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Are Newer Pharmaceuticals More Recalcitrant to Removal in Wastewater Treatment?

Jürg Oliver Straub¹, Julien Le Roux², Damien Tedoldi³

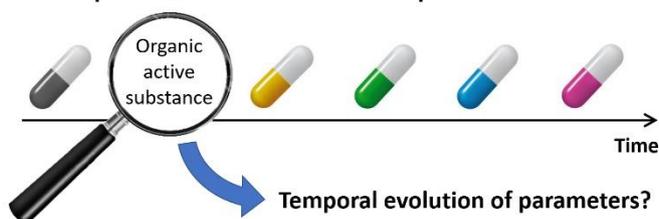
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Graphical abstract

Human pharmaceuticals from 1840 to present



Literature databases:
Experimental values
(248 pharmaceuticals)



EPI Suite™:
Modelled values
(1850 pharmaceuticals)

Over time:

- Molecular mass ↗
- Lipophilicity ↗
- Ready biodegradability ↘
- Removal in wastewater treatment plants ↗ (due to sorption to sludge)

Highlights

- mass, lipophilicity, biodegradability and removal of 1850 pharmaceuticals modelled
- corresponding experimental data for ≥ 248 pharmaceuticals was collated
- mass and lipophilicity globally increased over time, while biodegradability decreased
- Predicted removal in wastewater treatment increased, but only through adsorption
- EPISuite may be useful for environmental risk assessment of pharmaceuticals

Keywords. Pharmaceuticals – molecular mass – lipophilicity – ready biodegradability – wastewater treatment – removal – EPISuite

Abbreviations

API, active pharmaceutical ingredient; EPISuite, Estimation Programs Interface Suite (U.S.Environmental Protection Agency); ERA, environmental risk assessment; NRB, not readily biodegradable; OAS, organic active substance; RB, readily biodegradable; WWTP, wastewater treatment plant

1 Introduction

Pharmaceuticals in the environment have been a matter of ongoing scientific, public media and regulatory debate for the past 30-plus years (Straub & Hutchinson 2012). While only comparatively few active pharmaceutical ingredients (APIs) have been identified as posing a likely risk to environmental organisms in certain countries (Gunnarsson *et al.* 2019), many more have come under general suspicion of being environmentally hazardous and, in combination with sufficiently high concentrations and/or sufficiently long exposure in environmental compartments, causing risks as well (Küster & Adler 2014). In consequence, several jurisdictions, *e.g.*, the United States of America, the European Union and Switzerland, have developed guidelines for Environmental Risk Assessments (ERAs) for new pharmaceuticals as part of their regulatory registration package, while other countries, *viz.* Canada and Japan, are known to devise such guidance (Straub & Hutchinson 2012). At present, however, the necessity for such ERA information only relates to new APIs but not in general to existing, so-called ‘legacy’ APIs already on the market (U.S. FDA 1998, 2016; EMA 2015, 2018). Therefore, many experts are asking for environmentally relevant information to be developed for all APIs, in order to include legacy APIs into the considerations (*e.g.*, Küster & Adler 2014). On a political level, these requests have led to several APIs being added to the European Union Water Framework Directive Watch List (2022) based on potential PBT properties (environmental Persistence, Bioaccumulation, environmental Toxicity). The Watch List currently specifies sulfamethoxazole, trimethoprim, venlafaxine and O-desmethylvenlafaxine, several azole fungicides, clindamycin, ofloxacin, metformin and guanylurea to be monitored, quantified and reported on a regular basis in surface waters of all EU member states.

While more and more environmentally relevant information on APIs has come into the public scientific domain over the past two decades, from academic and pharmaceutical industry peer-reviewed publications, output of targeted research programmes, published pharmaceutical industry Safety Data Sheets or ERAs, the sheer number of APIs on the market is staggering. One of the best known, free public internet repositories on API information, DrugBank (Wishart *et al.*, 2017), listed 2730 different small-molecule APIs (*i.e.*, not including large, biotechnologically produced biologics or protein APIs) on June 21, 2021. Basic environmental data, even though mostly incomplete, *i.e.*, not covering both environmental fate and ecotoxicity to a satisfactory depth, may be available for approximately 300 APIs (iPiE*Sum 2019), meaning that for around 90% or more of all APIs in DrugBank there is insufficient data for even a very preliminary, but realistic, estimate of potential environmental risks.

Testing one small-molecule API according to the requirements of Phase I of the current European Medicines Agency Guideline for ERA of Human APIs (EMA 2015, 2018) needs experimental data elaborated according to the Organisation of Economic Co-operation and Development (OECD) Test Guidelines (TGs) (OECD TGs 2021) in accordance with Good Laboratory Practice (GLP) quality assurance (OECD GLP 2021). Human APIs are mainly excreted into sewage that (except in case of losses from the sewer system or combined sewer overflows) passes through a wastewater treatment plant (WWTP) whence it is discharged into surface waters. Therefore, the basic dataset focusses on the aquatic compartment and comprises at least the following properties: water solubility (OECD TG 105); lipophilicity (OECD TGs 107,

117 or 123); ad- and desorption to sediment and activated sludge (OECD TG 106); transformation in two aerobic water/sediment systems (OECD TG 308); activated sludge respiration inhibition (OECD TG 209); algal growth inhibition (OECD TG 201 with green algae, for antibiotic APIs to be performed with two different cyanobacteria); *Daphnia magna* reproduction (OECD TG 211); fish early life stage development test (OECD TG 210); and at least one chronic sediment toxicity test (several OECD TGs available). Note that this list does not encompass possible additional aquatic tests, *e.g.*, ready biodegradability (OECD TG 301 series) or wastewater treatment simulation (OECD TGs 303A or 314B), nor any specific soil fate or ecotoxicity tests nor bioaccumulation. However, just the above series of tests will need a minimum of 100 g of pure API with a certificate of analysis and an analytical method with a low limit of detection, *e.g.*, liquid chromatography–tandem mass spectrometry, which method needs to be set up and validated by the contract laboratory in accordance with GLP as well. At least two of these tests, OECD TGs 107 and 308, are usually performed with radio-labelled test material, which is expensive to buy or synthesise; however, without radio-labelled substance the analytical effort and expenditure may become prohibitive. The cost for one such series of tests may range between EUR 300,000 and 450,000, exclusive of radio-labelled test material and assuming no particular problems like very low water solubility and/or very strong adsorption – or endocrine activity –, all of which may increase testing effort and analytical costs considerably. Based on own, long-term experience, total costs for such a standard package for one single, ‘uncomplicated’ test substance, as quoted by competent, GLP-certified contract laboratories in Europe, would be between EUR 350,000 and 500,000. In addition, the time needed for processing such a series of tests is at the very least 6 months, more realistically around one year.

The costs and limited availability of GLP-certified contract laboratories shows that the demand for developing basic environmental data for all APIs on the market, or at least for those collated in DrugBank, is unrealistic. To process even 2000 legacy APIs at an assumed average cost of EUR 400,000 would cost about EUR 800 million. In addition, it would take at least 1500 lab-years to finish this work, on top of other, possibly more urgent tasks for priority substances like new APIs, veterinary drugs, agrochemicals, household chemicals and production intermediates, all of which need contract lab work as well.

In view of limited resources for testing, therefore, intelligent means of prioritising APIs are clearly needed, so that in the end only realistic candidates for potentially risk-causing APIs need be tested. New conceptual models and priority lists of APIs have been developed and published over the past two decades (*e.g.*, Huggett *et al.*, 2003; De Voogt *et al.*, 2009; Guillén *et al.*, 2012; Roos *et al.*, 2012; Oldenkamp *et al.*, 2013; Caldwell *et al.*, 2014; Kostich *et al.*, 2014; Burns *et al.*, 2018; Gunnarsson *et al.*, 2019), which are often based on use amounts, ecotoxicity, mode of action and/or bioaccumulation, beside additional criteria. These lists are of high value as they point out candidates for further environmental investigation. However, other substance-specific properties may be somewhat neglected, particularly removal in WWTPs (typically defined as the difference between inflow and outflow of a WWTP, divided by the inflow). Kümmerer pointed out repeatedly (*e.g.*, Kümmerer 2007; Kümmerer & Schramm 2008) that APIs that are removed in WWTPs will not reach the aquatic environment, which decreases the exposure of and thereby the potential risk to aquatic organisms. The current European Union ERA Guideline (EMA 2015) recognises Kümmerer’s

postulate insofar as APIs that are shown to be readily biodegradable (RB) do not require additional environmental fate testing in a sediment/water study (OECD TG 308) (OECD TG 2021); this can only mean that the substance is expected to be reliably removed in WWTPs and that it will not reach surface waters in sufficient amounts to be of concern. Therefore, information on biodegradability and removability in WWTPs is highly important in refining – and reducing – the API priority lists.

Long-term experience of the first author, while working as the environmental risk assessor for an international pharmaceutical company for 21 years, with existing and new, company-internal and peer-reviewed published, scientific environmental data for APIs, suggests that more recent APIs are less easily biodegradable than older ones and that the former are therefore removed to a lesser extent in WWTPs. Preliminary data to support this notion are provided by searching the quality-vetted, public iPiE*Sum (Intelligence-led Assessment of Pharmaceuticals in the Environment, a research European project co-sponsored by the European Union and the European pharmaceutical industry) database (iPiE*Sum 2019) for RB APIs: out of 6 RB APIs in iPiE*Sum, four (acetylsalicylic acid, hydrocortisol, prednisolone and dexamethasone) were first registered or put on the market between 1899 and 1958, while only two were registered later, cefepime in 1996 and abiraterone in 2011 (registration data from DrugBank database) (Wishart *et al.*, 2017). On the other hand, in a recent group of APIs, *viz.* 52 anti-cancer protein kinase inhibitors all first registered between 2001 and 2020 (Wishart *et al.*, 2017), all 9 out of 9 APIs with a reported biodegradability test result are not readily biodegradable (NRB) (iPiE*Sum 2019). Obviously, both of these results are only plausibility statements, but they do suggest that recent APIs may indeed be less biodegradable and they call for further investigation.

Therefore, the idea was developed to create a reasonably complete list of organic small-molecule APIs (as inorganic ones do not biodegrade and their sorption may be difficult to model) and to use experimental data where available, plus modelling data, to predict removal in WWTPs as well as other properties that inform on environmental fate. The working hypothesis for this investigation was that more recent APIs are indeed less well biodegradable and removed to a lesser extent in WWTPs than older APIs.

2 Materials and Methods

2.1 Data Sources and Revision

DrugBank (Wishart *et al.*, 2017) was selected for an initial list of small-molecule APIs. In browsing mode (<https://go.drugbank.com/drugs>), the single APIs are listed with name, molecular mass, summary formula, structure, description and categories, at 25 APIs per page; the name is a hyperlink that leads to the full entry for the API in question. As a free download of the above data for all the small-molecule APIs was not available (S. Swanek of DrugBank, pers. comm. by e-mail to JOS, 24 July 2020), single pages of 25 APIs each were serially copied into a Libre Office Calc (2020) spreadsheet until the whole dataset was downloaded manually. One peculiarity of the DrugBank browsing mode is that molecular mass and summary formula are placed in the same cell, meaning that these two properties had to be separated by hand into two different cells for each API after the download.

The initial list of APIs was reduced and simplified as follows. Many of the APIs in the full list are inorganic salts; as this investigation only addresses organics, all of the pure inorganics were deleted from the list. Some APIs contain metals that cannot be modelled by the common, free quantitative structure-property software Estimation Programs Interface Suite (EPISuite™) v4.11 (U.S. EPA 2017), *e.g.*, carboplatin containing platinum, or aurothioglucose containing gold; such substances had to be removed from the list. Prodrugs were eliminated as well, as these are not excreted but (as a simplified assumption) only the actual active metabolite is; the latter was newly entered into the spreadsheet if not already contained. Similarly, radio contrast agents were deleted as they are diagnostic substances that have no remarkable pharmacological or ecotoxicological activity (*e.g.*, Steger-Hartmann *et al.*, 1999, 2001; Nowak *et al.*, 2020). Redundancies, *e.g.*, different salts of the same organic moiety, were reduced to a single entry of that organic moiety. Last, nutritional supplements, peptides including glycopeptides, oligomers and polymers were eliminated from the spreadsheet as well. This whole procedure left just the actual organic active substances (OASs).

The resulting spreadsheet was then supplemented with all or part of the following data per OAS depending on their availability. Chemical Abstracts Services (CAS) number as a unique identifier; Simplified Molecular Input Line Entry Specification (SMILES) code for simple modelling; experimental *n*-octanol/water partition coefficient ($\log K_{ow}$); calculated/modelled $\log K_{ow}$; experimental biodegradation information; calculated/modelled biodegradability; predicted WWTP removal; and year of regulatory API approval or start of marketing. The additional data were collated from the following public web sources: DrugBank (Wishart *et al.*, 2021) single API pages, specifically the Chemical Identifiers and References sections, the latter with direct hyperlinks to ChemSpider (Royal Society of Chemistry 2021) information for the API in question, including in many cases modelling results plus some empirical data from EPISuite (U.S. EPA 2017); DrugCentral (2021); PubChem (2021); EU Reference Dates list (EURD 2021); vetted data from the iPiE*Sum database (iPiE*Sum 2019); European Union Chemicals Agency (ECHA 2021) data for biodegradability; as well as abbreviated ERAs and Safety Data Sheets published by some pharmaceutical companies, *viz.* AstraZeneca (2021), GlaxoSmithKline (2021) and F.Hoffmann-La Roche (2021a, b). In some very few cases, no information was found at all and EPISuite modelling proved impossible; such OASs were also eliminated from the final spreadsheet, which formed the basis for the evaluation of the OASs.

2.2 Statistics

In order to investigate the existence of a temporal evolution in the reported parameters (*e.g.*, molecular mass or $\log K_{ow}$), the dataset was split into time series groups to allow for comparisons. As most OASs that are still in use were registered after 1950, all OASs registered between 1840 and 1950 were grouped together, then successive 20-year periods were considered from 1950 to 2010, and the last group corresponded to the current period (2010–2022), resulting in five time series groups.

Since several parameters were found not to be normally distributed (Shapiro-Wilk and Lilliefors tests), non-parametric statistics were preferred over parametric statistics. The five groups were first compared globally with a Kruskal-Wallis test, then comparisons between two consecutive time series groups were

carried out using a Wilcoxon-Mann-Whitney test. The statistical model on which these tests are built assumes independent data and states that the distributions of the considered variable among the groups differ by a certain location shift δ , which under the null hypothesis is equal to zero (*i.e.*, all groups originate from the same parent distribution); in case of two variables X_1 and X_2 , the model may be formally stated as follows (equation 1):

$$F_{X_1}(x) = F_{X_2}(x + \delta) \quad (1)$$

where F_{X_i} is the cumulative distribution function of the variable X_i .

It was presently chosen *not* to interpret the results of these statistical tests in a dichotomous way, *i.e.*, concluding a “significant” or “non-significant” shift depending on the p -value of the test, but rather to use the p -value as a continuous indicator of the compatibility between the data and the aforementioned statistical model, as suggested *e.g.* by Greenland *et al.* (2016) and Amrhein, Greenland and McShane (2019). The smaller the p -value, the more unusual the observations would be if the location shift were indeed equal to zero under the model assumptions. Whenever a p -value was estimated to be lower than 10^{-6} , it was reported as “ $p < 10^{-6}$ ” considering both the larger uncertainty and low practical interest in the exact value.

When applying a Wilcoxon-Mann-Whitney test for the comparison of two variables X_1 and X_2 , different values of the location shift δ may be considered, besides zero. Testing alternative values leads to multiple comparisons between $X_1 + \delta$ and X_2 , to each of which a p -value can be attributed. This approach provides (1) a *point estimate* of δ , such that the comparison would lead to the highest p -value, being therefore most compatible with the data (under the model assumptions); and (2) a *95% confidence interval*, corresponding to the range of values that produced $p > 0.05$ (Greenland *et al.* 2016). These complementary indicators were calculated for each comparison and are presented as Supplementary Information.

Boxplots were used for most visualisations of the data. On these graphs, the thick central line represents the median value of the distribution and the two sides of the box represent the 25th and 75th percentiles; however, due to the skewedness of most distributions, the usual criteria for identifying outliers were not appropriate, hence it was chosen to represent the min and max values by the ends of the whiskers. The 90th and 95th percentiles were also displayed on the graphs using coloured lines.

Several proportions were estimated (*e.g.*, the proportion of RB molecules or the proportion of molecules with a logK_{ow} value higher than a certain threshold) among N compounds under consideration. For each proportion, a 90% confidence interval was computed by considering the smallest and greatest values of the probability q such that the number of molecules with the adequate property belongs to the 90% prediction interval of a binomial distribution $\mathcal{B}(N, q)$. All statistical analyses were performed using the R software (R Core Team, 2021).

3 Results

3.1 Final spreadsheet

The initial spreadsheet, completed on 24 August 2020, comprised 2730 APIs (not shown). Reducing this by deleting inorganics, eliminating radio contrast agents and multiples, and replacing prodrugs with their active metabolites, resulted in 1915 OASs (not shown). Finally, removing nutritional supplements, peptides,

oligomers and polymers, left a final OAS spreadsheet of 1850 substances (Supplementary Information) with a structure as detailed in Table 1.

Most OASs do not have a complete record. While identifiers were found for all, experimental data were much harder to trace. For experimental $\log K_{ow}$ values, many were available indirectly from Lab Network data (obtained from DrugBank via links to ChemSpider, which often cites Lab Network; Lab Network itself is not free), from literature as cited in EPISuite (U.S. EPA 2017) or from pharma company data (AstraZeneca 2021; GSK 2021; F.Hoffmann-La Roche 2021a) plus iPiE*Sum (2019). These experimental $\log K_{ow}$ values were separated into two columns, Lab Network and other experimental data, as on filling in the spreadsheet it was suspected that the many Lab Network data might be systematically different from the literature $\log K_{ow}$ values (see also Fig. S1). In case more than one $\log K_{ow}$ was located per column, the geometric mean of the values was entered, except in those rare cases where the $\log K_{ows}$ ranged from negative to positive values, where the arithmetic mean was used. Calculated $\log K_{ow}$ values were uniquely taken from EPISuite, mostly via DrugBank hyperlink to ChemSpider, which gives summary EPISuite reports for many OASs; in case of missing reports, EPISuite v4.11 (U.S. EPA 2017) was run without modifications with the corresponding SMILES code, resulting in 311 (16.8% of total OASs) additional calculated $\log K_{ow}$ values, beside biodegradation and WWTP removal predictions. In three cases, mostly charged organic moieties, no values were obtained as EPISuite could not interpret the SMILES. Some few of the calculated results presented improbably low, or high, $\log K_{ow}$ values, ranging from -16.60 for pentosan polysulfate (ionised) to 23.74 for ubiquinol. As the OECD TGs for determining the $\log K_{ow}$ (OECD TGs 107, 117 and 123) (OECD TG 2021) have a combined validity range from -2 to 8.4 , it was arbitrarily decided to eliminate calculated $\log K_{ow}$ values deviating more than 3 log units from these limits, *i.e.*, OASs with $\log K_{ow} < -5$ ($n = 35$ removed) or $\log K_{ow} > 11.4$ ($n = 7$ removed), from the list.

Experimental ready biodegradability data were taken from iPiE*Sum (2019) or from pharmaceutical company data (AstraZeneca 2021; GSK 2021; F.Hoffmann-La Roche 2021a) in the form of readily biodegradable (RB) or not readily biodegradable (NRB), according to the criteria of the particular OECD TG 301 series test guideline (OECD TG 2021) that was used. As it was noted that many ‘commodities’ (simple alcohols, sugar alcohols, simple acids, amino acids, sugars, *etc.*) were contained in the final list, the ECHA (2021) online database was consulted for RB information for these compounds. In case no ready biodegradability but inherent biodegradability (OECD TG 302 series) or WWTP simulation test (*e.g.*, OECD TGs 303A or 314B) (OECD 2021) results were available, any non-biodegradability in these tests was interpreted as NRB in view of higher inoculum concentrations and lower test substance concentrations in these latter tests. In the ECHA (2021) database, several compounds are described as RB based on modelling, *e.g.*, by EPISuite, or on read-across from structurally similar compounds; such data were not accepted as true RB but were marked ‘weight of evidence’ in the final list and they were not used for drawing conclusions. Calculated biodegradability was uniquely taken from the ‘Ready Biodegradability Prediction’ from EPISuite (U.S. EPA 2017), which contains several models to predict the biodegradability of substances; only if all five aerobic and one anaerobic degradation models concur will a compound be characterised as RB. The predicted WWTP removal was copied from the EPISuite summary reports.

Lastly, the year of registration or introduction was taken from DrugBank (Wishart *et al.*, 2017), DrugCentral (2021) or the EU Reference Dates (2020). In those cases where no year could be found, the search was widened to a general web search using Google, but even then, for 209 (including commodities like acetic acid, ethanol, guaiacol, propyl alcohol, sucrose, urea, *etc.*) no year could be located.

Table 1. Structure of the final spreadsheet of active organic substances with main references per property.

Property	Main References
Name	DrugBank (Wishart <i>et al.</i> , 2017)
Molecular formula	DrugBank (Wishart <i>et al.</i> , 2017); PubChem (2021)
Molecular mass, Da	DrugBank (Wishart <i>et al.</i> , 2017); PubChem (2021)
CAS number	DrugBank (Wishart <i>et al.</i> , 2017); ChemSpider (Royal Society of Chemistry, 2021); PubChem (2021)
SMILES code	DrugBank (Wishart <i>et al.</i> , 2017); ChemSpider (Royal Society of Chemistry, 2021); PubChem (2021)
logK _{OW} , experimental ¹	DrugBank (Wishart <i>et al.</i> , 2017) → ChemSpider → Properties → Lab Network
logK _{OW} , experimental ²	DrugBank (Wishart <i>et al.</i> , 2017) → ChemSpider → Properties → EPISuite; EPISuite (U.S. EPA, 2017); iPiE*Sum (iPiE*Sum, 2019); Pharma company data (AstraZeneca, 2021; GlaxoSmithKline (GSK, 2021); F.Hoffmann-La Roche (2021a, 2021b)
logK _{OW} , calculated	DrugBank (Wishart <i>et al.</i> , 2017) → ChemSpider → Properties → EPISuite; (U.S. EPA, 2017)
Biodegradability, experimental	iPiE*Sum (2019); Pharma company data (AstraZeneca, 2021; GlaxoSmithKline (GSK, 2021); F.Hoffmann-La Roche (2021a, 2021b); ECHA (2021)
Biodegradability, calculated	DrugBank (Wishart <i>et al.</i> , 2017) → ChemSpider → Properties → EPISuite; EPISuite (2017)
WWTP removal, predicted, %	DrugBank (Wishart <i>et al.</i> , 2017) → ChemSpider → Properties → EPISuite; EPISuite (2017)
Year of registration/introduction	DrugBank (Wishart <i>et al.</i> , 2017); Drug Central (2021); EU Reference Dates (EURD List, 2020)

CAS = Chemical Abstracts Services; ECHA = European Chemicals Agency; logK_{OW} = decadic logarithm of *n*-octanol/water partition coefficient; SMILES = Simplified Molecular Input Line Entry Specification; → = direct, clickable link from the source on the left side to the next source of data on the right side of the arrow.

^{1,2} Experimental logK_{OW} values were separated into two columns: (1) Available Lab Network data only (obtained from DrugBank via hyperlink to ChemSpider); and (2) logK_{OW} values from literature as cited in EPISuite or pharma company data including iPiE Sum; please see text (chapter 3.1) for explanation.

3.2 Year of introduction or registration of organic active substances vs. molecular mass

The final list contains a total of 1850 substances, of which Fig. 1 shows 1641 OASs vs. their year of registration or introduction. In all, 11 OASs that are still in use go back into the 19th century (Fig. 1a), the oldest of which, chloral hydrate, dates from 1840, followed by cocaine, ouabain, amyl nitrite, hyoscyamine, physostigmine, paraldehyde, diamorphine (= heroin), aminophenazone, salicylic acid and acetylsalicylic acid. Comparatively few OASs are listed from the first four decades of the 20th century, but from around 1940 the number introduced per year increases, from 1950 onwards even quite strongly, as evidenced by the density of the cloud of crosses in Fig. 1a (note that the most recent new OAS in the graph dates from 2021, due to completing and supplementing the initial list over several months).

Considering statistical distributions of molecular masses for the different time groups (as explained in section 3.1 and displayed in Fig. 1b), it appeared that the main parameters describing these distributions also displayed a global rising trend over time. For example, among all OASs registered before 1950, the median molecular mass was 245 Da, the 75th percentile amounted to 300 Da, and the 90th percentile to 372 Da, while these parameters rose to 440, 525, and 680 Da, respectively, for the current period (2010–2022). The heaviest 25 OASs in the present list, all of which have a molecular mass higher than 1000 Da, were introduced after 1960, and 15 of them were introduced after 1995 – the maximum being sugammadex registered in 2015 with ~2002 Da.

The comparison was further developed via statistical testing. First, a Kruskal-Wallis test strongly suggested the existence of a shift between (at least) two groups, with $p < 10^{-6}$. Subsequently, the Wilcoxon-Mann-Whitney test was applied to compare the distributions of molecular masses between successive periods (1840–1950 vs. 1950–1970, *etc.*): all comparisons were characterized by a p -value between $3 \cdot 10^{-4}$ and $< 10^{-6}$. For each comparison, testing different values for δ (as described in section 2.2) led to a point estimate and a 95% confidence interval which are presented in table S1. These intervals contained only positive values ranging from 13 to 85 Da, indicating that the most recent distribution was systematically shifted upwards. The results therefore support the aforementioned observation that molecular masses of OASs tended to rise over time.

Both increases over time, in number and average mass, may reflect two different aspects: on one hand, a deepening scientific understanding of molecular biological and pathophysiological processes and a huge accretion of pharmacological knowledge from the first half of the 20th century onward, leading to more targeted APIs; on the other hand, potentially the important increase from the rise of antibiotics and possibly also from additional, ‘me-too’ APIs, which are APIs with the same or similar mode of action compared with the first API in that class, that have subsequently been developed and registered by different companies. One example, already mentioned in the Introduction, are the 52 anti-cancer protein kinase inhibitors all first registered after the year 2000 in Drug Bank (Wishart *et al.*, 2017). Another may be a class of antibiotics, *viz.* the β -lactams characterised by the β -lactam double ring fragment, which from the original, natural penicillin discovered by Fleming in 1928 (Lalchandama 2020) to the recent ceftobiprole registered in 2018 (MHRA 2021) contain over 100 APIs (DrugBank) (Wishart *et al.*, 2017).

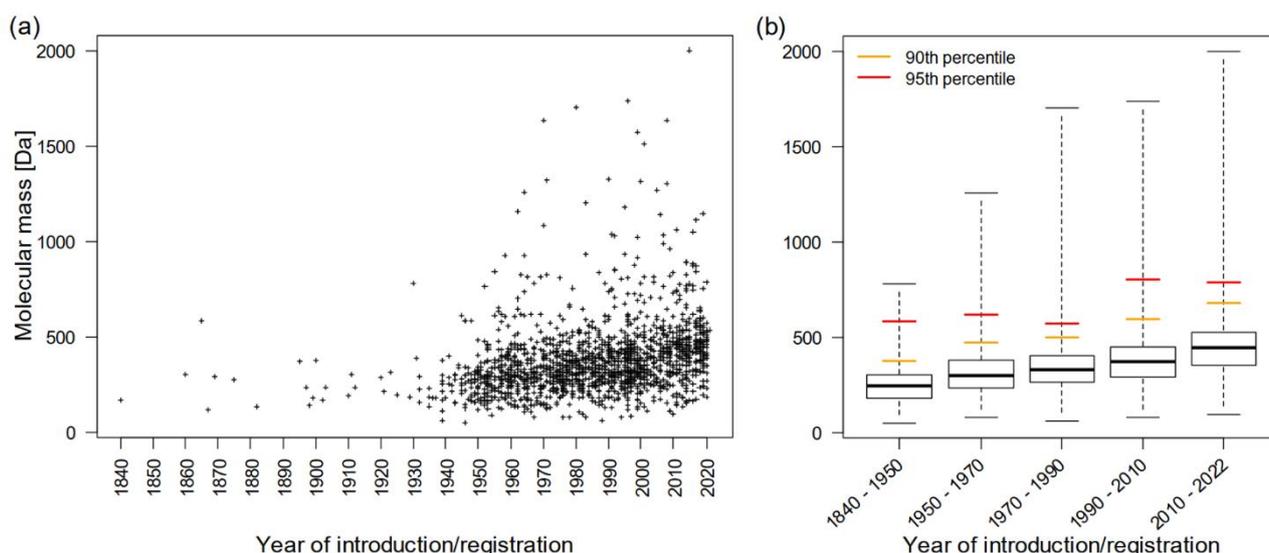


Fig. 1. Year of first registration or introduction to the market *versus* molecular mass: (a) 1641 organic active substances; (b) dataset split into time series groups (see text), with quantiles. Crosses = single substances. In fig. 1b: thick line = 50th percentile or median; lower and upper borders of rectangle = 25th and 75th percentiles; yellow whisker = 90th percentile; red whisker = 95th percentile; grey whiskers = minimum and maximum values.

3.3 Year of introduction/registration vs. *n*-octanol/water partition coefficient

An increasing trend in the molecular mass of OASs over time may also be reflected by increasing $\log K_{ow}$ values, as suggested by Fig. 2. These graphs were elaborated using values calculated by EPISuite (2017), as they form the most complete dataset ($n = 1809$ excluding values below $\log K_{ow} -5$ and above $\log K_{ow} 11.4$, see section 2.1, Methods). Correspondences between these predicted values and the experimental data, either from Lab Network or from other databases, are presented as Supporting information (figures S2 and S3) and further discussed in section 4.2.1. Fig. 2 depicts both (a) the statistical distributions of $\log K_{ow}$ values for the different time groups – on the same principle as Fig. 1b for molecular mass – and (b) the proportion of values among these groups that exceed the respective thresholds of 2, 3 and 4.

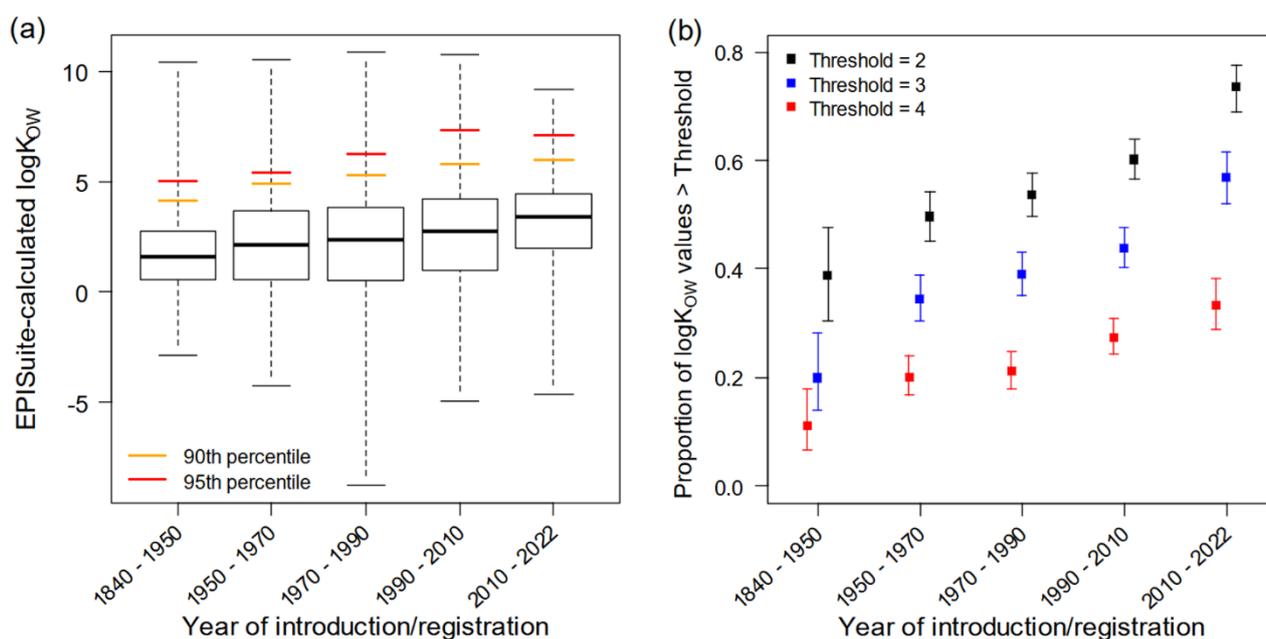


Fig. 2. Year of first registration or introduction to the market *versus* calculated *n*-octanol/water partition

coefficients ($\log K_{OW}$) of organic active substances: (a) dataset split into time series groups (see text), with quantiles; (b) Proportion of $\log K_{OW}$ values higher than 2, 3 and 4 in each time series group. 2a: Thick line = median; lower and upper borders of rectangle = 25th and 75th percentiles; yellow whisker = 90th percentile; red whisker = 95th percentile; grey whiskers = minimum and maximum values. 2b: Black symbols = $\log K_{OW}$ threshold 2; blue symbols = $\log K_{OW}$ threshold 3; blue symbols = $\log K_{OW}$ threshold 4; squares = average proportions; whiskers = 90% confidence interval.

Comparing the time series groups of $\log K_{OW}$ values, the medians, 75th and 90th percentiles of the groups appeared to increase monotonously, from 1.6, 2.8 and 4.1, respectively, before 1950, to 3.4, 4.5 and 6.0, respectively, after 2010. Similar to the previous observation with molecular masses, a Kruskal-Wallis test provided a strong indication of a shift in $\log K_{OW}$ distributions between (at least) two groups ($p < 10^{-6}$). Subsequently, Wilcoxon-Mann-Whitney tests were applied to investigate differences between two consecutive data groups (table S2). Comparisons between $\log K_{OW}$ distributions in the earlier periods (*i.e.*, 1840–1950 *vs.* 1950–1970 and 1950–1970 *vs.* 1970–1990) were characterized by p -values of 0.087 and 0.275, respectively; considering 95% confidence intervals, a shift ranging from a negative albeit relatively small value to a positive increase higher than $\frac{1}{2}$ log unit, appeared compatible with the data. Moving to more recent periods (*i.e.*, 1970–1990 *vs.* 1990–2010 and 1990–2010 *vs.* 2010–2022), the observed differences became less compatible with the hypothesis of same parent distributions ($p = 0.047$ and $1.6 \cdot 10^{-3}$, respectively). Additionally, when comparing the periods separated by two decades (1840–1950 *vs.* 1970–1990, 1950–1970 *vs.* 1990–2010 and 1970–1990 *vs.* 2010–2022), a rise in $\log K_{OW}$ values may be inferred with more certainty ($p = 0.011$, $2.3 \cdot 10^{-3}$ and $< 10^{-6}$ respectively). In other words, even if the incremental increase between two consecutive periods is not always entirely distinguishable from the internal variability of $\log K_{OW}$ values, it becomes clear when the whole trend is considered. The progression is also highlighted by the proportion of $\log K_{OW}$ values greater than 2 – rising from $39 \pm 9\%$ before 1950 to $74 \pm 4\%$ after 2010 – or any other $\log K_{OW}$ threshold from 2 to 4 (Fig. 2b).

3.4 Year of introduction/registration *vs.* removability in wastewater treatment

Removability of a given substance in a WWTP is defined as the influent concentration minus the effluent concentration, divided by the influent concentration; it is generally given as a percentage. EPISuite (U.S. EPA 2017) predicts the removal of a chemical in a typical activated sludge-based sewage treatment plant. Values are given for total removal and three processes that may contribute to removal: sorption to sludge, air stripping for volatile compounds and biodegradation. The program assumes a standard system design and set of default operating conditions. Depending on the substance properties, all three processes may play a role in WWTPs, hence EPISuite combines them as (1) total sludge adsorption plus (2) total volatilisation plus (3) total biodegradation, resulting in its prediction of total removal in WWTPs.

3.4.1 Sludge adsorption

Adsorption to organic particles in primary, in activated aerobic, potentially in anaerobic, and in settling sludge, depending on the type of WWTP, is characterised by the sludge adsorption constant (K_d) and the organic-carbon adsorption constant (K_{OC}). EPISuite has two algorithms for calculating a $\log K_{OC}$, the second starting from the $\log K_{OW}$ (taken from internal database, user-entered, or calculated), with corrections for molecular fragments. One common substitute to calculate both constants is the $\log K_{OW}$, where a higher

$\log K_{ow}$ (*i.e.*, increased lipophilicity) generally implies higher sorption. Thus, WWTP removal predictions rely heavily on lipophilicity.

3.4.2 Volatilisation

Volatilisation is not an important removal process for most OASs, as they are generally designed to remain in the patient's body and be excreted either with urine, meaning they are (or can be rendered) better water soluble, or through the faeces, meaning they are more lipophilic. Only few OASs are eliminated through the lungs, which in turn decreases the fraction excreted in urine or faeces. Still, for a few compounds, volatilisation in the aeration basin of a WWTP may be the primary removal process.

3.4.3 Ready biodegradability

There are two main sources of data for biodegradability: on one hand, the EPISuite predictions; on the other hand, experimental biodegradability data from iPiE*Sum (2019), pharma company Safety Data Sheets or ERAs (AstraZeneca 2021; GSK 2021; F.Hoffmann-La Roche 2021a). Out of the final list of 1850 OASs, EPISuite calculated the biodegradability of 1848 OASs, of which 162 (8.8%) were predicted to be RB and 1686 (91.2%) were predicted to be NRB. The RB percentage appears to be increased by the biodegradability of old 'commodities' like simple alcohols, acids, sugars, sugar alcohols or amino acids, which may not have been tested by pharma companies for their more recent registrations, in view of ERA requirements only going back to the late 1980s or 1990s (Straub & Hutchinson 2012). However, out of the 1850 OASs, the ECHA (2021) database inventories at least 81 such commodities with RB information and, indeed, out of those 81 compounds 36 (44%) are RB, significantly contributing to the overall RB share. Among those 36 RB commodities, EPISuite predicted 31 correctly to be RB, but 5 to be NRB. If the former 31 RB commodities are not included in the set of predicted RB compounds, there remain 131 RB OASs out of a remaining total of 1817, which corresponds to 7.2% of the total EPISuite-modelled substances. Thus, EPISuite predicts between 8.8% and 7.2% RB in the final OAS list, depending on the inclusion or not of commodities.

Experimental data were available for 248 OASs in the final list of 1850, of which 8 were weight of evidence, *i.e.*, marked RB in the ECHA (2021) database but without solid support by an experimental test and therefore not accepted here. Of the remaining 240 OASs, 44 (18.3%) were RB and 196 (81.7%) were NRB. Again, if the commodities are not accounted for (36 RB compounds among them), this leaves 204 non-commodity OASs, of which 8 (3.9%) are RB and 196 (96.1%) are NRB. Hence, the smaller set of experimental biodegradability data finds between 18.3% and 3.9% RB out of a total of 240 OASs, depending on the inclusion of commodities or not. The experimental range encompasses the EPISuite-predicted range. Both the upper and the lower figures for experimental data may be somewhat skewed by some compounds that are borderline RB, as some may have been accepted as RB in the ECHA (2021) database without matching the strict OECD TG 301 (OECD 2021) 10-day-window criterion, while others may have been designated NRB for not strictly meeting this criterion. Still, on the conservative side, it emerges that among current small-molecule OASs, between 3.9% and 7.2% may be expected to be RB.

Comparing the year of introduction or registration of an API or OAS with its experimental and predicted RB property suggests that for both the share of RB compounds is decreasing over time (Fig. 3). For each

period of time, the share of NRB molecules (among those for which biodegradability data was available) was calculated, considering both experimental data (blue symbols) and EPISuite-predicted characteristics (black symbols). A 90% confidence interval on each proportion was computed according to the procedure detailed in section 2.2. The large blue intervals for the first two periods are related to the scarcity of experimental data for the OASs introduced during these two periods ($n = 10$ for 1840–1950 and $n = 26$ for 1950–1970) leading to a higher uncertainty. The average share of NRB biodegradable compounds tends to increase monotonously with time, although the differences after 1970 are less marked than for the $\log K_{OW}$ values. Part of this trend may be explained by the relatively high share of commodities, which tend to have been introduced earlier than the majority of non-commodity OASs.

Looking at molecular mass in comparison with experimental RB properties shows a trend as well (Fig. 4a). As might be expected, smaller organic molecules are on the whole better biodegradable than bigger ones, confirming the results of Boethling *et al.* (2007). Also in this comparison, commodities may be crucial for the trend, as on the whole they have smaller molecular masses than the majority of OASs (not shown). RB compounds also show noticeably lower experimental $\log K_{OW}$ values compared to NRB molecules (Fig. 4b).

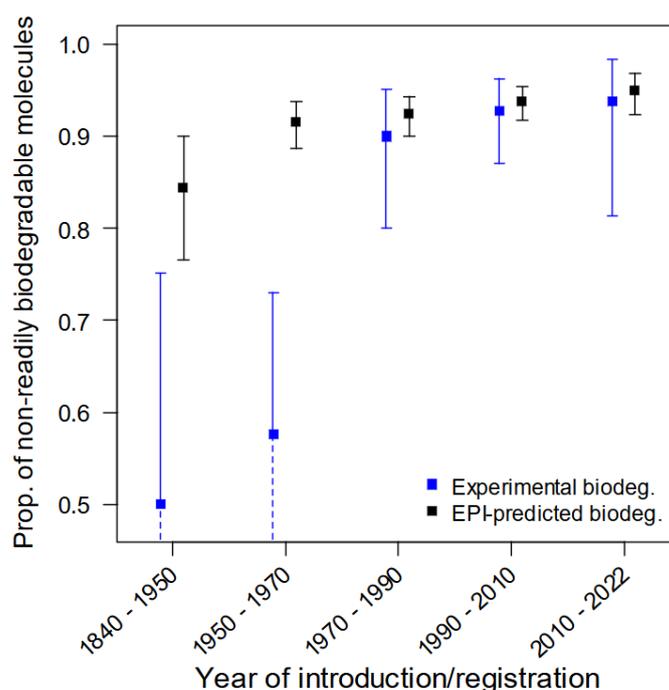


Fig. 3. Year of introduction or registration *versus* experimental or EPISuite-predicted biodegradability, analysed by time series groups. Blue symbols = experimental ready biodegradability; black symbols = EPISuite-predicted ready biodegradability; squares = average proportions; whiskers = 90% confidence interval.

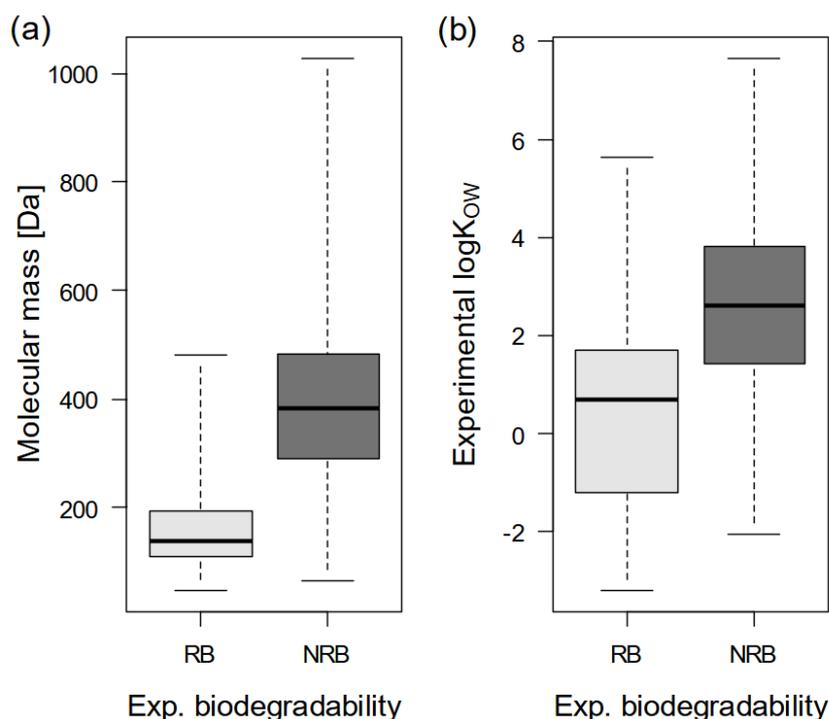


Fig. 4. Molecular mass (a) and experimental logK_{OW} (b) versus experimental biodegradability (RB: readily biodegradable, NRB: not readily biodegradable). Thick line = median; lower and upper borders of rectangle = 25th and 75th percentiles; whiskers = minimum and maximum values.

3.4.4 Total removal in WWTPs

A total of 1641 EPISuite WWTP removal predictions are available for OASs with a known year of introduction or registration from the final list, as for a few there is no date available. EPISuite has minimum and maximum limits for elimination through biodegradation and/or sorption to activated sludge, the minimum being 1.85% and the maximum ~94% removal (Fig. 5). Some very few exceptions to the latter limit apply for volatile substances, only 3 OASs from the final list, which may reach even higher removal rates, up to > 99% for dimethicone, due to both ~56% physical stripping on aeration, ~43% sorption and < 0.1% biodegradation, while two medicinal gases reach nearly all of their removal (desflurane > 98%; sevoflurane > 96%) through stripping. Overall, older, pre-1920s OASs are predicted to be badly removed (WWTP removal < 15%), but from around 1930 removal rates span the whole above range. Although median removals remained close to the minimum value of 1.85% for the first 4 periods (1840–2010), *i.e.*, half of the compounds introduced during these periods are expected to be marginally removed by WWTPs, the upper percentiles showed a monotonous increase over time. Hence, the 75th percentile rose from less than 5% before 1950 to more than 50% after 2010 and the 90th percentile rose from ~20% to more than 90%.

As mentioned above, EPISuite (U.S. EPA 2021) details WWTP removals by sorption to sludge, potential volatilisation (Henry constant) and biodegradation. Looking at the relative shares, it becomes clear that even in case of compounds predicted to be RB by EPISuite itself, removal by biodegradation is low compared to sorption. The reason behind this peculiar behaviour is that, while EPISuite calculations are based on a hydraulic retention time of 8 h in the aeration (biodegradation) basin in its ‘standard’ WWTP model, a worst-case biodegradation half-life of 10,000 h is set by default (see ‘Help’ button in EPISuite; U.S. EPA 2017). This means that, run in normal mode, EPISuite will *not* use its own biodegradability assessment from the

Biowin section; hence, no matter whether a substance is predicted to be RB or NRB, the very long default half-life will result in non-significant biological removal in a WWTP. The use of shorter half-lives can be set manually, but it was decided at the beginning (see ‘Methods’) to use EPISuite in its standard mode. In addition, despite updated, improved versions of EPISuite being made available from time to time (Card *et al.*, 2017), the prediction of biodegradability itself by various available models, be it primary or ultimate biodegradation, is obviously still quite unsatisfactory and this holds for EPISuite as well (Nolte & Ragas 2017).

The importance of lipophilicity-driven sorption in the EPISuite prediction of WWTP removal can indeed be easily identified by plotting the predicted WWTP removal *vs.* $\log K_{ow}$ values (see Fig. S4, SI). Whatever the source of $\log K_{ow}$ values (experimental from Lab Network, experimental as cited by EPISuite or from pharma industry, or predicted by EPISuite), the data points show comparable sigmoid patterns, with an increase from minimal to maximal predicted removal within a span of approximately 3–4 $\log K_{ow}$ units somewhere in-between $\log K_{ow}$ 0 and 6. Out of the 162 OASs predicted by EPISuite to be RB, fully 95 are expected to be removed only at the minimum of 1.85%; a further 44 substances show less than 10% removal, while 10 substances should be removed between 10% and 50% (not shown). But even among the remaining 13 OASs with total removals between 50% and 94%, none attains more than 1% of that removal through biodegradation, while all 13 compounds show more than 99% of the total removal through sorption. One RB-predicted substance, ethyl chloride, is expected to be mainly (~81%) removed through volatilisation/stripping; however, but even in this case the predicted biodegradation share is 20 times lower than the sorbed fraction, despite having the lowest calculated $\log K_{ow}$ with 1.58 among the 15 OASs, while all other 12 compounds have a $\log K_{ow} > 4$ (not shown).

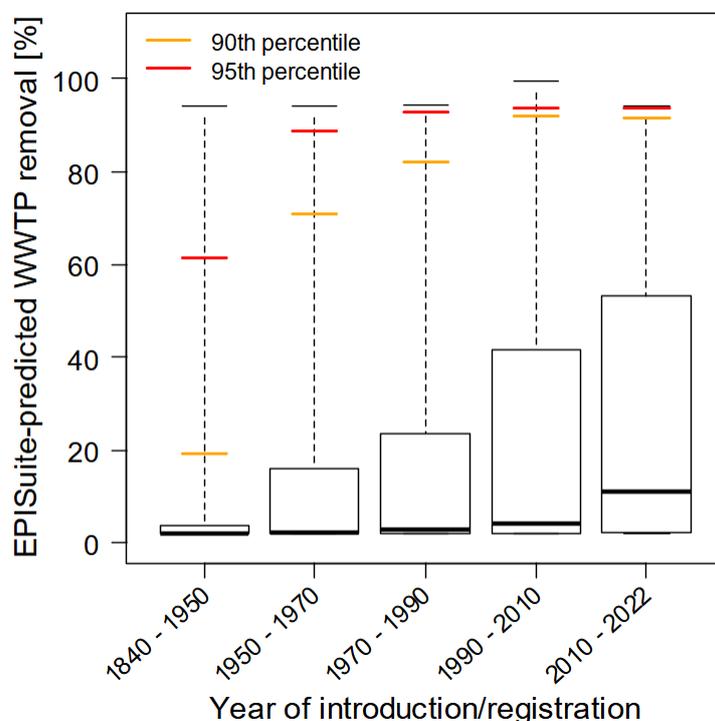


Fig. 5. Year of introduction or registration *versus* total EPISuite-predicted wastewater treatment plant removal for 1641 organic active substances. Thick line = median; lower and upper borders of rectangle = 25th

and 75th percentiles; yellow whisker = 90th percentile; red whisker = 95th percentile; grey whisker = maximum value (note the lower whiskers are not visible).

4 General discussion

4.1 *Number of organic active substances introduced per year*

While only few still existing OASs were introduced in the years 1840 to around 1950, the number registered per year after 1950 is much higher. To better illustrate this trend, Fig. 6 shows the cumulative distribution of registrations over time, which has a very interesting shape: after 1950, the number of compounds registered each year is approximately constant, leading to a nearly linear increase in the cumulative crosses per annum.

Despite major advances and breakthroughs in pathophysiological, molecular biological, genetical and pharmacological knowledge and understanding in the 70 years since the middle of the 20th century, the number of new registrations per time unit remains more or less the same. This is an astonishing finding for which, for the time being, we cannot offer any simple, cogent explanation. One would expect that, thanks to the above advances, the registrations per year would generally increase; hence, we suspect that there is one or several mechanisms introduced over the past 70 years that slow this increase. Potentially (but we have no proof for this speculation), this might be the repeated addition of more and more stringent requirements in the constantly evolving regulatory process for registrations worldwide. Also, the increasing development over the past 35 years (Mullard, 2021) of biologics, biotechnologically produced high-molecular-weight therapeutic proteins (which are not covered by the present investigation into OASs), may have drawn some effort away from research into new, small-molecule OASs.

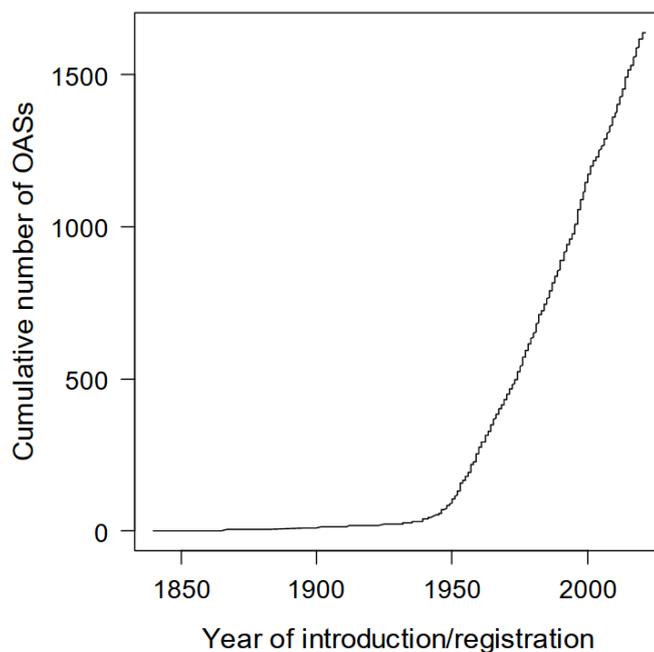


Fig. 6. Cumulative proportions of new pharmaceutical organic active substances (OASs) introduced or registered per year from 1840 (n = 1641 at the beginning of the year 2021).

4.2 *Assessment of EPISuite for prediction of biodegradability and WWTP removal*

The free software EPISuite (U.S. EPA 2017) has been used widely in this investigation, as experimental $\log K_{OW}$ values have been found for a maximum of 1186 out of the 1850 OASs under consideration (649 when considering other sources than Lab Network), and empirical RB or NRB classifications have only been retrieved for 240 of them. Therefore, it is necessary to give EPISuite a retrospective evaluation as to its reliability, specifically regarding human APIs or OASs.

4.2.1 *n-Octanol/Water partition coefficient*

The separation of experimental Lab Network $\log K_{OW}$ data from other empirical literature values, as cited in EPISuite (U.S. EPA 2017) or from pharma company data (AstraZeneca 2021; GSK 2021; F.Hoffmann-La Roche 2021a) plus iPiE*Sum (2019), was described above in section 3.1. As a reminder, the rationale for this separate treatment was that many Lab Network data were suspected to be systematically different from other experimental $\log K_{OW}$ values taken from literature. This observation is concretely illustrated in Fig. S1, showing that the former values are lower than the latter for ~75% of the compounds with both data available. A paired Wilcoxon-Mann-Whitney test also provided a strong indication in that sense ($p < 10^{-6}$), as well as the regression line for Lab Network values *vs.* other experimental values ($y = [0.78 \pm 0.05] x + [-0.15 \pm 0.15]$) that noticeably diverged from the first bisector ($y = x$). The reason for this deviation is unknown.

In the present investigation, only the EPISuite-predicted $\log K_{OW}$ has been used for calculating adsorption. EPISuite predictions appeared to be closer to the “other empirical” data (Fig. S2) than to the Lab Network values (Fig. S3) with respect to which they present a larger offset of almost 1 log unit. The observed difference between the “other” experimental and EPISuite-predicted $\log K_{OW}$ values was thus considered as reasonably small for the presently targeted applications.

4.2.2 *Biodegradability*

While EPISuite only has two summary characterisations, RB or NRB, the pharma data are more complex. In many cases there is information on ready biodegradability tests (OECD TG 301 series), but sometimes biodegradation results from other OECD tests are given, *e.g.*, inherent biodegradability (OECD TG 302 series) or WWTP simulation tests (OECDs 303A, 314B, etc.) (OECD TG 2021). For the present assessment, only those OASs that had successfully passed an RB test were assigned RB, but none of the inherent ones, and in case of the WWTP model tests only those that showed a substance half-life in activated sludge below 3 hours. The reasoning behind this cut-off is that in many simpler WWTPs the total hydraulic residence time is not more than 3 hours, meaning that compounds with a higher half-life are not expected to be biodegraded substantially.

Comparing predicted and experimental biodegradabilities pairwise, after eliminating 8 ‘weight of evidence’ RB OASs, results in Table 2. If predicted RB coincides with experimental RB, this is a true positive incidence; if the predicted NRB coincides with the experimental NRB, this is a true negative incidence; if the predicted NRB coincides with an experimental RB, this is a false negative incidence; and if the predicted RB coincides with an experimental NRB, this is a false positive incidence (Parik *et al.*, 2008). The sensitivity is the probability of a correct prediction as RB in case the substance is RB indeed; thus, the

sensitivity is estimated by the number of true positives divided by the sum of true positives plus false negatives (Parik *et al.*, 2008), *i.e.*, $33 \div (33+10)$, or 0.77. Applying the same approach as previously, a 90% confidence interval for the “true” value of this probability is 0.64–0.86. The specificity is the probability of a correct prediction as NRB in case the OAS is truly NRB; the specificity is estimated by the number of true negatives divided by the sum of true negatives plus false positives, *i.e.*, $176 \div (176+22)$, or 0.89 (90% confidence interval: 0.85–0.92).

Table 2. Sensitivity and specificity of EPISuite predictions in pairwise comparison with experimental data.

Experimental biodegradability	EPISuite-predicted biodegradability	
	Readily biodegradable	Not readily biodegradable
Readily biodegradable	true positive n = 33	false negative n = 10
Not readily biodegradable	false positive n = 22	true negative n = 176

Total n = 240 active organic substances.

For any conclusions based on these results, some particulars must be kept in mind that may influence the conclusions, (1) the relatively low number of pairwise comparisons due to the lack of experimental data (240 out of 1850 OASs, or only 13% of the final list); (2) possible inherent uncertainties in and variabilities between the different OECD TG 301 series RB tests themselves (OECD, 2021); (3) the fact that biodegradability is not only the property of a given substance but also of the environment or the exposure to micro-organisms where it degrades (Blok, 2001; Boethling *et al.*, 2007, 2009); (4) possible errors by the first author, or in the consulted databases, in assigning RB or NRB based on experimental test results; (5) potentially, a strong bias introduced by the higher RB proportion among the commodities (without the latter, the sensitivity goes down steeply to 12.5% while the specificity only diminishes minimally to 88.8%, due to one NRB commodity in the true negatives; not shown), and (6) a possible bias in EPISuite (2017) algorithms due to training with industrial chemicals, but not specifically with APIs. With these caveats, the elevated sensitivity in the whole dataset suggests that EPISuite is quite successful in predicting truly RB OASs; but the higher specificity, also after eliminating the commodities, implies that EPISuite is even better – if certainly not faultless – at identifying truly NRB substances. An analogous conclusion was reached by Boethling *et al.* (2004) for a small set of premanufacture notice API candidates in the United States, based on two out of the six EPISuite biodegradability predictions and stringent OECD 301C and 301D RB tests.

The overall satisfactory performance of EPISuite in distinguishing RB from NRB OASs may be useful in terms of pharmaceuticals ERA: an EPISuite-predicted NRB API will mostly prove to be NRB in an experimental study, hence it may not really be worthwhile to do an RB test but, if any test at all, possibly a higher-tier WWTP simulation test (*e.g.*, OECD 303A or 314B) (OECD TGs, 2021) instead. This is supported by the fact that even after eliminating the commodities, the specificity barely decreases. On the other hand, an RB prediction by EPISuite may make it worthwhile to perform a comparatively cheap RB test if a positive result may save other, more costly, environmental fate studies. Thus, while EPISuite was never

specifically intended to be utilised for pharmaceuticals ERA, it appears still as highly useful, not only for very basic initial RB or NRB estimations, but also to inform on certain decisions regarding ERA testing strategy. This relates not only to new OASs, but specifically also to customising and ideally minimising the testing for old ‘legacy’ substances.

4.2.3 WWTP Removal

The above results strongly suggest that – at least in EPISuite modelling – RB does not equate with removability in a WWTP, as sorption emerges as the most important removal pathway overall, while on the whole volatilisation or biodegradation seem to play minor roles. The latter in particular may be primarily dependent on the hydraulic retention time in the aerobic biodegradation basin, but EPISuite obviously uses a worst-case WWTP scenario with a very long biodegradation half-life, which explains the insignificant predicted removal even for RB compounds. For this reason, EPISuite is very conservative in assessing removal through biodegradation, as supported by a comparison between predicted and actually observed removal rates for a few common ‘legacy’ OASs in activated sludge WWTPs (Table 3), based on Boethling *et al.* (2009) and supplemented by the authors.

EPISuite-predicted WWTP removal correlates with lipophilicity, as already seen above, with removal rates increasing above 10% in the range of $\log K_{OW}$ 3.2–3.5, but not with RB or NRB classifications; however, actual removal rates are often clearly higher. Out of the above 32 OASs, 11 are removed to a higher extent than predicted by EPISuite, while only one OAS is removed to a lower extent; the remaining 20 OASs show actual removal ranges from smaller than, to approximately as predicted, to higher than predicted. In particular, the observed removal is not only higher for (potential borderline) RB OASs like acetylsalicylic acid (LUA, 2002) or paracetamol (Henschel *et al.*, 1997), where far-reaching removal would be expected in a WWTP, but also for others that are NRB (*e.g.*, clarithromycin, ethinyl oestradiol, norfloxacin), or for compounds that are not lipophilic (*e.g.*, atenolol, metformin, sulfamethoxazole, tetracycline). This strongly supports the notion that high lipophilicity is not the only driver for actual removal in WWTPs. Properties relating to the bacterial biomass in a WWTP, *e.g.*, adaptation of the activated sludge to OASs that occur regularly, or potentially co-metabolism (*i.e.*, existing degradation pathways and enzyme complements for other compounds in polyvalent sludge being employed to degrade ‘new’ OASs), the overall design of a specific WWTP with tertiary or quaternary treatment steps and hydraulic retention times in aerobic and anaerobic compartments of a given WWTP (*e.g.*, Boxall & Ericson, 2012; Gerrity & Snyder, 2012), may be as – or even more – important. As Blok (2001) emphasised, biodegradability in a given WWTP is not only a function of the substance properties, but of the whole system. In considering exclusively substance properties like lipophilicity (*i.e.*, sorption) and volatility, but hardly biodegradability, there is a systematic shortcoming of EPISuite, which, however, is comprehensible in view of the many different types of WWTPs that exist on one hand and on the in-built worst-case assumptions of EPISuite on the other.

4.3 Biodegradability and Removability in WWTPs over Time

The working hypothesis for this investigation was that more recent APIs are less well biodegradable and therefore removed to a minor extent in WWTPs than older APIs. The results presented above strongly suggest that both parts of this postulate need to be treated separately.

Table 3. Comparison between EPISuite-predicted and actual measured removal rates for some ‘common’ active organic substances in wastewater treatment plants.

Active organic substances	WWTP removal rate, %				Observed versus predicted removal	Reference for observed
	predicted		observed			
	logK _{ow}	removal	removal range	samples analysed, n		
Acetylsalicylic acid	1.13	1.91	81±12 to 99		>>	LUA 2002; Kasprzyk-Hordern <i>et al.</i> , 2009
Atenolol	-0.03	1.85	20 to 99±1	25	>>	Paffoni <i>et al.</i> , 2006; PILLS 2012; Guillossou <i>et al.</i> , 2019
Atorvastatin	6.36	93.21	18 to 98	12	<~	Gros <i>et al.</i> , 2010; Golovko <i>et al.</i> , 2021
Azithromycin	3.24	30.99	-213±126 to 68		<~	Paffoni <i>et al.</i> , 2006; Ghosh <i>et al.</i> , 2009; Guillossou <i>et al.</i> , 2019
Bezafibrate	4.25	42.58	-10 to 99		<>	Strenn <i>et al.</i> , 2004; Schneider, 2005
Carbamazepine	2.25	2.96	-124 to 21±25	22	<~	Dunlavey <i>et al.</i> , 2010; Guillossou <i>et al.</i> , 2019; Golovko <i>et al.</i> , 2021
Ciprofloxacin	0.00	1.85	-3 to 66±35	18	~>	Gros <i>et al.</i> 2010; PILLS, 2012
Clarithromycin	3.18	7.30	34±43 to 98	17	>>	PILLS, 2012
Cyclophosphamide	0.96	1.88	13 to 26±19	11	>>	PILLS 2012
Diazepam	2.70	4.42	-25 to <83	10	<>	Straub 2008 R
Diclofenac	4.02	56.55	-186 to 35±21	22	>~	PILLS 2012; Guillossou <i>et al.</i> , 2019; Golovko <i>et al.</i> , 2021
Erythromycin	2.48	6.23	-3±82 to >98	22	~>	PILLS 2012; Guillossou <i>et al.</i> , 2019
Ethinyl oestradiol	4.12	17.51	~65 to >90		>>	Andersen <i>et al.</i> , 2003; Clara <i>et al.</i> , 2004
5-Fluorouracil	-0.81	1.85	>87 to <LOD*	97	>>	Straub 2010 R
Fluoxetine	4.65	32.40	-54 to >90		<>	Golovko <i>et al.</i> , 2021; Zorita <i>et al.</i> , 2009
Gemfibrozil	4.77	69.1	<10-75 to 30-99	14	<>	Paxéus 2004; Gros <i>et al.</i> , 2010
Hydrochlorothiazide	-0.10	1.85	-23±20 to 66±8		<>	Castiglioni <i>et al.</i> , 2006; Radjenovic <i>et al.</i> , 2007; Guillossou <i>et al.</i> , 2019
Ibuprofen	3.79	28.72	58-90 to >99		>>	Ternes <i>et al.</i> , 1999; Thomas & Foster 2005; Guillossou <i>et al.</i> , 2019
Indomethacin	4.23	43.65	0-40 to 92->99	9	<>	Schneider, 2005; Zhou <i>et al.</i> , 2009
Ketoprofen	3.00	6.85	40-100, 93	13	>>	Paffoni <i>et al.</i> , 2006; Gros <i>et al.</i> , 2010; Guillossou <i>et al.</i> , 2019
Lidocaine	1.66	2.94	20 to 79±21	11	>>	PILLS 2012
Metformin	-1.40	1.85	(-103) to 99	≥84	~>	Straub <i>et al.</i> , 2019a R
Mycophenolic acid	4.22	40.99	(-73) to 95	11	<>	Straub <i>et al.</i> , 2019b R
Naproxen	3.10	7.55	15 to >99	24	~>	Straub & Stewart, 2007 R; Guillossou <i>et al.</i> , 2019
Norfloxacin	-0.31	1.85	30-98 to 75-97	11	>>	Gros <i>et al.</i> , 2010; Ghosh <i>et al.</i> , 2009
Oestradiol	3.94	30.52	40 to >47		~>	Carballa <i>et al.</i> , 2004; Zorita <i>et al.</i> , 2009
Paracetamol	0.27	1.86	96-100 to >99		>>	LUA 2002; Gros <i>et al.</i> , 2010; Guillossou <i>et al.</i> , 2019
Propranolol	3.48	12.58	-56 to 22		<~	Golovko <i>et al.</i> , 2021; Paffoni <i>et al.</i> , 2006

Active organic substances	WWTP removal rate, %				Observed <i>versus</i> predicted removal	Reference for observed
	predicted		observed			
	logK _{ow}	removal	removal range	samples analysed, n		
Sulfamethoxazole	0.48	1.88	(-1100) to >99	≥195	~>	Straub 2016 R; Guillosoou <i>et al.</i> , 2019
Tamoxifen	6.30	93.03	32 to 45		<<	Zhou <i>et al.</i> , 2009
Tetracycline	-1.33	1.85	39 to 40-89		>>	Paffoni <i>et al.</i> , 2006; Gros <i>et al.</i> , 2010
Trimethoprim	0.73	1.88	(-550) to >99	≥112	~>	Straub 2013 R; Guillosoou <i>et al.</i> , 2019

The range indicated shows lower and upper removal rates, as single measurements, ranges or as averages \pm standard deviation, from different WWTPs sampled. Removal rates were calculated as influent concentration minus effluent concentration, divided by influent concentration. Comparisons of observed *vs.* predicted removal rates are given by a range, with symbols denoting: <, << = observed removal smaller, or much smaller, than predicted; ~ = observed removal in range of predicted; >, >> = observed higher, or much higher, than predicted; <> = both smaller and higher removal rates have been observed.

LOD = Limit of detection; R = literature review; WWTP = wastewater treatment plant; *: 5-Fluorouracil has never been detected in a WWTP effluent, with LODs ranging from 1.7 down to 0.015 $\mu\text{g/L}$; () = values in brackets are considered outliers, see cited references.

4.3.1 Biodegradability

While the data suggest an overall trend toward lower biodegradability over time, it is only warranted as a very general observation. Among newer OASs there are both RB and NRB compounds, but similar to the whole dataset, the RB compounds are clearly in the minority. Specifically, experimental data suggest that, as a lower estimate, 3.9% of OASs comply with RB conditions, while EPISuite predicts 7.2% of OASs to be RB (both datasets not including commodities). On one hand, both figures show only a small fraction to be RB, thus, small-molecule OASs on the whole are not well biodegradable, with well >90% not fulfilling the RB conditions. On the other hand, even an RB share of <10% is not negligible: 3.9% does signify that 72 out of the 1850 OASs investigated here potentially conform to Kümmerer's (*e.g.*, Kümmerer, 2007; Kümmerer and Schramm, 2008) criterion that APIs that are removed in WWTPs will not reach the aquatic environment and therefore are not likely to cause significant environmental risks in surface waters. (This number nearly doubles to 133 OASs based on 7.2% RB as calculated by EPISuite). Thus, once their ready biodegradability has been confirmed, some of these APIs may need less or no in-depth testing for environmental fate and hazards, which in turn will reduce priority lists and subsequent testing effort and costs.

In addition, biologics, *i.e.*, biotechnologically produced large protein molecules, mostly monoclonal antibodies, have increased in number from zero to 100 registered APIs over the past 35 years (Mullard, 2021). Such biologics have been shown in a proof-of-concept investigation to be RB (Straub, 2010). While biologics will never completely replace small-molecule APIs, due to high cost and the need for application through infusion (*i.e.*, self-administration is not feasible for the time being), they do replace at least a part of older small-molecule APIs and thereby decrease the total load of nondegradable OASs excreted into sewage. In this way they help to lighten the load of potentially persistent compounds on WWTPs and surface waters.

4.3.2 Removability

Removability in WWTPs, however, does not simply equate with ready biodegradability. RB compounds may be reasonably predicted to be rapidly removed in a functional WWTP; this is accepted by the current European Union ERA Guideline (EMA 2015) that states that APIs that are shown to be RB do not need an additional environmental fate sediment/water test (OECD TG 308) (OECD TG, 2021), which in the end means that the substance is expected to be reliably removed in WWTPs. However, there are other means of removal, from sorption to sludge or, more seldom, volatilisation (both weighted highly by EPISuite), to biodegradation by competent, adapted activated aerobic or anaerobic sludge, or through co-metabolism (not weighted highly by EPISuite in standard mode). According to the outputs of EPISuite, the predicted WWTP removal is expected to increase over time despite a moderately decreasing share of RB OASs. This is indeed consistent with the increase of experimental and modelled logK_{ow} values over time, thus favouring the removal of OASs by sorption to sludge. Table 3, however, suggests that WWTP removal is often underestimated by EPISuite, which can be attributed to a more significant contribution of biodegradation processes under realistic conditions. As one example, acetylsalicylic acid (Aspirin) is predicted by all 7 Biowin models in EPISuite to be RB. Run in standard mode, EPISuite predicts a total WWTP removal of 1.91% (Table 3), of which only 0.09% is through biodegradation (not shown). However, manually overriding the default half-life of 10,000 h by selecting 'Use Biowin output and EPA draft method for assigning half-

lives' under the EPISuite 'STP' button, the re-calculated removal increases to 92.1%, of which 91.7% through biodegradation. This predicted value compares well to the removal range of 81±12% to 99% (LUA, 2002; Kasprzyk-Hordern *et al.*, 2009) cited in Table 3.

5 Conclusions

From 1840 to present, OASs tend to become more massive and more lipophilic overall, albeit with a high variability. This development may reflect, amongst others, an increase in scientific, molecular biological and pharmaceutical knowledge and understanding, which results in better targeted OASs. Contrary to molecular mass and logK_{ow}, however, ready biodegradability of new OASs shows a decrease over time; among all molecules under consideration, only a small fraction, less than 10% of OASs, are RB. But the available data suggest that despite this tendency, overall removal in WWTPs still increases over time, which will reduce the exposure of aquatic compartments to certain OASs, in line with Kümmerer's (2007) postulate. Conversely, however, the presence of less biodegradable OASs in WWTPs and more specifically in sludge (which should concomitantly increase in view of the trend of producing more lipophilic new OASs) may be of concern already now and possibly even more so in the future, especially with regards to sewage sludge valorisation as an agricultural fertiliser.

While EPISuite (U.S. EPA 2017) was not conceived specifically for environmental property modelling of pharmaceuticals, it still proves quite useful, in particular for predicting on RB or NRB characteristics and informing on ERA testing strategy. In addition, EPISuite RB predictions may also be used to refine, after due experimental confirmation, existing priority lists for legacy pharmaceuticals ERA. EPISuite in standard mode is very conservative in assigning the biodegradation share in total removal, hence sorption to sludge is modelled as the dominant process, which leads to higher removal rates following the increase of logK_{ow} values. EPISuite should be used with caution when estimating WWTP removal due to biodegradability, as this could be underestimated as compared with sorption processes; however, the biodegradation half-life used in the removal prediction may be altered manually, resulting in improved predictions. Lastly, EPISuite's elevated sensitivity and specificity of RB *versus* NRB OASs may suggest that, at least regarding ready biodegradability, pharmaceutical OASs are not that different from 'common' chemicals, the original training set for EPISuite.

The working hypothesis for this investigation was that more recent APIs are less well biodegradable and therefore removed to a lesser extent in WWTPs than older APIs. We conclude that this hypothesis needs to be separated: An overall decrease in ready biodegradability over time is confirmed while, contrary to expectation, the upper half of the predicted removal values in WWTPs increase, but only due to adsorption.

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Declaration of competing interest

Jürg Oliver Straub clearly stated that he used to work for F.Hoffmann-La Roche Ltd, a global pharmaceuticals company, until his retirement, that he draws a pension from this company and that he has shares in this company.

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Data availability

The basic data are given in the attached S1 spreadsheet.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scp.2022.100834>.

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