluation of heart rate modification of 6 dpf *D. rerio* (A) and *D. magna* (B) after 24 h exposure to furosemide, pyridinium of furosemide and saluamine. Heartbeats were counted manually over 15 seconds (daphnia), and 1 minute (zebrafish larvae). For D. magna; Control = reconstituted water; number of individuals = 14 per condition. For D. rerio; Control = E3 medium; number of individuals = 16 per condition. No significant effect of DMSO was observed for both experiments; * = p-value < 0.05; ** = p-value < 0.01; *** = 0.001 (non-parametric Kruskal-Wallis test).

Taken together, these results show all three compounds tend to have an antioxidant effect and can impact the heart rate of daphnia and zebrafish. The impact of SAL at low concentrations is of particular concern for aquatic organisms.

IV. Behavioral toxicity and algal toxicity

At environmental concentrations, more sensitive tests are required to assess impacts on aquatic organisms, which can potentially jeopardize the species survival in the long term. An exposure to chemical compounds, even at very low concentrations, can rapidly induce behavioral changes (Amiard-Triquet 2009). For example, concentrations 10 to 100 times lower than lethal concentrations presented an impact on zebrafish larvae behavior (Robinson, 2009), as well as exposures to environmental samples impact at (Sandré et al. 2022). Behavioral tests have also been shown to be relevant on *D. magna* at low concentrations (Dionisio et al. 2020). In response to contamination, organisms can modify their behavior (escape, avoidance) or in the opposite way their behavior is modified by the exposure to the contamination (displacement, feeding rate) (Boyd & Lipkowitz 2002). A light-dark transition stress test was then performed on *D. rerio* and *D. magna* after exposure to evaluate for the first time the impact of environmental concentrations of these 3 molecules (**figure 6**).

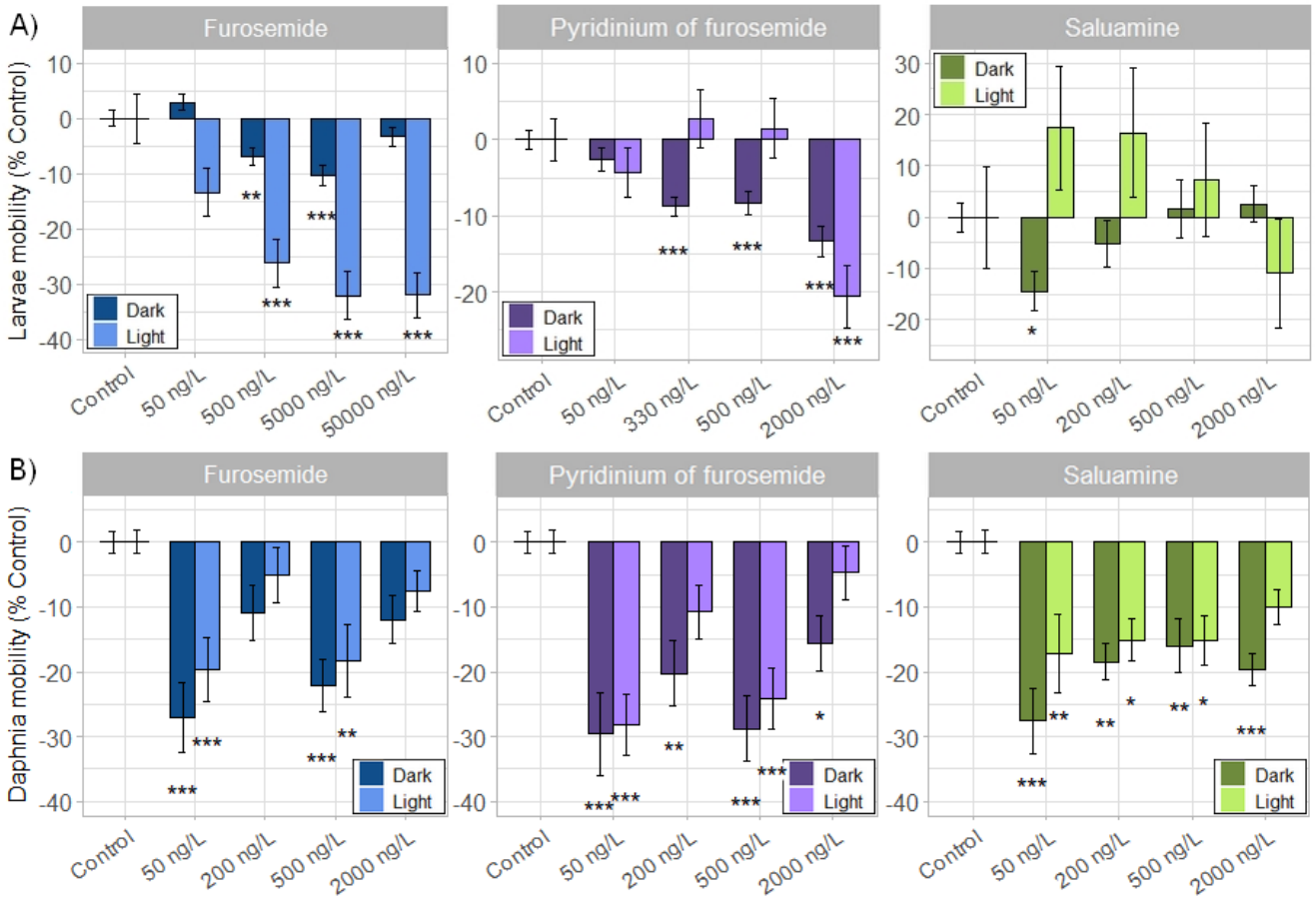


Figure 6. Impact of furosemide, pyridinium of furosemide and saluamine on the mobility of 6 dpf *D. rerio* larvae (A) and *D. magna* (B) during dark periods (stress) and light periods. Individual mobility was measured by the zebrabox during 3 periods of darkness of 5 min interspersed with 2 periods of light of 10min. For D. rerio; Control = E3 medium; number of individuals = 36 per condition. For D. magna; Control = reconstituted water; number of individuals = 24 per condition. For both sets of experiments, no effect of DMSO was observed.* = p-value < 0.05; ** = p-value < 0.01; *** = 0.001 (non-parametric Kruskal-Wallis test).

The difference in activity between light and dark periods was quite visible in zebrafish with an average movement of 3.01 ± 0.60 cm/min and 7.88 ± 1.39 cm/min, respectively. Peng et al. (2016) also noted that the distance traveled by *D. rerio* larvae was twice as great during dark periods reflecting stress response (Macphail et al. 2009). In Daphnia, the average activity was greater than in fish larvae, but there was less difference between activity during lighted periods (12.06 ± 1.89 cm/min) and dark periods (15.16 ± 2.08 cm/min). It is thus possible that *D. magna* is less responsive to this type of stress.

FUR significantly affected the movement of zebrafish larvae during the light period, reflecting an impact on overall larval mobility. From 500 ng/L, an inhibition of about 30% of the mobility of the larvae compared to the controls was observed. During the dark period, mobility was reduced by about 10% at 50 and 500 ng/L. In *D. magna*, a significant decrease of about 20% in the lighted period and about 25% in the dark period was noted at 50 and 500 ng/L. Villa et al. 2018 also observed significant inhibition of swimming distance in insect larvae (*Diamesa zernyi*) exposed 96 h to FUR but at high dose (500 mg/L). After exposure to PYR, a significant decrease in *D. rerio* larval movement about 10%, was observed from 330 ng/L, thus reflecting a reduced activity in response to stress. PYR also induced a reduction in activity during the light period for the highest concentration tested (2000 ng/L). In *D. magna*, a significant inhibition was observed for all concentrations in dark periods, similar to the observations on fish larvae, but also at 50 and 500 ng/L in light periods. Dysfunction in the mitochondria could lead to a disruption of energy metabolism and thus generate a

decrease in mobility (Zhuo et al. 2012). As PYR has been shown to impact mitochondria (Laurencé et al. 2019), this could explain its effect on the behavior of both organisms. SAL also negatively impacted larval mobility in the dark period at 50 ng/L in fish. In the light period, the responses of the larvae to SAL were strikingly variable, precluding any conclusions to be drawn. However, daphnia activity was significantly inhibited in all conditions after exposure to SAL.

Overall, exposure to the three compounds tended towards a reduction of both *D. rerio* larvae and *D. magna* activity. However, the decrease in daphnia mobility did not appear dose-dependent on this range of concentrations, as the percentages of inhibition are fairly close between the different concentrations. A decrease in the distance moved by 6 dpf *D. rerio* during the dark period was also measured after exposure to tramadol and citalopram (Bachour et al. 2020). Villa et al. (2018) hypothesized that as mechanisms that involve antioxidant and detoxifying enzymes require energy, they could lead to an energy deficit and thus to an inhibition of the behavior. It was also shown in insect larvae that FUR had an impact on the ATPase pump (Maddrell & O'Donnell 1992, Caruso-Neves and Lopes 2000). Behavioral changes may also be related to a neurological impact (Peng et al. 2016, Leuthold et al. 2019). Decreased activity during dark periods corresponds to an inhibition of the stress response, such as escaping predation. The response during the lighted period reflects the general activity. Thus, a decrease in the movement of young fish in the environment could have a negative impact on foraging or social interactions (alteration of aggression and dominance hierarchies, disruption of group interactions, mating) (Saaristo et al. 2018). Therefore, the reduction of activity may potentially compromise the survival of individuals or the population.

In order to better evaluate the impact on the aquatic environment, it was also interesting to take into account the impact on vegetal organisms, although the standardized acute toxicity tests in algae generally give rather high EC50 values. After 72 h of exposure to FUR, Christensen et al. (2009) obtained an EC50 on the growth of *Pseudokirchneriella subcapitata* at 142.0 mg/L and Guo (2015) obtained an EC50 on the growth of *Desmodesmus subspicatus* of 322.2 mg/L. Kuzmanovic et al. (2015) estimated (ECOSAR Model) the EC50 of FUR on *Pseudokirchneriella subcapitata* at 19.8 mg/L. These values are far from the actual concentrations in the environment. In the framework of this study, different tests, intended to be more sensitive, were then applied. Several parameters were monitored on the phytoplankton species *C. reinhardtii*, after exposure to FUR, PYR and SAL. First, the growth kinetics between 24 h and 120 h of exposure (**figure 7.A**), as well as the area of the algae after 120 h of exposure (**figure 7.B**) were measured to investigate whether the compounds had an inhibitory effect on algal growth. Finally, the mobility of the algae was evaluated after 120 h of exposure (**figure 7.C**).

Furosemide is known to be photodegradable (Hanamoto et al. 2014) and can transform to saluamine (Della Greca et al. 2004; Katsura et al. 2015, Jakimska et al. 2014, Sandré et al. 2023). To avoid biases due to furosemide degradation or the presence of multiple compounds, experiments on *C. reinhardtii* were performed in the dark. This algae being mixotrophic, photosynthesis is not essential, so it can draw the carbon necessary for its development by transforming the acetate present in abundance in the TAP medium used.

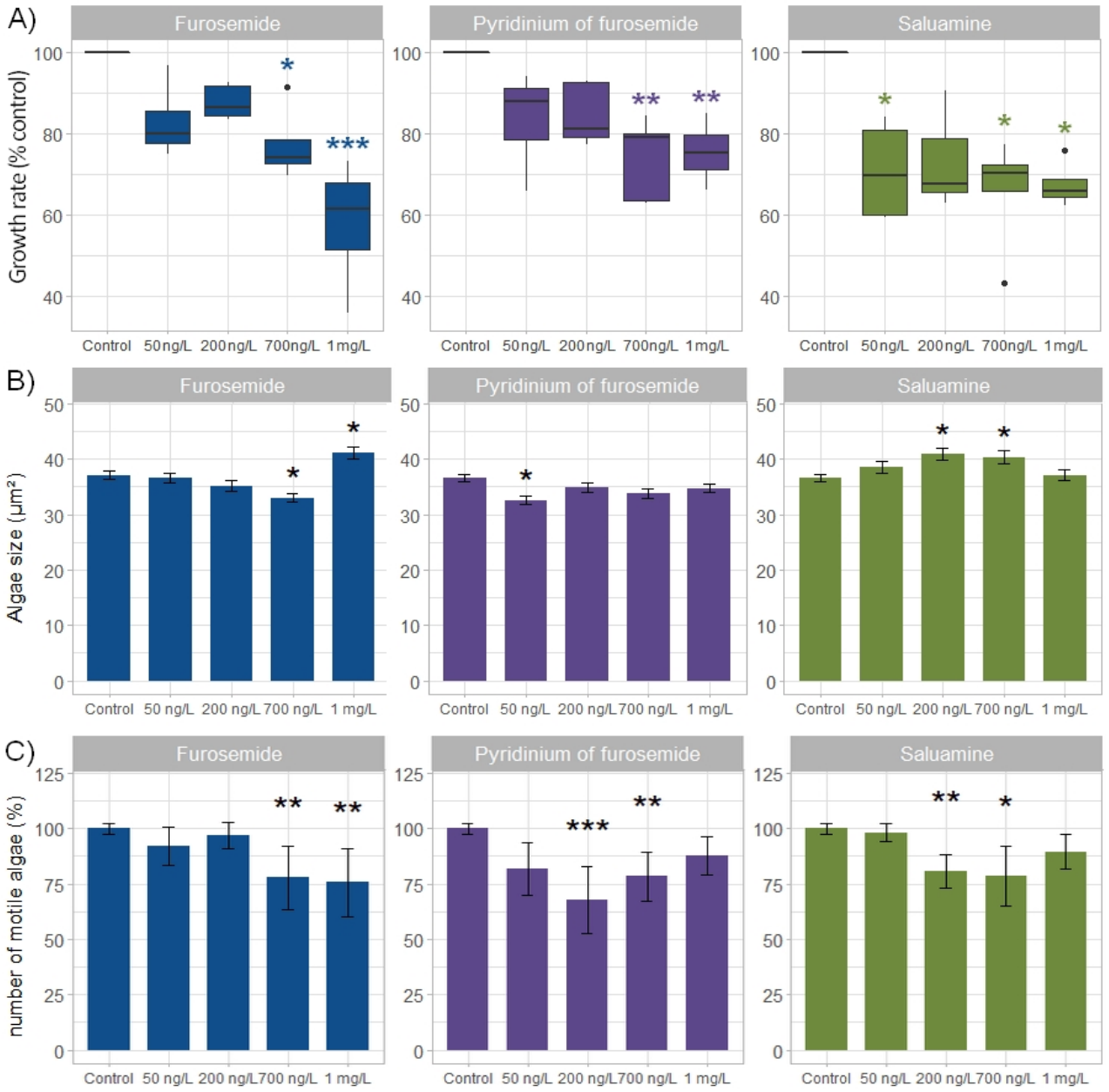


Figure 7. Effect of furosemide, pyridinium of furosemide and saluamine at 1 mg/L and environmental concentrations on growth rate (A), size (B), and mobility (C) of *Chlamydomonas Reinhardtii*. Control = TAP medium; for the growth test, 6 replicates per condition were performed; for the measurement of algal area, about 120 algae per condition were measured in 3 replicates, for algal mobility, 2 replicates were performed. No effect of methanol was observed.* = p-value < 0.05; ** = p-value < 0.01; *** = 0.001 (non-parametric Kruskal-Wallis test).

The experiments with *C. reinhardtii* revealed that a 1 mg/L concentration of any of the three compounds resulted in a growth rate inhibition (**figure 7A**), more pronounced for FUR. At a concentration of 700 ng/L, growth inhibition was also observed for all conditions, and even as low as 50 ng/L for SAL. The inhibition observed after 120 h could be due to a delay of the exponential phase as observed in Roy et al. (2009) after 72 h exposure to trace concentrations (17 ng/L) of palladium.

Results for algal size were somewhat more variable (**figure 7B**). For FUR, a slight inhibition of growth was observed at a concentration of 700 ng/L, while at 1 mg/L, an increase in size was observed, visible in all replicates. For PYR, the impact on size was limited, with a slight inhibition at 50 ng/L, but no visible effect at the other concentrations tested. For SAL, an increase in size was observed at environmental concentrations, and a decrease at 1 mg/mL. Three hypotheses can be made to interpret this observation : i) this response may

follow a hormesis-like curve with a stimulating effect on the size of algae at low doses, then a significant deleterious effect at higher concentrations, although concentrations above 1 mg/mL have not been tested. Wong (2000) already observed a stimulation of algal growth, photosynthesis and chlorophyll synthesis by low concentrations of 2,4-dichlorophenoxyacetic acid and glyphosate (active substances used in the composition of herbicides) but a complete inhibition of algal growth, photosynthesis and chlorophyll-a synthesis at higher concentrations (>20 µg/L). ii) Alternatively, this increase in size at low concentrations of SAL could be due to its structure. Indeed, Fries et al. (1974) studied the growth-promoting properties of several compounds, including p-hydroxybenzaldehyde, vanillin, syringaldehyde, 3-methylcatechol, 4-methylcatechol, 3,4-dihydroxybenzoic acid, caffeic acid and ferulic acid. The authors found that the growth-promoting properties were related to the molecular configuration, in particular to the presence of a carboxylic acid in the para position relative to a methyl, alcohol, aldehyde or carboxyl group. SAL, containing a carboxyl group, presents structural similarities with these molecules. iii) Another hypothesis is that the increase in size of *C. reinhardtii* could be due to a blockage of cell division as suggested by Rioux (2018). After exposure to platinum, these authors noted an increase in mean diameter, sometimes up to twice the normal size, and an inhibition of cell division (possibly blocked in G2 phase).

Finally, concerning the mobility experiment (**figure 7C**), the results showed an overall decrease when the algae were exposed to the three compounds. For FUR, the effect was again visible at 700 ng/L and 1 mg/L. PYR and SAL also showed a tendency to inhibit mobility, with significant effects at 200 and 700 ng/L. This loss of motility is due to the loss of flagella or their inactivity. Moreover, the culture of algae in the dark had no effect on this parameter, the control algae were 97.4% mobile. *C. reinhardtii* has two flagella allowing it to move toward or away from the light to optimize energy intake (Boyd et al. 2018). This loss of mobility could then affect the efficiency of the alga by decreasing its photosynthetic capacities, and/or restrict the formation of aggregates that are a protective/survival mechanism in case of stress (De Carpentier et al. 2019), or prevent sexual reproduction, that requires fusion (“sexual binding”) between the algae (Musgrave et al. 1979). In the aquatic environment, immobile algae should be less present in the water column making them less accessible to the organisms that feed on them.

Impacts of pharmaceuticals on algae at 1 mg/L have previously been noted by Pino et al. (2016). Paracetamol, ibuprofen, diclofenac and salicylic acid led to a decrease in photosynthetic yield. Joachim et al. (2021) also observed a decrease in photosynthetic pigments and an increase in oxygen reactive species after exposure to diclofenac. In our case, the measured effect excludes a possible impact on photosynthesis because the cultures and experiments on *C. reinhardtii* were performed in the dark. In the literature, algae have been shown to be particularly sensitive to exposure to pharmaceutical compounds (Guo 2015, Kosma et al. 2014). Our results showed that all three compounds studied significantly impacted algal growth, size, and mobility at environmental concentrations, which is of concern given that algae play an important ecological role such as oxygen production, nutrient cycling, and food supply (as the basis of food webs). Therefore, all these results suggest that these three compounds could induce deleterious effects on ecosystems.

V. Assessment of environmental risk

A. Assessment of lowest effect concentrations

The LOEC (Lowest Observed Effect Concentration) is a toxicological measure used to determine the lowest concentration of a chemical substance that produces an observable and statistically significant effect compared to a control group. Unlike the NOEC (No-Observed-Effect Concentration, defined by the WHO in 1990), the LOEC indicates the concentration at which a toxic effect is detected. It is therefore an important value for assessing the environmental risks associated with exposure to chemicals. The LOEC values obtained through the different bioassays carried out in this study have been summarized in **table 3**.

Tableau 3. Summary of the lowest concentrations of furosemide, pyridinium of furosemide and saluamine inducing effects on different organisms (LOEC).

Organism	Measured parameter	Exposure	LOEC FUR	LOEC PYR	LOEC SAL
C. reinhardtii	Growth kinetics	Environmental and high concentrations	700 ng/L	700 ng/L	50 ng/L
	Size (area)	Environmental and high concentrations	700 ng/L	50 ng/L	200 ng/L
	Mobility	Environmental and high concentrations	700 ng/L	200 ng/L	200 ng/L
A. salina	Lethality	High concentrations	42 mg/L*	108 mg/L*	66 mg/L*
D. magna	Lethality	High concentrations	41 mg/L*	23 mg/L*	60 mg/L*
	Cardiotoxicity	Environmental and high concentrations	225 mg/L	300 mg/L	70 mg/L
	Oxidative stress	Environmental	-	-	-
	Mobility	Environmental	50 ng/L	50 ng/L	200 ng/L
	Mobility (stress)	Environmental	50 ng/L	50 ng/L	50 ng/L
D. rerio	Cardiotoxicity	Environmental	-	2000 ng/L	50 ng/L
	Oxidative stress	Environmental and high concentrations	-	10 mg/L	100 ng/L
	Mobility	Environmental	500 ng/L	2000 ng/L	-
	Mobility (stress)	Environmental	500 ng/L	330 ng/L	50 ng/L

*These values correspond to EC10; - means that no impact was found at the tested concentrations.

The lowest LOEC for FUR was obtained in behavioral tests on *D. magna*. mobility was generally significantly reduced but also during periods of stress (dark periods) from 50 ng/L (**figure 6B**). For PYR and SAL, this parameter was also impacted at 50 ng/L, the lowest concentration tested, qualifying it as the most downgrading parameter. At this concentration, PYR also reduced *C. reinhardtii* areas and SAL induced an increase of *D. rerio* heart rate and a reduction of *C. reinhardtii* growth rate. The lowest concentration tested, which is fully relevant in the environment, thus showed an effect on at least one parameter for each organism.

The concentration of a chemical in the environment below which no toxic effects are expected for exposed organisms, called Predicted No Effect Concentrations (PNECs), can be determined using the LOEC values. A

Safety Factor (SF) is applied to the LOEC value to account for uncertainties in individual sensitivity, as well as uncertainties in toxicological and environmental data. We therefore calculated PNECs from our tests on *D. magna*, *A. salina*, *D. rerio*, and *C. reinhardtii,* by setting the SF at 100 because we have long-term values for at least 3 species belonging to different trophic levels (INERIS, 2014). The PNEC values for FUR, PYR and SAL are presented in **table 4**.

Table 4. Summary of the calculated indexes assessing the potential environmental risk of furosemide, pyridinium of furosemide and saluamine

	FUR	PYR	SAL
PNEC (ng/L)	0.5	0.5	0.5
RQ Upstream	0.6	4.9	1.3
RQ Downstream	3.7	1.2	1.9
RQ WWTP	16.2	4.7	9.5
Toxicity score	3.3	4.1	5.9

RQ = Risk quotient, PNEC = Predicted No Effect Concentration

Those values, calculated from sensitive tests performed at environmental concentrations, are very low. However, they take into account real impacts on organisms as opposed to the effect evaluated through classical acute toxicity tests. Although the observed effects are less deleterious than lethality, these parameters (mobility, oxidative stress, heart rate modification, …) can nevertheless affect the survival of organisms and therefore populations. For example, In another multi-organism study (in mesocosms) with several sensitive parameters measured (genotoxicity, oxidative stress,...), Joachim et al. (2021) also found very low effect concentrations up to 41 ng/L and notes an impact of diclofenac on fish and macrophyte populations and zooplankton and macroinvertebrate communities. Moreover, if only acute indicators such as lethality are considered, the risk is greatly underestimated. For example, the PNEC of FUR calculated from the EC50 values obtained earlier on *D. magna* (the lowest) is estimated at 19.55 µg/L (with a safety factor of 10000) while a significant effect at 50 ng/L has been shown. In the literature, two other PNEC values were determined at 1.56 µg/L and 45.15 µg/L for furosemide based on the EC50 of algal, crustacean and fish toxicity tests (Riva et al. 2019, Mendoza et al. 2015) close to the value calculated based on acute toxicity test.

Moreover, calculations of PNECs by using safety factors are probably not intended for such sensitive tests (ng/L) and give really low values. In this case, it is more relevant to calculate the environmental risk directly from the LOECs. Then, to assess the potential environmental risk associated with FUR, PYR and SAL (Risk Quotient, RQ), we compared the lowest LOEC and the measured environmental concentration (MEC). The MEC/LOEC ratio is similar to the PEC/PNEC ratio classically used, because it is a comparison between a measurement of the environmental concentration and a threshold value based on toxicity tests. From concentrations upstream, downstream of Paris and at the outlet of the Seine-centre WWTP (**table 2**) and the value of the most downgraded parameter calculated for each molecule (**table 3**), the RQ were calculated (**table 4**). For the three molecules, the toxic concentrations are lower than the concentrations measured in the environment, so they present an environmental risk in this context. The RQ calculated shows values systematically higher than 1 for the three molecules (except for FUR Upstream), thus indicating an

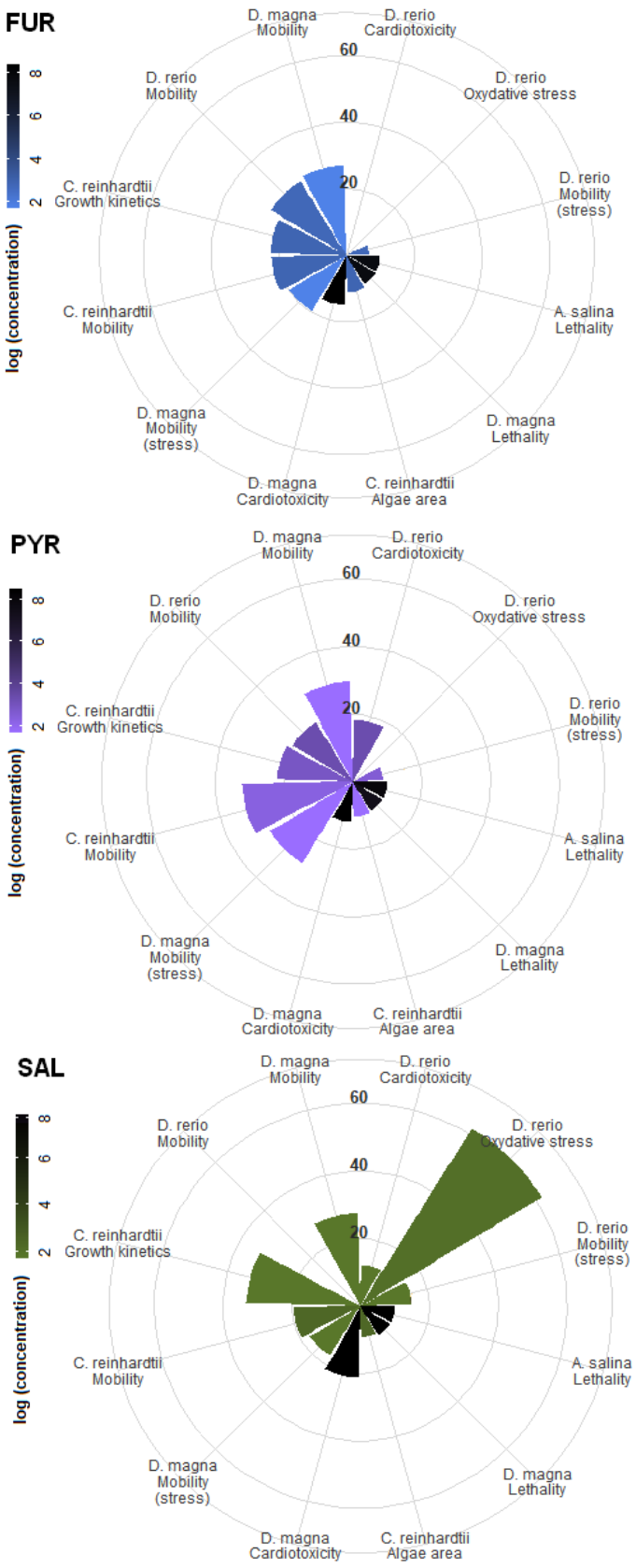


Figure 8. Toxicity of furosemide, pyridinium of furosemide and saluamine on the different endpoints measured on the four organisms. The length of the bars represents the percentage effect relative to the respective controls in each experiment. Dark colors (around log 8) represent high concentrations exposures (mg/L) and light colors (around log 2) represent environmental concentrations exposures (ng/L).

environmental risk for these molecules (Riva et al. 2019, Kosma et al. 2014) and especially more important in the output of WWTPs.

However, the risk appears lower for SAL than for the two others although this compound had marked effects on different parameters (in particular on mobility and oxidative stress). The calculation of the RQ does not take into account the impact on the different functions of an organism : it is based only on the parameter for which the effect concentration is the lowest. For a more representative risk assessment, it may be interesting to take into account the strength of the effect. The percentage effect relative to the control organisms for each of the tests performed in this study is shown in **figure 8**. On several parameters, a stronger effect is clearly observed for the degradation products. For FUR, as in the LOEC assessment (table 3), the most sensitive parameter is the mobility of daphnids, with a strong reduction in their movement. On the other hand, for PYR, it is the inhibition of algal mobility that is most affected. Finally, for SAL, the effect is more pronounced on oxidative stress in fish, with an important antioxidant activity.

This highlights the need to refine the calculations by integrating all available information and not only the most downgrading parameter. It would then be interesting to calculate toxicity scores taking into account the different parameters. In the literature, several authors established scores to assess toxic impact in order to simplify the comparison between molecules. Villa et al. (2018) suggested a Behavioral Stress Indicator (BSI) to evaluate chemicals that have an effect on behavior (compared to their control conditions) and Hagger et al. (2008) developed a Biomarker Index based on physiological (feeding, heart rate), cellular (micronucleus, neutral red, phagocytosis), molecular (protein) and exposure (AChE, metallothionein) endpoints (on *Mytillus edulis*) for

whole environmental samples. Ortiz de García et al. (2017) also developed Characterization Factors (CFs) to compare 27 pharmaceutical and personal care products using USEtox™ software that takes into account the properties of chemical substances and their behavior but requires access to databases. In our case, we wanted to include both the effect concentration and the impact. The scores are moderated by an impact coefficient that designates the importance of the effect (established arbitrarily). The calculated toxicity scores are presented in table 4. This new comparison shows this time the highest toxicity of SAL, then PYR, and finally, their parent molecule. This is not the first time that higher toxicity has been observed for processing products. For example Isidori et al. (2006) conducted a study on photodegradation product of furosemide and showed that EC50 values were lower for the degradation product than for furosemide for *Brachionus calyciflorus* (1.04 mg/L) and *Ceriodaphnia dubia* (0.57 mg/L) reflecting a higher toxicity.

Keeping in mind that these toxicity scores and integrative tests give important information on individual substances, they do not provide insights into real world situations where they coexist and interact with many other molecules (cocktail effect) as well as biotic and abiotic factors.

B. Cocktail effects

Cocktail effects of multiple chemicals are often poorly considered in environmental risk studies, even though these chemical mixtures are the norm in the environment. The combined effects of multiple substances may be more important than individual effects, highlighting the need to better understand the effects of chemical cocktails to assess environmental risks more accurately. Although initially the objective was to increase the efficacy of drug combinations while avoiding undesirable harmful effects to the patients due to unsafe drug interactions (Long et al. 2014), cocktail effects are increasingly studied in the environment and computational approaches have been proven useful to predict mixture toxicity (Lee et al. 2007). Using the Combenefit modeling software, it is possible to assess the environmental risks associated with simultaneous exposure to multiple chemicals based on statistical models and experimental data (Di Veroli et al. 2016). In this section, the antagonistic, additive, or synergistic effect of furosemide and its two by-products in a two-to-one mixture (FUR-PYR, FUR-SAL, PYR-SAL) was studied. Experiments on lethality at high concentrations (lower than EC50) and on mobility at environmental concentrations were performed on *D. magna* (the most sensitive model) after 24 h of exposure to each compound individually and by pair, following the protocols of the previous experiments. The experimental data were then compared to mathematical models of dose-response using the Combenefit software. Two different models (BLISS and LOEWE) were evaluated and the results of the BLISS model are presented in **figure 9**.

The LOEWE model is based on the assumption that the effects of the two compounds combined are additive. This means that the combined effect of the mixture is equal to the sum of the individual effects of each compound. It is also simpler to use because it does not require much input data. The BLISS model, on the other hand, is based on the assumption that the effects of the two compounds combined are multiplicative rather than additive. This means that the combined effect of the mixture is equal to the product of the individual effects of each compound. This model may require more input data to be accurate. These two models are the most used reference models for evaluating drug interactions (Lee et al. 2007). In our study, the BLISS model performed better at both high and environmental concentrations. It is then possible that the

BLISS model is more sensitive and/or better suited to the incoming data. The effectiveness of the BLISS model could also reflect the fact that the compounds have a multiplicative effect on the biological system rather than an additive one; it can better describe their interactions than the LOEWE model or other additive models.

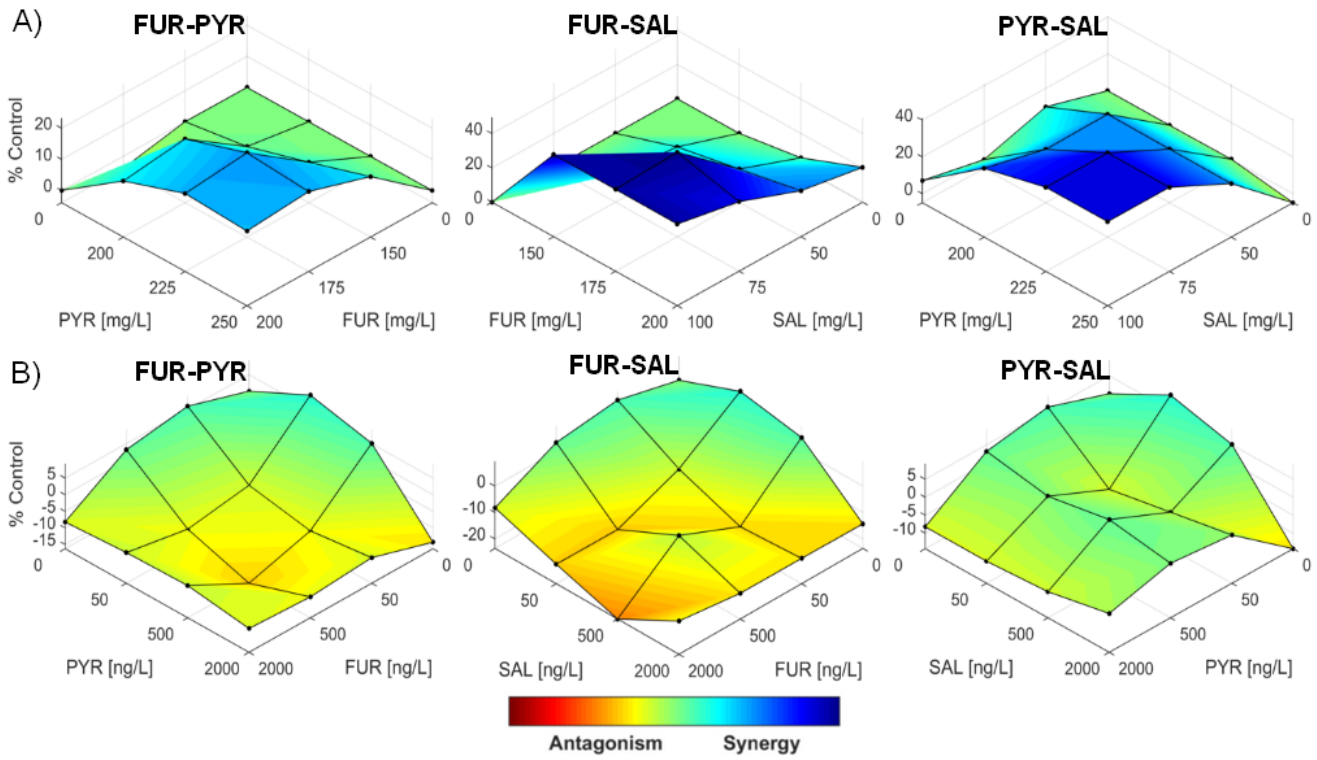


Figure 9. Effect of FUR-PYR, FUR-SAL, PYR-SAL mixtures at high (A) and low (B) concentrations on *D. magna* survival and mobility with the BLISS model. 3D representations of the effects of the FUR-PYR, FUR-SAL and PYR-SAL mixtures from the BLISS model estimates obtained from the Combenefit software. Two replicates were performed for high concentrations and 3 replicates for low concentrations, i.e. 24 and 36 daphnia per condition. Synergistic effects are represented in blue (positive values), antagonistic effects are in red (negative values). The results of the LOEWE model are presented in SI.VI

At high concentrations, the BLISS model showed a significant synergistic effect for all three mixtures, with the strongest effects obtained for the mixtures containing SAL. For low concentrations, the model shows this time a slight antagonistic effect, more visible in the FUR SAL combination. In *D. rerio*, Bachour et al. (2020) also showed an antagonistic effect of a tramadol and citalopram mixture on swimming activity, compared to the compounds alone (lower EC50), yet to be explained. Thus, the interactions between these compounds give quite different results depending on the concentration. These results underline the unpredictable character of interactions between molecules. These approaches are still limited by the choice of concentrations, the study of effects only by a pair of molecules, or the prerequisite of experimental data as in the QSAR models. As it is impossible to test all combinations of molecules, more efficient tools and strategies must be developed, such as coupling approaches in ecotoxicology, bioinformatics and non-targeted analysis chemistry (e.g. HRMS) to identify the different degradation products and their associated toxicity (Sandré et al 2022). Furthermore, a better characterization of the mechanisms underlying these effects on more refined levels of integrative biology, such as OMICs approaches, to enhance understanding of how drugs interact at the molecular level to produce observed behavioral effects. An AOP (Adverse Outcome Pathway) approach may also be of interest to better understand the effects of mixture. In addition to mixtures of pollutants, many external factors, such as dissolved oxygen depletion (or even hypoxia), temperature variation, food scarcity, and the presence of micro and macroorganisms, can impact the overall sensitivity of organisms. For example, hypoxia (which is enhanced by global warming) can amplify the toxic responses of compounds (Joachim et al. 2021).

Conclusion

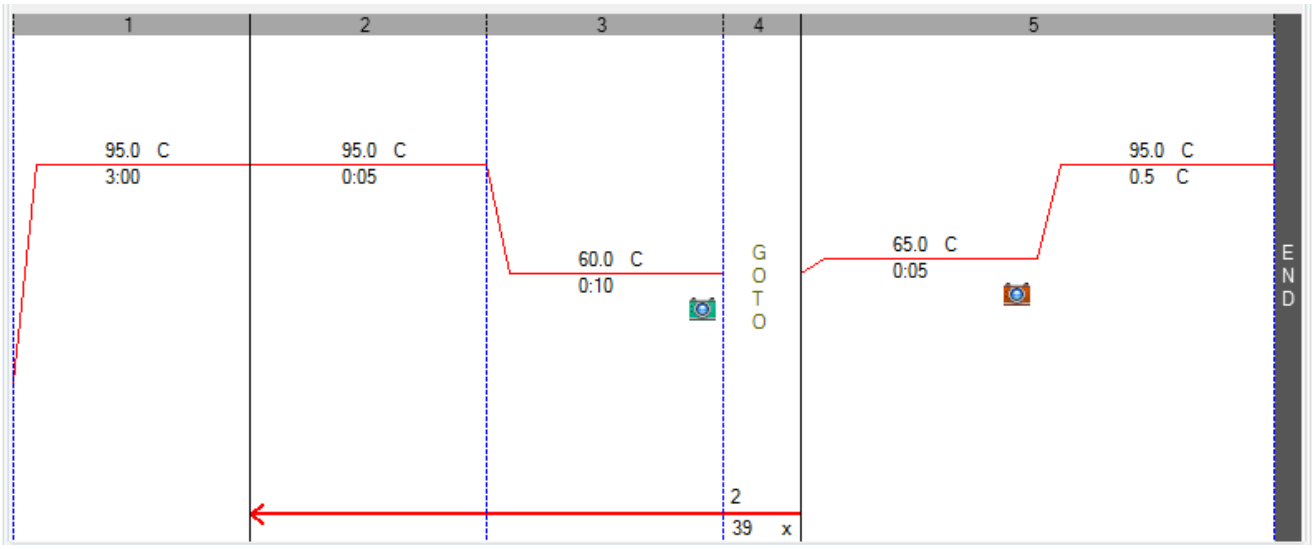
First, regarding the acute toxicity assessment, our results showed that SAL is more toxic than FUR. The acute toxicity value for FUR was close to the literature, with a toxic concentration of several hundred mg/L, which is far from environmental considerations. In the environment, the contamination levels of the three compounds were measured in the Seine river. Values close to the european rivers average (80 ng/L) were found for FUR. SAL and PYR were also measured for the first time in the environment, with concentrations of the same order of magnitude. At these concentrations, several bioassays showed an impact on key ecosystem organisms such as fish, crustaceans and green algae. The toxic effects of FUR, PYR and SAL on several parameters can potentially affect the survival of organisms and even entire populations. Indeed, multi-parameter impact has been shown to induce some fragility in organisms, such as increased susceptibility to endogenous bacterial pathogens and toxins from microalgae and macrophytes (Joachim et al. 2021). Daphnia mobility was found to be the most sensitive endpoint, with a LOEC of 50 ng/L for all three compounds. This is of interest for future environmental risk assessment. Indeed, the Risk Quotient, (greater than 1) indicated a potential environmental risk for the three compounds, taking into account the most downgrading parameter, thus lacking subtlety and nuance. When a toxicity score integrating all parameters, the intensity of the response and the effect concentration was calculated, the results brought to light the greater toxicity of SAL than PYR, itself more toxic than FUR, their parent molecule, at relevant environmental concentrations. Finally, it is important to note that a synergistic (multiplicative) effect of the three compounds in mixture was observed at high concentrations but antagonistic effects at environmental concentrations.

We previously anticipated that the persistence of SAL in the environment may potentially be problematic, due to its less efficient removal by sewage treatment plants (Sandré et al. 2023a). This hypothesis is now reinforced since this substance was found to be the most toxic in this study. Furthermore, its toxicity could also potentially be a problem for furosemide-treated patients because as for PYR, its effects at low dose in chronic exposure are not known, thus requiring further investigation concerning the exposure doses and urinary pharmacokinetics of these metabolites in patients. Moreover, given that PYR has been shown to induce the hallmarks of neurodegenerative diseases at high dose in mice (Laurencé et al 2019) and in the absence of information on PYR bioaccumulation/effects in the furosemide treated patients' body, determining whether the chronic exposure to FUR (thus to PYR) could increase the risk of Alzheimer or Parkinson diseases is essential. Finally, it should be noted that this study did not take into consideration the bioavailability and stability of these substances, which are important parameters for assessing their impact on the environment. It would be interesting to conduct mesocosm studies, as in the study by Joachim et al. (2021), that take into account the interactions between organisms.

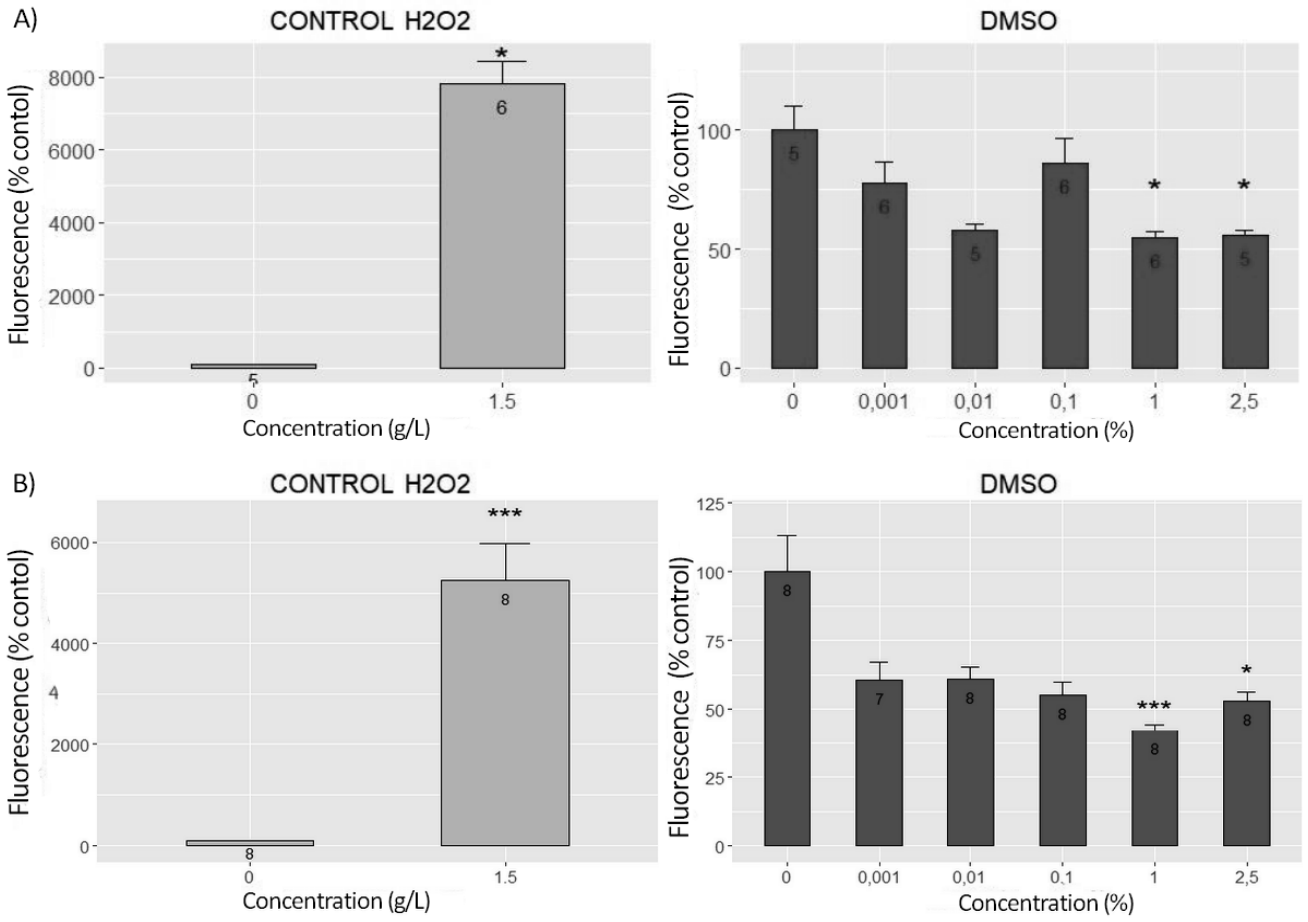
Acknowledgements: Thanks to Sadia Bagagnan and the OPUR Program for providing the Seine water samples and to Michael Rivard for the PYR synthesis. Thanks to the PRAMMICS platform (OSU-EFLUVE UMS 3563) for the analytical equipment (UPLC-IMS-QTOF and AutoTrace SPE) and to Emilie Caupos for her expertise in the analytical section. Thanks to Claire Thérial for her help on the biochemistry part. Thanks to Université Paris-Est Creteil and to the French ministry of higher education research and innovation for F. Sandré PhD fellowship.

Author contribution: Fidji Sandre: investigation, visualization, writing-original draft & editing; Aliénor Duval: investigation, writing, review & editing; Luna Grimault: investigation, review & editing; Alexis Golven: investigation; Christophe Morin: review & editing; Laure Garrigue-Antar: supervision, conceptualization, review & editing.

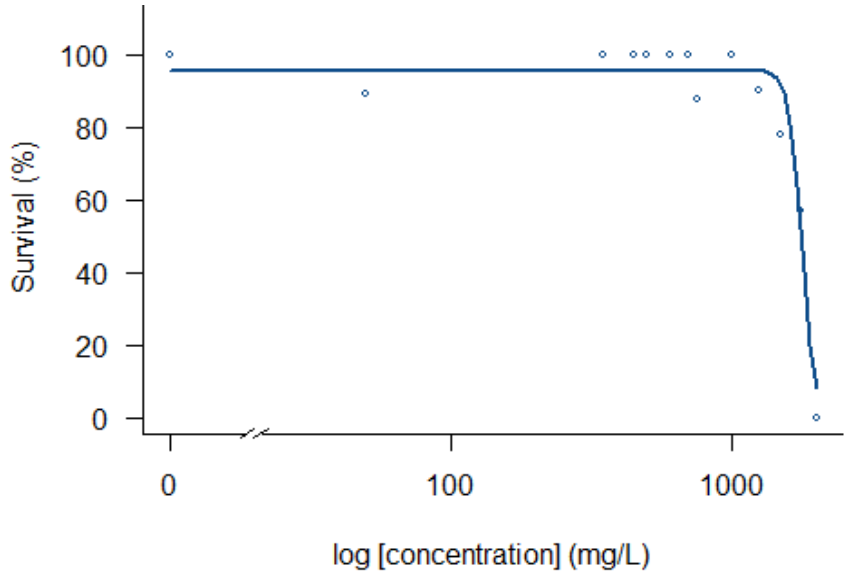
Supplementary Informations :



SI. figure S1. THERMAL PROFILE: qPCR and Melting-Curve

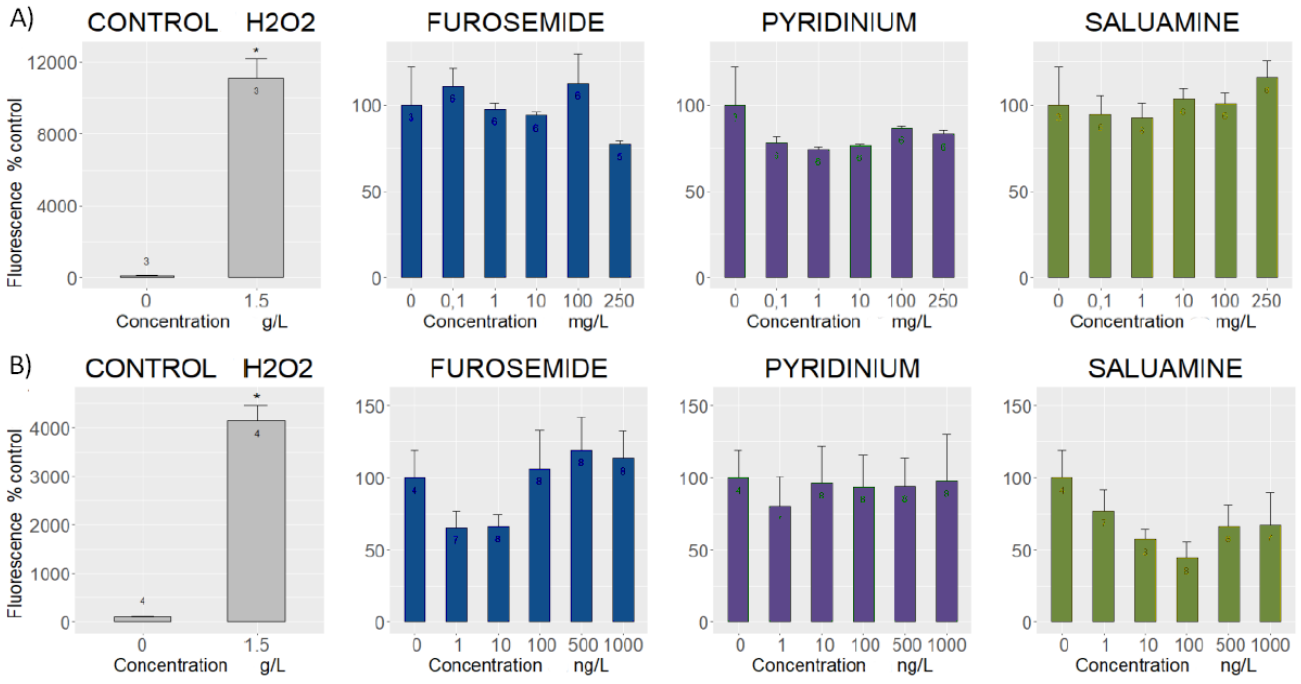


SI. figure S3. H_2O_2 and DMSO controls for oxidative stress experiments in *D. rerio* larvae for Chronic exposure (A) and acute exposure (B). Fluorescence emitted by zebrafish larvae following chronic exposure to 0.001 - 0.01 - 0.1 - 1 - 2.5% DMSO reported as a percentage of control; Controls in gray; DMSO in red; Bars correspond to standard error; Numbers in bars correspond to numbers of larvae; Kruskall-Wallis test; *: p-value < 0.05, **: p-value < 0.01, ***: p-value < 0.001.

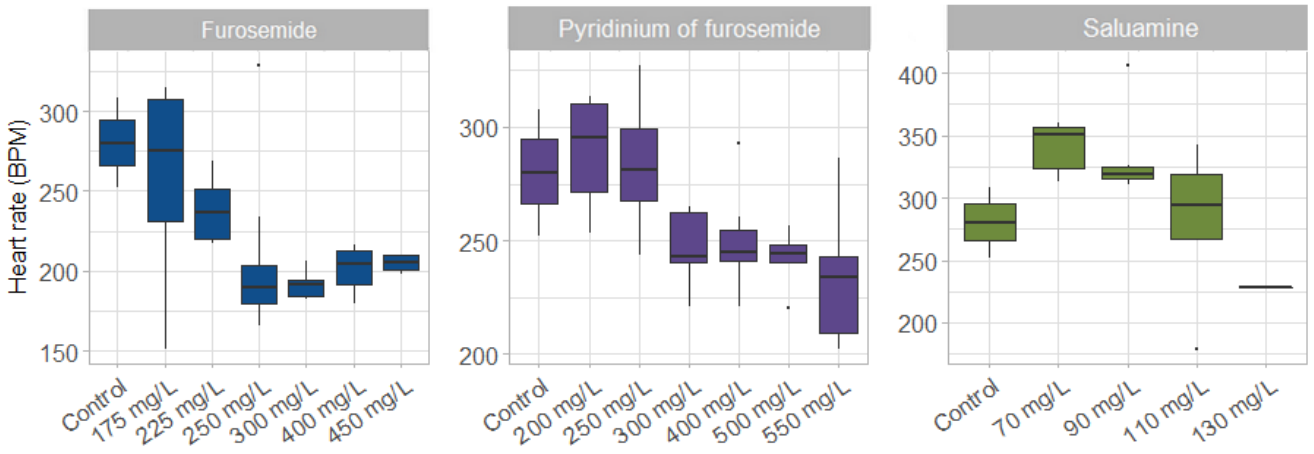


SI. figure S2. Dose-response curves after 96 h of exposure to furosemide on *D. rerio*.

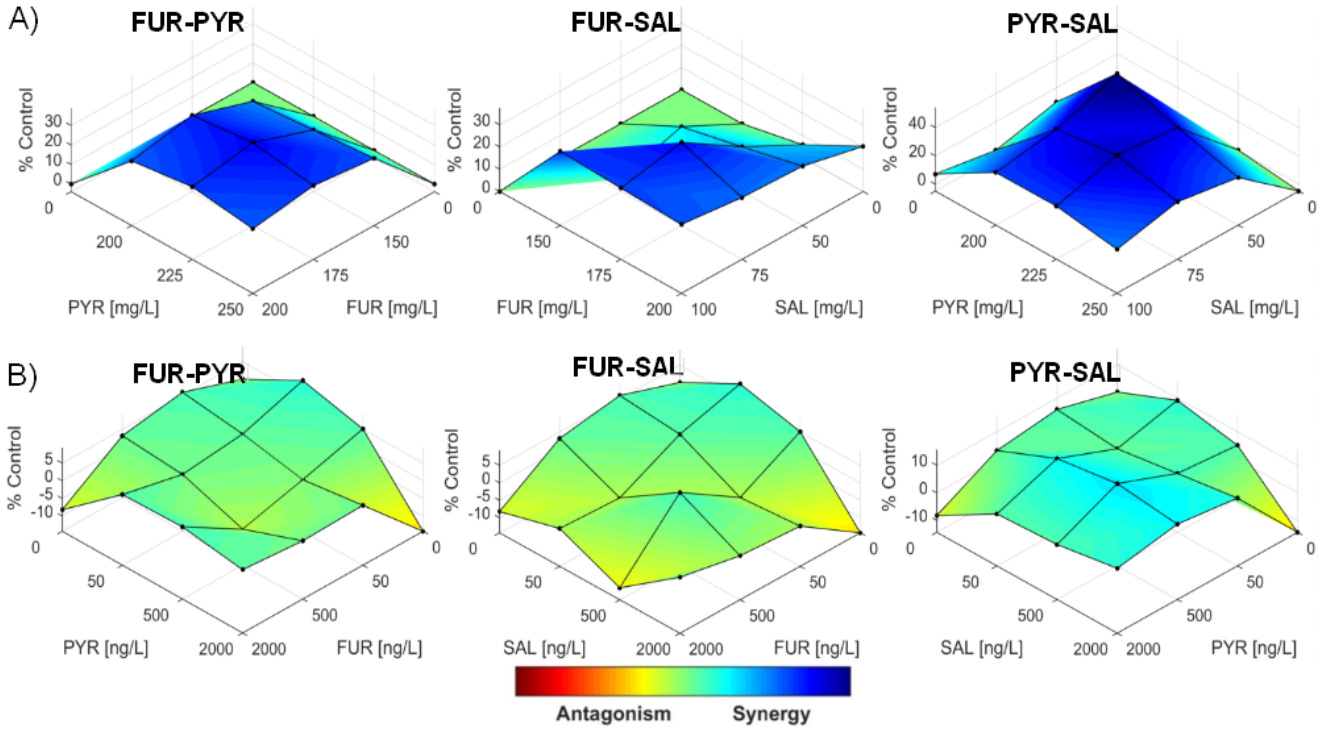
The EC50 could not be clearly determined due to issues with the solubility of FUR above 750 mg/L making the actual exposure concentration imprecise. The choice of not increasing the DMSO concentration due to possible bias was made. Therefore, due to lack of reference to compare to FUR, exposures to PYR and SAL were not extended.



SI. figure S4. Fluorescence emitted by *D. rerio* larvae following acute exposure to high (A) and environmental (B) concentrations. Fluorescence emitted by zebrafish larvae following acute exposure to high (0 - 0,1 - 1 - 10 - 100 - 250 mg/L) normalized to the acute DMSO control and environmental (1 - 10 - 100 - 500 - 1000 ng/L) concentrations of FUR, PYR, and SAL reported as percent of control; Controls in gray; Furosemide in blue; Furosemide pyridinium in green; Saluamine in yellow; Bars are standard error; Numbers in bars are numbers; Kruskall-Wallis test; *: p-value < 0.05, **: p-value < 0.01, ***: p-value < 0.001



SI. figure S5. Evaluation of heart rate modification of D. magna after 24 h exposure to furosemide, pyridinium of furosemide and saluamine. At the exposure concentrations, an effect of DMSO (used to solubilize the compounds) was observed. The number of Heart Beats Per Minute (BPM) is normalized to the effect of DMSO. Heartbeats were counted manually over 15 seconds; Control = reconstituted water; number of individuals = 14 per condition. * = p-value < 0.05; ** = p-value < 0.01; *** = 0.001 (non-parametric Kruskal-Wallis test).



SI. figure S6. Effect of FUR-PYR, FUR-SAL, PYR-SAL mixtures at high (A) and low (B) concentrations with the LOEWE model. 3D representations of the effects of the FUR-PYR, FUR-SAL and PYR-SAL mixtures from the BLISS model estimates obtained from the Combenefit software. Two replicates were performed for high concentrations and 3 replicates for low concentrations, i.e. 24 and 36 daphnia per condition. Synergistic effects are represented in blue (positive values), antagonistic effects are in red (negative values).

Références

Abbott, L.M., Kovacic, J., 2008. The pharmacologic spectrum of furosemide. J Veter Emer Crit 18, 26–39. https://doi.org/10.1111/j.1476-4431.2007.00267.x

Aherne, G.W., Briggs, R., 1989. The relevance of the presence of certain synthetic steroids in the aquatic environment. Journal of Pharmacy and Pharmacology 41, 735–736. https://doi.org/10.1111/j.2042-7158.1989.tb06355.x

Al-Omar, I.A., Al-Ashban, R.M., Shah, A.H., 2009. Toxicity Studies on 4-Chloro-5-Sulfamoylanthranilic Acid the Degradation Product of a Loop Diuretic Furosemide. Research Journal of Pharmacology 63–77, 3.

Amiard-Triquet, C., 2009. Behavioral Disturbances: The Missing Link between Sub-Organismal and Supra-Organismal Responses to Stress? Prospects Based on Aquatic Research. Human and Ecological Risk Assessment: An International Journal 15, 87–110. https://doi.org/10.1080/10807030802615543

Andreasen, F., Botker, H., Lorentzen, K., 1982. In vitro studies on the hydrolysis of frusemide in gastrointestinal juices. British Journal of Clinical Pharmacology 14, 306–309. https://doi.org/10.1111/j.1365-2125.1982.tb01984.x

Bachour, R.-L., Golovko, O., Kellner, M., Pohl, J., 2020. Behavioral effects of citalopram, tramadol, and binary mixture in zebrafish (Danio rerio) larvae. Chemosphere 238, 124587. https://doi.org/10.1016/j.chemosphere.2019.124587

Badia-Fabregat, M., Lucas, D., Gros, M., Rodríguez-Mozaz, S., Barceló, D., Caminal, G., Vicent, T., 2015. Identification of some factors affecting pharmaceutical active compounds (PhACs) removal in real wastewater. Case study of fungal treatment of reverse osmosis concentrate. Journal of Hazardous Materials 283, 663–671. https://doi.org/10.1016/j.jhazmat.2014.10.007

Badia-Fabregat, M., Lucas, D., Pereira, M.A., Alves, M., Pennanen, T., Fritze, H., Rodríguez-Mozaz, S., Barceló, D., Vicent, T., Caminal, G., 2016. Continuous fungal treatment of non-sterile veterinary hospital effluent: pharmaceuticals removal and microbial community assessment. Appl Microbiol Biotechnol 100, 2401–2415. https://doi.org/10.1007/s00253-015-7105-0

Battaglin, W.A., Meyer, M.T., Kuivila, K.M., Dietze, J.E., 2014. Glyphosate and Its Degradation Product AMPA Occur Frequently and Widely in U.S. Soils, Surface Water, Groundwater, and Precipitation. J Am Water Resour Assoc 50, 275–290. https://doi.org/10.1111/jawr.12159

Battaglin, W.A., Thurman, E.M., Kalkhoff, S.J., Porter, S.D., 2003. HERBICIDES AND TRANSFORMATION PRODUCTS IN SURFACE WATERS OF THE MIDWESTERN UNITED STATES. J Am Water Resources Assoc 39, 743–756. https://doi.org/10.1111/j.1752-1688.2003.tb04402.x

Besse, J.-P., Garric, J., 2008. Human pharmaceuticals in surface waters. Toxicology Letters 176, 104–123. https://doi.org/10.1016/j.toxlet.2007.10.012

Boyd, D.B., Lipkowitz, K.B., 2002. Appendix: Examination of the Employment Environment for Computational Chemistry, in: Reviews in Computational Chemistry. John Wiley & Sons, Ltd, pp. 293–319. https://doi.org/10.1002/0471433519.app

Boyd, M., Rosenzweig, F., Herron, M.D., 2018. Analysis of motility in multicellular Chlamydomonas reinhardtii evolved under predation. PLoS ONE 13, e0192184. https://doi.org/10.1371/journal.pone.0192184

Carlsson, G., Patring, J., Kreuger, J., Norrgren, L., Oskarsson, A., 2013. Toxicity of 15 veterinary pharmaceuticals in zebrafish (Danio rerio) embryos. Aquatic Toxicology 126, 30–41. https://doi.org/10.1016/j.aquatox.2012.10.008

Caruso-Neves, C., Lopes, A.G., 2000. Sodium pumps in the Malpighian tubule of Rhodnius sp. An. Acad. Bras. Ciênc. 72, 407–412. https://doi.org/10.1590/S0001-37652000000300014

Chen, X., Teng, M., Zhang, J., Qian, L., Duan, M., Cheng, Y., Zhao, F., Zheng, J., Wang, C., 2020. Tralopyril induces developmental toxicity in zebrafish embryo (Danio rerio) by disrupting the thyroid system and metabolism. Science of The Total Environment 746, 141860. https://doi.org/10.1016/j.scitotenv.2020.141860

Chiffre, A., Degiorgi, F., Buleté, A., Spinner, L., Badot, P.-M., 2016. Occurrence of pharmaceuticals in WWTP effluents and their impact in a karstic rural catchment of Eastern France. Environ Sci Pollut Res 23, 25427–25441. https://doi.org/10.1007/s11356-016-7751-5

Chinnaiyan, P., Thampi, S.G., Kumar, M., Mini, K.M., 2018. Pharmaceutical products as emerging contaminant in water: relevance for developing nations and identification of critical compounds for Indian environment. Environ Monit Assess 190, 288. https://doi.org/10.1007/s10661-018-6672-9

Chio, I.I.C., Tuveson, D.A., 2017. ROS in Cancer: The Burning Question. Trends in Molecular Medicine 23, 411–429. https://doi.org/10.1016/j.molmed.2017.03.004

Christensen, A.M., Markussen, B., Baun, A., Halling-Sørensen, B., 2009. Probabilistic environmental risk characterization of pharmaceuticals in sewage treatment plant discharges. Chemosphere 77, 351–358. https://doi.org/10.1016/j.chemosphere.2009.07.018

De Carpentier, F., Lemaire, S.D., Danon, A., 2019. When Unity Is Strength: The Strategies Used by Chlamydomonas to Survive Environmental Stresses. Cells 8, 1307. https://doi.org/10.3390/cells8111307

Della Greca, M., Iesce, M.R., Previtera, L., Rubino, M., Temussi, F., 2004. A new photoproduct of the drug furosemide in aqueous media. Environ Chem Lett 2, 155–158. https://doi.org/10.1007/s10311-004-0080-9

Di Nica, V., Villa, S., Finizio, A., 2017. Toxicity of individual pharmaceuticals and their mixtures to *Aliivibrio fischeri* : Experimental results for single compounds and considerations of their mechanisms of action and potential acute effects on aquatic organisms: Aquatic toxicity of pharmaceuticals. Environ Toxicol Chem 36, 807–814. https://doi.org/10.1002/etc.3568

Di Veroli, G.Y., Fornari, C., Wang, D., Mollard, S., Bramhall, J.L., Richards, F.M., Jodrell, D.I., 2016. Combenefit: an interactive platform for the analysis and visualization of drug combinations. Bioinformatics 32, 2866–2868. https://doi.org/10.1093/bioinformatics/btw230

Diaz-Sosa, V.R., Tapia-Salazar, M., Wanner, J., Cardenas-Chavez, D.L., 2020. Monitoring and Ecotoxicity Assessment of Emerging Contaminants in Wastewater Discharge in the City of Prague (Czech Republic). Water 12, 1079. https://doi.org/10.3390/w12041079

Dionísio, R., Daniel, D., Alkimin, G.D. de, Nunes, B., 2020. Multi-parametric analysis of ciprofloxacin toxicity at ecologically relevant levels: Short- and long-term effects on Daphnia magna. Environmental Toxicology and Pharmacology 74, 103295. https://doi.org/10.1016/j.etap.2019.103295

Duan, M., Zhang, J., Liu, J., Qian, L., Chen, X., Zhao, F., Zhao, W., Zhong, Z., Yang, Y., Wang, C., 2021. Toxic effects of broflanilide exposure on development of zebrafish (Danio rerio) embryos and its potential cardiotoxicity mechanism. Environmental Pollution 286, 117481. https://doi.org/10.1016/j.envpol.2021.117481

Fekete-Kertész, I., Kunglné-Nagy, Z., Molnár, M., 2016. Ecological impact of micropollutants on aquatic life determined by an innovative sublethal endpoint daphnia magna heartbeat rate. Carpathian Journal of Earth and Environmental Sciences 11, 345–354.

Finaud, J., Lac, G., Filaire, E., 2006. Oxidative Stress: Relationship with Exercise and Training. Sports Medicine 36, 327–358. https://doi.org/10.2165/00007256-200636040-00004

Fries, L., 1974. Growth stimulation of axenic red algae by simple phenolic compounds. Journal of Experimental Marine Biology and Ecology 15, 1–9. https://doi.org/10.1016/0022-0981(74)90058-6

Gros, M., Ahrens, L., Levén, L., Koch, A., Dalahmeh, S., Ljung, E., Lundin, G., Jönsson, H., Eveborn, D., Wiberg, K., 2020. Pharmaceuticals in source separated sanitation systems: Fecal sludge and blackwater treatment. Science of The Total Environment 703, 135530. https://doi.org/10.1016/j.scitotenv.2019.135530

Guo, J., 2015. Impact of pharmaceuticals on algal species. University of York.

Hammarlund-Udenaes, M., Benet, L.Z., 1989. Furosemide pharmacokinetics and pharmacodynamics in health and disease—An update. Journal of Pharmacokinetics and Biopharmaceutics 17, 1–46. https://doi.org/10.1007/BF01059086

Hanamoto, S., Kawakami, T., Nakada, N., Yamashita, N., Tanaka, H., 2014. Evaluation of the photolysis of pharmaceuticals within a river by 2 year field observations and toxicity changes by sunlight. Environ. Sci.: Processes Impacts 16, 2796–2803. https://doi.org/10.1039/C4EM00448E

Hughes, S.R., Kay, P., Brown, L.E., 2013. Global Synthesis and Critical Evaluation of Pharmaceutical Data Sets Collected from River Systems. Environ. Sci. Technol. 47, 661–677. https://doi.org/10.1021/es3030148

Isidori, M., Nardelli, A., Parrella, A., Pascarella, L., Previtera, L., 2006. A multispecies study to assess the toxic and genotoxic effect of pharmaceuticals: Furosemide and its photoproduct. Chemosphere 63, 785–793. https://doi.org/10.1016/j.chemosphere.2005.07.078

Jakimska, A., Śliwka-Kaszyńska, M., Reszczyńska, J., Namieśnik, J., Kot-Wasik, A., 2014. Elucidation of transformation pathway of ketoprofen, ibuprofen, and furosemide in surface water and their occurrence in the aqueous environment using UHPLC-QTOF-MS. Anal Bioanal Chem 406, 3667–3680. https://doi.org/10.1007/s00216-014-7614-1

Jelic, A., Fatone, F., Di Fabio, S., Petrovic, M., Cecchi, F., Barcelo, D., 2012. Tracing pharmaceuticals in a municipal plant for integrated wastewater and organic solid waste treatment. Science of The Total Environment 433, 352–361. https://doi.org/10.1016/j.scitotenv.2012.06.059

Joachim, S., Beaudouin, R., Daniele, G., Geffard, A., Bado-Nilles, A., Tebby, C., Palluel, O., Dedourge-Geffard, O., Fieu, M., Bonnard, M., Palos-Ladeiro, M., Turiès, C., Vulliet, E., David, V., Baudoin, P., James, A., Andres, S., Porcher, J.M., 2021. Effects of diclofenac on sentinel species and aquatic communities in semi-natural conditions. Ecotoxicology and Environmental Safety 211, 111812. https://doi.org/10.1016/j.ecoenv.2020.111812

Kang, M.-Y., Tsuchiya, M., Packer, L., Manabe, M., 1998. In *vitro* study on antioxidant potential of various drugs used in the perioperative period. Acta Anaesthesiologica Scandinavica 42, 4–12. https://doi.org/10.1111/j.1399-6576.1998.tb05073.x

Katsura, S., Yamada, N., Nakashima, A., Shiraishi, S., Furuishi, T., Ueda, H., 2015. Identification of Furosemide Photodegradation Products in Water–Acetonitrile Mixture. Chem. Pharm. Bull. 63, 617–627. https://doi.org/10.1248/cpb.c15-00122

Kołecka, K., Gajewska, M., Stepnowski, P., Caban, M., 2019. Spatial distribution of pharmaceuticals in conventional wastewater treatment plant with Sludge Treatment Reed Beds technology. Science of The Total Environment 647, 149–157. https://doi.org/10.1016/j.scitotenv.2018.07.439

Kosma, C.I., Lambropoulou, D.A., Albanis, T.A., 2014. Investigation of PPCPs in wastewater treatment plants in Greece: Occurrence, removal and environmental risk assessment. Science of The Total Environment 466–467, 421–438. https://doi.org/10.1016/j.scitotenv.2013.07.044

Kuzmanović, M., Ginebreda, A., Petrović, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. Science of The Total Environment 503–504, 289–299. https://doi.org/10.1016/j.scitotenv.2014.06.056

Lahet, J.J., Lenfant, F., Courderot-Masuyer, C., Ecarnot-Laubriet, E., Vergely, C., Durnet-Archeray, M.J., Freysz, M., Rochette, L., 2003. In vivo and in vitro antioxidant properties of furosemide. Life Sciences 73, 1075–1082. https://doi.org/10.1016/S0024-3205(03)00382-5

Laurencé, C., Rivard, M., Lachaise, I., Bensemhoun, J., Martens, T., 2011. Preparative access to transformation products (TPs) of furosemide: a versatile application of anodic oxidation. Tetrahedron 67, 9518–9521. https://doi.org/10.1016/j.tet.2011.10.006

Laurencé, C., Rivard, M., Martens, T., Morin, C., Buisson, D., Bourcier, S., Sablier, M., Oturan, M.A., 2014. Anticipating the fate and impact of organic environmental contaminants: A new approach applied to the pharmaceutical furosemide 7. http://dx.doi.org/10.1016/j.chemosphere.2014.05.036

Laurencé, C., Zeghbib, N., Rivard, M., Lehri-Boufala, S., Lachaise, I., Barau, C., Le Corvoisier, P., Martens, T., Garrigue-Antar, L., Morin, C., 2019. A new human pyridinium metabolite of furosemide, inhibitor of mitochondrial complex I, is a candidate inducer of neurodegeneration. Biochemical Pharmacology 160, 14–23. https://doi.org/10.1016/j.bcp.2018.12.007

Lee, J.J., Kong, M., Ayers, G.D., Lotan, R., 2007. Interaction Index and Different Methods for Determining Drug Interaction in Combination Therapy. Journal of Biopharmaceutical Statistics 17, 461–480. https://doi.org/10.1080/10543400701199593

Leenhardt, S., Mamy, L., Pesce, S., Sanchez, W., 2023. Impacts des produits phytopharmaceutiques sur la biodiversité et les services écosystémiques. éditions Quae. https://doi.org/10.35690/978-2-7592-3657-2

Leuthold, D., Klüver, N., Altenburger, R., Busch, W., 2019. Can Environmentally Relevant Neuroactive Chemicals Specifically Be Detected with the Locomotor Response Test in Zebrafish Embryos? Environ. Sci. Technol. 53, 482–493. https://doi.org/10.1021/acs.est.8b04327

Li, Z., Maier, M.P., Radke, M., 2014. Screening for pharmaceutical transformation products formed in river sediment by combining ultrahigh performance liquid chromatography/high resolution mass spectrometry with a rapid data-processing method. Analytica Chimica Acta 810, 61–70. https://doi.org/10.1016/j.aca.2013.12.012

Li, Z., Sobek, A., Radke, M., 2015. Flume Experiments To Investigate the Environmental Fate of Pharmaceuticals and Their Transformation Products in Streams. Environ. Sci. Technol. 49, 6009–6017. https://doi.org/10.1021/acs.est.5b00273

Liang, R., He, J., Shi, Y., Li, Z., Sarvajayakesavalu, S., Baninla, Y., Guo, F., Chen, J., Xu, X., Lu, Y., 2017. Effects of Perfluorooctane sulfonate on immobilization, heartbeat, reproductive and biochemical performance of Daphnia magna. Chemosphere 168, 1613–1618. https://doi.org/10.1016/j.chemosphere.2016.11.147

Long, G.V., Fung, C., Menzies, A.M., Pupo, G.M., Carlino, M.S., Hyman, J., Shahheydari, H., Tembe, V., Thompson, J.F., Saw, R.P., Howle, J., Hayward, N.K., Johansson, P., Scolyer, R.A., Kefford, R.F., Rizos, H., 2014. Increased MAPK reactivation in early resistance to dabrafenib/trametinib combination therapy of BRAF-mutant metastatic melanoma. Nat Commun 5, 5694. https://doi.org/10.1038/ncomms6694

MacPhail, R.C., Brooks, J., Hunter, D.L., Padnos, B., Irons, T.D., Padilla, S., 2009. Locomotion in larval zebrafish: Influence of time of day, lighting and ethanol. NeuroToxicology 30, 52–58. https://doi.org/10.1016/j.neuro.2008.09.011

Maculewicz, J., Kowalska, D., Świacka, K., Toński, M., Stepnowski, P., Białk-Bielińska, A., Dołżonek, J., 2022. Transformation products of pharmaceuticals in the environment: Their fate, (eco)toxicity and bioaccumulation potential. Science of The Total Environment 802, 149916. https://doi.org/10.1016/j.scitotenv.2021.149916

Maddrell, S.H.P., O'Donnell, M.J., 1992. Insect malpighian tubules: V-ATPase action in ion and fluid transport. Journal of Experimental Biology 172, 417–429. https://doi.org/10.1242/jeb.172.1.417

McCormick, J.M., Paiva, M.S., Häggblom, M.M., Cooper, K.R., White, L.A., 2010. Embryonic exposure to tetrabromobisphenol A and its metabolites, bisphenol A and tetrabromobisphenol A dimethyl ether disrupts normal zebrafish (Danio rerio) development and matrix metalloproteinase expression. Aquatic Toxicology 100, 255–262. https://doi.org/10.1016/j.aquatox.2010.07.019

Musgrave, A., van der Steuyt, P., Ero, L., 1979. Concanavalin A binding to Chlamydomonas eugametos flagellar proteins and its effect on sexual reproduction. Planta 147, 51–56. https://doi.org/10.1007/BF00384590

Narumiya, M., Nakada, N., Yamashita, N., Tanaka, H., 2013. Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. Journal of Hazardous Materials 260, 305–312. https://doi.org/10.1016/j.jhazmat.2013.05.032

Nihemaiti, M., Huynh, N., Mailler, R., Mèche-Ananit, P., Rocher, V., Barhdadi, R., Moilleron, R., Le Roux, J., 2022. High-Resolution Mass Spectrometry Screening of Wastewater Effluent for Micropollutants and Their Transformation Products during Disinfection with Performic Acid. ACS EST Water 2, 1225–1233. https://doi.org/10.1021/acsestwater.2c00075

Ogrunc, M., Di Micco, R., Liontos, M., Bombardelli, L., Mione, M., Fumagalli, M., Gorgoulis, V.G., d'Adda di Fagagna, F., 2014. Oncogene-induced reactive oxygen species fuel hyperproliferation and DNA damage response activation. Cell Death Differ 21, 998–1012. https://doi.org/10.1038/cdd.2014.16

Olvera-Vargas, H., Leroy, S., Rivard, M., Oturan, N., Oturan, M., Buisson, D., 2016. Microbial biotransformation of furosemide for environmental risk assessment: identification of metabolites and toxicological evaluation. Environ Sci Pollut Res 23, 22691–22700. https://doi.org/10.1007/s11356-016-7398-2

Osterauer, R., Kölher, H., 2008. Temperature-dependent effects of the pesticides thiacloprid and diazinon on the embryonic development of zebrafish (Danio rerio). Aquatic Toxicology 86, 485–494. https://doi.org/10.1016/j.aquatox.2007.12.013

Pascoe, D., Karntanut, W., Müller, C.T., 2003. Do pharmaceuticals affect freshwater invertebrates? A study with the cnidarian Hydra vulgaris. Chemosphere 51, 521–528. https://doi.org/10.1016/S0045-6535(02)00860-3

Peng, X., Lin, J., Zhu, Y., Liu, X., Zhang, Yinglan, Ji, Y., Yang, X., Zhang, Yan, Guo, N., Li, Q., 2016. Anxiety-related behavioral responses of pentylenetetrazole-treated zebrafish larvae to light-dark transitions. Pharmacology Biochemistry and Behavior 145, 55–65. https://doi.org/10.1016/j.pbb.2016.03.010

Persoone, G., Wells, P., 1987. Artemia in aquatic toxicology: a review. pp. 259–275.

Pino, M.R., Muñiz, S., Val, J., Navarro, E., 2016. Phytotoxicity of 15 common pharmaceuticals on the germination of Lactuca sativa and photosynthesis of Chlamydomonas reinhardtii. Environ Sci Pollut Res 23, 22530–22541. https://doi.org/10.1007/s11356-016-7446-y

Rioux, G., 2018. Spéciation du platine en présence de matière organique naturelle et biodisponibilité pour l'algue verte Chlamydomonas reinhardtii. (masters). Université du Québec, Institut national de la recherche scientifique, Québec.

Robinson, P.D., 2009. Behavioural toxicity of organic chemical contaminants in fish: application to ecological risk assessments (ERAs). Can. J. Fish. Aquat. Sci. 66, 1179–1188. https://doi.org/10.1139/F09-069

Rocco, L., Frenzilli, G., Fusco, D., Peluso, C., Stingo, V., 2010. Evaluation of zebrafish DNA integrity after exposure to pharmacological agents present in aquatic environments. Ecotoxicology and Environmental Safety 73, 1530–1536. https://doi.org/10.1016/j.ecoenv.2010.07.032

Roy, G., 2009. Les éléments du groupe platine (Pd, Pt et Rh) dans les eaux de surface et leur toxicité chez l'algue verte Chlamydonomas reinhardtii. (masters). Université du Québec, Institut national de la recherche scientifique, Québec.

Sandré, F., Moilleron, R., Morin, C., Garrigue-Antar, L. Pharmaceutical pollutants of emerging concern. Origin and fate of furosemide and its degradation products in the aquatic environment, a review.

Sandre, F., Huynh, N., Caupos, E., El-Mrabet, L., Partibane, C., Lachaise, I., Pommier, C., Rivard, M., Morin, C., Moilleron, R., Le Roux, J., Garrigue-Antar, L., 2023. Occurrence and fate of an emerging drug pollutant and its by-products during conventional and advanced wastewater treatment: Case study of furosemide. Chemosphere 322, 138212. https://doi.org/10.1016/j.chemosphere.2023.138212

Sandré, F., Huynh, N., Gromaire, M.-C., Varrault, G., Morin, C., Moilleron, R., Le Roux, J., Garrigue-Antar, L., 2022. Road Runoff Characterization: Ecotoxicological Assessment Combined with (Non-)Target Screenings of Micropollutants for the Identification of Relevant Toxicants in the Dissolved Phase. Water 14, 511. https://doi.org/10.3390/w14040511

Son, Y., Cheong, Y.-K., Kim, N.-H., Chung, H.-T., Kang, D.G., Pae, H.-O., 2011. Mitogen-Activated Protein Kinases and Reactive Oxygen Species: How Can ROS Activate MAPK Pathways? Journal of Signal Transduction 2011, 1–6. https://doi.org/10.1155/2011/792639

Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2007. The behaviour and ecology of the zebrafish, Danio rerio. Biological Reviews 83, 13–34. https://doi.org/10.1111/j.1469-185X.2007.00030.x

Świacka, K., Michnowska, A., Maculewicz, J., Caban, M., Smolarz, K., 2021. Toxic effects of NSAIDs in non-target species: A review from the perspective of the aquatic environment. Environmental Pollution 273, 115891. https://doi.org/10.1016/j.envpol.2020.115891

Thomas, K.V., Langford, K.H., 2010. Point Sources of Human Pharmaceuticals into the Aquatic Environment, in: Kümmerer, K., Hempel, M. (Eds.), Green and Sustainable Pharmacy. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 211–223. https://doi.org/10.1007/978-3-642-05199-9_14

Tkaczyk, A., Bownik, A., Dudka, J., Kowal, K., Ślaska, B., 2021. Daphnia magna model in the toxicity assessment of pharmaceuticals: A review. Science of The Total Environment 763, 143038. https://doi.org/10.1016/j.scitotenv.2020.143038

Villa, S., Di Nica, V., Pescatore, T., Bellamoli, F., Miari, F., Finizio, A., Lencioni, V., 2018. Comparison of the behavioural effects of pharmaceuticals and pesticides on Diamesa zernyi larvae (Chironomidae). Environmental Pollution 238, 130–139. https://doi.org/10.1016/j.envpol.2018.03.029

Wong, P.K., 2000. Effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll±a synthesis of Scenedesmus quadricauda Berb 614.

Zhuo, H.-Q., Huang, L., Huang, H.-Q., Cai, Z., 2012. Effects of chronic tramadol exposure on the zebrafish brain: A proteomic study. Journal of Proteomics 75, 3351–3364. https://doi.org/10.1016/j.jprot.2012.03.038

Cette étude met en évidence les impacts toxiques non seulement du furosémide, précédemment montré comme omniprésent dans le milieu aquatique, mais également de la saluamine et du pyridinium du furosémide, sur différents organismes aux concentrations environnementales mesurées dans la Seine. Ces organismes peuvent avoir des rôles essentiels dans les écosystèmes (Voir partie IV.) et un impact sur ces derniers peut avoir des répercussions importantes sur le milieu.

Des impacts négatifs ont été relevés sur le stress oxydant, la mobilité, la réponse au stress, le battement cardiaque des poissons et crustacés ainsi que sur la croissance, la taille et la mobilité des algues vertes. Le score de toxicité calculé pour les trois composés, prenant en compte tous les paramètres et les concentrations environnementales, montre que la saluamine est le composé le plus toxique, suivi du pyridinium du furosémide et enfin du furosémide. Il est important de noter que des effets synergiques ont été observés à des concentrations élevées, mais des effets antagonistes ont été constatés à des concentrations environnementales. Les résultats de notre étude montrent que la mobilité des daphnies est l'indicateur le plus sensible pour évaluer l'impact des trois composés, avec une LOEC de 50 ng/L pour chacun d'entre eux.

IV. Approche écosystémique

De nombreux services sont fournis par les organismes dans un environnement naturel : productions alimentaires et non alimentaires, services de régulation et de maintien du sol, qualité de l'eau, lutte biologique, pollinisation, habitat, biodiversité, régulation climatique, … (Leenhardt et al. 2023). Un impact sur ces organismes peut alors avoir des conséquences sur l'écosystème dans son ensemble. La présence de certaines espèces clés joue un rôle vital dans le maintien de l'organisation et de la diversité des communautés écologiques. Si ces espèces venaient à disparaître, cela pourrait avoir des répercussions significatives sur les processus écologiques et la composition spécifique des communautés (Lévêque & Paugy 2006).

Le rôle écologique des organismes peut varier en fonction des espèces mais quelques fonctions écosystémiques sont plus fréquemment évoquées pour les espèces étudiées lors de cette thèse. Les poissons, par exemple, qu'ils soient piscivores ou zooplanctonivores, ont un rôle dominant sur les chaînes trophiques. Par prédation/consommation, ils régulent la densité de population ou la distribution des espèces qu'ils consomment qui vont alors elles-mêmes moins consommer de ressources (cascade trophique). Ils peuvent également stocker (consommation), transporter et recycler des éléments nutritifs (excrétion d'azote ou de phosphore) (Lévêque & Paugy 2006). D'autres poissons perturbent le sédiment en cherchant leur nourriture, ce qui permet la remise en solution d'éléments nutritifs, et les poissons détritivores recyclent les éléments nutritifs des débris organiques.

Les daphnies occupent souvent une place centrale dans les écosystèmes des étangs et des lacs en tant que principal consommateur primaire. Elles se nourrissent de petites particules en suspension, en particulier d'algues unicellulaires, par filtration, et sont ainsi un maillon important des chaînes alimentaires aquatiques et contribuent de façon importante au recyclage de l'eau. En tant que proie pour les poissons

et les prédateurs invertébrés, leur rôle est crucial (Kattel 2022, Ebert 2022). Les artémias occupent un rôle similaire en milieu salin (Marden et al. 2020). Une diminution ou disparition de ces espèces peut par exemple contribuer à l'eutrophisation des milieux.

Les algues vertes sont une source importante de nourriture pour les petits organismes aquatiques, tels que les protozoaires et les rotifères. De plus, elles contribuent au cycle des nutriments en absorbant les nitrates et les phosphates de l'eau, qui peuvent être utilisés par d'autres organismes dans l'écosystème. Enfin, elles peuvent jouer un rôle dans la production de dioxygène par photosynthèse, contribuant ainsi à l'oxygénation de l'eau et à la survie des organismes qui en dépendent (Stevenson 2014).

Les impacts sur les organismes peuvent avoir des répercussions négatives sur l'écosystème dans son ensemble en altérant les fonctions écosystémiques associées. Si ces impacts touchent des organismes qui jouent un rôle clé dans le fonctionnement de l'écosystème et qu'ils sont les seuls à occuper ces fonctions, cela peut être préjudiciable. Un écosystème est alors plus résilient lorsque plusieurs espèces occupent un même rôle trophique (TEEB, 2012), d'où l'importance de l'existence de la biodiversité. Afin de sensibiliser et d'améliorer les politiques en faveur de la biodiversité, l'Évaluation française des écosystèmes et des services (EFESE), à été mis en place, pilotée par le ministère chargé de l'environnement (Puydarrieux et Beyou 2017). La protection de l'écosystème est inscrite dans le code de l'environnement et l'article L. 110-1-II précise que « la sauvegarde des services fournis (par la biodiversité) est considérée comme étant d'intérêt général». La notion de services écosystémiques (fonctions écosystémiques qui sont bénéfiques à l'homme) est en phase d'être introduite dans le domaine juridique, ce qui pourrait offrir une protection supplémentaire à la biodiversité (**figure 23**). Cependant, cette approche est peut être orientée par le fait que la biodiversité a un impact direct sur l'homme au travers des services écosystémiques fournis comme la production de nourriture (Holt et al. 2016) ou de services culturels (Leenhardt et al. 2023). L'usage de certains composés biocides impactent directement ces services (Chagnon et al. 2015).

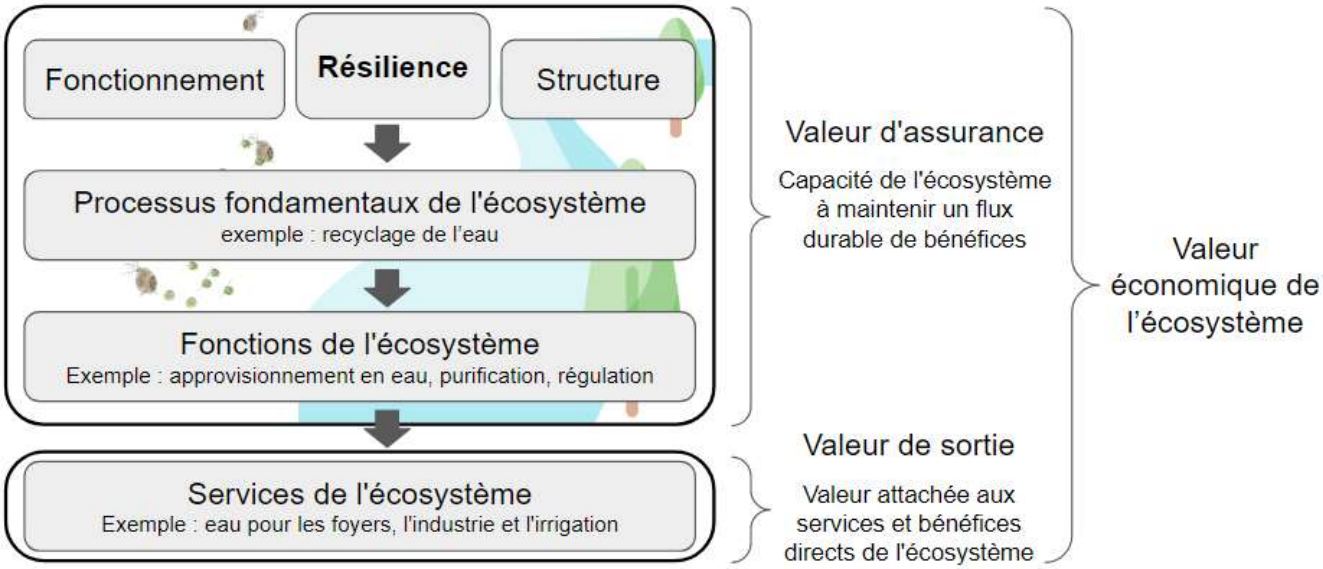


Figure 23. valeur d'assurance et de production en tant que composante de la valeur économique de l'écosystème. Adapté de TEEB 2012.

Plusieurs approches sont alors basées sur la valeur économique des biens fournis par l'écosystème (Leenhardt et al. 2023). Bien que les écosystèmes aquatiques contribuent grandement à la valeur économique de la biosphère (production de nourriture, purification de l'eau de l'eau, tourisme, régulation du climat), ils sont très mal étudiés par rapport aux écosystèmes terrestres (Martinez et al. 2007, Liquete et al. 2013). La méthodologie pour étudier ces systèmes aquatiques est plus limitée et le fait que le milieu aquatique soit un environnement très dynamique rend son étude plus difficile (Liquete et al. 2013). Plus d'études sur ces systèmes sont alors nécessaires pour combler ces manques car mieux appréhender l'écosystème aquatique est d'une grande importance pour la mise en œuvre pratique des politiques de conservation.

V. Conclusion

Les méthodes traditionnelles de protection de l'environnement et d'évaluation des risques se concentrent principalement sur la toxicité aiguë, mais ces approches présentent des limites. Les cadres réglementaires tels que la Directive Cadre sur l'Eau visent à atteindre un bon état chimique de l'eau en se basant sur des valeurs seuils déterminées à partir d'indicateurs peu sensibles tels que la létalité (partie I.). Des approches plus spécifiques ou plus intégratives prenant en compte les populations, les communautés, les réseaux trophiques existent (Leenhardt et al. 2023, Joachim et al. 2021), mais sont complexes, ce qui rend leur normalisation difficile (Leenhardt et al. 2023). Dans notre cas, l'approche multi-modèle nous a permis d'identifier certains paramètres sensibles aux concentrations environnementales. Parmi nos différents modèles, certains étaient plus sensibles ou plus faciles à mettre en place que d'autres (parties II. et III.).

Le modèle de poisson, et plus spécifiquement le poisson zèbre, est largement utilisé dans de nombreux domaines, notamment en écotoxicologie, offrant ainsi une base bibliographique solide. Les poissons sont des organismes complexes, procurant ainsi de nombreuses possibilités d'investigations et leurs œufs sont facilement disponibles et manipulables, avec une éclosion rapide, ce qui nous a permis de réaliser des expériences relativement sensibles, comme des tests comportementaux. Cependant, l'utilisation du poisson comme modèle expérimental peut être contraignante. La réglementation sur l'expérimentation animale, en particulier la directive 2010/63, limite l'expérimentation sur le poisson zèbre aux stades juvéniles, c'est-à-dire sous forme de larves non autonomes (sauf en cas de demande de saisine). Cependant, ces stades de développement sont particulièrement intéressants à étudier car ils sont très sensibles. En revanche, l'utilisation du modèle de poisson est lourde à mettre en place et à entretenir : elle requiert en effet un investissement important en terme de moyens humains et de matériel spécifique pour l'élevage, nécessaires au maintien d'une qualité de l'eau optimale et au bien être animal, et qui est très contraignant pour des petites structures comme la nôtre.

Moins contraignante à la fois pour ses conditions d'élevage et en termes de réglementation, qui ne concerne pas les crustacés, la daphnie s'est révélée être un modèle particulièrement intéressant dans notre étude. Également très étudiée en écotoxicologie, elle est bien documentée notamment dans le cadre d'études d'impacts de polluants, auxquels elle est très sensible. Ainsi les tests de comportement (transition lumière/obscurité) se sont révélés être les plus sensibles parmi tous ceux que nous avons

effectués (tous organismes confondus). Son temps de génération court la rend très pratique pour l'expérimentation et son mode de reproduction par parthénogenèse (et donc la production de clones) permet des expériences très répétables.

L'artémia est également un modèle simple à utiliser, et comme il est possible de se procurer des œufs déshydratés, il n'est même pas nécessaire de les maintenir en élevage. Cependant ces œufs proviennent d'élevages importants et sont issus de plusieurs "parents", la variabilité individuelle est alors plus importante. Peu d'expériences ont été réalisées sur ces organismes car la détermination des EC50 a nécessité de nombreux réplicats dû à l'importante variabilité.

L'algue verte a fourni des résultats assez différents et complémentaires des modèles animaux précédents, qui sont pertinents à exploiter en raison de la position de l'algue à la base de nombreuses chaînes trophiques. Les tests effectués ont également montré un impact de nos composés au ng/L. Comme les composés pharmaceutiques n'impactent pas les mêmes récepteurs que dans les modèles animaux, ces approches sont aussi intéressantes à prendre en compte. Comme les lignées cellulaires eucaryotes, la culture de *C. reinhardtii* nécessite des repiquages réguliers et un environnement stérile, la moindre contamination compromettant les résultats.

Les organismes, choisis ici en raison des différentes qualités évoquées dans la partie I, peuvent être critiqués pour leur pertinence par rapport au milieu étudié. Le poisson zèbre étant un poisson tropical, il n'est pas représentatif des rivières françaises pour lesquelles les concentrations de FUR, PYR et SAL ont été relevées dans cette étude. On assume cependant une certaine proximité avec les poissons présents localement (téléostéens). Etant un poisson considéré comme domestique (au même titre que la carpe koï, le guppy, le poisson combattant, et le poisson rouge), et contrairement aux espèces de poissons que l'on pourrait trouver par exemple dans la Seine (chevaine, épinoche …), il ne nécessite pas de certificat de capacité et ses conditions d'élevage sont bien connues. La daphnie, malgré sa représentation géographique importante, est un organisme vivant plutôt en eaux stagnantes, et l'espèce d'artémie utilisée dans ce travail est un organisme marin. Dans les conditions de notre étude, un modèle de crustacé d'eau douce pouvant se trouver dans les eaux des rivières, comme le gammare, serait plus pertinent pour de futures études. L'espèce d'algue choisie est très fréquente dans de nombreuses zones géographiques, en revanche, les conditions de culture en laboratoire sont loin des conditions environnementales (température et lumière contrôlées, milieu riche et stérile,..) mais dans l'optique de réaliser des tests répétables, peu d'alternatives sont possibles.

Finalement, au vu des avantages et des inconvénients de chacune des espèces utilisées, la daphnie semble être le meilleur modèle, notamment en termes de sensibilité au test de comportement. Il serait intéressant de maintenir ce test de transition lumière/obscurité réalisé dans la Zebrabox, dans le cadre de futures études d'évaluation de risque. Des approches plus spécifiques, par l'analyse de gènes biomarqueurs permettrait d'aller plus loin dans cette étude et de comprendre un peu mieux les cibles impactées par les polluants testés.

Dans ce chapitre, nous avons par ailleurs montré des effets délétères des trois composés testés à des concentrations environnementales sur ces différents organismes et nous avons notamment relevé une toxicité plus importante du pyridinium du furosémide et de la saluamine sur plusieurs paramètres suivis. Cette étude souligne l'intérêt de mieux caractériser les produits de transformation et de prendre en compte des approches plus sensibles dans les évaluations de risques. Avec des indicateurs basés sur la létalité, l'impact environnemental est largement sous-estimé. Par exemple, la PNEC du FUR calculée à partir de la valeur d'EC50 obtenue sur *D. magna* (la plus basse) est estimée à 195,5 µg/L (avec un facteur de sécurité de 1000) alors que nous avons relevé des effets importants au ng/L. Il est alors possible que les molécules aient des effets négatifs sur les écosystèmes, car elles peuvent altérer le comportement des organismes et ainsi affecter leur capacité à assurer leurs fonctions écosystémiques.

Chapitre IV.

Impact sur les cellules humaines

Chapitre IV.
Impact sur les cellules humaines

I. Approche protéomique

Le furosémide étant un médicament, avant sa mise sur le marché, son impact sur l'homme est extensivement étudié. De nombreuses études médicales sont publiées sur le sujet (Abbot & Kovacic 2018, Ahmed 2020, Huang et al. 2016, Antoine et al. 2007). En revanche, peu d'informations ont été retrouvées sur les produits de dégradation.

Dans ce chapitre, nous proposons d'étudier la toxicité du furosémide, du pyridinium du furosémide, de la saluamine et du furfural, ainsi que leur mélange, sur des cellules humaines. Des tests de toxicité aiguës (MTT) sont réalisés dans un premier temps pour déterminer des concentrations d'effets. Des lignées cellulaires humaines ont été sélectionnées en fonction des caractéristiques du furosémide et de ses métabolites. Le furosémide étant métabolisé par le foie et filtré par les reins, des cellules hépatiques HepG2 et la lignée cellulaire HEK 293, dérivée de rein embryonnaire humain, ont été choisies. De plus, comme le pyridinium du furosémide montre des évidences de neurotoxicité, le choix des cellules SH-SY5Y, classiquement utilisées pour étudier la maladie de Parkinson, la neurogenèse et d'autres processus caractéristiques du cerveau, est apparu pertinent.

Une étude de protéomique est ensuite réalisée, constituant une première approche pour l'étude des mécanismes de toxicité des métabolites du furosémide. La protéomique est une discipline scientifique et un outil puissant qui étudie l'ensemble des protéines exprimées par un génome dans une cellule ou un tissu. Elle permet d'analyser les protéines dans leur ensemble, leurs interactions et leurs modifications, afin de mieux comprendre leur rôle dans les processus biologiques et les maladies. Les données obtenues par la protéomique peuvent être exploitées pour identifier des biomarqueurs de maladies et mieux appréhender les processus physiopathologiques, ou pour étudier les voies de signalisation impliquées dans les processus biologiques. Elles peuvent aussi contribuer à la compréhension des mécanismes de la toxicité des médicaments et des produits chimiques. Les premiers résultats de notre étude sont présentés ci-après.

Study of the mechanisms of toxicity of furosemide and its degradation products: a proteomic approach.

Fidji Sandre [1], Sandrine Bourgoin-Voillard [2], Benoît Vallee [3], Damien Haber [3], José Courty [3], Christophe Morin [4], Laure Garrigue-Antar [1]

[1] Leesu - Univ Paris Est Creteil, Ecole des Ponts, Creteil, F-94010, France
[2] Proteomic platform Prométhée, EPSP - TIMC, University Grenoble Alpes, France
[3] IMRB - Univ Paris-Est Creteil - CHU Henri Mondor, France
[4] IUT - Sénart Fontainebleau, 36 Rue Georges Charpak, 77567 Lieusaint

Introduction

Every day, many molecules intended for therapeutic use are introduced in the market. The research for new drugs is centered around their potential therapeutic effects on a particular molecular target, and the requirement of an acceptable efficacy and safety profile. Information on drug metabolism is crucial for the determination of the pharmacological and toxicological effects in order to lower the risk of drug metabolism-induced toxicity (Zhang & Tang 2018). Over the past 30 years, major progress has been made in drug metabolism understanding by technological developments in molecular biology, genetics, and analytical chemistry. Mass spectrometry, in particular, has significantly improved the detection levels of compounds, unraveling new metabolites previously unknown (Baillie 2008; Laurencé et al. 2019). Furthermore, even more recent untargeted approaches such as proteomics or metabolomics, can now provide an integrative view by detecting as many as possible intermediates, being limited by the availability of pure standards for verification and quantification (Alarcon Barrera et al. 2022). Thus, many drug metabolites toxicity remain poorly studied, if at all, and along with their parent molecules, can potentially be problematic for human health. Indeed, they pose potential risks for several chronic diseases such as cancer (IARC Monographs n°108), cardiovascular diseases (Marcén 2009), neurodegenerative diseases (Parkinson's or Alzheimer's diseases), which cause the deaths of increasing numbers of patients worldwide, and represent financial and emotional burdens on their families. It is therefore necessary to better characterize the by-products of pharmaceutical molecules and to develop methods to better understand their interactions. In this context, furosemide, a drug marketed since 1964 (Stokes and Nunn 1964) used to treat heart and kidney failure or hypertension, seems to be a good study model. It is one of the most sold drugs in the world and is widely found in the aquatic environment, due to poor metabolization and elimination either unmodified or as glucuronide conjugate (Sandré et al. 2023b), where it has been shown to be toxic to aquatic life (Sandré et al. 2023a). It has also shown cytotoxic and hepatotoxic impacts in rodents (Williams et al. 2007; Peterson 2013). More than 40 by-products of furosemide have been identified in literature and in our previous work after degradation by wastewater treatment plants and oxidation treatments or by natural processes such as hydrolysis, solar degradation or biodegradation (Bundgaard et al. 1988, Starling et al. 2019, Laurencé et al. 2014, Olvera-Vargas et al. 2016, Sandre et al. 2023, Sandré et al. 2023b). Furthermore, during phase I metabolism, the furan ring of the drug is oxidized by cytochrome P450, resulting in the formation of reactive intermediates that can bind covalently to hepatic proteins (Williams et al. 2007). Three metabolites, saluamine, furfural and pyridinium of furosemide, somewhat better characterized, have also shown signs of toxicity on different organisms. Saluamine, which has been known for several decades (Hammarlund-Udenaes & Benet 1989, Andreasen et al. 1982), is the most

common degradation product of furosemide in the literature. It causes changes in certain body parameters in mice (increased alanine, aspartate aminotransferase (EC 2.6.1.1) and creatinine, as well as reduced blood glucose, liver and kidney congestion) (Al-Omar et al. 2009). Saluamine has also been shown to be the most toxic degradation product of furosemide in the aquatic environment (Sandré et al. 2023a). Furfural is a small, highly soluble molecule whose acute toxicity has been widely studied due to its use in the production of furfuryl alcohol in the metal casting industry, as a solvent for dyes, as a corrosion inhibitor, in flavors and fragrances, and also as a reagent for drug synthesis (IARC, 2016). Furfural is highly irritating to the skin and lungs in humans and can even induce pulmonary hemorrhage and edema in rats after oral or dermal exposure (Flek and Sedivěc 1978; Reed and Kwok 2014). Pyridinium of furosemide has been discovered more recently, but its occurrence in humans was suspected from experiments in which the degradation/oxidation of furosemide into Pyridinium of furosemide by rat liver microsomal fractions was attributed to cytochrome P450 (Chen & Burka 2007). Later, it has been identified by electrochemistry by Laurencé et al. (2011) and finally established as a genuine human metabolite in urine of furosemide-treated patients (Laurencé et al. 2019). Although fewer studies are available on its toxicity, it has been shown to induce several hallmarks of neurodegenerative diseases (death of dopaminergic neurons, inhibition of mitochondrial respiratory chain complex I, accumulation of alpha synuclein in neurons), making it a metabolite of concern (Laurencé et al. 2019). The mechanisms that generate these metabolites toxicity remain poorly understood to date, if at all. Pyridinium of furosemide has been shown to induce the intrinsic apoptosis cascade by the mitochondrial translocation of Bax and the activation of caspase-9 and caspase-3 in SH-SY5Y cells (Laurencé et al 2019). To better understand the biological actions of furosemide and its metabolites and therefore their impact, a better knowledge of the signaling pathways is necessary. OMICs approaches are therefore an interesting integrative approach, focused on biological mechanisms. Proteomics in particular, allowing access to protein functions, is a key approach to identify metabolic pathways or biomarkers associated with diseases or responses to stress.

The objective of this study was thus to unveil in cell lines some processes susceptible to occur following exposure to furosemide and its metabolites in humans. First, human cell lines were selected based on the characteristics of furosemide and its metabolites. As furosemide is metabolized by the liver and filtered by the kidneys, Hep-G2 liver cells and the human embryonic kidney-derived cell line HEK 293 were chosen. Furthermore, as the pyridinium of furosemide showed evidence of neurotoxicity, the choice of SH-SY5Y cells, classically used to study neurodegenerative diseases, neurogenesis and other physiological brain processes, appeared relevant. In a second step, a proteome analysis was performed on Hep-G2 cell line exposed to the metabolites and their mixture to identify possible biomarkers.

Material & methods

I. Cell lines, reagents and instruments

Cell lines. The Hep-G2 (derived from liver tissue of a patient with hepatocellular carcinoma) and HEK 293 (human embryonic kidney derived epithelial cells) cell lines were from Sigma-Aldrich. The SH-SY5Y cell line, derived from human neuroblastoma, was from the ATCC (CRL-2266, American Type Culture Collection).

Cell culture. Hep-G2 and HEK 293 lines were cultured in Dulbecco's Modified Eagles Medium - High (DMEM at 4.5 g/L glucose) with pyruvate. Dulbecco's Modified Eagles Medium - Low (DMEM at 1 g/L glucose) was used

for the SH-SY5Y line. Both media were supplemented with 10% of Fetal Calf Serum (FBS) (GIBCO), 1% Pénicilline-streptomycin (GIBCO), 5 mL of 100X Glutamine (Sigma-Aldrich) and 5 mL of 100X Non-Essential Amino Acids (NEAA) (Sigma-Aldrich). Cells were incubated at 37 °C in humidified atmosphere of 5% CO2 Cells were detached twice weekly using trypsin (GIBCO), counted with acridine orange using a dual fluorescence cell counter (LUNA-FL) and seeded at 1.10^6 cells /mL into a new flask until 80-90% confluency.

Exposure solutions. Furosemide and saluamine were purchased from Sigma-Aldrich. Pyridinium of Furosemide was synthesized locally at ICMPE as described by Laurencé et al. (2011) (>96% purity). Furfural was purchased from Eurisotop. Stock solutions of furosemide, pyridinium and saluamine were prepared in DMSO (Sigma-Aldrich). The solutions were filtered on sterile 0.22 µm syringe filters (Carl Roth) to ensure sterility and then diluted directly into culture medium.

MTT viability assay. MTT tetrazolium salt (or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was purchased from Sigma-Aldrich. Absorbance measurements were performed with a UV-visible spectrometer (Labsystems iEMS Reader MF).

Protein extraction. Before extraction, cells were rinsed with PBS (phosphate-buffered saline) buffer (GIBCO) and incubated on ice in RIPA (Radio-Immunoprecipitation Assay) lysis buffer (150 mM NaCl (EUROMEDEX), 0.5% NP40 (Sigma-Aldrich), 0.5% Sodium deoxycholate (Sigma-Aldrich), 20% SDS (EUROMEDEX), and 25 mM TRIS (EUROMEDEX) pH 7.4, in the presence of phosphatases and proteases inhibitors (ROCHE). After centrifugation (10min, 13000 g), supernatants were collected and stored at -20°C until use.

Protein assay. Protein quantification was done by Pierce BCA assay kit (Thermo Scientific Reference 23225).

Ultrafiltration and enzymatic digestion. Protein purification was performed on vivaspin columns by ultracentrifugation (vivaspin 500 centrifugal concentrators, Sigma-Aldrich). For enzymatic digestion, a 50mM ABC (ammonium bicarbonate) buffer with 0.2% Rapigest (from a 2% stock solution), 5 mM Dithiothreitol (DTT) (Sigma-Aldrich) was prepared. Trypsin and lysine-C were purchased from Promega.

Proteomics. Quantitative proteomics (label-free) is based on mass spectrometry. Samples were injected on NanoAcquity-SYNAPT G2Si (Waters), on a NanoACQUITY silica C18 column, 1.7 µm (Waters).

II. Acute toxicity test

A. Cells exposure

Detached cells were counted with acridine orange in a LUNA cell counter. 5000 cells were seeded in a volume of 100 µL per well in a 96-well plate (Laurencé et al. 2019). The plates were then incubated for 24 hours at 37°C, after which the exposure solutions (furosemide, saluamine, furosemide pyridinium, furfural, mixture or DMSO (vehicle control)) were added at the desired concentrations. The volume of each well was then made up to 200 µL and the plates were placed for 96h at 37°C in the incubator.

B. MTT test and results

MTT viability test was performed to evaluate the acute toxicity of the compounds as previously described (Laurencé et al 2019). Briefly, MTT tetrazolium salt , at 5 mg/ml, was added to the wells. for 2 h. The medium was then replaced by 100 µL of DMSO and placed under a rotating shaker to dissolve the crystals formed. Absorbance measurements were performed with a UV-visible spectrophotometer (Labsystems iEMS Reader MF) at 595 nm. The amount of formazan crystals produced being proportional to the number of living cells, the percentage of cell survival was calculated to obtain dose-response curves. Effect concentrations for 10% living cells (EC10) were then determined for each condition.

III. Label-free quantitative proteomic analysis

A. Cells exposure

To prepare cells for proteomic analysis, 6-well plates were seeded at 175,000 cells per well following the protocol in the previous section. After 24 h of incubation, the cells were exposed to EC10 of the compounds and then returned to the incubator for 96 h. For each cell line, each condition was performed in triplicate along with the associated DMSO controls.

B. Protein extraction

After 96 h of exposure, cell medium was removed and the cells were rinsed twice with cold PBS. 300µL of RIPA with phosphatases and proteases inhibitors was then added to each well for 15 min on ice. The cell lysates were collected with a cell scraper, and transferred to 1.5mL Eppendorf tubes. The extracts were sonicated 3 times for 10 seconds at an amplitude of 60 (WATTS) to disrupt DNA. The tubes were then centrifuged at 13,000 g for 10 minutes to remove debris. The supernatant was transferred to new Eppendorfs tubes. The protein concentration of each sample was determined colorimetrically using a BCA assay kit. The samples were then frozen at -20°C until use.

C. Protein digestion

Samples were first purified by ultrafiltration (Vivaspin Ultracentrifugation with LMCO 2 kDA) with 50 mM ABC (Ammonium Bicarbonate) buffer as buffer exchange. Resulting proteins were quantified by a BCA assay to prepare aliquots of 30 µg of proteins, dry them with a Speed Vacuum, store them at -20°C.

The protein digestion was performed as follows on 30 µg of proteins: i) proteins were diluted into 50 mM ABC (Ammonium Bicarbonate) buffer with 0.1% RapiGest SF solution (Waters), ii) proteins were reduced with dithiothreitol (DTT) (5mM) at 56°C for 1h, then alkylated with iodoacetamide (IAA) (15 mM) in the dark for 30 minutes. DTT was added to achieve a final concentration of 20mM. iii) the protein digestion was performed with trypsin/Lys-C enzymatic mix (Promega). iv) resulting peptides were spiked with enolase (ENO1-YEAST) used as an internal standard for quantitation analysis. v) TFA was added to achieve a pH lower than 2. In such an acidic medium, enzymatic digestion was stopped and samples may be desalted after an incubation at 37°C for 30 minutes and a centrifugation of 14,000g for 10 minutes. Supernatant containing desalted peptides was retrieved for LC/MS/MS analysis.

D. LC-MS/MS

Each sample was analyzed in triplicates by using the system NanoAcquity-ESI-SynaptG2-Si (Waters) operating in positive mode with a nano-LC-/HDMS^E method and a reverse phase nano-liquid chromatography. In more details, samples were loaded on a ACQUITY UPLC peptide BEH130 C18 nanoACQUITY^{TM} column, 100 µm x 100 mm with 1.7 µm diameter (Waters) at a flow rate of 0.45 µL/min. Peptides were separated with a gradient increasing from 1% Buffer B (acetonitrile with 0.1% formic acid)/99% Buffer A (H_2O with 0.1% formic acid) to 40% Buffer B/60% Buffer A, and then to 85% Buffer B/15% Buffer A, over 120 min. MS spectra were recorded in a 50-2,000 m/z mass range at 10,000-20,000 resolution. The nano LC/HDMS^E method included 120 min of acquisition time, positive polarity, MS^E acquisition range of 50-4,000 Da, collision energy range of 20-55 V. The samples were injected randomly to limit the analysis bias. In order to check the stability of the signal, samples of quality control (QC) were injected at the beginning, during and at the end of the analyses.

E. Bioinformatic analysis

Identification and quantification of proteins were performed by using the software Progenesis for proteomics QI (Waters) with the algorithm Ion accounting and the following parameters: trypsin enzyme of digestion; a maximum of 2 missed cleavages; a maximum of protein mass of 250 kDa: fixed modification: carbamidomethyl (C); variable modifications: oxidation (M), deamination (N, Q) and acetylation (N-ter); false discovery rate (FDR) of 1%, automatic mass tolerance, isotope filter of 2; Human UniprotKB/Swissprot database (UP000002494 - Release 2022_02; https://www.uniprot.org/) concatenated with the sequence of the internal standard (ENO1_YEAST, P00924). Identification of proteins was done by considering a 1% FDR (False discovery rate) with at least one unique peptide. Quantification of proteins was considered for proteins with at least two unique peptides and one peptide when the molecular weight was lower than 15 000 kDa. Proteins were considered significantly deregulated when P-values (ANOVA) were ≤ 0.05 with a fold change between each group compared ≥ 1.5-2. The fold change threshold is defined according to volcano plots of related quantitative data. A visualization of deregulated proteins is done through heatmap by using FunRich software (version 3.1.3) (Pathan et al. 2015). A gene ontology (GO) enrichment analysis of cellular compartments, biological processes and related diseases of quantified and deregulated proteins was performed by using David bioinformatics Resources (2022) and human database as the background of the analysis (Sherman et al. 2022). Networks of deregulated proteins were generated by using Cytoscape software (version 3.9.0) with String tool (version 1.7.0) and Omics Visualizer (version 1.3.0).

Results & discussion

I. Determination of effect concentrations

A first assessment of the toxicity of furosemide (FUR), pyridinium of furosemide (PYR), saluamine (SAL), furfural (FRF) and their mixture (MIX) was performed through an acute toxicity test (MTT). The tests were carried out initially for FUR at 24, 48, 72 and 96 h and the most contrasted responses appear at 96 h, which will be the exposure time used for all the exposures thereafter. The dose response curves obtained after SH-SY5Y, HEK 293 and Hep-G2 exposed 96 h to FUR, PYR, SAL, FRF and MIX are presented in **figure 1**.

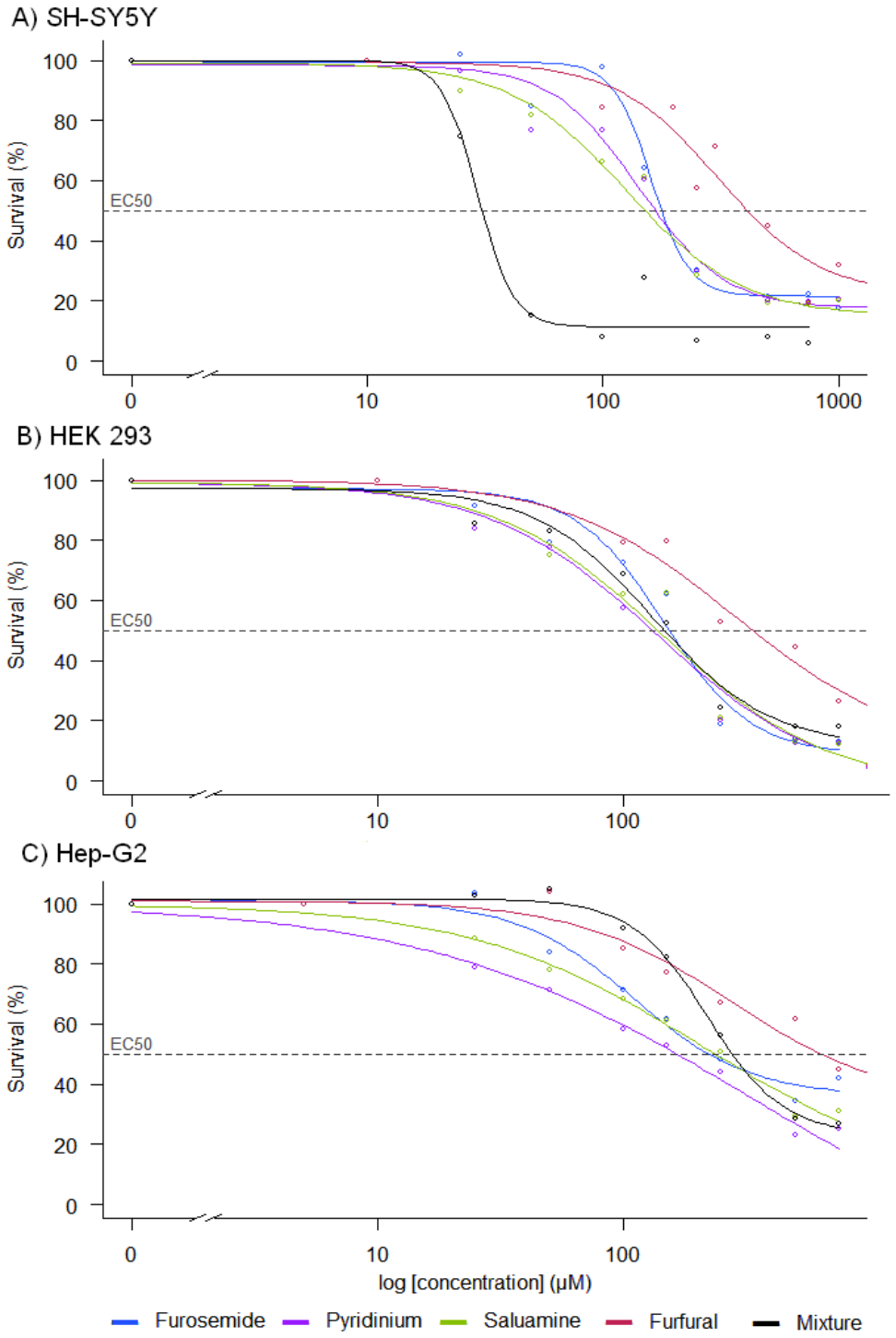


Figure 1. Dose response curves of SH-SY5Y (A), HEK 293 (B) and Hep-G2 (C) exposed for 96 h to FUR, PYR, SAL, FRF and MIX. *The dose-response curves were drawn using the R software with the drm function. The quality of the models was verified according to the definition of the slope, the high limit and the low limit. The EC50 were determined from the dose response curves. Each point represented the average value of 3 replicates for each cell line. Standard errors are not shown for clarity.*

Dose-response curves were determined for all compounds. DMSO had little effects on the three cell lines (not shown). Thus, effective concentrations for 50% of the cells (EC50) values for each compound and each cell line were normalized by the effect of DMSO (presented in **table 1**). For SH-SY5Y cells, the curves were well-defined. EC50 for FUR, PYR and SAL were close to each other around 140 µM but FRF EC50 was twice lower than FUR EC50, indicating lower toxicity to human neuroblastoma cells. However, the mixture (MIX) of the 4 compounds had an EC50 that was five times lower than the one observed for FUR, PYR, or SAL individually, indicating a

greater toxicity through a synergistic or at least additive effect of the compounds. In SH-SY5Y cells, Laurencé et al. (2019) also observed significant mortality after 96 h of exposure to PYR between 50 and 1000µM, but relatively little effect before 96 h. The authors hypothesized that the intracellular penetration of pyridinium occurs slowly because it is a polar molecule. Laurencé et al. (2019) observed a 30% effect at the highest tested concentrations of FUR (1 mM), while we observed a greater effect (> 75%) in our conditions, probably due to the different experimental settings (24-well plates, 80,000 cells/mL, different serum). A less pronounced toxicity was also observed for FRF, which was therefore the least lethal molecule on the three cell lines used in this study. In SH-SY5Y and HEK 293 cells, the EC50 values for the different compounds were generally quite similar. Unlike SH-SY5Y cells, the mixtures do not appear to be much more toxic than the individual compounds for Hep-G2 and HEK 293 cells. At the tested concentrations, no complete cell death was observed, especially for Hep-G2 cells, whose survival did not drop below 20%. It is possible that detoxification processes, which are typically located in the liver, are activated.

Table 1. Effect concentrations (µM) and robustness of dose-response models on cell lines.

Cell line	Exposure	EC50	sd	EC10	sd	model p-value
SH-SY5Y	FUR	160.28	14.86	107.11	14.79	2.71e-15 ***
	PYR	141.52	14.38	56.39	16.27	4.67e-14 ***
	SAL	124.84	14.51	35.73	9.21	5.29e-12 ***
	FRF	309.42	42.70	103.63	33.70	1.81e-09 ***
	MIX	29.37	1.96	20.04	1.90	2.20e-16 ***
HEK 293	FUR	145.73	10.35	58.94	13.37	2.20e-16 ***
	PYR	135.96	16.72	24.40	5.36	2.36e-12 ***
	SAL	143.70	19.85	26.47	6.15	1.55e-10 ***
	FRF	283.67	56.85	49.45	14.45	2.36e-06 ***
	MIX	131.35	15.75	39.78	13.82	3.24e-11 ***
Hep-G2	FUR	109.95	17.28	33.42	9.06	9.31e-09 ***
	PYR	298.79	**225.17**	11.59	3.96	**0.1873**
	SAL	208.95	96.85	19.16	4.88	0.03376 *
	FRF	276.09	80.44	55.33	13.02	0.0010452 **
	MIX	219.39	27.10	100.92	20.02	7.84e-11 ***

EC50 = Effect concentration 50%; EC10 = Effect concentration 10%. Each value was normalized by DMSO response. The quality of the models was verified according to the high limit, the low limit and the definition of the slope (model p-value column) ; Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

In the literature, FUR has already been reported as cytotoxic. Indeed, Lu et al. (2018) defined an EC50 of 17.6 µM after 24 hours of exposure to human astrocytes. However, we obtained much higher values after 96 hours of exposure, indicating less toxicity on our cell lines than on human astrocytes. The impact of furosemide has also been tested on hepatic rat cells, with EC50 between 1591 µM and 6048 µM (Fautrel et al. 1991, Biagini et al. 2006). These values are much higher than those we obtained on human liver cells. FUR has also been tested on fish cells, with 24-hour EC50 of 3420 µM on *Oncorhynchus mykiss* cell lines and 7789 µM on *Poeciliopsis lucida* cell lines (Caminada et al. 2006, Christensen et al. 2009).

The dose-response curves allowed us to choose the effective concentrations for the proteomics studies. Dose response models for SH-SY5Y and HEK 293 cells were well-defined (p-values <0.05), but for Hep-G2 cells, the models were less accurate for the by-products, and the EC50 of PYR in particular is very uncertain. The standard error was also generally higher for FRF. On the other hand, working concentrations at the EC10 instead of EC50 allowed for more precise values in the models while avoiding massive cell apoptosis which would partly mask other ongoing processes and strongly biased the proteome analysis results.

II. Proteomic study

We performed a proteomics study in the human cells that were exposed to furosemide and its degradation products. This approach enables the identification of expressed proteins and their relative quantities (under- or over-expressed). Proteomics can be used to shed light on the biological mechanisms involved in physiological or pathological processes, and to identify disease biomarkers or potential therapeutic targets. In this section, we aimed to identify which proteins may be involved in the observed toxicity on human cells, as well as the potential effects of the studied compounds.

The proteins from cells, exposed for 96 hours to the EC10 of FUR, PYR, SAL, FRF, and MIX (see above), were analyzed. For the HEK 293 cell line, 801 proteins were identified, of which 624 were quantified. For the SH-SY5Y cell line, 362 proteins were identified, of which 306 were quantified. Finally, for the Hep-G2 cell line, 1692 proteins were identified, and 1379 were quantified, making it the cell line with the largest number of exploitable proteins. In the following part of this study, we focused on the Hep-G2 cell line (**figure 2**).

Among the quantified proteins in the Hep-G2 cell line, 71 deregulated proteins were identified after exposure to FUR, 101 after exposure to PYR, 138 after exposure to SAL, 205 after exposure to FRF, and only 34 after exposure to the mixture (**figure 2**).

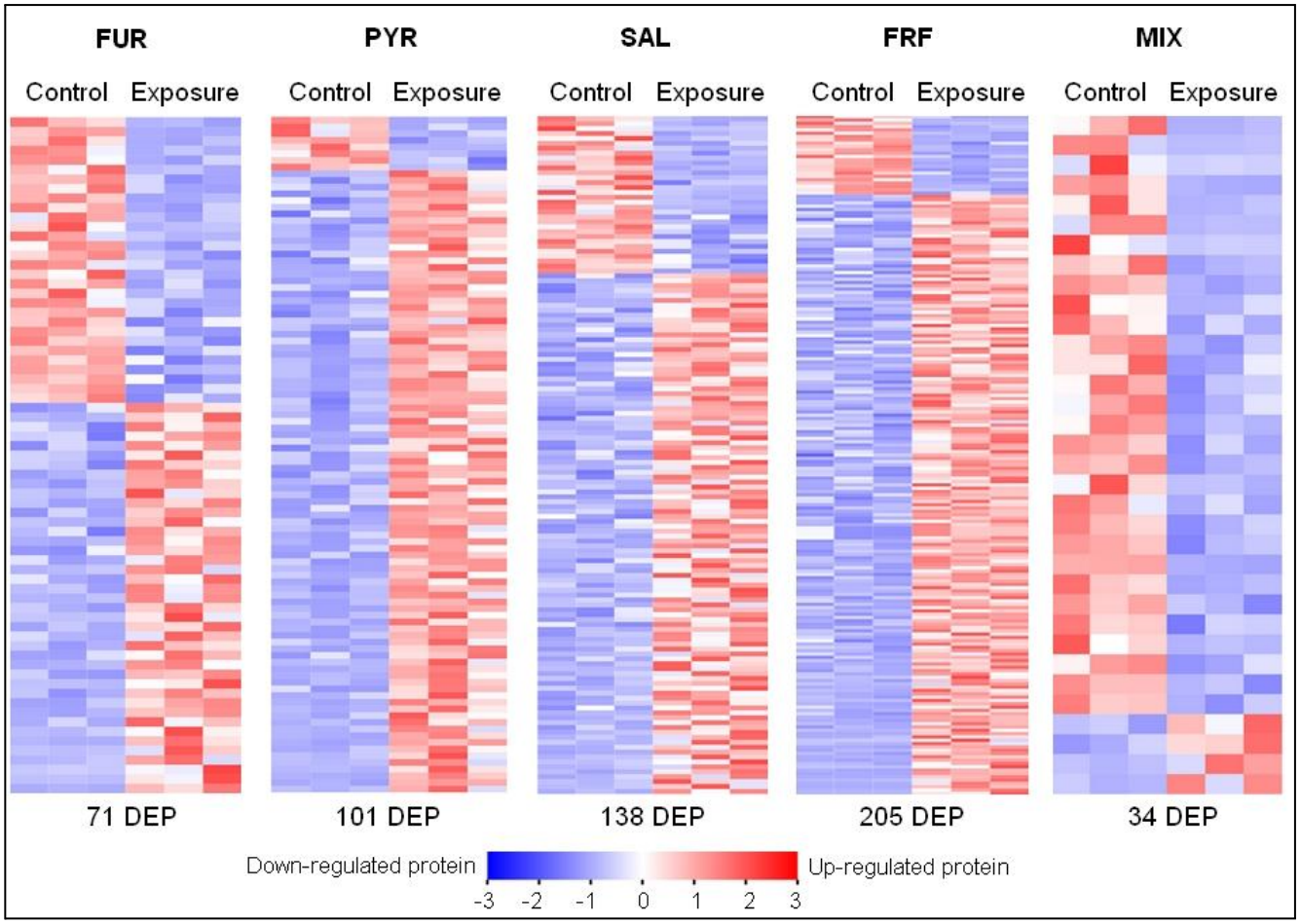


Figure 2. Distribution of deregulated proteins following exposure to FUR, PYR, SAL, FRF and to the mixture. *The down-regulated proteins are shown in blue on the heatmap, while the up-regulated ones are shown in red. DEP = deregulated protein. FUR = Furosemide ; PYR = Pyridinium of furosemide; SAL = Saluamine, FRF = Furfural; MIX= Mixture of all 4 compounds.*

The deregulated proteins were mostly up-regulated except for the mixture, where the opposite was observed. For each condition, the three replicates produced reproducible results. Among these proteins, the most robust ones (*i.e.* with more than two unique peptides and p-value <0.05) were compared (**Table SI.I**). Ultimately, few responses related to apoptosis were observed and a significant number of deregulated proteins were identified, which confirmed the choice of EC10 for the exposures.

A. Deregulated protein by FUR exposure

Based on the list of deregulated proteins, a gene ontology enrichment analysis of biological processes and related diseases was conducted using the David Bioinformatics Resources. For FUR, no relevant disease was identified, suggesting that the drug did not induce major deleterious effects on liver cells at the tested concentration. However, several metabolic processes were activated and numerous proteins potentially associated with them were deregulated.

Among the proteins dysregulated due to exposure to FUR (see Table SI.I), several of them have been associated with endocrine disruption. For instance, Estradiol 17-beta-dehydrogenase 11 is an enzyme involved in the metabolism of steroid hormones, particularly in the biosynthesis and inactivation of estrogen and androgen

(Corbould et al. 1997; Chai et al. 2003; Mindnich et al 2004). Underexpression of the associated protein could reduce the organism's ability to efficiently metabolize these compounds, which could disrupt hormones homeostasis. Also underexpressed, sulfotransferase 1A2, mainly expressed in the liver, as well as sulfotransferase 1A3, are enzymes involved in the metabolism of many chemicals, including drugs, and steroid hormones such as dehydroepiandrosterone (DHEA) and androsterone (Paul et al. 2012). Under-regulation of sulfotransferase 1A2 can lead to a decrease in the organism's ability to metabolize xenobiotics, which could result in deleterious effects, and may also lead to a decrease in the sulfation of DHEA, a precursor hormone for the synthesis of testosterone and estrogen (Traish et al. 2011), which can reduce the availability of these hormones and have effects on sexual function. DHEA is also involved in aging and neurological development (Greaves et al. 2018). Moreover, several proteins involved in the regulation of hormonal signaling (serine/threonine-protein phosphatase 2A catalytic subunit beta isoform (PP2A), Annexin A7, and progranulin) are also downregulated after exposure to FUR. Processes of hormonal signaling regulation include the production and release of hormones by endocrine glands, the transport of hormones in the blood, their binding to receptors on target cells, and the resulting intracellular signaling (Keen et al. 2005, Alauddin et al. 2020, Suzuki et al. 2009). PP2A also controls the cell cycle and cell death. It is considered as a tumor suppressor and is often functionally inactivated in cancer (Seshacharyulu et al 2013). The underexpression of these proteins can have negative health consequences, particularly regarding the regulation of growth and development, the regulation of sexual function, immune response and cancer onset. Moreover, these results appear to be consistent with the scientific literature. Indeed, Isidori et al. (2009) and Fent et al. (2006) showed a strong estrogenic potential of furosemide with the YES-assay on *Saccharomyces cerevisiae* with an EC50 of 0.99 mg/L and 469 mg/L, respectively, also showing that furosemide binds to the human estrogen receptor. This is consistent with an endocrine disruption caused by FUR.

Furthermore, several down-regulated proteins are involved in the regulation of cellular signaling and the response to cellular stress. For example, Nucleolysin TIAR, Annexin A7, Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform, and Dual specificity protein phosphatase 9 are known to play a role in the response to oxidative stress (Laura et al. 2021, Alauddin et al. 2020, Sommer et al. 2002, Chen et al. 2019). However, the underexpression of these proteins appears to contradict the findings of Lahet et al. (2003) and our previous work (Sandre et al. 2058 tox) which suggested an antioxidant effect of furosemide. Oxidative stress is a complex process, and there are numerous molecular mechanisms involved in its regulation. The antioxidant effect could also be due to the overexpression of thioredoxin reductase (observed after exposure to FUR, but not the other compounds), an enzyme that can reduce organic and inorganic peroxides, thereby acting as an antioxidant (Choi et al. 2011). Sulfotransferases 1A2 and 1A3 are involved in the metabolism of xenobiotics and also have a role in defense against oxidative stress (Paul et al. 2012), as do proteins that regulate the production of transcription factors such as G-rich sequence factor 1, which may be involved in regulating genes that code for antioxidant enzymes (Noh et al. 2019). A more in-depth analysis of the study or experimental situation is necessary to establish a clear link between the mentioned protein regulations and the antioxidant effect.

B. PYR, SAL and FRF-induced protein dysregulation and associated diseases

Several identified proteins are linked to liver toxicity after exposure to PYR, SAL and FRF, which is consistent in the context of the study. In addition, liver damage has already been reported in the literature for FRF exposure (Shimizu et al. 1989).

The enrichment analysis on the proteins dysregulated after exposure to PYR indicates that some have been reported in several diseases (table SI.II), such as the development of cancer or inflammatory diseases. Others, such as hermaphroditism, sex differentiation disorders, ambiguous genitalia, intersex conditions, pseudohermaphroditism, polycystic ovary syndrome and sclerocystic ovaries can be linked to endocrine disruption. It is therefore possible that the dysregulated proteins implicated in endocrine disruption observed for FUR (see previous section) may also be observed for PYR, which has a close molecular structure. Indeed, Sulfotransferase 1A2 expression, was modified in both conditions, although in opposite manner, (upregulated for FUR and downregulated in the case of PYR).

PYR is also associated with modification of cognitive traits and aging/telomere length (table SI.VI). Some cognitive traits may be related to neurological diseases and neurodegeneration, for example, Alzheimer's disease is characterized by progressive loss of memory and other cognitive functions (Joe & Ringman 2019), while Parkinson's disease can affect cognition, working memory, attention, and spatial perception (Balestrino & Schapira 2019). Studies have shown that telomere length shortening may be associated with an increased risk of neurological diseases such as Alzheimer's disease (Yu et al. 2021), but remain inconclusive to date for Parkinson's disease. Nevertheless, correlation has been made between telomere attrition and numerous age related disorders and chronic diseases (cardiovascular diseases, hypertension, arthritis, osteoporosis, diabetes, cancer) and neurological disorders (reviewed in Levstek et al. 2020), in association with oxidative stress or inflammation. These studies reinforce our previous study which suggested that PYR could be a potential inducer of neurodegenerative disease because it causes inhibition of the mitochondrial respiratory chain in the striatum, accumulation of phosphorylated alpha-synuclein at Serine129, a decrease in tyrosine hydroxylase in the striatum, and an accumulation of Tau in the hippocampus (Laurencé et al. 2019). The inhibition of the mitochondrial respiratory chain in the striatum can be influenced by several proteins on the list, including Citrate synthase, Cytochrome c oxidase subunit 4, ATP synthase subunit alpha, and NADH-cytochrome b5 reductase 3, all of which are involved in energy production in mitochondria (Annesley & Fisher 2019). The accumulation of phospho-Serine129 alpha-synuclein can be influenced by Eukaryotic translation initiation factor 3 subunit G, which is involved in alpha-synuclein regulation (Weng et al. 2015). Finally, the accumulation of Tau in the hippocampus may be related to Calmodulin-3, which is involved in the regulation of calcium signaling (Balshaw et al. 2002). Studies have suggested that the accumulation of Tau protein in the hippocampus may be associated with an alteration in the regulation of calcium, which is an important process for the normal functioning of neurons (Esteras & Abramov 2020)

For SAL, several diseases were associated with deregulated genes quantified in our study (Table SI.III), particularly numerous neurological diseases (Table SI.VII). The quantified proteins also appeared to be strongly involved in cancer. Although this observation is of interest, it should be kept in mind that the Hep-G2 cell line was isolated from an hepatocellular carcinoma, thus already presenting dysregulated cancer-related pathways. Some cardiovascular pathologies were also associated and a large number of diseases were linked to

genotoxicity. However, very few diseases potentially related to endocrine disruption seem to be linked to exposure to SAL. It is possible that its small size does not allow it to bind to estrogen receptors, unlike FUR. To our knowledge, only one article studied toxicity saluamine toxicity in literature. According to Al-Omar et al. (2009), saluamine causes glucose metabolism disturbances and hepatic inflammation, with an increase of aspartate and alanine aminotransferases and congestion in mice. The observed inflammatory response could involve Interleukin enhancer-binding factor 3 (ILF3) protein, which has possible roles in various pathologies like stroke, cancer, inflammation and dyslipidemia, although these roles remain poorly understood to date (Xie et al 2021).

The largest number of proteins identified was observed with exposure to FRF, but there were not many diseases directly associated with it based on the enrichment analysis. However, as for PYR and SAL, numerous identified proteins have been potentially linked to cancers, and a few with neurodegenerative diseases (Parkinson disease, cognitive trait, aging/telomere length).

C. MIX-induced protein dysregulation and associated diseases

Unexpectedly, exposure to the mixture of our four compounds led to significantly fewer deregulated proteins than for exposures to the compounds alone, suggesting the occurrence of compensatory or antagonistic effects. In addition, the enrichment analysis did not show any proteins linked with neurodegenerative diseases, whereas the exposures to single compounds did. On the other hand, several listed diseases are related to cardiovascular problems (Table SI.IX). However, as FUR has long been prescribed successfully to patients with heart failure and hypertension, it seems unlikely that the occurrence of these complications could have gone unnoticed for such a long time. Thus, it seems more realistic that the mix of compounds, at the concentrations used in the experiment, affects the expression of proteins involved in these pathologies, keeping in mind that nothing is known to date about PYR, SAL and FRF concentrations and pharmacology in the patients bodies. This gap of knowledge highlights the need for pharmacological studies of these metabolites, as well as on their long term effects at very low doses exposures in animal models.

Conclusion

Taken together, our results show close EC50 for FUR and its degradation products PYR and SAL on human cells, and a slightly lower toxicity with FRF. The mixture was found to be much more toxic than the single compounds on SH-SY5Y cells, which was not as marked on the two other cell lines HepG2 and HEK293. The proteins from the three cell types exposed to EC10 were extracted. Proteomic analysis was carried out on Hep-G2 cells, which showed a much larger number of deregulated proteins. Enrichment analysis revealed a few diseases that could be associated with the deregulated proteins following exposure to FUR, supporting the hypothesis of low toxicity of this commonly used drug. However, the same analysis revealed possible links with neurodegenerative diseases, endocrine disruption, cancer and oxidative stress after exposure to PYR, SAL, and FRF. For the mixture, fewer proteins were identified, and the diseases previously associated with the deregulated proteins by FUR metabolites were not retrieved, suggesting possible antagonistic effects. Finally, this study provides important preliminary data to improve the current understanding of furosemide impacts, as well as those of its by-products and their mixture on human cells. The identification of several deregulated proteins puts forward valuable leads to investigate potential mechanisms of action associated with observed toxic responses, with respect to organ specificities. Beyond the case of furosemide, our work further highlights

the urgent need to better take into account drug by-products during toxicological risk assessment, as they could have major deleterious impacts due to a potentially higher toxicity than their parent molecules.

Acknowledgement: Thanks to the OPUR program for proteomic funding. Thanks to Université Paris-Est Creteil and to the French ministry of higher education research and innovation for F. Sandré PhD fellowship.

Author contribution: Fidji Sandre: investigation, visualization, writing-original draft & editing; Sandrine Bourgoin-Voillard: investigation, conceptualization, review & editing; Benoît Vallee: investigation, review & editing; Damien Haber: investigation; José Courty: visualization; Christophe Morin: review & editing; Laure Garrigue-Antar: supervision, conceptualization, review & editing.

Supplementary informations :

Table SI.I. Furosemide deregulated proteins

40S ribosomal protein S21	Hsp90 co-chaperone Cdc37
UDP-glucose 4-epimerase	Cell division control protein 42 homolog
G-rich sequence factor 1	Leukocyte elastase inhibitor
5'-3' exoribonuclease 2	DnaJ homolog subfamily A member 2
Transmembrane protease serine 11A	Sulfotransferase 1A3
Leukotriene A-4 hydrolase	Keratin_ type I cytoskeletal 10
Nuclear migration protein nudC	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit
Dihydropteridine reductase	60S acidic ribosomal protein P1
Small nuclear ribonucleoprotein E	Barrier-to-autointegration factor
GTP cyclohydrolase 1 feedback regulatory protein	Thioredoxin-dependent peroxide reductase_ mitochondrial
26S proteasome regulatory subunit 6B	ATP synthase subunit f_ mitochondrial
Tetratricopeptide repeat protein 38	Kinectin
26S proteasome regulatory subunit 8	Partner of Y14 and mago
2-hydroxyacyl-CoA lyase 2	DnaJ homolog subfamily A member 3_ mitochondrial
NADH-cytochrome b5 reductase 3	Immunoglobulin lambda-like polypeptide 5
Succinate-semialdehyde dehydrogenase_ mitochondrial	40S ribosomal protein S10
Mitochondrial import receptor subunit TOM40 homolog	Protein SEC13 homolog
Keratin_ type II cytoskeletal 4	Actin-related protein 2/3 complex subunit 2
Keratin_ type II cytoskeletal 1b	BolA-like protein 2
Leucine-rich repeat-containing protein 59	Nucleolysin TIAR
Trifunctional enzyme subunit beta_ mitochondrial	Annexin A7
Hsp90 co-chaperone Cdc37	Peptidyl-prolyl cis-trans isomerase A-like 4G
Cell division control protein 42 homolog	Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform
Leukocyte elastase inhibitor	Dual specificity protein phosphatase 9
DnaJ homolog subfamily A member 2	Sulfotransferase 1A2
Sulfotransferase 1A3	Splicing factor 1
Keratin_ type I cytoskeletal 10	Acyl-coenzyme A thioesterase 1
Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	Aspartate--tRNA ligase_ cytoplasmic
60S acidic ribosomal protein P1	THO complex subunit 4
Barrier-to-autointegration factor	Alcohol dehydrogenase class-3
Thioredoxin-dependent peroxide reductase_ mitochondrial	Progranulin
ATP synthase subunit f_ mitochondrial	Splicing factor 3A subunit 3
Kinectin	Copine-1
Partner of Y14 and mago	60S ribosomal protein L9
DnaJ homolog subfamily A member 3_ mitochondrial	Cytochrome c oxidase subunit 4 isoform 1_ mitochondrial
Immunoglobulin lambda-like polypeptide 5	Sialic acid synthase

Protein S100-A9	Ras GTPase-activating-like protein IQGAP2
Immunoglobulin kappa variable 1D-13	14-3-3 protein eta
Spermidine synthase	Lymphokine-activated killer T-cell-originated protein kinase
ADP-ribosylation factor-like protein 8B	Estradiol 17-beta-dehydrogenase 11
D-dopachrome decarboxylase	Fatty acid-binding protein_ liver
Protein S100-A9	Transcription intermediary factor 1-beta
Immunoglobulin kappa variable 1D-13	Aldehyde dehydrogenase family 1 member A3
Spermidine synthase	Large neutral amino acids transporter small subunit 1
ADP-ribosylation factor-like protein 8B	Cell division cycle 5-like protein
D-dopachrome decarboxylase	

Red= Up-regulated protein; Blue =down-regulated protein; only protein with P-value ≤ 0.05 and fold change ≥ 1.75 are presented

Table SI.II. Pyridinium of furosemide deregulated proteins

Beta-enolase	Peroxiredoxin-6
Elongation factor 1-gamma	NHP2-like protein 1
Cofilin-2	Citrate synthase_ mitochondrial
GTP:AMP phosphotransferase AK3_ mitochondrial	Cytochrome c oxidase subunit 4 isoform 1_ mitochondrial
E3 ubiquitin-protein ligase LRSAM1	Putative protein FAM86JP
60S ribosomal protein L3	60S ribosomal protein L5
60S ribosomal protein L28	Purine nucleoside phosphorylase
Sodium/potassium-transporting ATPase subunit alpha-1	UDP-glucose 6-dehydrogenase
Ras-related protein Rab-37	60S ribosomal protein L27a
40S ribosomal protein SA	40S ribosomal protein S4_ X isoform
Keratin_ type II cytoskeletal 8	60S ribosomal protein L36
Fructose-bisphosphate aldolase A	Zinc finger HIT domain-containing protein 2
40S ribosomal protein S23	Neutral alpha-glucosidase AB
40S ribosomal protein S13	Sialic acid synthase
GTP-binding nuclear protein Ran	Serine/arginine-rich splicing factor 1
Ubiquitin carboxyl-terminal hydrolase 7	Myosin light chain 6B
THO complex subunit 4	Zinc finger and SCAN domain-containing protein 26
Phosphoserine aminotransferase	Retinal dehydrogenase 2
Minor histocompatibility antigen H13	Alpha-actinin-1
ATP-citrate synthase	High mobility group protein B1
Heterogeneous nuclear ribonucleoprotein M	4F2 cell-surface antigen heavy chain
Nucleosome assembly protein 1-like 1	Atlastin-2
Vimentin	Fascin
Glutathione S-transferase omega-1	Transferrin receptor protein 1
Phosphoglycerate kinase 1	NADH-cytochrome b5 reductase 3
Proteasome subunit alpha type-5	Serine/arginine-rich splicing factor 4
Neutrophil defensin 1	Eukaryotic initiation factor 4A-III
Gamma-enolase	Non-POU domain-containing octamer-binding protein
Chloride intracellular channel protein 1	60S ribosomal protein L23a
Serpin B6	Heat shock-related 70 kDa protein 2
Putative heat shock protein HSP 90-alpha A5	Heat shock protein 75 kDa_ mitochondrial
2_4-dienoyl-CoA reductase [(3E)-enoyl-CoA-producing]_ mitochondrial	Aldo-keto reductase family 1 member C1
Histone H1.1	Aldo-keto reductase family 1 member C2
Leucine-rich repeat-containing protein 59	Tumor protein D54
Lymphokine-activated killer T-cell-originated protein kinase	X-ray repair cross-complementing protein 6
Calmodulin-3	Prothymosin alpha
Acyl-CoA-binding protein	Ras-related protein Rab-1A
Agmatinase_ mitochondrial	ELAV-like protein 1
Protein DEK	60S ribosomal protein L11

60S ribosomal protein L27	Pyruvate carboxylase_ mitochondrial
Prelamin-A/C	L-xylulose reductase
Ribonucleoside-diphosphate reductase subunit M2	ATP synthase subunit alpha_ mitochondrial
Malonate--CoA ligase ACSF3_ mitochondrial	Eukaryotic translation initiation factor 3 subunit G
Heat shock 70 kDa protein 6	Pyrroline-5-carboxylate reductase 2
Ubiquitin-conjugating enzyme E2 variant 2	Galectin-7
60S ribosomal protein L23	Malectin
Polymeric immunoglobulin receptor	Peroxiredoxin-5_ mitochondrial
Aldehyde dehydrogenase family 1 member A3	Na(+)/H(+) exchange regulatory cofactor NHE-RF1
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1	Core histone macro-H2A.2
Sulfotransferase 1A2	Alanine aminotransferase 1
26S proteasome non-ATPase regulatory subunit 4	

Red= Up-regulated protein; Blue =down-regulated protein; only protein with P-value ≤ 0.05 and fold change ≥ 1.75 are presented

Table SI.III. Saluamine deregulated proteins.

Heterogeneous nuclear ribonucleoprotein U	Peptidyl-prolyl cis-trans isomerase B
ATP synthase subunit beta, mitochondrial	60S ribosomal protein L6
Heterogeneous nuclear ribonucleoprotein R	Histone H2B type 1-O
40S ribosomal protein S27-like	Galactokinase
Coronin-1C	Endoplasmic reticulum resident protein 29
Basigin	Glycerol kinase
14-3-3 protein eta	Cytosol aminopeptidase
60S ribosomal protein L7a	Poly [ADP-ribose] polymerase 1
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	Polypyrimidine tract-binding protein 1
Galectin-1	Interleukin enhancer-binding factor 3
Keratin, type II cytoskeletal 2 oral	Lamin-B1
Elongation factor 2	Alpha-2-macroglobulin
Phosphoenolpyruvate carboxykinase, cytosolic [GTP]	T-complex protein 1 subunit beta
Vomeronasal type-1 receptor 5	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
60S ribosomal protein L18	60S ribosomal protein L10a
Thiosulfate sulfurtransferase	Large structural phosphoprotein

Red= Up-regulated protein; Blue =down-regulated protein; only protein with P-value ≤ 0.05 and fold change ≥ 1.75 are presented

Table SI.IV. Furfural deregulated proteins

Peroxiredoxin-1	Keratin_ type I cytoskeletal 14
Parkinson disease protein 7	Keratin_ type I cuticular Ha6
Interferon-inducible double-stranded RNA-dependent protein kinase activator A	DNA replication licensing factor MCM7
Keratin_ type I cytoskeletal 13	Cofilin-2
Protein disulfide-isomerase	Histone H1.10
Vomeronasal type-1 receptor 5	Peptidyl-prolyl cis-trans isomerase FKBP1A
Keratin_ type I cytoskeletal 10	Tubulin alpha-1B chain
Hydroxyacyl-coenzyme A dehydrogenase_ mitochondrial	Tubulin alpha-1C chain
Serine hydroxymethyltransferase_ mitochondrial	Tubulin alpha-1A chain
Keratin_ type I cytoskeletal 18	Cytochrome b-c1 complex subunit 7
Beta-enolase	Keratin_ type II cytoskeletal 1
Methionine--tRNA ligase_ cytoplasmic	Prelamin-A/C
Constitutive coactivator of PPAR-gamma-like protein 1	ATP-citrate synthase
Histone H2B type 1-D	Leucine-rich PPR motif-containing protein_ mitochondrial
Heat shock 70 kDa protein 1B	GTP-binding nuclear protein Ran

Asparagine--tRNA ligase_ cytoplasmic	Poly(rC)-binding protein 1
Heterogeneous nuclear ribonucleoprotein A1	Aldo-keto reductase family 1 member C3
Lactotransferrin	Ras-related protein Rab-11A
GDP-mannose 4_6 dehydratase	Ras-related C3 botulinum toxin substrate 1
Peptidyl-prolyl cis-trans isomerase B	NADH-cytochrome b5 reductase 3
Tyrosine--tRNA ligase_ mitochondrial	NADH-ubiquinone oxidoreductase 75 kDa subunit_ mitochondrial
Putative 40S ribosomal protein S26-like 1	Glutaredoxin-1
40S ribosomal protein S26	Heterogeneous nuclear ribonucleoprotein C-like 3
WD repeat-containing protein 1	60S ribosomal protein L19
Probable phosphoglycerate mutase 4	Purine nucleoside phosphorylase
40S ribosomal protein S3a	2_4-dienoyl-CoA reductase [(3E)-enoyl-CoA-producing]_ mitochondrial
Phosphoglycerate mutase 1	Aldo-keto reductase family 1 member B15
Phosphoglycerate kinase 2	Monocarboxylate transporter 4
40S ribosomal protein S9	Transketolase
Serine/arginine-rich splicing factor 8	60S ribosomal protein L23
Tubulin alpha-3C chain	Sialidase-1
Proliferating cell nuclear antigen	Enoyl-CoA delta isomerase 2
Malate dehydrogenase_ mitochondrial	Sialic acid synthase
60S ribosomal protein L18a	Keratin_ type I cytoskeletal 27
Ras-related protein Rab-37	39S ribosomal protein L12_ mitochondrial
Nucleosome assembly protein 1-like 1	Splicing factor_ proline- and glutamine-rich
40S ribosomal protein S18	Heat shock 70 kDa protein 6
Probable ATP-dependent RNA helicase DDX17	Tumor protein D54
Trifunctional purine biosynthetic protein adenosine-3	Protein S100-A9
Immunoglobulin heavy constant alpha 1	Lipocalin-1
Serine/arginine-rich splicing factor 4	Low molecular weight phosphotyrosine protein phosphatase
Cytochrome c oxidase subunit 5B_ mitochondrial	Histone H2A.V
Histone H1t	Malonate--CoA ligase ACSF3_ mitochondrial
40S ribosomal protein S8	Glutathione S-transferase omega-1
40S ribosomal protein S23	40S ribosomal protein S21
UDP-glucose:glycoprotein glucosyltransferase 1	L-xylulose reductase
Heterogeneous nuclear ribonucleoprotein D0	T-complex protein 1 subunit zeta-2
14-3-3 protein theta	Lymphokine-activated killer T-cell-originated protein kinase
Transmembrane 9 superfamily member 4	Ubiquitin thioesterase OTUB1
Rootletin	Heterogeneous nuclear ribonucleoprotein M
Heat shock protein 75 kDa_ mitochondrial	Complement component C9
Clathrin heavy chain 2	Ras-related protein Rab-8B
Heterogeneous nuclear ribonucleoprotein F	Agmatinase_ mitochondrial
Sulfotransferase 2A1	DnaJ homolog subfamily A member 3_ mitochondrial
40S ribosomal protein SA	Aspartyl/asparaginyl beta-hydroxylase
Receptor of activated protein C kinase 1	DnaJ homolog subfamily A member 2
40S ribosomal protein S5	Tubulin beta-4B chain
Zinc finger HIT domain-containing protein 2	Putative heat shock protein HSP 90-alpha A5
Solute carrier family 2 facilitated glucose transporter member 1	Acyl-CoA synthetase short-chain family member 3_ mitochondrial
Lysozyme C	Phosphoserine aminotransferase
Aspartate aminotransferase_ cytoplasmic	Eukaryotic translation initiation factor 3 subunit E
40S ribosomal protein S27-like	AP-2 complex subunit mu
Adipocyte plasma membrane-associated protein	ATP synthase membrane subunit DAPIT_ mitochondrial
Aldo-keto reductase family 1 member B10	60S ribosomal protein L23a
Neutrophil defensin 1	Cofilin-1
Far upstream element-binding protein 1	Phosphoglycerate kinase 1
60S ribosomal protein L27	60S ribosomal protein L3
Glutaminase kidney isoform_ mitochondrial	Eukaryotic translation initiation factor 3 subunit H
Endoplasmin	Serine/threonine-protein phosphatase 4 regulatory subunit 4
Putative tubulin-like protein alpha-4B	Heterogeneous nuclear ribonucleoprotein K

Ninein	26S proteasome non-ATPase regulatory subunit 11
Transaldolase	Voltage-dependent anion-selective channel protein 3
26S proteasome non-ATPase regulatory subunit 4	Histone H1.1
Eukaryotic initiation factor 4A-I	Lamin-B2
Ubiquitin-conjugating enzyme E2 L3	Ribonucleoside-diphosphate reductase subunit M2
Potassium voltage-gated channel subfamily H member 5	Zinc finger and SCAN domain-containing protein 26
Eukaryotic initiation factor 4A-II	Calmodulin-3
ATP synthase subunit alpha_ mitochondrial	Acyl-CoA-binding protein
Opioid growth factor receptor	28S ribosomal protein S27_ mitochondrial
Fructose-bisphosphate aldolase A	Endothelial differentiation-related factor 1
Delta-1-pyrroline-5-carboxylate dehydrogenase_ mitochondrial	Pyridoxal kinase
Fatty acid-binding protein_ liver	Enoyl-CoA hydratase_ mitochondrial
Ras-related protein Rab-8A	Putative heat shock protein HSP 90-beta-3
Mitogen-activated protein kinase kinase kinase 1	Septin-2
Aldo-keto reductase family 1 member C4	60S ribosomal protein L14
Citrate synthase_ mitochondrial	Elongation factor 1-delta
Large neutral amino acids transporter small subunit 1	3-ketoacyl-CoA thiolase_ peroxisomal
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	Calmodulin-like protein 5
Alpha-actinin-1	Exportin-2
Cytochrome b-c1 complex subunit 1_ mitochondrial	Zinc-alpha-2-glycoprotein
Myosin light polypeptide 6	Guanine nucleotide-binding protein G(t) subunit alpha-1
Pseudouridine-5'-phosphatase	Tricarboxylate transport protein_ mitochondrial
ATP-dependent DNA helicase DDX11	Keratin_ type II cytoskeletal 79
Leucine-rich repeat-containing protein 59	60S ribosomal protein L10a
Spindlin-2A	Heat shock protein HSP 90-alpha A2
Differentially expressed in FDCP 6 homolog	3-mercaptopyruvate sulfurtransferase
ATP-dependent RNA helicase DDX55	Interleukin enhancer-binding factor 3
Shutoff protein	26S proteasome non-ATPase regulatory subunit 12
Pro-Pol polyprotein	Serpin H1
Aldehyde dehydrogenase family 1 member A3	Clathrin heavy chain 1
NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4	Alanine--tRNA ligase_ cytoplasmic
Syntaxin-7	Histone H1.0
Serpin B6	

Red= Up-regulated protein; Blue =down-regulated protein; only protein with P-value ≤ 0.05 and fold change ≥ 1.75 are presented

Table SI.V. Mixture deregulated proteins

Tubulin beta-2A chain	Spermidine synthase
Phosphatidylethanolamine-binding protein 1	Protein SCO1 homolog_ mitochondrial
Nucleolin	Insulin-like growth factor 2 mRNA-binding protein 3
60S acidic ribosomal protein P1	Cytochrome c1_ heme protein_ mitochondrial
Heterogeneous nuclear ribonucleoprotein K	Ras-related C3 botulinum toxin substrate 1
Lamin-B1	Heat shock 70 kDa protein 1-like
Cofilin-1	Putative heat shock protein HSP 90-alpha A4
Nucleoside diphosphate kinase A	Early E3A 11.6 kDa glycoprotein
Liver carboxylesterase 1	Gelsolin
Protein Wiz	Metaxin-2
Phosphoenolpyruvate carboxykinase_ cytosolic [GTP]	Mannose-P-dolichol utilization defect 1 protein
Pro-Pol polyprotein	ATP-binding cassette sub-family A member 9
Nucleoside diphosphate kinase B	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1
Adenomatous polyposis coli protein 2	Developmentally-regulated GTP-binding protein 2
40S ribosomal protein S20	3-ketoacyl-CoA thiolase_ peroxisomal

Heat shock protein 75 kDa_ mitochondrial	Heterogeneous nuclear ribonucleoprotein H3
Insulin-like growth factor 2 mRNA-binding protein 1	Heterogeneous nuclear ribonucleoproteins C1/C2

Red= Up-regulated protein; Blue =down-regulated protein; only protein with P-value ≤ 0.05 and fold change ≥ 1.75 are presented

Table SI.VI. Diseases related to deregulated proteins induced by PYR exposure

Category	Term	Count	P-value
Disruption of endocrine functions	Hermaphroditism	2	3,80E-02
	Sex Differentiation Disorders	2	3,80E-02
	Ambiguous Genitalia	2	3,80E-02
	Intersex Conditions	2	3,80E-02
	Pseudohermaphroditism	2	3,80E-02
	Polycystic Ovary Syndrome	6	4,80E-03
	Sclerocystic Ovaries	6	4,80E-03
Neurological diseases	cognitive trait	5	3,10E-03
	Aging/ Telomere Length	5	3,20E-03
Cancer	Adenocarcinoma of lung (disorder)	7	4,80E-03
	Neoplastic Cell Transformation	5	2,10E-02
	Neoplasms, Hormone-Dependent	2	2,30E-02
	prostate cancer	8	1,50E-02
liver diseases or injuries	Drug-Induced Acute Liver Injury	8	3,40E-02
	Chemically-Induced Liver Toxicity	8	3,40E-02
	Chemical and Drug Induced Liver Injury	8	3,40E-02
	Drug-Induced Liver Disease	8	3,40E-02
	Hepatitis, Drug-Induced	8	3,40E-02
	Hepatitis, Toxic	8	3,40E-02
inflammatory and autoimmune diseases	Degenerative polyarthritis	7	7,20E-05
	Osteoarthrosis Deformans	7	7,20E-05
	Anemia, Diamond-Blackfan	5	7,40E-05
	Acquired Immunodeficiency Syndrome	12	5,90E-03
	Diamond-Blackfan anemia	2	3,00E-02
Cardiological diseases	Myocardial Ischemia	6	1,10E-02
Other	Thumb deformity	2	1,50E-02
	Glycogen Storage Disease	3	1,80E-02
	Keloid	3	2,90E-02

Table SI.VII. Diseases related to deregulated proteins induced by SAL exposure

Category	Term	Count	P-value
Cancer	leukemia, acute myeloid	3	2,00E-03
	brain cancer	4	4,80E-03
	esophageal cancer	4	7,90E-03
	Multiple Myeloma	4	8,40E-03
	Carcinoma, Squamous Cell, Esophageal Neoplasms	3	9,90E-03
	Myeloid Leukemia	3	9,90E-03
	Lung Neoplasms	3	9,90E-03
	Lymphoma, Non-Hodgkin	5	1,00E-02

	Bladder Neoplasm	6	1,30E-02
	Malignant neoplasm of urinary bladder	6	1,30E-02
	Carcinoma, Squamous Cell, Head and Neck Neoplasms	3	1,80E-02
	Adenocarcinoma	5	2,90E-02
	Adenocarcinoma, Basal Cell	5	2,90E-02
	Adenocarcinoma, Oxyphilic	5	2,90E-02
	Adenocarcinoma, Tubular	5	2,90E-02
	Carcinoma, Cribriform	5	2,90E-02
	Carcinoma, Granular Cell	5	2,90E-02
	Adenoma, Carcinoma, Colorectal Neoplasms	2	3,00E-02
	Carcinoma, Hepatocellular, Hepatitis B, Chronic, Hepatitis C, Chronic, Liver Neoplasms	2	3,00E-02
	Liver carcinoma	11	3,00E-02
	breast cancer	10	3,40E-02
	Carcinoma, Squamous Cell, Head and Neck Neoplasms, Squamous cell carcinoma	3	3,50E-02
	esophageal carcinoma	2	3,70E-02
	Leukemia, Myeloid, Acute, Precursor Cell Lymphoblastic Leukemia-Lymphoma, Recurrence	2	3,70E-02
	Leukemia, myelodysplastic (TRLIMDS)	2	3,70E-02
	Leukemia|Prenatal Exposure Delayed Effects	2	3,70E-02
	malignant mesothelioma	2	3,70E-02
	Cecal Neoplasms	2	3,90E-02
	Malignant neoplasm of cecum	2	3,90E-02
	prostate cancer	9	3,90E-02
	Carcinoma, Hepatocellular|Liver Neoplasms	3	4,00E-02
	breast cancer	9	4,30E-02
	myelodysplastic syndrome	2	4,50E-02
Cardiological diseases	Arterial Fatty Streak	2	4,90E-02
	Atheroma	2	4,90E-02
	Fibroatheroma	2	4,90E-02
	Plaque, Atherosclerotic	2	4,90E-02
Endocrine functions	Infertility, Male|Varicocele	2	3,00E-02
	preterm delivery	3	3,40E-02
Genotoxicity	Micronuclei, Chromosome-Defective	4	8,80E-05
	Benzene toxicity	4	2,30E-03
	1-hydroxypyrene, urinary	3	3,60E-03
	DNA adducts	3	3,60E-03
	DNA Damage|	3	4,90E-03
	cytogenetic studies	3	2,20E-02
	1-hydroxypyrene, DNA adducts, aromatic; mutagenicity	2	3,00E-02
	1,3-butadiene	2	3,00E-02
	2-hydroxyethyl mercapturic acid	2	3,00E-02
	Acrylamide	2	3,70E-02
	exposure to 1,3-butadiene	2	3,70E-02

	Aneuploidy, Chromosome Aberrations, Chromosome abnormality, Trisomy	2	4,50E-02
	Chromosome Aberrations, Chromosome abnormality, DNA Damage	2	4,50E-02
	DNA damage, biomarkers	2	4,50E-02
	styrene toxicity	2	4,50E-02
	Black carbon exposure	2	4,50E-02
liver diseases or injuries	liver injury, drug-induced	2	3,00E-02
Neurological diseases	Cognitive trait	6	2,00E-03
	Aging/ Telomere Length	6	2,10E-03
	Neuropathy	3	2,50E-03
	Autosomal Dominant Juvenile Parkinson Disease	4	2,60E-03
	Autosomal Dominant Parkinsonism	4	2,60E-03
	Autosomal Recessive Parkinsonism	4	2,60E-03
	Familial Juvenile Parkinsonism	4	2,60E-03
	Parkinson disease 2, autosomal recessive juvenile	4	2,60E-03
	Parkinsonism, Experimental	4	2,60E-03
	Ramsay Hunt Paralysis Syndrome	4	2,60E-03
	Parkinsonian Disorders	4	3,20E-03
	Parkinsonism, Juvenile	4	3,20E-03
	Spinocerebellar ataxia 17	3	3,40E-03
	Parkinson's disease	6	1,90E-02
	Alzheimer's disease	6	3,50E-02
	Anterior Horn Cell Disease	2	3,90E-02
	Familial Motor Neuron Disease	2	3,90E-02
	Motor Neuron Disease	2	3,90E-02
	Motor Neuron Disease, Lower	2	3,90E-02
	Motor Neuron Disease, Secondary	2	3,90E-02
	Motor Neuron Disease, Upper	2	3,90E-02
	Encephalopathy, solvent-induced	2	4,50E-02
	Lateral Sclerosis	2	4,90E-02
inflammatory and autoimmune diseases	lymphoma; Hodgkin's disease	3	1,20E-03
	aplastic anemia, acquired	3	5,60E-03
	HIV Coinfection	5	2,00E-02
	HIV Infections	5	2,00E-02
	Diabetes Mellitus, Experimental	5	2,30E-02
	Contact Dermatitis	4	3,40E-02
	Contact hypersensitivity	4	3,40E-02
	Atrophy|Helicobacter Infections	2	3,70E-02
	Degenerative polyarthritis	6	2,30E-03
	Osteoarthrosis Deformans	6	2,30E-03
	manganism, susceptibility to occupational chronic	2	4,50E-02
Other	Acquired Immunodeficiency Syndrome	21	1,30E-05
	lung function	3	1,90E-02
	Alloxan Diabetes	5	2,30E-02
	Streptozotocin Diabetes	5	2,30E-02

	schizophrenia	10	2,70E-02
	Pulmonary Disease, Chronic Obstructive	4	2,80E-02
	Protein Deficiency	2	3,00E-02
	Balkan Nephropathy	2	3,70E-02
	Occupational Diseases|Respiratory Tract Diseases	2	3,70E-02
	Ureteral Calculi	2	3,90E-02
	bronchitis; pneumonia	2	4,50E-02
	Hodgkin's disease; non-Hodgkin's lymphoma	2	4,50E-02

Table SI.VIII. Diseases related to deregulated proteins induced by FRF exposure

Category	**Term**	**Count**	**P-value**
Cancer	Adenocarcinoma of lung (disorder)	10	2,00E-03
	Squamous cell carcinoma of esophagus	6	9,40E-03
	Animal Mammary Neoplasms	7	1,30E-02
	Mammary Carcinoma, Animal	7	1,30E-02
	Hereditary Diffuse Gastric Cancer	10	1,80E-02
	Mammary Neoplasms, Experimental	7	1,90E-02
	Stomach Neoplasms	10	2,00E-02
	Malignant neoplasm of stomach	10	2,10E-02
	Carcinoma, Spindle-Cell	7	2,40E-02
	Carcinomatosis	7	2,40E-02
	Anaplastic carcinoma	7	2,40E-02
	Undifferentiated carcinoma	7	2,40E-02
	Carcinoma	7	2,40E-02
	Liver carcinoma	13	4,30E-02
Disruption of endocrine functions	Abortion, Spontaneous	5	2,50E-02
Liver diseases or injuries	Chemically-Induced Liver Toxicity	12	2,20E-02
	Hepatitis, Toxic	12	2,20E-02
	Drug-Induced Acute Liver Injury	12	2,20E-02
	Hepatitis, Drug-Induced	12	2,20E-02
	Drug-Induced Liver Disease	12	2,20E-02
	Chemical and Drug Induced Liver Injury	12	2,20E-02
Neurological diseases	Parkinson Disease	5	2,80E-02
	cognitive trait	6	7,20E-03
	Aging/ Telomere Length	6	7,40E-03
Degenerative diseases	Degenerative polyarthritis	5	3,80E-02
	Post-Traumatic Osteoporosis	6	1,40E-03
	Osteoporosis, Age-Related	6	1,40E-03
	Osteoporosis, Senile	6	1,40E-03
	Osteoporosis	6	1,60E-03
Dermatological pathologies	Keratoderma, Palmoplantar	3	1,90E-02
	Congenital reticular ichthyosiform erythroderma	2	2,70E-02
	Erythrokeratoderma, Reticular	2	2,70E-02
	Ichthyosis, Cyclic, with Epidermolytic Hyperkeratosis	2	2,70E-02
Other	Anemia, Diamond-Blackfan	4	7,60E-03
	Osteoarthrosis Deformans	5	3,80E-02
	Acquired Immunodeficiency Syndrome|Disease Progression	37	7,10E-13

Table SI.IX. Diseases related to deregulated proteins induced by MIX exposure

Category	Term	Count	P-value
Cardiological diseases	Heart Failure, Right-Sided	4	2,10E-03
	Myocardial Failure	4	2,10E-03
	Left-Sided Heart Failure	4	2,10E-03
	Heart failure	4	2,10E-03
	Heart Decompensation	4	2,10E-03
	Congestive heart failure	4	2,10E-03
Cancer	Colorectal Neoplasms	4	2,70E-02
	Liver carcinoma	5	3,00E-02
	Colorectal Carcinoma	5	8,00E-02
	Adenocarcinoma of lung (disorder)	3	8,50E-02
	Neoplasm Metastasis	3	9,30E-02
	Mesothelioma	2	9,30E-02
Other	Acquired Immunodeficiency Syndrome	9	2,90E-04
inflammatory and autoimmune diseases	Diabetes mellitus type II, Metabolic Syndrome X|Obesity	2	6,00E-03
	Type 2 Diabetes| edema | rosiglitazone	9	6,10E-02

Références

Alarcon-Barrera, J.C., Kostidis, S., Ondo-Mendez, A., Giera, M., 2022. Recent advances in metabolomics analysis for early drug development. Drug Discovery Today 27, 1763–1773. https://doi.org/10.1016/j.drudis.2022.02.018

Alauddin, M., Salker, M.S., Umbach, A.T., Rajaxavier, J., Okumura, T., Singh, Y., Wagner, A., Brucker, S.Y., Wallwiener, D., Brosens, J.J., Lang, F., 2020. Annexin A7 Regulates Endometrial Receptivity. Front. Cell Dev. Biol. 8, 770. https://doi.org/10.3389/fcell.2020.00770

Al-Omar, I.A., Al-Ashban, R.M., Shah, A.H., 2009. Toxicity Studies on 4-Chloro-5-Sulfamoylanthranilic Acid the Degradation Product of a Loop Diuretic Furosemide. Research Journal of Pharmacology 63–77, 3.

Andreasen, F., Botker, H., Lorentzen, K., 1982. In vitro studies on the hydrolysis of frusemide in gastrointestinal juices. British Journal of Clinical Pharmacology 14, 306–309. https://doi.org/10.1111/j.1365-2125.1982.tb01984.x

Annesley, S.J., Fisher, P.R., 2019. Mitochondria in Health and Disease. Cells 8, 680. https://doi.org/10.3390/cells8070680

Baillie, T.A., 2008. Metabolism and Toxicity of Drugs. Two Decades of Progress in Industrial Drug Metabolism. Chem. Res. Toxicol. 21, 129–137. https://doi.org/10.1021/tx7002273

Balestrino, R., Schapira, A.H.V., 2020. Parkinson disease. Eur J Neurol 27, 27–42. https://doi.org/10.1111/ene.14108

Balshaw, D.M., Yamaguchi, N., Meissner, G., 2002. Modulation of Intracellular Calcium-Release Channels by Calmodulin. Journal of Membrane Biology 185, 1–8. https://doi.org/10.1007/s00232-001-0111-4

Biagini, C.P., Boissel, E., Borde, F., Bender, V.E., Bouskila, M., Blazy, F., Nicaise, L., Mignot, A., Cassio, D., Chevalier, S., 2006. Investigation of the hepatotoxicity profile of chemical entities using Liverbeads® and WIF-B9 in vitro models. Toxicology in Vitro 20, 1051–1059. https://doi.org/10.1016/j.tiv.2006.01.013

Bundgaard, H., Nørgaard, T., Nielsen, N.M., 1988. Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions. International Journal of Pharmaceutics 42, 217–224. https://doi.org/10.1016/0378-5173(88)90178-0

Caminada, D., Escher, C., Fent, K., 2006. Cytotoxicity of pharmaceuticals found in aquatic systems: Comparison of PLHC-1 and RTG-2 fish cell lines. Aquatic Toxicology 79, 114–123. https://doi.org/10.1016/j.aquatox.2006.05.010

Chai, Z., Brereton, P., Suzuki, T., Sasano, H., Obeyesekere, V., Escher, G., Saffery, R., Fuller, P., Enriquez, C., Krozowski, Z., 2003. 17β-Hydroxysteroid Dehydrogenase Type XI Localizes to Human Steroidogenic Cells. Endocrinology 144, 2084–2091. https://doi.org/10.1210/en.2002-221030

Chen, H.-F., Chuang, H.-C., Tan, T.-H., 2019. Regulation of Dual-Specificity Phosphatase (DUSP) Ubiquitination and Protein Stability. IJMS 20, 2668. https://doi.org/10.3390/ijms20112668

Chen, L.-J., Burka, L.T., 2007. Chemical and Enzymatic Oxidation of Furosemide: Formation of Pyridinium Salts. Chem. Res. Toxicol. 20, 1741–1744. https://doi.org/10.1021/tx700262z

Choi, H., Tostes, R.C., Webb, R.C., 2011. Thioredoxin Reductase Inhibition Reduces Relaxation By Increasing Oxidative Stress and S-Nitrosylation in Mouse Aorta: Journal of Cardiovascular Pharmacology 58, 522–527. https://doi.org/10.1097/FJC.0b013e31822d80a5

Christensen, A.M., Markussen, B., Baun, A., Halling-Sørensen, B., 2009. Probabilistic environmental risk characterization of pharmaceuticals in sewage treatment plant discharges. Chemosphere 77, 351–358. https://doi.org/10.1016/j.chemosphere.2009.07.018

Corbould, A.M., 1998. Expression of Types 1, 2, and 3 17 -Hydroxysteroid Dehydrogenase in Subcutaneous Abdominal and Intra-Abdominal Adipose Tissue of Women. Journal of Clinical Endocrinology & Metabolism 83, 187–194. https://doi.org/10.1210/jc.83.1.187

Esteras, N., Abramov, A.Y., 2020. Mitochondrial Calcium Deregulation in the Mechanism of Beta-Amyloid and Tau Pathology. Cells 9, 2135. https://doi.org/10.3390/cells9092135

Fautrel, A., Chesné, C., Guillouzo, A., de Sousa, G., Placidi, M., Rahmani, R., Braut, F., Pichon, J., Hoellinger, H., Vintézou, P., Diarte, I., Melcion, C., Cordier, A., Lorenzon, G., Benicourt, M., Vannier, B., Fournex, R., Peloux, A.F., Bichet, N., Gouy, D., Cano, J.P., Lounes, R., 1991. A multicentre study of acute in vitro cytotoxicity in rat liver cells. Toxicology in Vitro 5, 543–547. https://doi.org/10.1016/0887-2333(91)90090-Z

Fent, K., Escher, C., Caminada, D., 2006. Estrogenic activity of pharmaceuticals and pharmaceutical mixtures in a yeast reporter gene system. Reproductive Toxicology 22, 175–185. https://doi.org/10.1016/j.reprotox.2006.04.010

Flek, J., Sedivěc, V., 1978. The Absorption, metabolism and excretion of furfural in man. Int. Arch Occup Environ Heath 41, 159–168. https://doi.org/10.1007/BF00572888

Greaves, R.F., Wudy, S.A., Badoer, E., Zacharin, M., Hirst, J.J., Quinn, T., Walker, D.W., 2019. A tale of two steroids: The importance of the androgens DHEA and DHEAS for early neurodevelopment. The Journal of Steroid Biochemistry and Molecular Biology 188, 77–85. https://doi.org/10.1016/j.jsbmb.2018.12.007

Hammarlund-Udenaes, M., Benet, L.Z., 1989. Furosemide pharmacokinetics and pharmacodynamics in health and disease—An update. Journal of Pharmacokinetics and Biopharmaceutics 17, 1–46. https://doi.org/10.1007/BF01059086

IARC, 2016. Monographs On The Evaluation Of Carcinogenic Risks To Humans (No. 108). International Agency for Research on Cancer.

Isidori, M., Bellotta, M., Cangiano, M., Parrella, A., 2009. Estrogenic activity of pharmaceuticals in the aquatic environment. Environment International 35, 826–829. https://doi.org/10.1016/j.envint.2008.11.006

Joe, E., Ringman, J.M., 2019. Cognitive symptoms of Alzheimer's disease: clinical management and prevention. BMJ l6217. https://doi.org/10.1136/bmj.l6217

Keen, J.C., Zhou, Q., Park, B.H., Pettit, C., Mack, K.M., Blair, B., Brenner, K., Davidson, N.E., 2005. Protein Phosphatase 2A Regulates Estrogen Receptor α (ER) Expression through Modulation of ER mRNA Stability. Journal of Biological Chemistry 280, 29519–29524. https://doi.org/10.1074/jbc.M505317200

Lahet, J.J., Lenfant, F., Courderot-Masuyer, C., Ecarnot-Laubriet, E., Vergely, C., Durnet-Archeray, M.J., Freysz, M., Rochette, L., 2003. In vivo and in vitro antioxidant properties of furosemide. Life Sciences 73, 1075–1082. https://doi.org/10.1016/S0024-3205(03)00382-5

Laura, D., Anna, P., Nicola, F., Loriano, B., Rigers, B., Gianfranco, S., 2021. Stress granules in Ciona robusta: First evidences of TIA-1-related nucleolysin and tristetraprolin gene expression under metal exposure. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 243, 108977. https://doi.org/10.1016/j.cbpc.2021.108977

Laurencé, C., Rivard, M., Lachaise, I., Bensemhoun, J., Martens, T., 2011. Preparative access to transformation products (TPs) of furosemide: a versatile application of anodic oxidation. Tetrahedron 67, 9518–9521. https://doi.org/10.1016/j.tet.2011.10.006

Laurencé, C., Rivard, M., Martens, T., Morin, C., Buisson, D., Bourcier, S., Sablier, M., Oturan, M.A., 2014. Anticipating the fate and impact of organic environmental contaminants: A new approach applied to the pharmaceutical furosemide 7. http://dx.doi.org/10.1016/j.chemosphere.2014.05.036

Laurencé, C., Zeghbib, N., Rivard, M., Lehri-Boufala, S., Lachaise, I., Barau, C., Le Corvoisier, P., Martens, T., Garrigue-Antar, L., Morin, C., 2019. A new human pyridinium metabolite of furosemide, inhibitor of mitochondrial complex I, is a candidate inducer of neurodegeneration. Biochemical Pharmacology 160, 14–23. https://doi.org/10.1016/j.bcp.2018.12.007

Levstek, T., Kozjek, E., Dolžan, V., Trebušak Podkrajšek, K., 2020. Telomere Attrition in Neurodegenerative Disorders. Front. Cell. Neurosci. 14, 219. https://doi.org/10.3389/fncel.2020.00219

Lu, Y., Yuan, L., Chen, X., Zhang, A., Zhang, P., Zou, D., 2019. Systematic analysis and identification of unexpected interactions from the neuroprotein drug interactome in hydrocephalus pharmacological intervention. J. Bioinform. Comput. Biol. 17, 1950002. https://doi.org/10.1142/S0219720019500021

Marcén, R., 2009. Immunosuppressive Drugs in Kidney Transplantation: Impact on Patient Survival, and Incidence of Cardiovascular Disease, Malignancy and Infection. Drugs 69, 2227–2243. https://doi.org/10.2165/11319260-000000000-00000

Mindnich, R., Möller, G., Adamski, J., 2004. The role of 17 beta-hydroxysteroid dehydrogenases. Molecular and Cellular Endocrinology 218, 7–20. https://doi.org/10.1016/j.mce.2003.12.006

Noh, J.H., Kim, K.M., Pandey, P.R., Noren Hooten, N., Munk, R., Kundu, G., De, S., Martindale, J.L., Yang, X., Evans, M.K., Abdelmohsen, K., Gorospe, M., 2019. Loss of RNA-binding protein GRSF1 activates mTOR to elicit a proinflammatory transcriptional program. Nucleic Acids Research 47, 2472–2486. https://doi.org/10.1093/nar/gkz082

Olvera-Vargas, H., Leroy, S., Rivard, M., Oturan, N., Oturan, M., Buisson, D., 2016. Microbial biotransformation of furosemide for environmental risk assessment: identification of metabolites and toxicological evaluation. Environ Sci Pollut Res 23, 22691–22700. https://doi.org/10.1007/s11356-016-7398-2

Pathan, M., Keerthikumar, S., Ang, C.-S., Gangoda, L., Quek, C.Y.J., Williamson, N.A., Mouradov, D., Sieber, O.M., Simpson, R.J., Salim, A., Bacic, A., Hill, A.F., Stroud, D.A., Ryan, M.T., Agbinya, J.I., Mariadason, J.M., Burgess, A.W., Mathivanan, S., 2015. FunRich: An open access standalone functional enrichment and interaction network analysis tool. Proteomics 15, 2597–2601. https://doi.org/10.1002/pmic.201400515

Paul, P., Suwan, J., Liu, J., Dordick, J.S., Linhardt, R.J., 2012. Recent advances in sulfotransferase enzyme activity assays. Anal Bioanal Chem 403, 1491–1500. https://doi.org/10.1007/s00216-012-5944-4

Peterson, L.A., 2013. Reactive Metabolites in the Biotransformation of Molecules Containing a Furan Ring. Chem. Res. Toxicol. 26, 6–25. https://doi.org/10.1021/tx3003824

Reed, N.R., Kwok, E.S.C., 2014. Furfural, in: Encyclopedia of Toxicology. Elsevier, pp. 685–688. https://doi.org/10.1016/B978-0-12-386454-3.00147-0

Sandré, F., Duval, A., Grimault, L., Golven, A., Morin, C., Garrigue-Antar, L., 2023a. First approach for the ecotoxicological assessment of a ubiquitous pharmaceutical compound (furosemide) and its transformation products.

Sandre, F., Huynh, N., Caupos, E., El-Mrabet, L., Partibane, C., Lachaise, I., Pommier, C., Rivard, M., Morin, C., Moilleron, R., Le Roux, J., Garrigue-Antar, L., 2023. Occurrence and fate of an emerging drug pollutant and its by-products during conventional and advanced wastewater treatment: Case study of furosemide. Chemosphere 322, 138212. https://doi.org/10.1016/j.chemosphere.2023.138212

Sandré, F., Moilleron, R., Morin, C., Garrigue-Antar, L., 2023b. Pharmaceutical pollutants of emerging concern. Origin and fate of furosemide and its degradation products in the aquatic environment, a review.

Seshacharyulu, P., Pandey, P., Datta, K., Batra, S.K., 2013. Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer. Cancer Letters 335, 9–18. https://doi.org/10.1016/j.canlet.2013.02.036

Sherman, B.T., Hao, M., Qiu, J., Jiao, X., Baseler, M.W., Lane, H.C., Imamichi, T., Chang, W., 2022. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Research 50, W216–W221. https://doi.org/10.1093/nar/gkac194

Shimizu, A., Nakamura, y, Harada, M., Ono, T., Sato, K., Inoue, T., Kanisawa, M., 1989. Positive Foci of Glutathione S-Transferase Placental Form in the Liver of Rats Given Furfural by Oral Administration. Jpn J Cancer Res 80, 608–611.

Sommer, D., Coleman, S., Swanson, S.A., Stemmer, P.M., 2002. Differential susceptibilities of serine/threonine phosphatases to oxidative and nitrosative stressq. Archives of Biochemistry and Biophysics.

Starling, M.C.V.M., Souza, P.P., Le Person, A., Amorim, C.C., Criquet, J., 2019. Intensification of UV-C treatment to remove emerging contaminants by UV-C/H2O2 and UV-C/S2O82−: Susceptibility to photolysis and investigation of acute toxicity. Chemical Engineering Journal 376, 120856. https://doi.org/10.1016/j.cej.2019.01.135

Stokes, W., Nunn, L., 1964. A New Effective Diuretic -Lasix. British Medical Journal 2, 910–914.

Suzuki, M., Lee, H.-C., Kayasuga, Y., Chiba, S., Nedachi, T., Matsuwaki, T., Yamanouchi, K., Nishihara, M., 2009. Roles of Progranulin in Sexual Differentiation of the Developing Brain and Adult Neurogenesis. J. Reprod. Dev. 55, 351–355. https://doi.org/10.1262/jrd.20249

Traish, A.M., Kang, H.P., Saad, F., Guay, A.T., 2011. Dehydroepiandrosterone (DHEA)—A Precursor Steroid or an Active Hormone in Human Physiology (CME). The Journal of Sexual Medicine 8, 2960–2982. https://doi.org/10.1111/j.1743-6109.2011.02523.x

Weng, Y.C., Chen, C.M., Chen, Y.C., Fung, H.C., Chang, C.W., Chang, K.H., Wu, Y.R., 2016. Eukaryotic translation initiation factor 4-γ, 1 gene mutations are rare in Parkinson's disease among Taiwanese. Journal of the Formosan Medical Association 115, 728–733. https://doi.org/10.1016/j.jfma.2015.07.020

Williams, D.P., Antoine, D.J., Butler, P.J., Jones, R., Randle, L., Payne, A., Howard, M., Gardner, I., Blagg, J., Park, B.K., 2007. The Metabolism and Toxicity of Furosemide in the Wistar Rat and CD-1 Mouse: a Chemical and Biochemical Definition of the Toxicophore. J Pharmacol Exp Ther 322, 1208–1220. https://doi.org/10.1124/jpet.107.125302

Xie, F., Cui, Q., Wang, Z., Liu, B., Qiao, W., Li, N., Cheng, J., Hou, Y., Dong, X., Wang, Y., Zhang, M., 2021. ILF3 is responsible for hyperlipidemia-induced arteriosclerotic calcification by mediating BMP2 and STAT1 transcription. Journal of Molecular and Cellular Cardiology 161, 39–52. https://doi.org/10.1016/j.yjmcc.2021.07.011

Yu, G., Lu, L., Ma, Z., Wu, S., 2021. Genetically Predicted Telomere Length and Its Relationship With Alzheimer's Disease. Front. Genet. 12, 595864. https://doi.org/10.3389/fgene.2021.595864

Zhang, Z., Tang, W., 2018. Drug metabolism in drug discovery and development. Acta Pharmaceutica Sinica B 8, 721–732. https://doi.org/10.1016/j.apsb.2018.04.003

Les premiers résultats de notre étude montrent que le FUR et ses produits de dégradation (PYR et SAL) ont une toxicité proche sur les cellules humaines, tandis que le FRF est légèrement moins toxique. Le mélange de ces composés est beaucoup plus toxique que les composés individuels sur les cellules SH-SY5Y, mais pas autant sur les cellules HepG2 et HEK293. L'analyse protéomique des cellules exposées à des concentrations faibles révèle que le furosémide seul n'a qu'une faible toxicité, mais que les produits de dégradation sont potentiellement liés à des maladies neurodégénératives, à des perturbations endocriniennes, à des cancers et au stress oxydatif. En outre, cette étude souligne l'importance de prendre en compte les produits de dégradation des médicaments dans l'évaluation du risque toxicologique, car ils peuvent avoir des impacts majeurs et potentiellement plus toxiques que leurs molécules parentes. Les résultats de l'analyse protéomique sur les cellules SH-SY5Y et HEK 293 (en cours de traitement) permettront de compléter nos observations sur les Hep-G2 en révélant les effets communs mais aussi et surtout les spécificités cellulaires des impacts du FUR et ses métabolites.

Conclusion générale & perspectives

Conclusion générale & perspectives

Au cours de ce travail, nous avons dégagé plusieurs axes de réflexions autours des différents chapitres.

Dans le **Chapitre I : Le furosémide, un polluant d'intérêt émergent**, nous avons documenté plusieurs aspects du furosémide au travers d'une revue.

- C'est le diurétique le plus vendu dans le monde et il est prescrit en Europe entre 1 à 26 tonnes par an mais sa consommation est amenée à augmenter dans le futur en raison du vieillissement de la population et de l'augmentation des maladies cardiovasculaires.
- Il est présent en forte concentration dans les rejets des établissements médicalisés, les eaux usées non traitées et les boues de STEU qui représentent les principales sources dues à la consommation humaine. Il est en partie éliminé par les STEU, ce qui entraîne une concentration plus faible dans les eaux usées traitées mais il est malgré tout rejeté dans l'environnement et se retrouve dans les rivières et les sédiments. On en trouve en revanche peu dans les eaux souterraines et potables.
- Il peut être métabolisé (principalement par le foie et les reins) et dégradé par différents procédés de traitement des eaux usées et par des processus naturels (photodégradation, biodégradation, hydrolyse). Sa dégradation est donc possible dans le milieu récepteur. Environ 45 produits de transformation ont été identifiés.
- La toxicité aiguë du furosémide n'est pas très importante, avec des PNEC assez élevées pour de nombreux organismes. Cependant, il a montré des propriétés génotoxiques, cytotoxiques et hépatotoxiques sur des modèles animaux ainsi qu'une activité œstrogénique. De plus, certains produits de transformation peuvent être plus toxiques que le furosémide lui-même, tels que le pyridinium du furosémide, un hydroxykétone et un photoproduit, mais il y a peu d'informations sur la toxicité globale de ces produits.

Cette première approche nous a permis de mieux comprendre l'ampleur de la contamination par le furosémide, qui est très présent et très consommé, et de mieux cerner les informations manquantes sur lui et ses produits de dégradation. La saluamine et le pyridinium du furosemide en particulier ont été identifiés comme métabolites, produits de photodégradation, et produits d'oxydation.

Dans le **Chapitre II : Origine et devenir du furosémide et de ses produits de dégradation dans le milieu aquatique**, nous avons discuté plus en détail du devenir du furosémide et de ses produits de dégradation, au travers d'un article de recherche et d'une étude sur le bassin de la Seine-Normandie.

- La présence de PYR et SAL à des concentrations de plusieurs milliers de ng/L dans les eaux de rejets des EHPAD, mais également à des concentrations proches dans des eaux usées urbaines à été relevée pour la première fois.

- Une bonne élimination de FUR (70%), SAL (80%) et PYR (90%) a été constatée dans la STEU Seine-centre. Cependant, plusieurs centaines de ng/L de ces substances sont tout de même rejetées dans le milieu aquatique.

- L'ozonation et la chloration étaient particulièrement efficaces pour éliminer le furosémide et le pyridinium, mais ont produit de la saluamine. L'UV/H_2O_2 était bien moins efficace. Deux nouveaux sous-produits d'oxydation sont également apparus.

- La contamination du bassin versant Seine-Normandie par le FUR est assez diffuse avec moins de 20% des sites échantillonnés présentant une concentration supérieure à 20 ng/L. Cependant, des concentrations de plus de 100 ng/L ont été relevées à proximité de sources ponctuelles liées à l'activité humaine (présence de STEU à proximité des EHPAD, hôpitaux, etc.). Le FUR contribue de manière non négligeable au flux de polluants dans la Seine au niveau de la station de Poissy (650 kg/an).

- D'après les données extraites de la base de données OPENMEDIC, environ 30 tonnes de FUR ont été prescrites en France en 2019, 2020 et 2021, ce qui est relativement important par rapport aux autres pays Européens.

- Des concentrations importantes de FUR et de SAL ont été mesurées dans les urines de festivaliers, probablement liées à la consommation de drogues récréatives, ce qui représente une nouvelle source de contamination jusqu'alors non prise en compte.

Ce chapitre nous a permis de mieux comprendre l'origine des produits de dégradation du furosémide, leurs concentrations environnementales, et de dresser un portrait "chimique" plus complet du furosémide. Nous avons également eu un aperçu de la contamination des rivières françaises, en particulier sur le bassin versant Seine-Normandie.

Le **Chapitre III : Evaluation de l'écotoxicité par une approche multi-modèle,** visait à mieux caractériser la toxicité de FUR, PYR et SAL pour lesquels peu d'informations sont disponibles et d'estimer le risque que représentent les trois composés pour le milieu récepteur.

- La toxicité aiguë (tests d'immobilisation sur *D. magna* et *A. salina)* de PYR est du même ordre de grandeur que celle de FUR tandis que la SAL a montré une toxicité deux fois plus importante sur les deux modèles. Il a été constaté que le FUR peut traverser le chorion des œufs de poissons zèbre engendrant ainsi une exposition dès les stades très précoces du développement.

- Les concentrations dans la Seine en amont et en aval de l'agglomération parisienne ont été mesurées et sont comprises entre 30 et 250 ng/L, donnant un ordre de grandeur pour les concentrations dites "environnementales".

- Les trois composés ont provoqué des effets délétères sur la mobilité, la fréquence cardiaque et le stress oxydant de *D. rerio* et *D. magna* et sur la mobilité, la vitesse de croissance, la mobilité et la taille de *C. reinhardtii*.

- Les concentrations sans effet prédites (PNEC) pour les 3 composés sont estimées à 0,5 ng/L. Ces valeurs sont très basses car elles sont basées sur des tests sensibles au ng/L. Les Quotients de Risques (RQ) calculés sont systématiquement supérieur à 1 pour les trois composés indiquant un risque important pour l'environnement. Des scores de toxicité basés sur le pourcentage et les concentrations d'effets ont montré une toxicité plus importante de SAL, puis de PYR et enfin de FUR.

- Le mélange des 3 composés présente des effets synergiques à fortes concentrations mais antagonistes à concentrations environnementales. Ces expériences ont souligné le caractère imprévisible des mélanges et montre la nécessité de développer des nouveaux outils pour leur étude.

- La daphnie s'est révélée être un modèle particulièrement intéressant car elle est sensible, les expériences sont répétables et elle est facile à maintenir en laboratoire. Les tests de comportement ont montré des résultats dès les plus basses concentrations testées (50 ng/L) pour les trois composés.

- L'importance de l'approche multi-modèle pour l'évaluation du risque environnemental a bien été confirmée car elle prend en compte la sensibilité de plusieurs organismes appartements à différents niveaux trophiques et occupant différentes fonctions pour l'écosystème. Les fonctions écosystémiques des organismes étudiées (régulation de la chaîne trophique, recyclage de l'eau, production de nourriture, stockage de carbone, ...) peuvent être altérées par FUR, PYR et SAL.

Dans ce chapitre, nous avons montré des effets délétères des trois composés testés à des concentrations environnementales sur différents organismes et nous avons notamment relevé une toxicité plus importante du pyridinium du furosémide, mais surtout de la saluamine sur plusieurs paramètres suivis.

Finalement, dans le **Chapitre IV : Impact sur les cellules humaines**, nous avons mené une première approche pour étudier la toxicité de ces métabolites humains sur les cellules humaines.

- La toxicité aiguë de FUR, PYR, SAL, FRF et leur mélange a été déterminée sur des lignées cellulaires humaines de foie, de rein et de neuroblastome. L'EC50 de FUR est assez proche de celle de ses sous produits excepté le furfural qui montre une toxicité moins importante. Le mélange des trois composés est cinq fois plus toxique que les composés seuls sur les cellules de neuroblastome.

- Lors de l'étude protéomique, un nombre plus important de protéines dérégulées a été identifié pour la lignée cellulaire de foie et en particulier pour les produits de dégradation. Ces protéines sont impliquées dans la perturbation endocrinienne, les maladies neurodégénératives et le cancer. Moins de protéines dérégulées ont été relevées après l'exposition au mélange mais ces protéines sont liées à des pathologies cardiaques.

Ce dernier chapitre nous a permis de suspecter certaines cibles d'action des composés et de leur mélange au travers de l'expression de protéines dérégulées.

Cette thèse met en avant la nécessité d'adopter une approche plus sensible et multi-modèles pour mieux comprendre l'impact des polluants pharmaceutiques sur l'environnement et de développer des stratégies de traitement plus efficaces et durables pour limiter leur présence dans les eaux usées et les rejets industriels. Nous avons également contribué à caractériser des polluants encore peu étudiés. Les résultats fournis lors des différents chapitres nous permettent de répondre à la problématique initiale :

Est-ce le furosémide et ses produits de dégradation présentent un risque pour l'écosystème aquatique, à des concentrations pertinentes dans l'environnement ?

En plus des rares études dans la littérature scientifique montrant la génotoxicité et/ou la cytotoxicité du **furosémide** sur des organismes aquatiques (Rocco et al. 2010 et Isidori et al. 2006), nous ajoutons un impact sur le cœur et sur le comportement de la daphnie et du poisson zèbre ainsi que sur la croissance et la mobilité de chlamydomonas, aux concentrations trouvées dans l'environnement. La PNEC (très basse) et le Quotient de Risque (supérieur à 1) obtenus dans cette étude confirment également le risque important que représente le furosémide sur l'environnement aquatique.

Le **pyridinium du furosémide** a montré des effets similaires. De plus, les précédents travaux réalisés sur ce composé avaient montré le développement de marqueurs précoces caractéristiques de maladies neurodégénératives ainsi qu'une inhibition de la chaîne respiratoire mitochondriales (Laurencé et al. 2019). Ces résultats pourraient être en lien avec l'effet observé sur le comportement mais à concentrations environnementales cette fois ce qui pourrait être largement problématique pour les organismes aquatiques.

Enfin, la **saluamine** est classée comme le composé le plus toxique dans cette thèse avec une EC50 deux fois plus basse que le furosémide sur les tests de toxicité aiguë sur les crustacés et une toxicité plus marquée sur plusieurs paramètres en concentrations environnementales. De plus, à forte concentration, les mélanges présentant les effets synergiques les plus importants étaient ceux contenant de la saluamine. La saluamine s'est également montrée persistante; d'une part, elle n'est pas complètement dégradée par les STEU, mais en plus elle peut être produite par la dégradation du furosémide et du pyridinium du furosémide (à la fois dans les STEU, mais aussi dans l'environnement par photodégradation ou bioconversion), ce qui en fait le composé le plus probable dans l'environnement parmi ceux étudiés. Elle pourrait également être accumulée dans les sédiments au vu de son coefficient de partage important (kd) dans la phase particulaire des échantillons d'EHPAD mais une étude sur les sédiments serait nécessaire pour confirmer cette hypothèse.

Il semblerait que le furosémide et ses sous-produits, et en particulier la saluamine, soient en effet problématiques.

Notre étude présente quelques limites, notamment le faible nombre d'échantillons environnementaux analysés, ce qui peut entraîner une approximation importante des concentrations environnementales. Réaliser davantage d'analyses pourrait cependant facilement résoudre ce problème. De plus, les modèles d'étude choisis ne sont pas nécessairement les plus représentatifs de l'environnement étudié. Des études

sur des organismes tels que le gammare, plus cohérent dans les rivières, ou des espèces de poissons présents dans la Seine seraient intéressants pour rapprocher les conditions expérimentales des conditions réelles. Enfin, des études mécanistiques seraient également nécessaires pour comprendre les mécanismes de toxicité impliqués dans les réponses observées, pour lesquels nous avons pu émettre des hypothèses, notamment à l'aide d'approches telles que les AOP. Enfin, nous avons identifié ces composés comme étant problématiques, il serait maintenant intéressant d'engager la réflexion sur les solutions potentielles pour maîtriser le transfert de ce type de polluants dans les milieux aquatiques.

Au niveau réglementaire, nous avions précédemment discuté de certaines mesures mises en place concernant les composés pharmaceutiques. On retrouve notamment le plan national sur les micropolluants qui vise à réduire les émissions (à la source) de toutes les molécules susceptibles de polluer les ressources en eau en accord avec les objectifs fixés par la DCE. Il est cependant difficilement applicable pour les composés pharmaceutiques, dû à leur fonction thérapeutique indispensable pour la santé humaine. Nous avions également parlé de l'autorisation de mise sur le marché, obligatoire pour tous les médicaments, et comprenant depuis 2005, une évaluation du Risque Environnemental également basé sur des Quotients de Risque. Cependant, cette évaluation n'est pas prise en compte lors de la commercialisation de la molécule et contraint seulement à préciser les conditions de stockage et d'élimination des composés. De plus, les évaluations ne prennent pas en compte les produits de dégradation. La **prise en compte de ces évaluations** pourrait déjà représenter un pas vers des pratiques plus respectueuses de l'environnement. Il serait également intéressant de **suivre l'évolution de ces molécules dans les milieux récepteurs** en les **incluant aux listes de polluants suivis** sur les bassins versants. Le furosémide est par exemple déjà suivi sur le bassin Rhône-Méditerranée, il pourrait être justifié d'inclure la saluamine.

Avec une méthodologie assez proche de notre étude, le projet européen REMPHARMAWATER (2000-2003) avait pour objectif de mieux comprendre l'impact de certains résidus de médicaments dans l'eau (ibuprofène, naproxène, diclofénac, propranolol, aténolol, fluoxétine, sertraline, carbamazépine, gabapentine, losartan, valsartan, sulfaméthoxazole, triméthoprime, éthinylestradiol, estrone, levonorgestrel, iopromide, iomeprol) sur la santé humaine et l'environnement. Ses auteurs ont mis en évidence la présence de ces médicaments, ainsi que de leurs métabolites, dans les eaux usées et dans les eaux de surface. Ils ont montré que ces composés peuvent affecter le développement et le comportement des organismes aquatiques, ainsi que leurs systèmes endocrinien et immunitaire et que ces substances pouvaient persister dans l'environnement. Ils en avaient conclu que les effluents de station de traitement des eaux usées doivent être mieux traités avant d'être rejetés dans l'environnement pour réduire leur impact environnemental. Depuis la fin du projet en 2003, il y a eu quelques améliorations dans le traitement des eaux usées:

- En 2006, la directive européenne sur les eaux résiduaires urbaines[37] a été modifiée, obligeant les États membres à collecter et traiter les eaux usées urbaines de manière adéquate en exigeant la réduction des substances dangereuses (certains métaux lourds, composés organiques toxiques, pesticides et détergents).

[37] Circulaire du 8 décembre 2006 relative à la mise en conformité de la collecte et du traitement des eaux usées des communes en application de la directive n° 91/271/CEE du 21 mai 1991

- En 2008, la Commission européenne a publié une recommandation sur la surveillance et le contrôle des substances dangereuses dans les eaux[38]. Cette recommandation a conduit à l'adoption de normes de qualité environnementale pour les eaux de surface en Europe, qui incluent des critères de qualité pour certains polluants.

Cependant, 20 ans plus tard, nous constatons que les eaux usées ne sont toujours pas suffisamment traitées et que les composés pharmaceutiques ne sont pas pris en compte.

En revanche, les procédés de traitement des eaux usées dans les STEU ont connu des améliorations significatives au cours des dernières années, notamment grâce à l'utilisation de procédés de traitement biologique plus efficaces pour dégrader les matières organiques, la mise en œuvre de systèmes de traitement des eaux usées à faible consommation d'énergie et la mise en place de systèmes de surveillance en temps réel pour contrôler la qualité de l'eau traitée et ajuster les processus de traitement (Zejda et al. 2020, Ching et al. 2021, Cardoso et al. 2021). De plus, des méthodes telles que la MBR (membrane bioreactor), l'ozonation ou l'oxydation par l'acide performique, qui sont généralement utilisées pour la désinfection, ont émergé dans le but d'éliminer les micropolluants (Kosek et al. 2020, Programme OPUR[39]). Cependant, bien que de nouveaux procédés soient intéressants à tester, il est important d'identifier et d'évaluer les sous-produits qu'ils engendrent. De ce fait, certains processus pourraient être plus avantageux car ils retiennent les composés au lieu de les dégrader. L'ultrafiltration par exemple est un procédé efficace sur les micropolluants, mais son coût et son entretien pour le remplacement et le nettoyage des membranes peuvent être élevés (Ajo et al. 2018, Kosec et al. 2020). Le charbon actif est également pertinent car il est capable d'adsorber les molécules, mais il faut recycler le charbon une fois saturé (Guillossou, 2019). D'autres pistes peuvent également être intéressantes pour les nouveaux médicaments. Le projet EDIFIS, par exemple, vise à développer une méthode pour rendre les médicaments plus respectueux de l'environnement. Pour cela, ses auteurs cherchent à créer des médicaments qui se désintègrent facilement dans la nature une fois qu'ils ont été éliminés. Cette meilleure biodégradabilité est recherchée en modifiant la structure des médicaments. Le défi ici est de maintenir leur efficacité thérapeutique.

A l'issue de cette thèse, plusieurs questions se posent sur nos trois composés : Quelle est la fraction disponible pour les organismes aquatiques ? Est-ce qu'ils peuvent se bioaccumuler ? Est-ce qu'ils auraient des propriétés de perturbateurs endocriniens comme cela a été montré *in vitro* pour le furosémide ? (Isidori et al. 2009, Fent et al. 2006). A quelle vitesse se dégrade la saluamine en conditions environnementales? Il serait pertinent de confirmer l'affinité de la saluamine avec les sédiments et si c'est le cas d'étudier l'impact sur des organismes vivants sur/dans ces sédiments. Il serait également intéressant d'étudier la stabilité, puis la toxicité des autres sous-produits du furosémide, identifiés dans le premier chapitre. Cependant les standards ne sont pas disponibles sur le marché, comme pour le pyridinium du furosémide, il faut réussir à les synthétiser. L'analyse dirigée par l'effet (EDA) serait un outil intéressant pour étudier la toxicité des produits de dégradation d'une molécule, car elle permettrait d'identifier les composants responsables des effets toxiques observés dans des échantillons environnementaux complexes. Nous pourrions aussi limiter l'expérimentation animale pour étudier ces composés en

[38] Directive 2013/39/UE du parlement européen et du conseil du 12 août 2013 modifiant les directives 2000/60/CE et 2008/105/CE en ce qui concerne les substances prioritaires pour la politique dans le domaine de l'eau
[39] https://www.leesu.fr/opur/

employant des approches *in silico*. Les QSAR (Quantitative Structure-Activity Relationships) par exemple, sont des modèles mathématiques utilisés pour prédire l'activité biologique d'une molécule, sa toxicité, sa solubilité, son coefficient de partage octanol/eau et de nombreux autres paramètres. Ces modèles se sont montrés efficaces sur des familles de composés (Gramatica, 2020).

Enfin, nous avons montré au travers de notre étude protéomique, un impact sur les cellules humaines. De nombreuses protéines dérégulées sont impliquées dans des maladies neurodégénératives. Nos premiers résultats indiquent clairement qu'il est nécessaire d'étudier le risque des produits de dégradation du furosémide en exposition chronique sur l'Homme. Ce travail de thèse s'insère dans une stratégie complète d'évaluation du furosémide et de ses métabolites, comportant également l'étude de leur pharmacocinétique urinaire chez une cohorte de patients traités par ce médicament. De plus, en absence d'information sur la bioaccumulation du pyridinium du furosémide dans le corps humain, il est important de déterminer si une exposition chronique au furosémide peut augmenter le risque de maladies d'Alzheimer et de Parkinson, par une étude épidémiologique. Ce modèle furosémide/métabolites, qui combine des approches en santé environnementale et humaine, permet de prendre en compte l'ensemble des systèmes biologiques impactés par l'utilisation de ce médicament. Cette approche systématique "OneHealth" de l'étude d'un médicament de sa consommation par le patient jusqu'à l'environnement, via les réseaux d'assainissement, forme la base d'une stratégie intégrée transférable à d'autres polluants émergents.

Bibliographie

Bibliographie

Abe, R., Toyota, K., Miyakawa, H., Watanabe, H., Oka, T., Miyagawa, S., Nishide, H., Uchiyama, I., Tollefsen, K.E., Iguchi, T., Tatarazako, N., 2015. Diofenolan induces male offspring production through binding to the juvenile hormone receptor in Daphnia magna. Aquatic Toxicology 159, 44–51. https://doi.org/10.1016/j.aquatox.2014.11.015

AESN, 2022. Programme de mesures - SDAGE 2022-2027. Agence de l'Eau Seine-Normandie.

Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM), 2013. Analyse des ventes de médicaments en France en 2013. 36.

Ahmed, N.J., 2020. The Outpatient Use of Furosemide in a Public Hospital. JPRI 79–83. https://doi.org/10.9734/jpri/2020/v32i730463

Ajo, P., Preis, S., Vornamo, T., Mänttäri, M., Kallioinen, M., Louhi-Kultanen, M., 2018. Hospital wastewater treatment with pilot-scale pulsed corona discharge for removal of pharmaceutical residues. Journal of Environmental Chemical Engineering 6, 1569–1577. https://doi.org/10.1016/j.jece.2018.02.007

Al-Omar, I.A., Al-Ashban, R.M., Shah, A.H., 2009. Toxicity Studies on 4-Chloro-5-Sulfamoylanthranilic Acid the Degradation Product of a Loop Diuretic Furosemide. Research Journal of Pharmacology 63–77, 3.

Andreasen, F., Botker, H., Lorentzen, K., 1982. In vitro studies on the hydrolysis of frusemide in gastrointestinal juices. British Journal of Clinical Pharmacology 14, 306–309. https://doi.org/10.1111/j.1365-2125.1982.tb01984.x

Antoine, D.J., Williams, D.P., Regan, S.L., Park, B.K., 2007. Formation of cytotoxic protein reactive metabolites from furosemide: Biological consequences of drug metabolism. Toxicology 240, 157. https://doi.org/10.1016/j.tox.2007.06.079

Beffagna, G., 2019. Zebrafish as a Smart Model to Understand Regeneration After Heart Injury: How Fish Could Help Humans. Front. Cardiovasc. Med. 6, 107. https://doi.org/10.3389/fcvm.2019.00107

Belanger, S.E., Balon, E.K., Rawlings, J.M., 2010. Saltatory ontogeny of fishes and sensitive early life stages for ecotoxicology tests. Aquatic Toxicology 97, 88–95. https://doi.org/10.1016/j.aquatox.2009.11.020

Beyers, H., Malan, S.F., van der Watt, J.G., de Villiers, M.M., 2000. Structure-Solubility Relationship and Thermal Decomposition of Furosemide. Drug Development and Industrial Pharmacy 26, 1077–1083. https://doi.org/10.1081/DDC-100100271

Bindschedler, M., Degen, P., Flesch, G., de Gasparo, M., Preiswerk, G., 1997. Pharmacokinetic and pharmacodynamic interaction of single oral doses of valsartan and furosemide. European Journal of Clinical Pharmacology 52, 371–378. https://doi.org/10.1007/s002280050303

Bora, F., Yılmaz, F., Bora, T., 2016. Ecstasy (MDMA) and its effects on kidneys and their treatment: a review. Iran J Basic Med Sci 19.

Brennecke, A., Villar, L., Wang, Z., Doyle, L.M., Meek, A., Reed, M., Barden, C., Weaver, D.F., 2020. Is Inhaled Furosemide a Potential Therapeutic for COVID-19? The American Journal of the Medical Sciences 360, 216–221. https://doi.org/10.1016/j.amjms.2020.05.044

Bundgaard, H., Nørgaard, T., Nielsen, N.M., 1988. Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions. International Journal of Pharmaceutics 42, 217–224. https://doi.org/10.1016/0378-5173(88)90178-0

Cardoso, B.J., Rodrigues, E., Gaspar, A.R., Gomes, Á., 2021. Energy performance factors in wastewater treatment plants: A review. Journal of Cleaner Production 322, 129107. https://doi.org/10.1016/j.jclepro.2021.129107

Chagnon, M., Kreutzweiser, D., Mitchell, E.A.D., Morrissey, C.A., Noome, D.A., Van der Sluijs, J.P., 2015. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environ Sci Pollut Res 22, 119–134. https://doi.org/10.1007/s11356-014-3277-x

Ching, P.M.L., So, R.H.Y., Morck, T., 2021. Advances in soft sensors for wastewater treatment plants: A systematic review. Journal of Water Process Engineering 44, 102367. https://doi.org/10.1016/j.jwpe.2021.102367

Chiffre, A., Degiorgi, F., Buleté, A., Spinner, L., Badot, P.-M., 2016. Occurrence of pharmaceuticals in WWTP effluents and their impact in a karstic rural catchment of Eastern France. Environ Sci Pollut Res 23, 25427–25441. https://doi.org/10.1007/s11356-016-7751-5

Cizmas, L., Sharma, V.K., Gray, C.M., McDonald, T.J., 2015. Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. Environ Chem Lett 13, 381–394. https://doi.org/10.1007/s10311-015-0524-4

De Carpentier, F., Lemaire, S.D., Danon, A., 2019. When Unity Is Strength: The Strategies Used by Chlamydomonas to Survive Environmental Stresses. Cells 8, 1307. https://doi.org/10.3390/cells8111307

Delli Compagni, R., Gabrielli, M., Polesel, F., Turolla, A., Trapp, S., Vezzaro, L., Antonelli, M., 2020. Risk assessment of contaminants of emerging concern in the context of wastewater reuse for irrigation: An integrated modelling approach. Chemosphere 242, 125185. https://doi.org/10.1016/j.chemosphere.2019.125185

Ebert, D., 2022. Daphnia as a versatile model system in ecology and evolution. EvoDevo 13, 16. https://doi.org/10.1186/s13227-022-00199-0

Fabien, E., Sabine, H., Florent, L., Tristan, M., Marjolaine, D., Christine, A., Florent, B., Joël, A., Agrocapi, 2022. Projet Agrocapi – Étude de filières de valorisation agricole d'urinofertilisants p55.

Flahou, T., 2022. Développement d'une méthode d'analyse des pharmaceutiques dans les urines destinées à l'épandage de cultures.

Godoy, A.A., Kummrow, F., Pamplin, P.A.Z., 2015. Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment - A review. Chemosphere 138, 281–291. https://doi.org/10.1016/j.chemosphere.2015.06.024

Gramatica, P., 2020. Principles of QSAR Modeling: Comments and Suggestions From Personal Experience. International Journal of Quantitative Structure-Property Relationships 5, 61–97. https://doi.org/10.4018/IJQSPR.20200701.oa1

Gros, M., Ahrens, L., Levén, L., Koch, A., Dalahmeh, S., Ljung, E., Lundin, G., Jönsson, H., Eveborn, D., Wiberg, K., 2020. Pharmaceuticals in source separated sanitation systems: Fecal sludge and blackwater treatment. Science of The Total Environment 703, 135530. https://doi.org/10.1016/j.scitotenv.2019.135530

Grosse, Y., Loomis, D., Guyton, K.Z., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Mattock, H., Straif, K., 2017. Some chemicals that cause tumours of the urinary tract in rodents. The Lancet Oncology 18, 1003–1004. https://doi.org/10.1016/S1470-2045(17)30505-3

Guillossou, R., 2019. Élimination des micropolluants organiques dans les eaux résiduaires urbaines par adsorption sur charbon actif : compréhension des processus et implications opérationnelles (These de doctorat). Paris Est.

Hezari, M., Davis, P.J., 1992. N-dealkylation of Furosemide to yield the mammalian metabolite CSA using Cunninghamella elegans. Drug Metabolism and Disposition 20, 92.

Holt, A.R., Alix, A., Thompson, A., Maltby, L., 2016. Food production, ecosystem services and biodiversity: We can't have it all everywhere. Science of The Total Environment 573, 1422–1429. https://doi.org/10.1016/j.scitotenv.2016.07.139

Houdou, G., 2004. Analyse et usage des substances psychotropes en milieu festif : intérêt du suivi des consommations. Université de CAEN.

Huang, X., Dorhout Mees, E., Vos, P., Hamza, S., Braam, B., 2016. Everything we always wanted to know about furosemide but were afraid to ask. American Journal of Physiology-Renal Physiology 310, F958–F971. https://doi.org/10.1152/ajprenal.00476.2015

Isidori, M., Bellotta, M., Cangiano, M., Parrella, A., 2009. Estrogenic activity of pharmaceuticals in the aquatic environment. Environment International 35, 826–829. https://doi.org/10.1016/j.envint.2008.11.006

Isidori, M., Nardelli, A., Parrella, A., Pascarella, L., Previtera, L., 2006. A multispecies study to assess the toxic and genotoxic effect of pharmaceuticals: Furosemide and its photoproduct. Chemosphere 63, 785–793. https://doi.org/10.1016/j.chemosphere.2005.07.078

ISO 6341, 2012. Détermination de l'inhibition de la mobilité de Daphnia magna Straus (Cladocera, Crustacea) - Essai de toxicité aiguë.

ISO 14669, 1999. Détermination de la toxicité létale aiguë vis-à-vis de copépodes marins (Copepoda, Crustacea) - Essai de toxicité aiguë.

Joachim, S., Beaudouin, R., Daniele, G., Geffard, A., Bado-Nilles, A., Tebby, C., Palluel, O., Dedourge-Geffard, O., Fieu, M., Bonnard, M., Palos-Ladeiro, M., Turiès, C., Vulliet, E., David, V., Baudoin, P., James, A., Andres, S., Porcher, J.M., 2021. Effects of diclofenac on sentinel species and aquatic communities in semi-natural conditions. Ecotoxicology and Environmental Safety 211, 111812. https://doi.org/10.1016/j.ecoenv.2020.111812

Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., Craddock, C., Kyzar, E.J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., Gilman, C.P., Pittman, J., Rosemberg, D.B., Gerlai, R., Echevarria, D., Lamb, E., Neuhauss, S.C.F., Weng, W., Bally-Cuif, L., Schneider, and the Zebrafish Neuros, H., 2013. Towards a Comprehensive Catalog of Zebrafish Behavior 1.0 and Beyond. Zebrafish 10, 70–86. https://doi.org/10.1089/zeb.2012.0861

Kattel, G.R., 2022. Climate warming in the Himalayas threatens biodiversity, ecosystem functioning and ecosystem services in the 21st century: is there a better solution? Biodivers Conserv 31, 2017–2044. https://doi.org/10.1007/s10531-022-02417-6

Kevorkian, J.-P., Riveline, J.-P., Vandiedonck, C., Girard, D., Galland, J., Féron, F., Gautier, J.-F., Mégarbane, B., 2021. Early short-course corticosteroids and furosemide combination to treat non-critically ill COVID-19 patients: An observational cohort study. Journal of Infection 82, e22–e24. https://doi.org/10.1016/j.jinf.2020.08.045

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of embryonic development of the zebrafish. Dev. Dyn. 203, 253–310. https://doi.org/10.1002/aja.1002030302

Kumar, P. (Ed.), 2012. The Economics of Ecosystems and Biodiversity: Ecological and Economic Foundations. Routledge, London. https://doi.org/10.4324/9781849775489

Laurencé, C., Rivard, M., Lachaise, I., Bensemhoun, J., Martens, T., 2011. Preparative access to transformation products (TPs) of furosemide: a versatile application of anodic oxidation. Tetrahedron 67, 9518–9521. https://doi.org/10.1016/j.tet.2011.10.006

Laurencé, C., Rivard, M., Martens, T., Morin, C., Buisson, D., Bourcier, S., Sablier, M., Oturan, M.A., 2014. Anticipating the fate and impact of organic environmental contaminants: A new approach applied to the pharmaceutical furosemide 7. http://dx.doi.org/10.1016/j.chemosphere.2014.05.036

Laurencé, C., Zeghbib, N., Rivard, M., Lehri-Boufala, S., Lachaise, I., Barau, C., Le Corvoisier, P., Martens, T., Garrigue-Antar, L., Morin, C., 2019. A new human pyridinium metabolite of furosemide, inhibitor of mitochondrial complex I, is a candidate inducer of neurodegeneration. Biochemical Pharmacology 160, 14–23. https://doi.org/10.1016/j.bcp.2018.12.007

Lee, B.-Y., Choi, B.-S., Kim, M.-S., Park, J.C., Jeong, C.-B., Han, J., Lee, J.-S., 2019. The genome of the freshwater water flea Daphnia magna: A potential use for freshwater molecular ecotoxicology. Aquatic Toxicology 210, 69–84. https://doi.org/10.1016/j.aquatox.2019.02.009

Leenhardt, S., Mamy, L., Pesce, S., Sanchez, W., 2023. Impacts des produits phytopharmaceutiques sur la biodiversité et les services écosystémiques. éditions Quae. https://doi.org/10.35690/978-2-7592-3657-2

Lemaire, S.D., Hours, M., Gerard-Hirne, C., Trouabal, A., Roche, O., Jacquot, J.-P., 1999. Analysis of light/dark synchronization of cell-wall-less Chlamydomonas reinhardtii (Chlorophyta) cells by flow cytometry. European Journal of Phycology 34, 279–286. https://doi.org/10.1080/09670269910001736332

Lévêque, C., Paugy, D., 2006. Les poissons des eaux continentales africaines : diversité, écologie, utilisation par l'homme.

Liquete, C., Piroddi, C., Drakou, E.G., Gurney, L., Katsanevakis, S., Charef, A., Egoh, B., 2013. Current Status and Future Prospects for the Assessment of Marine and Coastal Ecosystem Services: A Systematic Review. PLoS ONE 8, e67737. https://doi.org/10.1371/journal.pone.0067737

Magyary, I., 2018. Recent advances and futur trends in zebrafish bioassays for aquatic ecotoxicology. Ecocycles 4, 12–18. https://doi.org/10.19040/ecocycles.v4i2.108

Mandalika, A., Qin, L., Sato, T.K., Runge, T., 2014. Integrated biorefinery model based on production of furans using open-ended high yield processes. Green Chem. 16, 2480–2489. https://doi.org/10.1039/C3GC42424C

Marchand-Pilard, M., 2022. Propositions d'évolutions de l'encadrement juridique des rejets de médicaments humains dans l'eau. Université Paris 8 Vincennes-Saint-Denis.

Marden, B., Brown, P., Bosteels, T., 2020. Great Salt Lake Artemia: Ecosystem Functions and Services with a Global Reach, in: Baxter, B.K., Butler, J.K. (Eds.), Great Salt Lake Biology: A Terminal Lake in

a Time of Change. Springer International Publishing, Cham, pp. 175–237. https://doi.org/10.1007/978-3-030-40352-2_7

Martínez, M.L., Intralawan, A., Vázquez, G., Pérez-Maqueo, O., Sutton, P., Landgrave, R., 2007. The coasts of our world: Ecological, economic and social importance. Ecological Economics 63, 254–272. https://doi.org/10.1016/j.ecolecon.2006.10.022

Merchant, S.S., Prochnik, S.E., Vallon, O., Harris, E.H., Karpowicz, S.J., Witman, G.B., Terry, A., Salamov, A., Fritz-Laylin, L.K., Maréchal-Drouard, L., Marshall, W.F., Qu, L.-H., Nelson, D.R., Sanderfoot, A.A., Spalding, M.H., Kapitonov, V.V., Ren, Q., Ferris, P., Lindquist, E., Shapiro, H., Lucas, S.M., Grimwood, J., Schmutz, J., Cardol, P., Cerutti, H., Chanfreau, G., Chen, C.-L., Cognat, V., Croft, M.T., Dent, R., Dutcher, S., Fernández, E., Fukuzawa, H., González-Ballester, D., González-Halphen, D., Hallmann, A., Hanikenne, M., Hippler, M., Inwood, W., Jabbari, K., Kalanon, M., Kuras, R., Lefebvre, P.A., Lemaire, S.D., Lobanov, A.V., Lohr, M., Manuell, A., Meier, I., Mets, L., Mittag, M., Mittelmeier, T., Moroney, J.V., Moseley, J., Napoli, C., Nedelcu, A.M., Niyogi, K., Novoselov, S.V., Paulsen, I.T., Pazour, G., Purton, S., Ral, J.-P., Riaño-Pachón, D.M., Riekhof, W., Rymarquis, L., Schroda, M., Stern, D., Umen, J., Willows, R., Wilson, N., Zimmer, S.L., Allmer, J., Balk, J., Bisova, K., Chen, C.-J., Elias, M., Gendler, K., Hauser, C., Lamb, M.R., Ledford, H., Long, J.C., Minagawa, J., Page, M.D., Pan, J., Pootakham, W., Roje, S., Rose, A., Stahlberg, E., Terauchi, A.M., Yang, P., Ball, S., Bowler, C., Dieckmann, C.L., Gladyshev, V.N., Green, P., Jorgensen, R., Mayfield, S., Mueller-Roeber, B., Rajamani, S., Sayre, R.T., Brokstein, P., Dubchak, I., Goodstein, D., Hornick, L., Huang, Y.W., Jhaveri, J., Luo, Y., Martínez, D., Ngau, W.C.A., Otillar, B., Poliakov, A., Porter, A., Szajkowski, L., Werner, G., Zhou, K., Grigoriev, I.V., Rokhsar, D.S., Grossman, A.R., 2007. The *Chlamydomonas* Genome Reveals the Evolution of Key Animal and Plant Functions. Science 318, 245–250. https://doi.org/10.1126/science.1143609

Míguez, L., Esperanza, M., Seoane, M., Cid, Á., 2021. Assessment of cytotoxicity biomarkers on the microalga Chlamydomonas reinhardtii exposed to emerging and priority pollutants. Ecotoxicology and Environmental Safety 208, 111646. https://doi.org/10.1016/j.ecoenv.2020.111646

Mittmann, B., Ungerer, P., Klann, M., Stollewerk, A., Wolff, C., 2014. Development and staging of the water flea Daphnia magna (Straus, 1820; Cladocera, Daphniidae) based on morphological landmarks. EvoDevo 5, 12. https://doi.org/10.1186/2041-9139-5-12

Nebot, C., Falcon, R., Boyd, K.G., Gibb, S.W., 2015. Introduction of human pharmaceuticals from wastewater treatment plants into the aquatic environment: a rural perspective. Environ Sci Pollut Res 22, 10559–10568. https://doi.org/10.1007/s11356-015-4234-z

Newman, M., Ebrahimie, E., Lardelli, M., 2014. Using the zebrafish model for Alzheimer's disease research. Front. Genet. 5. https://doi.org/10.3389/fgene.2014.00189

Obreshkove, V., Fraser, A.W., 1940. Growth and differentiation of daphnia magna eggs in vitro. The Biological Bulletin 78, 428–436. https://doi.org/10.2307/1537664

OCDE, 2013. Ligne directrice n° 236 : Poisson, essai de toxicité aiguë au stade embryonnaire.

OCDE, 2012. Ligne Directrice n° 211 : Daphnia magna, essai de reproduction.

OCDE, 2004. Ligne directrice n° 202 : Daphnia magna, test d'immobilisation aigue.

Olvera-Vargas, H., Leroy, S., Rivard, M., Oturan, N., Oturan, M., Buisson, D., 2016. Microbial biotransformation of furosemide for environmental risk assessment: identification of metabolites and toxicological evaluation. Environ Sci Pollut Res 23, 22691–22700. https://doi.org/10.1007/s11356-016-7398-2

Paijens, C., Bressy, A., Frère, B., Moilleron, R., 2020. Biocide emissions from building materials during wet weather: identification of substances, mechanism of release and transfer to the aquatic

environment. Environ Sci Pollut Res 27, 3768–3791. https://doi.org/10.1007/s11356-019-06608-7

Persoone, G., Wells, P., 1987. Artemia in aquatic toxicology: a review. pp. 259–275.

Peterson, L.A., 2013. Reactive Metabolites in the Biotransformation of Molecules Containing a Furan Ring. Chem. Res. Toxicol. 26, 6–25. https://doi.org/10.1021/tx3003824

Poureetezadi, S.J., Wingert, R.A., 2016. Little fish, big catch: zebrafish as a model for kidney disease. Kidney International 89, 1204–1210. https://doi.org/10.1016/j.kint.2016.01.031

Puydarrieux, P., Beyou, W., 2017. Cadre conceptuel pour l'Evaluation Française des Ecosystèmes et des services Ecosystemiques. Ministère de l'Environnement, de l'Energie et de la Mer.

Rocco, L., Frenzilli, G., Fusco, D., Peluso, C., Stingo, V., 2010. Evaluation of zebrafish DNA integrity after exposure to pharmacological agents present in aquatic environments. Ecotoxicology and Environmental Safety 73, 1530–1536. https://doi.org/10.1016/j.ecoenv.2010.07.032

Saleem, S., Kannan, R.R., 2018. Zebrafish: an emerging real-time model system to study Alzheimer's disease and neurospecific drug discovery. Cell Death Discov. 4, 45. https://doi.org/10.1038/s41420-018-0109-7

Sandre, F., Huynh, N., Caupos, E., El-Mrabet, L., Partibane, C., Lachaise, I., Pommier, C., Rivard, M., Morin, C., Moilleron, R., Le Roux, J., Garrigue-Antar, L., 2023. Occurrence and fate of an emerging drug pollutant and its by-products during conventional and advanced wastewater treatment: Case study of furosemide. Chemosphere 322, 138212. https://doi.org/10.1016/j.chemosphere.2023.138212

Sandré, F., Huynh, N., Gromaire, M.-C., Varrault, G., Morin, C., Moilleron, R., Le Roux, J., Garrigue-Antar, L., 2022. Road Runoff Characterization: Ecotoxicological Assessment Combined with (Non-)Target Screenings of Micropollutants for the Identification of Relevant Toxicants in the Dissolved Phase. Water 14, 511. https://doi.org/10.3390/w14040511

Santos, J.L.F., Zanardi, P., Alo, V., Rodriguez, M., Magdaleno, F., De Langhe, V., Dos Santos, V., Murialdo, G., Villoldo, A., Coria, M., Quiros, D., Milicchio, C., Garcia Saiz, E., 2021. Pulmonary Edema in COVID-19 Treated with Furosemide and Negative Fluid Balance (NEGBAL): A Different and Promising Approach. JCM 10, 5599. https://doi.org/10.3390/jcm10235599

Sarah, Q.S., Anny, F.C., Misbahuddin, M., 2017. Brine shrimp lethality assay. Bangladesh J Pharmacol 12. https://doi.org/10.3329/bjp.v12i2.32796

Schröder, H., Gebhardt, W., Thevis, M., 2010. Anabolic, doping, and lifestyle drugs, and selected metabolites in wastewater-detection, quantification, and behaviour monitored by high-resolution MS and MS n before and after sewage treatment. Anal Bioanal Chem 398, 1207–1229. https://doi.org/10.1007/s00216-010-3958-3

Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2007. The behaviour and ecology of the zebrafish, Danio rerio. Biological Reviews 83, 13–34. https://doi.org/10.1111/j.1469-185X.2007.00030.x

Starling, M.C.V.M., Souza, P.P., Le Person, A., Amorim, C.C., Criquet, J., 2019. Intensification of UV-C treatment to remove emerging contaminants by UV-C/H2O2 and UV-C/S2O82−: Susceptibility to photolysis and investigation of acute toxicity. Chemical Engineering Journal 376, 120856. https://doi.org/10.1016/j.cej.2019.01.135

Stevenson, J., 2014. Ecological assessments with algae: a review and synthesis. Journal of Phycology 50, 437–461. https://doi.org/10.1111/jpy.12189

Stokes, W., Nunn, L., 1964. A New Effective Diuretic -Lasix. British Medical Journal 2, 910–914.

Strähle, U., Scholz, S., Geisler, R., Greiner, P., Hollert, H., Rastegar, S., Schumacher, A., Selderslaghs, I., Weiss, C., Witters, H., Braunbeck, T., 2012. Zebrafish embryos as an alternative to animal experiments—A commentary on the definition of the onset of protected life stages in animal welfare regulations. Reproductive Toxicology 33, 128–132. https://doi.org/10.1016/j.reprotox.2011.06.121

TEEB, 2012. TEEB - The Economics of Ecosystems and Biodiversity: Ecological and Economic Foundations.

Toyota, K., Hiruta, C., Ogino, Y., Miyagawa, S., Okamura, T., Onishi, Y., Tatarazako, N., Iguchi, T., 2016. Comparative Developmental Staging of Female and Male Water Fleas Daphnia pulex and Daphnia magna During Embryogenesis. Zoological Science 33, 31. https://doi.org/10.2108/zs150116

Völkel, P., Dupret, B., Le Bourhis, X., Angrand, P.-O., 2018. Le modèle poisson zèbre dans la lutte contre le cancer. Med Sci (Paris) 34, 345–353. https://doi.org/10.1051/medsci/20183404016

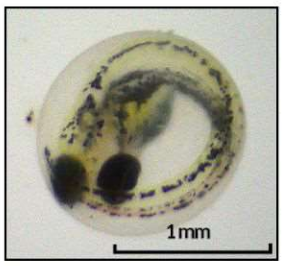
Weill, A., Drouin, J., Desplas, D., Cuenot, F., Dray-Spira, R., Zureik, M., 2020. Usage des médicaments de ville en France durant l'épidémie de la Covid-19 – point de situation après les 8 semaines de confinement et une semaine de post-confinement - Étude pharmaco-épidémiologique à partir des données de remboursement du SNDS, EPIPHARE. ANSM & Assurance maladie.

Zejda, V., Máša, V., Václavková, Š., Skryja, P., 2020. A Novel Check-List Strategy to Evaluate the Potential of Operational Improvements in Wastewater Treatment Plants. Energies 13, 5005. https://doi.org/10.3390/en13195005

Zoumpouli, G.A., Zhang, Z., Wenk, J., Prasse, C., 2021. Aqueous ozonation of furans: Kinetics and transformation mechanisms leading to the formation of α,β-unsaturated dicarbonyl compounds. Water Research 203, 117487. https://doi.org/10.1016/j.watres.2021.117487

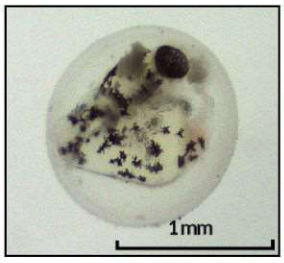
Annexes

Annexes

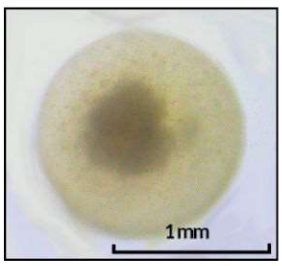


1. Oeuf sain pigmenté

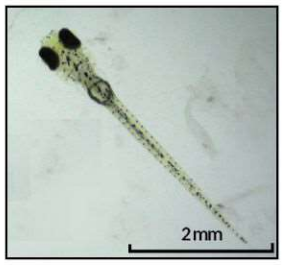
2. Oeuf sain albinos



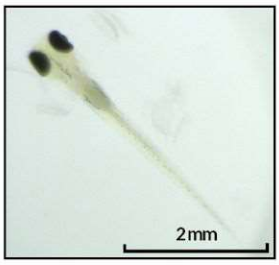
3. Oeuf avec un oedème



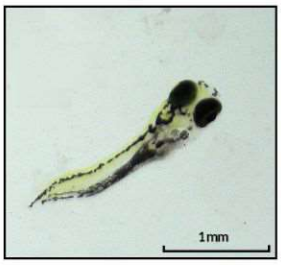
4. Oeuf coagulé



5. Larve normale

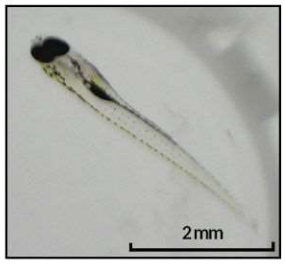


6. Larve Albinos



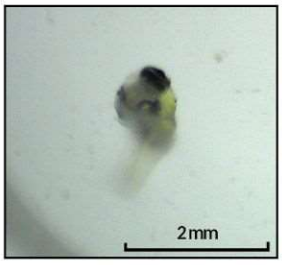
7. Larve de petite taille

8. Larve petite taille tordue

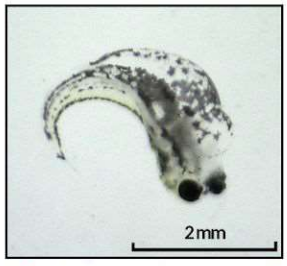


9. Larve flottante (côté)

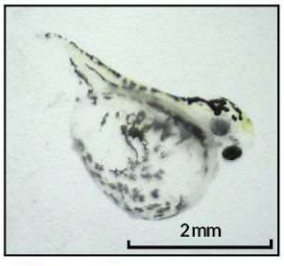
10. Larve flottante (Haut)



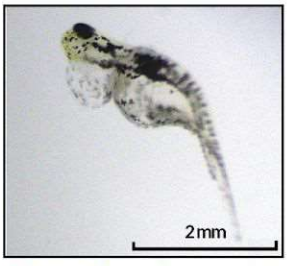
11. Larve flottante (bas)+tordue



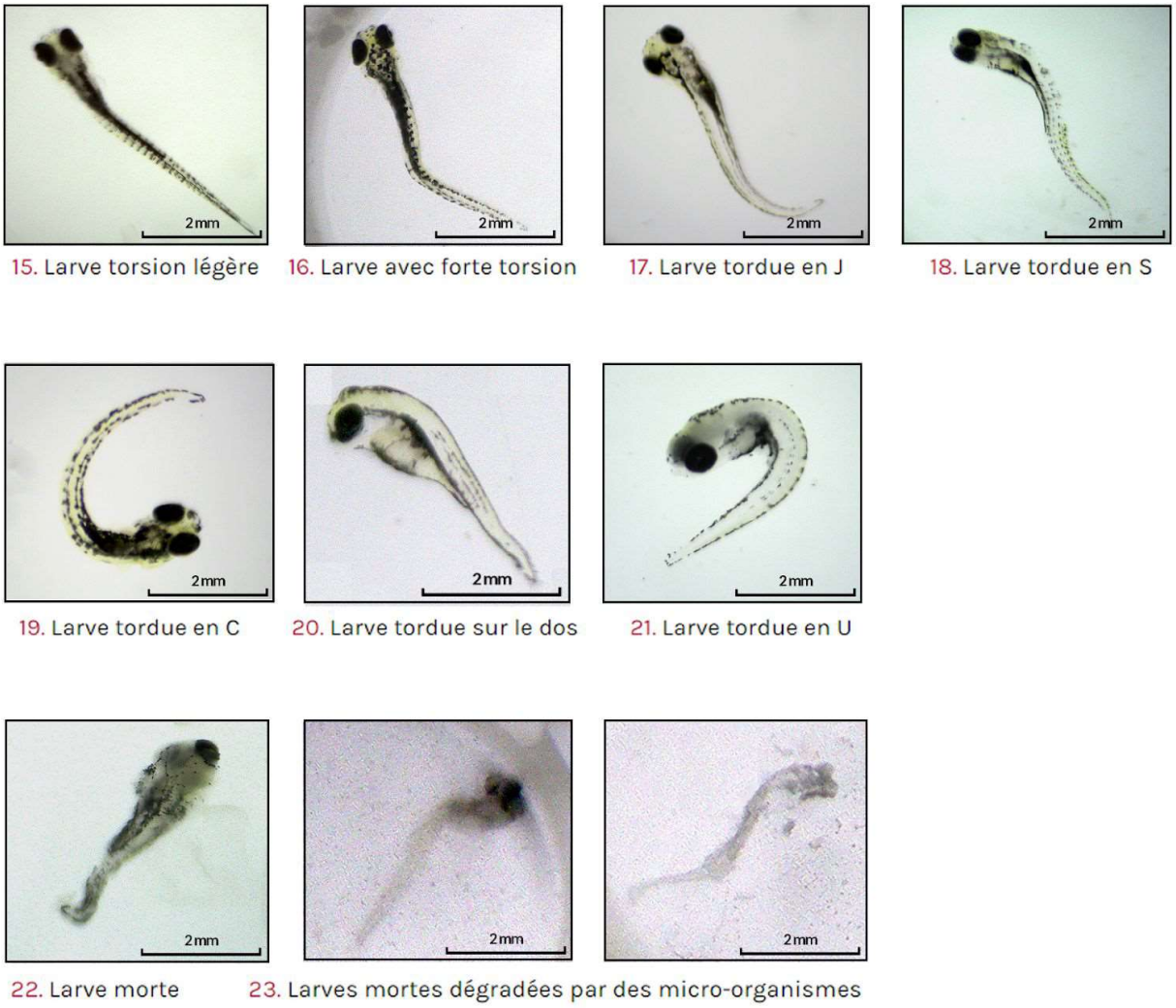
12. Oedème du sac vitellin



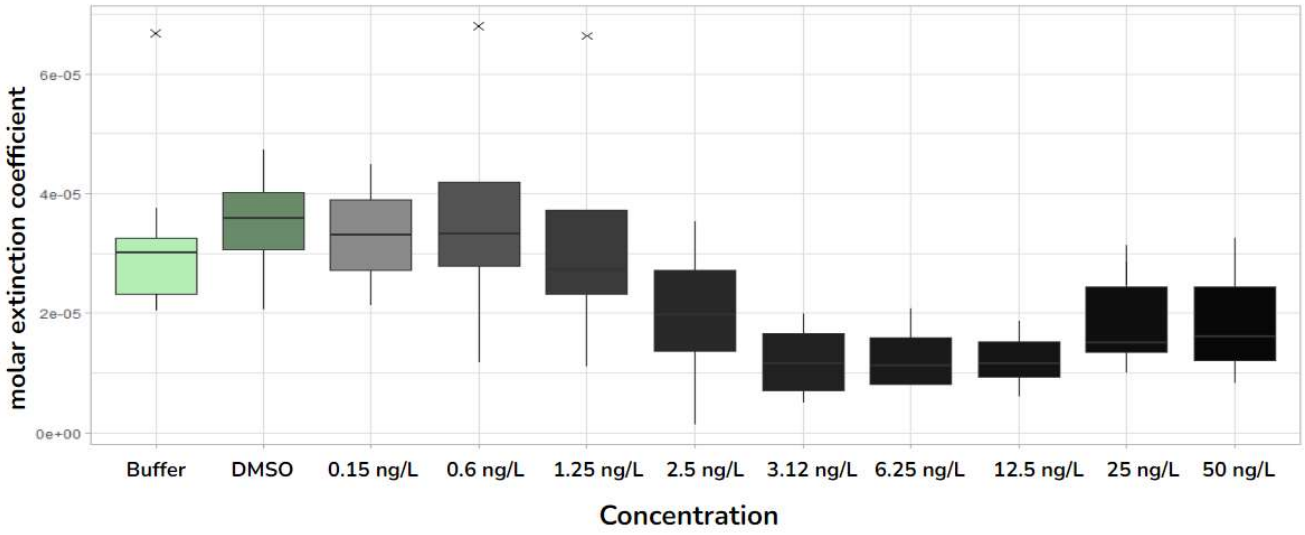
13. Doubles oedèmes sac vitellin et péricardiaque



14. Double oedème (côté)



Annexe 1. Photographies d'œufs et de larves de poissons zèbres à différents stades de développement présentant des déformations.



Annexe 2. Inhibition de la chaîne respiratoire mitochondriale après exposition à différentes concentrations de Pyridinium du furosémide.