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Halogenation of Pharmaceuticals Is an Impediment to Ready Biodegradability

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Abstract: For pharmacological reasons many active organic pharmaceutical substances (AOPSs) are singly or multiply halogenated. Halogenation can confer optimised steric fitting of an AOPS to its molecular receptor; moreover, by increasing the lipophilicity of a compound, passive permeation through bilipid membranes into target cells is enhanced. As halogenation is widely suspected to inhibit biodegradability in wastewater treatment plants, the relationship of halogenation vs. ready biodegradability was investigated. Among 230 AOPSs with empirical ready biodegradability data, all 70 halogenated AOPSs are not readily biodegradable, and halogenation is confirmed to be an impediment to ready biodegradability. As a counterexample to halogenation, hydrophilic substitutions (hydroxy, carboxylic-acid or terminal-amine groups) are positively correlated with ready biodegradability. Regarding halogenation, therefore, pharmacological goals stand in stark contrast to environmental goals. Possible ideas toward solutions for this contradiction are discussed.

Keywords: pharmaceuticals; halogenation; ready biodegradability; wastewater treatment plants; adsorption to sludge; removal; co-metabolism

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1. Introduction

The presence and potential risks of pharmaceuticals in the environment (PIE) have been a widely discussed topic in environmental sciences since the 1980s [1]. Hundreds of academic publications detail adverse effects of active organic pharmaceutical substances (AOPSs) against environmental organisms on different levels of biological organisation, from molecular to cellular, organ, whole-organism, reproductive and population up to ecosystem effects. However, effects of PIE can only occur subsequent to exposure, meaning that the concentration levels of AOPSs in environmental compartments are as important for risk assessment as the effects are, and vice versa, that an abatement or the absence of AOPSs in the environment will reduce or prevent risk, as emphasised repeatedly by Kümmerer and colleagues [2–4]. If AOPSs from human excretions are removed to a high extent in wastewater treatment plants (WWTPs), exposure to PIE in surface waters or sediments will decrease, as will the risks for aquatic organisms. Therefore, removal in WWTPs through biodegradation or sorption to activated sludge (or rarely volatilisation) is a crucial parameter for the aquatic risk assessment of AOPSs.

Many human AOPSs are halogenated. Halogens are a group of non-metal, reactive atoms that comprise bromine, chlorine, fluorine and iodine (plus two unstable elements, astatine and tennessine, both of which are not used as halogens in AOPSs) [5]. The bond energy, i.e., the binding strength of the halogens, is strongest for fluorine and serially weaker for chlorine, bromine and iodine [6,7]. While halogens in the form of X2 are quite reactive, once they are covalently bound to non-halogens, they are hard to separate again; the fluorine-to-carbon bond in particular needs a very high energy to break. In pharmacology, halogenation, the addition of one or several halogen atoms to a molecule,

is used to improve the affinity of an AOPS to a given receptor on the surface of or inside cells. The binding, which functions in an analogous way to a hydrogen bond, is directional and specific between a covalently bound halogen atom and a nucleophile fragment of the target molecule [8]. Moreover, halogenation also increases the hydrophobicity of an AOPS; thereby enhancing the passive permeation through biological bilipid-layer membranes [6,9]. As most AOPSs need to get into the target cells in order to exert their pharmacological functions, enhanced affinity and improved membrane permeation are specifically targeted pharmacological properties and constitute very frequent modifications of AOPSs, that are often achieved through halogenation. In 2014, Smith et al. [10] presented over 230 different human chlorinated AOPSs. Fluorination has also become a widespread feature of small molecule AOPSs [11]; recently, Inoue et al., [12] counted over 300 fluorinated AOPSs on the market.

Due to their binding strength, the halogens are hard to cleave from the AOPSs, both in human metabolism and in WWTPs or other environmental compartments. However, halogenated compounds can be biodegraded through dehalogenation, preferentially in anaerobic, reducing environments [13–18]. In addition, as halogenated AOPSs are on the whole more lipophilic than the same, non-halogenated compounds—hence they are prone to adsorption to activated sludge [19]—, it may be more difficult for enzymes in sludge to bind to, dehalogenate and further degrade AOPSs. Hence, halogenation is expected to result in reduced biodegradability. In WWTPs, therefore, halogenation may have two simultaneous effects on AOPSs, namely decreased biodegradability and increased sorption to sludge. Further, due to increased lipophilicity, the tendency of halogenated AOPSs to bioaccumulate may increase in parallel [7]. As a counterexample to halogenation, substitutions that increase hydrophilicity, e.g., hydroxy, carboxylic-acid or terminal-amino residues, are generally expected to be associated with improved biodegradability [20].

Thus, low biodegradability seems to be a recurring property of halogenated AOPSs. This contribution aims to investigate the general validity of this statement using a dataset of halogenated (more lipophilic) and non-halogenated (more hydrophilic), specifically terminal-amino-, hydroxy- and carboxylic-acid-substituted AOPSs with empirical data on ready biodegradability. Substances are considered readily biodegradable (RB) if they fulfil the criteria defined in a ready biodegradability test performed according to Organisation for Economic Co-operation and Development (OECD) test guidelines of the OECD 301 series [21] (see Section 4.1, Ready Biodegradability, in the Discussion). If a substance conforms to RB criteria, far-reaching removal through biodegradation may be assumed in WWTPs. In addition, the removal of AOPSs in WWTPs through both biodegradation and adsorption to sludge (plus volatilisation or loss to air in rare cases) will be modelled using EPISuite (Estimation Programs Interface Suite) v4.11 [22], allowing a comparison between these two major removal pathways.

The aim of this study is to correlate empirical ready biodegradability data and modelled removal pathways in WWTPs for a large set of AOPSs with either halogen substitutions or hydrophilic substitutions. The working hypothesis for this investigation is that halogenated AOPSs are less well RB than non-halogenated ones.

2. Materials and Methods

2.1. Basic Data

A dataset of 1850 unique small-molecule AOPSs was collated during 2020 and 2021 (Supplemental Information in ref. [23]), comprising international nonproprietary name, Chemical Abstracts Services (CAS) number, molecular formula, molecular mass, Simplified Molecular Input Line-Entry System (SMILES) code of the molecule, empirical lipophilicity ($\log K_{OW}$) and experimental ready biodegradability where available, as well as in silico modelled properties: lipophilicity, biodegradability and WWTP removal, calculated by EPISuite [22]. EPISuite contains different estimation programs for quantitative structure-property modelling, based on the substance SMILES code as a minimal input, of various environmentally relevant properties of the molecules under consideration (e.g., KOWWIN

for lipophilicity, HENRYWIN for Henry's Law Constant and BIOWIN for aerobic and anaerobic biodegradability of organic chemicals); EPISuite also has a limited internal database for such properties from the literature.

Experimental biodegradability information was collated for that subset of AOPSs for which documented RB results were found in the above dataset (Supplemental Information in ref. [23]). All compounds with only inherent or WWTP model biodegradability data were assigned not readily biodegradable (NRB) status, while substances that were proposed to be RB based solely on weight-of-evidence considerations were excluded from the dataset. 'Commodities', i.e., common, low-molecular-weight organics that are being used and therefore listed as AOPSs, e.g., simple alcohols, sugars, phenol, etc. [23], were excluded from the list. Note that all radiocontrast agents, many of which contain iodine, were also excluded from the above dataset, as they have no pharmacological function.

The retrieved AOPSs were then scanned for the presence of halogens; their type (Br, Cl, F or I) and number per molecule were entered into a Libre Office [24] Calc spreadsheet that already contained the above information. Hydroxy, carboxylic-acid and terminal-amino substitutions were also recorded.

In order to model the different removal pathways in WWTPs, EPISuite was run for all AOPSs from the above list (i.e., with empirical RB/NRB information). Where available, empirical $\log K_{OW}$ data were entered, else this field was left blank and EPISuite estimated a $\log K_{OW}$ value. For AOPSs known to be RB, half-lives were set manually in both the aeration vessel and the settling tank in the STP (=WWTP) tab in EPISuite to 1 h, corresponding to rapid biodegradation, while the primary clarifier half-life was left blank. For AOPSs known to be NRB, the default 'Use 10,000 h half-life' button was cleared and the 'Use BIOWIN output and EPA draft method for assigning half-lives' option was selected in the STP parameters. The predicted shares in per cent of loss to air, sorption to sludge, biodegradation and resulting total removal were recorded in the same spreadsheet. The removal pathways, sorption to sludge and biodegradation, were compared between RB and NRB AOPSs. In order to further elucidate the role of halogenation, all halogen atoms were deleted from the SMILES codes of the halogenated AOPSs, and EPISuite was run again with the dehalogenated forms with the aim of comparing the different resulting removal pathways.

2.2. Statistics

The halogenated and hydrophilic-substituents datasets were compared using the non-parametric Fisher's Exact Test to investigate whether substitution and ready biodegradability are independent properties. The approach considers the contingency tables reporting, for each substituent under study, the number of substituted or non-substituted substances that are RB or NRB. For the present application, the Fisher's Exact Test was more appropriate than the χ^2 test because (i) for certain halogens, the row (or column) totals were particularly unbalanced, and (ii) the observed counts in some categories were close, if not equal, to zero.

The null hypothesis of the test states that there is no association between the two properties, i.e., the probability of being RB among the substituted molecules is equal to the probability of being RB among the non-substituted ones. This is equivalent to stating that the true Odds Ratio (OR) of the populations of substituted and non-substituted molecules from which the observations were drawn is 1 (one). An OR > 1 means ready biodegradability is likelier to occur in the first group, while an OR < 1 means it is likelier to occur in the second group. Under the null hypothesis, and considering that the margins (i.e., row and column totals) are fixed—as a result of which there is only one degree of freedom—, the probability of the observed allocation in the contingency table can be deduced from the hypergeometric distribution. This distribution also enables the identification of all allocations that would be equally or less probable than the observed one: the sum of their individual probabilities provides the *p*-value of the test.

In addition, the EPISuite-predicted removal at different stages of the wastewater treatment process (sorption to sludge, biodegradation and total WWTP removal including losses to air) was compared between groups: (i) RB vs. NRB compounds and (ii) halogenated vs. in silico dehalogenated compounds. Due to the non-Gaussian distribution of most variables under study, non-parametric statistics were preferred over parametric statistics: comparisons between RB and NRB AOPSs were therefore carried out using a Wilcoxon–Mann–Whitney test, while comparisons between paired data from halogenated AOPSs and their dehalogenated analogues were performed with a Wilcoxon signed-rank test. The tests were performed in Python3 [25] (under Linux® Mint [26]) as well as R, version 4.2 [27] (under Windows®) to check the consistency of the results.

It was chosen not to interpret the results of these statistical tests in a dichotomous way, i.e., jumping from a 'significant' to a 'non-significant' conclusion 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 underpinning statistical model, e.g., as suggested by Greenland et al., [28] and Amrhein et al., [29]. The smaller the *p*-value, the more unusual the observations would be under the null hypothesis. 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 impact of the exact value. To refine the picture given by the *p*-value of Fisher's Exact Tests, a 95% confidence interval on the odds ratio was calculated in each case.

3. Results

3.1. Dataset

Empirical ready biodegradability data have been retrieved for 230 unique AOPSs (Supplementary Table S1), of which 33 (14.3%) are RB and 197 (85.7%) are NRB (Table 1). Ten commodities comprising acetic acid, benzyl alcohol, ethanol, ethanolamine, glycerin, lactitol, phenol, propyl alcohol, triethanolamine and urea were excluded, all of which are RB and would have biased the share of RB compounds if left in the dataset. Out of these 230 AOPSs, 70 are singly or multiply halogenated: 38 are chlorinated, 39 fluorinated, 3 brominated and 2 iodinated. Of the 70 halogenated compounds, none (0%) proved to be RB. Among the 160 non-halogenated APOSs, 33 (20.6%) are RB and 127 (79.4%) are NRB.

Table 1. Relationships of halogen and hydrophilic substituents in a set of 230 active organic pharmaceutical substances (AOPSs) with experimental ready biodegradability data.

Molecular Fragment		**Empirical Biodegradability**					**Fisher's Exact Test**	
Groups Substituents	Fragments per molecule, n	AOPSs, n	RB, n	RB, %	NRB, n	NRB, %	*p*-Value	Odds Ratio (95% CI)
All AOPSs	NA	230	33	14.3	197	85.7	NA	NA
Non-halogenated AOPSs	NA	160	33	20.6	127	79.4	NA	NA
Halogenated AOPSs								
Fluorine	1–7	39	0	0	39	100	0.0020	0 (0–0.51)
Chlorine	1–6	38	0	0	38	100	0.0021	0 (0–0.53)
Bromine	1–2	3	0	0	3	100	1	0 (0–14.7)
Iodine	1–4	2	0	0	2	100	1	0 (0–32.1)
Sum of all halogens	1–7	70	0	0	70	100	0.0000025	0 (0–0.22)
Hydrophilic substituents								
Hydroxy group	1–13	102	24	23.5	78	76.5	0.00053	4.1 (1.7–10.4)
Carboxylic acid	1–8	37	12	32.4	25	67.6	0.0017	3.9 (1.5–9.6)
Terminal amine	1–2	42	8	19.0	34	81.0	0.34	1.5 (0.55–3.9)
Sum of above hydro philic substituents	1–15	142	33	23.2	109	76.8	$<10^{-6}$	∞ (6.5–∞)

Note(s): Considering the contingency table comparing the readily biodegradable fraction of the halogen- or hydrophilic-substituted AOPSs against the respective non-substituted AOPSs, the *p*-value is the probability of all allocations equally or more extreme than observed, under the null hypothesis (absence of association between substitution and ready biodegradability); note, $p = 1$ indicates too small a sample size to compute a probability. For Odds Ratios, please refer to the text. CI = Confidence interval; NA = not applicable; NRB = not readily biodegradable; RB = readily biodegradable; ∞ = infinite (for practical purposes) Odds Ratio.

In the dataset of 230 compounds with experimental RB data, 142 AOPSs containing one or more hydroxy, carboxylic-acid or terminal-amine substituents, but no halogens, were identified, out of which 33 (23.2%) are RB and 109 (76.8%) are NRB. Regarding the

single substituents, out of 102 hydroxylated AOPSs, 24 (23.5%) are RB; among 37 with carboxylic acid groups, 12 (32.4%) are RB; and out of 42 compounds with terminal amine substituents, 8 (19.0%) are RB.

3.2. Comparing Halogenated and Hydrophilic Pharmaceuticals

Comparing the halogenated and the hydrophilic AOPSs with those compounds that do not have the respective substituent using Fisher's Exact Test, results in the finding that for both chlorinated, fluorinated and the sum of all halogenated AOPSs biodegradability is clearly different from the non-halogenated set (Table 1; for contingency tables and Python3 program for Fisher's Exact Test see Supplementary Table S2).

Based solely on the *p*-values, no such conclusion can be drawn directly for the brominated and iodinated AOPSs, which is due to their very low numbers among the dataset. However, the ORs computed for all halogenated AOPSs, singly or combined, are all 0, and for chlorinated, fluorinated and the sum of all halogenated AOPSs the computed 95% confidence intervals on these values are quite far from 1 (Table 1). Overall, the results suggest that the RB property is very unlikely in all of the halogenated groups.

Conversely, among the hydrophilic substituents, based on the *p*-values and ORs (Table 1), both hydroxy and carboxylic-acid groups are positively related to ready biodegradability, while the terminal amines by themselves do not show a visible association with the latter property (even though the estimated OR is >1). Looking at the whole set of AOPSs with any one or several of the three hydrophilic substituents, there is again a strongly positive relationship with ready biodegradability.

3.3. Comparing Wastewater Treatment Plant Removal Pathways

EPISuite was run for all 230 AOPSs with RB/NRB information, the WWTP removal results are given in Supplementary Table S3. The comparison between RB and NRB compounds of the modelled removal pathways sorption to sludge, biodegradation and total removal (including losses to air) by Mann–Whitney–Wilcoxon tests is presented in Table 2. All statistical data suggest clear differences between the two groups. For RB AOPSs, the range of values confirms that, overall, sorption to sludge is a minor removal pathway compared to biodegradation (under the ideal situation of rapid biodegradation in the aeration vessel and settling tank, as entered into EPISuite in paragraph 2.1); the median predicted total removal among this group of compounds is very high with ~91%. The predicted total removal in WWTPs is much higher for RB than for NRB AOPSs, which suggests that the complementary removal pathway for many NRB compounds, sorption to sludge, cannot fully compensate for the lower biodegradability. Additionally, for NRB AOPSs, sorption to sludge and biodegradation are predicted to have relatively similar importance in total removal. Other removal pathways are not important overall: >1% removal through losses to air is predicted for only one compound, ethyl chloride (monochlorethane), with ~29% to air.

Table 2. Comparison between the EPISuite-predicted removal pathways in wastewater treatment plants (sorption to sludge, biodegradation and total removal) between the readily and not readily biodegradable active organic pharmaceutical substances (AOPSs).

Removal Pathway	AOPSs				M-W-W Test *p*-Value
	Readily Biodegradable		Not Readily Biodegradable		
	Median	1st–9th Deciles	Median	1st–9th Deciles	
AOPSs, n	33		197		
Sorption to sludge, %	0.4	0.4–6.6	2.9	0.6–61.0	$<10^{-6}$
Biodegradation, %	90.2	88.9–90.3	14.0	0.1–84.7	$<10^{-6}$
Total removal, %	90.7	90.6–95.5	27.8	7.7–92.1	$<10^{-6}$

Note(s): Data are reported as median, 1st and 9th deciles of the removal rate due to each process, among the n AOPSs in each category; the *p*-value is associated with the Mann–Whitney–Wilcoxon (M-W-W) test for group comparisons.

3.4. Comparing Halogenated and In Silico Dehalogenated Pharmaceuticals

To further elucidate the role of halogenation, all halogen atoms were removed in silico from the SMILES codes of the 70 halogenated AOPSs and EPISuite was run again with the dehalogenated analogues. A comparison of the halogenated and dehalogenated results regarding predicted ready biodegradability and WWTP removal pathways (sorption to sludge, biodegradation and total removal including losses to air) is given in Table 3 (full data in Supplementary Table S4).

Table 3. Pairwise comparison between the wastewater treatment plant removal pathways for the original 70 halogenated active organic pharmaceutical substances (AOPSs) with their dehalogenated analogues, both modelled by EPISuite. The *p*-value is associated with the (paired) Wilcoxon signed-rank (WSR) test.

Results	AOPSs				WSR Test *p*-Value
	Original Halogenated		In Silico Dehalogenated		
	Median Value	1st–9th Deciles	Median Value	1st–9th Deciles	
AOPSs, n	70		70		
Readily biodegradable, n	0 (exp.), 4 (pred.) [a]		6 (pred.) [b]		
Sorption to sludge, %	5.0	1.6–87.6	4.8	1.0–60.6	1.5×10^{-4}
Biodegradation, %	0.8	0.1–28.9	22.0	0.6–80.3	$<10^{-6}$
Total removal, %	26.1	3.0–92.2	45.9	9.1–95.3	0.015

Note(s): [a] None are empirically RB, but 4 are falsely predicted to be RB by EPISuite; [b] 6 are predicted to be RB by EPISuite. exp. = experimental; pred. = predicted.

Among the 70 halogenated AOPSs, none are experimentally RB. EPISuite correctly predicts 66 (94.3%) to be NRB and falsely estimates 4 (5.7%) to be RB. The latter (bronopol, chlorphenesin, epichlorohydrin and ethyl chloride) are all relatively small molecules with masses ranging from 64.5 to 202.6 Da, while the average molecular mass of the 230 AOPSs with RB data is 357.2 Da and the average mass of the 1850 compounds in [23] is 368.1 Da (not shown). The EPISuite RB predictions were previously shown to be reasonably accurate: sensitivity = 0.77, 90% confidence interval (90% CI) = 0.64–0.86; specificity = 0.89, 90% CI = 0.85–0.92 [23]. Among the dehalogenated forms, six (8.6%) are predicted by EPISuite to be RB. This comparison further supports the finding that halogenation does impair ready biodegradability.

As assumed, in 51 cases out of 70 (72.9%), the predicted sorption to sludge in WWTPs is higher for the halogenated AOPSs than for their dehalogenated analogues (see Figures S1–S3 in the Supporting Information), with a positive difference between 0.1 and 81.5% removal (median: 9.1%). Thus, according to the results of the Wilcoxon signed-rank test, removal through sorption can be considered to be greater among the former than among the latter group, even though the median value does not differ a lot (Table 3). On the contrary, EPISuite predicts a lower removal through biodegradation for 57 out of 70 (81.4%) of the halogenated AOPSs compared to their dehalogenated analogues, with a negative difference between –0.1 and –70.1% removal (median: –20.5%). The Wilcoxon signed-rank test emphasises the shift between the two groups even more than for sorption (Table 3). However, the total removal in WWTPs is not as clearly different between the two groups, which reflects the complementarity of the removal pathways of higher sorption for halogenated vs. higher biodegradation for dehalogenated forms. However, as found above, sorption to sludge of halogenated AOPSs cannot fully compensate for their lack of ready biodegradability.

Ethyl chloride was earlier predicted to be the only AOPS in the dataset with significant losses to air (29%), while its dehalogenated analogue, ethane, is calculated to reach 81% losses to air. Considering a Henry's Law constant K_H of 0.011 atm $\times$ m^3 $\times$ mol^{-1} at 24.8 °C for ethyl chloride [30] and a 45 times higher K_H of 0.5 atm $\times$ m^3 $\times$ mol^{-1} for ethane (both K_Hs empirical), as cited by EPISuite [22], this strong increase in losses to air is plausible.

4. Discussion

4.1. Ready Biodegradability

There are six ready biodegradability test guidelines in OECD 301 (301A to 301F) [21], all based on a similar principle: the substance under consideration is declared RB if its biodegradation exceeds a certain threshold within a given duration. However, these six tests specify different concentrations of inoculum and test substance as well as different endpoints and criteria for RB. Therefore, a classification of "RB" in one 301 test need not mean exactly the same as "RB" in another. However, even following the same OECD 301X test guideline, the results may vary, due to the microbial inocula that will differ between test labs. As a consequence, test results may show RB in one lab but NRB in another. Further, the NRB qualification does not distinguish between substances that might reach the required criterion after a couple of hours, a couple of days, a couple of months or even years after the temporal limit for the criterion. Additionally, the 301 tests require that the test substance is the only organic substrate added to the rinsed bacterial inoculum, which itself has a low concentration of $\leq$30 mg/L. Hence, co-metabolism, i.e., the availability of catabolic enzymes that were originally selected or evolved for the degradation of other substances [31], is largely excluded in these tests. This minimal-degrading-enzyme condition in the OECD 301 tests is extremely strict and therefore RB substances are accepted to be removed to a high degree in WWTPs. However, the conditions in an RB test are manifestly different from the normal situation in WWTPs, where all kinds of different natural and synthetic compounds occur as a mixture in the wastewater, where the concentration of activated sludge is much higher and where, therefore, co-metabolism may be expected to be the rule rather than an exception. As Kennes-Veiga et al., [30] recently formulated, "Among the scientific community, it is widely assumed that the main biotransformation mechanism in real environmental conditions is co-metabolism". Therefore, while RB compounds are predicted to be removed to a high extent in WWTPs through biodegradation, an NRB test result does not necessarily mean that no removal will take place in practice.

4.2. Experimental Ready Biodegradability Dataset

A total of 230 small-molecule AOPSs, excluding commodities, with empirical ready biodegradability information was investigated for the presence of halogen or hydrophilic substituents and their likely influence on ready biodegradability. While 230 compounds may not be very much, considering at least 1850 different small-molecule AOPSs are on the market that were collated from DrugBank [23], it is still more than one-tenth of that total. To our knowledge, no dataset of such a size has been collated so far in order to elucidate the influence of simple substituents on ready biodegradability. Out of these 230 AOPSs, 33 are experimentally RB, while 197 are NRB. The RB fraction of 14.3% (Figure 1) most probably overestimates the actual share of RB compounds among all AOPSs, which was recently estimated between 3.9% (experimental data only, without commodities) and 7.2% (EPISuite-predicted data) among the larger dataset with 1850 AOPSs [23]. In the present entire dataset, the predicted RB fraction is slightly higher at 7.9%.

Considering the results detailed in Sections 3.2 and 3.4, while halogenation is certainly not the only property of a molecule to impact biodegradability, our data support the conclusion that it is an important one. This is further underpinned by the comparison of the EPISuite-predicted WWTP removal pathways between the original halogenated AOPSs and their in silico dehalogenated counterparts. Overall, for most of the latter, sorption to sludge is predicted to be lower than for the halogenated ones while the biodegradation share is plainly higher. Increased sorption to sludge for the halogenated AOPSs, however, cannot fully compensate for the much higher removal through biodegradation for the dehalogenated forms. It is recognised that this kind of modelling is fraught with uncertainty; but, except for time- and resource-consuming actual synthesis and testing of dehalogenated AOPSs, it is the only possibility to compare halogenated and non-halogenated compounds with the same basic structure.

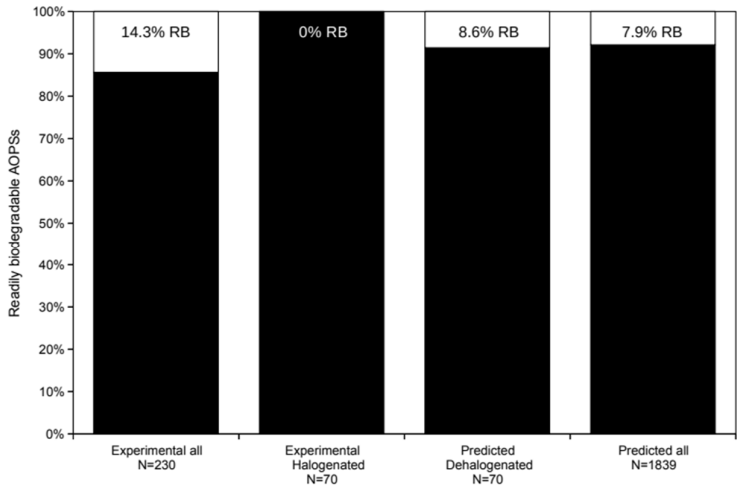

Figure 1. Experimental and EPISuite-predicted ready biodegradability of active organic pharmaceutical substances (AOPSs). White: readily biodegradable (RB) fraction; black: not readily biodegradable share. For 'Predicted Dehalogenated' see text.

4.3. Representativity of the Ready Biodegradability plus Halogenated Dataset

Another question concerns the representativity of the 230 AOPSs with empirical RB data, of which 70 are halogenated; among the latter, 38 (54.3%) are chlorinated, 39 (55.7%) fluorinated, 3 (4.3%) brominated and 2 (2.9%) iodinated. Is the ratio of 70 halogenated out of 230 (30.4%) realistic for all pharmaceuticals? Among the 1850 AOPSs in [23], 566 (30.6%) are singly or multiply halogenated: 282 (49.8%) are chlorinated, 243 (42.9%) fluorinated, 30 (5.3%) brominated and 11 (1.9%) iodinated. The shares of halogenated AOPSs among the two totals are very close together, while the fractions of the different halogens show a higher variability. Thus, the figure of just over 30% halogenated AOPSs overall seems reasonable, while the shares of the different substituents vary somewhat more.

4.4. Halogenation vs. Biodegradability, What Options Are There?

Halogenation impairs ready biodegradability of AOPSs, which is in stark contrast to Green Pharmacy [2–4], Green Chemistry [32] and Safe and Sustainable by Design [33] goals, which strive to discover, develop and produce AOPSs that are biodegradable, i.e., not persistent, and not bioaccumulating. Together, the two properties of impaired biodegradability and tendency to bioaccumulate increase the likelihood of a halogenated AOPS being PBT (persistent, bioaccumulative and eco-toxic), which is a highly undesirable classification in terms of environmental properties and risks [34]. In contrast, the purpose of pharmaceuticals is not in the first place to develop environmentally compatible substances, but to prevent, treat, mitigate or cure illnesses, or to support medical procedures. The function of halogenation is to improve the affinity of an AOPS to a given receptor and to enhance its uptake into cells through cell membranes [6,8,9]. Thus, if halogenation is important for an improved function of AOPSs, but detrimental to ready biodegradability as suggested by this work, what is to be done?

There is no single, simple solution to this contradiction between pharmacological and environmental goals; however, two strategic measures to address this predicament may be envisaged. The first concerns new small-molecule AOPSs in development, where optimising the steric configuration of a compound is highly important in order to achieve the best possible interaction with its target. Ideally, this might be attained without, or at least with less, halogenation of the AOPSs. However, even if optimising the steric configuration means maintaining halogenation, an optimal 'fit' of a given AOPS with its molecular target may mean that less of the active compound will be needed to attain its pharmacological goal—and less of a compound administered means less will be excreted overall; in such cases the relative pros and cons should be assessed. Computer-assisted or -directed molecular design is already widely used in pharmaceutical research and can

further support this goal, potentially also in replacing halogens with some other substituents (which would then need testing and assessing for their impact on biodegradability, beside toxicity).

For existing AOPSs, however, it must be recognised that the lifecycle of pharmaceuticals does not favour any modification of molecules: The pharmaceutical research industry develops new AOPSs over several years, conducting extensive preclinical and clinical safety and efficacy studies. Once an AOPS reaches marketing authorisation, the company will then use the remaining time of patent protection to earn enough for a return on the investment, including for the many substances that did not make it to market. Once patent protection has lapsed, generics companies will start producing and co-marketing successful AOPSs. However, one thing generics companies will not do is modify existing molecules, as that would necessitate conducting the whole package of preclinical and clinical studies, effectively as for a new compound, at comparable expenditure of cost and time. The original research pharma company, on the other hand, has little incentive to modify an existing AOPS, as a 'successor' would compete with their own original product. For existing and generic AOPSs, which make up the vast bulk of medicines used around the world, minimising or optimising halogenation is not realistic. Hence, other ways to address the reduced biodegradability of halogenated AOPSs must be sought.

One measure to pursue is to retrofit and upgrade WWTPs to include an anaerobic degradation compartment with a sufficiently long hydraulic residence time, to achieve denitrification and, ideally, also dehalogenation, which latter is reported to occur preferentially in anoxic environments [13–18]. Feng et al., [35] have demonstrated that enzymatic processes have the potential to be efficient for dehalogenation; this may be achieved, for example, with general haloalkane dehalogenases [36]. Kim et al., [37] showed that co-metabolic degradation was responsible for the anaerobic dehalogenation of trichloroacetic acid. Co-metabolism suggests that relatively unspecific dehalogenase enzymes are involved, which may also work with other halogenated compounds, such as AOPSs [31]. Further, at least for one common fluorinated AOPS, 5-fluorouracil (5-FU), a host of experimental data for aerobic biodegradation (reviewed in ref. [38]) shows no significant removal in standard OECD biodegradation tests with comparatively high, but guideline default, 5-FU concentrations of 30–100 mg/L. However, various WWTP simulation biodegradation tests and measurements in actual WWTPs, with 5-FU concentrations ranging from 10 mg/L to 5 µg/L or lower, showed quite rapid degradation and dehalogenation. Finally, 5-FU has never been detected in effluents or surface waters, supporting efficient removal in WWTPs [38].

More experimental work will be needed to define optimised operating parameters for WWTPs, in particular for anaerobic degradation that is targeted at enabling dehalogenation in addition to denitrification. Furthermore, anaerobic digestion of surplus activated sludge may additionally enhance dehalogenation, in particular in those instances where the sludge will ultimately be spread on arable land as a fertiliser. Where land-spreading is not applied, incineration of surplus sludge, digested or not, will prevent halogenated AOPSs from reaching the environment through leaching from a landfill.

4.5. Reality Check

The 5-FU example suggests that the relatively high test substance concentrations in strict accordance with the OECD guidelines may, at least in certain cases, impact the degrading micro-organisms through toxic effects [39]. At lower, but realistic substance concentrations, however, biodegradation and dehalogenation (besides adsorption to sewage sludge) may occur without impediment under anaerobic and aerobic conditions. Some halogenated AOPSs measured in actual WWTPs in different countries, that have been shown to be removed at least in part, may illustrate this (Table 4). Note that in most studies only a summary removal rate is given (as influent concentration minus effluent concentration, divided by influent concentration, in per cent), i.e., without differentiation between biodegradation, adsorption to sludge or, theoretically, loss to air. In the current guideline for

regulatory environmental risk assessment for pharmaceuticals in the European Union [40], a positive (RB) test outcome voids the normal requirement for an elaborate, costly water/sediment environmental fate test. The fact that NRB substances can be removed to a high extent in WWTPs may suggest that an additional WWTP simulation test that includes co-metabolism, e.g., OECD test guidelines 303A or 314B [21], and is performed at realistic substance concentrations, might also be introduced into the regulatory scheme; in case of significant removal shown in such a test, the requirement for a sediment/water environmental fate test might be dropped as well, and the removal in WWTPs might be factored into further considerations.

Table 4. Measured removal rates of brominated, chlorinated or fluorinated active organic pharmaceutical substances (AOPSs) in actual and pilot wastewater treatment plants.

AOPS Name	Halogens, n			Removal Pathways					References
	Br	Cl	F	Aerobic	Co-Metabolic	Anaerobic	Sorption	Total, %	
Bezafibrate		1		+	?	?	+	–10 to 99%	[41,42]
Bromazepam	1			?	?	?	?	up to 72% [a]	[41–45]
Chlorhexidine		2		?	?	–	?	60 to 100%	[46]
Ciprofloxacin			1	?	?	+	+	62 to 87%	[47,48]
Diazepam		1		+	?	+	?	0 to 99%	[49]
Diclofenac		2		?	?	?	+	0 to 99%	[41,44,50–52]
5-Fluorouracil			1	+	?	?	–	100%	[38]
Fluoxetine			3	?	?	?	+	negative	[45]
Glyburide		1		?	?	?	+	45 to 96%	[44,50,53–55]
Hydrochlorothiazide		1		?	?	?	+	0 to 76%	[53,56]
Indomethacin		1		?	?	?	?	0 to 99%	[41,49,55]
Nord(i)azepam	1			+	?	?	+	NQ	[42]
Norfloxacin			1	?	?	?	+	30 to 98%	[43,44]
Ofloxacin			1	?	?	?	+	20 to 99%	[41,44]
Oxazepam	1			+	?	?	?	20 to 24%	[52,53]
Triclosan		3		?	?	?	+	up to 95%	[56–58]

Note(s): In this limited literature search, no WWTP removal information was located for iodinated AOPSs. [a] WWTP with additional UV treatment [42]. Removal pathway symbols: + = demonstrated or likely; ? = unknown; – = unlikely.

These few examples show varying total removal rates ranging from negative up to 100%, which confirms that the finding of halogenated AOPSs being NRB does not translate to non-removal in working WWTPs. Many compounds can be expected to adsorb to sewage sludge based on lipophilicity, but microbiological dehalogenation and degradation have been analytically demonstrated, at least for a few compounds. In most cases, however, the principal removal pathway remains unknown. The observed negative removal is interpreted to show deconjugation of glucuronidated or sulphated human metabolites in the WWTP, (e.g., [49,59]), resulting in additional amounts of the original AOPS in the effluent; else, in some cases sampling issues and analytical uncertainties may suggest negative removal. Additionally, the wide ranges of removal rates for single AOPSs show that removal is not only a function of biodegradability and absorbability of single substances, but also of the construction type, composition of the influent, substance concentrations, aerobic and anaerobic sludge microbiology as well as hydraulic residence time of different WWTPs [60,61]. These findings further support the need for more targeted experimental investigation into WWTP removal for certain AOPSs including halogenated compounds.

5. Conclusions

The working hypothesis for this investigation was that halogenated AOPSs are less RB than non-halogenated ones and are therefore removed in WWTPs more through sorption to sludge than through biodegradation. Based on experimental and modelling data, this conjecture is supported by appropriate statistics; however, sorption to sludge for halogenated compounds may not fully compensate for their reduced biodegradability. Conversely, AOPSs with hydrophilic substitutions are more likely to be RB and be removed through biodegradation rather than sorption. As existing halogenated AOPSs are unlikely to be replaced by better degradable ones, other options must be investigated on how to improve WWTP layout and management for their enhanced removal.

6. Limitations

Sumpter et al. [62] have recently argued that scientific papers should include a 'Limitations' section, where shortcomings in the sources, methods or conclusions should be listed by the authors. The first limitation of this work is the (still) comparatively low number of AOPSs, with or without halogenation, with ready biodegradability data. Additionally, there is some inherent uncertainty about the equivalence of the tests, as discussed above. Apart from the tests, other properties of compounds besides halogenation will undoubtedly influence biodegradability. In addition, both WWTP characteristics and realistic influent concentrations will have an impact on substance removal.

Further, while representativeness has been checked regarding the occurrence of halogenated compounds in the dataset, it is not clear whether this dataset is representative of all human pharmaceuticals regarding disease areas covered; on scanning the list, several salicylates (pain and inflammation), "-tinib" tyrosine kinase inhibitors (cancer) and benzodiazepines (neurology and anaesthesia) catch the eye, all of which may be overrepresented, while there are not too many antibiotics or specific ophthalmologicals, for instance. Last, it is unknown whether the most prevalent chemical substance groups or the major AOPSs by market volume are suitably represented.

Assuming that most wastewaters containing excreted human AOPSs will be treated in a WWTP is reasonable overall for western Europe and North America, with the (not so exceptional) exception of combined sewer overflows leading to discharge of untreated wastewater. However, this assumption cannot be maintained for many countries in the tropics, as shown by the risk of faecal contamination of drinking water in sub-Saharan Africa [63].

The distribution between the removal pathways in a WWTP relies on modelling. While EPISuite is a rational and accepted tool, it is not the same as actual measurement (see also ref. [23]); on the other hand, using such models does allow the development and comparison of different scenarios as in the present work. Hai et al. [19] showed that, for halogenated compounds, lipophilicity alone does not sufficiently describe sorption, but that some factoring-in of halogenation is needed; further, Oh and Seo [64], working on adsorption of halogenated compounds to biochar, found that not only lipophilicity but also the pH of the medium is highly important for correctly describing sorption; to our knowledge, EPISuite does not specifically consider any halogenation factor nor the *p*H in its algorithms.

Last but not least, statistical results describe strengths of associations and correlations but do not thereby demonstrate causations.

Therefore, while the results presented here are likely to be valid and correct, it should be borne in mind that there are uncertainties and, in particular, that halogenation is certainly not the only determinant of biodegradability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15132430/s1, Table S1: Basic data; Table S2: Fisher's Exact Test; Table S3: WWTP fate; Table S4: WWTP halog vs. dehalog; Figures S1–S3: EPISuite-predicted WWTP removal through sorption, biodegradation and total removal for the original halogenated AOPSs and their in silico dehalogenated analogues.

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Conflicts of Interest: Until his regular retirement, J.O.S. used to work for the pharmaceuticals company F. Hoffmann-La Roche Ltd.; he draws a pension from and has shares in this company. The other authors declare no conflicts of interest.

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