Accepted Manuscript

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PII: S0304-3894(19)30804-0
DOI: https://doi.org/10.1016/j.jhazmat.2019.120851
Article Number: 120851

Reference: HAZMAT 120851

To appear in: *Journal of Hazardous Materials*

Received date: 25 January 2019
Revised date: 25 June 2019
Accepted date: 29 June 2019

Please cite this article as: Jaén-Gil A, Buttiglieri G, Benito A, Gonzalez-Olmos R, Barceló D, Rodríguez-Mozaz S, Metoprolol and metoprolol acid degradation in UV/H_2O_2 treated wastewaters: An integrated screening approach for the identification of hazardous transformation products, *Journal of Hazardous Materials* (2019), https://doi.org/10.1016/j.jhazmat.2019.120851

Metoprolol and metoprolol acid degradation in UV/H_2O_2 treated wastewaters: an integrated screening approach for the identification of hazardous transformation products

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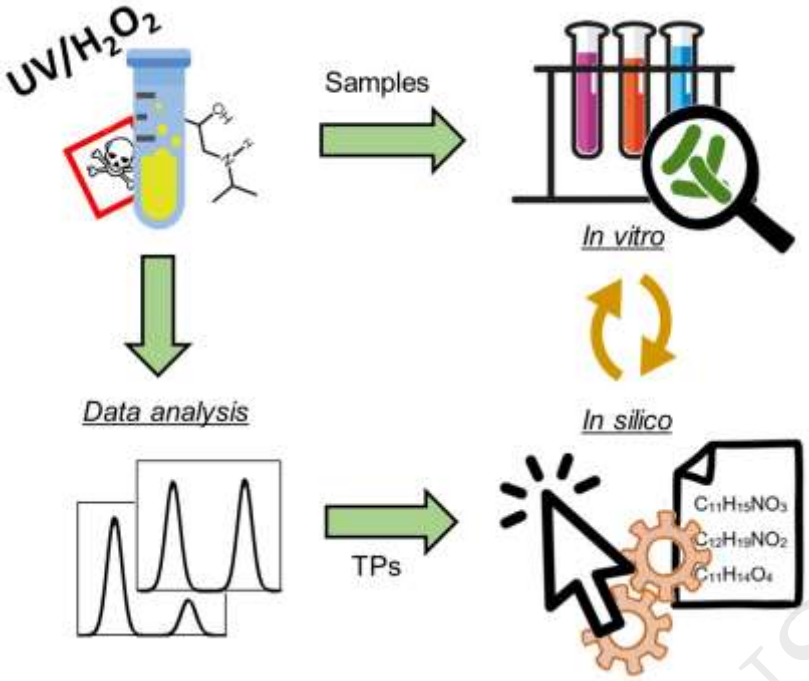
Graphical abstract



Highlights:

- Development of an integrated screening methodology for detection of hazardous TPs.
- Degradation and transformation of MTP and MTPA in UV/H_2O_2 experiments.
- Tentative identification of 24 TPs from MTP and MTPA photo-oxidation.
- Overview of the TPs generated in pure water, hospital and industrial wastewater.
- Ecotoxicity of the compounds identified using *in silico* and *in vitro* experiments.

ABSTRACT

Advancements on analytical strategies to determine the chemicals present in treated wastewater are necessary to clearly link their occurrence with the ecotoxicity of such effluents. This study describes the development of an integrated screening approach to

determine the highest number of pharmaceutical transformation products (TPs) in a single run. The identification of TPs was based on the comparison of detected features with literature sources, compound prediction tools, in-house libraries and reference standards using high resolution mass spectrometry (HRMS). This integrated approach allowed a better estimation (*in silico*) of the ecotoxicological contribution of the individual TPs identified. As a proof of concept, this methodology was applied for identification of the TPs generated from metoprolol and its main human metabolite (metoprolol acid) in pure water, hospital wastewater and industrial wastewater treated by UV/H_2O_2. Twenty-four TPs with potential ecotoxicological implications were identified and their presence was pinpointed as a function of the treated wastewater. An integrated screening approach has been developed using four different screening methodologies in the same run. Additionally, the metabolite MTPA has been considered as a target pollutant in UV/H_2O_2 experiments.

Keywords: *Metoprolol; Metoprolol acid; Advanced oxidation processes; Suspect screening; Hazard assessment.*

1. Introduction

A large number of pharmaceuticals compounds generated from industrial and domestic activities are present in wastewater effluents and released into the natural aquatic environment [1–3], where they can pose a long-term risk for aquatic organisms and human health [4–6]. Since conventional wastewater treatment plants (WWTPs) are not designed to eliminate these contaminants completely [3], the development of alternative and polishing wastewater treatment processes has become of high interest in order to attain appropriate quality status on treated water. Much time and efforts have been invested to monitor the removal efficiencies of selected pharmaceuticals by

means of alternative wastewater treatments [7–11]. In this context, advanced oxidation processes (AOPs) are among the most investigated, and suggested to be included in the wastewater treatment trains [12].

Among the pharmaceuticals present in wastewater, metoprolol (MTP) is a highly consumed β-blocker [13] detected in wastewater in the range of 160-2000 ng/L [14,15], with low removal rates in conventional WWTPs (usually between 0 and 36%) [15–17]. After human consumption, 10% of metoprolol is excreted unchanged in urine [14], whereas up to 60–65% of MTP initial dose is excreted as metoprolol acid (MTPA) as well as other metabolites (although at much lower concentration) such as O-desmethylmetorpolol (O-DMTP), α-hydroxymetoprolol (α-HMTP) and deaminated MTP [18–20]. According to the guidelines on environmental risk assessment of the European Medicines Agency, MTPA should be considered as a relevant MTP metabolite in monitoring studies being excreted at ≥ 10% of the administered dose [21]. Additionally, MTPA is pointed out to be also a transformation product (TP) of MTP in WWTPs and sometimes more recalcitrant than MTP itself [17]. The generation of this metabolite from atenolol biodegradation in activated sludge (CAS) has also been demonstrated [22].

Typically, sensitive and selective analytical methods have been developed for monitoring the elimination of target pollutants, driving studies to a limited number of chemicals [23]. The use of this approach becomes incomplete when applying to wastewater effluents, where the formation on unknown chemicals coming from biological and physicochemical transformation processes appears to be extensive [19]. The presence of TPs are of high concern since they may be more toxic and/or persistent than the parent compounds [18]. Therefore, the application of advanced analytical instrumentation based on high-resolution mass spectrometry (HRMS) becomes crucial for the detection and identification of unknown TPs in treated wastewater effluents. Different analytical strategies have been successfully applied for the screening of TPs, considering that the analytical reference standards of such TPs are not always available for confirmation [24–26]. Among them, non-target analysis with the selection of the most intense detected peaks represents the simplest applied strategy to prioritize compound identification [27]. However, the presence of hundreds of TPs coming from

several contaminants within a single sample points out post-acquisition data processing as a tedious, time-consuming and challenging task [28,29]. Suspect screening approaches have partially overcome this challenge, where the information on tentative compounds can be collected from software prediction tools or databases containing a broad number of compounds to be likely detected [30–33]. Therefore, the integration of these screening strategies in a single step may allow accounting for a greater proportion of TPs present in samples.

In recent years, hazard-oriented studies have been applied to assess the risk of compound mixtures of TPs using both *in vitro* bioassays and *in silico* studies [34–38]. So far, the most common applications for *in silico* modeling are the quantitative structure-activity relationships (QSAR) based methodologies. QSAR allows to estimate the ecotoxicological effects of the selected chemicals by quantitative association of their structural parameters (or physicochemical properties) with their biological activity [39]. The combination of these bioanalytical and computational tools may represent a holistic approach for a comprehensive assessment of the potential risks in treated wastewater effluents.

The aim of the present study is to develop an integrated screening methodology for comprehensive detection and identification of hazardous TPs in hospital (HWW) and industrial wastewater (IWW). A customized overview of MTP and MTPA transformation in the selected wastewater matrices treated by UV/H_2O_2 photo-oxidation is provided as a proof of concept. The ecotoxicity of the samples was determined by using an *in vitro* bioassay, as well as theoretically estimated using *in silico* QSAR models for all the individual compounds identified. This study highlights the utmost importance to perform an advanced and integrated screening approach for proper identification of hazardous TPs in wastewater effluents.

2. Experimental

2.1. Chemicals and reagents

Metoprolol tartrate salt (MTP) was purchased from Sigma-Aldrich (Barcelona, Spain); metoprolol acid (MTPA), O-desmethylmetoprolol (O-DMTP), and α-hydroxymetoprolol (α-HMTP) were supplied by Toronto Research Chemicals Inc. (North York, Canada) at

high purity grade (> 98%). Ultra-pure water, acetonitrile and methanol LiChrosolv grade were supplied by Merck (Darmstad, Germany).

2.2 Experimental set-up

UV/H_2O_2 photo-oxidation experiments were carried out under laboratory conditions at 25 ˚C using a UV Laboratory Reactor System from UV-Consulting Peschl® with a total working volume of 550 mL, approximately. The UV lamp consisted in a low-pressure mercury vapor lamp 15 W Heraeus Noblelight TNN 15/32 emitting at 254 nm. Preliminary experiments were performed in order to optimize the best AOPs conditions. H_2O_2 consumption was first optimized in pure water fortified at 10 mg/L of MTP and treated with UV, H_2O_2 and UV+H_2O_2 at 25, 100, 250 and 1000 mg/L. The optimized H_2O_2 concentration and the final experimental time (25 mg/L H_2O_2 and 10 min of reaction) were selected to further evaluate the elimination of MTP, MTPA and the generated TPs. Additionally, sodium thiosulfate was added to interrupt oxidation reaction (with stoichiometric excess of 20%). Then, individual degradation experiments at the optimized AOP conditions selected (25 mg/L H_2O_2 and 10 min of reaction) were launched to describe degradation kinetics in pure water of MTP and MTPA (spiked at 2.5 mg/L each).

Afterwards, three sets of experiments were performed in duplicate for the determination of TPs in: (a) pure water fortified with 2.5 mg/L of MTP and MTPA as a reference sample; (b) hospital wastewater (HWW) from the sewer manifold of Sant Joan de Déu Hospital (Barcelona, Catalonia) fortified with 2.0 µg/L of MTP and MTPA to assure the presence of the target pollutants at concentrations commonly detected in wastewater; and (c) industrial wastewater (IWW) from a pharmaceutical industry containing MTP at 33.0 mg/L. The samples were collected in duplicate at initial and final time (10 min) adding 20% in excess of sodium thiosulfate to stop oxidation reaction. Detailed information is presented in Supplementary Material, S1.

2.3 Sample analysis and data processing

The samples collected from the three sets of UV/H_2O_2 experiments as well as the reference samples (mix of individual standards available spiked at 2.5 mg/L) were analyzed using a liquid-chromatography system coupled to a (LTQ)-Orbitrap mass

spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) as described previously [40]. Detailed information of sample analysis is presented in Supplementary Material, S1.

A comprehensive screening methodology using Compound Discoverer 2.0 connected to Mass Frontier 7.0 software (Thermo Fisher Scientific Inc., Waltham, MA) was applied in a single run to the data collected after MS acquisition from pure water, HWW and IWW samples. The scheme containing the workflow procedure used for data treatment is presented in Fig. 1. Prior to automatic software data processing, input files (chromatograms and mass spectra files) were loaded together with two different lists containing suspected compounds to be present in samples: the 1st list containing compound exact masses from literature sources and the 2nd list (the in-house library) containing compound exact masses and retention times (R_t) obtained from previous experiments [40]. Additionally, MTP and MTPA chemical structures were pinpointed as well as tentative chemical transformations to further create the 3rd list of tentative predicted TPs by the software. Additional information is presented in Table S1.

Automatic data processing starts with MS data filtering between 50 and 400 Da and from 1 to 12 min with a S/N ratio of 3 (Fig. 1, Table S1). To compensate small differences in retention times, chromatographic alignment was performed by using a mass tolerance error of 5 ppm and a maximum retention time shift of 0.3 min. All those masses present in non-spiked pure water (control blank sample) were deducted from all matrix samples, by applying a mass and a retention time tolerance of 5 ppm and of 0.3 min, respectively. Immediately after, data processing was performed in two different steps: a) by detection of unknown compounds (where features above a S/N of 10 with a minimum peak intensity of 10^4 counts were selected) and b) by detection of expected compounds from compound prediction (where more complete MS full scan data was required without being filtered out). Then, the three lists of TPs previously indicated (from literature, in-house library and the one automatically created by the software) were used to identify the TPs generated from MTP and MTPA, jointly with the data acquired from the spiked control samples at a mass tolerance error of 5 ppm. This procedure was performed throughout four identification strategies, in accordance with the clarification scheme previously reported by Schymanski *et al.*, 2014 [41]: (1) the list from the literature (Table S2) was used to identify *unequivocal molecular formulas* (identification factor 1, IF=1) by

comparison of compound exact masses; (2) the list of predicted TPs automatically created from the software (Table S3) was used to identify *tentative structures* (identification factor 2, IF=2) by comparison of compound exact masses and predicted MS/MS scans; (3) the in-house library (Table S4) was used to identify *probable structures* (identification factor 3, IF=3) by comparison of reported TP exact masses, experimental retention times and MS/MS ion spectra; (4) *confirmed structures* (identification factor 4, IF=4) were identified with reference standards through comparison with MS exact masses, retention time and MS/MS ion fragmentation pattern from control files. Since most of the compounds were identified from more than one identification strategy, the maximum confidence attained for each compound was assigned as follows: *unequivocal molecular formulas* (IF=1) < *tentative structures* (IF=2) < *probable structures* (IF=3) < *confirmed structures* (IF=4).

All information provided by the software was manually checked (to avoid false positives hits) and the compounds with reasonable confidence (IF≥2) were further included into the existing in-house library for the detection of MTP and MTPA TPs in future studies. Then, transformation pathways were suggested and TPs were classified as 1st, 2nd and ≥ 3rd generation regarding the number of chemical transformations applied to the MTP chemical structure (1, 2 or ≥ 3, respectively).

2.4. *In silico* and *in vitro* toxicological assessment

Since no reference standards are commercially available for most of the identified TPs, the software EPI Suite™ through ECOSAR™ model was applied to predict the following acute toxicity endpoints (expressed in mg/L) for each compound: 48-h *Daphnia* LC_{50}, 96-h fish LC_{50} and 96-h green algae EC_{50}. Acute Toxicity Estimation (*ATEmix*) was calculated to evaluate the toxicity contribution of all identified chemicals present in each mixture sample, in comparison with the estimated toxicity at the initial time (Eq. 1) [42]. Potential synergistic and antagonistic effects between the compounds are excluded in this equation. C_i denotes the presence of a compound present in a mixture (in %) and ATE_i accounts for the acute toxicity estimated for an ingredient (EC_{50} or LC_{50}).

$$\frac{100}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i} \qquad \text{(Eq. 1)}$$

The *in silico* estimations were tentatively correlated with the individual ecotoxicological contribution of the parent compounds (MTP and MTPA) and the TPs identified using *in vitro* bioassays. The ISO 11348-3 protocol ([43] and Supplementary Material, S1) for testing bacterial bioluminescence of wastewater matrices was used to assess toxicity throughout Microtox® Model 500 Toxicity Analyzer (Strategic Diagnostics Inc. Newark, DE, US). The percentage of decay on emitted light was measured when samples were in contact 15 min with the bioluminescent bacterium *V. fischeri* at a final experimental time of 10 min. The presence of sodium thiosulfate in bioassay was tested and had no toxic effect on luminescent bacteria at the added concentration.

Additional parameters were also evaluated in accordance with the individual structural properties of the detected emerging TPs such as bioaccumulation factor, mutagenicity and developmental toxicity using the Toxicity Estimation Software Tool (T.E.S.T.) v. 4.2.1 program (consensus method). Chemical biodegradability, carcinogenicity and toxicological hazards according to the Cramer classification scheme [44] were evaluated using Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) v. 3.1.0 (Ideaconsult Ltd, Sofia, Bulgaria).

3. Results and Discussion

3.1 MTP and MTPA degradation kinetics

The preliminary experiments in fortified pure water (MTP at 10 mg/L) with UV, H_2O_2 and UV+H_2O_2 (at 25, 100, 250 and 1000 mg/L) promoted high removal efficiencies of MTP up to 99% after few minutes in most of the cases (Fig. S1). While H_2O_2 alone had no effect on MTP degradation, UV and UV+H_2O_2 experiments provided increasing MTP degradation rates with increasing H_2O_2 concentration (Fig. S2). Since a very high removal was already achieved at low H_2O_2 dosages, further experiments were performed at 25 mg/L of H_2O_2 and 10 min of reaction. Afterwards, the removal of MTP and MTPA (at an initial concentration of 2.5 mg/L each) was monitored in separated experiments (Fig. S3). The fast removals of MTP and MTPA fitted quite well ($R^2 > 0.98$) pseudo first-order kinetics (Fig. S4) with K_{obs} of 1.95 min^{-1} and 2.39 min^{-1} for MTP and MTPA, respectively. Additional is provided in Supplementary Material, S3.

Finally, dissimilar results were obtained regarding MTP and MTPA removal for the three matrices tested in TP determination experiments. They were both eliminated almost 100% in pure water (initial concentration 2.5 mg/L each), whereas the elimination rates in hospital wastewater were 71.6 ± 0.8% for MTP and 88.7 ± 1.1% for MTPA (initial concentration 2.0 µg/L each). In contrast, only 11.1 ± 1.5% of MTP (initial concentration 33.0 mg/L) was eliminated in industrial wastewater. These findings indicate that many other factors are involved (e.g. organic matter, bacteria, pollutant concentration and in general matrix effect, among others, Table S5) and seemed to interfere in MTP and MTPA elimination by the AOP technology. Moreover, some recalcitrant by-products might be formed which could not be completely degraded under the selected UV/H_2O_2 conditions. Thus, the elucidation and identification of their transformation pathways as well as the evaluation of their toxicity in the different matrices are required to provide a comprehensive overview of the treatment technology performance.

3.2 Detection and identification of TPs

Characterization of MTP and MTPA transformation through UV/H_2O_2 advanced oxidation processes was performed by applying the methodology described in Section 2.3. in pure water, HWW and IWW matrices. Peak filtering resulted in a total of 2,194 features of interest to be further processed through the four identification strategies selected (Fig. 2). After data processing, 85 candidates were finally pinpointed as potential TPs from MTP and MTPA (Table S6), which highlights a dramatic data reduction of 96%.

Among them, 88% (75 features) were detected by automatic comparison with the selected compound exact masses, collected from the literature list in Table S2 (32 exact masses out of 39 compounds were detected at different retention times, Table S6) and their predicted isotopic patterns. Since the molecular formula was the only identification factor that could be considered for each compound (IF=1), the chance of false positives was especially significant for this suspect screening strategy. For instance, the presence of *m/z* 284.18563 (α-HMTP) was found at five different retention times along the same chromatogram, indicating poor selectivity on peak detection. Among the 75 compounds detected, 92.9% were detected matching two isotopic ions from the predicted pattern,

while a 6.7% and a 0.4% were matched with three and four isotopic ions. These TP candidates were classified as *unequivocal molecular formulas* (IF=1).

Another set of compounds (22 compounds out of the 85 final candidates; 26%) (Fig. 2 and Table S6) was detected based on the comparison of the compound exact masses and fragmentation spectra of the TPs predicted by the software (Table S3) with the data acquired (IF=2). The total number of predicted candidates automatically generated and included into the prediction list was 357 (264 for MTP and 93 for MTPA, Table S3), meaning that only a small percentage of them was detected in the samples. Even though this strategy provides valuable information to rapidly identify *tentative structures*, manual inspection was always required to avoid false positive hits. Chemical structures were classified as features when the predicted MS/MS spectra included at least 3 characteristic fragments and/or FISh (Fragment Ion Search) coverages ≥ 65% [45].

The identification using in-house libraries (Table S4) allowed the detection of 15 compounds (18% of the 85 total suspected candidates; Fig. 2 and Table S6), having the same compound exact masses, experimental retention times and product fragmentation patterns as in previous MTP and MTPA degradation studies [40]. For instance, the fragmentation spectra of TP284, previously reported in fungal experiments at R_t of 7.31 min [40], was also detected in the present study with UV/H_2O_2 treatment at the same retention time. These features summed an additional identification factor (IF=3) to be classified as *probable structures*.

Finally, 5% (4 compounds) of the 85 TP candidates were classified as *confirmed structures* after comparison with analytical standards (Fig. 2 and Table S6), being this strategy overly restrictive (IF=4). Due to the overall limited availability of chemical standards of contaminant TPs, the application of other screening strategies based on literature information, compound prediction and in-house libraries are necessary to attain an enhanced overview of the TPs generated.

The obtained results highlight the increase in the number of features with the decrease of identification factors number. The four compounds confirmed with reference standards were also detected through the other three strategies (in-house library, compound prediction and literature information). The use of the in-house library

allowed the detection of 11 additional compounds. However, 4 out of the 15 compounds identified using in-house libraries were not detected using software compound prediction: two of them were not predicted by the software (e.g. *m/z* 238.14376 and *m/z* 240.15940) while the other two were not intense enough to perform MS/MS ion fragmentation (e.g. *m/z* 254.13868 and *m/z* 316.17545). Since no MS/MS confirmation was possible, these 4 TPs were classified as *tentative structures* through TP exact mass and retention time comparison only (IF=2). Moreover, 12 out of 37 compounds present in the literature list were not included into the software predicted list either. On the other hand, the use of compound prediction strategy allowed the inclusion of 345 tentative exact masses not present into the ready-made literature list. The obtained results indicate that the combination of different suspect screening strategies is required to account for the highest number of TPs.

After compound identification (Table S7), MTP and MTPA transformation pathways were suggested taking into account the 26 compounds with IF ≥ 2 from the 85 initial candidates (Fig. 3). Finally, the new generated information was included into the in-house database to perform faster and more reliable screening analysis of TPs in future studies. In comparison with other studies previously reported [45–47], this methodology limited the presence of false positives at a higher extent, reducing time and efforts invested in data processing.

3.3 MTP and MTPA transformation in wastewater effluents

The removal percentages of MTP and MTPA and the relative abundance of photo-oxidation intermediates were calculated at the final experimental time of 10 min for each of the considered water matrices (Fig. 4). Since no references standards were available for all the intermediates identified (to quantify losses on SPE extraction) and their chemical structure were similar to the parent compound (MTP), the same recovery and matrix effect were considered for all TPs identified in the suggested semi-quantification approach.

The highest removal rates were achieved with MTP and MTPA spiked in pure water (2.5 mg/L) as indicated in section 3.1. The absence of other interfering contaminants and organic matter led to extremely high elimination rates (≥ 99%). A similar pattern was

observed in the elimination of the generated intermediates, with 82% of them classified as ≥ 3rd generation TPs (Fig. 4). These compounds are mainly described as residual TPs (TP114, TP116, TP121, TP134 and TP150) indicating that the treatment process is close to attain total compound mineralization. For instance, TP114 (corresponding to the lowest molecular mass identified in the analyzed samples) was detected at a relative abundance of 72%.

The results of the experiments performed with UV/H_2O_2 treating HWW (spiked with MTP and MTPA at the realistic concentration of 2.0 µg/L) were quite different (Fig. 4): 28% of MTP remained in the samples at the end of the treatment (MTP removal of 72%). Similar removal rates were observed for MTPA (89%). The higher HWW matrix complexity reduced the efficiency of the UV/H_2O_2 treatment in comparison with pure water experiments. There was, in fact, higher relative percentage of 1st and 2nd generation TPs (up to 39% and 53%, respectively) and lower percentages of those ≥ 3rd generation, confirming the delay in terms of global degradation rates. Higher proportion of the recalcitrant intermediates α-HMTP and TP240 were also found in comparison with pure water experiments, attaining percentage of about 39% and 47%, respectively. Among them, the α-HMTP was reported as a persistent TP in activated sludge [17] while both of them were also detected in fungi experiments [40].

Finally, the last experiments in IWW were characterized by a high content of organic matter (Table S5) and the extremely high MTP concentration (33.0 mg/L). This source was collected from a pharmaceutical industry producing MTP, whereas no MTPA was detected. The efficiency in terms of MTP elimination was much lower than in previous cases (only 11%). The degradation pathways of MTP were also affected, leading to a large increase in terms of number and presence of 1st generation TPs (64% of the total compounds detected in IWW). This is for example the case of TP300, a 2nd generation TP found in HWW and less present in IWW while TP284, 1st generation TP and intermediate in the formation of TP300 (Fig. 3), was present at higher concentration in IWW (Fig. 4). Likewise, TP240 (2nd generation TP) was more present in HWW than in IWW whereas O-DMTP, 1st generation TP and intermediate in the formation of TP240 (Fig. 3), was present at higher concentration in IWW (Fig.4). It is important to mention that O-DMTP has also been reported as a compound of environmental concern [17].

These results emphasize the difficulties in treating this kind of matrices with UV/H_2O_2, as expected, but interestingly shade lights also on TP generation.

As a conclusion, maintaining the same UV/H_2O_2 conditions, different removal profile of MTP and MTPA was observed, as a function of the water matrix and the initial concentration(s) of the parent compound(s). Extremely different scenarios were also observed in terms of presence of the identified intermediates (Fig. 4), also due to the influence of the different organic matter content and other interfering compounds of the water matrix on degradation mechanisms. In contrast with other reported AOP experiments (Fenton, photo-Fenton, ozonation and Fe^{2+}/ozonation [48–50]), it is important to remark that MTPA was highly eliminated by UV/H_2O_2 photo-oxidation not only in pure water but also in such a complex matrix like HWW.

3.4 Ecotoxicological impacts of the generated TPs

The detection and identification of known and unknown intermediates of target compounds provided the possibility to focus on those compounds of potential concern. While the removal of MTP and MTPA decreased from pure water to HWW and IWW experiments (Fig. 5a), the calculated *in silico* acute toxicity, relative to the toxicity estimated at the initial time, increased after AOP treatment up to 35% in IWW (Fig. 5b) and decreased up to 100% and 43% in pure water and HWW, respectively. This fact might be related to the low degradability of MTP in IWW but also to the TPs generated. The presence of some non-residual TPs such as TP176, TP218, TP250 (estimated EC_{50} and LC_{50} lower than MTP for some end-points, Table S8) in IWW might be correlated to the estimated increase in toxicity after UV/H_2O_2 treatment. Actually, an increase in toxicity in the *V. fischeri* bioassay (*in vitro* toxicity test) was also observed after AOP treatment of real IWW (data not shown). However, it cannot only be attributed to the generation of MTP TPs but also to the generation of intermediates from all the compounds present, apart from MTP. In the case of pure water, no luminescence inhibition in *V. fischeri* bioassays was observed neither before nor after AOP treatment. The absence of measured toxicity in fortified pure water, also at the initial time before the treatment, prevents us to validate the decrease in toxicity observed by the *in silico* estimations (a reduction of almost 100%, Fig. 5b). This decrease in *in silico* toxicity would

be explained by the almost total removal of MTP and MTPA and to the relative low presence of detected intermediates (0.9%).

Additionally, the TPs identified in the three treated matrices were qualitatively evaluated in terms of structure-activity to predict if they might be persistent, bioacummulative, carcinogenic, mutagenic or generate adverse effects on the development of the organism (Fig. 6 and Table S9). Although the highest degradation of parent compounds and TPs was achieved treating fortified pure water, the majority of these TPs belong to ≥ 3rd generation TPs, containing α,β-unsaturated aldehydes and carbonyls groups (TP114) as well as aliphatic secondary amines, likely to increase the hazards of treated water (TP114, TP150 and TP134). The identified compounds in treated fortified pure water were less persistent (2%) and bioaccumulative (16%) than in HWW and IWW but more carcinogenic, mutagenic and developmental toxic (up to 81%), being most of them above the Threshold of Toxicological Concern (TTC, Cremer classification class III). This might suggest significant toxicity with appreciable risk to human health. However, it is important to mention that these qualitative analyses do not directly consider the relative presence of TPs (TPs presence in pure water was only 0.9%). Moreover, the parent compound MTP was, in fact, the most bioaccumulative compound present in the samples (Table S9). Total bioaccumulation and persistence of TPs in HWW and IWW resulted similar but the number and concentration of TPs were extremely different among them. Finally, the presence of the carcinogenic TP238 and TP252 (related to aliphatic and aromatic aldehydes in the molecular structures) should be considered of high concern in IWW also because of the high total presence of TPs in this matrix (up to 47.8% of the initial MTP concentration, 33.0 mg/L).

Although treated IWW was the most toxic matrix with persistent transformation products, those found in treated pure water were more degraded (2nd or ≥ 3rd generation) but also more hazardous in terms of mutagenicity and carcinogenicity. The wide differences in the presence and distribution of TPs in the tested treated matrices highlight the importance of performing individual and comprehensive studies to determine all by-products after water and wastewater treatment.

4. Conclusion

An integrated screening approach was applied as a proof of concept for the rapid characterization of metoprolol and metoprolol acid transformation products after UV/H_2O_2 photo-oxidation in spiked pure water, hospital wastewater and industrial wastewater. Among the total features detected, 88% were matched with those extracted from literature sources, 26% from compound prediction tools, 18% from in-house libraries and 5% were confirmed with reference standards. Finally, twenty-six compounds were selected for further discussion of their occurrence in the different matrices tested. Depending on the treated water matrix, extremely different scenarios were observed concerning the generation of hazardous TPs (*in silico*): while treated industrial wastewater was the most toxic matrix (containing persistent and less degraded TPs), pure water contained more degraded TPs but also more hazardous in terms of mutagenicity and carcinogenicity (though present at a lower concentration). However, further experiments would be required to better evaluate *in vitro* toxicity effects of TPs, *e.g.* increasing MTP and MTPA concentration and/or considering more appropriate bioassays.

Acknowledgements

This work has been funded by the Spanish Ministry of Economy and Competitiveness (projects CTM 2013 48545-C2-1-R and CTM2017-85385-C2-1-R) and partly supported by the Generalitat de Catalunya (Consolidated Research Groups 2017-SGR-14, 2017-SGR-1404, 2017-SGR-1124, 2017-SGR-1318 and 2017-SGR-1016). S.R.M and R.G.O acknowledge the “Redes de Excelencia 2015” program (CTM2015-71054-REDT). S.R.M and G.B. acknowledge the Ramon y Cajal research fellowships (RYC-2014- 16707 and RYC-2014-16754) from the Spanish Ministry of Economy and Competitiveness. A.J.G. acknowledges the predoctoral grant from AGAUR (2018FI_B1_00212). R.G.O thanks to “Obra Social La Caixa” for receiving funding to carry out this research through the Intensification Research Fellowship 2017-URL-IR2Q-023.

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Figure Captions

Fig. 1. Screening workflow containing the four different identification strategies used: identification from literature sources, software compound prediction, in-house libraries and analytical reference standards (IF = identification factor).

Figure 1.

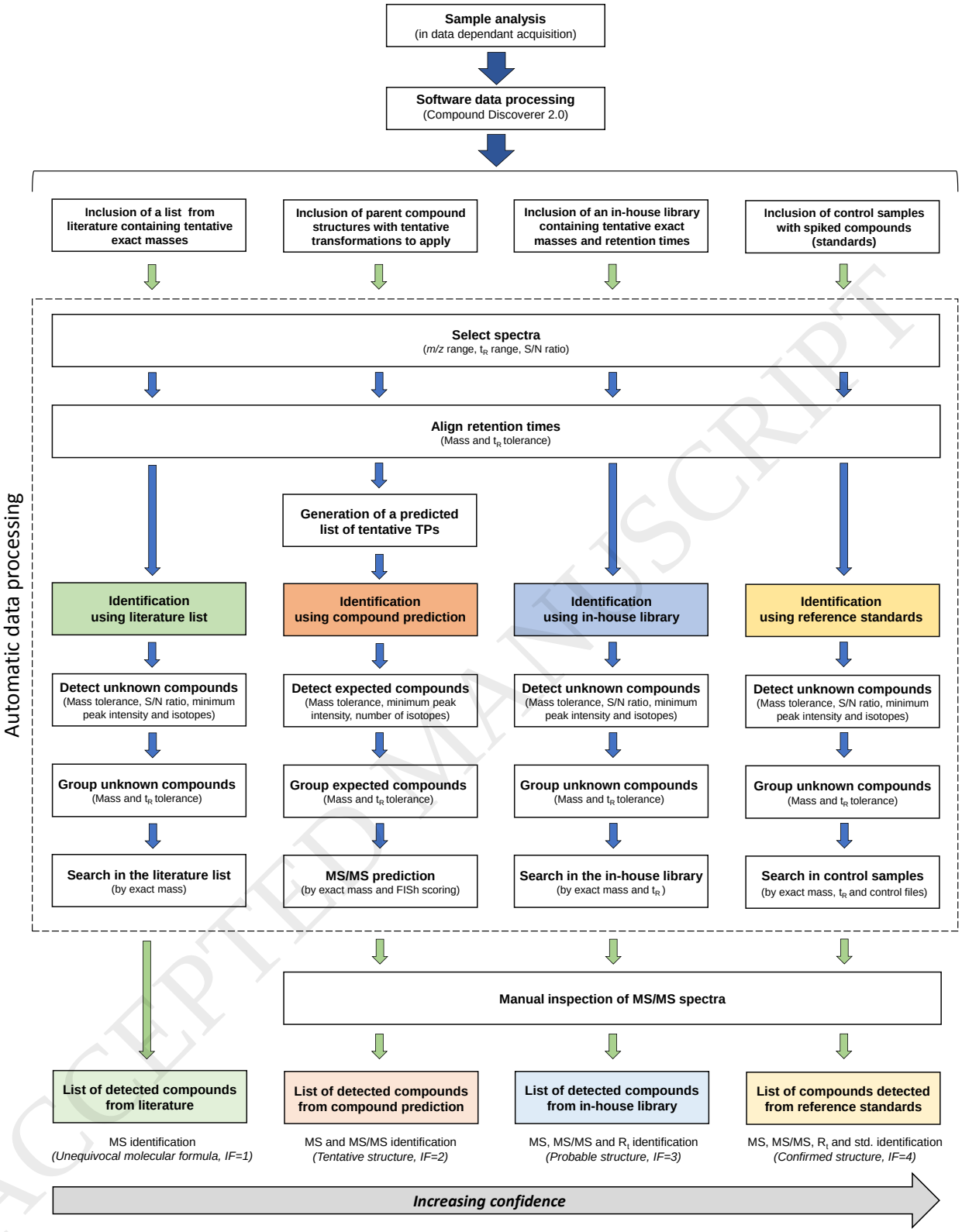

Fig. 2. Total ion features $[M+H]^+$ detected in pure water, HWW and IWW after data filtering grouped by molecular weight and retention time (grey dots). Identified features using the four strategies presented in Fig.1: literature (green dots), software compound prediction (red dots), in-house libraries (blue dots) and analytical reference standards (yellow dots).

Figure 2.

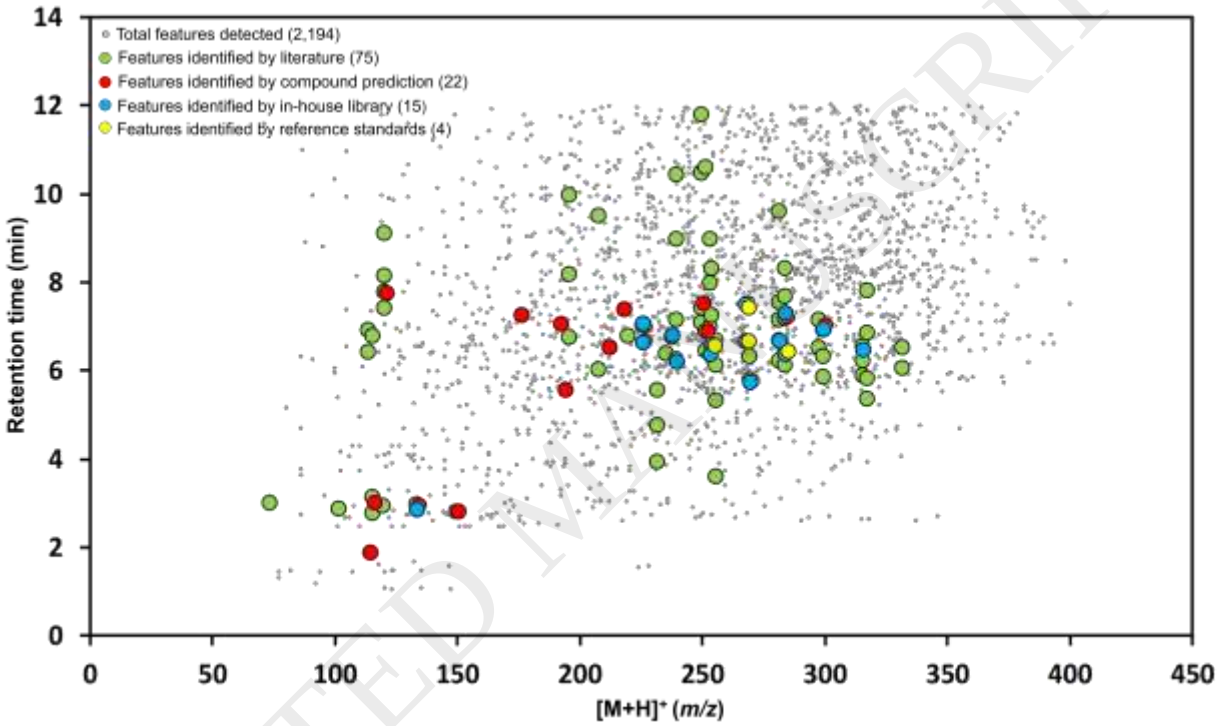

Fig. 3. TPs identified in pure water, HWW and IWW effluents though UV/H_2O_2 treatment: *tentative structures,* IF=2 (red); *probable structures,* IF=3 (blue); and *confirmed structures*, IF=4 (yellow).

Figure 3.

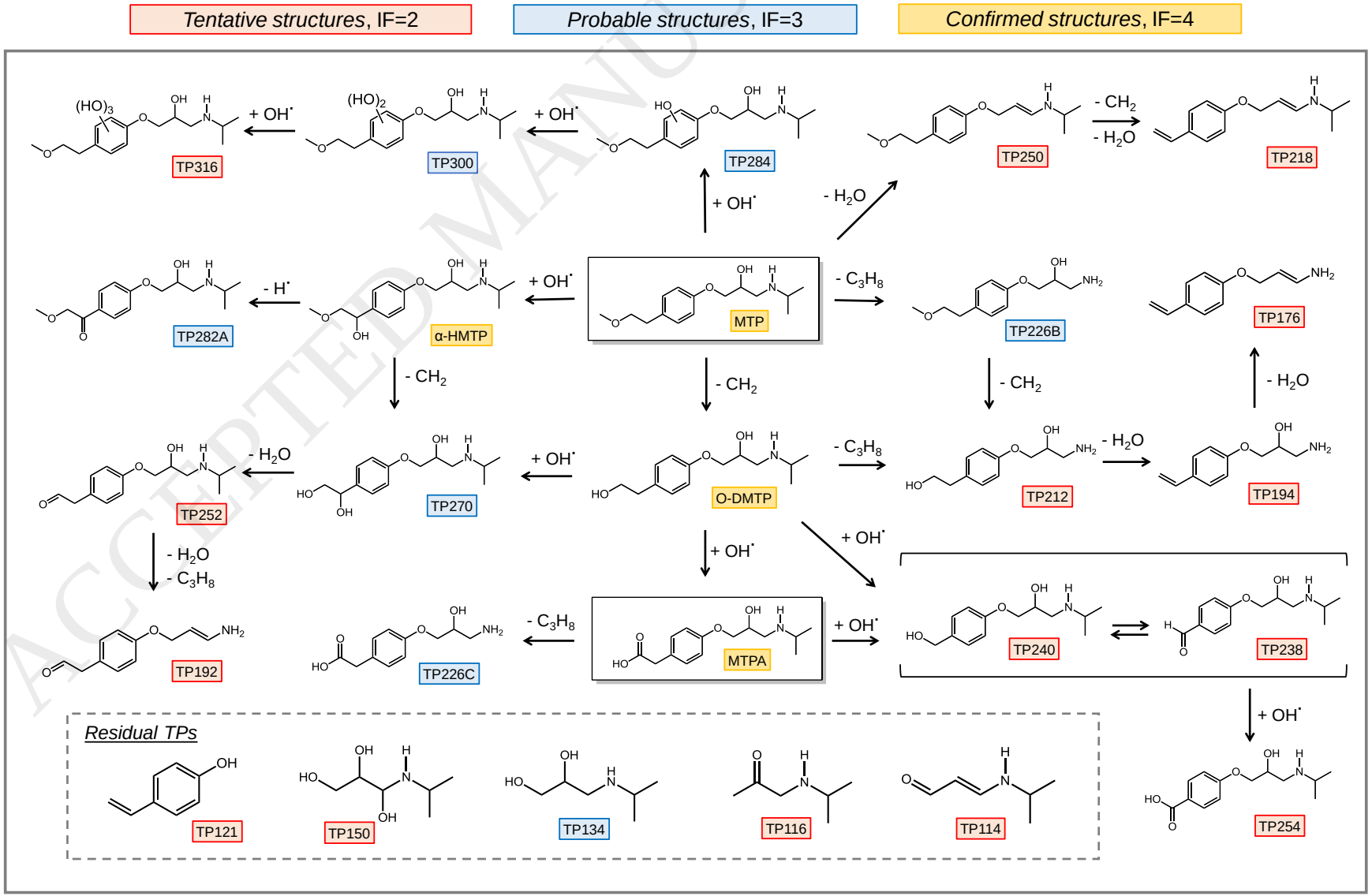

Fig. 4. Presence contribution ($Area^{t}_{TP}/\Sigma Area^{t}_{TPs}$)-(%) of the TPs identified in pure water, HWW and IWW though UV/H_2O_2 treatment at experimental final time of 10 min. TPs are classified as 1st generation (dark brown), 2nd generation (brown), and ≥ 3rd generation (light brown). Initial concentration, MTP and MTPA removal and TP presence as ($\Sigma Area^{t}_{TPs}/\Sigma Area^{0}_{MTP+MTPA}$)-(%) is also included.

Figure 4

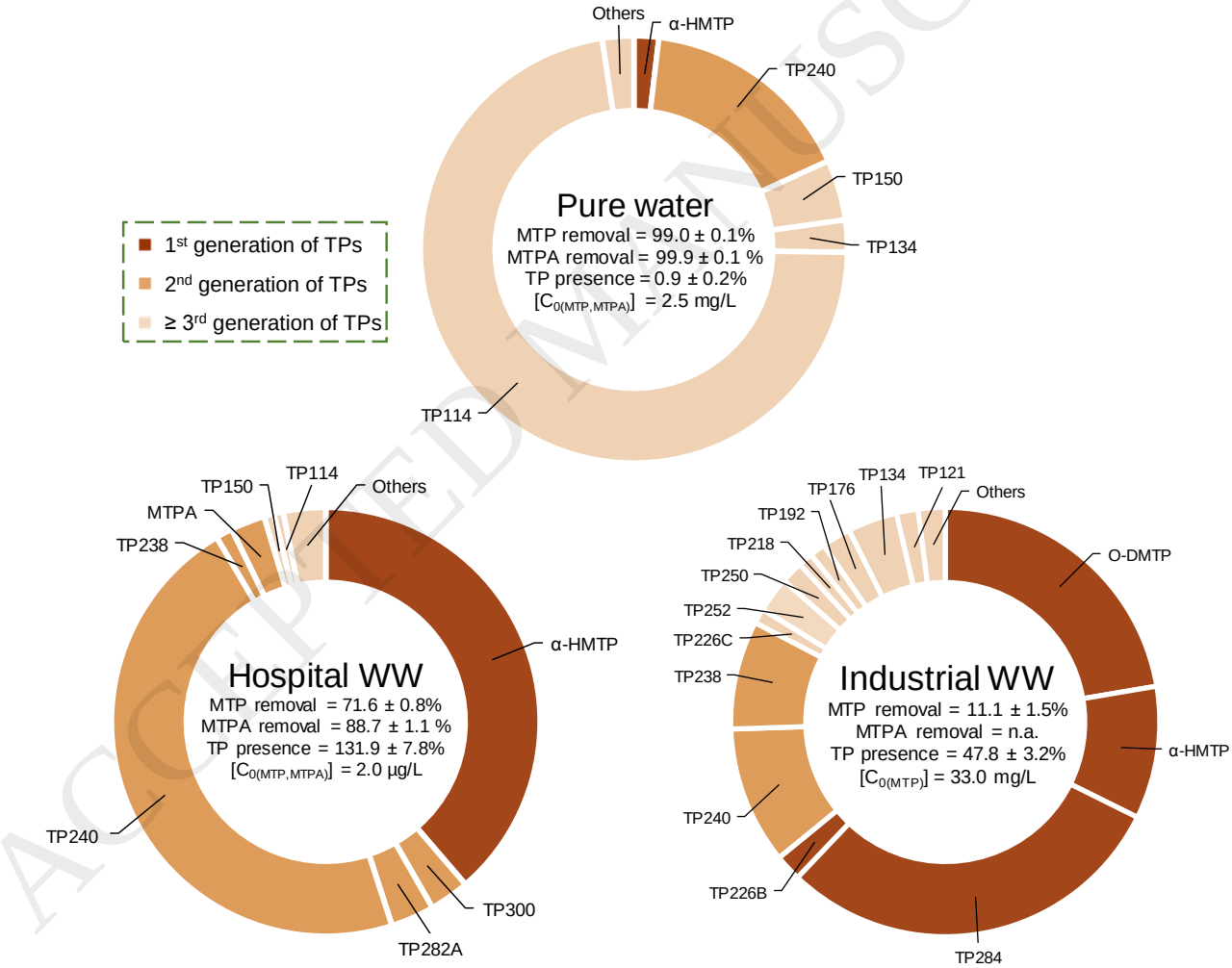

Fig. 5. a) Relative presence of MTP and MTPA in pure water, HWW and IWW after treatment of UV/H_2O_2. TP presence is included as ($\Sigma Area^{t}_{TPs}/\Sigma Area^{0}_{MTP+MTPA}$)-(%). b) Predicted *in silico* fish, *Daphnia* and green algae toxicities of the treated effluents using Eq. 1. Negative values indicate the decrease in toxicity along UV/H_2O_2 treatment.

Figure 5

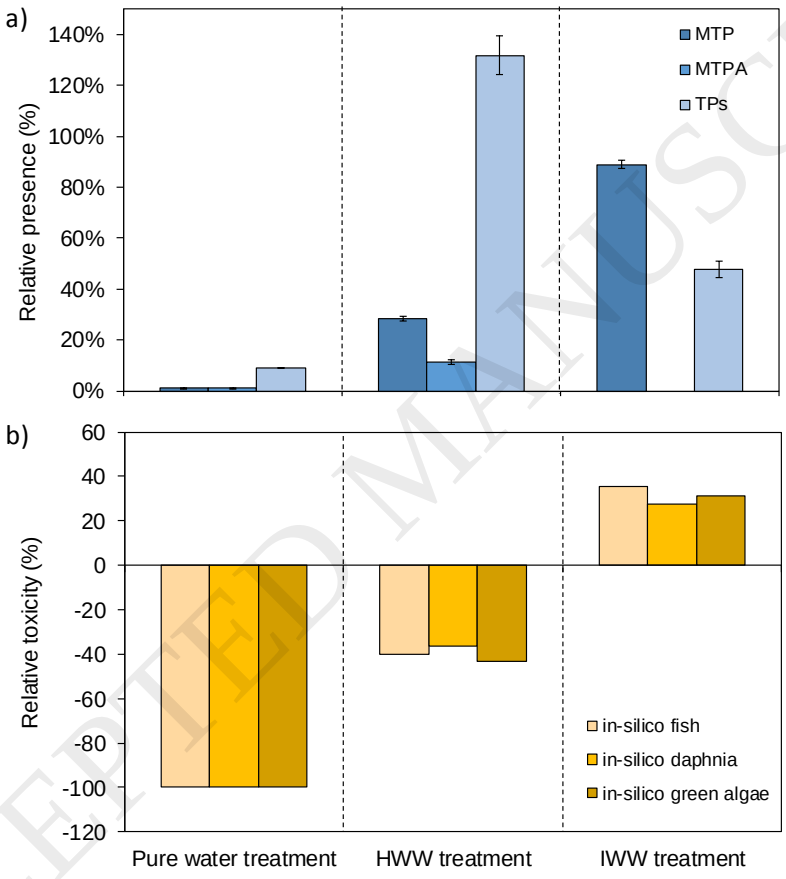

Fig. 6. Presence contribution ($Area^{t}_{TP}/\Sigma Area^{t}_{TPs}$)-(%) of the TPs detected in Fig. 4 grouped as persistent, bioaccumulated, carcinogenic, mutagenic and developmental toxic as well as Cramer hazard classification (Class III). TP presence is included as ($\Sigma Area^{t}_{TPs}/\Sigma Area^{0}_{MTP+MTPA}$)-(%).

Figure 6.