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Comprehensive prioritisation scheme for active pharmaceutical ingredients in Denmark

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Active pharmaceutical ingredients (APIs) can cause severe adverse effects if released into the environment. In response to the 2574 APIs approved in Denmark and costly environmental monitoring initiatives, a prioritisation scheme is presented to identify candidate substances for further investigation, based on their hazard, use and risk. The proposed prioritisation scheme consists of three subsequent filters; human toxicology and ecotoxicology followed by reported Danish use and lastly measured or predicted environmental concentration and risk ratios based on these. To generate a final list of 300 APIs, actions such as assigning scores for missing data were implemented. These substances may inform future monitoring campaigns focused on pharmaceutical contamination in aquatic environments through effluent discharge. All in all, 84% among the top-50 priority APIs have not previously been included in chemical analysis of any environmental samples in Denmark. These APIs belonged to therapeutic groups such as treatments for hypertension, antibiotics, antifungals, antineoplastics, and medicines affecting the nervous system. Of particular concern is metformin, clindamycin and clotrimazole as these are all amongst the highest ranked based on risk, and also appear on the EU commission's watch list of substances for EU-wide monitoring in the field of water policy. The scheme can be updated, adapted and implemented in other geographical regions.

Sustainability spotlight

There are about 2000 active pharmaceutical ingredients (APIs) which are being administered worldwide in prescription medicines, non-prescription drugs and veterinary drugs, the residues of which are of increasing environmental concern among countries. In 2019 the European Union developed a strategic approach to pharmaceuticals in the environment due to the overwhelming evidence that traces of pharmaceuticals in the environment could have an adverse impact on ecosystems. The approach is part of the zero pollution ambition for a toxic-free environment, as expressed in the European Green Deal, aiming to protect both public health and ecosystems through avoiding negative effects of chemical substances, including certain pharmaceutical residues in water. To effectively address and mitigate the environmental concerns there is need to prioritize APIs of greatest environmental concern as also stressed by The Organization for Economic Cooperation and Development (OECD) to prioritize chemicals and hereunder APIs to protect environmental health. This paper adapts a prioritization approach to cover APIs by combining both human and environmental hazard data, exposure information to support prioritization of APIs of increased environmental concern. This allows an objective prioritization of individual as well as classes of APIs for risk management, among others the targeting removal of prioritized APIs in wastewater treatment technologies, aid considerations of greening the use and development of APIs. This will support the risk management of the more than 2000 APIs used today globally and thereby support the UN SDGs #3 (good health); #6 (clean water); #12 (responsible production); #14 (life under water).

1 Introduction

Effective pharmaceuticals are essential for the treatment of many different diseases in humans and animals. Simultaneously, the contamination of the environment, such as water and soil, by some active pharmaceutical ingredients (APIs) through use and/or disposal as well as in rare cases by the manufacturing process, has during the past decade been identified an emerging problem in the EU.[1] This led to the requirement of API removal in the new urban wastewater treatment directive (Directive (EU) 2024/3019 of the European Parliament and of the Council of 27 November 2024 concerning urban wastewater treatment, OJ, 2024, 3019, 1-59) with cost distributed by the polluter pays principle. APIs are designed to elicit biological responses at extremely low concentrations, often in the nanogram per liter (ng L^{-1}) range—for example, hormones, meaning that even extremely low environmental concentrations have the potential to cause adverse effects such as interfering with biochemical, cellular physiological and behavioural processes in plants and animals.[2] Moreover, there is an extensive body of evidence that this pollution has a vast adverse impact on wildlife such as birds, fish, and insects, and has the potential to affect entire ecosystems.[3–5] Given the

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thousands of APIs that are approved for use by the EU, and therefore potentially emitted to the environment, a prioritisation process is an efficient strategy to identify candidate APIs most likely to cause severe adverse environmental effects for initiating effective and low-cost monitoring campaigns.

One of the major concerns associated with pharmaceutical contamination of the environment is antibiotic resistance, where the environmental exposure of antibiotics ultimately causes a reduction in therapeutic efficacy in humans and animals by promoting the spread of bacterial antibiotic resistance genes.[6,7] There are several sources of environmental contamination of antibiotics, most notability the incomplete removal in wastewater treatment plants (WWTPs) after use.[6] Although the simplest solution to reduce environmental antibiotic concentrations is to minimize their use relative to the needed effect, but due to oversubscription and misuse of antibiotics in different countries and regions[8] some, other actions are clearly necessary like including them in the urban wastewater treatment directive (EU Directive 2024/3019),[9] which also argue that there is a need to increase the removal efficiency of antibiotics in the WWTPs.[10] In order to optimise removal processes, there is a need to perform a science-based risk assessment to identify and prioritise the antibiotics that may cause the most serious environmental harm.

Additionally, there have been reports of an increase of use of antineoplastic APIs, mainly used for chemotherapy.[11] The common denominator for antineoplastic APIs is that they are cytotoxic inducing cell death (apoptosis) and/or inhibit cellular growth.[12] There are currently large data gaps in terms of environmental concentrations of these APIs and potential adverse effects on wildlife and humans. Another group of APIs that may cause adverse effects when found in environmental matrices, are the antidepressants selective serotonin reuptake inhibitors (SSRIs), are amongst the most globally prescribed pharmaceuticals[13] and have previously been described in various environmental compartments such as wastewaters as well as surface, ground and drinking waters.[14] Moreover, SSRIs have been found in sediment and biota.[15] Due to the same mode of action for all these APIs, a concentration addition model has been demonstrated for mixtures of SSRIs.[16] SSRIs are potent compounds, and even low environmental concentrations have been shown to indirectly affect survival in non-target organisms such as algae, plants and could be bioaccumulated in fish by causing delays in physiological development, a decrease in aggressiveness, and inhibition of feeding responses.[17]

The EU Green Deal zero pollution and toxic free action plan and the urban wastewater treatment EU Directive 2024/3019 demonstrates the importance of monitoring and managing pharmaceutical pollutants. This underscores the need to develop a human and environmental risk-based prioritisation systems to aid cost-effective environmental monitoring and risk management of APIs. Adverse aquatic effect investigations are initiated if the predicted environmental concentration (PEC) of an API exceeds 0.01 μg L^{-1} in the EU[18] and if the Expected Environmental Concentration surpasses 0.1 μg L^{-1} in the US[19] Furthermore, highly lipophilic APIs ($\log K_{ow} \geq 4.5$ in the EU and $\log K_{ow} \geq 3.5$ in the US) or APIs defined as a potential endocrine disruptors, are also considered reasons for increased concern in both the EU and US, independent of any exposure information.[18–21] Thus, there is strong need for a careful characterization of the environmental concentration for all APIs currently in use, to support risk-based informed tools and practices.

The aim for this study was to develop a prioritisation scheme to cover all potential APIs that may enter the aquatic environment in Denmark. It is based on previous prioritisation schemes for emerging contaminants[22] and adapted to APIs, and might also be used to distribute costs *via* the polluter pays principle. The focus has been on initial investigation and prioritisation of the API, or the parent drug prescribed to humans, and not any of the thousands of metabolites reported for medicines in environmental samples.[23] The scheme was designed around a tiered approach, where continuous update of the data, weights and filters can be done, to enable translation of the scheme for application by other users and geographical areas.

2 Materials and methods

2.1 Conceptual approach

The prioritisation process described in this paper is a risk-based prioritisation method creating an objective ranking of APIs prescribed to people of concern in order to prioritise further environmental monitoring and/or risk management actions. The scheme was applied to all human prescription based active pharmaceutical ingredients (APIs) that may potentially enter and contaminate the Danish surface waters and environment through WWTP effluents. While in an earlier paper we worked on the prediction of API discharges including the metabolization in the human body and the WWTP[24] and a risk assessment,[25] in this paper we focus on an alternative approach for the ranking of the prescription APIs for human use without measurements. Based on the prescription data, the proposed method consists of a series of three filters where APIs qualify for subsequent tiers on the basis of a score, they gain from comparing parameters from different sources. We applied three and progressive prioritisation filters (Table 1) to develop the list of ranked compounds based on both hazard and risk, which will be described in detail below. All APIs were scored and ranked but the prioritization highlights the APIs of primary concern. We are not including veterinary pharmaceuticals nor over-the-counter pharmaceuticals in the assessment.

Compounds were scored using a standardized approach, ranging from 0 to a maximum of 3 based on the assessed severity of a particular property relative to the other compounds in the study. Moreover, the weighting of this score (ranging from 0 to a maximum of 10; see Table 1) was determined based on factors such as the origin and quality of the data (uncertainty of the parameter), the assessed severity and relevance to human health and environmental impact as well as in certain cases geographical closeness to Denmark for the collection of monitoring data. The sole exception to the maximum weighting of 10 was for compounds listed on the European Commission's 2022/1307 watch list.[26] Compounds appearing on this list was given

Table 1 Overview of the lists, number of entries in the list, scoring parameters for the three filters, weighting factor and source used in the prioritisation scheme

List	Number of entries	Filter 1 a scoring parameter	Filter 1b scoring parameter	Filter 2 scoring parameter	Filter 3a scoring parameter	Filter 3b scoring parameter	Weighting factor	Source
REACH article 59 candidate list	194	Reasons for inclusion Hazard statement code(s)	Hazard statement code(s)-environmental hazard				10	https://echa.europa.eu/en/candidate-list-table
REACH annex XIV authorisation list	55	Reasons for inclusion					1	https://echa.europa.eu/da/authorisation-list
REACH annex XVII restriction list	125	Reasons for inclusion					10	https://echa.europa.eu/da/substances-restricted-under-reach
Community rolling action plan (CoRAP) list of the european chemical agency (ECHA)	307	Remarks	Hazard statement code(s)-environmental hazard				5	https://echa.europa.eu/da/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table
Substitute it now! (SIN) list	761	Reasons for inclusion Hazard class and category code(s)	Reasons for inclusion				7 10	https://chemsec.org/buisness-tool/sin-list
List of possible endocrine disrupting compounds (EDC)	430	Category					3	https://edlists.org
Authorised medicinal products and new medicinal products in Denmark	2574	ATC[a] code (first three characters)					5	https://laegemiddelstyrelsen.dk/en/licensing/licensing-of-medicines/lists-of-authorised-and-deregistered-medicines/how-to-use-the-list-of-authorised-medicinal-products/
Roos *et al.* 2012	582	Pregnancy cat.1					8	*https://www.sciencedirect.com/science/article/pii/S0048969712000824?via%3Dihub*
			$\log K_{ow}$				1	
						PEC/PNEC	5	
EU Commission's watch list of substances for union-wide monitoring in the field of water policy	26		Included				100	https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32022D1307
Measured API toxicity (Sanderson *et al.* 2012)	194		LD_{50}/LC_{50}				2	https://link.springer.com/article/10.1007/s00128-012-0921-3
			$\log K_{ow}$				1	
ECOSAR (Sanderson *et al.* 2003)	2842		$\log K_{ow}$				1	*https://www.sciencedirect.com/science/article/pii/S0273230004000029?via%3Dihub#TBL1*
						PEC/PNEC	3	
							6	

Table 1 (*Contd.*)

List	Number of entries	Filter 1 a scoring parameter	Filter 1b scoring parameter	Filter 2 scoring parameter	Filter 3a scoring parameter	Filter 3b scoring parameter	Weighting factor	Source
Annual sale in Danish pharmacies				Number of patients using API annually				**https://www.esundhed.dk/Emner/Laegemidler/Apotekernes-salg-af-laegemidler**
Umweltbundesamt (UBA) database – pharmaceuticals in the environment	276 895				All countries Sweden, Finland, Norway, Germany Denmark		1	**https://www.umweltbundesamt.de/en/Database-pharmaceuticals-in-the-environment-0**
NORMAN ecotoxicology database (experimental)	757				Lowest PNEC that are based on experimental data		2	**https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php?checkSelect=2**
COWI report (2021)	136				Maximum MEC/PNEC Average MEC/PNEC In hospital effluent?		10 2	Personal correspondence
NORMAN ecotoxicology database (predicted)	31148					Lowest PNEC that are based on predicted data	1	**https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php?checkSelect=2**
Calculated PEC	84						3	See Section 3b

[a] Anatomical Therapeutic Chemical (ATC) classification system from WHO collaborating centre for drug statistics.

a weighting score of 100, in order to ensure that these compounds were prioritized highly to reflect the risk management priorities.

2.2 Establishment of a curated list of APIs sold in Denmark

Initially a list of all approved APIs was downloaded from the Danish Medicines Agency's (DMA) webpage in September 2022. In total, 2574 unique APIs were approved for use in Denmark (see Fig. 1). Additionally, the number of APIs was reduced based on their presence in Medicinpriser.dk, a database administered by DMA containing prices for all available medicines in Denmark, as only APIs currently available for sale in Denmark was considered relevant for the scope of this study. This database is updated every fortnight. By using information regarding availability on the Danish market retrieved from Medicinpriser.dk, the number of entries were decreased from the original 2574 APIs to 1506 individual APIs.

Successively, this initial list was further curated by excluding certain entries such as vaccines, inorganic salts as well as plant or animal extracts (chemical substances of unknown or variable composition, complex reaction products and biological materials (UVCBs)), as they were considered as out of scope or too complex in their chemical composition for this prioritisation scheme. For instance, plant and animal extracts used in certain medication are complex biological matrices, and we are therefore in this stage unable to evaluate them according to the steps presented in this prioritisation scheme. These exclusions reduced the initial list from 1506 APIs to 1024 APIs (see Fig. 1), generating a curated list suitable for the prioritisation framework.

2.3 Filter 1a: hazard-human toxicity filter

The initial prioritisation step was based on existing lists of chemicals for example established under REACH, presented in Table 1. These lists address several different human hazard properties such as the potential for endocrine disruption or carcinogenicity, mutagenicity, and reproductive toxicity (CMR). The human toxicology filter also scored compounds according to hazard statements such as H350 – may cause cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard), or H360 – may damage fertility or the unborn child.

Moreover, this filter also contained scoring APIs according to their Anatomical Therapeutic Chemical (ATC) classification, where for instance antibacterial use, sex hormones and other compounds with use in the endocrine system, antifungal and/or antiparasitic effects as well antineoplastic and immunomodulating agents were deemed as highly prioritised and thus scored high, see SI. Compounds with ATC codes linked to psychoactive effects were considered less significant and were given a lesser score. Moreover, APIs with ATC codes concerning for example topical products, diabetes treatment and antinauseants were considered not highly significant for the prioritisation scheme and therefore not given any score for this particular parameter.

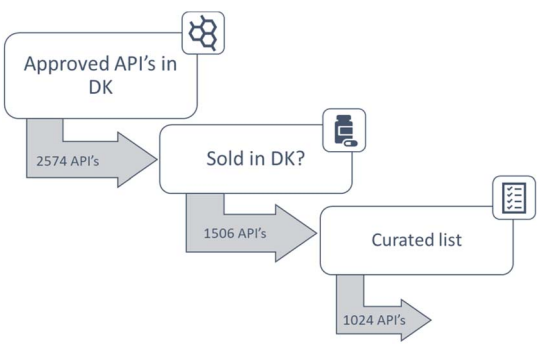

Fig. 1 Schematic over the initial steps for establishing a list over approved APIs in use in Denmark.

2.4 Filter 1b: hazard – ecotoxicology filter

Totalling the overall scoring for this filter, scores for environmental hazard classifications such as H410 – very toxic to aquatic life with long lasting effects as well as scores based on available data on physico-chemical properties, such as *n*-octanol/water partition coefficient ($\log K_{ow}$) were included. The $\log K_{ow}$ is defined as the ratio of the concentration of a substance in *n*-octanol and water at equilibrium at a specified temperature. Moreover, scores for the toxicity parameters LD_{50} and LC_{50}, defined as the lethal dose for 50% of a test population and the lethal concentration (in an environmental matrix such as water) for 50% of a test population respectively, in living organisms such as rats, fish, and algae were added together. In the case of LD_{50}, data obtained from rats (*R. norvegicus*) were chosen as a well-established model organism to represent mammals. As for LC_{50}, fish and algae species were chosen as model organisms for determining toxicity in an aquatic environment.

The initial prioritisation step was based on the following existing lists presented in Table 1. The compounds listed on the EU Commission's watch list of substances for union-wide monitoring in the field of water policy were considered very highly prioritised and given a score of 100, ensuring them to pass through all filters and to be included on the final list of prioritised compounds. The EU watch list contains pharmaceuticals where already available data has indicated that they "*may pose significant risk, at Union level, to or via the aquatic environment, but for which monitoring data are insufficient to come to a conclusion on the actual risk posed*".[26]

2.5 Filter 2: use of medicine in DK

The use amount is an important starting point for understanding and quantifying potential exposure concentrations in the environment, and it was therefore selected as the second filter. The database used for this filter was the official Danish database for sales of medicines in registered pharmacies in 2021 operated by The Danish Health Data Authority. We selected statistics from 2021 as we considered this data would most accurately reflect the use of medicines in Denmark in 2022. Unfortunately, there is no official statistic for 2021 for the sale of over-the-counter medicines by establishments that are

not registered pharmacies, thus especially ibuprofen, diclofenac and paracetamol are underrepresented. The last year for official statistics were in 2016, and this was deemed as being too inaccurate to be relevant for the purpose of this study. For instance, the Danish legislation for selling medicines over the counter was updated in late 2017, resulting in for instance more stringent control of availability for over-the counter medicines.[27]

Another important factor for the filter is the measurement of amount or use of medicines in Denmark. The two most suitable and possible options for calculations of the use were defined daily dose (DDD) or the number of persons using the medicine. DDD is the assumed average maintenance dose for a drug used by an adult person. However, some groups of medications, such as antineoplastic agents, anaesthetics, and dermatological products, do not have DDDs established. As some of the subcategories for the medications (D0, L01 and N01) was considered prioritised ATC groups (see SI), the use of DDDs for estimating Danish medicines use were therefore considered unsuitable for evaluating the use of medicines in Denmark. Hence, filter 2 was quantified based on the number of persons reportedly using a medicine on a quarterly basis, which were tallied together to constitute a year, thus adding up to the number of people using the medicine in Denmark annually. It is also important to note, that because an API is available for sale on the Danish market (as indicated by the data from medicinpriser.dk), does not necessarily mean that it was used by a significant number of patients. The scores for use in Denmark are presented in SI.

2.6 Filter 3a measured values – including risk

The last tier of the applied prioritisation scheme consisted of a risk evaluation using measured (MEC) (Filter 3a) or predicted (PEC) (Filter 3b) data for environmental concentrations. For Filter 3a, the APIs passing the hazard and use filters described above are placed in context based on measured exposure concentrations to derive an appreciation of potential risks. In one of the few studies measuring APIs in Danish waters, The Danish Water Sector (DANVA) commissioned a report by the consultancy firm COWI in 2021. This report lists 136 APIs that have been detected in Danish waters, and which are incorporated into this prioritisation scheme.

To address the scarcity of data from Denmark, the third prioritisation step utilised existing datasets comprising measured API exposure concentrations, as detailed in Table 1. These datasets were derived from global monitoring data; however, environmental measurements from geographically proximate Nordic countries and Germany, were assigned greater weight than those from more distant regions. Additionally, monitoring data from other parts of the world were incorporated into the analysis. In the approach described in this paper, the usually multiple entries in the database from the German EPA, Umweltbundesamt (UBA), was added and given a score depending on how frequently monitored the API had been found in the past. In the first tier the number of occurrences were combined for all countries. In the second tier, countries close to Denmark, such as the Nordic countries and Germany were combined to give a score with a higher weighting factor. Subsequently, the monitoring data from Denmark was scored even higher (see SI).

The risk was evaluated by the risk characterisation ratio (RCR) according to eqn (1) below:

$$\mathrm{MEC/MNEC} = \frac{\text{Measured ecological concentration(MEC)}}{\text{Measured no-effect concentration(MNEC)}} \quad (1)$$

2.7 Filter 3b: risk – predicted environmental concentration (PEC)

This filter addresses a scoring of a combined assessment of predicted toxicological values and risk ratios based on predicted environmental concentrations. For instance, the NORMAN Ecotoxicology Database (see Table 1) used in this filter lists the lowest predicted Predicted No-Effect Concentration (PNECs) based on Quantitative Structure–Activity Relationship (QSAR) models that were being evaluated by experts prior to become included in the database.

3 Results and discussion

3.1 Establishment of a curated list of APIs in Denmark

The first step in curating the initial list of 2574 APIs approved in Denmark was to filter APIs based on their actual presence on the Danish market. The number of entries was reduced from the original 2574 APIs to 1506 individual APIs. The justification for this step is that we find it unlikely that a medicine not consistently available on the Danish market is used and emitted through effluent water from WWTPs in such quantities that it would have a potentially adverse effect on the environment. Moreover, the list was further curated from 1506 APIs to 1024, by removing entries such as vaccines, inorganic salts and plant and animal extracts, as these were considered either too chemically complex or out of scope for the prioritisation scheme (Fig. 1).

3.2 Filter 1a: hazard-human toxicity filter

The initial filter step applied to the APIs in the curated list is a combined score from studies in human toxicology (1a) as well ecotoxicology (1b). We combine the hazard-human toxicity as an initial filter criterion with the environmental hazard (1b) – as the focus is on the environmental impacts APIs may have in the prioritisation scheme. These traits are nonetheless considered very important and form one part of the initial filter (Filter 1a). Furthermore, the weighting (see Table 1) reflects the severity of the parameters evaluated by this filter.

3.3 Filter 1b: hazard – ecotoxicology filter

Due to both the extensive use of pharmaceuticals as well as the incomplete removal in WWTPs, these compounds are introduced into the water continuously. Furthermore, most APIs have a comparatively high polarity and relatively low volatility,

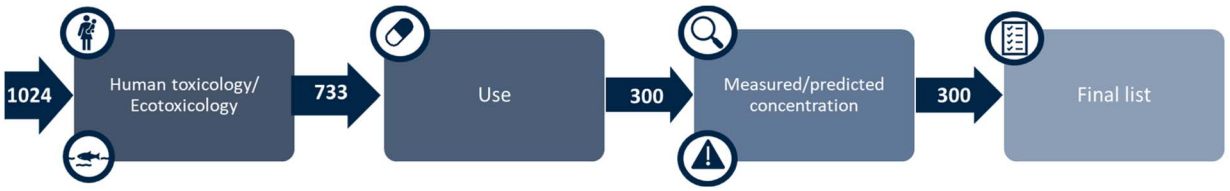

Fig. 2 Results from the prioritisation filters resulting in the final list of prioritised APIs.

causing them to migrate into the water environment. Once in the water matrix, the toxicity of the APIs is determined by several factors such as exposure time, type and developmental stage of the organism, water temperature and pH, API concentration and potency as well as the physico-chemical properties of the API. As a parameter in the prioritisation scheme, $\log K_{ow}$ has an ecotoxicological significance, since it then can be used as a relative indicator of the tendency of an organic compound to be absorbed by living organisms.

Additionally, this filter evaluated toxicity data, including LC50 values based on experimental data from model organisms such as rats, representing mammals, as well as fish, daphnia and algae, for determining toxicity in an aquatic environment. However, these studies are both laborious and time-consuming, which is reflected in the relatively low number of APIs with measured LC_{50} in comparison to the large amount of commercially available APIs. For instance, out of the 194 compounds on the list by Sanderson *et al.* (2012),[28,29] 43 were also found on the curated list of APIs commercially available in Denmark.

In total, 733 of the 1024 curated APIs advanced to the next tier of the prioritisation scheme, Filter 2: Use of medicine in Denmark (Fig. 2). The APIs that progressed are therefore to be considered as potentially problematic since several are classified as carcinogenic/mutagenic/reproductive (CMR) compounds, endocrine disruptors (EDs) or exhibiting properties relevant for ecotoxicity such as a high $\log K_{ow}$ or low LD_{50}/LC_{50} value.

3.4 Filter 2: use of medicine in DK

Since there are currently no official statistics of the prevalence of importing particular medicines or amount of APIs from the EU, it was assumed for the purpose of this study that this occurrence is so low that it would not significantly impact the results from this study. Additionally, the private import of medicines from a third country is in most cases illegal according to Danish legislation (BEK nr 1360 af 18/12/2012)[29] and the amount imported and used, is for the purpose of this study, assumed to be negligible in comparison to the legal use of medicines in Denmark.

However, by only having access to data from medicines sold in pharmacies, and not over-the counter sales statistics, there is a risk that the use of certain APIs with a known ecotoxicological profile, such as diclofenac, is underestimated due to the assumed high sales numbers in establishments not registered as pharmacies. After applying Filter 2: Use in Denmark, we concluded that out of the 733 APIs that passed through filter 1a + b, evaluating human and environmental hazard, 300 APIs are currently in use in Denmark (Fig. 2).

3.5 Filter 3a measured values – including risk

For Filter 3a, the APIs passing the hazard and usage filters described above are investigated with a filter consisting of a combined evaluation of measured concentrations, measured toxicological values and risk ratios based on measured values. In terms of information of concentration of APIs in the Danish environment the available monitoring data are limited. There are limited datasets for API in Danish waters, even though in some projects pharmaceuticals have been analysed in wastewater[25] which was a one-time monitoring of practically all WWTPs in Denmark. However, there are by now a multitude of data generated in various contexts, mostly research related projects, where data are published sparsely. Examples of such datasets are the COWI report (2021),[30] see Table 1. To overcome this lack of data from Denmark, the third prioritisation step was based on existing lists containing measured API exposure concentrations, presented in Table 1. Moreover, environmental measurements from countries considered geographically adjacent to Denmark (such as the Nordic countries, and Germany) was weighted higher than measurements from countries considered further away from Denmark. All in all, out of 300 APIs passing through filter 2 – Use of medicines in Denmark, 112 had measured data retrieved from any of the lists in Table 1.

3.6 Filter 3b: predicted values – including risk

Filter 3b addresses a scoring of a combined assessment of predicted toxicological values and risk ratios based on predicted environmental concentrations (PECs). The scores for risk of APIs with calculated risk characterization ratios from eqn (1), where PEC substitutes MEC, were used. All in all, 25 APIs out of 300 entering filter 3 did not have any monitored or predicted environmental data. These were given a penalty score corresponding to 25% (6.75) of the total possible score for Filter 3b.

By applying the three tiers of filters, starting with hazard filters, and further use in Denmark and lastly risk, described in the Materials and Methods section above, we generated a final list of 300 APIs (see Fig. cc). These compounds were then given a total score based on the scores from the individual filters.

3.7 Top-50 ranked APIs in Denmark

A total score was calculated for all 300 APIs that passed the three filters. Table 2 displays the top-50 highest ranked APIs in Denmark for future prioritisation for monitoring in Danish surface

Table 2 Top 50 prioritised APIs in Denmark, indicated in grey are APIs previously analysed in Danish environmental samples

Preferred name	CAS number	Therapeutic use	Comment – therapeutic use	Total score	Prioritisation group	Previously analysed in DK?	On the 4 EU watch list
Metformin	657-24-9	Drugs used in diabetes	Antihyperglycemic (lowers blood sugar levels)	162,3	1	No	Yes
Estradiol	50-28-2	Sex hormones and modulators of the genital system	Female steroid hormone	150,0	1	Yes	No
Trimethoprim	738-70-5	Antibacterials for systemic use	Antibiotic	144,6	1	Yes	Yes
Carbamazepine	298-46-4	Antiepileptics	Anticonvulsant medication	144,2	1	Yes	No
17-Alpha-estradiol	57-63-6	Sex hormones and modulators of the genital system	Female steroid hormone	132	1	Yes	No
Fipronil	12006837-3	Psycholeptics	CNS GABA-A inhibitor	130,5	1	No	Yes
Clindamycin	18323-44-9	Antibacterials for systemic use	Antibiotic	125,4	1	No	Yes
Miconazole	22916-47-8	Stomatological preparations	Antifungal	124,3	1	No	Yes
Imazalil	35554-44-0	Radiopharmaceuticals	Contrast	122,4	1	No	Yes
Citalopram	59729-33-8	Psychoanaleptics	Antidepressant	121,6	1	Yes	No
Clotrimazole	23593-75-1	Gynecological antiinfectives and antiseptics	Antifungal	114,8	1	No	Yes
Diclofenac	15307-86-5	Other dermatological preparations	Nonsteroidal anti-inflammatory drug (NSAID)	91,6	2	Yes	No
Amoxicillin	26787-78-0	Antibacterials for systemic use	Antibiotic	90,2	2	Yes	No
Warfarin	81-81-2	Antithrombotic agents	Anticoagulant (blood thinner)	81,4	2	Yes	No
Tiotropium bromide	136310-93-5	Drugs for obstructive airway diseases	Anti-asthmatic	80,6	2	No	No
Metoprolol	51384-51-1	Beta blocking agents	Treat high blood pressure (hypertension)	80,0	2	Yes	No
Diazepam	439-14-5	Psycholeptics	Benzodiazepine (anxiolytic)	79,6	2	No	No
Sertraline	79617-96-2	Psychoanaleptics	Anti-depressant (selective serotonin reuptake inhibitor (SSRI))	79,4	2	Yes	No
Midazolam	59467-70-8	Psycholeptics	Benzodiazepine (anxiolytic)	78,0	2	No	No
Lisinopril	76547-98-3	Agents acting on the renin-angiotensin system	Treat high blood pressure (hypertension)	75,8	3	No	No
Propylthiouracil	51-52-5	Thyroid therapy	Tretment of hyperthyroidism	75,1	3	No	No
Carbidopa	28860-95-9	Antiepileptics	Used in treatment for Parkinson disease	74,0	3	No	No
Etoricoxib	202409-33-4	Psychoanaleptics	COX-2 selective inhibitor for treatment of pain	74,0	3	No	No
Moxonidine	75438-57-2	Antihypertensives	Treat high blood pressure (hypertension)	73,5	3	No	No
Risperidone	106266-06-2	Psycholeptics	Anti-psyhcotic	73,0	3	No	No
Oxycodone	76-42-6	Analgesics	Opiod	72,3	3	No	No
Misoprostol	59122-46-2	Drugs for acid related disorders	Also used to terminate pregnancies	72,1	3	No	No
Pregabalin	128013-69-4	Antiepileptics	Anticonvulsant medication	72,0	3	No	No
Lamotrigine	84057-84-1	Antiepileptics	Anticonvulsant medication	71,6	3	Yes	No
Bumetanide	28395-03-1	Diuretics	Treat high blood pressure (hypertension)	71,0	3	No	No

Table 2 (*Contd.*)

Preferred name	CAS number	Therapeutic use	Comment – therapeutic use	Total score	Prioritisation group	Previously analysed in DK?	On the 4 EU watch list
Zonisamide	68291-97-4	Antiepileptics	Anticonvulsant medication	70,6	3	No	No
Olsalazine	15722-48-2	Antidiarrheals	Used to treat inflammatory bowel disease (IBS)	70,1	3	No	No
Ibandronic acid	114084-78-5	Antithrombotic agents	Treatment of osteoporosis	70,1	3	No	No
Naproxen	22204-53-1	Gynecological antiinfectives and antiseptics	Antifungal	69,9	3	Yes	No
Enalapril	75847-73-3	Agents acting on the renin-angiotensin system	Treat high blood pressure (hypertension)	68,8	3	Yes	No
Felodipine	72509-76-3	Calcium channel blockers	Treat high blood pressure (hypertension)	68,5	3	No	No
Desogestrel	54024-22-5	Sex hormones and modulators of the genital system	Female steroid hormone (synthetic)	68,1	3	No	No
Trandolapril	87679-37-6	Agents acting on the renin-angiotensin system	Treat high blood pressure (hypertension)	67,8	3	No	No
Anastrozole	120511-73-1	Endocrine therapy	Used to decrease estrogen levels (breast cancer)	67,6	3	No	No
Losartan	114798-26-4	Agents acting on the renin-angiotensin system	Treat high blood pressure (hypertension)	66,9	3	Yes	No
Atorvastatin	134523-00-5	Lipid modifying agents	Used to lower lipid levels	66,5	3	No	No
Clioquinol	130-26-7	Antibacterials for systemic use	Antifungal	66,3	3	No	No
Budesonide	51333-22-3	Antidiarrheals	Anti-asthmatic	65,5	3	No	No
Mirtazapine	61337-67-5	Psychoanaleptics	Anti-depressant	65,1	3	No	No
Mycophenolate mofetil	128794-94-5	Immunosuppressants	Immunosuppressant (prevent the rejection of organs)	65,1	3	No	No
Levocabastine	79516-68-0	Calcium channel blockers	Antihistamine	63,8	3	No	No
Risedronic acid	105462-24-6	Drugs for treatment of bone diseases	Treatment of osteoporosis	63,8	3	No	No
Droperidol	548-73-2	Anti-depressants	Anti-psyhcotic	63,8	3	No	No
Salicylic acid	69-72-7	Painkiller	Nonsteroidal anti-inflammatory drug (NSAID)	63,4	3	Yes	No
Conestat alfa	80295-38-1	Blood forming organs	Hemotological agent	61,8	3	No	No

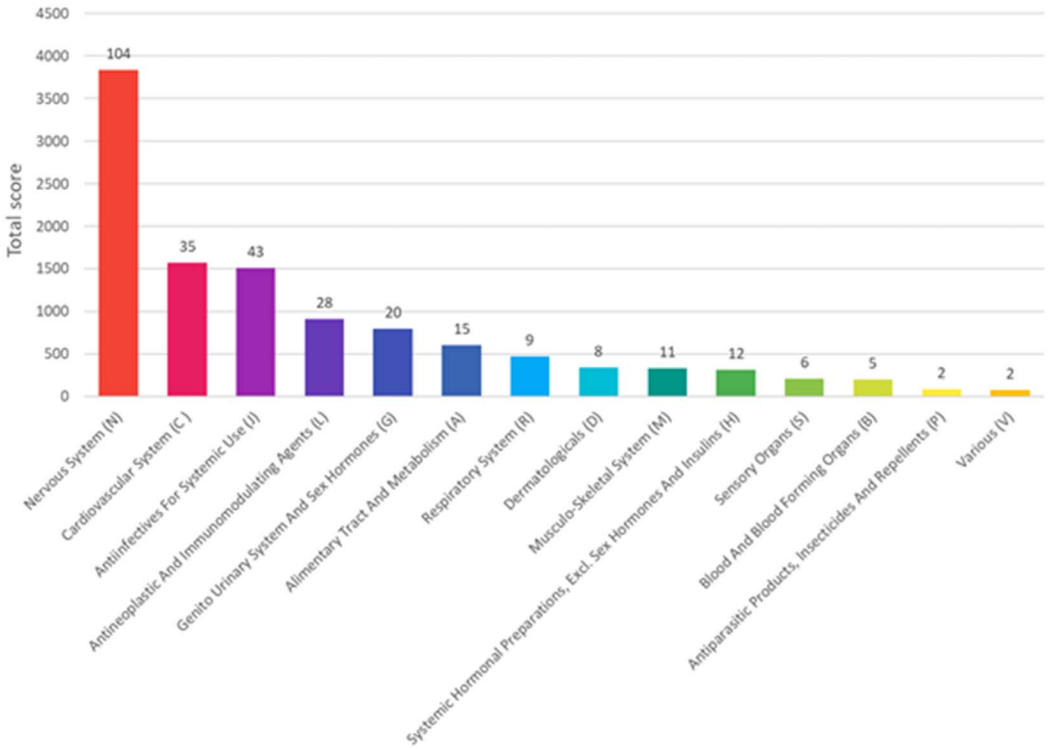

Fig. 3 Total score for APIs within each ATC code. The total number of entries for each ATC code is indicated above each respective bar.

waters. A list of all APIs included in the study and their scoring are presented in SI. The top-50 highest ranked APIs in Denmark have various therapeutical uses, such as antibiotics, treatment for high blood pressure, anticonvulsant agents, steroid hormones, as well as several different types of antidepressant and anxiolytic medicines. In total, 84% ($n = 42$) of the APIs appearing amongst the top-50 highest scorers have not previously been measured in Denmark, indicating that the application of the suggested prioritisation scheme into national monitoring programmes will provide valuable information regarding data gaps.

APIs of particular concern are the ones in bold (on the EU Commission's watch list of substances for Union-wide monitoring) and/or analysed in Danish waters in italic in group 1 with a score >100 (Trimethoprim). The second priority group 2 are the ones with a score >78 and the last priority group 3 with scores >61.8 in Table 2. For instance, metformin which is widely recommended as the first-line choice of pharmaceutical to be used to treat type II diabetes by lowering the blood glucose levels. Metformin as the highest ranked API it does not undergo any metabolism in the liver and is excreted unchanged *via* the urine to the wastewater system. Hence, metformin is one of the most commonly found drugs in aquatic environments and despite metformin does not have a hormone-like structure, it can affect the endocrine system in vertebrates *via* the steroid production in fish and other animals[31] thus causing impacts.

Moreover, from Tables 2 and, it can be concluded that the most abundant therapeutic uses are either pharmaceuticals used treat hypertension (20%, $n = 10$) such as sartans or anticonvulsants (14%, $n = 7$). With the exception of valsartan and enalapril, none of the APIs used to treat hypertension have been measured in Danish environmental samples. Moreover, four of the anticonvulsant APIs, pregabalin, lamotrigine and zonisamide, have not previously been reported as measured in Danish environmental samples.[32]

3.8 Groups of APIs of particular concern

In Fig. 3, the total score for the APIs within each ATC code is presented. From this bar chart it can be concluded that the ATC code group with the highest total score is the "nervous system" including both anti-depressants such as carbamazepine, gabapentin and the selective serotonin reuptake inhibitor (SSRI) citalopram, pharmaceuticals in the benzodiazepines (BZD) class, such as diazepam, and medicines for pain relief such as several different opioids (oxycodone, morphine and codeine). The second highest scoring group of APIs are pharmaceuticals used to treat illnesses regarding the cardiovascular system, such as the aforementioned pharmaceuticals used to treat hypertension such as metoloprol and losartan. Moreover, "antiinfectives for systemic use" including antibiotics such as clarithromycin and "antineoplastic and immunomodulating agents", such as chemotherapeutic pharmaceuticals are amongst the highest scoring groups of APIs. In general, these total scores are relatively well correlated with the number of entries for each ATC code.

3.8.1 Nervous system pharmaceuticals. One of the subgroups of pharmaceuticals belonging to the "Nervous system" ATC code is SSRIs. These antidepressants are highly

prescribed pharmaceuticals, globally and have previously been detected in various environmental compartments such as wastewaters as well as surface-, ground-, and drinking waters. SSRIs are potent compounds, and even low environmental concentrations have been shown to indirectly affecting survival in non-target organisms such as algae, plants and could be bioaccumulated in. The effects for fish, molluscs, and other aquatic invertebrates after exposure to SSRIs include delays in physiological development, a decrease in aggressiveness, and inhibition of feeding responses.[17]

Also assigned to the "Nervous system" ATC code are the benzodiazepines (BZDs) such as diazepam and midazolam (see Table 2). These APIs act as central nervous system depressants and are primarily used to treat anxiety but can also be used to treat other types of disorders such as seizures (carbamazepine, gabapentin) and alcohol withdrawal syndrome[33] BZDs are somewhat hydrophobic compounds found in wastewater effluent, due to continued release, as well as sediment for prolonged times. It has been shown that exposure to high aquatic concentrations of BZDs alters critical behaviours in wild fish, such as prolonging the appropriate response to predators.[34] Neither diazepam nor midazolam (see Table 2) have previously been analysed in Danish monitoring campaigns – they have been found in water in Germany and Sweden according to the UBA list.

Another large subgroup of pharmaceuticals assigned to the "nervous system" ATC code are the pain killing opioids oxycodone (see Table 2), morphine, tramadol and codeine, used for the treatment of acute and chronic pain. Moreover, there is also uncertainty around the illicit use opioids for recreational purposes in most countries, that this prioritisation scheme does not account for due to lack of reliable use statistics. Several different opioids have been measured in treated wastewater due to incomplete removal, however it has been suggested that the current monitoring methods are not sufficient enough to understand the environmental fate and possible transformation[35] Furthermore, it has been shown that some of the opioid metabolites or transformation products might, in some cases, have a greater ecotoxicity than the parent compound.[36] Some of the opioids (tramadol) have previously been reported as included in previous Danish monitoring campaigns.

3.8.2 Treatment of hypertension (high blood pressure). In terms of treatment of hypertension with pharmaceuticals, there are several different mechanisms of actions, such as diuretics, angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor blocker (ARB) and the beta-blockers. The use of pharmaceuticals to treat hypertension has nearly doubled in Western countries in the last two decades [and has been detected in hospital effluent as well as municipal wastewaters.[24,25,37] There is currently a large knowledge gap in terms of the ecotoxicological effect of most pharmaceuticals used to treat hypertension upon their release in the aquatic environment, with the exception of beta-blockers. Angiotensin receptor blockers such as the sartans losartan and irbesartan (see Table 2) have been implicated to affect various physiological functions in animals, such as cardiovascular regulation, metabolism, and growth.[37]

3.8.3 Antineoplastic pharmaceuticals. There are several different types of drugs used in chemotherapy with various modes of action. The common denominator for all these APIs is that they are cytotoxic, meaning that they at cellular level induce cell death (apoptosis) and/or inhibit cellular growth.[12] Due to this cytotoxic property, as well as an increased use due to a rising cancer incidence, antineoplastic pharmaceuticals have over the last decade been predicted to become one of the emerging chemical classes in ecotoxicology.[11] Yet, even after more than a decade, data on the occurrence of antineoplastics in the aquatic environment and their subsequent toxicity to aquatic wildlife are still relatively sparse in comparison with other pharmaceuticals such as antidepressants belonging to the SSRIs.[38] For instance, anastrozole (see Table 2) has previously been reported as analysed in Denmark.

4 Conclusions

The aim for this study was to develop a risk-based prioritisation scheme for APIs contaminating water in Denmark in support of a sustainable management of them. There are currently no systematic datasets investigating APIs available for neither wastewater nor surface water in Denmark, and the results from the prioritisation scheme shows that 84% of the top-50 scoring APIs have not previously been reported in Danish environmental monitoring campaigns. A key conclusion for future Danish API monitoring is the inclusion of API substances such as *e.g.* metformin that appear on the EU Commission's watch list and where there is a current use in Denmark but currently no monitoring in prioritization group 1 in Table 2.

There are five ATC classes of potential higher concern than others: "nervous system", "cardiovascular system", "anti-infectants for systemic use", "antineoplastics and immunomodulating agents" and "genito urinary system and sex hormones" (Fig. 3). Several of the top scorers in these classes have not been included in Danish monitoring campaigns until now.

All antineoplastic APIs, such as anastrozole and methotrexate, are despite their different modes of action highly cytotoxic. There is a trend for increased use of antineoplastics both globally and in Denmark due to increasing cancer rates and improved treatment, yet the adverse effects on the environment from these cytotoxic APIs requires further investigation. Neither of the high scoring anastrozole nor methotrexate in this prioritisation scheme have previously been reported as analysed in Danish environmental samples.

In this study, the focus is on investigating and prioritise the API (parent compound), and not any of the thousands of transformation products (metabolites) reported for medicines in environmental samples. However, we believe that an investigation into the development of a prioritisation scheme for metabolites, would be beneficial in the future prioritisation efforts.

Moreover, another data gap that needs to be further explored is to incorporate the statistics of medicines bought over the counter (OTC) in Denmark to the existing prioritisation scheme, in order to properly assess the exposure of for instance known environmental contaminants such as diclofenac. It is likely that

the sale of medicines containing this API is significantly underestimated when only using data from sale at pharmacies. Inclusion of veterinary pharmaceuticals in addition to the OTC drugs would complete the overview of the relative risk of APIs in surface waters and thus better inform the monitoring needs.

Author contributions

HS: Funding acquisition; conceptualization; writing – review and editing; formal analysis; methodology; LB: writing – review and editing; formal analysis; visualisation; PF: writing – review and editing; formal analysis; PNC: writing – review and editing; KB: writing – review and editing; MH: writing – review and editing; MYN: writing – review and editing; PL: writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

All data provided and used in the paper is fully accessible from the references. In addition, we have provided the whole database of the APIs as supplementary information (SI). See DOI: **https://doi.org/10.1039/d5su00120j**.

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References

1 EU, Regulation (EU) no 1235/2010 of the European Parliament and of the Council of 15 December 2010, 2010, Retrieved from **https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32010R1235**.
2 T. G. Bean and B. A. Rattner, *Health Care and Environ. Contam.*, 2018, **43**(3), 87–122.
3 D. J. Caldwell, F. Mastrocco, L. Margiotta-Casaluci and B. W. Brooks, *Chemosphere*, 2014, **115**, 4–12.
4 P. Grenni, V. Ancona and A. B. Caracciolo, *Microchem. J.*, 2018, **136**, 25–39.
5 J. P. Berninger and B. W. Brooks, *Toxicol. Lett.*, 2010, **193**, 69–78.
6 S. Zhou, C. Di Paoloa, X. Wuc, Y. Shao and T. B. Seiler, *Environ. International*, 2019, **128**(1), 1–10.
7 M.-C. Danner, A. Robertson, V. Behrends and J. Reiss, *Sci. Total Environ.*, 2019, **10**, 793–804.
8 A. Pruden, D. Larsson, A. Amézquita, P. Collignon, K. Brandt, D. Graham and Y. Zhu, *Environ. Health Perspect.*, 2013, **121**(8), 878–885.
9 EU 2924, **https://eur-lex.europa.eu/eli/dir/2024/3019/oj/eng**.
10 R. Gothwal and T. Shashidhar, *Clean:Soil, Air, Water*, 2015, **43**(4), 479–489.
11 A. Johnson, M. Jürgens, R. Williams, K. Kümmerer, A. Kortenkamp and J. Sumpter, *J. Hydrol.:X*, 2008, **348**(1–2), 167–175.
12 P. Nygren, *Acta Oncol.*, 2001, **40**(2–3), 166–174.
13 OECD, *Health at a Glance 2021: OECD Indicators*, OECD Publishing, Paris, 2021.
14 J. Argaluza, S. Domingo-Echaburu, G. Orive, J. Medrano, R. Hernandez and U. Lertxundi, *World J. Biol. Psychiatry*, 2021, **11**(10), 791–804.
15 D. J. Johnson, H. Sanderson, R. A. Brain, C. J. Wilson, K. J. Bestari and K. R. Solomon, *Regul. Toxicol. Pharmacol.*, 2005, **42**(3), 313–323.
16 L. J. Silva, M. P. Pereira, L. M. Meisel, C. M. Lino and A. Pena, *Environ. Pollut.*, 2015, **197**, 127–143.
17 D. G. Moreira, A. Aires and M. de Lourdes Pereira, *Comp. Biochem. Physiol., C*, 2022, **256**, 109322.
18 European Medicines Agency, Guideline on the Environmental Risk Assessment of Medicla Products for Human Use, 2006, Retrieved February 22, 2023, from **https://www.ema.europa.eu/en/environmental-risk-assessment-medicinal-products-human-use-scientific-guideline**.
19 US Food and Drug Administration, Guidance for Industry Environmental Assessment of Human Drug and Biologics Applications - FDA-1998-D-0278, 1998, **https://www.fda.gov/regulatory-information/search-fda-guidance-documents/environmental-assessment-human-drug-and-biologics-applications**.
20 European Medicines Agency, Questions and answers on 'Guideline on the environmental risk assessment of medicinal products for human use' - EMA/CHMP/SWP/44609/2010 Rev, 2016, **1**, Retrieved from, **https://www.ema.europa.eu/en/questions-answers-guideline-environmental-risk-assessment-medicinal-products-human-use**.
21 US Food and Drug Administration, Environmental Assessment: Questions and Answers Regarding Drugs With Estrogenic, Androgenic, or Thyroid Activity Guidance for Industry, 2016, Retrieved from **https://www.fda.gov/regulatory-information/search-fda-guidance-documents/environmental-assessment-questions-and-answers-regarding-drugs-estrogenic-androgenic-or-thyroid**.
22 H. Sanderson, P. Fauser, & K. Vorkamp, Prioritization of emerging contaminants for a Nordic screening study - Scientific Report from DCE – Danish Centre for Environment and Energy No. 446, 2021, Retrieved from **https://dce.au.dk/udgivelser/vr/nr-401-450/abstracts/no-446-prioritization-of-emerging-contaminants-for-a-nordic-screening-stud**.
23 US Food and Drug Administration, Guidance for Industry Environmental Assessment of Human Drug and Biologics Applications - FDA-1998-D-0278, 1999.
24 V. Kisielius, L. Äystö, T. Lehtinen, S. Kharel, M. Stapf, V. Zhiteneva and K. Bester, *J. Hazard. Mater.*, 2024, **476**, 134998.
25 F. Spilsbury, V. Kisielius, K. Bester and T. Backhaus, *Sci. Total Environ.*, 2024, **906**, 167440.

26 H. Sanderson, P. Fauser, L. Bengtström and K. Vorkamp, *RSC Sust.*, 2024, **2**(2), 558–566.
27 EC, Commission Implementing Decision (EU) 2022/1307 of 22 July 2022 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council, 2022, Retrieved from **https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2020.257.01.0032.01.ENG&toc=OJ:L:2020:257:TOC**.
28 H. Sanderson, *Curr. Drug Saf.*, 2012, **7**(4), 309–312.
29 BK nr 1274 af 27/11/2017, Bekendtgørelse om forhandling af håndkøbslægemidler uden for apotek, 2022, Retrieved from **https://www.retsinformation.dk/eli/lta/2017/1274**; BEK nr 1360 af 18/12/2012.Bekendtgørelse om fremstilling, indførsel og distribution af aktive stoffer til fremstilling af lægemidler, 2012, Retrieved from **https://www.retsinformation.dk/eli/lta/2012/1360**.
30 COWI 2021, **https://www.danva.dk/media/7775/medicinrester-i-spildevand-og-vandmiljoe_2021.pdf**.
31 E. P. Ambrosio-Albuquerque, L. F. Cusioli and A. A. Rosângela Bergamasco, *Environ. Toxicol. Pharmacol.*, 2021, **83**, 33460803.
32 Medicin.dk, 2023, Retrieved Feb 28, 2023, from, **https://pro.medicin.dk/Laegemiddelgrupper/Grupper/239010**; Middeldatabasen, 2023, Retrieved Feb 28, 2023, from, **https://middeldatabasen.dk/Chemical.asp?ChemicalID=715**.
33 T. Brodin, J. Nordling, A. Lagesson, J. Klaminder, G. Hellström, B. Christensen and J. Fick, *J. Toxicol. Environ. Health, Part A*, 2017, **80**(16–18), 963–970.
34 K. Demeestere, M. Petrović, M. Gros, J. Dewulf, H. Van Langenhove and D. B. Anal, *Anal. Bioanal. Chem.*, 2010, **396**(2), 825–837.
35 H. Hennies, E. Friderichs and J. Schneider, *Arzneim. Forsch.*, 1988, **38**(7), 877–880.
36 OECD, *Health Statistics*, 2019, Retrieved from **https://www.oecd-ilibrary.org/social-issues-migration-health/data/oecd-health–statisticshealth-data-en**.
37 K. Zhang, Y. Zhao and K. Fent, *Sci. Total Environ.*, 2020, **729**, 32361434.
38 A. Wormington, M. D. María, H. Kurita, H. B. Joseph, N. Denslow and C. Martyniuk, *Environ. Toxicol. Chem.*, 2020, **39**(15), 967–985.