University of the Aegean

Department of Environment

PhD dissertation

Environmental risk assessment associated with the occurrence of emerging organic micropollutants in Sewage Treatment Plants

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Abstract

The term ‘Emerging Organic Contaminants’ (EOCs) includes a broad spectrum of chemicals that have not yet been regulated. Amongst others, they include pharmaceuticals (PhCs), illicit drugs (IDs), endocrine disrupting compounds (EDCs), perfluorinated compounds (PFCs), benzotriazoles (BTRs), benzothiazoles (BTHs), artificial sweeteners (ASs) and siloxanes (SLXs). These compounds are contained in everyday products and they are detected in domestic wastewater worldwide. Due to their physicochemical properties, some of them tend to adsorb onto the suspended solids during wastewater treatment, and are thus transferred to sewage sludge and soil; whereas others are discharged through treated wastewater to the aquatic environment.

Sufficient data concerning the concentration levels of EOCs in effluents and sewage sludge, as well as data regarding the toxicity of certain groups of EOCs in different groups of (micro)organisms, is now available in the literature. However, to date, the environmental risk associated with their presence in Sewage Treatment Plants (STPs) has not been sufficiently assessed. In order to achieve this purpose, the European Union (EU) has proposed a methodology based on Risk Quotients (RQs) calculation. This methodology has been applied, so far, to specific EOCs or/and individual rivers or lakes receiving wastewater. On the other hand, it is well known that a large number of EOCs coexist in STPs and via effluents or/and sewage sludge end up in the environment, worldwide. The main objective of this PhD thesis is to develop and implement an environmental risk assessment methodology based on RQs calculation in two phases. Initially, among all substances for which data are available, the potential most hazardous ones are identified. In a second tier, a more intensive risk assessment is applied for these specific micropollutants, while the relative uncertainty is quantified. In order to achieve the aforementioned main objective of this dissertation, the research was carried out in three steps, while the specific objectives were respectively: a) to estimate the possible environmental risks associated with the existence of EOCs in treated wastewater, on a country level and identify those substances that seem to pose the highest risk to Greek rivers receiving effluents from STPs, b) to assess the potential environmental risks from the disposal of sewage sludge containing EOCs in soil and identify those compounds that seem to present the

highest risk for Greece and c) to evaluate the risk associated with triclosan (TCS) released from STPs in European rivers using a probabilistic risk assessment approach.

In the first step of the study, the concentrations of all EOCs determined in Greek STPs were recorded through literature review. Acute toxicity data (EC50/LC50 values) was collected after literature review or using ECOSAR, and risk quotients (RQs) were calculated for treated wastewater and 25 Greek rivers, for 3 different aquatic organisms (fish, *Daphnia magna*, algae). According to the results, monitoring data was available for 207 micropollutants belonging to 8 different classes. RQ > 1 was calculated for 34 compounds in secondary treated wastewater. TCS (in algae), decamethylcyclopentasiloxane and tetradecamethylhexasiloxane (in fish) presented RQ > 1 for all studied rivers; decamethylcyclopentasiloxane (in *Daphnia magna*), caffeine (in algae) and nonylphenol (in fish) presented RQ > 1 in rivers with dilution factors (DF) equal or lower to 1910, 913 and 824, respectively. The classes of EOCs that present the greatest threat due to single or mixture toxicity were EDCs and SLXs. The mixture of microcontaminants seems to pose a great ecological risk, even in rivers with DF higher than 2300.

In the second step, data on the concentration levels of EOCs in sewage sludge of Greek STPs was collected after literature review. Risk assessment was based on both terrestrial and aquatic acute toxicity data, using both the maximum and the average measured concentrations of the target compounds. EC50/LC50 values were collected through literature review or using the ECOSAR program in cases that experimental values were not available. TCS (EDCs) seems to pose an environmental risk on the terrestrial organisms, as its RQ value exceeded 1, both in terrestrial and aquatic toxicity data based risk assessment. Calculations based on aquatic toxicity data showed that another 11 compounds had risk quotients higher than 1, most of them belonging to the classes of EDCs and SLXs. Tetradecamethylhexasiloxane presented the highest quotient among the evaluated compounds, while high quotients were also calculated for decamethylcyclopentasiloxane and caffeine. No environmental risk for the terrestrial environment is expected due to the individual action of IDs, PFCs and BTRs. Although the estimated threat due to nonylphenolic compounds showed significant variation depended on the sludge source and the day of sampling, these factors did not affect the estimated risk for SLXs, caffeine and ofloxacin. The mixture's RQ_{soil}, calculated using either the maximum or the average concentration

values, far exceeded 1 (253 and 209, respectively), thus indicating that a threat to the terrestrial environment due to the baseline toxicity of specific compounds may be presumed. It is worth mentioning that four SLXs contributed significantly (more than 90%) to this result.

In the last part, a probabilistic risk assessment was applied to investigate the environmental risks for the European aquatic environment associated with TCS occurrence in treated wastewater. The concentrations of TCS in effluents of STPs were recorded through literature review, while toxicity data was collected for three groups of aquatic organisms (algae, *Daphnia magna* and fish). RQs were calculated for risk characterization, while Monte Carlo simulation was applied to quantify the associated uncertainty. TCS monitoring data was available for 349 STPs located in 15 out of the 50 European countries. Its mean concentrations in STPs effluents ranged between 2.2 ng L^{-1} and 47,800 ng L^{-1}. Higher TCS concentrations were observed in primarily treated wastewater, whereas no differences among countries or among secondary and tertiary effluents on the basis of the whole set of collected data were found. The 95th percentile of RQ for TCS was higher than 1 (in algae) for rivers with dilution factors (DFs) equal to or lower than 100, when the maximum concentration values were used, whereas the 95th percentile of RQ exceeded 1 for rivers with DFs up to 10, in cases where the calculations were based on mean concentration values. The probability that RQ exceeds 1 in rivers (for algae) ranged from 0.2% (DF = 1000) to 45% (DF = 2), when calculations are based on mean concentration values. The corresponding probabilities in rivers with DFs equal to 2 for *Daphnia magna* and fish were 0.7% and 0.4%, respectively.

This thesis is divided into four chapters. Chapter 1 includes a short literature review on the target groups of micropollutants investigated, the Environmental Risk Assessment (ERA) process and the relevant legislation in the European Union, as well as the objectives and the outline of this PhD thesis. In Chapter 2, the materials and methods are described. In Chapter 3, the results of this study are presented and discussed, while Chapter 4 summarizes the most important conclusions, as well as suggestions for future research. Subsequently, in the Annex, supplementary data is presented.

Keywords

Emerging organic contaminants (EOCs), Micropollutants, Sewage, River water, Sludge-amended soil, Environmental risk assessment, Risk quotient, Probabilistic risk assessment, Monte Carlo simulation

Περίληψη

Ο όρος ‘Αναδυόμενοι Οργανικοί Μικρορρύποι’ (ΑΟΜ) περιλαμβάνει ένα ευρύ φάσμα χημικών ενώσεων, οι οποίες δεν καλύπτονται από την υπάρχουσα νομοθεσία και μεταξύ άλλων περιλαμβάνουν φαρμακευτικές ουσίες, ναρκωτικά, ενδοκρινικούς διαταράκτες, υπερφθοριωμένες ενώσεις, βενζοτριαζόλια, βενζοθειαζόλια, συνθετικές γλυκαντικές ύλες και σιλοξάνια. Οι ενώσεις αυτές περιέχονται σε προϊόντα καθημερινής χρήσης και ανιχνεύονται σε αστικά απόβλητα σε όλο τον κόσμο. Εξαιτίας των φυσικοχημικών ιδιοτήτων τους, ορισμένες από αυτές παρουσιάζουν την τάση να προσροφώνται στα αιωρούμενα στερεά κατά τη διάρκεια της επεξεργασίας των υγρών αποβλήτων και για το λόγο αυτό καταλήγουν μέσω της ιλύος στο χερσαίο περιβάλλον, ενώ άλλες διοχετεύονται μέσω των επεξεργασμένων υγρών αποβλήτων στο υδατικό περιβάλλον.

Παρά το γεγονός ότι στη βιβλιογραφία υπάρχουν πλέον αρκετά διαθέσιμα δεδομένα για τα επίπεδα συγκεντρώσεων των ΑΟΜ στα επεξεργασμένα απόβλητα και στην ιλύ, καθώς επίσης και δεδομένα για την τοξικότητά ορισμένων ΑΟΜ σε διαφορετικές κατηγορίες (μικρό)οργανισμών, μέχρι σήμερα δεν έχει εκτιμηθεί επαρκώς ο περιβαλλοντικός κίνδυνος από την παρουσία τους στις Μονάδες Επεξεργασίας Λυμάτων (ΜΕΛ). Για την επίτευξη του συγκεκριμένου στόχου, η Ευρωπαϊκή Ένωση έχει προτείνει μία μεθοδολογία που στηρίζεται στον υπολογισμό πηλίκων επικινδυνότητας (RQ). Η συγκεκριμένη μεθοδολογία μέχρι σήμερα έχει εφαρμοστεί για συγκεκριμένους ΑΟΜ ή/και μεμονωμένους αποδέκτες που δέχονται εκροές από τις ΜΕΛ. Από την άλλη, είναι γνωστό ότι στις ΜΕΛ, παγκοσμίως, συνυπάρχει ένας μεγάλος αριθμός ΑΟΜ που καταλήγουν μέσω των υγρών αποβλήτων ή/και της ιλύος στο περιβάλλον. Κύριος στόχος της παρούσας διδακτορικής διατριβής είναι η ανάπτυξη και η εφαρμογή μίας μεθοδολογίας εκτίμησης περιβαλλοντικού κινδύνου που βασίζεται στα πηλίκα επικινδυνότητας και περιλαμβάνει σε πρώτο στάδιο τον εντοπισμό των πιθανών πλέον επικίνδυνων ΑΟΜ από το σύνολο των ουσιών για τις οποίες υπάρχουν διαθέσιμα δεδομένα και σε δεύτερο στάδιο τη συστηματικότερη εκτίμηση κινδύνου συγκεκριμένων ρύπων, με παράλληλη ποσοτικοποίηση της σχετικής αβεβαιότητας που προκύπτει από τους εν λόγω υπολογισμούς. Για να επιτευχθεί ο προαναφερόμενος κύριος στόχος της διδακτορικής διατριβής η έρευνα υλοποιήθηκε σε τρία στάδια, οι επιμέρους στόχοι των οποίων ήταν αντίστοιχα: α) να εκτιμηθούν οι πιθανοί περιβαλλοντικοί κίνδυνοι που συνδέονται με την ύπαρξη ΑΟΜ

στα επεξεργασμένα υγρά απόβλητα σε επίπεδο χώρας και να εντοπιστούν οι ουσίες οι οποίες φαίνεται να παρουσιάζουν τη μεγαλύτερη επικινδυνότητα για τα ελληνικά ποτάμια που δέχονται εκροές από τις ΜΕΛ, β) να εκτιμηθούν οι πιθανοί περιβαλλοντικοί κίνδυνοι από τη διάθεση της ιλύος που περιέχει ΑΟΜ στο έδαφος και να εντοπιστούν εκείνες οι ουσίες που παρουσιάζουν τη μεγαλύτερη επικινδυνότητα για την περίπτωση της Ελλάδας, γ) να αξιολογηθεί ο περιβαλλοντικός κίνδυνος που σχετίζεται με την απελευθέρωσή της ουσίας τρικλοζάνης μέσω των ΜΕΛ στα ευρωπαϊκά ποτάμια, χρησιμοποιώντας μια προσέγγιση πιθανολογικής εκτίμησης κινδύνου.

Συγκεκριμένα, στο πρώτο στάδιο της παρούσας εργασίας, οι συγκεντρώσεις όλων των ΑΟΜ που έχουν ανιχνευτεί στις ελληνικές ΜΕΛ καταγράφηκαν, μετά από βιβλιογραφική ανασκόπηση. Τα δεδομένα οξείας τοξικότητας (EC50/LC50) για τις υπό μελέτη ουσίες συλλέχθηκαν είτε από τη βιβλιογραφία, είτε με χρήση του σχετικού μοντέλου ECOSAR και τα πηλίκα επικινδυνότητας (RQ) υπολογίστηκαν στα επεξεργασμένα υγρά απόβλητα και σε 25 ελληνικά ποτάμια, για 3 κατηγορίες οργανισμών που ζουν στο υδάτινο περιβάλλον (ψάρια, δαφνίδες, φύκη). Σύμφωνα με τα αποτελέσματα, δεδομένα συγκέντρωσης βρέθηκαν για 207 μικρορρύπους, οι οποίοι ανήκουν σε 8 διαφορετικές κατηγορίες. Τιμές για τα RQ μεγαλύτερες από τη μονάδα υπολογίστηκαν για 34 ενώσεις στα εξερχόμενα υγρά απόβλητα δευτεροβάθμιας επεξεργασίας. Για την τρικλοζάνη (στα φύκη) και τα δεκαμέθυλοκυκλο πεντασιλοξάνιο και τετραδεκαμέθυλο εξασιλοξάνιο (στα ψάρια) υπολογίστηκαν RQ > 1 σε όλα τα ποτάμια που μελετήθηκαν, ενώ για το δεκαμέθυλοκυκλο πεντασιλοξάνιο (στις δαφνίδες), την καφεΐνη (στα φύκη) και την εννεϋλοφαινόλη (στα ψάρια) υπολογίστηκαν RQ > 1 σε ποτάμια με συντελεστή αραίωσης ίσο ή μικρότερο από 1910, 913 και 824, αντίστοιχα. Οι κατηγορίες των ΑΟΜ που παρουσιάζουν μεγαλύτερη απειλή λόγω τοξικότητας των μεμονωμένων ουσιών ή των μειγμάτων είναι οι ενδοκρινικοί διαταράκτες και τα σιλοξάνια. Το μείγμα των μικρορρύπων φαίνεται ότι αποτελεί σημαντικό οικολογικό κίνδυνο, ακόμα και σε ποτάμια με συντελεστή αραίωσης μεγαλύτερο του 2300.

Στο δεύτερο στάδιο της έρευνας, συλλέχθηκαν από τη βιβλιογραφία τα επίπεδα συγκέντρωσης των ΑΟΜ στην επεξεργασμένη ιλύ των ελληνικών ΜΕΛ. Η εκτίμηση επικινδυνότητας πραγματοποιήθηκε με βάση δεδομένα οξείας τοξικότητας, τόσο για χερσαίους, όσο και για υδάτινους οργανισμούς, με χρήση τόσο της μέγιστης, όσο και

της μέσης συγκέντρωσης των υπό μελέτη ουσιών. Οι τιμές EC50/LC50 συλλέχθηκαν είτε μέσω βιβλιογραφικής ανασκόπησης, είτε με χρήση του προγράμματος ECOSAR, στις περιπτώσεις που τα πειραματικά δεδομένα τοξικότητας δεν ήταν διαθέσιμα. Η τρικλοζάνη φαίνεται να συνιστά περιβαλλοντικό κίνδυνο για τους χερσαίους οργανισμούς, αφού η τιμή RQ ήταν μεγαλύτερη της μονάδας, τόσο στην περίπτωση εκτίμησης επικινδυνότητας που βασίστηκε σε χερσαίους, όσο και σε αυτήν που βασίστηκε σε υδάτινους οργανισμούς. Οι υπολογισμοί με βάση τα δεδομένα υδάτινης τοξικότητας έδειξαν ότι ακόμη 11 ουσίες είχαν RQ > 1 και οι περισσότερες από αυτές ανήκαν στις κατηγορίες των ενδοκρινικών διαταρακτών και των σιλοξανίων. Το τετραδεκαμέθυλο εξασιλοξάνιο παρουσίασε το μεγαλύτερο πηλίκο επικινδυνότητας σε σχέση με όλες τις άλλες ουσίες, ενώ μεγάλες τιμές πηλίκων υπολογίστηκαν επίσης και για το δεκαμέθυλοκυκλο πεντασιλοξάνιο και την καφεΐνη. Δε φαίνεται να υπάρχει κίνδυνος για το χερσαίο περιβάλλον από τη μεμονωμένη δράση των ουσιών που ανήκουν στις κατηγορίες των ναρκωτικών, των υπερφθοριωμένων ενώσεων και των βενζοτριαζολίων. Παρόλο που ο εκτιμώμενος κίνδυνος από τις εννεϋλοφαινολικές ενώσεις έδειξε σημαντική διακύμανση εξαρτώμενη από την πηγή της ιλύος και τη μέρα της δειγματοληψίας, οι παράγοντες αυτοί δε φαίνεται να επηρεάζουν τον εκτιμώμενο κίνδυνο από τα σιλοξάνια, την καφεΐνη και την οφλοξασίνη. Το RQ του εδάφους για το μείγμα των ενώσεων, το οποίο υπολογίστηκε τόσο με τις μέγιστες, όσο και τις μέσες τιμές συγκεντρώσεων, υπερέβη κατά πολύ τη μονάδα (253 και 209, αντίστοιχα), γεγονός που υποδηλώνει πιθανή απειλή για το χερσαίο περιβάλλον, εξαιτίας της βασικής τοξικότητας (baseline toxicity) των συγκεκριμένων ουσιών. Είναι αξιοσημείωτο ότι 4 σιλοξάνια συνέβαλαν σημαντικά (σε ποσοστό άνω του 90%) στο συγκεκριμένο αποτέλεσμα.

Στο τελευταίο στάδιο της παρούσας διατριβής εφαρμόστηκε πιθανολογική εκτίμηση κινδύνου (probabilistic risk assessment) για τη διερεύνηση της επικινδυνότητας για το ευρωπαϊκό υδάτινο περιβάλλον, η οποία σχετίζεται με την ύπαρξη της τρικλοζάνης στα επεξεργασμένα υγρά απόβλητα. Οι συγκεντρώσεις της τρικλοζάνης στα εξερχόμενα υγρά απόβλητα των ευρωπαϊκών ΜΕΛ καταγράφηκαν μετά από βιβλιογραφική ανασκόπηση, ενώ συλλέχθηκαν δεδομένα τοξικότητα για 3 κατηγορίες υδάτινων οργανισμών (φύκη, δαφνίδες, ψάρια). Το πηλίκο RQ υπολογίστηκε για το χαρακτηρισμό του κινδύνου, ενώ η προσομοίωση Monte Carlo εφαρμόστηκε για την ποσοτικοποίηση της σχετικής αβεβαιότητας. Οι τιμές

συγκεντρώσεων της τρικλοζάνης ήταν διαθέσιμες για 349 ΜΕΥΑ που βρίσκονται σε 15 από τις 50 ευρωπαϊκές χώρες. Οι μέσες τιμές συγκεντρώσεων στα εξερχόμενα υγρά απόβλητα κυμαίνονται ανάμεσα στα 2.2 ng L^{-1} και 47,800 ng L^{-1}. Μεγαλύτερες τιμές συγκεντρώσεων καταγράφηκαν στα απόβλητα πρωτοβάθμιας επεξεργασίας, ενώ δεν παρατηρήθηκαν στατιστικά σημαντικές διαφορές συγκεντρώσεων μεταξύ των χωρών ή μεταξύ των μονάδων δευτεροβάθμιας ή τριτοβάθμιας επεξεργασίας. Το 95ο εκατοστημόριο του RQ ήταν μεγαλύτερο του 1 (στα φύκη) για ποτάμια με συντελεστή αραίωσης ίσο ή μικρότερο του 100, στην περίπτωση που χρησιμοποιήθηκαν οι μέγιστες τιμές συγκεντρώσεων στους υπολογισμούς, ενώ το αντίστοιχο εκατοστημόριο υπερέβη την τιμή 1 για ποτάμια με τιμή αραίωσης μέχρι την τιμή 10, στην περίπτωση που οι υπολογισμοί βασίστηκαν στις μέσες τιμές συγκεντρώσεων. Η πιθανότητα το RQ (για τα φύκη) να υπερβαίνει το 1 στα ποτάμια κυμαίνεται από 0.2% (συντελεστής αραίωσης 1000) μέχρι 45% (συντελεστής αραίωσης 2), όταν οι υπολογισμοί βασίζονται στις μέσες τιμές συγκέντρωσης. Οι αντίστοιχες πιθανότητες σε ποτάμια με συντελεστή αραίωσης 2, για τις δαφνίδες και τα ψάρια, ήταν 0.7% και 0.4%, αντίστοιχα.

Τα ακόλουθα κεφάλαια δομούν την παρούσα διατριβή: το Κεφάλαιο 1 περιλαμβάνει μια σύντομη βιβλιογραφική ανασκόπηση σχετική με τις κατηγορίες των ΑΟΜ που μελετήθηκαν, τη διαδικασία της Εκτίμησης Περιβαλλοντικού Κινδύνου και τη σχετική ευρωπαϊκή νομοθεσία, καθώς επίσης και τους στόχους της εργασίας. Στο Κεφάλαιο 2 περιγράφεται η μεθοδολογία του ακολουθήθηκε. Στο Κεφάλαιο 3 παρουσιάζονται τα ευρήματα της μελέτης, ενώ στο Κεφάλαιο 4 συνοψίζονται τα βασικά συμπεράσματα και παρουσιάζονται προτάσεις για μελλοντική έρευνα. Στο τέλος της διατριβής, στο Παράρτημα, παρατίθενται σε πίνακες διάφορα συμπληρωματικά στοιχεία.

Λέξεις-κλειδιά

Αναδυόμενοι οργανικοί μικρορρύποι, Υγρά απόβλητα, Ποτάμια, Έδαφος, Εκτίμηση περιβαλλοντικού κινδύνου, Πηλίκο επικινδυνότητας, Πιθανολογική εκτίμηση κινδύνου, Προσομοίωση Monte Carlo.

List of publications

Papers in Scientific Journals

Paper I Thomaidi V.S., Stasinakis A.S., Borova V.L., Thomaidis N.S., 2015. Is there a risk for the aquatic environment due to the existence of emerging organic contaminants in treated domestic wastewater? Greece as a case-study. *Journal of Hazardous Materials*, 283, 740-747.

Paper II Thomaidi V.S., Stasinakis A.S., Borova V.L., Thomaidis N.S., 2016. Assessing the risk associated with the presence of emerging organic contaminants in sludge-amended soil: A country-level analysis. *Science of the Total Environment*, 548-549, 280-288.

Paper III Thomaidi V.S., Matsoukas C., Stasinakis A.S., 2017. Risk assessment of triclosan released from sewage treatment plants in European rivers using a combination of Risk Quotient methodology and Monte Carlo simulation. *Science of the Total Environment*, 603-604, 487-494.

Papers in Scientific Conferences

I. Thomaidi V.S., Mazioti A.A., Stasinakis A.S., Thomaidis N.S. Risk assessment associated with the presence of emerging organic micropollutants originated from municipal wastewater in the aquatic environment: the case of Greek rivers, 25th Annual Meeting, Society of Environmental Toxicology and Chemistry (SETAC Europe), 3-7 May 2015, Barcelona, Spain (Abstr. Code TU204).

II. Thomaidi V.S., Stasinakis A.S., Borova V.L., Thomaidis N.S. Occurrence of emerging organic contaminants released from wastewater treatment plants in the aquatic environment and effects on aquatic life: the case of Greece, 14th International Conference on Environmental Science and Technology, CEST 2015, September 3-5, 2015, Rhodes, Greece (Abstr. Code cest2015_00076).

III. Thomaidi V.S., Stasinakis A.S., Borova V.L., Thomaidis N.S. Risk assessment associated with the presence of emerging organic contaminants released from wastewater treatment plants in sludge amended soil and effects on terrestrial life: Greece as a case study, 15th International Conference on Environmental Science and Technology, CEST 2017, 31 August to 2 September, 2017, Rhodes, Greece (Abstr. Code cest2017_00425).

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AF: assessment factor

AP: alkylphenol

APEO: alkylphenol ethoxylate

AS: artificial sweetener

BCF: bioconcentration factor

BPA: bisphenol A

BTH: benzothiazole

BTR: benzotriazole

DF: dilution factor

E1 : estrone

E2: 17β-estradiol

EC50: median effect concentration

EC: European Commission

ECHA: European Chemical Agency

EDC: endocrine disrupting compound

EE2: 17α-ethinyl-estradiol

EEA: European Environment Agency

EFSA: European Food Safety Authority

EOC: emerging organic contaminant

EQS: environmental quality standard

ERA: environmental risk assessment

EU: European Union

FR: flame retardant

ID: illicit drug

LC50: median lethal concentration

MCS: Monte Carlo simulation

MEC: measured environmental concentration

NAS: National Academy of Sciences

NOEC: no observed effect concentration

NP: nonylphenol

NRC: National Research Council

OP: octylphenol

PAH: polycyclic aromatic hydrocarbon

PBDE: polybrominated diphenyl ether

PBT: persistence, bioaccumulation, toxicity

PCB: polychlorobiphenyl

PCP: personal care product

PEC: predicted environmental concentration

PFC: perfluorinated compound

PhC: pharmaceutical

PNEC: predicted no effect concentration

PPCPs: pharmaceuticals and personal care products

PRA: probabilistic risk assessment

STP: sewage treatment plant

SLX: siloxane

T: testosterone

TCS: triclosan

TGD: Technical Guidance Document

TR: Toxic Ratio

UK: United Kingdom

USA: United States of America

USEPA: United States Environmental Protection Agency

WFD: Water Framework Directive

WTP: water treatment plant

1. Literature Review

1.1. Emerging Organic Contaminants

1.1.1. General introduction

Organic micropollutants are released into the environment via effluents originating from Sewage Treatment Plants (STPs). These compounds may pose an ecological risk to aquatic and terrestrial organisms and might adversely affect human health via the food chain. Increasing concern about the potential hazard they pose to biota has triggered a great deal of research on this issue.

The term *emerging organic contaminants* (EOCs) refers to organic microcontaminants that have long been present in the environment, but have not gained scientific attention until recently (Wu et al., 2010). They are used in large quantities in our daily life and include a wide variety of compounds such as personal care products (PCPs), endocrine disrupting compounds (EDCs), pharmaceuticals (PhCs), illicit drugs (IDs), flame retardants (FRs), industrial additives and reagents, artificial sweeteners (ASs), perfluorinated compounds (PFCs), benzotriazoles (BTRs), benzothiazoles (BTHs), siloxanes (SLXs), and water disinfection by-products (Figure 1). Previous studies have shown that most of these compounds present partial or no removal during sewage treatment (Bletsou et al., 2013; Stasinakis et al., 2013) and as a result they are often detected in treated wastewater and the aquatic environment, worldwide (Farré et al., 2008; Kokotou and Thomaidis, 2013; Santos et al., 2013; Robles-Molina et al., 2014).

Although some of the EOCs have been regulated for water quality monitoring in the last few years (Barbosa et al., 2016), for the majority of them there are no legal environmental discharge limits, as they are not covered under worldwide routine monitoring programs. Results of toxicity studies and data concerning their occurrence and fate in the environment will determine whether the aforementioned compounds should be included in relevant regulations and legislation.

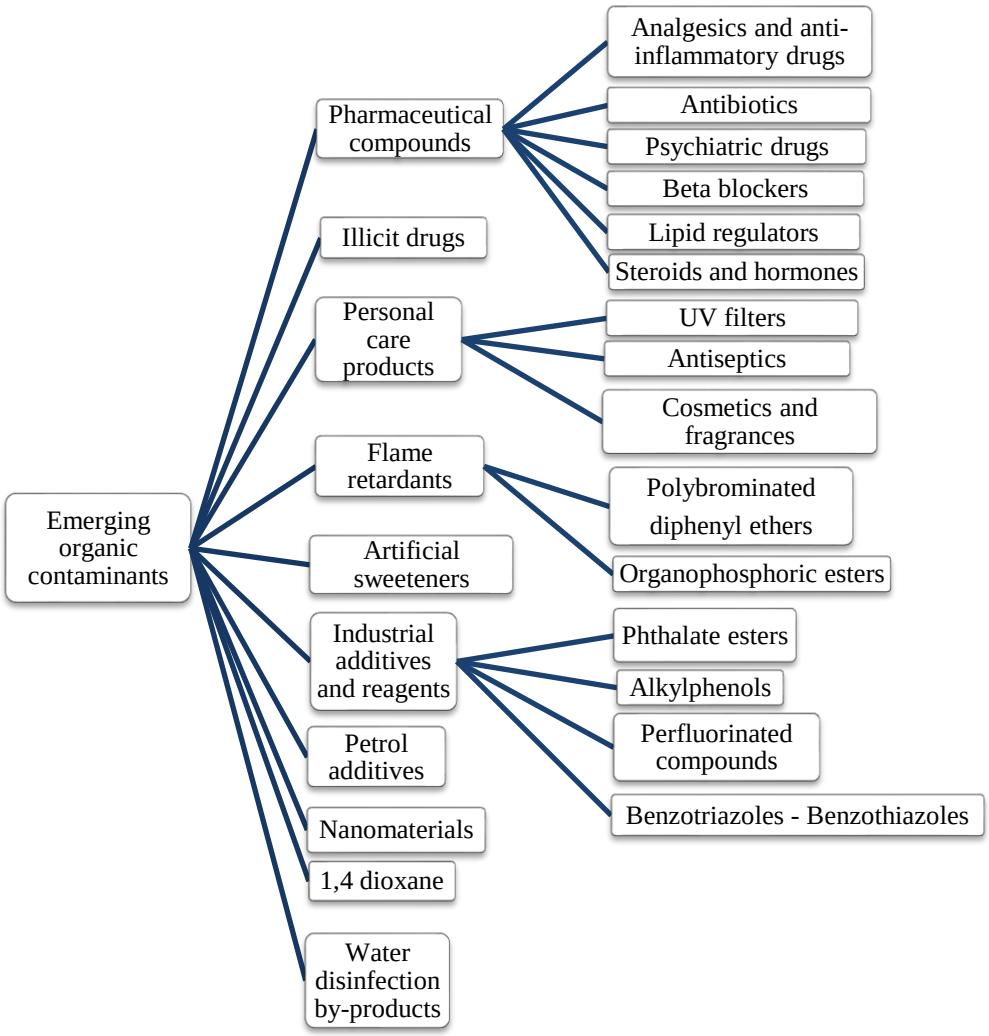


Figure 1: Prominent classes of emerging organic contaminants

1.1.2. Sources and occurrence in the environment

The majority of the EOCs are contained in widely used everyday products, PCPs and drugs. For instance, 17α-ethynyl-estradiol (EE2), a type of synthetic estrogen, is used as an oral contraceptive for women, bisphenol A (BPA) is used in industrial processes as a plasticizer, while triclosan (TCS), one of the most widespread EDCs, is a broad-spectrum antimicrobial agent contained in personal hygiene products, as well as in kitchen utensils, toys, textiles, socks and trash bags (Bester, 2003; Roberts et al., 2014; Gao et al., 2015; Kim et al., 2017). In addition, certain chemicals such as paracetamol, acetylsalicylic acid, ibuprofen, naproxen and diclofenac are the active substances of widely used drugs (Samaras et al., 2011; Kosma et al., 2014); whereas

artificial sweeteners are used in the food industry to sweeten foods and beverages (Kokotou and Thomaidis, 2013). It is worth mentioning that certain EOCs belonging to the class of EDCs are natural substances. For example, the female hormones estrone (E1) and 17β-estradiol (E2) are extensively widespread in aquatic ecosystems, due to their continuous excretion by females (Grover et al., 2011; Rahman Kabir et al., 2015).

EOCs can enter the environment through several pathways, such as STPs, hospitals, landfills, aquaculture areas and runoff from animal husbandry and agriculture (Figure 2) (Stuart et al., 2012; Yang et al., 2017). Hospitals are important sources of EOCs, as a variety of compounds can be found in the faeces and urine of patients or are the result of diagnostic, laboratory and research activities. Among others, the most common EOCs originating from hospitals are drugs and their metabolites, disinfectants, sterilization products, radioactive markers and iodinated contrast media. Moreover, veterinary drugs, excreted by animals, enter the ecosystem, initially polluting the terrestrial environment and then, as a consequence, surface, underground and drinking water (Farré et al., 2008; Hu et al., 2010; Bártíková et al., 2016; Wei et al., 2016).

One of the most significant sources of EOCs in the environment are STPs. Previous studies have shown that most of these compounds present partial or no removal during sewage treatment (Bletsou et al., 2013; Stasinakis et al., 2013; Luo et al., 2014), while their transformation products and metabolites may exhibit greater toxicity than the parent compounds (Farré et al., 2008; Fatta-Kassinos et al., 2011). The main reason for the EOCs' insufficient removal during wastewater treatment processes is the fact that the treatment processes that are usually applied (i.e. activated sludge process), have been designed for the removal of conventional pollutants such as organic matter, suspended solids, nitrogen and phosphorus and not for the elimination of organic micropollutants. The physicochemical properties of EOCs (solubility, volatility, adsorbability, absorbability, biodegradability, polarity and stability) differ from one another, and as a result their behavior and fate in STPs is difficult to predict. Finally, the fact that their concentration values are much lower than those of conventional pollutants makes their removal during sewage treatment even more difficult (Verlicchi et al., 2010; Yang et al., 2017).

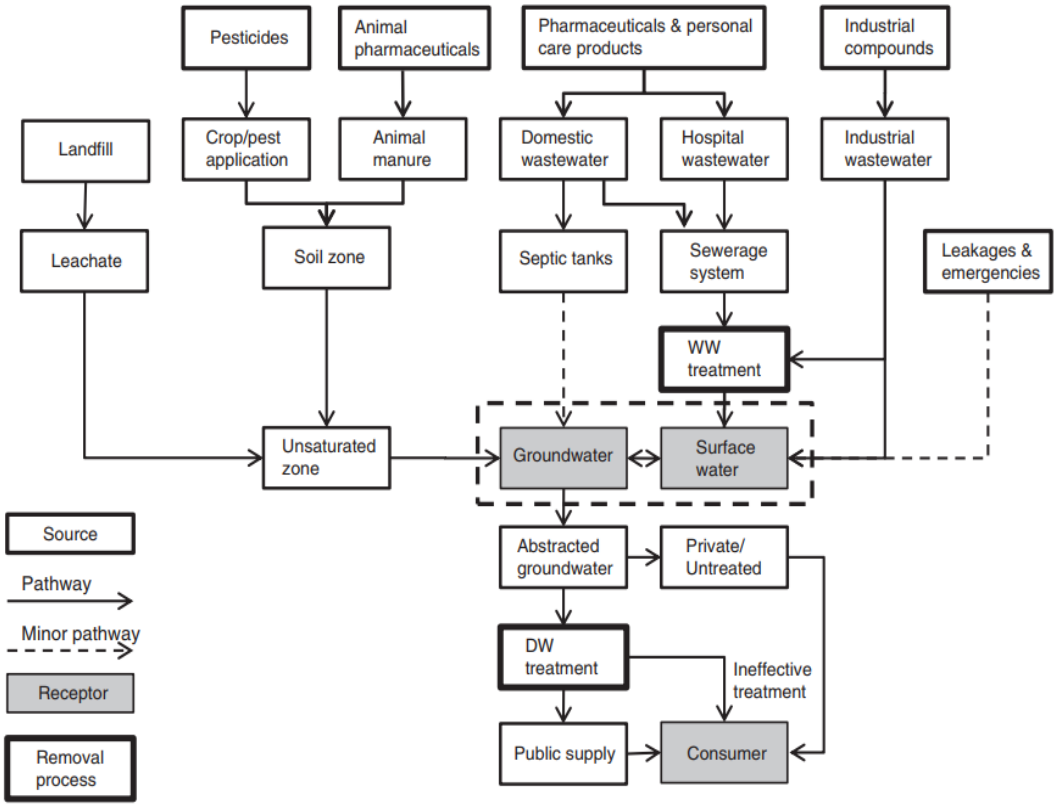


Figure 2: Potential pathways of some EOCs in receptors and aquatic environment (Stuart et al., 2012)

A significant number of studies have been conducted in recent decades monitoring the concentrations of several EOCs in the aquatic (surface water, groundwater) and terrestrial environment (Yoon et al., 2010; Chen et al., 2011; Bu et al., 2013; Meffe and de Bustamande, 2014; Pal et al., 2014; Tijani et al., 2016; Zhao et al., 2016; Yang et al., 2017). The detected concentrations in water vary from less than 1 ng L^{-1} to some μg L^{-1}; whereas concentrations in the range of μg g^{-1} dw have been determined in sludge amended soils. The groups of EOCs that are more commonly detected are PhCs and EDCs. Previous studies have also revealed that the aforementioned compounds have been detected in drinking water, worldwide (e.g. Canada, USA, Italy, France, Germany and United Kingdom). For instance, concentration values for carbamazepine (PhC) up to 601 ng L^{-1} have been reported in drinking water, while the corresponding value for BPA (EDC) is 99 ng L^{-1} (Kleywegt et al, 2011; Vulliet et al, 2011; Tijani et al., 2016; Yang et al., 2017). It is worth mentioning that as EOCs are contained in everyday products, the ones that detected in treated wastewater and sewage sludge are generally the same in all developed countries and their

concentration values cover a similar range of similar levels (González et al., 2010; Arvaniti and Stasinakis, 2015).

1.1.3. Transformation and fate in the environment

Once released into the environment, EOCs follow several pathways according to their physicochemical properties, such as their solubility in water and hydrophobicity. Some of these compounds remain stable for a long period; whereas others undergo several transformations at different rates. Transformation products may present different behavior and sometimes exhibit greater toxicity. For example, the biodegradation product of nonylphenol ethoxylates, nonylphenol (NP), and the photolysis products of acesulfame and sucralose are more persistent and more toxic than their parent compounds (Farré et al., 2008; Richardson and Kimura, 2017).

Attenuation and transformation of EOCs in the environment can be carried out in a multitude of ways: natural procedures, chemical processes or microbial degradation. In the first case, the dilution observed when released into bodies of water (lakes, rivers, torrents, open seas) reduces their concentration levels and the environmental hazards they might pose to aquatic organisms (Gros et al., 2010). Other physical mechanisms, such as dispersion and sorption onto sediments and suspended solids, also play a significant role in the natural removal of pollutants from the aquatic environment (Lin et al., 2006; Farré et al., 2008).

Photolysis is one of the main chemical transformation procedures in the aquatic environment and can contribute to the attenuation of EOCs, especially in surface water. The whole process is rather complex, depends on several factors (e.g., season, pH, humic acids, nitrate) and leads to a variety of products, which are sometimes more or less toxic than the initial compounds. In cases that pollutants are adsorbed onto solid phase, they are not exposed to solar radiation and, therefore, the majority of them do not participate in photochemical reactions. In such cases, microbial degradation is the dominant fate pathway of EOCs in surface water, where microbes either use them as a carbon and energy source or degrade these compounds through co-metabolism (Stenuit and Agathos, 2010; Li et al., 2014; Koumaki et al., 2015; Petrie et al., 2014; Richardson and Kimura, 2017).

Fate and transformation of EOCs in rivers have been studied by several researchers. It is believed that the multiple processes that take place inside the rivers contribute to the attenuation and, sometimes, elimination of the polluting load, in a natural way. Although it is quite difficult to delve into the removal mechanisms, relevant studies have indicated that sedimentation, biotransformation and/or phototransformation have the most important role in determining the fate of chemicals in rivers. According to Quandrud et al. (2004), a 60% reduction of estrogenic activity was observed along a 40 km stretch of the Santa Cruz River, Arizona (United States of America, USA), while all three previously mentioned mechanisms are involved in the removal processes. Similar studies conducted in British and Swiss rivers revealed that the dominant mechanisms reducing pollutant concentration were sedimentation/biotransformation and phototransformation, respectively (Kari and Giger, 1995; Williams et al., 2003).

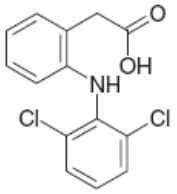
EOCs can be retained in high concentrations in soil surroundings. However, as they have different physicochemical properties, their fate and transport in sludge amended soils will vary. Compounds with high hydrophobicity exhibit greater potential for retention in the soil matrix; whereas those with high water solubility present hydrophilic mobility, which may result in plant uptake or their transport to bordering surface water and groundwater. In general, biodegradation processes are more intense in soil, as there is a significant population of microorganisms which are essential to biodegradation reactions. Aerobic biodegradation is the main removal mechanism of organic micropollutants, whilst, depending on the pollutant nature, some other routes for degradation may also occur, such as soil photolysis and soil hydrolysis (Li et al., 2014; Clarke and Cummins, 2015; Petrie et al., 2014).

1.2. Classes and toxicity of emerging organic contaminants

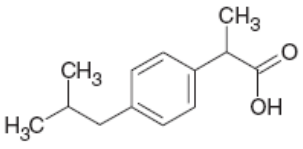
EOCs include a broad range of compounds mainly derived from the discharge of municipal wastewater effluents. Subsequently, brief information will be provided on some categories of EOCs that are often detected in the environment due to their presence in STPs.

1.2.1. Pharmaceuticals

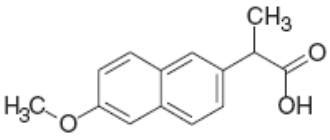
PhCs consist a class of EOCs that are used in human and veterinary medicine for the prevention and treatment of diseases. Their presence in the environment is an issue of major concern due to their negative effects on humans and ecosystems (Verlicchi and Zambello, 2015; Tijani et al., 2016; Wilkinson et al., 2016; Ebele et al., 2017). In the European Union (EU), about 3,000 different substances are used in medicine, primarily analgesics and anti-inflammatory drugs, contraceptives, antibiotics, beta-blockers, lipid regulators, antiepileptics and antidepressants. Also, a large number of PhCs are used in veterinary medicine, mainly antibiotics and anti-inflammatory drugs. Worldwide, more than 5,000 substances have been dispensed and launched for human and veterinary consumption. The most frequently consumed PhCs belong to the class of non-steroidal anti-inflammatory drugs (Figure 3) and include the substances acetylsalicylic acid, paracetamol, naproxen, ibuprofen and diclofenac, with annual consumption in UK and Germany ranging from 26 to 836 t (Fent el al., 2006; Tijani et al., 2016).



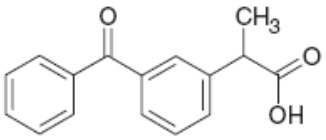
Diclofenac



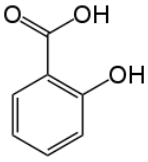
Ibuprofen



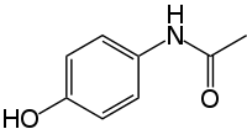
Naproxen



Ketoprofen



Acetylsalicylic acid



Paracetamol

Figure 3: Chemical structures of the principal non-steroidal anti-inflammatory drugs

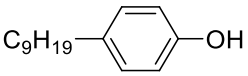
PhCs are released into the environment either as parent compounds, or as their metabolites. The main pathway starts from humans via excretion and terminates in the environment through STPs. Therefore, STPs consists the major route for these substances to the ecosystems, which, after treated wastewater and sludge disposal, end up in rivers, lakes, soil, groundwater and drinking water (Fatta-Kassinos et al., 2011; Ziylan and Ince, 2011). Other emission sources of PhCs in the environment are hospitals, the pharmaceutical industry, livestock farming, fish farming and unconsumed drugs via solid waste (Houtman, 2010; Tijani et al., 2016; Ebele et al., 2017).

When released into the environment PhCs and/or their transformation products may cause adverse effects on aquatic and terrestrial organisms, which are difficult to predict. Usually, their environmental concentrations are low, yet, due to their continuous release into ecosystems, it is more likely to exhibit chronic than acute toxicity; whereas their mixtures may exert considerable toxicity, as well. Nevertheless, the lack of chronic toxicity data renders their adequate risk assessment rather intractable (Fent et al., 2006; Farré et al., 2008; Wilkinson et al., 2016; Ebele et al., 2017). Toxicity tests on terrestrial and aquatic organisms revealed that many PhCs, including diclofenac, ibuprofen, naproxen, clofibric acid, carbamazepine, tetracycline, paracetamol and acetylsalicylic acid, might exhibit either chronic or acute toxicity on exposed organisms (Henschel et al., 1997; Cleuvers, 2003; Isidori et al., 2005; Fent et al., 2006; Flippin et al., 2007; Farré et al., 2008; Tijani et al., 2016; Ebele et al., 2017.

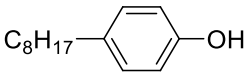
1.2.2. Endocrine disrupting compounds

EDCs are chemicals that can cause negative effects on the endocrine system of humans and animals. This broad category includes natural estrogens, such as E1 and E2, natural androgens, such as testosterone (T), synthetic estrogens and androgens, such as EE2, phytoestrogens such as isoflavonoides, as well as various industrial compounds, including alkylphenols (APs), polychlorinated biphenyls, certain pesticides, phthalates and BPA. These substances are often detected in municipal and industrial effluents, landfills, soil, sediments, groundwater, surface water, and even in drinking water (Liu et al., 2009; Zhang et al., 2014; Huang et al., 2014; Zhang et al., 2016). Among the great variety of compounds belonging to EDCs, APs (mainly

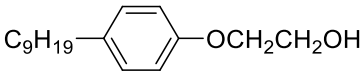
octylphenols, OPs and nonylphenols, NPs), alkylphenol ethoxylates (APEOs), TCS and BPA are of particular interest (Figure 4). APEOs belong to the category of non-ionic surfactants and, since 1950, they have been widely used in industrial, agricultural and household applications, namely detergents, emulsifiers, wetting agents, dispersants or solvents. It is estimated that the annual production of APEOs amounts to around 500,000 t (Pothitou και Voutsa, 2008; David et al., 2009). TCS is a broad-spectrum antimicrobial agent contained in personal hygiene products and it has been placed on the list of the 10 most frequently detected organic micropollutants in the aquatic environment (Huang et al., 2016; Zhang et al., 2016) as, only in Europe, approximately 350 t of TCS are produced per year for commercial applications (Stasinakis et al., 2008). BPA is a monomer used in the production of adhesives, food and beverage protective coatings, compact disks, contact lenses, thermal paper, building materials, etc. Its global production exceeds the tremendous amount of 2,300,000 t per year (Staples et al., 1998; Yang et al., 2006).



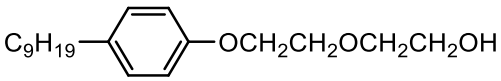
Nonylphenol



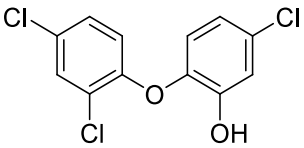
4-t-octylphenol



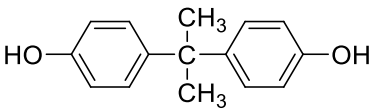
Nonylphenol monoethoxylate



Nonylphenol diethoxylate



Triclosan



Bisphenol A

Figure 4: Chemical structures of the principal endocrine disrupting compounds

EDCs and their degradation products have been detected in effluents of several municipal STPs, worldwide (Ying et al., 2002; Voutsa et al., 2006; Stasinakis et al., 2008; Manickum and John, 2014; Xu et al., 2014), thus they are discharged continuously into the environment. Their disposal can cause serious health problems for humans and adversely affects plants, aquatic and terrestrial organisms and wildlife as well. Several studies address the effects of EDCs on various organisms, such as reptiles, amphibians, crustaceans, birds and mammals, although most of them have focused on fish. In general, surveys in various countries (UK, Italy, Spain, Netherlands, Denmark, Switzerland and USA) report cases of endocrine disorders and, in particular, "feminization" of fish (Reinen et al., 2010; Kabir et al., 2015). NP, in particular, included in Directive 2000/60/EC (EC, 2001), is considered an extremely toxic chemical and classified as a priority substance; while several studies refer to the toxicity of APEOs, TCS and BPA (Orvos et al., 2002; Ying et al., 2002; Stasinakis et al, 2008).

1.2.3. Perfluorinated compounds

PFCs are chemicals consisting of a fully fluorinated hydrophobic alkyl chain of varying length (usually C4 to C16) and a hydrophilic end group (Figure 5). They are known to be chemical and thermal stable compounds and have been widely used in industrial and household applications for over 50 years (EFSA, 2008; Arvaniti and Stasinakis, 2015). Their best-known applications are non-stick pans and cookware, surface coatings for textiles and furniture, paper products, fire-fighting foams and shampoos. They are also used in the manufacture of paints, adhesives, polishing materials and electronic components (Ahrens, 2011; Arvaniti et al., 2012).

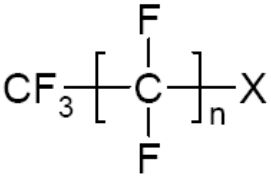


Figure 5: General chemical structure of perfluorinated compounds (EFSA, 2008)

PFCs are widespread in the aquatic environment and have been detected in precipitation, groundwater, surface water, and even in drinking water. Wastewater is considered to be one of the most important routes of these compounds to the

environment, while treated sludge and landfills contribute to their transport to the soil and thus to plants and animals (Ahrens, 2011). Several researchers have reported their potential hazard to humans, biota and wildlife, even to Arctic animals, such as seagulls, polar bears and polar foxes (Letcher et al., 2010; Rosal et al., 2010; Rahman et al., 2014).

1.2.4. Benzotriazoles – Benzothiazoles

BTRs and BTHs consist of a benzene ring fused with a triazole and thiazole ring, respectively (Figure 6). They are highly polar compounds that are used in a variety of applications, at both a household and an industrial level. They are used as corrosion inhibitors for the protection of metals, in deicing fluids for aircrafts and cars, brake fluids, industrial cooling systems and dishwashing detergents, industrial cooling systems, vulcanization accelerators in rubber production and biocides in paper and leather manufacturing (Weiss and Reemtsma, 2005; Jover et al., 2009; Reemtsma et al., 2010). As they are highly resistant to biodegradation, they are expected to be present in STPs effluents and, consequently, in surface water, sediments and groundwater. Studies conducted in the EU and the USA, revealed the existence of BTRs and BTHs in treated wastewater, rivers, groundwater and drinking water (van Leerdam et al., 2009; Janna et al., 2011; Asimakopoulos et al., 2013).

Benzotriazoles Benzothiazoles

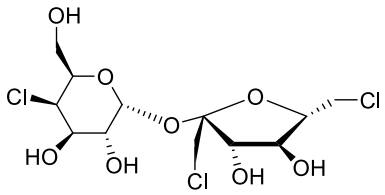
Figure 6: General chemical structures of benzotriazoles and benzothiazoles (Weiss and Reemtsma, 2005)

Although BTRs and BTHs are high production volume chemicals, they did not catch the attention of the scientific community until 1990, when they were associated with toxicological effects on fish (Janna et al., 2011). Related studies have shown that BTRs could adversely affect the nervous and endocrine system and inhibit the

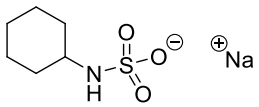
synthesis of proteins, enzymes and RNA in mammals (Castro et al., 2005). On the other hand, BTHs have been classified as potential neurotoxic substances, while their mixtures can cause mortality, growth inhibition and serious damage to brain and eyes cells of young fish (Evans et al., 2000). In general, they are considered toxic substances, but only at concentrations higher than environmentally encountered (Herrero et al., 2014). Further research is required to evaluate their toxicity to living organisms.

1.2.5. Artificial sweeteners

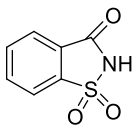
ASs are substances that are mainly used in the production of low-calorie foods and beverages, but also in drugs and sanitary products (Scheurer et al., 2009; Kokotou and Thomaidis, 2013). The list of authorized ASs varies from country to country (Zygler et al., 2009). The most popular ASs are acesulfame, cyclamate, neohesperidin dihydrochalcone, saccharine and sucralose (Figure 7). Some of them are excreted from the human body without being metabolized and via STPs enter the environment, where they have been extensively detected in surface water, groundwater, soil and drinking water (Scheurer et al., 2009; Kokotou and Thomaidis, 2013; Lim et al., 2017; Richardson and Kimura, 2017).



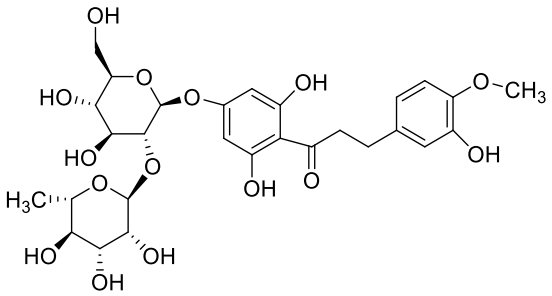
Sucralose



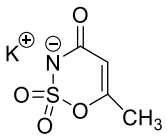
Cyclamate



Saccharine



Neohesperidin dihydrochalcone



Acesulfame

Figure 7: Chemical structures of the principal artificial sweeteners

There is a limited number of published studies concerning the toxicity of ASs. Certain researchers have investigated the effects of these compounds on rats, coming to the conclusion that saccharine may not pose serious risk to humans (Udem and Nwobodo, 2011). However, concerns exist for adverse impacts on other living organisms; namely, algae, crustaceans, plants and worms (Kobetičová et al., 2016; Amy-Sagers et al., 2017; Richardson and Kimura, 2017). More research should be carried out related to the acute and chronic toxicity of ASs on aquatic and terrestrial organisms.

1.2.6. Siloxanes

SLXs are organosilicon compounds with a linear or cyclic chain, whose molecules contain Si-O-Si linkages (Figure 8). They are widely used in industrial applications and consumer products (cosmetics, shampoos, detergents, paper coatings, textiles, concrete etc.) (Bletsou et al., 2013; Wang et al., 2013; Capela et al., 2017). Due to their low water solubility and high sorption coefficients, they tend to adsorb on suspended solids during wastewater treatment. Therefore, their concentration values in sludge are expected to be high, although their presence and fate in STPs have not yet been studied in detail (Bletsou et al., 2013; Surita and Tansel, 2014).

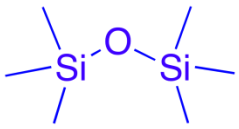


Figure 8: The siloxane Si-O-Si linkage

To date, a few studies have been conducted concerning the toxicity of the aforesaid compounds. Published articles in scientific journals indicate that their environmental concentrations (water, sediments, soil) are low enough to cause toxicity to living organisms. However, their tendency to bioaccumulate in aquatic organisms, such as invertebrates and fish, indicates the need for long-term environmental monitoring of these compounds, as well as research into their fate and distribution in the aquatic environment (Wang et al., 2013; Mackay et al., 2015).

1.3. Environmental risk assessment

1.3.1. General introduction

In the last decades thousands of chemicals have been released into the environment from urban and industrial sources. Since the early sixties, the scientific community, media and general public have become aware of the probable short- and long-term negative effects of these compounds on humans and aquatic and terrestrial ecosystems, as well (Van der Oost et al., 2003). Recently, implementation of relevant legislative measures by authorities has forced companies and industries to take measures in order to degrade pollutant emissions. In this direction, methodologies assessing the impacts of exposure to chemicals, for both humans and ecosystems, had to be institutionalized. Among these methods, risk assessment is one of the most widely used and nowadays, it is considered as the most objective and reliable tool to inform risk management researchers, regulators and policy makers (Syberg and Hansen, 2016).

1.3.2. The environmental risk assessment process

Environmental or ecological risk assessment (ERA) is a process that estimates the probability and extent of an adverse effect of chemicals or a mixture of chemicals on non-human populations, communities and ecosystems. ERA process usually entails a sequence of steps (Figure 9); namely, hazard identification, exposure assessment, effects or dose-response assessment and risk characterization (Van Leeuwen and Hermens, 1996; Calow, 2001; Van der Oost et al., 2003; Simon, 2014).

In the phase of *hazard identification*, the adverse effects which a chemical or a mixture of chemicals, have an inherent capacity to cause, are identified. This step is a qualitative determination of whether or not a certain agent is related to effects of adequate importance to justify further scientific research. It involves gathering and evaluating data on the types of effects that may be provoked by a substance and exposure conditions under which environmental harm will be caused. The likelihood of harm due to exposure distinguishes risk from hazard. For instance, a toxic substance that is hazardous to an aquatic or terrestrial population poses no risk to the

particular population, unless the organisms are exposed to it (Van Leeuwen and Hermens, 1996; European Commission, EC, 2003; Van der Oost et al., 2003).

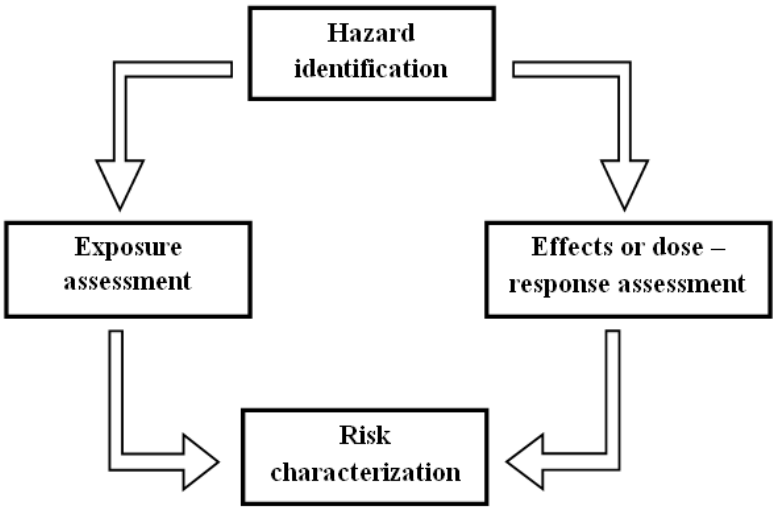


Figure 9: Steps in the environmental risk assessment process (Van Leeuwen and Hermens, 1996)

Effects or dose-response assessment aims to provide a quantitative estimation of the relationship between dose or level of exposure to a chemical and its effects which are potentially hazardous to the assessment endpoint. Most effects assessments are based on toxicity testing (Suter II et al., 1993; Van Leeuwen and Hermens, 1996; European Commission, EC, 2003). As it is impossible to assess the effects of chemicals on all species and all procedures taking place in an ecosystem, data is usually obtained from experimental laboratory toxicity studies on microorganisms, plants and animals, and, more rarely, from experimental field studies. The tiered approach illustrated in Figure 10 is used when authorities must determine toxicological hazards and request initial and additional tests to be carried out for new and existing chemicals. Testing occurs in a series of steps (tiers) of increasing complexity, progressing from acute to chronic and, finally, to field tests. In such an approach, testing results increase and existing knowledge gaps are filled gradually, in order to minimize extra costs and unneeded testing (Calow, 2001).

Exposure assessment is the estimation of the concentrations or doses to which environmental matrices (water, sediments, soil, air) are or may be exposed. The exposure profile characterizes the environmental compartment in which the hazard

agent may exist and the biota that may be exposed to this agent (Calow, 2001). For existing chemicals, analytical measurements can be used and exposure can be assessed by measuring concentration (measured environmental concentration, MEC), while for new chemicals, chemical-related data, empirical environmental data and established environmental fate models are used, to estimate a predicted environmental concentration (PEC) (Van Leeuwen and Hermens, 1996; European Commission, EC, 2003; Van der Oost et al., 2003).

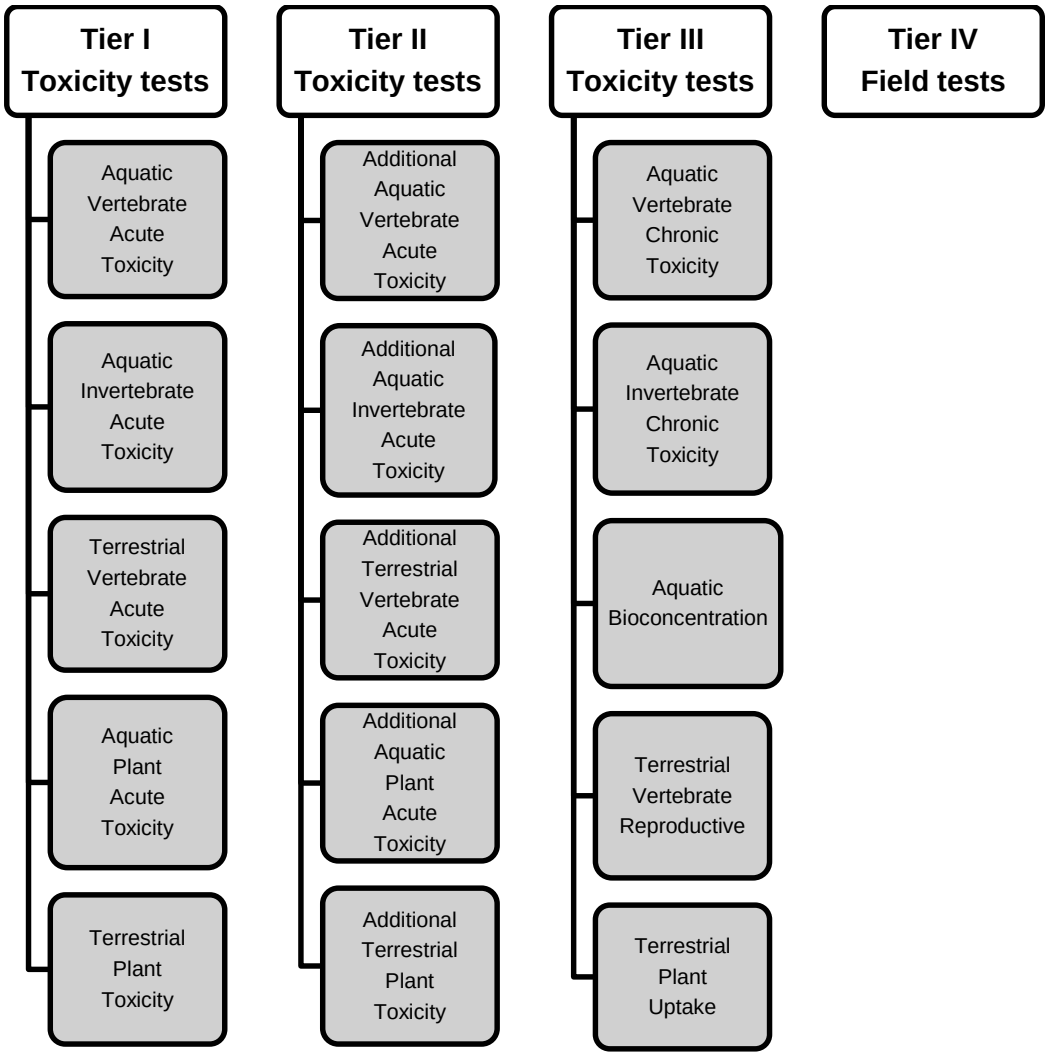


Figure 10: The tiered approach used in the effects assessment of chemicals (Calow, 2001)

Risk characterization is the final step of risk assessment and combines the information generated in the hazard identification, effects assessment and exposure

assessment phases, in order to provide an environmental risk estimation, which is usually expressed as a risk quotient or a risk probability (Calow, 2001). In other words, risk characterization is the estimation of the incidence and severity of the hazard effects likely to occur in an environmental compartment due to the measured or predicted exposure to a chemical and is, therefore, a key step in the final decision making process (European Commission, EC, 2003; Van der Oost et al., 2003). It should be pointed out that accurate risk assessments are difficult to exist and scientists usually differ in the conclusions they come to, even they deploy the same set of data (Van Leeuwen and Hermens, 1996).

In the following sections more information about the ecotoxicity tests conducted during the effects assessment step are given, whereas a brief historical retrospect of the ERA process in the USA and the EU is presented. A detailed description of the methodology of the EU ERA process then follows.

1.3.3. Ecotoxicity testing

The endpoints of the acute toxicity bioassays are usually expressed by LC50 (median lethal concentration) and/or EC50 (median effect concentration) values, which are defined as the concentrations of the chemical in an environmental compartment (water, sediment, soil, etc.) that kill 50% or cause a specific negative effect in 50% of the test organisms, respectively, during the observation period. In the case of chronic toxicity testing, the endpoints are expressed by NOEC (no observed effect concentration) value, which theoretically corresponds to the LC10 value. NOEC is, namely, the concentration in an environmental matrix below which a hazardous effect is unlikely to be observed (Hansen, 2007).

The toxicity tests are conducted using a variety of organisms. Specifically for the aquatic environment, the most commonly used organisms are freshwater fish; namely zebrafish (*Danio rerio*), rainbow trout (*Oncorhynchus mykiss*), Japanese medaka (*Oryzias latipes*), fathead minnow (*Pimephales promelas*), bluegill (*Lepomis macrochirus*) και sheephead minnow (*Cyprinodon variegates*). The prominence of fish in aquatic ecotoxicity testing is due to several reasons. Fish play a significant role in food chains and are an important source of food for humans. In addition, they are used as water quality indicators, accidents leading to the death of fish are visible to

the public and sociologically, indicate the need for water protection from chemicals. Finally, significant recreational value is attached to fishing in many cultures (Lammer et al., 2009). Apart from fish, in the aquatic environment, invertebrate species and microorganisms are commonly used for toxicity studies, such as crustaceans (e.g. *Daphnia magna*, *Ceriodaphnia dubia*, *Gammarus pulex* (L.), *Hyalella azteca*, *Thamnocephalus platyurus* etc.), algae (e.g., *Selenastrum capricornutum*, *Scenedesmus subspicatus*, *Scenedesmus vacuolatus*, *Pseudokirchneriella subcapitata*, *Nitzschia palea* etc.), bacteria (e.g., *Vibrio fischeri*, *Caulobacter crescentus*, *Anabaena flos-aquae*, *Microcystis Aeruginosa* etc.) and protozoa (*Tetrahymena pyriformis*, *Tetrahymena thermophila* etc.) (Janssen et al., 2000). The endpoints measured in these studies could include any response that an organism or population may exhibit as a result of a chemical stimulus. However, the endpoints most commonly used in acute toxicity studies using fish and crustacean are mortality and immobilization, as they are easily determined, have obvious biological and ecological significance and can be expressed in statistically rigid manner (Van Leeuwen and Hermens, 1996).

Although the terrestrial environment is crucial for the human population, the soil has only recently become an important topic for ecotoxicologists. Bacteria (e.g., *Photobacterium phosphoreum*) are by far the most numerous organisms in soil, and are thus commonly used in terrestrial toxicity testing. Other test species selected for bioassays are plants, such as wheat, oat and mustard (e.g., *Triticum aestivum*, *Avena sativa*, *Brassica alba* etc.) and various soil invertebrates, such as earthworms (e.g. *Eisenia fetida*, *E. Andrei* etc.), nematodes, arthropods, isopods, collembolan and millipedes (Van Leeuwen and Hermens, 1996; Höss et al., 2009). For the chronic toxicity assessment of certain chemicals, several higher animal species, such as mammals, are also used; namely, rats, mice, guinea pigs, dogs and monkeys (Verma et al., 2014). In general, the types of the selected organisms are determined by their specific properties, such as abundance, collection convenience, resistance, ease of development in laboratory conditions, knowledge of their genetic composition and sensitivity to various chemicals.

1.3.4. Environmental risk assessment in the USA

Federal agencies in the USA began to apply chemical risk assessment processes in the 1970s to estimate the cancer-causing potential of chemicals in commerce. In 1983, the National Academy of Sciences (NAS) published the landmark report entitled 'Risk Assessment in the Federal Government: Managing the Process' (NRC, 1983), commonly referred to as the 'Red Book', which presented the framework for conducting risk assessment. In the 1990s, the United States Environmental Protection Agency (USEPA) issued a number of relevant guidelines, initially for human health and subsequently for plants, animals and whole ecosystems. The processes presented in the aforementioned guidelines consist of the steps described in section 1.3.2 (Simon, 2014). As for the approaches and calculations used for conducting ERA, they include hazard quotients to quantify risk and various parameters to determine the levels of exposure to a chemical for a specific plant or animal, such as area use, food ingestion rate, bioaccumulation rates, bioavailability and stage of life (USEPA, 2017).

1.3.5. Environmental risk assessment in the EU

Member States of the EU had not set up any provisions relating directly to environmental protection until 1972, when an Environmental Action Program was established. This was an agreement by the Member States to collaborate on measures to protect their national environments and, consequently, that of the Community. Over the next years, hundreds of environmental measures were adopted and a series of guidelines relevant to ERA were issued (Calow, 2001). Nowadays, ERA is carried out in the EU by different advisory bodies, for instance European Chemical Agency (ECHA), European Environment Agency (EEA) and European Food Safety Authority (EFSA). However, the basic guideline describing, in detail, the methodology for ERA used in the regulation of new and existing chemicals in the EU, is the EU Technical Guidance Document (TGD) on Risk Assessment (European Commission, EC, 2003). According to the EU TGD, three approaches can be used for conducting ERA: the qualitative procedure, the PBT (Persistence, Bioaccumulation, Toxicity) assessment and the quantitative estimation, also known as risk quotient (RQ) methodology.

The *qualitative approach* is applied only in cases that the quantitative assessment of the effects and/or exposure is not possible, as with the air compartment and remote

marine areas, where no standardised biotic testing systems are available at present. Moreover, for some chemicals data for their environmental release is so scarce that the PEC values cannot be estimated quantitatively with precision, while in some cases toxicity data cannot be derived. Finally, for new substances and, sometimes, for existing chemicals as well, information about their degradation products is unlikely to be available, thus only a qualitative approach would usually be possible (European Commission, EC, 2003).

The *PBT assessment* concerns the potential of a substance to remain in the environment, accumulate in the organisms and pose toxicity, in conjunction with an estimation of its sources and emissions. It has been developed to identify those cases where the quantitative approach lacks conviction that the target populations are protected, especially those ecosystems where the risks are more difficult to estimate. Specifically for the marine environment, PBT assessment is conducted, as risks cannot be adequately estimated by the traditional risk assessment methodologies. In general, PBT approach is carried out for those chemicals which have a great tendency to persist and bioaccumulate in biota and exhibit toxicity effects after a long period. The criteria that a substance has to fulfill in order to be regarded as a PBT substance are presented in the EU TGD and include parameters such as half-life in marine water and sediment or freshwater and sediment, bioconcentration factor (BCF), chronic NOEC, etc. (European Commission, EC, 2003).

Information on the quantitative estimation methodology is given in detail in the following paragraph 1.3.6.

1.3.6. The risk quotient (RQ) methodology

According to the quantitative approach described in the EU TGD, ERA is conducted calculating the risk quotient (RQ) $\frac{PEC}{PNEC}$ or $\frac{MEC}{PNEC}$, where PNEC is the predicted no effect concentration; namely, the concentration below which unacceptable effects on organisms are unlikely to occur (Figure 11).

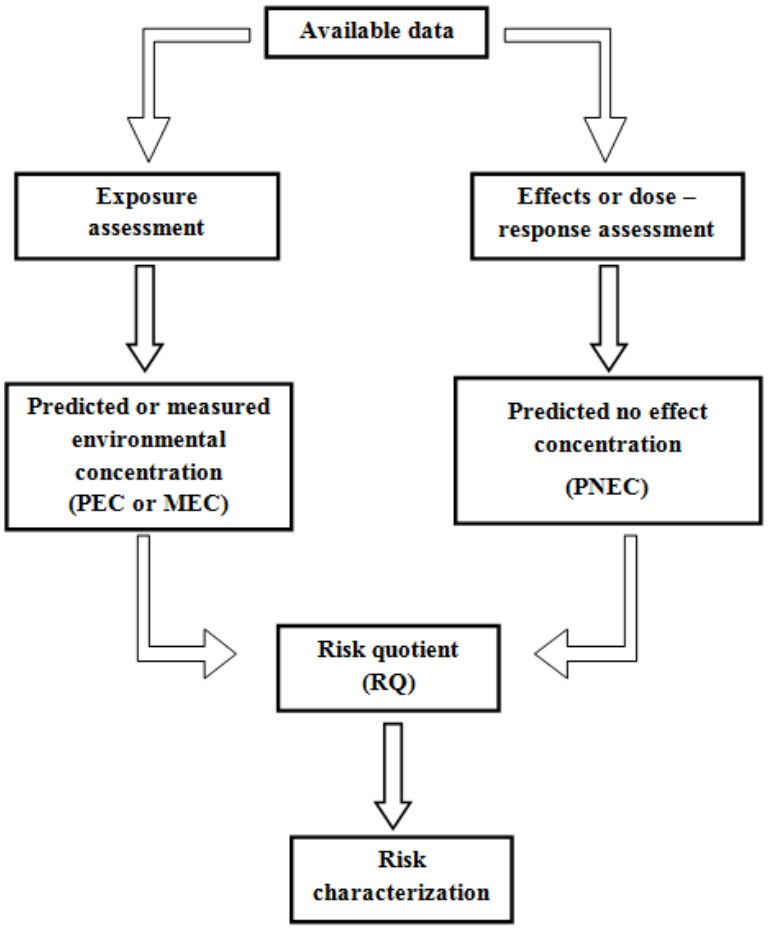


Figure 11: Steps in the risk quotient (RQ) methodology (Van Leeuwen and Hermens, 1996)

PNEC is derived by dividing the EC50/LC50 values of a chemical with an assessment factor (AF). The size of the AF depends on the confidence with which a PNEC value can be derived from the available data. When more toxicity data are available, the confidence increases and lower AFs are used. The proposed AFs by the EU TGD are presented in Table 1. The AF equal to 1000 is a conservative and protective factor and is used to ensure that chemicals with the potential to pose significant ecological risk are identified in the effect assessment. Although a variation in data may lead to a raised or lowered AF, an AF lower than 100 should not be used in deriving a PNEC for the aquatic environment, when ERA is based on acute toxicity data (European Commission, EC, 2003).

Table 1: Assessment factors to derive a PNEC for the aquatic and the terrestrial environment (European Commission, EC, 2003)

Available data	Assessment factor
Aquatic environment	
At least one short-term LC50/EC50 from each of three trophic levels of the base-set (fish, *Daphnia magna* and algae)	1000
One long-term NOEC (either fish or *Daphnia magna*)	100
Two long-term NOECs from species representing two trophic levels (fish and/or *Daphnia magna* and/or algae)	50
Long-term NOECs from at least three species (normally fish, *Daphnia magna* and algae) representing three trophic levels	10
Terrestrial environment	
LC50/EC50 short-term toxicity test(s) (e.g. plants, earthworms or microorganisms)	1000
NOEC for one long-term toxicity test (e.g. plants)	100
NOEC for additional long-term toxicity tests of two trophic levels	50
NOEC for additional long-term toxicity tests for three species of three trophic levels	10

In cases that RQ is less than 1, no ecological risk is indicated and no further testing is required; whereas, in cases that RQ is higher than 1, ecological risk for the environment is indicated and measures to reduce the risk need to be taken (Figure 12).

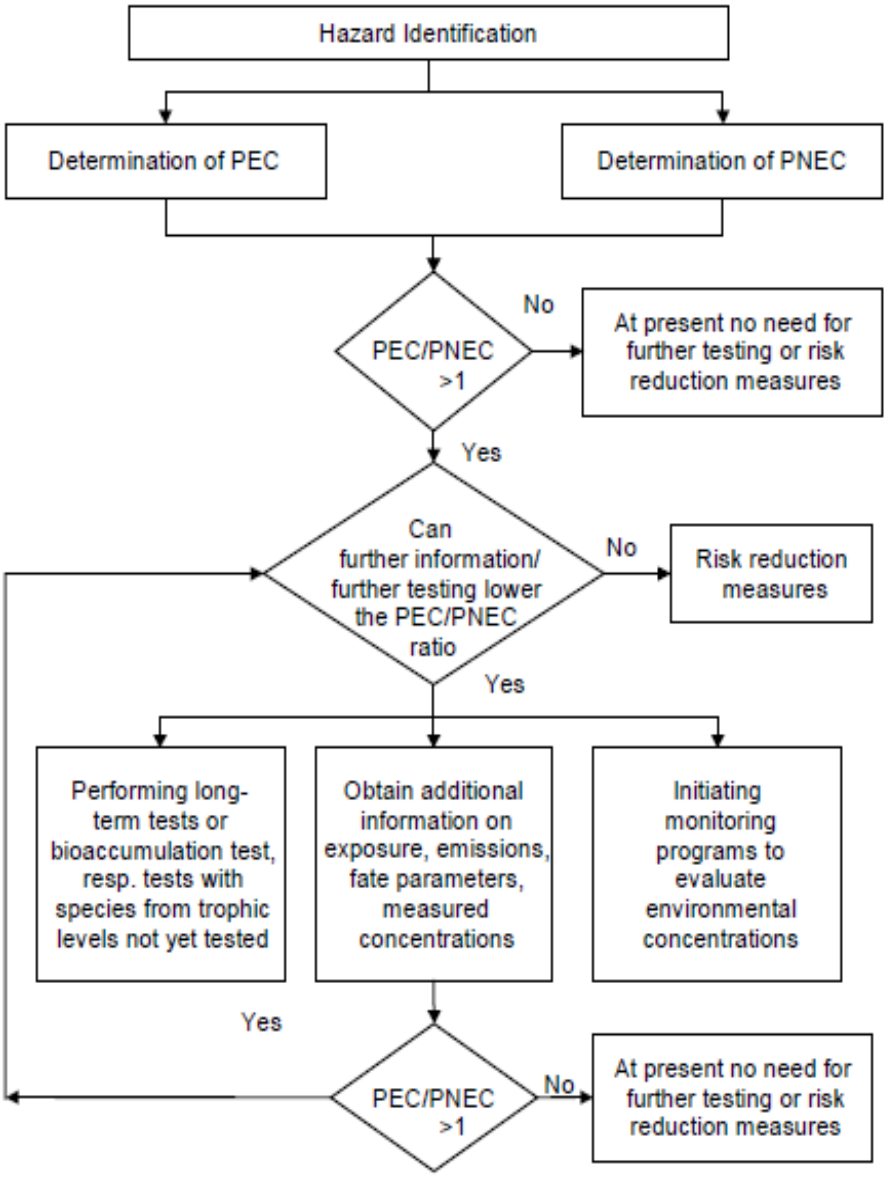


Figure 12: General procedure for environmental risk assessment based on the risk quotient methodology (European Commission, EC, 2003)

As far as the aquatic environment is concerned, the aforementioned methodology has so far been applied, either for a limited number of compounds and specific groups of micropollutants (e.g. antimicrobials, EDCs) or for specific rivers and lakes receiving wastewater, as well as specific pollution sources (e.g. hospitals). For example, Escher et al. (2011) evaluated the toxicological risk of 100 PhCs occurring in the wastewater of a general hospital and a psychiatric center in Switzerland, while Al Aukidy et al. (2014) quantified the environmental risk associated with 32 PhCs contained in the effluents of three hospitals in Italy. Stasinakis et al. (2012) investigated the ecological

risk of 4 PhCs and 4 EDCs in a Greek river receiving municipal and industrial wastewater. In another study conducted in Greece, the ecotoxicological risk, generated by 18 pharmaceuticals and personal care products (PPCPs) containing in hospital and municipal effluents released by 8 STPs, located in the northwestern Greece, was estimated (Kosma et al. 2014). On the other hand, it is obvious that the risk assessment is more realistic if a large number of substances is taken into account. Except for the USA (Diamond et al., 2011), so far, the potential risk from the presence of a large number of EOCs contained in treated wastewater has not been estimated at country level. Moreover, the contribution of individual pollutants to the estimated toxicity has not been assessed. Assuming that the EOCs that are detected in different countries are generally the same and that their concentration levels are ranged at similar levels, the above information could help water resource scientists to evaluate sites were EOCs may pose risk and be useful for policymakers as concerns the choice of micropollutants that should be included in future legislation and on the measures that should be taken for their efficient removal in STPs.

So far, in the terrestrial environment literature has focused on the environmental threats due to the existence of heavy metals and pathogens in sludge-amended soils (Lewis and Gattie, 2002; Smith, 2009; Pritchard et al., 2010). Based on this information, the EU and several countries have set limit values and have suggested practices to prevent harmful effects on soil, vegetation, animals and humans (Alvarenga et al., 2015; Verlicchi and Zambello, 2015), as agricultural reuse of treated sludge is one of the most common sludge management practices. Specifically, in EU-27, 21 Member States have adopted agricultural reuse, while 53% of the total produced sludge is recycled in agriculture directly or after composting (Kelessidis and Stasinakis, 2012). In the USA and Canada, more than 50% and 40%, respectively, of the produced biosolids are applied to land (Citulski and Farahbaksh, 2010; Venkatesan et al., 2015), while in China the land application of treated sewage sludge is suggested as the optimal solution for efficient sludge management (Yang et al., 2015). On the other hand, there is much less information on the environmental risk to the terrestrial environment due to the occurrence of EOCs in sewage sludge. Most of the relevant studies concern specific STPs and a limited number of compounds or specific groups of contaminants. Additionally, due to the limited available soil toxicity data, in most of the relevant articles the potential risk for the soil environment

has been estimated using only aquatic toxicity data and the methodology proposed by EC (2003); namely, González et al. (2010) evaluated the toxicological risk of 3 EDCs in the terrestrial environment in the South of Spain, while Martín et al. (2012b, 2015) assessed the ecological hazard associated with the presence of 16 and 22 PhCs respectively in sludge-amended soil in the same area. Three related studies have also been conducted in China. The potential risk due to the presence of 4 EDCs and 5 PhCs (Chen et al., 2011), 4 PhCs (Wu et al., 2014) and 2 synthetic musks and 2 SLXs (Liu et al., 2014) was estimated in sludge-amended soil, in the North, South and East of China, respectively. Although risk assessment is more reliable if a large number of compounds belonging to different classes are taken into account, to the best of our knowledge, so far, there is only one relevant study estimating the risk from the occurrence of a significant number of PhCs and PCPs in sewage sludge (Verlicchi and Zambello, 2015). On the other hand, the potential risk related to the presence of individual EOCs in sludge, as well as with their mixture toxicity on the terrestrial environment, has not been estimated at country level. Bearing in mind that the EOCs that are detected in sewage sludge are generally the same compounds in all developed countries and their concentration values cover a similar range of levels (González et al., 2010; Arvaniti and Stasinakis, 2015), studies that would clarify the above risk-related issues could be useful for researchers and policymakers in identifying those micropollutants that have to be a) removed more efficiently during wastewater and sludge treatment, b) periodically monitored in national sludge campaigns and c) included in relevant future legislations.

From the opposing point of view, the quantitative RQ approach described above, is tempting in its simplicity, but it should only be seen as a first attempt to estimate the ecological threat to aquatic and terrestrial organisms, due to the existence of EOCs in the environment. A number of uncertainties are contained, as time-dependent processes, such as degradation and transportation are not taken into account and for the involved groups of substances, less is known as far as their mode of action is concerned. Additionally, as RQ values are usually calculated based on the maximum PEC or MEC and the lowest PNEC values, this methodology provides information for the worst-case scenario, while no information is given for the uncertainty of the method and the possibility that RQ values exceed 1. The aforementioned drawbacks have led to the development of statistical extrapolation techniques in this area

(probabilistic risk assessment, PRA). PRA is a tool for the quantitative estimation of risk and associated uncertainties and Monte Carlo simulation (MCS) is the primary method used for conducting PRA (De Laender et al., 2010; Wu et al., 2011; Gottschalk and Nowack, 2012; García-Santiago et al., 2016). MCS is a specific probabilistic method that uses computer simulation to combine multiple probability distributions in an equation. The steps involved in a MCS include: (a) defining the statistical distributions of input parameters, (b) randomly sampling from these distributions, (c) performing repeated model simulations using the randomly selected sets of parameters and (d) analyzing the output (Figure 13) (Suter II et al., 1993; Van Leeuwen and Hermens, 1996; Simon, 2014).

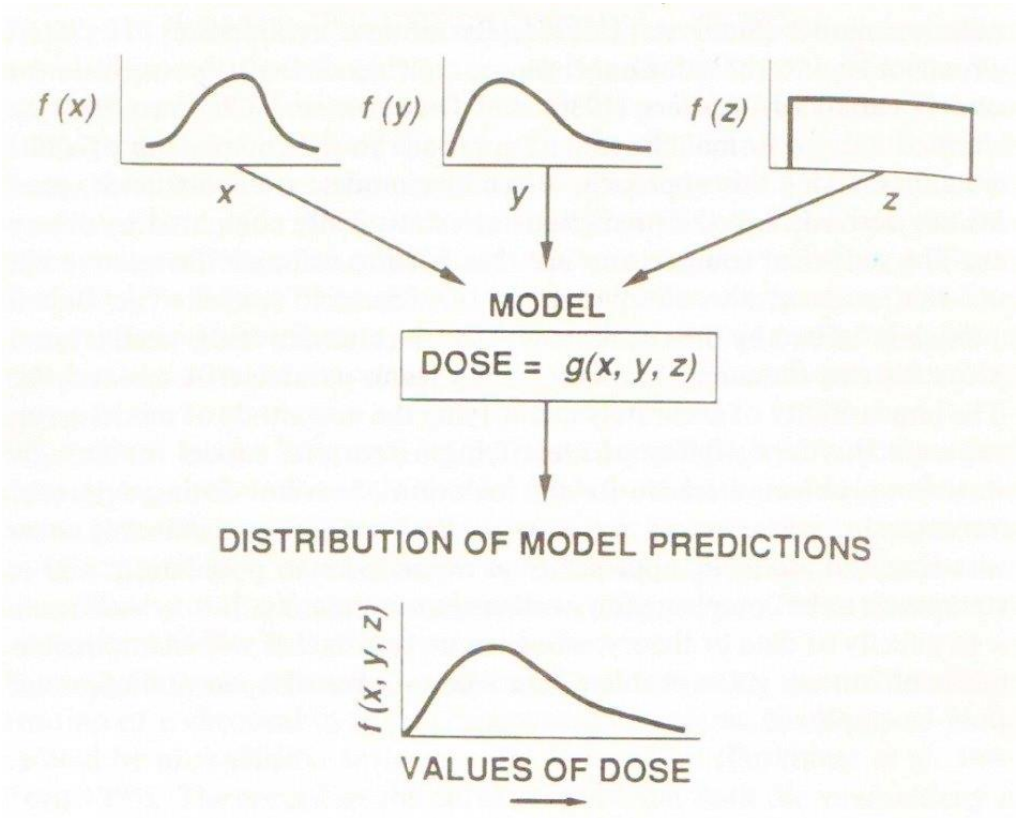


Figure 13: Steps involved in a Monte Carlo analysis (Suter II et al, 1993)

However, the conventional RQ method is likely to remain the basic tool for lower tiers of risk assessment, as it is simple, rapid and appropriate for use as screening tool, provided it is sufficiently conservative. Probabilistic methods constitute one of several approaches that may be used for higher tier assessments (Hart, 2001).

1.4. Legislation in the European Union

1.4.1. Water

Over the last four decades, the Member States of the EU, realizing the need for ensuring good quality for groundwater, surface, coastal and marine water, have established a legal framework for action in the field of water policy. Over these decades, various directives have been adopted by the Member States, in order to maintain and continuously improve the aquatic environment of the Community.

- In 1976, the Council Directive 76/464/EEC (EEC, 1976) on pollution caused by certain dangerous substances discharged into aquatic environment of the Community, requires the Member States to take appropriate measures to eliminate and reduce the pollution of surface, coastal and groundwater derived from certain chemicals (Annex, Lists I and II).
- The Council Directive 80/68/EEC (EEC, 1980) refers to the protection of groundwater from pollution caused by certain dangerous substances (Annex, Lists I and II). According to the aforementioned Directive, the Member States are required to apply all necessary steps to prevent the pollution of groundwater and check or eliminate the consequences of pollution that have already occurred.
- Over the following 20 years, the Member States through various actions (resolutions, reports, announcements and proposals) confirmed the need for action on the qualitative and quantitative protection of the Community waters. In particular, Council Directive 91/271/EEC (EEC, 1991) and its amending Community Directive 98/15/EC (EC, 1998) concern the collection, treatment and disposal of urban and industrial effluents, in order to protect the environment from the adverse effects of the abovementioned wastewater discharges.
- In October 2000, Directive 2000/60/EC (Water Framework Directive, WFD) (EC, 2000), establishing a framework for Community action in the field of water policy, was adopted by the EU. The Directive aims at maintaining and improving the aquatic environment in the Community and contributes to the progressive reduction of emissions of hazardous substances to water. Member States should prevent further deterioration in the quality of water and

protect/improve the status of inland surface, transitional, coastal waters and groundwater. The list of the priority substances, as finally determined by the Decision 2455/2001/EC (EC, 2001), includes 33 individual or groups of organic substances, including organohalogen compounds, persistent hydrocarbons, cyanides, organophosphorous and organotin compounds, pesticides, metals and their compounds, arsenic and its compounds, nonylphenols and octylphenols. Member States must implement the necessary measures with the aim of progressively reducing pollution from priority substances and monitoring the status of waters.

- The Council Directive 2008/105/EC (EC, 2008) sets environmental quality standards (EQSs) in the field of water policy for priority substances and some other pollutants, and defines annual average and maximum allowable concentration values for the aforementioned chemicals, to achieve good chemical status of surface water. Member States should improve the knowledge and data available on sources of priority substances and ways in which pollution occurs in order to identify targeted and effective control options.
- In 2013, a list of proposed emerging compounds was introduced to European legislation (in addition to WFD 2000/60/EC). The new EU Directive (2013/39/EC) (EC, 2013) has added some additional priority substances to the existing WFD priority substance list. By 14 September 2014, Member States had been required to establish a monitoring list of those substances for which the available evidence indicates that they may pose a significant Union-level risk to the aquatic environment. The first monitoring list includes the PhC diclofenac and the EDCs E2 and EE2. The priority substances in the new Directive are 45 in number, with particular reference to PhCs.
- In 2015 the watch list of substances for EU-wide monitoring was amended in Decision 2015/495/EU (EU, 2015; Barbosa et al., 2016). Apart from diclofenac, E2 and EE2, another 14 organic micropollutants were included in the new monitoring list; namely, the PhCs azithromycin, clarithromycin and erythromycin, the EDC E1, the pesticides methiocarb, oxadiazon, imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetamiprid and triallate, the UV filter 2-ethylhexyl-4-methoxycinnamate and the antioxidant 2,6-di-tert-butyl-4-methylphenol, commonly used as food additive. In May

2017, a technical report containing the results from the 1st year of monitoring was published by the European Commission's Joint Research Centre (JRC). In the light of these results, diclofenac, oxadiazon, 2,6-di-tert-butyl-4-methylphenol and triallate are proposed to be deselected from the watch list (EC, 2017).

Emerging pollutants are often detected in aquatic ecosystems, but as their removal in conventional STPs is rather inefficient, the majority of these substances are not included in the list of priority substances of the above Directives, with the exception of nonylphenols, octylphenols and perfluorooctane sulfonic acid and its derivatives, whereas only nonylphenols are designated as priority hazardous pollutants. This may be due to the absence of enough toxicological data and risk assessment studies for EOCs at this time.

1.4.2. Soil

The disposal of sewage sludge on land intended for agricultural uses (land farming) is of particular concern, as various micropollutants, such as polybromodiphenyl ethers (PBDEs), polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organotins and heavy metals accumulate in sludge and are transferred to the terrestrial environment. In order to limit soil contamination by micropollutants, both European and national regulations have been established, which mainly concern heavy metals, PAHs, and PCBs (Mailler et al., 2014).

- The main legislative text on sludge management is Council Directive 86/278/EEC (EEC, 1986), the so called Sewage Sludge Directive. This Directive encourages the safe use of sludge in agriculture, in order to avoid any harmful effects on soil, vegetation, animals and humans. Among other provisions, it establishes rules for sampling and analysis of sludge and soil, as well as limit values for heavy metals in sludge and soil. Member States are able to apply stricter provisions than those set out in the Directive, as happens in several cases for heavy metals, organic micropollutants and pathogenic microorganisms. In particular, 16 out of the 27 EU Member States have established stringent concentration limits for heavy metals in sludge, while the thresholds for heavy metal concentration in soil are stricter in 10 out of the 27

Member States. Regarding organic micropollutants, 9 out of the 27 Member States have set limits for certain priority substances, but no reference is made to EOCs, with the exception of nonylphenols (Kelessidis and Stasinakis, 2012).

- The Council Directive 91/271/EEC (EEC, 1991) concerning urban wastewater treatment, states that the recycling of sewage sludge should be encouraged and sludge disposal to surface waters should be gradually eliminated. Its final discharge to surface waters is prohibited after 31 December 1998. In general, sludge disposal should carried out in an environmentally acceptable manner, for the purpose of minimizing negative environmental impacts.
- The Council Directive 99/31/EC (EC, 1999) on the landfill of waste, establishes measures, procedures and guidelines for the prevention or reduction of negative environmental impacts resulting from the landfill of sewage. The above Directive aims to reduce biodegradable municipal waste destined for landfill to 35% of the total amount of biodegradable municipal waste, over a period of 15 years. Member States should take measures in order that liquid, hospital, corrosive, oxidizing and flammable waste, as well as used tyres, are not accepted in a landfill.
- According to the Commission Decision 2001/118/EC (EC, 2001), sewage sludge is enlisted in non-hazardous waste; whereas a specific waste management hierarchy is applied: a. prevention, b. preparing for reuse, c. recycling, d. other recovery (e.g. energy recovery) and e. disposal (Kelessidis and Stasinakis, 2012).
- In 2012, a technical report was published by the European Commission's Joint Research Centre (JRC). The report includes the results of a screening of sewage sludge samples in a pan-European dimension. 22 minor and trace elements and 92 organic compounds were analyzed in 63 samples originating from 15 countries. According to the results, the monitored concentrations do not justify the introduction of new limit values for the considered parameters within the Sewage Sludge Directive. However, the report encourages the Member States to monitor emerging contaminants, as the available database is inadequate (EC, 2012; Clarke and Cummins, 2015).

1.5. Novelty of the thesis

Based on the available literature data reported above, there is limited (or no) information on the following topics concerning the environmental risk assessment associated with the occurrence of EOCs in STPs:

The RQ methodology has been applied for the aquatic environment, so far, either for a limited number of compounds and groups of EOCs (e.g. antimicrobials, EDCs) or for specific effluent receivers, as well as specific pollution sources (e.g. hospitals) (Escher et al., 2011; Stasinakis et al., 2012; Al Aukidy et al., 2014; Kosma et al., 2014). Except for one study in the USA (Diamond et al., 2011), no research has been conducted to estimate the potential risk from the presence of a large number of EOCs contained in effluents, at country level. Moreover, the contribution of the individual pollutants to estimated mixture toxicity has not been assessed.

There is much less information concerning the risk to the terrestrial environment due to the occurrence of EOCs in sewage sludge. Most of the relevant studies refer to specific STPs and a limited number of compounds or specific groups of contaminants (González et al., 2010; Martín et al., 2012b; Liu et al., 2014; Martín et al., 2015). Additionally, due to the lack of soil toxicity data, the potential risk for the soil environment has been mainly estimated using only aquatic toxicity data and the methodology proposed by the EC (2003). There is only one study estimating the risk from the occurrence of a significant number of PPCPs in sewage sludge (Verlicchi and Zambello, 2015). Moreover, ERA related to the presence of individual EOCs and/or their mixture in the terrestrial environment has not been conducted at country level.

Beside the fact that the PRA methodology has been used for PNEC deduction of specific EOCs (Capdevielle et al., 2008; Lyndall et al., 2010; Gottschalk and Nowack, 2012; Durán and Beiras, 2017), so far there is no study evaluating the risk associated with the presence of a particular EOC, released from European STPs, for the aquatic environment at a European level. Furthermore, to the best of our knowledge, the PRA methodology, combined with the RQ method, has not been applied, so far, to calculate the uncertainty of the estimated risk due to the presence of EOCs released from the STPs in the aquatic environment.

1.6. Aims and outline of the thesis

The main objective of this study is to develop and implement an ERA methodology using a combination of RQ method and Monte Carlo simulation. Specifically, in a first tier, using the RQ methodology, the EOCs that present a possible threat for the aquatic and terrestrial environment are identified, whereas, for these specific micropollutants, in a second tier, a PRA process is applied to quantify the uncertainty resulting from RQ calculations. The specific objectives as well as the outlines of this PhD thesis are reported below:

Specific objectives

1. Recording the concentration levels of all EOCs detected in effluents and sewage sludge from Greek STPs.
2. Recording acute toxicity data of the target compounds for specific aquatic and terrestrial organisms.
3. Assessment of the potential environmental risk associated with the presence of the individual EOCs for the Greek aquatic and terrestrial environment.
4. Estimation of the possible threat due to the occurrence of mixtures of EOCs for the Greek aquatic and terrestrial environment.
5. Recording the concentration levels of triclosan (TCS) in European STPs' effluents. According to the results of the first tier risk assessment, this specific EOC seems to pose the highest hazard among all target compounds for the aquatic and terrestrial environment.
6. Recording the toxicity data of triclosan for specific aquatic organisms.
7. Estimation of the threat related to the presence of triclosan for the European aquatic environment using a probabilistic risk assessment methodology.

To achieve these goals, the following three studies were conducted:

1. Estimation of the possible environmental risks associated with the existence of EOCs in treated wastewater, at country level. On this aspect, Greece was chosen as a case study. A literature review was conducted to record the concentration levels of all EOCs determined in Greek STPs during the last decade. Acute toxicity data was collected from peer-reviewed literature or estimated using the predictive ECOSAR model for three classes of aquatic organisms (fish, *Daphnia magna* and algae) and the

potential environmental risk due to the disposal of treated wastewater to Greek rivers was estimated for the individual compounds and their mixture as well, using the RQ methodology.

2. Assessment of the potential environmental risks from the disposal of sewage sludge containing EOCs in soil, selecting Greece as a case study. For this purpose, soil and aquatic toxicity data were collected and the possible threat due to the occurrence of single compounds and mixture of EOCs was estimated using RQ approach. The effect of daily and source-origin variation in concentrations of selected EOCs on estimated threat was investigated, while the role of maximum and average measured concentrations of target compounds in calculated RQ values was checked.

3. Probabilistic risk assessment of TCS, originating from STPs' effluents, in the European aquatic environment. The RQ methodology applied at country level (using Greece as a case study) in objectives (1) and (2) indicated a presumable threat for the Greek aquatic and terrestrial environment due to the existence of TCS in STP effluents and sludge, respectively. Thus, TCS concentration levels in treated wastewater reported in the literature since 2002, for all European countries, were compiled and toxicity data from peer-reviewed literature for algae, *Daphnia magna* and fish were collected. To estimate the threat associated with the presence of TCS in European rivers, four scenarios were developed, based on different dilutions of the treated wastewater (2, 10, 100 and 1000). In order to underpin the reliability of the RQ methodology, an uncertainty analysis was conducted using Monte Carlo simulation.

2. Materials and methods

2.1. Concentration data collection

An extended literature review was initially conducted using the Scopus database to investigate the EOCs that have been detected in Greek STPs' effluents and dewatered sewage sludge during the last decade. The search terms were "emerging organic contaminants OR organic micropollutants" AND "concentration OR occurrence OR monitoring" AND "wastewater OR effluents OR sewage OR sludge" AND "Greece". The literature data concerning effluents concentration was collected from 20 international articles, dated from 2003 to 2014, while the corresponding sewage sludge data was collected from 8 international articles, dated from 2008 to 2015. The effluents and sludge concentration values were derived from 19 and 11 Greek STPs, respectively, whereas literature data was available for 57 EOCs in effluents and 49 EOCs in sewage sludge samples. In addition to the literature data found via Scopus database, unpublished data for the concentrations levels of 150 PhCs and IDs in secondary treated wastewater samples and 50 PhCs and IDs in sludge samples were kindly provided by the Laboratory of Analytical Chemistry of the Department of Chemistry, National and Kapodistrian University of Athens. All concentration values were recorded and the maximum concentration was selected for each substance in order to estimate ecological threat for the worst-case scenario. Information was also collected for the type and number of samples, the period of sampling and the analyzed phase (dissolved/particulate).

Another comprehensive literature review was, consequently, conducted to collect monitoring data of TCS in treated wastewater of European STPs. The review was carried out for all European countries (50 in total), including those that are not members of the European Union. Data from 69 international articles, dated from 2002 to 2015, was retrieved using the Scopus database. The search terms were "triclosan" AND "concentration OR occurrence OR monitoring" AND "wastewater OR effluents OR sewage" AND "the name of the country". The studies covered a total of 349 STPs. The minimum, maximum, mean and median TCS concentration values were recorded for each study, as well as the type (grab or composite) and number of samples, the type of sewage treatment and the affiliation country.

2.2. Toxicity data collection

2.2.1. Aquatic toxicity data

In order to perform ERA in the aquatic environment, acute toxicity data (EC50 or LC50) was collected from the literature for the target compounds and for three different trophic levels (algae, *Daphnia magna* and fish). The search terms were "algae OR *Daphnia magna* OR fish" AND "EC50 OR LC50 OR acute toxicity" AND "the name of the micropollutant". Toxicity data was collected from 61 international articles, dated from 1989 to 2013. According to the TGD (EC, 2003), PNEC was calculated by dividing the LC50 or EC50 value by an appropriate assessment factor (Equation (1)). Since only short-term toxicity data were available, an assessment factor of 1000 was applied on the lowest LC50 or EC50 value:

$$PNEC_{water} = \frac{EC50 \vee LC50}{1000} \qquad (1)$$

For those micropollutants that more than one toxicity data was available, the lowest value was chosen in order to estimate ecological threat for worst-case scenario. In cases that there was no experimental toxicity data in the literature, ECOSAR program was used (US EPA). This program is widely used to predict the toxicity of various compounds under aqueous conditions (Gros et al. 2010; Sanderson et al., 2003), based on the similarity of structure to other compounds whose toxicity in aquatic environment has been previously estimated. Most of the ECOSAR calculations are based on several physicochemical constants and especially on the octanol/water partition coefficient (K_{ow}) (Sanderson et al., 2003). However, there are certain groups of chemicals (e.g. PFCs, polyfluorinated compounds) whose toxicity cannot be predicted by ECOSAR model, as a) their physicochemical properties are vastly different from their non-substituted analogs, b) their water solubility cannot be accurately estimated due to their chemical properties or c) react with water and they are converted to other substances. For these groups of chemicals, EC50/LC50 values were not calculated by ECOSAR and risk assessment was performed only for the compounds that experimental toxicity data was available in the literature.

2.2.2. Terrestrial toxicity data

According to the TGD (EC, 2003), ERA in soil could be based on the short-term toxicity data of terrestrial organisms, such as plants, earthworms or/and soil microorganisms. Thus, experimental acute toxicity data (EC50 or LC50) for these groups of organisms were collected through literature review and the lowest value was chosen in order to estimate the environmental risk for worst-case scenario, as well. The search terms were "plant OR earthworm OR soil microorganism" AND "EC50 OR LC50 OR acute toxicity" AND "the name of the micropollutant". Toxicity data was collected from 12 international articles, dated from 2006 to 2015. EC50/LC50 values given in mg L^{-1} were converted to mg kg^{-1}, using the equilibrium partitioning method (EC, 2003):

$$E(L)C50(mg\ kg^{-1}) = E(L)C50(mg\ L^{-1}) \times K_d = E(L)C50(mg\ L^{-1}) \times K_{oc} \times f_{oc} \quad (2)$$

Where K_d is the soil-water partition coefficient (as L kg^{-1}), K_{oc} is the organic carbon partition coefficient (as L kg_{oc}^{-1}), calculated by the PCKOCWIN model (US EPA) and f_{oc} the organic carbon fraction in soil, which is equal to 0.02 kg_{oc}/kg_{solid} (EC, 2003).

Predicted no Effect Concentrations (PNECs) of the target substances based on terrestrial toxicity data ($PNEC_{soil,terrestrial}$, as ng g^{-1}) were calculated by dividing the lowest acute toxicity data (EC50 or LC50 value) by a factor equal to 1000 (EC, 2003):

$$PNEC_{soil,terrestrial} = \frac{EC50 \vee LC50}{1000} \quad (3)$$

As the experimental toxicity data for the effects of EOCs on terrestrial organisms is limited, PNEC values were also calculated using aquatic toxicity data ($PNEC_{soil,aquatic}$ as ng g^{-1}), according to Equations (1) and (4) (EC, 2003; González et al., 2010; Martín et al., 2012b; Wu et al., 2014):

$$PNEC_{soil,aquatic} = PNEC_{water} \times K_d = PNEC_{water} \times K_{oc} \times f_{oc} \quad (4)$$

For those substances that no terrestrial experimental toxicity data was available in the literature, the corresponding EC50 or LC50 values were calculated *via* the ECOSAR model, as well.

2.2.3. TCS toxicity data

The literature data on ecotoxicological acute and chronic effects of TCS on different groups of aquatic organisms was collected from 24 international articles, dated from 1986 to 2016. EC50/LC50 and NOEC values obtained for algae, *Daphnia magna* and fish were collected. Additional information, concerning the target aquatic organism species, the type and the duration of the toxicity test was also recorded. The selection of the studied organism groups and the dose descriptors was consistent with the TGD (EC, 2003) and the RQ methodology applied in the literature for estimating the ecological threat due to the existence of micropollutants in wastewater (Stasinakis et al., 2012; Frédéric and Yves, 2014; Carbajo et al., 2015; Chen et al., 2016).

2.3. Environmental risk assessment process

2.3.1. Aquatic environment

The risk assessment based on the hypothesis that the wastewater discharged by Greek STPs contains all the detected emerging contaminants. This assumption seems realistic as these chemicals are contained in every day products or/and excreted by humans, and their existence in domestic wastewater is considered independent from the size of the settlements.

The RQs for the individual substances were calculated for treated wastewater, dividing the maximum Measured Environmental Concentration (MEC) to Predicted No Effect Concentration (PNEC), for 3 different aquatic organisms; fish, *Daphnia magna* and algae, by Equation (5):

$$RQ = \frac{MEC}{PNEC} \qquad (5)$$

In cases that RQ is less than 1, no ecotoxicological risk for the aquatic environment is indicated, while in cases that RQ is greater than 1, ecotoxicological risk for the aquatic environment is indicated and further research is required (EC, 2003).

To estimate the risk due to the simultaneous presence of all micropollutants in wastewater, RQ_{mix} was calculated using the Equation (6) (Cleuvers et al., 2004; Escher et al., 2011):

$$RQ_{mix} = \sum_{i=1}^{n} RQ_i = \sum_{i=1}^{n} \frac{MEC_i}{PNEC_i} \qquad (6)$$

The above equation can be applied only if the components of the mixture exhibit their toxic action in the same way. According to the funnel hypothesis (Warne and Hawker, 1995) in mixtures containing a large number of chemicals, the compounds are more possible to exhibit a baseline mechanism of action, thus the risk assessment should be based on the hypothesis of concentration addition. Having in mind that emerging contaminants belong to various groups of chemicals and act via different modes of toxic action, baseline toxicity from ECOSAR program (also known as narcosis or nonspecific toxicity) was used for PNEC calculations (Escher et al., 2002; Öberg, 2004; Escher et al., 2011). It is necessary to note that the group of PFCs was not taken into account for the calculation of RQ_{mix}, since, as it has been mentioned before, the toxicity of these chemicals cannot be predicted by ECOSAR model.

To calculate risk quotients in Greek rivers (RQ_r), data about the average effluents flows of 25 Greek STPs and the average water flows of the corresponding rivers were collected and the relevant dilution factors (DF) were calculated (Equations (7) and (8)).

$$DF = \frac{Q_r}{Q_e} \qquad (7)$$

$$RQ_r = \frac{RQ}{DF} \qquad (8)$$

Where Q_e is the average flow of treated wastewater from a Greek STP ($m^3\ d^{-1}$) and Q_r is the average water flow of the corresponding river ($m^3\ d^{-1}$).

To estimate the possible environmental hazard of the mixture of micropollutants when wastewater is released in Greek rivers, the $RQ_{mix,river}$ was calculated using Equation (9):

$$RQ_{mix,\ river} = \frac{RQ_{mix}}{DF} \qquad (9)$$

2.3.2. Terrestrial environment

Risk assessment in soil was carried out, according to the quantitative approach described in the TGD (EC, 2003). RQs calculation was based on PEC and PNEC of the evaluated substances, for both the individual compounds and their mixture. The risk assessment was based on the hypothesis that the dewatered sludge produced in Greek STPs contains all the detected EOCs. This assumption seems realistic as the target compounds are contained in everyday products or/and excreted by humans, and their occurrence in sludge is not affected by the size of STP. In Greece, industrial activity is limited, thus 95% of the STPs sources are domestic and the type of the sludge treatment systems is similar, containing anaerobic digestion (for the largest STPs) and sludge dewatering using filter press. The population fraction served by the studied STPs was about 44% of the total population.

It should be mentioned that once the sludge is released into the terrestrial environment, the micropollutants undergo several processes, such as fixation, degradation, inactivation and transportation (Petrie et al., 2014; Verlicchi and Zambello, 2015). However, in this study these processes were not taken into account due to the lack of available data on the fate of these compounds after sludge disposal to soil. To estimate the ecological threat derived from the existence of the individual EOCs in soil, risk quotients ($RQ_{soil,terrestrial}$ or $RQ_{soil,aquatic}$) were calculated, according to Equation (10):

$$RQ_{soil} = \frac{PEC_{soil}}{PNEC_{soil}} \qquad (10)$$

Where PEC_{soil} (ng g^{-1} dw) is the concentration of the compounds in soil, estimated one year after a single sludge application (EC, 2003; Martín et al., 2012b).

The PEC_{soil} values were calculated for the worst case scenario applying the Equation (11), as recommended by the TGD of the European Commission (2003):

$$PEC_{soil} = \frac{\mathrm{MEC_{sludge} \times APPL_{sludge}}}{\mathrm{DEPTH_{soil} \times RHO_{soil}}} \qquad (11)$$

Where MEC_{sludge} (ng g^{-1} dw) is the maximum concentration values of the EOCs in sludge samples, $APPL_{sludge}$ is the dry-sludge application rate (0.5 kg m^{-2} $year^{-1}$, for agricultural soil), $DEPTH_{soil}$ is the mixing depth of soil (0.20 m, for agricultural soil)

and RHO_{soil} is the bulk density of wet soil (1700 kg m^{-3}, for agricultural soil) (González et al., 2010; Martín et al., 2012b).

If RQ_{soil} value is lower than 1, no ecological risk is expected, whereas when values are equal or greater than 1, adverse effect on terrestrial organisms is probable and further research is required (EC, 2003). For the EOCs that $RQ_{soil,terrestrial}$ or $RQ_{soil,aquatic}$ values were higher than 1, $PEC_{soil,average}$ values were also calculated for the average measured concentrations ($MEC_{sludge,average}$ as ng g^{-1} dw) using Equation 12, in order to investigate the possible threat for the terrestrial environment under more realistic conditions.

$$PEC_{soil,average} = \frac{\mathrm{MEC_{sludge,average} \times APPL_{sludge}}}{\mathrm{DEPTH_{soil} \times RHO_{soil}}} \qquad (12)$$

It is well known that chemicals in a mixture may either not interact, or interact synergistically or antagonistically (Backhaus and Faust, 2012). According to the funnel hypothesis (Warne and Hawke, 1995), in mixtures which contain a large number of chemicals, there is a greater possibility that the compounds exhibit a baseline mechanism of action and the risk assessment should be based on the hypothesis of concentration addition. So far, limited data is available on the effects of chemical mixtures on the terrestrial environment. Assuming that soil and water are affected comparably by toxicants (Warne, 2003), baseline toxicity or narcosis, predicted *via* the ECOSAR model only for aquatic organisms, was used to calculate $PNEC_{soil,aquatic}$ values (Öberg, 2004; Escher et al., 2011) and the possible hazard due to the presence of the mixture of chemicals ($RQ_{soil,\ mix}$) was estimated using Equation (13) (Escher et al., 2011):

$$RQ_{soil,mix} = \sum_{i=1}^{n} RQ_{soil,aquatic(i)} = \sum_{i=1}^{n} \frac{PEC_{soil(i)}}{PNEC_{soil,aquatic(i)}} \qquad (13)$$

It should be mentioned that in Equation 13, PEC_{soil} values were calculated using the maximum concentration values of the EOCs in sludge samples (worst case scenario) as well as for the average concentration values of the EOCs in sludge samples. For the estimation of the mixture toxicity, PFCs were not taken into account, since they could not be profiled by the ECOSAR program.

2.4. Probabilistic risk assessment of TCS

In order to assess the potential risk associated with the presence of TCS in the aquatic environment, the RQ calculations were based on the MEC values of the target compound in treated wastewater, the PNEC values for 3 different aquatic organisms - algae, *Daphnia magna* and fish - and the DF the effluents might undergo when released into the aquatic ecosystem (Equation (14)):

$$\text{RQ} = \frac{MEC}{PNEC\ x\ DF} \qquad (14)$$

According to the TGD of the European Commission (EC, 2003), PNEC was calculated by dividing the LC50 or EC50 value by an appropriate AF (Equation (15)):

$$\text{PNEC} = \frac{EC50 \text{v} LC50}{AF} \qquad (15)$$

The values of the AF depend on the diversity of the toxicity data and the variety of species covering the taxonomic groups of the base-set species (EC, 2003). An AF value equal to 1000 is commonly used in prioritization of chemicals, in cases where short-term toxicity data is available from three trophic levels (algae, D*aphnia magna* and fish) of the base-set. According to the TGD, the increasing data availability of the base-set species may lead to the decrease of the AF value. Thus, in this case, an AF value equal to 100 is used, as other authors have suggested (Wu et al., 2011; Grill et al., 2016; Sun et al., 2016). It should be mentioned that as long-term toxicity data (NOEC values) for TCS in the aquatic environment was scarce, the risk assessment was based exclusively on acute toxicity values.

In order to underpin the reliability of the risk assessment methodology, an uncertainty analysis was conducted. A Monte Carlo simulation with 70,000,000 iterations was applied to quantify the uncertainties of RQs (Wu et al., 2011; Federle et al., 2014) and estimate the uncertainty of the risk posed by TCS to the aquatic organisms. Both mean and maximum concentration values on an STP basis were used to calculate the descriptive statistics of TCS risk quotients, RQ_{mean} and RQ_{max}, respectively. Each parameter that affects the RQ values (MEC and EC50/LC50 for algae, *Daphnia magna* and fish) was associated with the lognormal probability distribution. The lognormality of MEC and EC50/LC50 values was examined *via* Kolmogorov-

Smirnov test and it could not be rejected at the 95% confidence level. A sensitivity analysis was also employed to assess the contribution of the forenamed input parameters to the variance of the acquired results, by calculating the Spearman rank correlation coefficient.

To estimate the threat associated with the presence of TCS in the European rivers, four scenarios were developed, based on the different dilution the effluents might undergo when released in the aquatic ecosystem. A recent study conducted in Germany by Link et al. (2017) revealed DFs equal to 2 for about 10% of streams. Besides, according to Keller et al. (2014), the annual median DFs vary by about 3 orders of magnitude across European rivers, as they range between the values 9 (Belgium) and 5,650 (Russia). Based on the above, the Monte Carlo simulation was applied for DFs equal to 2, 10, 100 and 1000.

3. Results and discussion

3.1. ERA of EOCs in Greek aquatic environment

3.1.1. Occurrence of EOCs in Greek STPs' effluents

As the literature review indicated, so far, there are a sufficient number of published articles in scientific journals (20) concerning the presence of emerging pollutants in the effluents of Greek STPs (Annex, Table S1). Most of these studies contain data for pharmaceuticals (11 papers) and EDCs (13 papers), while BTRs, BTHs, PFCs, ASs and SLXs have also been detected in Greek treated wastewater. As it is a common practice both in Greece and abroad, in 12 out of the 20 papers, analyses have been done only in the dissolved fraction of wastewater. Having in mind the low suspended solids concentrations in treated wastewater, this action could slightly underestimate the concentration levels of the compounds that exhibit a high tendency to adsorb onto particulate phase such as TCS and NP (Samaras et al., 2011). Most of the analyzed samples for PhCs (7 out of 11 papers) and EDCs (11 out of 13 papers) were grab, while all samples of BTRs, BTHs, PFCs, ASs and SLXs were composite.

As it has been mentioned in Paragraph 2.1, during the present study the concentration values of further 150 compounds (PhCs and IDs) provided by the Laboratory of Analytical Chemistry (Department of Chemistry, National and Kapodistrian University of Athens) were included in the ERA process (Annex, Table S2). According to the literature and experimental data, information on a total for 207 EOCs was available for the treated wastewater in Greece. As shown in Figure 14, the maximum concentration levels of detected micropollutants ranged from less than 1 ng L^{-1} (PhCs) to some tens of μg L^{-1} (ASs). The highest concentrations for each group of contaminants were 17292 ng L^{-1} for valproic acid (PhCs), 261 ng L^{-1} for codeine (IDs), 17400 ng L^{-1} for nonylphenol diethoxylate (EDCs), 1281 ng L^{-1} for perfluoroundecanoic acid (PFCs), 5773 ng L^{-1} for tolytriazole (BTRs), 616 ng L^{-1} for benzothiazole (BTHs), 27200 ng L^{-1} for acesulfame (ASs) and 6020 ng L^{-1} for decamethylcyclopentasilane (SLXs) (Annex, Table S3).

The concentration levels of the emerging pollutants in Greek STPs were in most cases in agreement with those of other countries (Siclair and Kannan, 2006; Voutsa et al., 2006; Buerge et al., 2009; Kasprzyk-Hordern et al., 2009; Verlicchi et al., 2010; Sanchís et al., 2013). Some deviations were observed for specific compounds such as caffeine, salicylic acid, valsartan, and triclosan that detected at higher concentrations in Greek STPs, while the concentrations of codeine, amphetamine, tramadol, 4-t-octylphenol and 1H-benzotriazole were lower comparing to those detected abroad (Voutsa et al., 2006; Kasprzyk-Hordern et al., 2009; Verlicchi et al., 2010; Sui et al., 2011).

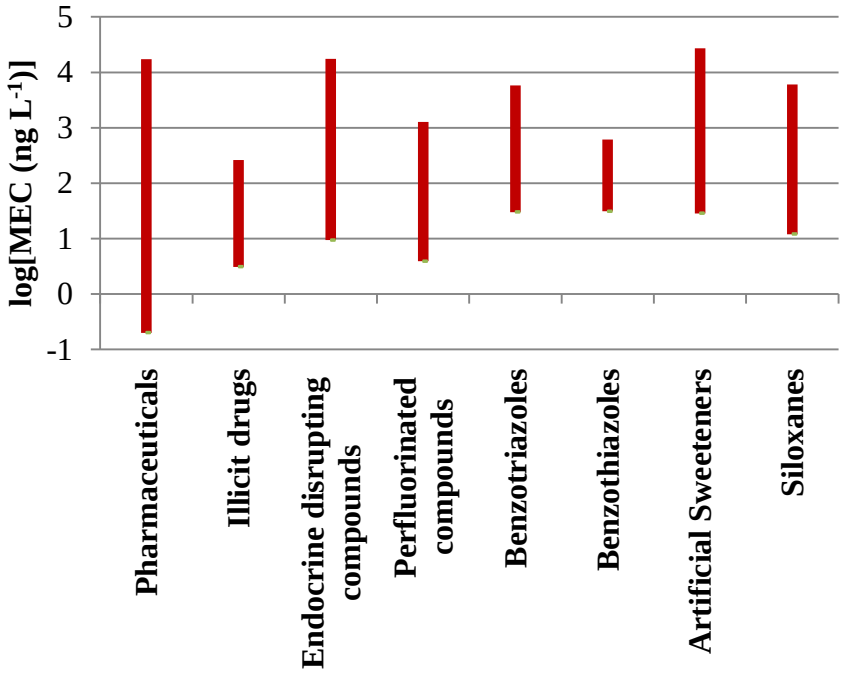


Figure 14: Maximum oncentration levels of eight (8) classes of emerging organic contaminants in secondary treated wastewater obtained from Greek STPs

3.1.2. Environmental risk due to the individual emerging contaminants

To estimate the environmental risks associated with emerging contaminants to the Greek aquatic environment, RQ values were initially calculated for single compounds. For 105 out of the 175 detected compounds, there was no experimental toxicity data in the literature; EC50 or LC50 values were found for 66 of them *via* ECOSAR

(Annex, Table S4). The classes of the emerging organic contaminants with limited experimental toxicity data were IDs, PFCs, BTRs, ASs and SLXs.

According to the results, 87% (in fish), 90% (in *Daphnia magna*) and 80% (in algae) of the compounds detected in the effluents of STPs do not seem to pose risks to all aquatic organisms as their RQ values were lower than 1 (Figure 15). On the other hand, RQ higher than 1 were calculated for 34 compounds in secondary treated wastewater (Table 2). As it was expected, for most emerging contaminants, the most sensitive aquatic organisms were algae. The classes of emerging contaminants that seem to present the greatest threat to aquatic organisms were EDCs and SLXs. According to Table 2, all EDCs had RQ > 1 for fish, while very high RQ values were calculated for TCS (4914, in algae) and NP (835, in fish). On the other hand, seven compounds belonging to the group of SLXs seem to pose environmental risks to aquatic organisms, as, for the majority of them, the RQ values were significantly high. Amongst them, tetradecamethylhexasiloxane had the highest RQ value (60370, in fish). Regarding PhCs, caffeine presented the highest RQ (927, in algae); whereas a possible threat was also noticed for 18 other compounds of this class (Table 2). All IDs, PFCs and BTHs had RQ < 1; whereas one compound from the class of BTRs (tolytriazole) and one compound from the class of ASs (sucralose) had RQ higher than 1.

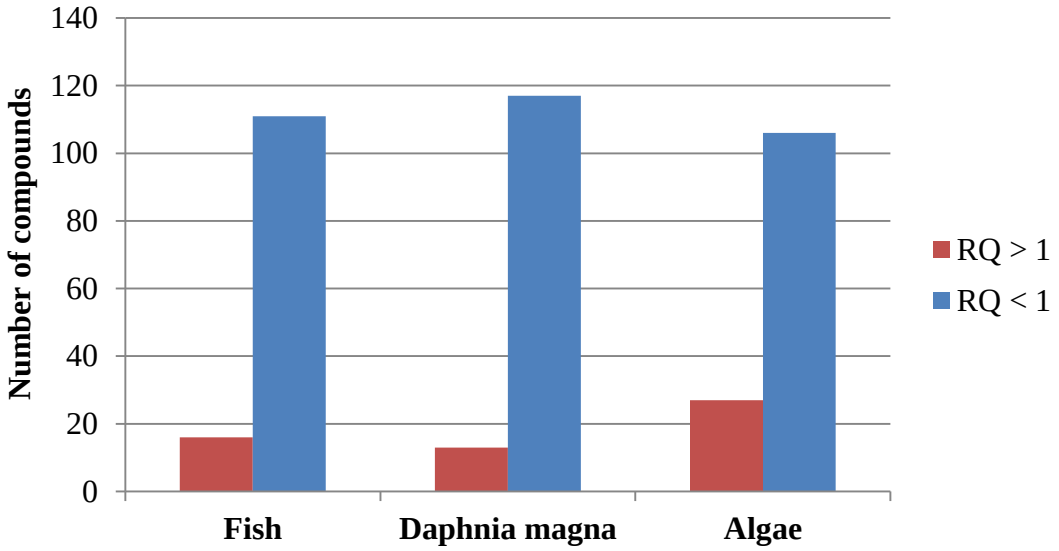


Figure 15: Emerging organic contaminants that present RQ values higher than 1 and lower than 1, in fish, *Daphnia magna* and algae

Table 2: Estimation of Risk Quotients, RQ (MEC/PNEC) for the emerging organic contaminants contained in treated wastewater. (For all other micropollutants RQ values were below 1 in all target aquatic organisms).

Emerging Contaminants	RQ values		
	Fish	***Daphnia magna***	**Algae**
Pharmaceuticals			
Amoxicillin	< 1	< 1	**44**
Atorvastatin	NA[1]	**2.4**	NA
Azithromycin	< 1	< 1	**15**
Caffeine	< 1	< 1	**927**
Clarithromycin	< 1	< 1	**31**
Clofibric acid	< 1	**1.9**	< 1
Diclofenac	**1.3**	< 1	< 1
Fluoxetine	< 1	< 1	**1.2**
Gemfibrozil	**1.9**	< 1	< 1
Ofloxacin	< 1	< 1	**9.8**
Pentobarbital	< 1	< 1	**38**
Phenobarbital	< 1	< 1	**18**
Sertraline	< 1	< 1	**2.4**
Sulfamethoxazole	< 1	< 1	**3.5**
Theophylline	< 1	< 1	**38**
Tramadol	**7.5**	**13**	**1.0**
Tylosin	NA	< 1	**1.2**
Valsartan	< 1	< 1	**2.4**
Venlafaxine	< 1	< 1	**1.1**
Endocrine Disrupting Compounds			
4-t-octylphenol	**1.4**	< 1	< 1
Bisphenol A	**7.0**	< 1	**1.1**
Nonylphenol	**835**	**67**	**30**
Nonylphenol diethoxylate	**54**	**24**	**31**
Nonylphenol monoethoxylate	**32**	**21**	**22**
Triclosan	**27**	**18**	**4914**
Benzotriazoles			
Tolytriazole	< 1	< 1	**1.5**

Artificial Sweeteners			
Sucralose	< 1	< 1	**113**
Siloxanes			
Hexamethylcyclotrisiloxane	**2.6**	**3.3**	**1.1**
Octamethylcyclotetrasiloxane	**20**	**18**	**3.9**
Decamethylcyclopentasiloxane	**4210**	**2076**	**602**
Dodecamethylcyclohexasilane	**366**	**337**	**30**
Decamethyltetrasiloxane	**132**	**131**	**17**
Dodecamethylpentasiloxane	**261**	**228**	NA[1]
Tetradecamethylhexasiloxane	**60370**	NA[1]	NA[1]

[1] NA: Not available

Risk assessment in treated wastewater presents interest only in cases of streams with small flows or during the summer season when the water of some rivers is reduced significantly and the greatest part of the flow is due to discharged treated wastewater. On the other hand, risk assessment in rivers with higher DFs is a matter of particular interest, as a potential toxicity of some substances may affect the balance in the aquatic ecosystems. It should be mentioned that apart from the dilution, the micropollutants undergo several processes, when they are released in the aquatic environment, such as adsorption to sediments, biotransformation and/or phototransformation (Farré et al., 2008). However, in this study, these processes were not taken into account.

Concerning the 25 Greek rivers that receive treated wastewater, DF ranging between 2 to 2388 was calculated (Table 3). Calculation of RQ values taking into account wastewater dilution showed RQ higher than 1 for 20 micropollutants in algae, 12 micropollutants in D*aphnia magna* and 13 micropollutants in fish (Figure 16). As it was expected, the rivers with DF equal to 2 and 3 presented the highest possibility for ecological threat due to the presence of 28 and 25 emerging contaminants, respectively; whereas a possible threat was also observed for 21 compounds and DF lower or equal to 101 (Table 4). Amongst target compounds, TCS (in algae), decamethylcyclopentasiloxane and tetradecamethylhexasiloxane (in fish) presented RQ > 1 for all studied rivers, indicating a possible ecological risk regardless wastewater dilution (Figures 16a, c). Additionally, for 24/25 rivers (DF ≤ 1910), 23/25 rivers (DF ≤ 913) and 22/25 rivers (DF ≤ 824), decamethylcyclopentasiloxane

(in D*aphnia magna*), caffeine (in algae) and NP (in fish) presented RQ values higher than 1, respectively (Figure 16 and Table 4).

Table 3

Estimation of risk quotients for the mixture of the emerging organic contaminants in treated wastewater (RQ_{mix}) and in 25 Greek rivers ($RQ_{mix,river}$). Dilution factors (DF) ranged from 2 to 2388.

	Dilution factor, DF	**RQ_{mix} / $RQ_{mix,river}$**		
		Fish	**Daphnia magna**	**Algae**
Treated wastewater	1	300000	300000	102000
River Siasiaki (Naousa)	2	150000	150000	50900
River Soulou (Ptolemaida)	3	100000	99900	33900
River Aisonas (Katerini)	11	27300	27200	9250
River Lithaios (Trikala)	11	27300	27200	9250
River Erkynas (Leivadia)	14	21500	21400	7270
River Sakoulevas (Florina)	15	20000	20000	6780
River Vozvozis (Komotini)	16	18800	18700	6360
River Evrotas (Sparti)	18	16700	16700	5650
River Aggitis (Drama)	22	13700	13600	4630
River Kalamas (Ioannina)	49	6130	6110	2080
River Peneios (Karditsa)	101	2970	3000	1010
River Karpenisiotis (Karpenisi)	133	2260	2250	765
River Peneios (Larisa)	142	2120	2110	717
River Loudias (Giannitsa)	230	1310	1300	442
River Peneios (Kalampaka)	273	1100	1100	373
River Strymonas (Serres)	286	1050	1050	356
River Alfeios (Pyrgos)	318	944	942	320
River Aliakmonas (Veroia)	608	494	493	167
River Titarisios (Tyrnavos)	750	400	399	136
River Gallikos (Kilkis)	790	380	379	129
River Aheloos (Agrinio)	824	364	364	124
River Arahthos (Arta)	873	344	343	117
River Aliaknonas (Kastoria)	913	329	328	111
River Alfeios (Krestena)	1910	157	157	53
River Evros (Orestiada)	2388	126	125	43

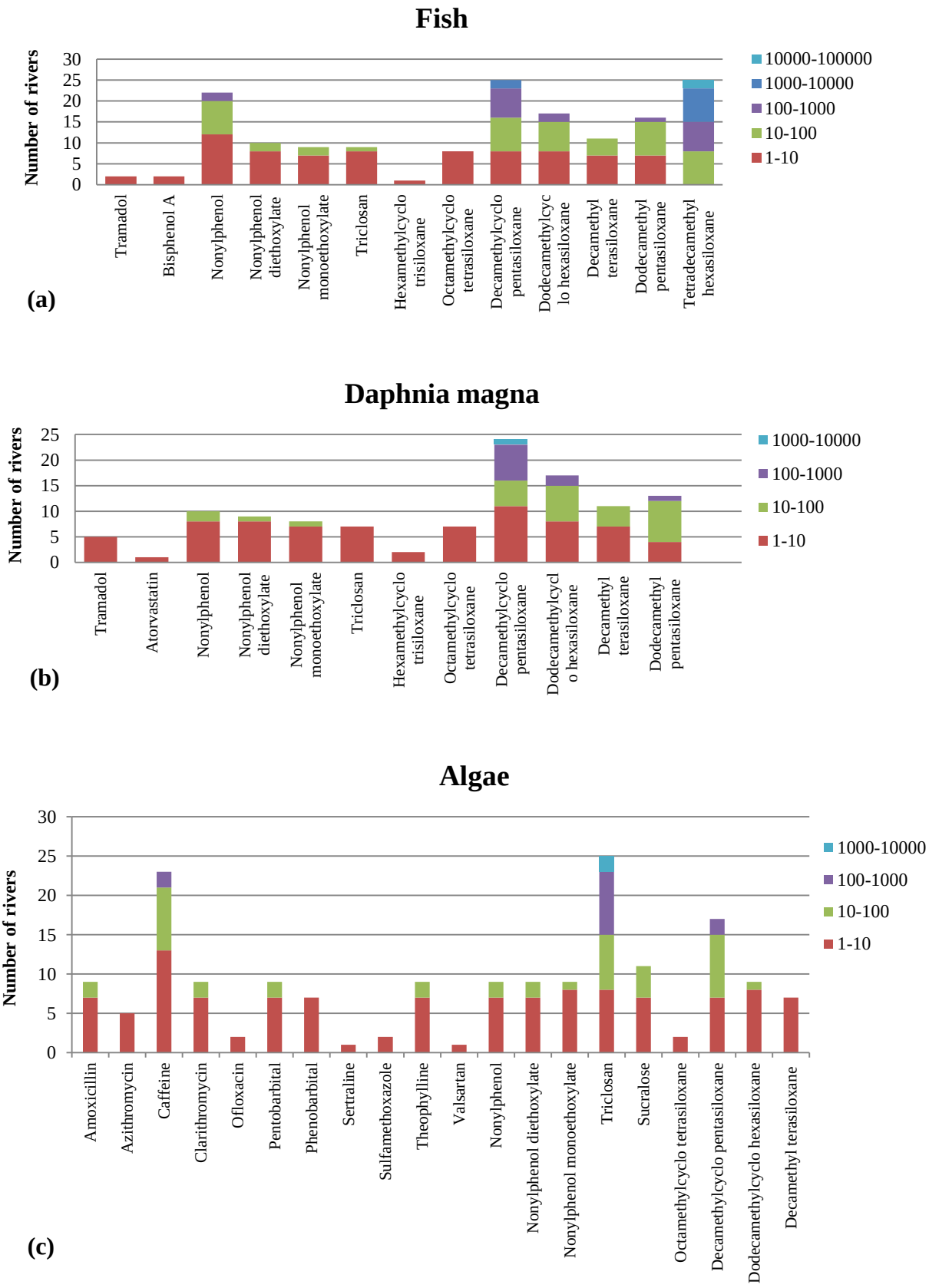


Figure 16: Emerging organic contaminants that present RQ values higher than 1 in 25 Greek rivers receiving treated wastewater. Results for fish (a), *Daphnia magna* (b) and algae (c)

Table 4

Maximum dilution factors (DF_{max}) for which the emerging organic contaminants present environmental risk (RQ > 1), in fish, *Daphnia magna* and algae.

	Fish		***Daphnia magna***		**Algae**	
Target compounds	DF	RQ	DF	RQ	DF	RQ
Pharmaceuticals						
Amoxicillin	NR[1]		NR		22	2.0
Atorvastatin	NR		2	1.2	NR	
Azithromycin	NR		NR		14	1.1
Caffeine					913	1.0
Clarithromycin					22	1.4
Ofloxacin					3	3.3
Pentobarbital					22	1.7
Phenobarbital					16	1.1
Sertraline					2	1.2
Sulfamethoxazole					3	1.2
Theophylline					22	1.7
Tramadol	3	2.5	11	1.2	NR	
Valsartan	NR		NR		2	1.2
Endocrine disrupters						
Bisphenol A	3	2.3	NR		NR	
Nonylphenol	824	1.0	49	1.4	22	1.4
Nonylphenol diethoxylate	49	1.1	22	1.1	22	1.4
Nonylphenol monoethoxylate	22	1.4	18	1.2	22	1.0
Triclosan	22	1.2	16	1.1	2388	2.1
Artificial sweeteners						
Sucralose	NR		NR		101	1.1
Siloxanes						
Hexamethylcyclotrisiloxane	2	1.3	3	1.1	NR	
Octamethylcyclotetrasiloxane	18	1.1	16	1.1	3	1.3
Decamethylcyclopentasiloxane	2388	1.8	1910	1.1	318	1.9
Dodecamethylcyclohexasiloxane	318	1.15	318	1.1	22	1.3
Decamethyltetrasiloxane	101	1.3	101	1.3	16	1.0
Dodecamethylpentasiloxane	203	1.1	142	1.8	NR	
Tetradecamethylhexasiloxane	2388	25.3	NR		NR	

[1]NR: No risk is presented in Greek rivers (RQ < 1)

Similarly to treated wastewater, the classes of emerging pollutants with the highest ecological risk for studied rivers were EDCs and SLXs, since 4 EDCs (NP, nonylphenol diethoxylate, nonylphenol monoethoxylate and TCS) and 5 SLXs (decamethylcyclopentasiloxane, dodecamethylcyclohexasiloxane, decamethyltetrasiloxane, dodecamethylpentasiloxane and tetradecamethylhexasiloxane) presented high RQ values even after wastewater dilution (Figure 16 and Table 4).

Taking into account that the persistence of the organic micropollutants in the aquatic environment may affect the risk they exhibit (Diamond et al., 2011), the chemical persistence of emerging organic contaminants that present environmental risk in rivers was calculated using ECOSAR (Figure 17). According to the results, 68% of the substances had half-lives in water less than 60 days, while 16% of them (azithromycin, clarithromycin, ofloxacin) had half-lives equal or greater than 180 days. Having in mind that the studied microcontaminants are continuously released in the aquatic environment through treated wastewater, the risk seems significant, even for those compounds that have the tendency to decompose quickly in rivers.

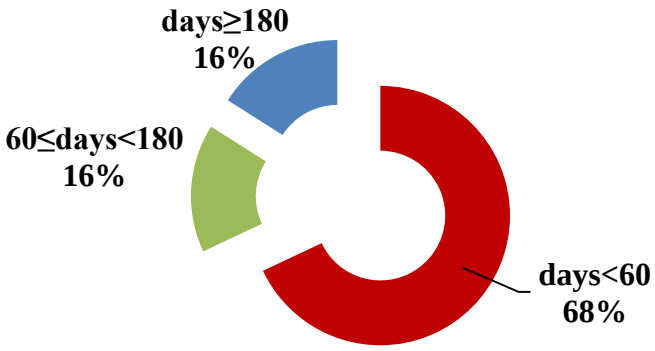


Figure 17: Chemical persistence of emerging organic contaminants that present environmental risk in rivers (RQ_r > 1). Half-lives were estimated using ECOSAR

Finally, it should be pointed out, that in order to have more reliable results in the future, more toxicity studies should be carried out, so abundant experimental data would be available to assess the toxicity of the target compounds. The lack of the

experimental data, especially the chronic one, is a major obstacle to a more accurate prediction of the threat due to these substances in the aquatic environment.

3.1.3. Environmental risk due to the mixture of emerging contaminants

To estimate possible ecological threat to treated wastewater and river water due the mixture of emerging contaminants, the risk quotients RQ_{mix} and $RQ_{mix,river}$ were calculated according to Equations 6 and 9, respectively. It should be mentioned that PFCs and 16 other emerging contaminants were not included in RQ_{mix}/$RQ_{mix,river}$ calculations as their baseline toxicities were not available by ECOSAR (Annex, Table S4).

According to the results (Table 3), the most sensitive aquatic organisms in the presence of the mixture was fish (RQ_{mix} = 300322), followed by *Daphnia magna* (RQ_{mix} = 299602) and algae (RQ_{mix} = 101766). The class of emerging contaminants that seem to have the highest contribution to mixture's toxicity was EDCs (Figure 18). Specifically, 98% and 97% of the toxicity of the mixture was due to NP, nonylphenol diethoxylate, nonylphenol monoethoxylate and TCS (in *Daphnia magna* and algae, respectively), while for the fish the contribution of these substances came up to 77%. Treated wastewaters remain an ecological hazard for aquatic organisms, even after they are released into rivers. As resulting from Figure 19 and Table 3, even in rivers where the dilution is very high (DF = 2388), the mixture of micropollutants poses a great ecological risk in aquatic organisms with $RQ_{mix,river}$ higher than 126, 125 and 43 for fish, *Daphnia magna* and algae, respectively.

To investigate whether the use of the baseline toxicity values lead to an underestimation of RQ_{mix} values as emerging contaminants exhibit specific mode of toxic action to the organisms, the Toxic Ratio (TR) was calculated using the Equation (16) (Escher et al., 2011; Iatrou et al., 2014):

$$TR = \frac{EC50/LC50_{\ baseline}}{EC50/LC50_{\ experimental}} \qquad (16)$$

Where $EC50/LC50_{baseline}$ is the baseline toxicity predicted by the ECOSAR model and $EC50/LC50_{experimental}$ is the acute EC50 or LC50 value, obtained from toxicological studies.

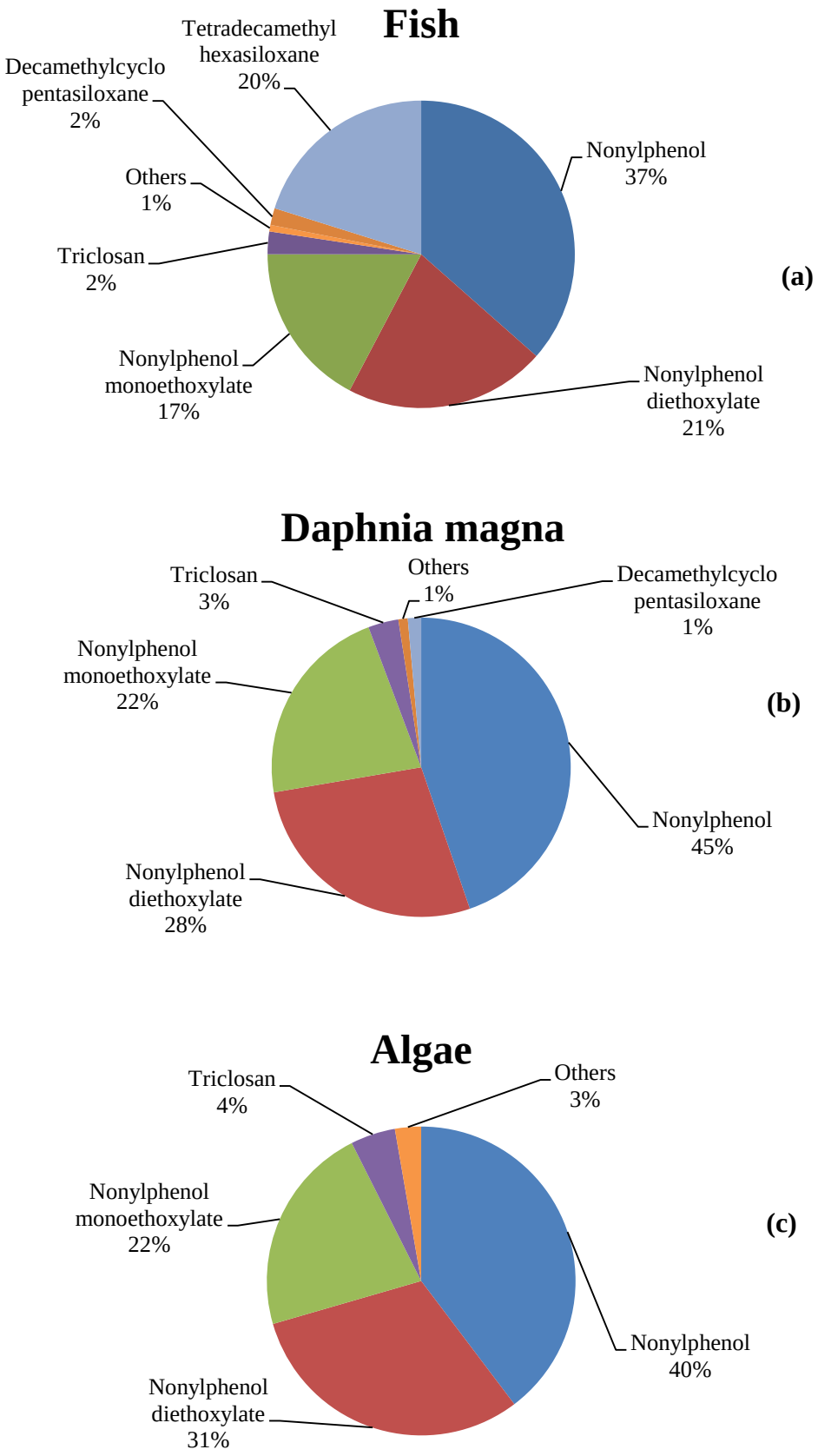


Figure 18: Contribution (%) of nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate, triclosan and other emerging contaminants to RQ_{mix} calculated for treated wastewater. Results for fish (a), *Daphnia magna* (b) and algae (c)

According to Verhaar et al. (1992), for TR > 10 the compound is likely to have a specific mode of toxic action; whereas if TR ≤ 10, it exhibits merely baseline toxicity. As it is shown in Annex (Table S5), 30% of the compounds have a TR value higher than 10 (in calculations for fish), while the corresponding rates for *Daphnia magna* and algae are 42% and 72%, respectively. Based on the above, a higher risk than estimated here, due to existence of these compounds in mixtures, cannot be excluded.

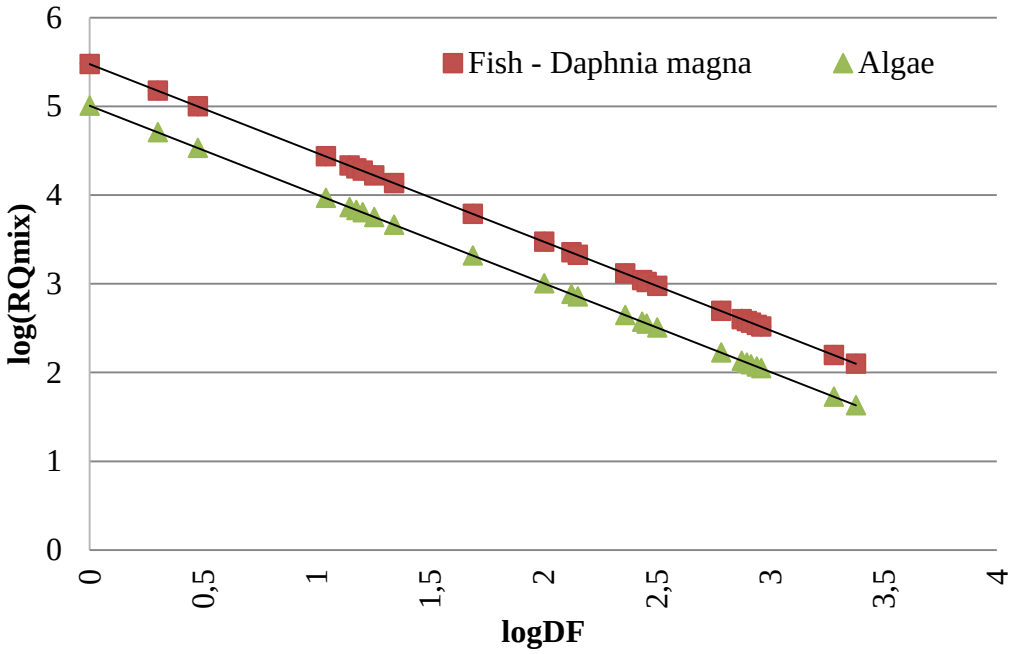


Figure 19: Effect of dilution factor (DF) on risk due to the existence of mixture of emerging organic contaminants in Greek rivers ($RQ_{mix,\ river}$)

3.1.4. Future directions for policy makers

The aforementioned results indicated that future national monitoring programs should include specific emerging contaminants that seem to possess an environment risk to surface water (Fig. 16). Candidate substances are TCS, nonylphenols, caffeine, sucralose and selected SLXs (e.g. decamethylcyclopentasiloxane, tetradecamethylhexasiloxane) and PhCs (e.g. tramadol, amoxicillin, clarithromycin, pentobarbital, theophylline). The expected dilution of discharged wastewater to surface water should be taken into account for micropollutants selection. Some of the emerging organic pollutants should be included in the relevant legislation and limit

values should be set for treated wastewater and surface water. Measures as those recently adopted in Switzerland for upgrading existed STPs (Eggen et al., 2014) and restrictions on industrial use of specific chemicals could also be adopted to minimize the ecological threat for the aquatic environment due to the existence of emerging microcontaminants. It is obvious that these procedures should be flexible as new substances and their metabolites are continuously detected and new toxicological data is raised.

Especially for NP, nonylphenol diethoxylate and nonylphenol monoethoxylate, their high RQ values indicate that there is work to do for the national authorities in order to control the use of these compounds, as their concentration levels exceeds the levels referred in the relevant Directives of the European Union (EC, 2003).

3.2. ERA of EOCs in Greek terrestrial environment

3.2.1. Occurrence of EOCs in Greek STPs' sewage sludge and soil

According to the literature data collected in this study, 8 articles have been published concerning the presence of EOCs in sewage sludge in Greek STPs (Annex, Table S6). Most of these studies contain data for PhCs (3 papers) and EDCs (4 papers), while IDs, BTHs, BTRs, PFCs and SLXs have also been detected in Greek sewage sludge samples. Samplings took place in the period between the years 2006 and 2013 and all analyzed samples were grab ones. As mentioned in Section 2.1, during the present study, additional sewage sludge samples were collected and analyzed for PhCs and IDs in the Laboratory of Analytical Chemistry of the Department of Chemistry, National and Kapodistrian University of Athens. Their concentration levels are reported in Annex, Table S7.

According to the literature and experimental data, 99 EOCs belonging to 7 different classes have been detected in Greek sewage sludge samples. As shown in Figure 20, their maximum concentrations ranged from less than 10 ng g^{-1} dw (octylphenol monoethoxylate and some PFCs) to some tens of μg g^{-1} dw (nonylphenolic EDCs). The highest concentrations for each group of micropollutants were 5,460 ng g^{-1} dw for naproxen (PhCs), 138 ng g^{-1} dw for THCA (IDs), 41,300 ng g^{-1} dw for nonylphenol monoethoxylate (EDCs), 3,209 ng g^{-1} dw for perfluoroundecanoic acid (PFCs), 412

ng g^{-1} dw for 1H-benzotriazole (BTRs), 312 ng g^{-1} dw for 2-hydroxybenzothiazole (BTHs) and 17,500 ng g^{-1} dw for decamethylcyclopentasiloxane (SLXs) (Annex, Table S8). As far as the estimated concentrations of target micropollutants in sludge-amended soil were concerned, their levels ranged between 0.008 ng g^{-1} dw (PFCs) and 60.7 ng g^{-1} dw (EDCs) (Annex, Table S8).

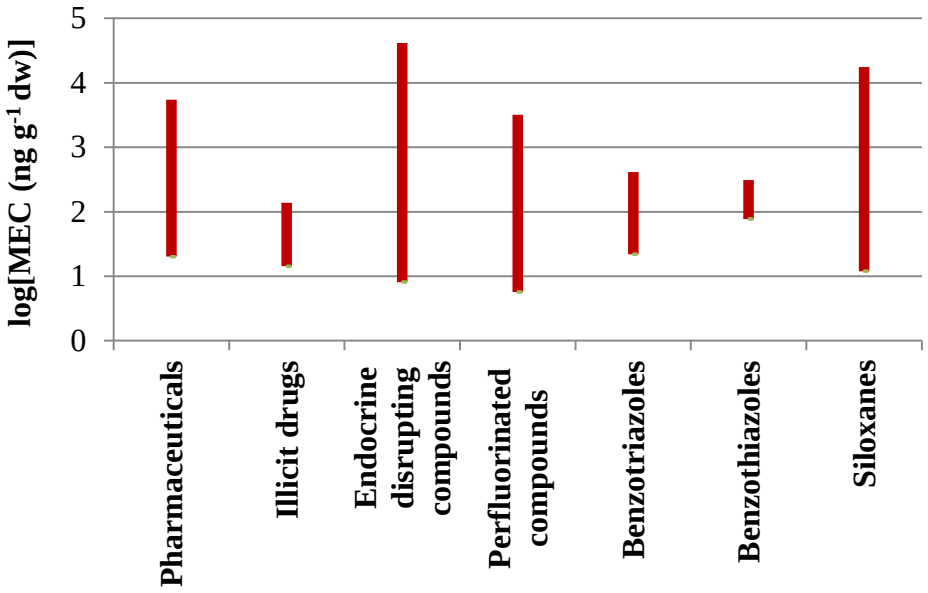


Figure 20: Maximum concentrations ($MEC_{sludge)}$ variation for seven (7) classes of emerging organic contaminants (EOCs) in dewatered sewage sludge obtained from Greek STPs

For certain groups of chemicals (e.g. EDCs, SLXs), the majority of the compounds presents high concentration values in sludge and consequently, in sludge-amended soil. This is probably not only due to their widespread domestic and industrial use, but also to their physicochemical properties that enhance their accumulation onto sludge, such as low water solubility and high sorption coefficients. Specifically, González et al. (2010) reported $LogK_{oc}$ values equal to 3.97, 4.94 and 5.06 for NP, nonylphenol monoethoxylate and nonylphenol diethoxylate, respectively, while the PCKOCWIN model predicted $LogK_{oc}$ values for SLXs ranging from 3.35 (hexamethylcyclotrisiloxane) to 6.22 (tetradecamethylhexasiloxane).

For most EOCs, the concentrations detected in Greek sewage sludge samples were similar or lower than those reported in the literature for other countries (González et al., 2010; Clarke and Smith, 2011; Martín et al., 2012b; Liu et al., 2014; Martín et al.,

2015). Higher concentrations than those in the literature (Martín et al., 2012b; Petrie et al., 2014; Martín et al., 2015) were found for few compounds, namely naproxen (5,460 ng g^{-1} dw sludge), fluoxetine (80.1 ng g^{-1} dw sludge) and BPA (3,910 ng g^{-1} dw sludge), indicating their higher use in Greece.

3.2.2. Risk assessment in sludge-amended soil based on terrestrial toxicological data of individual EOCs – worst case scenario

In order to assess the ecotoxicological risks associated with the presence of EOCs in sludge-amended soil, RQs' calculations were initially based on terrestrial toxicological data. The literature review revealed the lack of terrestrial toxicity data for most EOCs, as experimental EC50/LC50 values were available only for 18 out of the 99 target compounds. Most of the toxicity studies (10) were related to plants, while 7 and 4 studies were related to earthworms and soil microorganisms, respectively (Annex, Table S9). For 23 micropollutants, acute toxicity values in earthworms were also predicted using ECOSAR model. It is worth mentioning that, except for valproic acid, these values were not taken into account for the calculations of $RQ_{soil,terrestrial}$, as the predicted EC50/LC50 values were higher than the solubility of the target compounds. Although experimental toxicity data was available for atorvastatin, risk was not estimated for this micropollutant, as K_{oc} value was not available to apply the equilibrium partitioning method (Annex, Table S9).

Based on the aforementioned data, risk assessment was carried out for 18 EOCs; specifically 12 PhCs, 3 EDCs, 2 PFCs and 1 SLX. According to the results, TCS (EDCs) presented an $RQ_{soil,terrestrial}$ value equal to 8.1, indicating a possible ecological risk for earthworms. The rest of the target compounds seems to pose no environmental risk to the terrestrial organisms (plants, earthworms, soil microorganisms), as their $RQ_{soil,terrestrial}$ values were lower than 1 (Figure 21). To the best of our knowledge, this is the first time that risk assessment, based on terrestrial toxicity values, was carried out to such an extent in sludge-amended soil. Previous studies reported no risk for the terrestrial organisms due to TCS, decamethylcyclopentasiloxane and octamethylcyclotetrasiloxane, as they presented risk quotients lower than 1 (Chen et al., 2011; Liu et al., 2014).

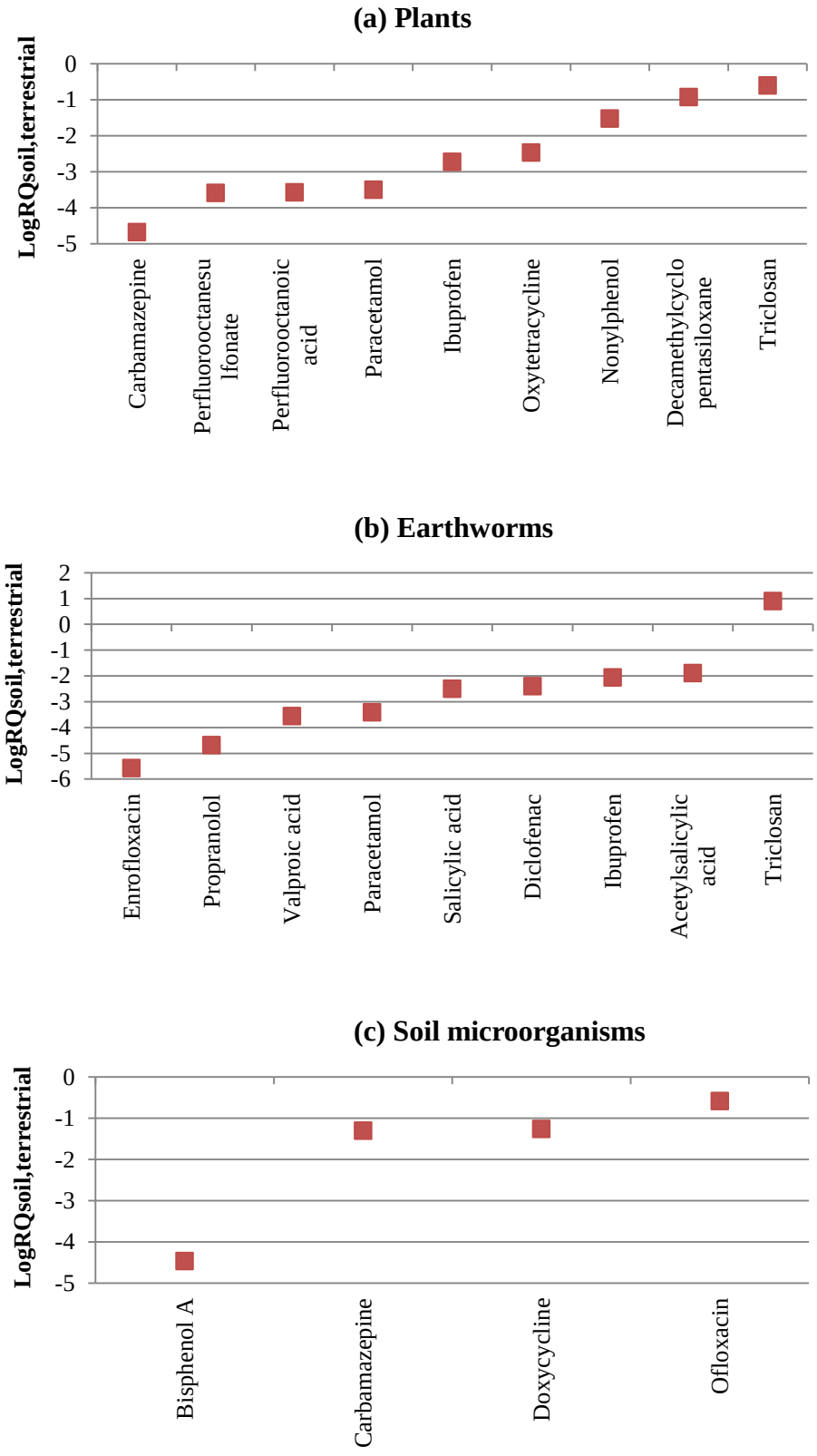


Figure 21: Risk quotients ($RQ_{soil,terrestrial}$) of 18 emerging organic contaminants (EOCs) in sludge-amended soil. Their calculations were based on terrestrial acute toxicity data for plants (a), earthworms (b) and soil microorganisms (c), as well as for the worst-case scenario

3.2.3. Risk assessment in sludge-amended soil based on aquatic toxicological data of individual EOCs – worst case scenario

As the terrestrial toxicity data was limited and covered a small part of the studied compounds, a risk assessment based on aquatic acute toxicity values was also conducted. In order to calculate the $PNEC_{soil,aquatic}$ values, organic carbon partition coefficients (K_{oc}) and short-term toxicity data (EC50 or LC50) were collected. For 77 out of the 99 detected micropollutants, K_{oc} values were predicted by the PCKOCWIN model, while for one micropollutant (octamethylcyclotetrasiloxane) K_{oc} value was found in the literature. For the rest 21 EOCs (mainly PFCs and SLXs), no K_{oc} values were available. K_{oc} values ranged from 1.00 L kg^{-1} (acetylsalicylic acid, caffeine, oxolinic acid and 2-hydroxybenzothiazole) to 1.68 x 10^6 L kg^{-1} (tetradecamethylhexasiloxane) (Annex, Table S10). On the other hand, for 45 substances experimental toxicity data was available, while EC50 or LC50 values were estimated for other 27 microcontaminants *via* the ECOSAR program. No information on their toxicity could be obtained for the remaining 27 compounds, mainly belonging to PFCs and SLXs (Annex, Table S10). Based on these facts, the environmental risk assessment was carried out for 68 out of 99 detected EOCs.

According to the results, for 12 out of the 68 target compounds (18%), $RQ_{soil,aquatic}$ higher than 1 were calculated (Figure 22). Most of these compounds belong to the classes of SLXs and EDCs, while the highest quotients were calculated for caffeine, tetradecamethylhexasiloxane, ofloxacin, decamethylcyclopentasiloxane and TCS, equal to 88, 66, 53, 51 and 29, respectively (Table 5). On the other hand, all IDs, PFCs, BTRs and most of the PhCs had $RQ_{soil,aquatic} < 1$, indicating no individual environmental threat due to their occurrence in sewage sludge. To the best of our knowledge, for the majority of the target compounds, no risk assessment in soil has been carried out in the past, except for certain PhCs and SLXs, NP and nonylphenol ethoxylates. According to González et al. (2010), NP, nonylphenol monoethoxylate and nonylphenol diethoxylate also presented a toxicological risk for the terrestrial organisms, while previous studies estimating the possible hazard for caffeine and decamethylcyclopentasiloxane reported RQ_{soil} values lower than 1 (Martín et al, 2012b; Liu et al., 2014; Martín et al, 2015).

3.2.4. Risk assessment in sludge-amended soil based on average environmental concentrations of individuals EOCs

In order to obtain information for the possible threat to the terrestrial environment under more realistic conditions, for those EOCs that presented ecological threats *via* the worst-case scenario (Table 5, Figure 21, Figure 22), $RQ_{soil,terrestrial}$ and $RQ_{soil,aquatic}$ values were also calculated using the average environmental concentrations reported in Annex (Table S11). According to the results of risk assessment (Table 6), 9 out of the 12 aforementioned EOCs, exhibited risk quotients higher than 1, whereas only the endocrine disrupting compounds NP, nonylphenol monoethoxylate and nonylphenol diethoxylate seem to pose no hazard to the terrestrial organisms when mean concentrations were used. The highest quotients were calculated for tetradecamethylhexasiloxane, decamethylcyclopentasiloxane and caffeine, equal to 58, 43 and 17, respectively, while TCS had both $RQ_{soil,terrestrial}$ and $RQ_{soil,aquatic}$ values higher than 1 (1.5 and 5.2, respectively). The above results reinforced the argument that these pollutants should be included in the national monitoring programs, in order to export more reliable conclusions regarding their toxicity in sludge-amended soil.

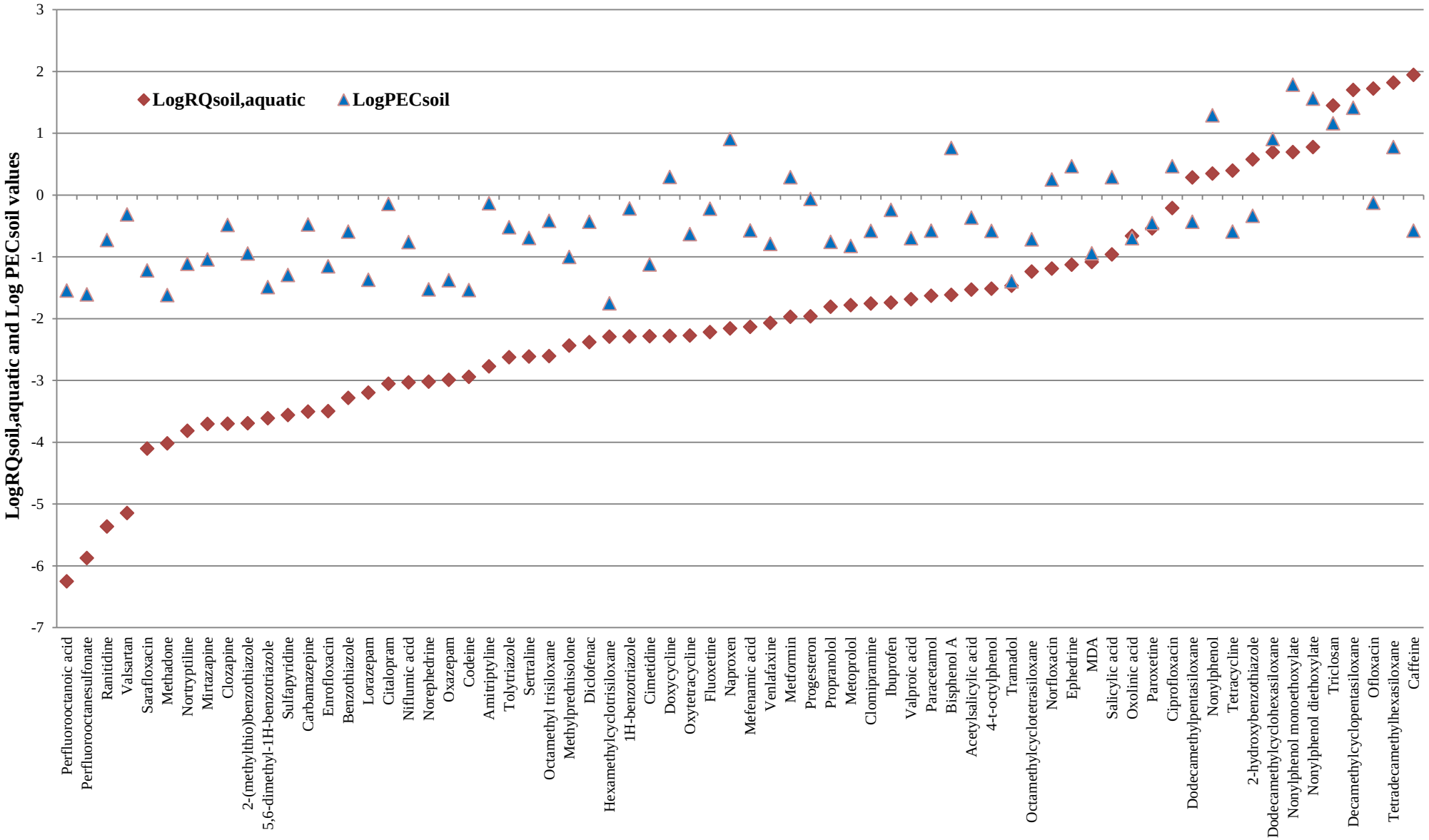


Figure 22: Risk quotients ($RQ_{soil,aquatic}$) and predicted soil concentration (PEC_{soil}) levels of 67 emerging organic contaminants (EOCs) in sludge-amended soil ($RQ_{soil,aquatic}$ values are ranked with increasing value; their calculations were based on aquatic acute toxicity data and worst-case scenario).

Table 5

Estimation of Risk Quotients, $RQ_{soil,aquatic}$ ($PEC_{soil}/PNEC_{soil,aquatic}$) for the emerging organic contaminants (EOCs) contained in sludge-amended soil. ($RQ_{soil,aquatic}$ values' calculation was based on maximum measured concentration in sludge (MEC_{sludge}) and aquatic acute toxicity data; for all other micropollutants, $RQ_{soil,aquatic}$ values were below 1).

Emerging Contaminants	**MEC_{sludge} (ng g^{-1} dw)**	**PEC_{soil} (ng g^{-1} dw)**	**$PNEC_{soil,aquatic}$ (ng g^{-1} dw)**	**$RQ_{soil,aquatic}$**
Pharmaceuticals				
Caffeine	93.1	0.14	0.003	88
Ofloxacin	159	0.23	0.014	53
Tetracycline	191	0.28	0.10	2.8
Endocrine disrupting compounds				
Nonylphenol	13200	19	8.7	2.2
Nonylphenol diethoxylate	24700	36	6.1	5.9
Nonylphenol monoethoxylate	41300	61	12	5.1
Triclosan	9850	15	0.52	29
Benzothiazoles				
2-hydroxybenzothiazole	312	0.46	0.12	3.8
Siloxanes				
Decamethylcyclopentasiloxane	17500	26	0.51	51
Dodecamethylcyclohexasiloxane	5490	8.1	1.6	5.1
Dodecamethylpentasiloxane	250	0.37	0.19	2.0
Tetradecamethylhexasiloxane	4070	6.0	0.091	66

Table 6

Estimation of Risk Quotients ($RQ_{soil,terrestrial}$ and $RQ_{soil,aquatic}$) based on average measured concentrations ($MEC_{sludge,average}$) for the emerging organic contaminants (EOCs) contained in sludge-amended soil and exhibit environmental threats *via* the worst-case scenario.

Emerging Contaminants	**$MEC_{sludge,average}$ (ng g^{-1} dw)**	**$PEC_{soil,average}$ (ng g^{-1} dw)**	**$PNEC_{soil,aquatic}$ (ng g^{-1} dw)**	**$RQ_{soil,terrestrial}$[1] or $RQ_{soil,aquatic}$[2]**
Pharmaceuticals				
Caffeine	34.0	0.05	0.003	**17**[2]
Ofloxacin	80.9	0.12	0.014	**8.6**[2]
Tetracycline	65.0	0.10	0.10	**1.0**[2]
Endocrine disrupting compounds				
Nonylphenol	4421	6.5	8.7	0.74[2]
Nonylphenol diethoxylate	2758	4.1	6.1	0.67[2]
Nonylphenol monoethoxylate	3552	5.2	12	0.43[2]
Triclosan	1831	2.7	1.8	**1.5**[1]
Triclosan	1831	2.7	0.52	**5.2**[2]
Benzothiazoles				
2-hydroxybenzothiazole	98.7	0.15	0.12	**1.3**[2]
Siloxanes				
Decamethylcyclopentasiloxane	15100	22	0.51	**43**[2]
Dodecamethylcyclohexasiloxane	5030	7.4	1.6	**4.6**[2]
Dodecamethylpentasiloxane	220	0.32	0.19	**1.7**[2]
Tetradecamethylhexasiloxane	3630	5.3	0.091	**58**[2]

3.2.5. Effect of variations in EOCs' sludge concentrations on the predicted environmental risk

For those compounds that exhibited environmental hazards *via* the worst-case scenario (Paragraphs 3.2.2 and 3.2.3), additional calculations of RQ_{soil} were conducted using all the available concentration values in Greek sludge samples in order to check whether variations in concentration due to the sludge source and day of sampling affect the predicted environmental risk. The group of EOCs for which the most measurements were available was EDCs that have been detected in 6 Greek STPs, during 4 sampling periods. The class of micropollutants with limited concentrations data was SLXs, as they have been detected only in one STP during one sampling period. Data for caffeine, ofloxacin and tetracycline was available from 2 Greek STPs and for 2-hydroxybenzothiazole from one STP in two sampling periods. Detailed information about the number of samples, the STPs and the sampling periods for all target compounds are presented in Table 7.

In Figure 23, box-and-whisker plots of $LogRQ_{soil}$ values for the target compounds are shown. It is worth mentioning that all the calculations are based on aquatic toxicity data, excepting TCS values which are based both on aquatic and terrestrial toxicity data. Standard deviations range from 3%, in the case of dodecamethylcyclohexasiloxane, to 85%, in the case of NP. According to the results, among EDCs, TCS seems to pose the most significant hazard to the terrestrial environment, as 91% and 60% of the analyzed samples presented $RQ_{soil,aquatic}$ and $RQ_{soil,terrestrial}$ values, respectively, higher than 1. The corresponding rate for NP, nonylphenol monoethoxylate and nonylphenol diethoxylate was 21%. As far as PhCs concerned, caffeine and ofloxacin presented $RQ_{soil,aquatic} > 1$ for all analyzed samples indicating that despite the daily differences in concentrations levels and the differences in plant capacity and sludge treatment processes in both STPs, a threat to the terrestrial environment seems possible for these compounds. Regarding SLXs, all sludge samples showed results of $RQ_{soil,aquatic}$ values higher than 1, indicating that the probable environmental threat due to the occurrence of these compounds in sludge is not affected by daily variations in concentration levels.

Table 7

Sampling data for the emerging organic contaminants (EOCs) that present RQ_{soil} values higher than 1.

Emerging Contaminants	Number of samples	Number of STPs (Location)	Number of sampling periods (Years)
Pharmaceuticals			
Caffeine	13	2 (Athens, Santorini Island)	2 (2010, 2013)
Ofloxacin	13	2 (Athens, Santorini Island)	2 (2010, 2013)
Tetracycline	13	2 (Athens, Santorini Island)	2 (2010, 2013)
Endocrine disrupting compounds			
Nonylphenol	64	6 (Athens, Mytilene, Chalkida, Nafplion, Herakleion, Kallikratia)	4 (2006, 2007, 2009, 2010-2011)
Nonylphenol diethoxylate	64	6 (Athens, Mytilene, Chalkida, Nafplion, Herakleion, Kallikratia)	4 (2006, 2007, 2009, 2010-2011)
Nonylphenol monoethoxylate	64	6 (Athens, Mytilene, Chalkida, Nafplion, Herakleion, Kallikratia)	4 (2006, 2007, 2009, 2010-2011)
Triclosan	64	6 (Athens, Mytilene, Chalkida, Nafplion, Herakleion, Kallikratia)	4 (2006, 2007, 2009, 2010-2011)
Benzothiazoles			
2-hydroxybenzothiazole	16	1 (Athens)	2 (2010-2011, 2012)
Siloxanes			
Decamethylcyclopentasiloxane	7	1 (Athens)	1 (2012)
Dodecamethylcyclohexasiloxane	7	1 (Athens)	1 (2012)
Dodecamethylpentasiloxane	7	1 (Athens)	1 (2012)
Tetradecamethylhexasiloxane	7	1 (Athens)	1 (2012)

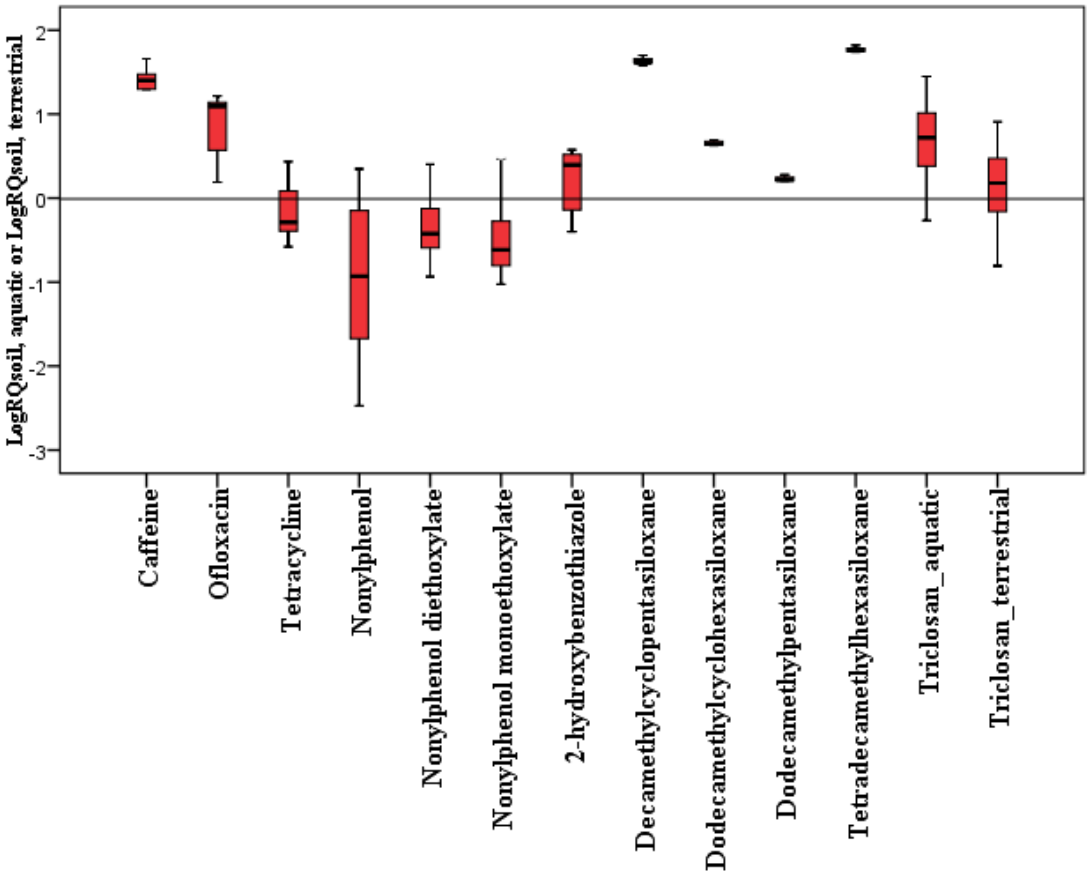


Figure 23: Box-and-whisker plots of $logRQ_{soil}$ values of those emerging organic compounds (EOCs) that had RQ_{soil} values higher than 1 for the worst-case scenario. All the results are based on aquatic toxicity data, excepting triclosan values which are based both on aquatic and terrestrial toxicity data. (All available concentration measurements have been used; the horizontal black line in the boxes represents the median value, the low and upper lines in each box correspond to the lower and upper quartile, the lines extending from each box show the highest and lowest $logRQ_{soil}$ values)

3.2.6. Risk assessment in sludge-amended soil due to the mixture of EOCs

In order to estimate the mixture toxicity of all EOCs in sludge-amended soil, baseline toxicity data was used and their risk quotients were summed up according to Equation (13). It should be pointed out that all PFCs and 26 other EOCs were not included in $RQ_{soil,\ mix}$ calculations, as their baseline toxicity and/or their organic carbon partition coefficient were not available through ECOSAR and PCKOCWIN programs, respectively (Annex, Table S10).

To the best of our knowledge, this is the first time that the results of such a study have been presented, as the risk assessment associated with the presence of a mixture of EOCs in sludge-amended soil has not been estimated before. According to the results obtained when the worst-case scenario was applied, the risk quotient of the mixture ($RQ_{soil,\ mix}$) was equal to 253, thus, it could be assumed that the combination of the micropollutants present a serious ecological hazard for terrestrial organisms in sludge-amended soil. The group of the target compounds that seem to make the highest contribution to the toxicity of the mixture was SLXs. As it is shown in Fig. 24a, 92% of the mixture's toxicity was due to decamethylcyclopentasiloxane, dodecamethylcyclohexasiloxane, dodecamethylpentasiloxane and tetradecamethylhexasiloxane, while the contribution of EDCs reached 7.7% and was due to nonylphenol diethoxylate and nonylphenol monoethoxylate. All the other classes of EOCs (PhCs, IDs, BTRs, BTHs) had minimal contribution to $RQ_{soil,\ mix}$, equal to 0.3%.

As the above approach was based on the maximum concentration values, a more reasonable scenario was applied, using the average concentrations of the studied compounds (Annex, Table S11). According to this approach, $RQ_{soil,\ mix}$ was equal to 209; lower than the one calculated *via* the worst case scenario, but still far above 1, indicating the possible environmental threat due to the presence of the mixture of the EOCs in sludge-amended soil. As far as the contribution of the SLXs to the mixture's toxicity is concerned, it is higher than the one obtained *via* the worst case scenario (99%), while the contribution of EDCs (due to nonylphenol diethoxylate and nonylphenol monoethoxylate) was lower, reaching a rate equal to 0.9% (Fig. 24b).

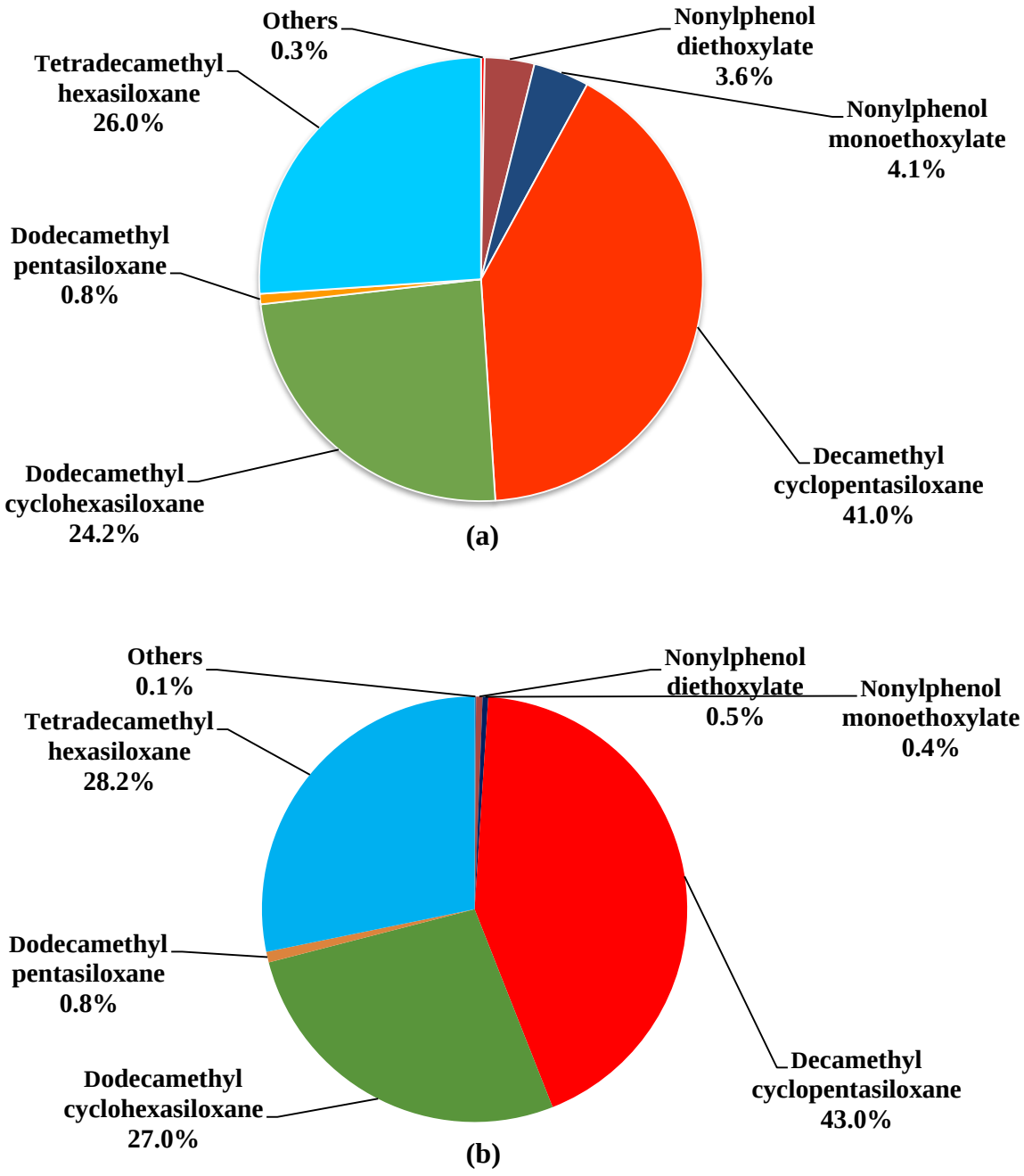


Figure 24: Contribution (%) of several emerging contaminants to $RQ_{soil,mix}$ calculated for sludge-amended soil using (a) maximum concentration values and (b) average concentration values ($RQ_{soil,mix}$ calculation was based on baseline toxicity predicted by ECOSAR program)

3.2.7. Suggestions and limitations

The basic legislative text concerning the sludge management in EU is Sewage Sludge Directive 86/278/EEC (EEC, 1986). Concerning EOCs, apart from NP, which has been included in some national legislation (Austria, Belgium, Denmark, France, Germany, Sweden, Czech Republic and Slovenia), no limit values have been set for them. The results of this study indicate that there is an urgent need for the countries that reuse sewage sludge for agricultural purposes to include some EOCs such as SLXs, nonylphenols, TCS, caffeine and ofloxacin in their national monitoring programs.

On the other hand, the quantitative approach taken in this study is tempting in its simplicity, but it should only be seen as a first attempt to estimate the ecological threat for the terrestrial organisms, due to the existence of EOCs in the terrestrial environment. A number of uncertainties are included, as the use of aquatic toxicity due to the lack of terrestrial experimental toxicity data for most of the EOCs. Moreover, the application of ECOSAR is adding to the uncertainties. Thus, more studies should be carried out to provide more data on the concentration levels and the toxic effects of EOCs in sludge-amended soils. The aforementioned actions may lead the EU and countries concerned to update current legislations.

3.3. ERA of TCS released from STPs in European rivers using a combination of RQ method and MC simulation

3.3.1. Occurrence of TCS in the European STPs

To date, there is a considerable number of published articles in scientific journals (68) concerning the presence of TCS in European STP treated wastewater. However, most of these studies refer to a small number of countries: namely, Spain (27 papers), Greece (12 papers), UK (8 papers), Germany (6 papers), France (4 papers) and Sweden (3 papers), while such studies have also been conducted once or twice in Italy, Poland, Switzerland, Czech Republic, Cyprus, Denmark, Norway, Portugal and Romania (Figure 25). No data is available for the remaining 35 European countries, including 6 ‘old’ Member States (Austria, Belgium, Finland, Ireland, Luxembourg and Netherlands) and 9 ‘new’ Member States (Bulgaria, Croatia, Estonia, Hungary, Latvia, Lithuania, Malta, Slovakia and Slovenia).

Regarding the type of available data, 52 out of the 68 articles (75%) report the mean concentration values of TCS in studied STPs, while detailed information for the minimum, maximum, average and median concentrations is given in 18 papers (26%). As many of the articles contain data for more than one STP, a total of 137 mean and 85 maximum concentration values have been reported for different European STPs, while the total number of STPs that has been studied for TCS occurrence is 349. Regarding the sampling procedure that has been followed in these studies, 45 (65%) and 51 (74%) papers contain data for the number and the type of samples (grab or composite), respectively. Specifically, the number of samples collected and analyzed per STP range from 1 to 48, while for the majority of the STPs (78%) they are less than or equal to 3 (Annex, Table S12). Furthermore, in 208 out of the 349 studied STPs (60%) the collected samples are grab; in 101 STPs (29%) they are composite; whereas for the remainder (11%) no relevant information is available. The type of final treatment provided in each studied STP is reported in 59 papers (86%). Most of the articles contain data for effluents originating from secondary treatment (219 out of 349 STPs) or tertiary treatment (107 out of 349 STPs), while less data is available for STPs with primary treatment (9 out of 349 STPs). The majority of secondary-treatment plants are equipped with activated sludge process, while tertiary treatment usually included coagulation, flocculation, filtration, and disinfection by UV. In a few tertiary STPs, the treatment includes chlorination, reverse osmosis, membrane bioreactors, trickling filters, rotating biological contactors, reed beds, powered activated carbon and ozonation; while the secondary step mainly included activated sludge process. As regards additional information concerning the operation of the STPs (capacity, flow rate, hydraulic retention time and sludge retention time), only a marginal number of studies include relevant data.

In Figure 26, box-and-whisker plots of MEC_{mean} and MEC_{max} values for TCS in European countries are shown. The lowest mean concentration value has been measured in Sweden (2.2 ng L^{-1}) and the highest one in Spain (47,800 ng L^{-1}), while the corresponding maximum values were 11 ng L^{-1} (UK) and 269,000 ng L^{-1} (Spain). The extremely high concentration values presented in Figure 26 for Spain (out and far-out values) originated from STPs that applied solely primary treatment.

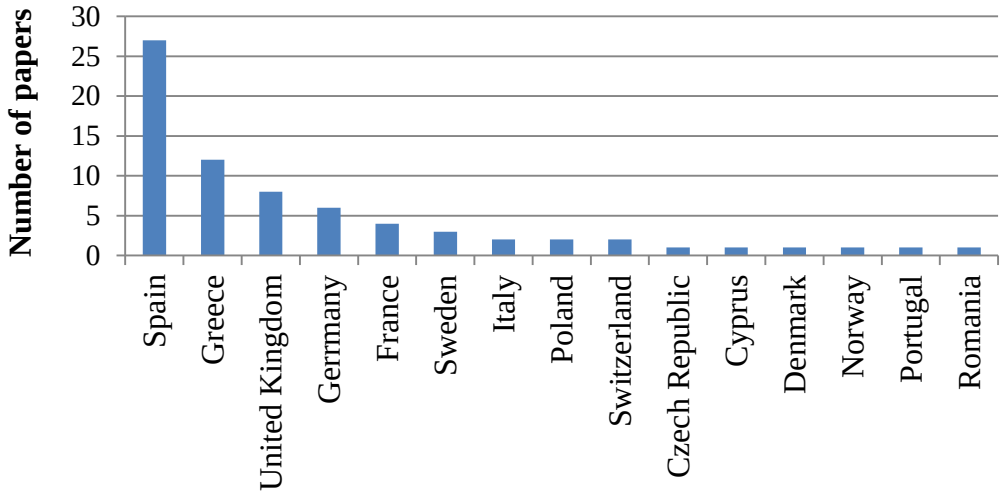


Figure 25: Number of papers published in scientific journals between 2002 and 2015 concerning the presence of TCS in treated wastewater originated from European Sewage Treatment Plants (STPs).

In order to determine whether there are any statistically significant differences between the effluents' concentrations determined in different countries, a one-way analysis of variance (Anova) was conducted using IBM SPSS Statistics Base 24. A similar analysis was carried out to investigate the statistically significant differences between TCS concentration in treated wastewater of STPs offering primary, secondary or tertiary treatment. According to the results for both average and maximum concentrations, at the 95% significance level there is no difference between the means of TCS concentration values among studied European countries. This conclusion was foreseeable, as TCS is contained in everyday products that are widely consumed in Europe and, so far, no specific ban exists on national level for TCS use. Comparison with the non-European literature showed that the concentration levels of TCS in European STPs were in most cases in agreement with those reported for other countries, worldwide. Specifically, monitoring studies of STPs of the USA (Barber et al., 2015), Canada (Arlos et al., 2015), Australia (Kookana et al., 2013), Japan (Nakada et al., 2006) and China (Chen et al., 2016) have reported mean and maximum TCS concentrations ranging from 10 to 600 ng L^{-1} and from 60 to 1400 ng L^{-1}, respectively.

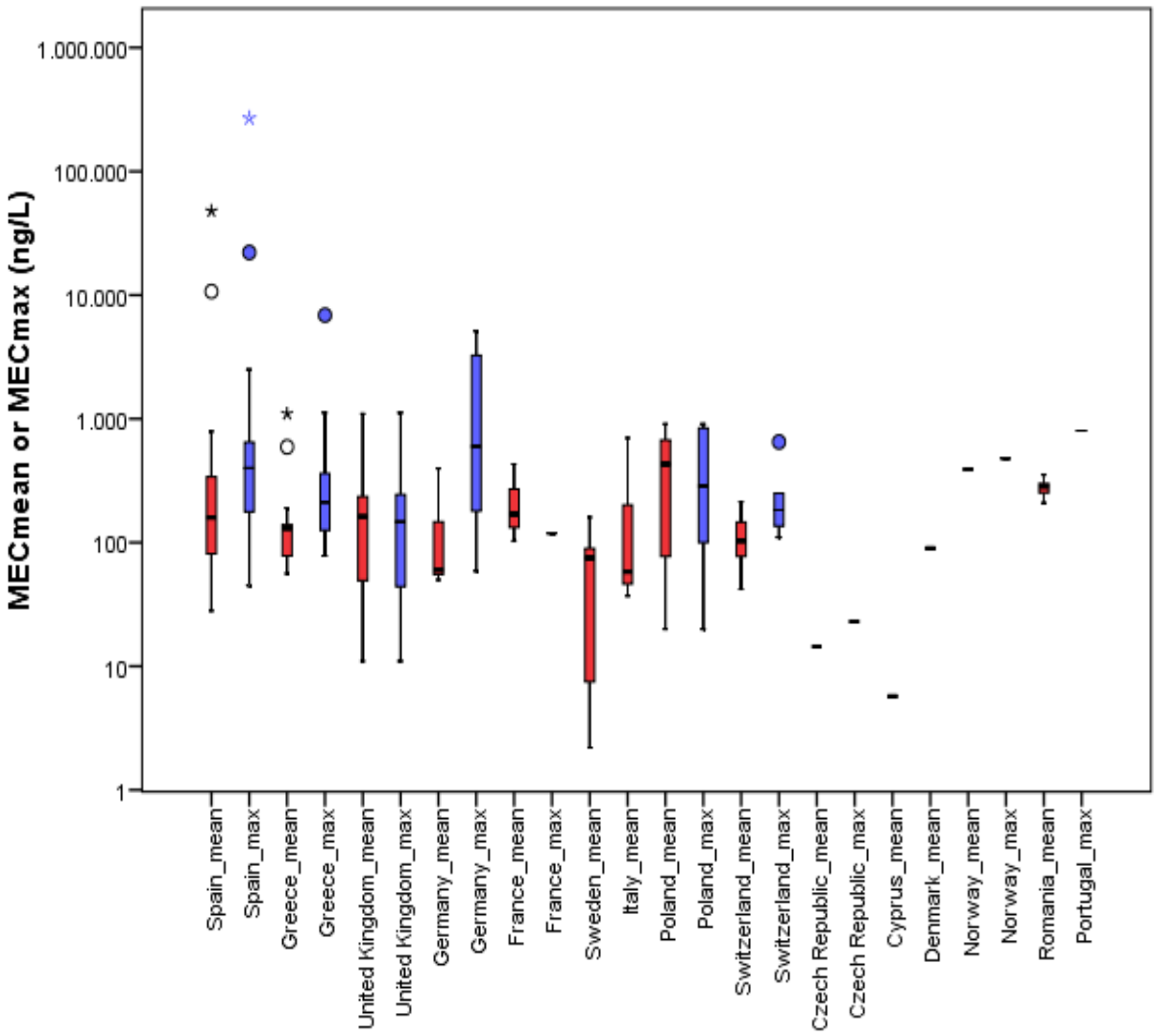


Figure 26: Box-and-whisker plots of MEC_{mean} and MEC_{max} values of TCS in treated wastewater from European STPs. Data is available for 14 and 10 countries respectively. The boxes represent the interquartile (IQ) range which contains the middle 50% of the records. The horizontal black line in the boxes represents the median value; the lines extending from each box show the highest and lowest MEC_{mean} and MEC_{max} values which are no greater than 1.5 times IQ range. The symbols ^{o} and * represent the "out" (between 1.5 and 3 times the IQ range) and "far out" or "extreme" (more than 3 times the IQ range) values, respectively

Regarding the role of sewage treatment type on TCS concentrations, the MEC_{mean} and MEC_{max} in treated wastewater are presented for different types of treatment in Figure 27. The MEC_{mean} values range from 81 ng L^{-1} to 47,800 ng L^{-1} for the STPs which

apply primary treatment, from 16 ng L^{-1} to 1,100 ng L^{-1} for those applying secondary treatment, while the corresponding concentration values are 2.2 ng L^{-1} and 650 ng L^{-1} for the tertiary European STPs. As far as the MEC_{max} values are concerned, the concentration of TCS in effluents range from 480 to 269,000 ng L^{-1}, 18 to 6,800 ng L^{-1} and 11 to 1,100 ng L^{-1} for primary, secondary and tertiary STPs, respectively. The analysis of variances indicated that at the 95% significance level higher TCS concentrations were observed after primary treatment compared to secondary or tertiary treatment. On the other hand, there was no difference between the means of TCS effluent concentration values among the European STPs applying secondary and tertiary treatment. The limited number of available papers reporting the exact type of applied tertiary treatment processes does not allow firm conclusions for possible differences between different tertiary processes (e.g. ozonation, powered activated carbon, membranes) on TCS removal. Further data is needed from full-scale STPs to evaluate the performance of different tertiary processes on TCS removal.

3.3.2. Acute and chronic aquatic toxicity data of TCS

According to the results of the literature survey, 24 peer-reviewed papers collated data on acute and chronic toxicity values of TCS on the aquatic organisms (algae, *Daphnia magna* and fish). Literature data referring to the species of the organisms, the type and the duration of the assays and the dose descriptor values (EC50/LC50 and NOEC), is reported in Annex (Table S13).

Concerning long-term toxicity, there is scarce data in the literature, as NOEC values have been reported only in 4 out of the 24 papers - reporting 1, 1 and 8 NOEC values for algae, D*aphnia magna* and fish, respectively. On the other hand, there is more data for the short-term toxicity of TCS. Specifically, 23 out of the 24 papers presented EC50/LC50 values for algae, *Daphnia magna* and fish; whereas the numbers of the dose descriptor values reported were 24, 13 and 24, respectively.

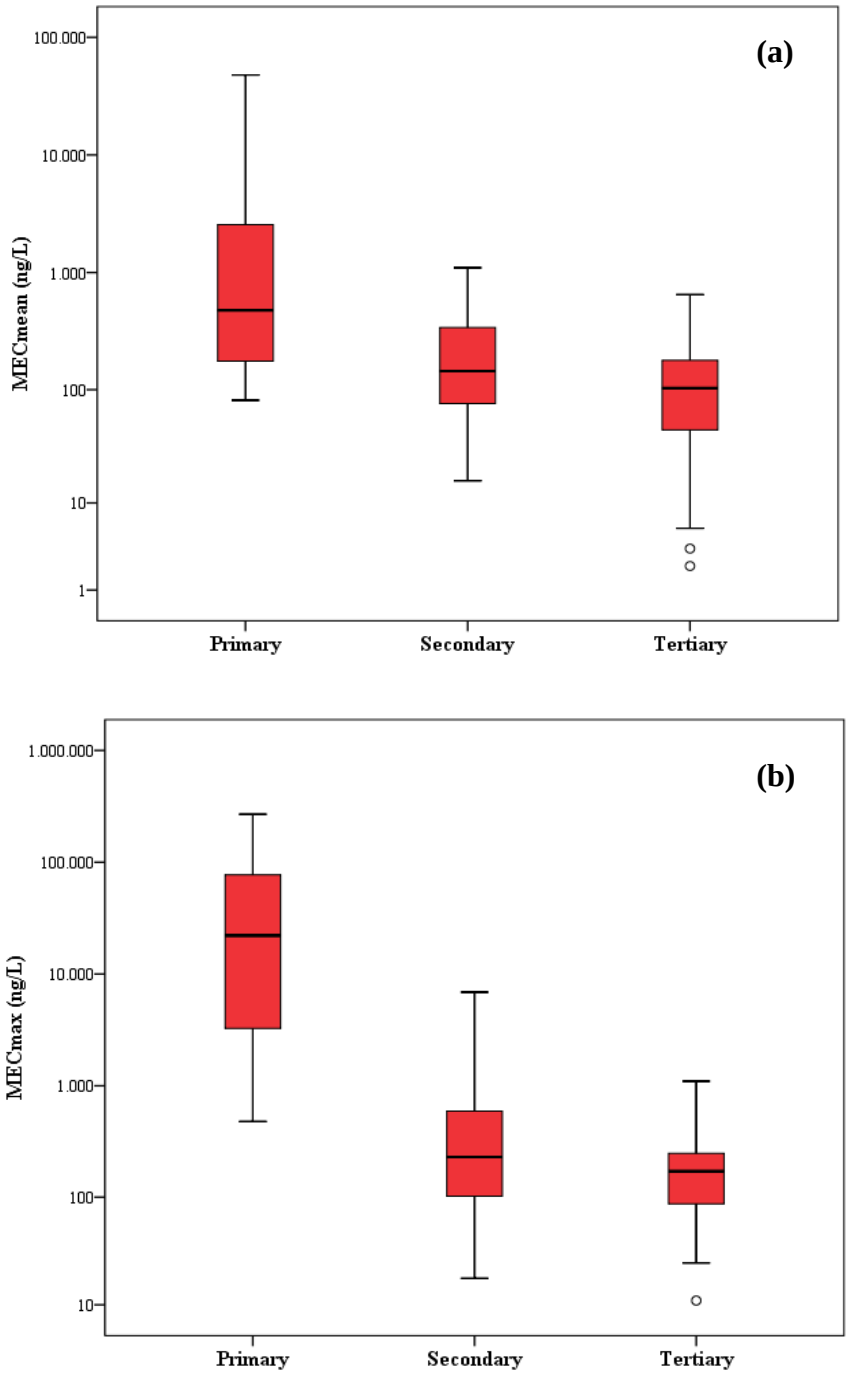


Figure 27: Box-and-whisker plots of (a) MEC_{mean} and (b) MEC_{max} values of TCS in primary, secondary and tertiary treated wastewater obtained from European STPs. The boxes represent the interquartile (IQ) range which contains the middle 50% of the records. The horizontal black line in the boxes represents the median value; the lines extending from each box show the highest and lowest MEC_{mean} and MEC_{max} values which are no greater than 1.5 times IQ range. The symbol ^{o} represents the "out" (between 1.5 and 3 times the IQ range) values

The most sensitive aquatic organisms were algae, presenting the lowest EC50/LC50 values (Figure 28, Annex, Table S13). Additionally, the group with the highest EC50/LC50 value variation was algae; the lowest EC50/LC50 value was reported for *Pseudokirchneriella subcapitata* (0.53 μg L^{-1}), while the highest for *Nitzschiapalea* (430 μg L^{-1}). Regarding daphnids and fish, EC50/LC50 values range from 52 to 857 μg L^{-1} and from 45 to 1,839 μg L^{-1}, respectively.

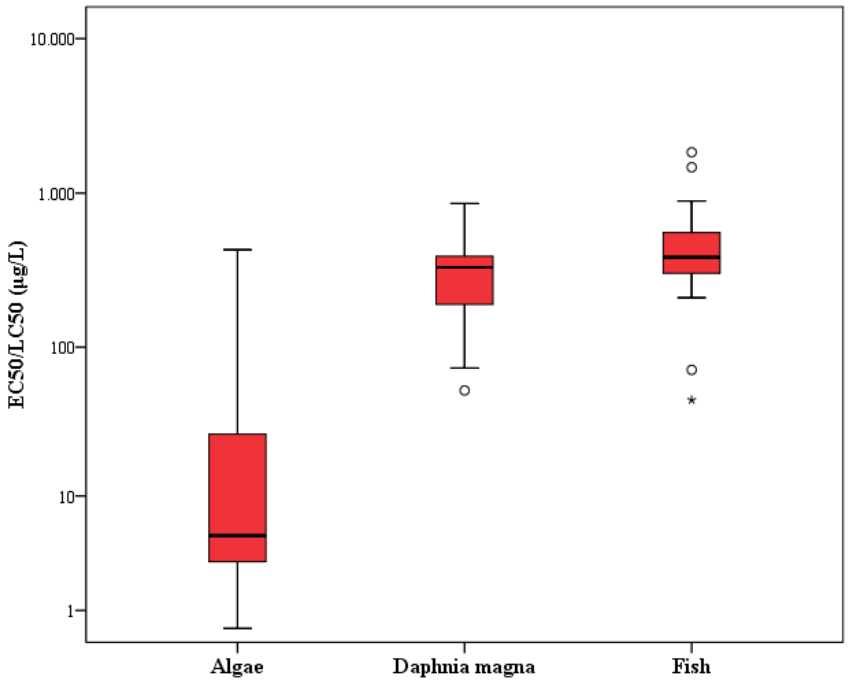


Figure 28: Box-and-whisker plots of EC50/LC50 values of TCS for algae, D*aphnia magna* and fish. The boxes represent the interquartile (IQ) range which contains the middle 50% of the records. The horizontal black line in the boxes represents the median value; the lines extending from each box show the highest and lowest MEC_{mean} and MEC_{max} values which are no greater than 1.5 times IQ range. The symbols ^{o} and * represent the "out" (between 1.5 and 3 times the IQ range) and "far out" or "extreme" (more than 3 times the IQ range) values, respectively

As presented in Annex (Table S13), there are significant differences among the calculated EC50/LC50 values, due to the diverse experimental conditions (pH, duration), the different types of assay and the variety of the species used for the experiments. Furthermore, considerable differences exist even in cases where the same

species are used and/or the same experimental conditions are applied. For instance, the reported LC50 values for 48-h mortality test of *Daphna magna* range from 190 to 390 μg L^{-1}, while the EC50 value for 48-h immobilization test of the same organism range from 52 to 856.8 μg L^{-1} (Annex, Table S13).

3.3.3. Environmental risk characterization

To estimate the possible ecological threat associated with the presence of TCS to STP effluents and river water, RQ values were calculated *via* MC simulation for mean and maximum concentrations reported in the literature and different groups of organisms. The probability distributions of concentration and acute toxicity data were analyzed *via* the Kolmogorov-Smirnov test. Our null hypotheses were that the MEC values, as well as the EC50/LC50 values for the target groups of organisms were individually described by lognormal distributions. The Kolmogorov-Smirnov test failed to reject all aforementioned null hypotheses for lognormality at the 95% confidence level. Even though sample sizes for each of the tests are rather small, the hypotheses that all datasets belong to lognormal distributions are relatively robust. The p-values of the Kolmogorov-Smirnov tests are 0.51, 0.41, 0.81 and 0.33 for MEC and EC50/LC50 for algae, *Daphnia magna* and fish, respectively. Therefore, for the MC simulations we draw our random samples of MEC and EC50/LC50 from lognormal distributions fitting the data collected from the literature. Since the Anova test indicated that there is no statistically significant difference between the means of concentration values among European countries, all available MEC values were lumped together in the derivation of their lognormal distribution and in the assessment of the ecological risk of TCS in the aquatic environment.

The descriptive statistics obtained for the RQ_{mean} and RQ_{max} values in treated wastewater and rivers with different DFs are listed in Tables 8 and 9. As shown in Table 8, for algae, the mean value of RQ_{mean} is higher than 1 in rivers with DFs equal to 2 and 10 (10 and 2.0, respectively), indicating a risk for the specific organisms in surface water; whereas it is lower than 1 in rivers with DF higher or equal to 100. For the other two groups of aquatic organisms the mean values of RQ_{mean} are below 1 in all rivers, regardless of the DF. Similar results were obtained when MEC_{max} values are used (Table 9), with the difference that the RQ_{max} are higher, reaching for algae the values of 27 and 5.4 in rivers with DF equal to 2 and 10, respectively. We should note

that in both Tables 8 and 9 all values for a specific DF should equal the corresponding value for the effluents, divided by this DF. However, because of the randomness introduced by the MC method, values may stray from the result based solely on the effluent value.

The maximum RQ values derived from MC simulations are not statistically robust and change with each MC run, because they correspond to a sample size of one. Therefore, in order to quantify the possible large risk cases, we report the 95th percentile value of RQ instead of its maximum, as other authors have also suggested (García-Santiago et al., 2016). As observed in Table 8, the 95th percentile RQ_{mean} values in effluents for algae, *Daphnia magna* and fish are 64, 0.62 and 0.42, respectively, while in rivers with DFs equal to 2 and 10 the corresponding 95th percentile RQ_{mean} values for algae are still above 1 (32 and 6.4, respectively). As expected, the most threatened aquatic organisms are algae; whereas no threat seems to occur for the other groups of aquatic organisms in rivers, regardless the DFs' value. Concerning the 95th percentile RQ_{max} values, algae seem to face a risk even in rivers with high flows. Specifically, the 95th percentile RQ_{max} values for algae are 74, 15 and 1.5 in rivers with DFs equal to 2, 10 and 100, respectively (Table 9). On the other hand, for *Daphnia magna* and fish the 95th percentile RQ_{max} values are lower than 1 in all rivers, regardless of the DF used.

Quantitative sensitivity analysis was performed to define the contribution of MEC and EC50/LC50 values to the risk assessment, using the Spearman rank correlation coefficient, because of the nonlinearity between RQ, MEC and EC50/LC50 in Equations 14 and 15. According to the results, for the groups of *Daphnia magna* and fish, the MEC value was the most influential variable, with an average contribution to output variance around 75%, compared with only around 25% introduced by the EC50/LC50 variance. On the contrary, for algae, approximately 65% of the variance in the estimated RQ is associated with the toxicity values (EC50/LC50).

Table 8

Descriptive statistics of TCS risk quotients, RQ_{mean} (mean TCS concentrations were used) for STPs' effluents and river water with different Dilution Factors (DF). The 50% column corresponds to the 50th percentile (median RQ value); while the 75% and 95% columns correspond to the 75th and 95th percentiles, respectively. Calculations were conducted for three groups of aquatic organisms.

Aquatic organisms	**Mean**	**SD**	**50%**	**75%**	**95%**
Effluents (DF = 1)					
Algae	20	270	1.5	6.9	64
Daphnia magna	0.16	0.46	0.053	0.15	0.62
Fish	0.11	0.31	0.036	0.10	0.42
Rivers (DF = 2)					
Algae	10	120	0.73	3.4	32
Daphnia magna	0.081	0.23	0.027	0.073	0.31
Fish	0.055	0.16	0.018	0.049	0.21
Rivers (DF = 10)					
Algae	2.0	28	0.15	0.69	6.4
Daphnia magna	0.016	0.046	$5.4 \cdot 10^{-3}$	0.015	0.062
Fish	0.011	0.031	$3.6 \cdot 10^{-3}$	$9.8 \cdot 10^{-3}$	0.042
Rivers (DF = 100)					
Algae	0.20	2.7	0.015	0.069	0.64
Daphnia magna	$1.6 \cdot 10^{-3}$	$4.6 \cdot 10^{-3}$	$5.4 \cdot 10^{-4}$	$1.5 \cdot 10^{-3}$	$6.2 \cdot 10^{-3}$
Fish	$1.1 \cdot 10^{-3}$	$3.1 \cdot 10^{-3}$	$3.6 \cdot 10^{-4}$	$9.8 \cdot 10^{-4}$	$4.2 \cdot 10^{-3}$
Rivers (DF = 1000)					
Algae	0.020	0.28	$1.5 \cdot 10^{-3}$	$6.9 \cdot 10^{-3}$	0.064
Daphnia magna	$1.6 \cdot 10^{-4}$	$4.6 \cdot 10^{-4}$	$5.4 \cdot 10^{-5}$	$1.5 \cdot 10^{-4}$	$6.2 \cdot 10^{-4}$
Fish	$1.1 \cdot 10^{-4}$	$3.1 \cdot 10^{-4}$	$3.6 \cdot 10^{-5}$	$9.8 \cdot 10^{-5}$	$4.2 \cdot 10^{-4}$

Table 9

Descriptive statistics of TCS risk quotients, RQ_{max} (maximum TCS concentration were used) for STPs' effluents and river water with different Dilution Factors (DF). The 50% column corresponds to the 50th percentile (median RQ value); while the 75% and 95% columns correspond to the 75th and 95th percentiles, respectively. Calculations were conducted for three groups of aquatic organisms.

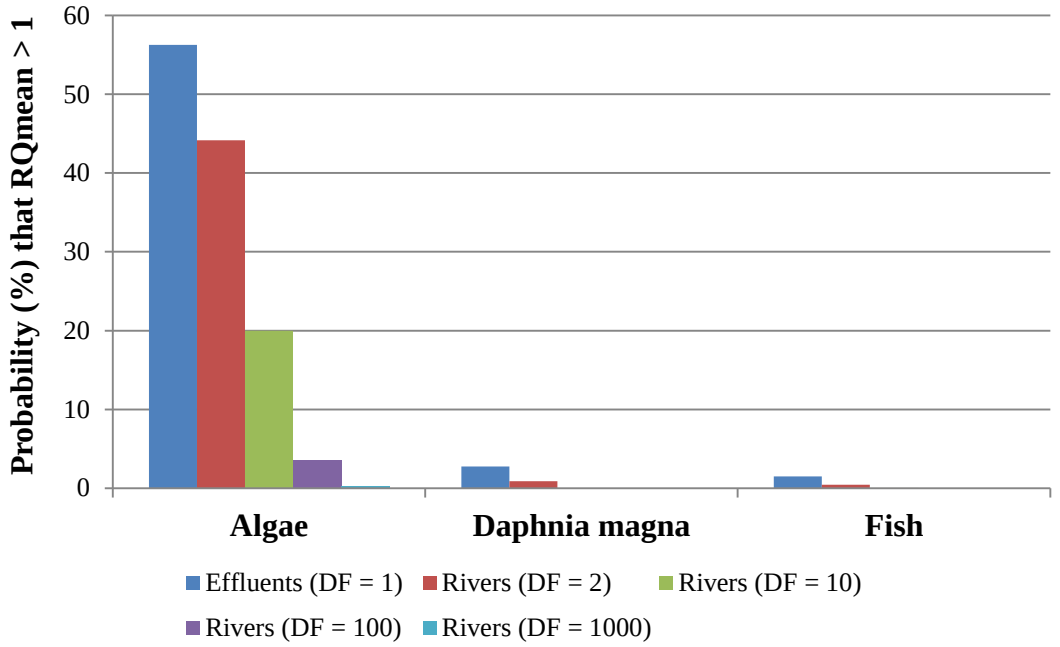
Aquatic organisms	**Mean**	**SD**	**50%**	**75%**	**95%**
Effluents (DF = 1)					
Algae	54	980	2.6	14	150
Daphnia magna	0.42	1.8	0.096	0.31	1.6
Fish	0.29	1.2	0.064	0.21	1.1
Rivers (DF = 2)					
Algae	27	490	1.3	6.8	74
Daphnia magna	0.21	0.90	0.048	0.15	0.82
Fish	0.14	0.61	0.032	0.10	0.55
Rivers (DF = 10)					
Algae	5.4	94	0.26	1.4	15
Daphnia magna	0.042	0.19	$9.6 \cdot 10^{-3}$	0.031	0.16
Fish	0.029	0.12	$6.4 \cdot 10^{-3}$	0.021	0.11
Rivers (DF = 100)					
Algae	0.54	10	0.026	0.14	1.5
Daphnia magna	$4.2 \cdot 10^{-3}$	0.018	$9.6 \cdot 10^{-4}$	$3.1 \cdot 10^{-3}$	0.016
Fish	$2.9 \cdot 10^{-3}$	0.012	$6.4 \cdot 10^{-4}$	$2.1 \cdot 10^{-3}$	0.011
Rivers (DF = 1000)					
Algae	0.054	1.1	$2.6 \cdot 10^{-3}$	0.014	0.15
Daphnia magna	$4.2 \cdot 10^{-4}$	$1.8 \cdot 10^{-3}$	$9.6 \cdot 10^{-5}$	$3.1 \cdot 10^{-4}$	$1.6 \cdot 10^{-3}$
Fish	$2.9 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	$6.4 \cdot 10^{-5}$	$2.1 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$

The probabilities of RQ_{mean} and RQ_{max} exceeding the acceptable risk value 1 were calculated from the MC simulations and are presented in Figure 29, for algae, *Daphnia magna* and fish. The probability that RQ_{mean} and RQ_{max} for algae is higher than 1, ranges from 0.2% and 0.8%, respectively, in rivers with DF = 1000, to 45% and 54%, in rivers with DF = 2. The corresponding probabilities in rivers with DFs equal to 10, 100 and 1000 are practically 0% for *Daphnia magna* and fish, while in rivers with DF equal to 2 the probabilities for these organisms range from 0.7% to 4%, for RQ_{mean} and RQ_{max}, respectively. Taking into consideration that a significant number of European STPs release their effluents in streams with DFs lower than 10 (Link et al., 2017), the aforementioned results indicate that TCS may pose a serious ecological risk to the aquatic ecosystems and efforts should be made to reduce its concentration levels in treated wastewater and receiving surface water.

3.3.4. Future requirements

An extended discussion is ongoing in the scientific community concerning the need for STPs upgrading in order to achieve efficient micropollutant removal and several papers have studied the mass balance of TCS in conventional and full-scale wastewater treatment systems (Heidler and Halden, 2007; Lozano et al., 2013). However, no comparative data is available from full-scale systems for TCS removal using different secondary and tertiary treatment processes.

Nowadays, the basic legislative text establishing the framework for EU action in the field of water policy is Directive 2000/60/EC. The list of priority substances, as finally adopted by Decision 2455/2001/EC and Directive 2013/39/EC includes 45 individual or groups of organic substances. According to the recent Directive 2013/39/EC, by September 14, 2014, the Member States had to develop monitoring lists for those pollutants where there was evidence indicating that they may pose a significant risk to the aquatic environment. Although in the aforementioned Directives no mention of TCS has been made, the results of the current study indicate that TCS seems to pose a serious environmental risk to small rivers. Monitoring programs should be expanded and a comprehensive overview of the results presented in previous studies should also be taken in to account to decide whether the specific micropollutant should be included in the European relevant legislation.



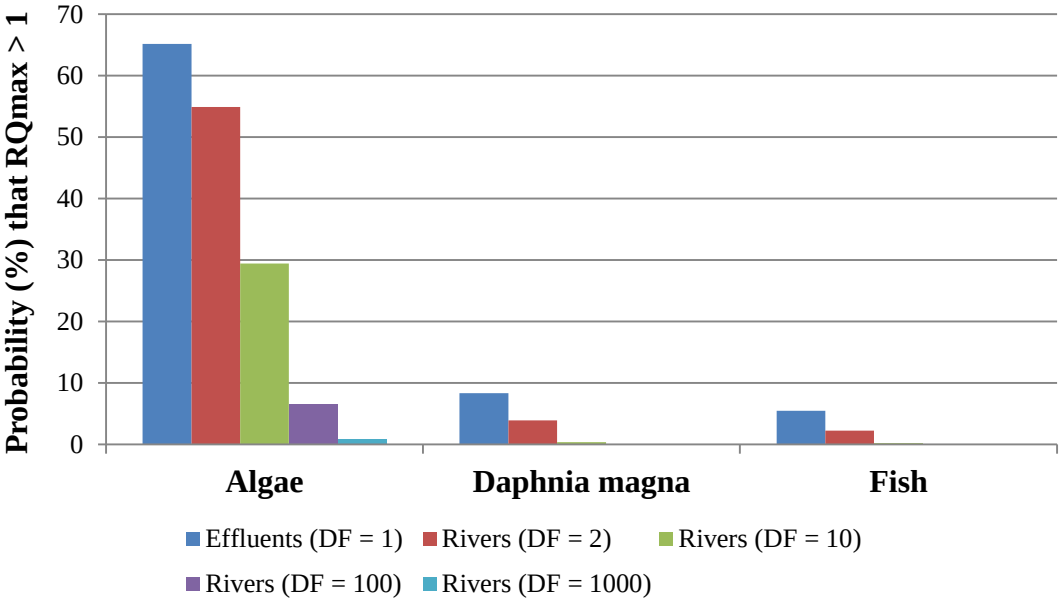


Figure 29: Probabilities (%) that (a) RQ_{mean} and (b) RQ_{max} exceed 1 in river waters for different Dilution Factors (DF). Results for algae, *Daphnia magna* and fish.

4. Conclusions and future research

4.1. Conclusions

This study developed and applied a procedure for investigating the environmental risk associated with the occurrence of emerging organic micropollutants in STPs. Specifically: i) the possible risk for the aquatic environment due to the existence of EOCs in treated wastewater, on country level, was estimated, choosing Greece as a case study, ii) the potential environmental risk for the terrestrial environment from the disposal of sewage sludge containing EOCs in soil was assessed, selecting Greece as a case study and iii) a probalistic risk assessment of TCS, originating from STPs' effluents, in the European aquatic environment was conducted. The most important results of this study are briefly presented below:

ERA of EOCs in the Greek aquatic environment

- Information on a total of 207 EOCs was available for the treated wastewater in Greece. The majority of the analyzed compounds were PhCs, IDs and EDCs, while few data were available for PFCs, BTRs, BTHs, SLXs and ASs.
- Maximum concentration levels of detected micropollutants ranged from less than 1 ng L^{-1} (PhCs) to some tens of μg L^{-1} (ASs).
- The concentration levels of EOCs in Greek STPs were in most cases in line with those of other countries.
- For 105 out of the 175 detected compounds, there was no experimental toxicity data in the literature; EC50 or LC50 values were found for 66 of them *via* ECOSAR. The classes of EOCs with limited experimental toxicity data were IDs, PFCs, BTRs, ASs and SLXs.
- RQ higher than 1 were calculated for 34 compounds in secondary treated wastewater.
- The rivers with DF equal to 2 and 3 presented the highest possibility for ecological threat due to the presence of 28 and 25 EOCs, respectively; whereas a possible threat was also observed for 21 compounds and DF lower or equal to 101. However, a possible ecological threat cannot be excluded even for rivers with high dilution factors (up to 2388).

- EDCs and SLXs presented the highest risk of all EOCs in both wastewater and rivers.
- TCS (in algae) and NP (in fish) had the highest RQs among EDCs, tetradecamethylhexasiloxane and decamethylcyclopentasiloxane (in fish) had the highest RQs among SLXs, while caffeine (in algae) had the highest RQ of all studied PhCs.
- TCS (in algae), tetradecamethylhexasiloxane and decamethylcyclopentasiloxane (in fish) presented RQ > 1 for all studied rivers, indicating a possible ecological risk regardless of wastewater dilution.
- The mixture of the micropollutants seems to exhibit a serious threat to aquatic organisms, as it shows an RQ_{mix} value far above 1. The class of emerging contaminants that had the highest contribution to the mixture toxicity, in both wastewater and rivers, was EDCs.

ERA of EOCs in the Greek terrestrial environment

- A total of 99 EOCs have been detected in sewage sludge in Greece. The majority of the analyzed compounds were PhCs, EDCs, PFCs and SLXs while few data are available for IDs, BTRs, BTHs.
- Maximum concentrations ranged from less than 10 ng g^{-1} dw (octylphenol monoethoxylate and some PFCs) to some tens of μg g^{-1} dw (nonylphenolic EDCs).
- For most EOCs, the concentrations detected in Greek sewage sludge samples were similar or lower than those reported in the literature for other countries. Higher concentrations than those in the literature were found for few compounds, namely naproxen, fluoxetine and BPA, indicating their greater use in Greece.
- There is a lack of terrestrial toxicity data for most EOCs, as experimental EC50/LC50 values were available only for 18 out of the 99 target compounds. For 23 micropollutants, acute toxicity values in earthworms were also predicted using ECOSAR model.
- EDCs and SLXs presented the highest risk of all EOCs in sludge-amended soil.
- TCS seems to pose a serious environmental hazard to the terrestrial organisms, as both $RQ_{soil,aquatic}$ and $RQ_{soil,terrestrial}$ values exceeded 1.
- The highest $RQ_{soil,aquatic}$ were calculated *via* the worst-case scenario for caffeine, ofloxacin, tetradecamethylhexasiloxane and decamethylcyclopentasiloxane.

- All SLXs and the PhCs caffeine and ofloxacin presented $RQ_{soil,\ aquatic}$ higher than 1, despite the daily variation in concentrations and the differences in plant capacity and treatment processes.
- The mixture of the micropollutants seems to exhibit a serious threat to terrestrial species, as it shows an $RQ_{soil,\ mix}$ value far above 1. The class of EOCs that had the highest contribution to the mixture toxicity was SLXs.

Probabilistic risk assessment of TCS in the European aquatic environment

- TCS monitoring data in European STP treated wastewater was available for 15 out of the 50 European countries.
- At the 95% significance level, there was no statistical difference in TCS concentrations determined in different European countries.
- Higher TCS concentrations in treated wastewater were observed after primary treatment, whereas, at the 95% significance level, there was no difference in STPs applying secondary and tertiary treatment.
- There is scarce experimental chronic aquatic toxicity data in the literature; whereas there is more data for the short-term toxicity of TCS.
- There are significant differences among the calculated EC50/LC50 values, due to the diverse experimental conditions (pH, duration), the different types of assay and the variety of the species used for the experiments. Furthermore, considerable differences exist even in cases where the same species are used and/or the same experimental conditions are applied.
- The most threatened aquatic organisms from TCS seem to be algae, while the major risk is expected for rivers with DFs lower or equal to 10.
- For algae, the mean values of RQ_{mean} and RQ_{max} were higher than 1 in rivers with DFs equal to 2 and 10.
- The 95th percentile RQ_{mean} values for algae were above 1 in rivers with DFs equal to 2 and 10, while, concerning the 95th percentile RQ_{max} values, algae seemed to face a risk even in rivers with high flows (DFs up to 100).
- The probability that RQ_{mean} and RQ_{max} for algae is higher than 1, ranges from 0.2% and 0.8%, respectively, in rivers with DF = 1000, to 45% and 54%, in rivers with DF = 2. In rivers with DF equal to 2 the probabilities for *Daphnia magna* and fish range from 0.7% to 4%, for RQ_{mean} and RQ_{max}, respectively.

4.2. Future research

Based on the results of this study and the literature review on occurrence and toxicity of EOCs some points for future research are proposed.

As the literature review indicated, there is a lack of monitoring data for specific classes of EOCs (IDs, PFCs, BTRs, BTHs, SLXs and ASs) in treated wastewater and sewage sludge. Especially for TCS, the data is mainly derived from a limited number of countries (Spain, Greece, UK, Germany, France and Sweden), while too little or no data is available for the remaining European countries. Thus, further work should be done on the analysis of EOCs concerning their occurrence in treated wastewater and sewage sludge, in order to underpin certainty over the analytical results and, therefore, to increase data for the exposure assessment step of ERA.

The literature review revealed that there is a lack of experimental aquatic and terrestrial toxicity data for a significant number of EOCs and especially for those belonging to the groups of IDs, PFCs, BTRs, SLXs and ASs. Particularly for the terrestrial environment, this deficiency poses a serious obstacle to the credibility of risk assessment results. Therefore, experiments should be conducted in order to calculate more EOCs' aquatic and terrestrial acute toxicity (EC50 and LC50) values. Furthermore, more research should be carried out, related to the chronic toxicity of the target compounds, as well as their mixture toxicity on the terrestrial and aquatic organisms of different trophic levels.

There is also an urgent need for studies aiming to investigate EOCs' mode of toxic action on aquatic and terrestrial organisms, as well as for studies concerning the effects of by-products and metabolites of emerging contaminants produced during wastewater treatment. Moreover, time-dependent processes, such as degradation and transportation of EOCs in sludge and soil should be investigated, as the aforementioned actions will provide more data on toxic effects of EOCs in sludge-amended soils.

Regarding TCS, the lack of enough chronic aquatic experimental toxicity data constitutes a serious obstacle to a more precise assessment of the risk associated with the specific compound in aquatic ecosystems and future efforts on the elaboration of such experiments should be made. Moreover, the absolute scarcity of terrestrial toxicity data makes it entirely impossible to conduct a reliable risk assessment

concerning the specific micropollutant for the terrestrial organisms. Thus, further research is needed to focus on the terrestrial toxicity data, so more accurate results can be exported on the toxicity of the particular substance. In addition, further study on the transformation patterns of TCS during wastewater treatment is necessary, as its transformation byproducts may also have toxic effects on the biota.

Finally, the quantitative approach of RQ calculation applied in paragraphs 3.1 and 3.2 of this study should be seen as the first step in screening the ecological threat for the aquatic and terrestrial environment due to the existence of a great number of EOCs in STPs and arrive at a smaller number of compounds that need deeper investigation. Additional research using PRA methods should be carried out for those compounds that seem to pose an environment risk to aquatic and terrestrial environment, in order to have more precise results concerning possible risks. Specifically, according to the results of the present study, apart from TCS, other candidate compounds for future PRA could be NP, tetradecamethylhexasiloxane, decamethylcyclopentasiloxane and caffeine. Moreover, another PRA approach, for example, Species Sensitivity Distribution (SSD), could be applied for TCS at European level, to compare its results with those of the present study.

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Annex

Table S1

Classes of emerging organic contaminants that have been detected in Greek Sewage Treatment Plants (STPs). Information is also given for the type and number of samples, the period of sampling and the analyzed phase (dissolved/particulate).

STPs	Number of analyzed compounds	Number of samples	Type of samples	Analyzed phase	Years of sampling	References
Pharmaceuticals						
Athens, Thessaloniki, Ioannina, Heraklion	5	11	NR[1]	dissolved	NR	Koutsouba et al, 2003
Athens, Halkida, Korinthos	7	6	composite	dissolved	2005-2006	Botitsi et al, 2007
Hania	2	NR	grab	dissolved	2006-2007	Antoniou et al, 2009
Ioannina[2]	10	32	composite and grab	dissolved	2006-2007	Kosma et al, 2010
Mytilene[2]	4	9	grab	dissolved	2008	Samaras et al, 2010

Athens	4	1	composite	dissolved	2009	Samaras et al, 2011
Katerini	4	6	grab	dissolved	2008	Stasinakis et al, 2012
Agrinio	10	3	grab	dissolved	2007-2008	Stamatis and Konstantinou, 2013
Athens and Mytilene	4	9	composite and grab	dissolved and particulate	2009	Samaras et al, 2013
Ioannina, Arta, Preveza, Agrinio, Grevena, Kozani, Veroia[2]	17	32	composite and grab	dissolved	2010-2011	Kosma et al, 2014
Athens	4	14	composite	dissolved and particulate	2010-2011	Stasinakis et al, 2013
Athens	130	8	composite	dissolved and particulate	2012	Laboratory of Analytical Chemistry of the Department of Chemistry, National and Kapodistrian University of Athens

Illicit drugs						
Athens	20	8	composite	dissolved and particulate	2012	Laboratory of Analytical Chemistry of the Department of Chemistry, National and Kapodistrian University of Athens
Endocrine disrupting compounds						
Mytilene[2]	5	1	grab	dissolved	2006	Gatidou et al, 2007
Athens, Mytilene, Halkida[2]	5	30 (6 plants)	composite and grab	dissolved and particulate	2006	Stasinakis et al, 2008
Thessaloniki[3]	14	NR	grab	dissolved and	2005-2006	Arditsoglou and Voutsa, 2010

				particulate		
Kallikratia	13	5	grab	dissolved[4]	2007	Pothitou and Voutsa, 2008
Hania	8	NR	grab	dissolved	2006-2007	Antoniou et al, 2009
Ioannina[2]	1	32	composite and grab	dissolved	2006-2007	Kosma et al, 2010
Hania	4	NR	grab	dissolved	2008	Klontza et al, 2009
Katerini	4	6	grab	dissolved	2008	Stasinakis et al, 2012
Athens	5	1	composite	dissolved	2009	Samaras et al, 2011
Athens and Mytilene	5	9	composite and grab	dissolved and particulate	2009	Samaras et al, 2013
Agrinio	1	3	grab	dissolved	2007-2008	Stamatis and Konstantinou, 2013

Ioannina, Arta, Preveza, Agrinio, Grevena, Kozani, Veroia[2]	1	32	composite and grab	dissolved	2010-2011	Kosma et al, 2014
Athens	5	14	composite	dissolved and particulate	2010-2011	Stasinakis et al, 2013
Benzotriazoles						
Athens	4	14	composite	dissolved and particulate	2010-2011	Stasinakis et al, 2013
Athens	4	2	composite	dissolved and particulate	2012	Asimakopoulos et al, 2013

Benzothiazoles						
Athens	4	14	composite	dissolved and particulate	2010-2011	Stasinakis et al, 2013
Athens	4	2	composite	dissolved and particulate	2012	Asimakopoulos et al, 2013
Perfluorinated Compounds						
Athens and Mytilene	18	6	composite	dissolved and particulate	2009-2010	Arvaniti et al, 2012
Athens	18	14	composite	dissolved and particulate	2010-2011	Stasinakis et al, 2013
Artificial sweeteners						

Athens	8	7	composite	dissolved and particulate	2012	Kokotou and Thomaidis, 2013
Siloxanes						
Athens	17	7	composite	dissolved and particulate	2012	Bletsou et al, 2013

[1] NR: not reported [2] municipal and hospital [3] municipal and industrial [4] mean values

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Table S2

Concentrations of pharmaceuticals and illicit drugs in secondary treated wastewater samples (ng L^{-1}) from Athens STP, Greece (N = 6).

Analytes	Method LOD (ng L^{-1})	[N][1]>LOD	Mean	Median	Min	Max
Pharmaceuticals						
7-aminoflunitrazepam	7.0	4	7.6	<LOD	<LOD	14
8-OH mirtazapine	6.5	6	13	15	<LOD	20
9-OH Risperidone	1.7	6	5.1	5.3	3.9	6.2
Acetylsalicyclic acid	50	4	79	53	<LOD	199
Alprazolam	2.3	6	5.4	5.6	<LOD	8.7
Amitriptyline	0.7	6	30	30	19	42
Amoxicillin	4.6	6	57	42	23	164
Atenolol	6.2	6	890	926	753	997
Atorvastatin	4.5	6	142	157	46	209
Azithromycin	19.3	6	175	149	94	280
Betamethasone	30	0	<LOD	<LOD	<LOD	<LOD
Bromazepam	2.7	6	18	18	6.8	32
Caffeine	7.0	6	1995	2160	664	3104
Carbamazepine	1.1	6	1116	1093	842	1533
Cefaclor	3.8	3	19	<LOD	<LOD	68
Cefadroxil	8.3	5	12	12	<LOD	24
Cefalexine	7.5	0	<LOD	<LOD	<LOD	<LOD
Cefazolin	4.4	0	<LOD	<LOD	<LOD	<LOD
Chloramphenicol	5.0	6	20	16	<LOD	40
Chlordiazepoxide	1.5	2	<LOD	<LOD	<LOD	2.9
Chlorpromazine	3.6	0	<LOD	<LOD	<LOD	<LOD
Chlotetracycline	7.4	0	<LOD	<LOD	<LOD	<LOD
Cimetidine	15.1	6	51	49	31	69
Ciprofloxacin	5.4	6	937	974	791	1088
Citalopram	1.2	6	328	311	251	465
Clarithromycin	1.9	6	697	587	148	1415

Clobazam	3.4	2	<LOD	<LOD	<LOD	4.8
Clofibric acid	6.0	0	<LOD	<LOD	<LOD	<LOD
Clomipramine	2.1	6	5.2	5.8	<LOD	8.7
Clozapine	2.1	6	69	70	36	94
Cortisole	16.0	4	54	30	<LOD	193
Cortisone	10.0	2	<LOD	<LOD	<LOD	18
Diazepam	1.1	5	1.8	1.6	<LOD	4.4
Diclofenac	21	6	927	827	576	1683
Dicloxacillin	34	3	<LOD	<LOD	<LOD	115
Difloxacin	9.9	0	<LOD	<LOD	<LOD	<LOD
Doxepin	1.6	6	5.4	4.2	<LOD	12
Doxycycline	14.9	6	49	48	38	63
Enrofloxacin	7.4	0	<LOD	<LOD	<LOD	<LOD
Ephedrine	10.3	6	2246	2307	966	3442
Fentanyl	1.4	0	<LOD	<LOD	<LOD	<LOD
Florfenicol	1.4	1	4.2	<LOD	<LOD	29
Flumequine	2.5	0	<LOD	<LOD	<LOD	<LOD
Flunitrazepam	25	0	<LOD	<LOD	<LOD	<LOD
Fluoxetine	1.6	6	19	18	8.2	28
Furosemide	21	3	310	<LOD	<LOD	1580
Gemfibrozil	2.6	6	177	182	55	284
Hydrochlorthiazide	9.2	6	2373	2384	2004	2668
Ibuprofen	15.5	6	301	277	165	457
Imipramine	1.1	0	<LOD	<LOD	<LOD	<LOD
Indapamine	71	4	<LOD	<LOD	<LOD	112
Ketamine	3.1	4	<LOD	<LOD	<LOD	4.9
Ketoprofen	3.8	6	146	139	74	225
Lamotrigine	12.2	6	462	470	390	514
Levetiracetam	4.4	6	27	24	16	57
Lidocaine	2.9	6	293	316	214	357
Lincomycin	5.9	6	28	30	17	34
Lorazepam	3.8	6	84	79	57	126

Marbofloxacin	5.1	2	<LOD	<LOD	<LOD	8.2
Mefenamic acid	67	4	<LOD	<LOD	<LOD	114
Meloxicam	6.5	2	7.6	<LOD	<LOD	29
Metformin	211	0	<LOD	<LOD	<LOD	<LOD
Methylprednisolone	18.6	2	<LOD	<LOD	<LOD	36
Metoprolol	54	6	853	859	775	899
Metronidazol	2.4	6	317	321	223	399
Midazolam	2.0	3	<LOD	<LOD	<LOD	3.8
Mirtazapine	1.3	6	78	79	66	93
Naproxen	8.0	6	265	236	160	464
Niflumic acid	5.3	6	554	512	412	794
Nitrazepam	6.4	0	<LOD	<LOD	<LOD	<LOD
Norclozapine	1.5	6	23	26	8.0	28
Nordiazepam	5.4	0	<LOD	<LOD	<LOD	<LOD
Norephedrine	5.1	4	<LOD	<LOD	<LOD	8.7
Norfentanyl	1.4	6	8.7	8.1	6.7	12
Norfloxacin	7.2	6	165	161	141	202
Norketamine	0.9	6	1.1	1.0	<LOD	2.0
Norsertraline	0.7	6	15	16	<LOD	34
Nortriptyline	1.0	6	7.9	8.5	3.9	11
Ofloxacin	1.7	6	144	149	123	157
Olanzapine	1.3	6	3.6	3.4	<LOD	6.8
Omeprazole	1.1	6	75	77	60	93
Oxazepam	1.3	6	71	71	54	89
Oxolinic acid	2.4	6	9.0	8.1	5.4	15
Oxytetracycline	7.0	0	<LOD	<LOD	<LOD	<LOD
Paracetamol	22	6	770	817	203	1149
Paroxetine	10.1	4	<LOD	<LOD	<LOD	15
Pentobarbital	180	5	265	249	<LOD	640
Phenobarbital	15.5	6	114	76	19	301
Phenytoin	104	0	<LOD	<LOD	<LOD	<LOD
Primidone	7.0	6	113	112	69	159

Progesterone	8.2	6	143	163	63	221
Propranolol	5.2	6	145	140	117	190
Ranitidine	7.7	6	95	42	26	327
Risperidone	0.3	6	2.4	2.0	1.6	4.0
Ronidazol	1.1	6	13	13	<LOD	28
Salicyclic acid	3.1	6	360	268	219	872
Sarofloxacin	1.9	0	<LOD	<LOD	<LOD	<LOD
Sertraline	5.4	6	18	17	7.7	29
Simvastatin	27	0	<LOD	<LOD	<LOD	<LOD
Sulfachloropyridazine	19.0	6	21	20	<LOD	39
Sulfaclozine	21	1	<LOD	<LOD	<LOD	27
Sulfadiazine	14.0	6	32	32	21	46
Sulfadimethoxine	10.1	6	14	15	<LOD	22
Sulfadimidine	12.2	6	17	19	<LOD	25
Sulfadoxine	18.9	0	<LOD	<LOD	<LOD	<LOD
Sulfaguanidine	8.6	0	<LOD	<LOD	<LOD	<LOD
Sulfamerazine	11.0	6	15	15	<LOD	24
Sulfamethizole	22	0	<LOD	<LOD	<LOD	<LOD
Sulfamethoxazole	15.7	6	87	87	50	104
Sulfamethoxypyridazine	6.6	5	9.8	11	<LOD	19
Sulfamonomethoxine	7.7	6	15	13	9.4	26
Sulfamoxole	17.3	0	<LOD	<LOD	<LOD	<LOD
Sulfapyridine	9.6	6	13	1	<LOD	21
Sulfathiazole	18.3	0	<LOD	<LOD	<LOD	<LOD
Sulfisoxazole	13.6	1	<LOD	<LOD	<LOD	18
Temazepam	1.3	6	8.3	8.2	3.6	12
Tetracycline	23	0	<LOD	<LOD	<LOD	<LOD
Theophylline	5.5	6	353	344	149	533
Thiamphenicol	5.0	6	122	74	<LOD	273
Thiopental	77	0	<LOD	<LOD	<LOD	<LOD
Tiamuline	9.8	0	<LOD	<LOD	<LOD	<LOD
Topiramate	21	6	489	493	338	650

Tramadol	6.2	6	892	888	828	978
Triamterene	4.3	0	<LOD	<LOD	<LOD	<LOD
Trimethoprim	1.7	6	241	224	208	358
Tylosin	28	2	<LOD	<LOD	<LOD	40
Valproic acid	17.5	6	7627	7658	142	17292
Valsartan	8.9	6	5673	5013	4358	8082
Venlafaxine	0.8	6	612	602	496	732
Zopiclone	2.8	2	<LOD	<LOD	<LOD	4.5
Illicit Drugs						
Analytes	Method LOD (ngL^{-1})	[N]>LOD	Mean	Median	Min	Max
6-monoacetylmorphine	5.5	0	<LOD	<LOD	<LOD	<LOD
Amphetamine	1.6	3	<LOD	<LOD	<LOD	3.1
Benzoylecgonine	1.0	6	92	74	63	183
Buprenorphine	3.6	5	9.3	6.8	<LOD	24
Cocaine	1.5	6	22	19	15	35
Codeine	4.5	6	202	191	180	261
EME	1.4	6	90	91	64	135
EDDP	2.1	6	40	40	37	42
Heroin	3.7	4	<LOD	<LOD	<LOD	8.2
LSD	2.3	0	<LOD	<LOD	<LOD	<LOD
LSD-OH	15.5	5	21	23	<LOD	38
MDA	2.4	2	<LOD	<LOD	<LOD	3.4
MDEA	3.3	0	<LOD	<LOD	<LOD	<LOD
MDMA	1.5	6	8.1	8.2	3.8	17
Methadone	3.1	6	23	23	20	26
Methamphetamine	1.8	6	6.3	6.0	4.6	8.3
Morphine	3.6	6	40	48	<LOD	79
Oxycodone	1.5	6	5.7	4.4	2.2	15
THC	70	0	<LOD	<LOD	<LOD	<LOD
THCA	79	4	83	<LOD	<LOD	205

[1] [N]: Number of samples with concentrations higher than LOD.

Table S3

Maximum measured environmental concentrations (MEC) of emerging organic contaminants in treated wastewater originated from Greek STPs (in ng L^{-1}).

Target Compounds	Sampling Area	Number of samples	Type of sample	MEC (ng L^{-1})	References
Pharmaceuticals					
7-aminoflunitrazepam	Athens	8	composite	14	*
8-OH mirtazapine	Athens	8	composite	20	*
9-OH-Risperidone	Athens	8	composite	6.2	*
Acetylsalicylic acid	Athens	8	composite	199	*
Alprazolam	Athens	8	composite	8.7	*
Amitriptyline	Athens	8	composite	42	*
Amoxicillin	Athens	8	composite	164	*
Atenolol	Athens	8	composite	997	*
Atorvastatin	Athens	8	composite	209	*
Azithromycin	Athens	8	composite	280	*
Bezafibrate	Ioannina[2]	3	grab	344.2[1]	Kosma et al, 2014
Bromazepam	Athens	8	composite	32	*

Budesonide	Ioannina[2]	3	grab	610.8[1]	Kosma et al, 2014
Caffeine	Ioannina	32	composite	13900[1]	Kosma et al, 2010
Carbamazepine	Athens	8	composite	1533	*
Cefaclor	Athens	8	composite	68	*
Cefadroxil	Athens	8	composite	24	*
Chloramphenicol	Athens	8	composite	40	*
Chlordiazepoxide	Athens	8	composite	2.9	*
Cimetidine	Athens	8	composite	69	*
Ciprofloxacin	Athens	8	composite	1088	*
Citalopram	Athens	8	composite	465	*
Clarithromycin	Athens	8	composite	1415	*
Clobazam	Athens	8	composite	4.8	*
Clofibric acid	Agrinio	3	grab	203[1]	Stamatis and Konstantinou, 2013
Clomipramine	Athens	8	composite	8.7	*
Clozapine	Athens	8	composite	94	*
Cortisole	Athens	8	composite	193	*
Cortisone	Athens	8	composite	18	*
Diazepam	Athens	8	composite	4.4	*

Diclofenac	Katerini	6	grab	7003[1]	Stasinakis et al, 2011
Dicloxacillin	Athens	8	composite	115	*
Doxepin	Athens	8	composite	12	*
Doxycycline	Athens	8	composite	63	*
Ephedrine	Athens	8	composite	3442	*
Florfenicol	Athens	8	composite	29	*
Fluoxetine	Athens	8	composite	28	*
Furosemide	Athens	8	composite	1580	*
Gemfibrozil	Ioannina[2]	32	grab	1700[1]	Kosma et al, 2010
Hydrochlorthiazide	Athens	8	composite	2668	*
Ibuprofen	Ioannina	32	composite	2600[1]	Kosma et al, 2010
Indapamine	Athens	8	composite	112	*
Ketamine	Athens	8	composite	4.9	*
Ketoprofen	Katerini	6	grab	1574[1]	Stasinakis et al, 2011
Lamotrigine	Athens	8	composite	514	*
Levetiracetam	Athens	8	composite	57	*
Lidocaine	Athens	8	composite	357	*
Lincomycine	Athens	8	composite	34	*
Lorazepam	Athens	8	composite	126	*

Marbofloxacin	Athens	8	composite	8.2	*
Mefenamic acid	Athens	8	composite	114	*
Meloxicam	Athens	8	composite	29	*
Methylprednisolone	Athens	8	composite	36	*
Metoprolol	Athens	8	composite	899	*
Metronidazol	Athens	8	composite	399	*
Midazolam	Athens	8	composite	3.8	*
Mirtazapine	Athens	8	composite	93	*
Naproxen	Veroia	3	grab	1076.0[1]	Kosma et al, 2014
Niflumic acid	Athens	8	composite	794	*
Norclozapine	Athens	8	composite	28	*
Norephedrine	Athens	8	composite	8.7	*
Norfentanyl	Athens	8	composite	12	*
Norfloxacin	Athens	8	composite	201	*
Norketamine	Athens	8	composite	2.0	*
Norsertraline	Athens	8	composite	34	*
Nortryptiline	Athens	8	composite	11	*
Ofloxacin	Athens	8	composite	157	*
Olanzapine	Athens	8	composite	6.8	*

Omeprazole	Athens	8	composite	93	*
Oxazepam	Athens	8	composite	89	*
Oxolinic acid	Athens	8	composite	15	*
Paracetamol	Ioannina[2]	3	grab	7400[1]	Kosma et al, 2010
Paroxetine	Athens	8	composite	15	*
Phenazon	Ioannina[2]	32	grab	700[1]	Kosma et al, 2010
Pentobarbital	Athens	8	composite	640	*
Phenobarbital	Athens	8	composite	301	*
Primidone	Athens	8	composite	159	*
Progesterone	Athens	8	composite	221	*
Propranolol	Athens	8	composite	190	*
Ranitidine	Athens	8	composite	327	*
Risperidone	Athens	8	composite	4.0	*
Ronidazole	Athens	8	composite	28	*
Salicylic acid	Ioannina[2]	32	grab	14600[1]	Kosma et al, 2010
Sertraline	Athens	8	composite	29	*
Sulfachloropyridazine	Athens	8	composite	39	*
Sulfaclozine	Athens	8	composite	27	*
Sulfadiazine	Athens	8	composite	46	*

Sulfadimethoxine	Athens	8	composite	22	*
Sulfadimidine	Athens	8	composite	25	*
Sulfamerazine	Athens	8	composite	24	*
Sulfamethoxazole	Ioannina[2]	3	grab	481.3[1]	Kosma et al, 2014
Sulfamethoxypyridazine	Athens	8	composite	19	*
Sulfamonomethoxine	Athens	8	composite	26	*
Sulfapyridine	Athens	8	composite	21	*
Sulfisoxazole	Athens	8	composite	18	*
Temazepam	Athens	8	composite	12	*
Theophylline	Athens	8	composite	533	*
Thiamphenicol	Athens	8	composite	273	*
Topiramate	Athens	8	composite	650	*
Tramadol	Athens	8	composite	978	*
Trimethoprim	Ioannina[2]	3	grab	533.2[1]	Kosma et al, 2014
Tylosin	Athens	8	composite	40	*
Valproic acid	Athens	8	composite	17292	*
Valsartan	Athens	8	composite	8082	*
Venlafaxine	Athens	8	composite	732	*
Zopiclone	Athens	8	composite	4.5	*

Illicit drugs					
Amphetamine	Athens	8	composite	3.1	*
Benzoylecgonine	Athens	8	composite	183	*
Buprenorphine	Athens	8	composite	24	*
Cocaine	Athens	8	composite	35	*
Codeine	Athens	8	composite	261	*
Ecgonine methylester (EME)	Athens	8	composite	135	*
EDDP	Athens	8	composite	42	*
Heroin	Athens	8	composite	8.2	*
LSD-OH	Athens	8	composite	38	*
MDA	Athens	8	composite	3.4	*
MDMA	Athens	8	composite	17	*
Methadone	Athens	8	composite	26	*
Methamphetamine	Athens	8	composite	8.3	*
Morphine	Athens	8	composite	79	*
Oxycodone	Athens	8	composite	15	*
THCA	Athens	4	composite	205	*
Endocrine Disrupting Compounds					
4-t-octylphenol	Kallikrateia	5	grab	40.00[1,4]	Pothitou and Voutsa, 2008

Bisphenol A	Halkida	3	grab	1100	Stasinakis et al, 2008
Nonylphenol	Athens	14	composite	6015	Stasinakis et al, 2013
Nonylphenol diethoxylate	Mytilene[2]	3	grab	17400	Stasinakis et al, 2008
Nonylphenol monoethoxylate	Mytilene[2]	3	grab	6890	Stasinakis et al, 2008
Octylphenol diethoxylate	Kallikrateia	5	grab	28.00[1,4]	Pothitou and Voutsa, 2008
Octylphenol monoethoxylate	Kallikrateia	5	grab	9.40[1,4]	Pothitou and Voutsa, 2008
Triclosan	Mytilene[2]	3	grab	6880	Stasinakis et al, 2008
Perfluorinated Compounds					
Perfluoropentanoic acid	Athens	6	composite	209.4	Arvaniti et al, 2012
Perfluorohexanoic acid	Athens	14	composite	8.1	Stasinakis et al, 2013
Perfluoroheptanoic acid	Athens	6	composite	11.5	Arvaniti et al, 2012
Perfluorooctanoic acid	Athens	14	composite	468	Stasinakis et al, 2013
Perfluorononanoic acid	Athens	6	composite	10.3	Arvaniti et al, 2012
Perfluorodecanoic acid	Athens	6	composite	15.9	Arvaniti et al, 2012
Perfluoroundecanoic acid	Athens	14	composite	1281	Stasinakis et al, 2013
Perfluorododecanoic acid	Athens	6	composite	33.9	Arvaniti et al, 2012
Perfluorotridecanoic acid	Athens	6	composite	46.6	Arvaniti et al, 2012
Perfluorotetradecanoic acid	Athens	6	composite	62.4	Arvaniti et al, 2012
Perfluorobutanosulfonate	Athens	14	composite	3.9	Stasinakis et al, 2013

Perfluorohexanesulfonate	Athens	14	composite	8.7	Stasinakis et al, 2013
Perfluoroheptanesulfonate	Athens	6	composite	8.6	Arvaniti et al, 2012
Perfluorooctanesulfonate	Athens	14	composite	25.3	Stasinakis et al, 2013
Perfluorodecanesulfonate	Athens	6	composite	35.1	Arvaniti et al, 2012
Perfluorooctane sulfonamide	Athens	6	composite	7.1	Arvaniti et al, 2012
Benzotriazoles					
1H-benzotriazole	Athens	14	composite	548	Stasinakis et al, 2013
1-hydroxybenzotriazole	Athens	14	composite	182	Stasinakis et al, 2013
Xylytriazole	Athens	14	composite	30	Stasinakis et al, 2013
Tolytriazole	Athens	14	composite	5773	Stasinakis et al, 2013
Benzothiazoles					
2-(methylthio)benzothiazole	Athens	14	composite	368	Stasinakis et al, 2013
2-aminobenzothiazole	Athens	14	composite	31	Stasinakis et al, 2013
2-hydroxybenzothiazole	Athens	14	composite	514	Stasinakis et al, 2013
Benzothiazole	Athens	14	composite	616	Stasinakis et al, 2013
Artificial Sweeteners					
Acesulfame	Athens	7	composite	27200	Kokotou and Thomaidis, 2013
Cyclamate	Athens	7	composite	4480	Kokotou and Thomaidis,

					2013
Neohesperidin dihydrochalcone	Athens	7	composite	28.5	Kokotou and Thomaidis, 2013
Saccharine	Athens	7	composite	270	Kokotou and Thomaidis, 2013
Sucralose	Athens	7	composite	26700	Kokotou and Thomaidis, 2013
Siloxanes					
Hexamethylcyclotrisiloxane (D3)	Athens	7	composite	256	Bletsou et al, 2013
Octamethylcyclotetrasilane (D4)	Athens	7	composite	197	Bletsou et al, 2013
Decamethylcyclopentasilane (D5)	Athens	7	composite	6020	Bletsou et al, 2013
Dodecamethylcyclohexasilane (D6)	Athens	7	composite	59	Bletsou et al, 2013
Tetradecamethylcycloheptasilane (D7)	Athens	7	composite	16	Bletsou et al, 2013
Decamethyl tetrasiloxane (L4)	Athens	7	composite	99	Bletsou et al, 2013
Dodecamethylpentasiloxane (L5)	Athens	7	composite	12	Bletsou et al, 2013
Tetradecamethylhexasiloxane (L6)	Athens	7	composite	163	Bletsou et al, 2013
$L7^3$	Athens	7	composite	310	Bletsou et al, 2013
$L8^3$	Athens	7	composite	343	Bletsou et al, 2013
$L9^3$	Athens	7	composite	484	Bletsou et al, 2013

L10[3]	Athens	7	composite	500	Bletsou et al, 2013
L11[3]	Athens	7	composite	634	Bletsou et al, 2013
L12[3]	Athens	7	composite	85	Bletsou et al, 2013
L13[3]	Athens	7	composite	35	Bletsou et al, 2013
L14[4]	Athens	7	composite	13	Bletsou et al, 2013

[1]Dissolved concentrations; [2]Hospital effluents; [3]Polydimethylsiloxanes; [4]mean values

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Table S4

Acute toxicity data (EC50/LC50) of studied compounds on fish, *Daphnia magna* and algae (the lowest value, obtained from toxicological studies, is presented).

Analytes	**References**	**EC50/LC50 (mg L^{-1})**		
		Fish	***Daphnia magna***	**Algae**
Pharmaceuticals				
7-aminoflunitrazepam	Predicted by ECOSAR	286.046	2.081	6.293
8-OH mirtazapine	NA[1]			
9-OH-Risperidone	NA[1]			
Acetylsalicylic acid	F[4]: Feng et al., 2013; D, A: Cleuvers, 2004	178.00	72.80	104.40
Alprazolam	Predicted by ECOSAR	2.499	2.845	1.064
Amitriptyline	Predicted by ECOSAR	0.616	0.103	0.043
Amoxicillin	F, D[4]: Predicted by ECOSAR; A: Holten Lützhøft et al., 1999	370.208	28.890	0.0037

Atenolol	F, A[4]: Yamamoto et al., 2007; D: Fraysse and Garric, 2005	1800.00	33.40	110.00
Atorvastatin	F, A: NA[1] ; D: Santos et al., 2013		0.086	
Azithromycin	F: Predicted by ECOSAR; D: Montforts, 2005 ; A: Harada et al., 2008	18.822	120.000	0.019
Bezafibrate	Hernando et al., 2007	5.300	30.000	18.000
Bromazepam	Predicted by ECOSAR	106.042	120.599	3.285
Budesonide	Predicted by ECOSAR	42.223	28.214	15.649
Caffeine	F, D: Fernández et al., 2010 ; A: Predicted by ECOSAR	87.500	182.000	0.015
Carbamazepine	F: Kim et al., 2007 ; D, A: Fernández et al., 2010	35.400	13.800	33.600
Cefaclor	Predicted by ECOSAR	8578.976	844.093	1018.020
Cefadroxil	Predicted by ECOSAR	1013.848	46.522	498.020
Chloramphenicol	F, D: Predicted by ECOSAR ; A: Lai et al., 2009	38.821	72.084	4.000
Chlordiazepoxide	Predicted by ECOSAR	103.168	60.900	53.268
Cimetidine	F, A: Predicted by ECOSAR; D: Kim et al, 2007	80.402	271.300	0.787
Ciprofloxacin	F: Predicted by ECOSAR; D: Santos et al., 2013; A: Yang et al., 2008	13131.424	12.800	6.700

Citalopram	F: Predicted by ECOSAR; D: Henry et al., 2004; A: Christensen, 2007	4.467	3.900	1.600
Clarithromycin	F: Predicted by ECOSAR; D: Isidori et al., 2005; A: Yang et al., 2008	17.364	18.660	0.046
Clobazam	Predicted by ECOSAR	119.930	143.162	3.632
Clofibric acid	F, D: Ginebreda et al., 2010; A: Sanderson and Thomsen, 2009	53	0.110	86.000
Clomipramine	Predicted by ECOSAR	0.241	0.044	0.016
Clozapine	Predicted by ECOSAR	17.666	2.321	1.579
Cortisole	Predicted by ECOSAR	80.776	52.860	28.836
Cortisone	Predicted by ECOSAR	60.749	40.038	21.965
Diazepam	Sanderson and Thomsen, 2009	12.7	4.300	3.100
Diclofenac	F: Brandhof and Montforts, 2010 ; D, A: Ginebreda et al., 2010	5.3	22.000	14.500
Dicloxacillin	Predicted by ECOSAR	65.427	30.539	3.075
Doxepin	Predicted by ECOSAR	2.639	0.397	0.207
Doxycycline	Predicted by ECOSAR	27.425	2.893	3.367
Ephedrine	Predicted by ECOSAR	232.743	23.805	26.591

Florfenicol	F: NA[1]; D: Kolodziejska et al., 2013; A: Lai et al., 2009		337.000	1.300
Fluoxetine	Brooks et al., 2003	0.705	0.820	0.024
Furosemide	F, A: Christensen et al, 2009 ; D: Isidori et al., 2006	497	60.620	142.000
Gemfibrozil	Hernando et al., 2007	0.9	10.400	4.000
Hydrochlorthiazide	F, D: Predicted by ECOSAR; A: Fernández et al., 2010	2808.512	8125.047	34.350
Ibuprofen	F: Predicted by ECOSAR; D, A: Ginebreda et al., 2010	42.036	9.020	4.000
Indapamine	NA[1]			
Ketamine	Predicted by ECOSAR	8.344	1.134	0.722
Ketoprofen	F, A: Predicted by ECOSAR; D: Fernández et al., 2010	264.080	64.000	179.455
Lamotrigine	Predicted by ECOSAR	357.865	3.760	2.478
Levetiracetam	Predicted by ECOSAR	2050.566	488.364	1.850
Lidocaine	F, D, A: Escher et al., 2011	106	112.000	760.000
Lincomycine	F: Predicted by ECOSAR; D: Isidori et al., 2005; A: Santos at al., 2010	1040.222	13.980	0.070
Lorazepam	Predicted by ECOSAR	49.008	44.712	1.683

Marbofloxacin	NA[1]			
Mefenamic acid	F, D: Kim et al., 2009; A: Feng et al., 2013	8.04	3.950	4.330
Meloxicam	Predicted by ECOSAR	1.392	3.944	0.184
Methylprednisolone	Predicted by ECOSAR	62.242	41.036	22.519
Metoprolol	F: van den Brandhof and Montforts, 2010; D: Santos et al., 2010; A: Cleuvers, 2003	31	63.900	7.300
Metronidazol	F, A: Madden et al, 2009; D: Predicted by ECOSAR	1060	12.068	3.440
Midazolam	Predicted by ECOSAR	0.519	0.532	0.116
Mirtazapine	Predicted by ECOSAR	11.124	1.389	0.814
Naproxen	F: Predicted by ECOSAR; D, A: Cleuvers, 2004	193.337	166.300	625.500
Niflumic acid	Predicted by ECOSAR	10.710	7.731	15.542
Norclozapine	NA[1]			
Norephedrine	Predicted by ECOSAR	275.365	27.614	32.074
Norfentanyl	Predicted by ECOSAR	70.248	8.087	3.352
Norfloxacin	F, D: Predicted by ECOSAR; A: Verlicchi et al, 2012	20081.355	1830.796	15.000

Norketamine	NA[1]			
Norsertraline	NA[1]			
Nortryptiline	Predicted by ECOSAR	0.805	0.132	0.058
Ofloxacin	F: Verlicchi et al, 2012 ; D: Isidori et al., 2005 ; A: Ferrari et al., 2004	10	31.750	0.016
Olanzapine	Predicted by ECOSAR	0.246	4.281	1.931
Omeprazole	Predicted by ECOSAR	2.054	1.271	0.210
Oxazepam	Predicted by ECOSAR	50.358	47.787	1.698
Oxolinic acid	F: Predicted by ECOSAR; D: Wollenberger et al., 2000 ; A: Holten Lützhøft et al., 1999	4466.764	4.600	16.000
Paracetamol	F, A: Henschel et al., 1997 ; D: Kuhn et al., 1989	378	9.200	134.000
Paroxetine	F: Predicted by ECOSAR; D: Henry et al., 2004 ; A: Christensen et al., 2009	3.864	0.580	0.140
Phenazon	Predicted by ECOSAR	5.781	36.797	1.346
Pentobarbital	F: Cunningham et al., 2006 ; D, A: Predicted by ECOSAR	49.5	7.641	0.017
Phenobarbital	F: Sanderson and Thomsen, 2009 ; D: Martins et al., 2007 ; A: Predicted by ECOSAR	484	1400.300	0.017
Primidone	Predicted by ECOSAR	531.259	1052.044	12.692

Progesteron	F: Escher et al., 2011 ; D, A: Predicted by ECOSAR	0.5	6.782	5.573
Propranolol	F: Stanley et al., 2006 ; D: Yamamoto et al., 2007 ; A: Ferrari et al., 2004	1.21	0.460	0.668
Ranitidine	Predicted by ECOSAR	797.927	78.001	95.290
Risperidone	Montforts, 2005	6.000	6.000	10.000
Ronidazole	Predicted by ECOSAR	242.023	19.445	1.080
Salicylic acid	F: Fernández et al., 2010; D: Cunningham et al., 2006 ; A: Predicted by ECOSAR	37	118.000	235.760
Sertraline	F, D: Minagh et al., 2009 ; A: Johnson et al., 2007	0.38	1.300	0.0121
Sulfachloropyridazine	F, D: Kim et al., 2007 ; A: Białk-Bielińska et al., 2011	535.7	233.500	32.250
Sulfaclozine	Predicted by ECOSAR	613.528	2.113	8.194
Sulfadiazine	F: Predicted by ECOSAR ; D: De Liguoro et al., 2009 ; A: De Orte et al., 2013	1516.102	212.000	0.110
Sulfadimethoxine	F: Predicted by ECOSAR ; D: Kim et al., 2007 ; A: Eguchi et al., 2004	166.297	204.500	2.300
Sulfadimidine	F, D: Predicted by ECOSAR ; A: Białk-Bielińska et al., 2011	291.394	2.045	19.520
Sulfamerazine	F: Predicted by ECOSAR ; D: De Liguoro et al., 2009 ; A: Białk-Bielińska et al., 2011	665.605	277.000	11.900
Sulfamethoxazole	F: Kim et al., 2007 ; D: Isidori et al., 2005 ; A: Fernández et al., 2010	562.5	25.200	0.030

Sulfamethoxypyridazine	F, D: Predicted by ECOSAR ; A: Białk-Bielińska et al., 2011	719.037	2.085	3.820
Sulfamonomethoxine	Predicted by ECOSAR	719.037	2.085	8.562
Sulfapyridine	F, D: Predicted by ECOSAR ; A: Białk-Bielińska et al., 2011	377.595	1.841	5.280
Sulfisoxazole	F, D: Predicted by ECOSAR ; A: Białk-Bielińska et al., 2011	180.221	1.952	18.980
Temazepam	Predicted by ECOSAR	70.230	72.175	2.281
Theophylline	Predicted by ECOSAR	223.802	17.796	0.014
Thiamphenicol	F, D: Predicted by ECOSAR ; A: Eguchi et al., 2004	211.345	286.165	8.860
Topiramate	Predicted by ECOSAR	3022.28	762.237	3.316
Tramadol	F, D: Montforts, 2005 ; A: Predicted by ECOSAR	0.13	0.073	0.959
Trimethoprim	F: Predicted by ECOSAR ; D: De Liguoro et al., 2012 ; A: Holten Lützhøft et al., 1999	317.910	8.210	16.000
Tylosin	F: NA[1] ; D: Wollenberger et al., 2000 ; A: Halling-Sørensen, 2000		680.000	0.034
Valproic acid	F: Lammer et al., 2009 ; D, A: Predicted by ECOSAR	20.189	100.976	108.510
Valsartan	F, A: Predicted by ECOSAR, 2013 ; D: Escher et al., 2011	13.495	580.000	3.322
Venlafaxine	Predicted by ECOSAR	7.678	1.062	0.653

Zopiclone	Predicted by ECOSAR	4.670	2.912	0.620
Illicit drugs				
Amphetamine	F: Madden et al., 2009 ; D, A: Predicted by ECOSAR	28.8	4.357	3.803
Benzoylecgonine	Predicted by ECOSAR	33458.809	6805.164	12041.672
Buprenorphine	Predicted by ECOSAR	0.509	0.187	0.079
Cocaine	Predicted by ECOSAR	32.290	5.482	4.350
Codeine	Predicted by ECOSAR	7.438	0.976	18.345
Ecgonine methylester	NA[1]			
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	NA[1]			
Heroin	Predicted by ECOSAR	2.935	11.217	7.636
2-oxo-3-hydroxy lysergic acid diethylamide (LSD-OH)	NA[1]			
MDA	NA[1]			

MDMA	NA[1]			
Methadone	Predicted by ECOSAR	2.242	0.344	0.172
Methamphetamine	Predicted by ECOSAR	20.511	2.509	1.967
Morphine	Predicted by ECOSAR	8.601	1.078	16.318
Oxycodone	Predicted by ECOSAR	458.553	46.786	52.515
11-nor-9-carboxy-tetrahydrocannabinol (THCA)	NA[1]			
Endocrine Disrupting Compounds				
4-t-octylphenol	F: Segner et al., 2003 ; D: Isidori et al., 2006 ; A: Predicted by ECOSAR	0.028	0.218	0.503
Bisphenol A	F: Brian et al., 2005 ; D: Duan et al., 2008 ; A: Staples et al., 1998	0.158	3.900	1.000
Nonylphenol	F: Brian et al., 2005 ; D: Brennan et al., 2006 ; A: Liu et al., 2010	0.00702	0.090	0.200
Nonylphenol diethoxylate	F, D: TenEyck and Markee, 2007 ; A: Predicted by ECOSAR	0.323	0.716	0.555
Nonylphenol monoethoxylate	F, D: TenEyck and Markee, 2007 ; A: Predicted by ECOSAR	0.218	0.328	0.307
Octylphenol diethoxylate	NA[1]			

Octylphenol monoethoxylate	NA[1]			
Triclosan	Orvos et al., 2002	0.260	0.390	0.0014
Perfluorinated Compounds				
Perfluoropentanoic acid	NA[2]			
Perfluorohexanoic acid	NA[2]			
Perfluoroheptanoic acid	NA[2]			
Perfluorooctanoic acid	F: Ye et al., 2009 ; D: Li, 2008 ; A: Rosal et al., 2010	328	181.00	96.20
Perfluorononanoic acid	NA[2]			
Perfluorodecanoic acid	NA[2]			
Perfluoroundecanoic acid	NA[2]			
Perfluorododecanoic acid	NA[2]			
Perfluorotridecanoic acid	NA[2]			
Perfluorotetradecanoic acid	NA[2]			
Perfluorobutanosulfonate	NA[2]			

Perfluorohexanesulfonate	NA[2]			
Perfluoroheptanesulfonate	NA[2]			
Perfluorooctanesulfonate	F: Ye et al., 2009 ; D: Ji et al., 2008 ; A: Rosal et al., 2010	9.14	37.36	35.00
Perfluorodecanesulfonate	NA[2]			
Perfluorooctane sulfonamide	NA[2]			
Benzotriazoles				
1H-benzotriazole	Predicted by ECOSAR	28.321	66.766	5.904
1-hydroxybenzotriazole	Predicted by ECOSAR	114.637	308.834	18.960
5,6-dimethyl-1H-benzotriazole (xylytriazole)	Predicted by ECOSAR	9.376	19.253	2.484
Tolytriazole	Predicted by ECOSAR	16.386	36.053	3.851
Benzothiazoles				
2-(methylthio)benzothiazole	F: Predicted by ECOSAR ; D: Nawrocki et al., 2005 ; A: Predicted by ECOSAR	11.831	12.700	8.943
2-aminobenzothiazole	Predicted by ECOSAR	21.349	1.074	1.707

2-hydroxybenzothiazole	F: Predicted by ECOSAR ; D: Nawrocki et al., 2005 ; A: Predicted by ECOSAR	3.786	15.100	0.611
Benzothiazole	F: Evans et al., 2000 ; D: Nawrocki et al., 2005 ; A: Predicted by ECOSAR	41.900	24.600	35.879
Artificial Sweeteners				
Acesulfame	Predicted by ECOSAR	1.320E+05	55082.266	11495.213
Cyclamate	Predicted by ECOSAR	2.120E+06	7.850E+05	99866.023
Neohesperidin dihydrochalcone	NA[1]			
Saccharine	Predicted by ECOSAR	1.333	1.758	0.377
Sucralose	Predicted by ECOSAR	2360.532	12788.485	0.236
Siloxanes				
Hexamethylcyclotrisiloxane (D3)	Predicted by ECOSAR	0.098	0.078	0.232
Octamethylcyclotetrasiloxane (D4)	F: Redman et al, 2012 ; D, A: Predicted by ECOSAR	0.010	0.011	0.050
Decamethylcyclopentasiloxane	D: Redman et al, 2012 ; F, A: Predicted by ECOSAR	0.00143	0.0029	0.010

(D5)				
Dodecamethylcyclohexasiloxane (D6)	F, D: Predicted by ECOSAR ; A: Redman et al, 2012	0.000161	0.000175	0.002
Tetradecamethylcyclo-heptasiloxane (D7)	NA[2]			
Decamethyltetrasiloxane (L4)	Predicted by ECOSAR	0.000752	0.000754	0.006
Dodecamethylpentasiloxane (L5)	F, D: Predicted by ECOSAR; A: NA[2]	4.6E-05	5.27E-05	
Tetradecamethylhexasiloxane (L6)	F: Predicted by ECOSAR; D, A: NA[2]	2.7E-06		
L7[3]	NA[2]			
L8[3]	NA[2]			
L9[3]	NA[2]			
L10[3]	NA[2]			
L11[3]	NA[2]			

L12[3]	NA[2]			
L13[3]	NA[2]			
L14[3]	NA[2]			

[1] ECOSAR program does not recognize the compound CAS number

[2] The compounds' toxicity is not possible to be predicted by ECOSAR model

[3] Polydimethylsiloxanes

[4] F: Fish, D: *Daphnia magna,* A: Algae

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Table S5

Toxic Ratio (TR = $EC50_{baseline}/EC50_{experimental}$) for the emerging organic contaminants that acute experimental toxicity data are available.

Emerging contaminants	Toxic Ratio (TR)		
	Fish	**Daphnia magna**	**Algae**
Acetylsalicylic acid	5.04	6.46	2.40
Amoxicillin	NA[1]	NA	176176
Atenolol	8.02	203	21.3
Azithromycin	NA	0.25	1889
Bezafibrate	0.53	0.07	0.19
Caffeine	82.5	19.0	NA
Carbamazepine	3.28	4.89	1.65
Chloramphenicol	NA	NA	157
Cimetidine	NA	7.26	NA
Ciprofloxacin	NA	629	417
Citalopram	NA	1.24	4.39
Clarithromycin	NA	1.83	864
Clofibric acid	5.81	1718	2.26
Diazepam	4.33	7.75	10.5
Diclofenac	7.11	1.17	2.86
Erythromycin	NA	5.92	5949
Flumequine	NA	NA	189
Flunitrazepam	3.66	NA	NA
Fluoxetine	1.51	0.94	65.8
Furosemide	0.29	1.37	0.49
Gemfibrozil	7.48	0.47	2.64
Hydrochlorthiazide	NA	NA	85.1
Ibuprofen	NA	3.09	10.3
Ketoprofen	NA	2.57	NA
Lidocaine	3.68	1.92	0.19
Lincomycine	NA	398	30879

Mefenamic acid	0.28	0.44	1.04
Metformin	NA	9015	NA
Metoprolol	13.3	3.57	20.72
Metronidazol	8.35	NA	421
Naproxen	NA	0.73	0.22
Norfloxacin	NA	NA	291
Ofloxacin	2806	409	261481
Oxolinic acid	NA	47.5	9.09
Oxytetracycline	NA	142226	89473
Paracetamol	11.8	234	6.19
Pentobarbital	3.72	NA	NA
Phenobarbital	1.56	0.29	NA
Progesteron	16.5	NA	NA
Propranolol	51.2	80.8	52.1
Salicylic acid	1.85	0.34	NA
Sulfachloropyridazine	14.5	16.1	45.6
Sulfadiazine	NA	55.8	32815
Sulfadimethoxine	NA	3.62	174
Sulfadimidine	NA	NA	36.0
Sulfadoxine	NA	NA	438
Sulfaguanidine	NA	49672	2927
Sulfamerazine	NA	15.3	134
Sulfamethizole	NA	NA	47.3
Sulfamethoxazole	8.50	93.7	32866
Sulfamethoxypyridazine	NA	NA	450
Sulfapyridine	NA	NA	171
Sulfaquionoxaline	NA	401	2702
Sulfathiazole	NA	17.7	52.5
Sulfisoxazole	NA	NA	22.9
Tetracycline	1618	3325	345567
Thiamphenicol	NA	NA	574
Tramadol	205	227	NA
Triamterene	191	126	42.6

Trimethoprim	NA	203	47.8
Valproic acid	8.06	NA	NA
Amphetamine	6.40	NA	NA
4-t-octylphenol	6.93	0.69	NA
Bisphenol A	39.7	1.06	5.78
Nonylphenol	7.84	0.50	0.75
Nonylphenol diethoxylate	0.85	0.30	NA
Nonylphenol monoethoxylate	0.61	0.32	NA
Triclosan	3.71	1.80	1030
2-(methylthio)benzothiazole	NA	0.59	NA
2-hydroxybenzothiazole	NA	2.32	NA
Benzothiazole	1.87	1.84	NA
Octamethylcyclotetrasiloxane	1.20	NA	NA
Decamethylcyclopentasiloxane	NA	0.48	NA
Dodecamethylcyclohexasiloxane	NA	NA	0.98

[1] NA: Not available

Table S6

Classes of emerging organic contaminants (EOCs) that have been detected in dewatered sludge samples originating from Greek Sewage Treatment Plants (STPs). Information is also given for the type and the number of samples and the period of sampling.

STPs	Number of analyzed compounds	Number of samples	Type of samples	Years of sampling	References
Pharmaceuticals					
Athens and Mytilene	4	9	grab	2009	Samaras et al, 2013
Athens	4	14	grab	2010-2011	Stasinakis et al, 2013
Santorini Island	129	5	grab	2013	Gago-Ferrero et al., 2015
Athens	46	8	grab	2010	Present study
Illicit drugs					
Santorini Island	19	5	grab	2013	Gago-Ferrero et al., 2015
Athens	4	8	grab	2010	Present study
Endocrine disrupting compounds					
Athens, Mytilene, Chalkida, Nafplion, Herakleion	4	27 (5 plants)	grab	2006	Stasinakis et al, 2008

Kallikratia[1]	13	5	grab	2007	Pothitou and Voutsa, 2008
Athens and Mytilene	5	9	grab	2009	Samaras et al, 2013
Athens	5	14	grab	2010-2011	Stasinakis et al, 2013
Benzotriazoles					
Athens	4	14	grab	2010-2011	Stasinakis et al, 2013
Athens	4	2	grab	2012	Asimakopoulos et al, 2013
Benzothiazoles					
Athens	4	14	grab	2010-2011	Stasinakis et al, 2013
Athens	4	2	grab	2012	Asimakopoulos et al, 2013
Perfluorinated Compounds					
Athens and Mytilene	18	6	grab	2009-2010	Arvaniti et al, 2012
Athens	18	14	grab	2010-2011	Stasinakis et al, 2013
Siloxanes					
Athens	17	7	grab	2012	Bletsou et al, 2013

[1] mean values

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Table S7

Concentrations of the detected pharmaceuticals (PhCs) and illicit drugs (IDs) in dewatered sludge samples (ng g^{-1} dw) from Athens STP, Greece (N = 8). All analyzed samples had concentration values higher than method limit of detection (MLOD).

Analytes	Mean	Median	Min	Max
8-OH mirtazapine	14.9	16.5	9.1	22.1
Acetylsalicylic acid	149	150	85.4	215
Amitriptyline	110	123	75.7	159
Atorvastatin	24.0	27.4	12.1	44.4
Azithromycin	122	165	91.4	204
Caffeine	59.8	59.9	40.5	180
Carbamazepine	71.4	65.0	63.0	84.1
Ciprofloxacin	95.7	91.5	80.3	107
Citalopram	119	133	102	151
Clarithromycin	63.1	92.9	42.4	122
Clomipramine	27.3	24.0	15.7	47.5
Clozapine	70.3	76.0	45.3	112
Diclofenac	27.5	37.2	13.0	50.6
Doxycycline	126	120	102	146
Enrofloxacin	11.9	7.9	5.4	20.7
Ephedrine	202	226	168	247
Fluoxetine	46.6	49.7	22.7	80.1
Lorazepam	24.5	14.8	13.3	46.9
Mefenamic acid	119	102	82.4	227
Metformin	177	192	147	237
Methylprednisolone	28.7	16.6	12.0	67.5
Metoprolol	32.2	50.7	15.3	81.7
Mirtazapine	51.0	50.9	42.1	59.7
Niflumic acid	82.9	74.2	68.6	105.7
Norclozapine	36.7	45.5	19.8	51.8
Norephedrine	11.1	10.9	2.9	20.2
Norfloxacin	196	177	149	242
Norsertraline	88.0	115	27.9	151

Nortriptyline	24.9	35.1	9.0	42.5
Ofloxacin	134	120	119	510
Oxazepam	18.4	17.1	6.0	29.4
Oxolinic acid	64.3	96.5	42.0	136
Oxytetracycline	32.6	40.2	20.0	40.3
Paracetamol	75.4	37.4	27.6	180
Paroxetine	29.9	32.8	10.6	55.0
Progesterone	135	103	69.0	273
Propranolol	38.3	40.5	30.4	46.9
Ranitidine	16.6	24.9	10.3	26.4
Salicyclic acid	208	163	115	350
Sarafloxacine	22.8	20.9	11.5	41.0
Sertraline	118	141	62.3	179
Tetracycline	30.4	32.3	18.6	37.2
Tramadol	25.6	18.3	20.5	42.7
Valproic acid	161	160	134	185
Valsartan	172	144	143	227
Venlafaxine	79.7	73.3	54.7	100
Codeine	19.3	17.2	14.3	32.1
MDA	23.9	30.5	10.8	31.6
Methadone	10.7	12.2	7.5	14.3
THCA	118	123	74.6	138

Table S8

Maximum measured environmental concentrations (MEC_{sludge}) of emerging organic contaminants (EOCs) in dewatered sewage sludge obtained from Greek STPs (in ng g^{-1} dw) and the corresponding predicted concentrations (PEC_{soil}) in soil one year after a single sludge application (all sludge samples were grab).

Target Compounds	**Sampling Area**	**Number of samples**	**MEC_{sludge} (ng g^{-1} dw)**	**PEC_{soil} (ng g^{-1} dw)**	**References**
Pharmaceuticals					
8-OH mirtazapine	Athens	8	22.1	0.033	*
Acetylsalicylic acid	Santorini Island	5	244	0.36	Gago-Ferrero et al., 2015
Amitriptyline	Santorini Island	5	227	0.33	Gago-Ferrero et al., 2015
Atorvastatin	Athens	8	44.4	0.065	*
Azithromycin	Santorini Island	5	267	0.39	Gago-Ferrero et al., 2015
Caffeine	Athens	8	93.1	0.14	*
Carbamazepine	Santorini Island	5	113	0.17	Gago-Ferrero et al., 2015
Cimetidine	Santorini Island	5	51.0	0.075	Gago-Ferrero et al., 2015
Ciprofloxacin	Santorini Island	5	115	0.17	Gago-Ferrero et al., 2015
Citalopram	Santorini Island	5	168	0.25	Gago-Ferrero et al., 2015
Clarithromycin	Athens	8	122	0.18	*
Clomipramine	Santorini Island	5	67.1	0.10	Gago-Ferrero et al., 2015

Clozapine	Athens	8	112	0.17	*
Diclofenac	Athens	9	250	0.37	Samaras et al, 2013
Doxycycline	Santorini Island	5	179	0.26	Gago-Ferrero et al., 2015
Enrofloxacin	Athens	8	20.7	0.030	*
Ephedrine	Athens	5	247	0.36	*
Fluoxetine	Athens	8	80.1	0.12	*
Ibuprofen	Athens	9	390	0.57	Samaras et al, 2013
Lorazepam	Athens	8	46.9	0.069	*
Mefenamic acid	Athens	8	227	0.33	*
Metformin	Athens	8	237	0.35	*
Methylprednisolone	Athens	8	67.5	0.099	*
Metoprolol	Athens	8	81.7	0.12	*
Mirtazapin	Athens	8	59.7	0.088	*
Naproxen	Athens	9	5460	8.0	Samaras et al, 2013
Niflumic acid	Athens	8	106	0.16	*
Norclozapine	Athens	8	51.8	0.076	*
Norepherdine	Athens	8	20.2	0.030	*
Norfloxacin	Athens	8	242	0.36	*
Norsertraline	Athens	8	151	0.22	*

Nortryptiline	Athens	8	42.5	0.063	*
Ofloxacin	Athens	8	159	0.23	*
Oxazepam	Athens	8	29.4	0.043	*
Oxolinic acid	Athens	8	136	0.20	*
Oxytetracycline	Santorini Island	5	159	0.23	Gago-Ferrero et al., 2015
Paracetamol	Athens	8	180	0.27	*
Paroxetine	Athens	8	55.0	0.081	*
Progesterone	Athens	8	273	0.40	*
Propranolol	Athens	8	46.9	0.069	*
Ranitidine	Santorini Island	5	32.7	0.049	Gago-Ferrero et al., 2015
Salicylic acid	Athens	8	350	0.52	*
Sarafloxacin	Athens	8	41.0	0.060	*
Sertraline	Athens	8	179	0.26	*
Sulfapyridine	Santorini Island	5	34.5	0.051	Gago-Ferrero et al., 2015
Tetracycline	Santorini Island	5	191	0.28	Gago-Ferrero et al., 2015
Tramadol	Athens	8	42.7	0.063	*
Valproic acid	Athens	8	185	0.27	*
Valsartan	Athens	8	227	0.33	*
Venlafaxine	Athens	8	100	0.15	*

Illicit drugs					
Codeine	Athens	8	32.1	0.047	*
MDA	Santorini Island	5	77.3	0.11	Gago-Ferrero et al., 2015
Methadone	Athens	8	14.3	0.021	*
THCA	Athens	8	138	0.20	*
Endocrine disrupting compounds					
4-t-octylphenol	Kallikrateia	5	179[1]	0.26	Pothitou and Voutsa, 2008
Bisphenol A	Athens	9	3910	5.8	Samaras et al, 2013
Nonylphenol	Mytilene	9	13200	19.4	Samaras et al, 2013
Nonylphenol diethoxylate	Mytilene	27 (5 plants)	24700	36.3	Stasinakis et al, 2008
Nonylphenol monoethoxylate	Mytilene	27 (5 plants)	41300	60.7	Stasinakis et al, 2008
Octylphenol diethoxylate	Kallikrateia	5	16.1[1]	0.024	Pothitou and Voutsa, 2008
Octylphenol monoethoxylate	Kallikrateia	5	8.1[1]	0.012	Pothitou and Voutsa, 2008
Triclosan	Mytilene	3	9850	14.5	Stasinakis et al, 2008
Perfluorinated Compounds					
Perfluoropentanoic acid	Athens	6	45.2	0.067	Arvaniti et al, 2012
Perfluorohexanoic acid	Athens	6	19.4	0.029	Arvaniti et al, 2012
Perfluoroheptanoic acid	Athens	6	16.4	0.024	Arvaniti et al, 2012
Perfluorooctanoic acid	Athens	6	19.4	0.029	Arvaniti et al, 2012

Perfluorononanoic acid	Athens	6	13.5	0.020	Arvaniti et al, 2012
Perfluorodecanoic acid	Athens	14	15.2	0.022	Stasinakis et al, 2013
Perfluoroundecanoic acid	Athens	14	3209	4.7	Stasinakis et al, 2013
Perfluorododecanoic acid	Athens	6	9.8	0.014	Arvaniti et al, 2012
Perfluorotridecanoic acid	Athens	6	19.6	0.029	Arvaniti et al, 2012
Perfluorotetradecanoic acid	Athens	14	6.1	0.009	Stasinakis et al, 2013
Perfluorohexanesulfonate	Athens	6	18.3	0.027	Arvaniti et al, 2012
Perfluoroheptanesulfonate	Athens	6	13.3	0.020	Arvaniti et al, 2012
Perfluorooctanesulfonate	Athens	14	16.7	0.025	Stasinakis et al, 2013
Perfluorooctane sulfonamide	Athens	6	5.7	0.008	Arvaniti et al, 2012
Benzotriazoles					
1H-benzotriazole	Athens	14	412	0.61	Stasinakis et al, 2013
Xylytriazole	Athens	14	22	0.032	Stasinakis et al, 2013
Tolytriazole	Athens	14	205	0.30	Stasinakis et al, 2013
Benzothiazoles					
2-(methylthio)benzothiazole	Athens	14	77	0.11	Stasinakis et al, 2013
2-hydroxybenzothiazole	Athens	14	312	0.46	Stasinakis et al, 2013
Benzothiazole	Athens	14	174	0.26	Stasinakis et al, 2013
Siloxanes					

Hexamethylcyclotrisiloxane (D3)	Athens	7	12	0.018	Bletsou et al, 2013
Octamethylcyclotetrasiloxane (D4)	Athens	7	130	0.19	Bletsou et al, 2013
Decamethylcyclopentasiloxane (D5)	Athens	7	17500	25.7	Bletsou et al, 2013
Dodecamethylcyclohexasiloxane (D6)	Athens	7	5490	8.1	Bletsou et al, 2013
Tetradecamethylcycloheptasiloxane (D7)	Athens	7	920	1.4	Bletsou et al, 2013
Octamethyl trisiloxane (L3)	Athens	7	260	0.38	Bletsou et al, 2013
Decamethyl tetrasiloxane (L4)	Athens	7	63	0.093	Bletsou et al, 2013
Dodecamethylpentasiloxane (L5)	Athens	7	250	0.37	Bletsou et al, 2013
Tetradecamethylhexasiloxane (L6)	Athens	7	4070	6.0	Bletsou et al, 2013
L7[2]	Athens	7	7330	10.8	Bletsou et al, 2013
L8[2]	Athens	7	9530	14.0	Bletsou et al, 2013
L9[2]	Athens	7	11700	17.2	Bletsou et al, 2013
L10[2]	Athens	7	12400	18.2	Bletsou et al, 2013
L11[2]	Athens	7	8650	12.7	Bletsou et al, 2013
L12[2]	Athens	7	3710	5.5	Bletsou et al, 2013
L13[2]	Athens	7	1220	1.8	Bletsou et al, 2013
L14[2]	Athens	7	490	0.72	Bletsou et al, 2013

[1]Mean values

[2] Polydimethylsiloxanes

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Table S9

Terrestrial acute toxicity data (EC_{50}/LC_{50}) of the studied compounds and Risk Quotients ($RQ_{soil,terrestrial}$) in sludge amended soil. The lowest EC50/LC50 value for each organism group (plants, earthworms and soil microorganisms), obtained from toxicological studies or ECOSAR model, is presented. EC50/LC50 values given in mg L^{-1} or mM were converted to mg kg^{-1}, using equilibrium partitioning method. $RQ_{soil,terrestrial}$ values' calculation was based on maximum measured concentration in sludge (MEC_{sludge}).

Emerging Contaminants	**Terrestrial acute toxicity data**			**$RQ_{soil,terrestrial}$**
	Organism	**EC50/LC50**	**References**	
Pharmaceuticals				
Acetylsalicylic acid	Plant	NA[1]		
	Earthworm	44850.684 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	140 mg L^{-1}	Tobajas et al., 2015	0.013
Atorvastatin	Plant	0.1729 mg L^{-1}	Hillis et al., 2008	NA[3]
	Earthworm	NA[1]		
	Soil microorganism	0.0418 mg L^{-1}	Hillis et al., 2008	NA[3]
Azithromycin	Plant	NA[1]		
	Earthworm	3880.976 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Carbamazepine	Plant	0.447 mM	Jos et al., 2003	2.1E-05
	Earthworm	NA[1]		

	Soil microorganism	0.0436 mg L^{-1}	Hillis et al., 2008	0.050
Clarithromycin	Plant	NA[1]		
	Earthworm	4068.436 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Diclofenac	Plant	NA[1]		
	Earthworm	90.49 mg kg^{-1}	Pino et al., 2015	4.1E-03
	Soil microorganism	NA[1]		
Doxycycline	Plant	NA[1]		
	Earthworm	9807.580 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	0.0369 mg L^{-1}	Hillis et al., 2008	0.055
Enrofloxacin	Plant	NA[1]		
	Earthworm	11010 mg kg^{-1}	Li et al., 2015	2.7E-06
	Soil microorganism	NA[1]		
Ibuprofen	Plant	293.70 mg kg^{-1}	González-Naranjo and Boltes, 2014	1.9E-03
	Earthworm	64.8 mg kg^{-1}	Pino et al., 2015	8.8E-03
	Soil microorganism	NA[1]		
Mefenamic acid	Plant	NA[1]		
	Earthworm	1617.666 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		

Naproxen	Plant	NA[1]		
	Earthworm	3076.510 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Niflumic acid	Plant	NA[1]		
	Earthworm	2637.133 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Ofloxacin	Plant	NA[1]		
	Earthworm	NA[1]		
	Soil microorganism	1 mg L^{-1}	Tobajas et al., 2015	0.26
Oxytetracycline	Plant	34.7 mg L^{-1}	An et al., 2009	3.4E-03
	Earthworm	41807.324 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Paracetamol	Plant	668.8 mg L^{-1}	An et al., 2009	3.2E-04
	Earthworm	693.5 mg kg^{-1}	Pino et al., 2015	3.9E-04
	Soil microorganism	NA[1]		
Propranolol	Plant	NA[1]		
	Earthworm	3298.63 mg kg^{-1}	Pino et al., 2015	2.1E-05
	Soil microorganism	NA[1]		
Salicylic acid	Plant	NA[1]		

	Earthworm	162.68 mg kg^{-1}	Pino et al., 2015	3.2E-03
	Soil microorganism	NA[1]		
Tetracycline	Plant	NA[1]		
	Earthworm	9481.616 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Valproic acid	Plant	NA[1]		
	Earthworm	1993.75 mg L^{-1}	ECOSAR	2.8E-04
	Soil microorganism	NA[1]		
Endocrine disrupting compounds				
4-t-octylphenol	Plant	NA[1]		
	Earthworm	8.773 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Bisphenol A	Plant	NA[1]		
	Earthworm	NA[1]		
	Soil microorganism	115 mg L^{-1}	Tobajas et al., 2015	3.4E-05
Nonylphenol	Plant	650 mg kg^{-1}	Roberts et al., 2006	0.03
	Earthworm	5.130 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Nonylphenol diethoxylate	Plant	NA[1]		

	Earthworm	243.781 mg L^{-1}	ECOSAR	NA^{2}
	Soil microorganism	NA^{1}		
Nonylphenol monoethoxylate	Plant	NA^{1}		
	Earthworm	195.712 mg L^{-1}	ECOSAR	NA^{2}
	Soil microorganism	NA^{1}		
Triclosan	Plant	57 mg kg^{-1}	Liu et al., 2009	0.25
	Earthworm	1.79 mg kg^{-1}	Lin et al., 2014	**8.1**
	Soil microorganism	NA^{1}		
Perfluorinated Compounds				
Perfluorooctanoic acid	Plant	107 mg kg^{-1}	Zhao et al., 2011	2.7E-04
	Earthworm	NA^{1}		
	Soil microorganism	NA^{1}		
Perfluorooctanesulfonate	Plant	95 mg kg^{-1}	Zhao et al., 2011	2.6E-04
	Earthworm	NA^{1}		
	Soil microorganism	NA^{1}		
Benzothiazoles				
2-(methylthio)benzothiazole	Plant	NA^{1}		
	Earthworm	235.249 mg L^{-1}	ECOSAR	NA^{2}
	Soil microorganism	NA^{1}		

Siloxanes				
Hexamethylcyclotrisiloxane (D3)	Plant	NA[1]		
	Earthworm	162.055 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Octamethylcyclotetrasiloxane (D4)	Plant	NA[1]		
	Earthworm	164.386 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Decamethylcyclopentasiloxane (D5)	Plant	209 mg kg^{-1}	Velicogna et al., 2012	0.12
	Earthworm	156.329 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA[1]		
Dodecamethylcyclohexasiloxane (D6)	Plant	NA[1]		
	Earthworm	142.719 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA		
Octamethyl trisiloxane (L3)	Plant	NA		
	Earthworm	133.769 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA		
Decamethyl tetrasiloxane (L4)	Plant	NA		
	Earthworm	124.137 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA		

Dodecamethyl pentasiloxane (L5)	Plant	NA		
	Earthworm	108.637 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA		
Tetradecamethylhexasiloxane (L6)	Plant	NA		
	Earthworm	91.542 mg L^{-1}	ECOSAR	NA[2]
	Soil microorganism	NA		

[1]Experimental EC_{50}/LC_{50} values were not available in the literature and they could not be predicted *via* the ECOSAR model.

[2]EC_{50}/LC_{50} value was not taken into account for $RQ_{soil,terrestrial}$ values' calculation, as the predicted value was higher than the solubility of the target compound.

[3]Compound's K_{oc} value was not available to apply the equilibrium partitioning method.

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Table S10

Partition Coefficients (K_{oc}) predicted by the PCKOCWIN model and aquatic acute toxicity data of the studied compounds (the lowest EC50/LC50 value, obtained from toxicological studies or ECOSAR model, is presented).

Analytes	**Partition Coefficient**	**Acute toxicity data**				
	K_{oc} (L kgoc^{-1})	**References**	**Organism**	**EC50/LC50 (ng mL^{-1})**	**PNEC$_{water}$ (ng mL^{-1})**	**PNEC$_{soil,aquatic}$ (ng g^{-1} dw)**
Pharmaceuticals						
8-OH mirtazapine	NA[1]	NA[2]				
Acetylsalicylic acid	1.000E+01	Cleuvers, 2004	Daphnia magna	72.8	72.8	14.6
Amitriptyline	5.047E+05	Predicted by ECOSAR	Algae	0.043	0.043	434
Atorvastatin	NA[1]	Santos et al., 2013	Daphnia magna	0.086	0.086	*
Azithromycin	NA[1]	Harada et al., 2008	Algae	0.019	0.019	*
Caffeine	1.000E+01	Predicted by ECOSAR	Algae	0.015	0.015	0.003
Carbamazepine	3.871E+03	Fernández et al., 2010	Daphnia magna	13.8	13.8	1068
Cimetidine	9.187E+02	Kim et al., 2007	Algae	0.787	0.787	14.5
Ciprofloxacin	3.551E+01	Yang et al., 2008	Algae	6.70	6.70	4.76
Citalopram	2.537E+04	Christensen, 2007	Algae	1.60	1.60	812

Clarithromycin	NA[1]	Yang et al., 2008	Algae	0.046	0.046	*
Clomipramine	4.677E+04	Predicted by ECOSAR	Algae	0.016	0.016	15.0
Clozapine	5.212E+04	Predicted by ECOSAR	Algae	1.579	1.579	1646
Diclofenac	8.333E+02	Brandhof and Montforts, 2010	Fish	5.30	5.30	88.3
Doxycycline	6.463E+03	Predicted by ECOSAR	Daphnia magna	2.893	2.893	374
Enrofloxacin	8.349E+01	Santos et al., 2010	Daphnia magna	131.7	131.7	220
Ephedrine	8.220E+01	Predicted by ECOSAR	Daphnia magna	23.805	23.805	39.1
Fluoxetine	2.074E+05	Brooks et al., 2003	Algae	0.024	0.024	99.6
Ibuprofen	3.943E+02	Ginebreda et al., 2010	Algae	4.0	4.0	31.5
Lorazepam	1.995E+03	Predicted by ECOSAR	Algae	1.683	1.683	67.2
Mefenamic acid	4.612E+02	Kim et al., 2009	Daphnia magna	3.95	3.95	36.4
Metformin	1.409E+02	Cleuvers, 2003	Daphnia magna	64.0	64.0	180
Methylprednisolone	6.012E+01	Predicted by ECOSAR	Algae	22.519	22.519	27.1
Metoprolol	6.224E+01	Cleuvers, 2003	Algae	7.30	7.30	9.09
Mirtazapine	2.821E+04	Predicted by ECOSAR	Algae	0.814	0.814	459
Naproxen	3.493E+02	Cleuvers, 2004	Daphnia magna	166.3	166.3	1162
Niflumic acid	1.204E+03	Predicted by ECOSAR	Daphnia magna	7.731	7.731	186
Norclozapine	7.487E+05	NA[2]				*
Norephedrine	5.663E+01	Predicted by ECOSAR	Daphnia magna	27.614	27.614	31.3

Norfloxacin	9.205E+01	Verlicchi et al, 2012	Algae	15.0	15.0	27.6
Norsertraline	2.357E+05	NA[2]				*
Nortryptiline	4.346E+05	Predicted by ECOSAR	Algae	0.058	0.058	504
Ofloxacin	4.444E+01	Ferrari et al., 2004	Algae	0.010	0.010	0.014
Oxazepam	1.207E+03	Predicted by ECOSAR	Algae	1.698	1.698	41.0
Oxolinic acid	1.000E+01	Wollenberger et al., 2000	Daphnia magna	4.60	4.60	0.92
Oxytetracycline	9.720E+01	Isidori et al., 2005	Daphnia magna	22.64	22.64	44.0
Paracetamol	6.172E+01	Kuhn et al., 1989	Daphnia magna	9.20	9.20	11.4
Paroxetine	4.320E+02	Christensen et al., 2009	Algae	0.140	0.140	1.21
Progesteron	7.987E+03	Escher et al., 2011	Fish	0.5	0.5	79.9
Propranolol	1.218E+03	Yamamoto et al., 2007	Daphnia magna	0.460	0.460	11.2
Ranitidine	2.776E+04	Predicted by ECOSAR	Daphnia magna	78.001	78.001	43306
Salicylic acid	2.396E+01	Fernández et al., 2010	Fish	37	37	17.7
Sarafloxacin	2.395E+03	Holten Lützhøft et al., 1999	Algae	16	16	766
Sertraline	3.421E+05	Johnson et al., 2007	Algae	0.0121	0.0121	82.8
Sulfapyridine	3.455E+02	Białk-Bielińska et al., 2011	Algae	5.28	5.28	184
Tetracycline	5.759E+01	Halling-Sørensen, 2000	Algae	0.09	0.09	0.104
Tramadol	8.037E+02	Montforts, 2005	Daphnia magna	0.073	0.073	1.17
Valproic acid	2.406E+01	Lammer et al., 2009	Fish	20.189	20.189	9.72

Valsartan	1.024E+06	Escher et al., 2011	Daphnia magna	580.000	580.000	68034
Venlafaxine	1.464E+03	Predicted by ECOSAR	Algae	0.653	0.653	19.1
Illicit drugs						
Codeine	1.305E+03	Predicted by ECOSAR	Daphnia magna	0.976	0.976	25.5
3 , 4 - methylenedioxyamphetamine (MDA)	3.455E+02	Predicted by ECOSAR	Algae	0.200	0.200	1.382
Methadone	7.279E+04	Predicted by ECOSAR	Algae	0.172	0.172	250
THCA	NA[1]	NA[2]				*
Endocrine disrupting compounds						
4-t-octylphenol	1.546E+04	Segner et al., 2003	Fish	0.028	0.028	8.66
Bisphenol A	7.519E+04	Brian et al., 2005	Fish	0.158	0.158	238
Nonylphenol	6.216E+04	Brian et al., 2005	Fish	0.00702	0.00702	8.73
Nonylphenol diethoxylate	9.400E+02	TenEyck and Markee, 2007	Fish	0.323	0.323	6.07
Nonylphenol monoethoxylate	2.811E+03	TenEyck and Markee, 2007	Fish	0.218	0.218	12.3
Octylphenol diethoxylate	2.387E+02	NA[2]				*
Octylphenol monoethoxylate	6.992E+02	NA[2]				*
Triclosan	1.842E+04	Orvos et al., 2002	Algae	0.0014	0.0014	0.516
Perfluorinated compounds						

Perfluoropentanoic acid	2.699E+02	NA[3]				*
Perfluorohexanoic acid	1.247E+03	NA[3]				*
Perfluoroheptanoic acid	5.761E+03	NA[3]				*
Perfluorooctanoic acid	2.662E+04	Rosal et al., 2010	Algae	96.20	96.20	51217
Perfluorononanoic acid	1.230E+05	NA[3]				*
Perfluorodecanoic acid	NA[1]	NA[3]				*
Perfluoroundecanoic acid	NA[1]	NA[3]				*
Perfluorododecanoic acid	NA[1]	NA[3]				*
Perfluorotridecanoic acid	NA[1]	NA[3]				*
Perfluorotetradecanoic acid	NA[1]	NA[3]				*
Perfluorohexanesulfonate	NA[1]	NA[3]				*
Perfluoroheptanesulfonate	NA[1]	NA[3]				*
Perfluorooctanesulfonate	1.009E+05	Ye et al., 2009	Fish	9.14	9.14	18445
Perfluorooctane sulfonamide	1.271E+06	NA[3]				*
Benzotriazoles						
1H-benzotriazole	9.962E+02	Predicted by ECOSAR	Algae	5.904	5.904	118
5,6-dimethyl-1H-benzotriazole (xylytriazole)	2.668E+03	Predicted by ECOSAR	Algae	2.484	2.484	133
Tolytriazole	1.647E+03	Predicted by ECOSAR	Algae	3.851	3.851	127

Benzothiazoles						
2-(methylthio)benzothiazole	3.118E+03	Predicted by ECOSAR	Algae	8.942	8.942	558
2-hydroxybenzothiazole	1.000E+01	Predicted by ECOSAR	Algae	0.611	0.611	0.122
Benzothiazole	9.962E+02	Nawrocki et al., 2005	Daphnia magna	24.600	24.600	490
Siloxanes						
Hexamethylcyclotrisiloxane (D3)	2.221E+03	Predicted by ECOSAR	Daphnia magna	0.078	0.078	3.46
Octamethylcyclotetrasiloxane (D4)	1.660E+04[1,4]	Redman et al, 2012	Fish	0.010	0.010	3.32
Decamethylcyclopentasiloxane (D5)	8.846E+03	Redman et al, 2012	Daphnia magna	0.0029	0.0029	0.513
Dodecamethylcyclohexasiloxane (D6)	4.086E+04	Redman et al, 2012	Algae	0.002	0.002	1.63
Tetradecamethylcyclo-heptasiloxane (D7)	1,888E+05	NA[3]				*
Octamethyl trisiloxane (L3)	7.712E+05	Predicted by ECOSAR	Daphnia magna	0.010	0.010	154
Decamethyl tetrasiloxane (L4)	NA[1]	Predicted by ECOSAR	Fish	0.000752	0.000752	*
Dodecamethylpentasiloxane	2.078E+05	Predicted by ECOSAR	Fish	4,6E-05	4,6E-05	0.191

(L5)						
Tetradecamethylhexasiloxane (L6)	1.680E+06	Predicted by ECOSAR	Fish	2,7E-06	2,7E-06	0.091
L7[5]	NA[1]	NA[3]				*
L8[5]	NA[1]	NA[3]				*
L9[5]	NA[1]	NA[3]				*
L10[5]	NA[1]	NA[3]				*
L11[5]	NA[1]	NA[3]				*
L12[5]	NA[1]	NA[3]				*
L13[5]	NA[1]	NA[3]				*
L14[5]	NA[1]	NA[3]				*

[1] PCKOCWIN program does not predict the compound K_{oc} value

[2] ECOSAR program does not recognize the compound CAS number

[3] The compounds' toxicity is not possible to be predicted by ECOSAR model

[4] Surita and Tansel, 2014

[5] Polydimethylsiloxanes

* $PNEC_{soil,aquatic}$ value was not calculated, as K_{oc} or/and EC_{50} values were not available

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Table S11

Average measured environmental concentrations ($MEC_{sludge,\ average}$) of emerging organic contaminants (EOCs) in dewatered sewage sludge obtained from Greek STPs (in ng g^{-1} dw) and the corresponding predicted concentrations ($PEC_{soil,\ average}$) in soil one year after a single sludge application (all sludge samples were grab).

Target Compounds	**Sampling Area**	**Number of samples**	**$MEC_{sludge,\ average}$ (ng g^{-1} dw)**	**$PEC_{soil,\ average}$ (ng g^{-1} dw)**
	Pharmaceuticals			
8-OH mirtazapine	Athens and Santorini Island	13	9.0	0.013
Acetylsalicylic acid	Athens and Santorini Island	13	179	0.26
Amitriptyline	Athens and Santorini Island	13	113	0.17
Atorvastatin	Athens	8	24.0	0.035
Azithromycin	Athens and Santorini Island	13	139	0.20
Caffeine	Athens and Santorini Island	13	34.0	0.05
Carbamazepine	Athens and Santorini Island	13	56.6	0.083
Cimetidine	Athens and Santorini Island	13	15.7	0.023
Ciprofloxacin	Athens and Santorini Island	13	87.1	0.13
Citalopram	Athens and Santorini Island	13	127	0.19
Clarithromycin	Athens and Santorini Island	13	40.6	0.060
Clomipramine	Athens and Santorini Island	13	24.7	0.036

Clozapine	Athens and Santorini Island	13	42.9	0.063
Diclofenac	Athens, Santorini Island and Mytilene	45	60.1	0.088
Doxycycline	Athens and Santorini Island	13	118	0.17
Enrofloxacin	Athens	8	11.9	0.018
Ephedrine	Athens and Santorini Island	13	118	0.17
Fluoxetine	Athens and Santorini Island	13	32.0	0.047
Ibuprofen	Athens and Mytilene	32	168	0.25
Lorazepam	Athens	8	24.5	0.036
Mefenamic acid	Athens	8	119	0.18
Metformin	Athens and Santorini Island	13	121	0.18
Methylopredisolone	Athens	8	28.7	0.042
Metoprolol	Athens and Santorini Island	13	19.3	0.028
Mirtazapin	Athens and Santorini Island	13	37.0	0.054
Naproxen	Athens and Mytilene	32	541	0.80
Niflumic acid	Athens and Santorini Island	13	63.2	0.092
Norclozapine	Athens	8	36.7	0.054
Norepherdine	Athens	8	11.1	0.016
Norfloxacin	Athens and Santorini Island	13	124	0.18
Norsertraline	Athens	8	88.0	0.13

Nortryptiline	Athens and Santorini Island	13	25.1	0.037
Ofloxacin	Athens and Santorini Island	13	80.9	0.12
Oxazepam	Athens	8	18.4	0.027
Oxolinic acid	Athens	8	64.3	0.095
Oxytetracycline	Athens and Santorini Island	13	51.2	0.075
Paracetamol	Athens	8	75.4	0.11
Paroxetine	Athens	8	29.9	0.44
Progesterone	Athens	8	135	0.20
Propranolol	Athens and Santorini Island	13	24.2	0.036
Ranitidine	Athens and Santorini Island	13	15.7	0.023
Salicylic acid	Athens and Santorini Island	13	113	0.17
Sarafloxacin	Athens	8	22.8	0.034
Sertraline	Athens and Santorini Island	13	88.2	0.130
Sulfapyridine	Athens and Santorini Island	13	24.5	0.036
Tetracycline	Athens and Santorini Island	13	65.0	0.096
Tramadol	Athens and Santorini Island	13	28.3	0.042
Valproic acid	Athens and Santorini Island	13	127	0.187
Valsartan	Athens	8	172	0.253
Venlafaxine	Athens and Santorini Island	13	47.0	0.069

	Illicit drugs			
Codeine	Athens and Santorini Island	13	19.2	0.028
MDA	Athens and Santorini Island	13	25.4	0.037
Methadone	Athens	8	10.7	0.016
THCA	Athens	8	118	0.174
	Endocrine disrupting compounds			
4-t-octylphenol	Kallikrateia	5	179	0.26
Bisphenol A	Athens, Mytilene, Chalkida, Nafplion, Herakleion and Kallikrateia	64	703	1.03
Nonylphenol	Athens, Mytilene and Kallikrateia	64	4421	6.5
Nonylphenol diethoxylate	Athens, Mytilene, Chalkida, Nafplion, Herakleion and Kallikrateia	64	2758	4.06
Nonylphenol monoethoxylate	Athens, Mytilene, Chalkida, Nafplion, Herakleion and Kallikrateia	64	3552	5.2
Octylphenol diethoxylate	Kallikrateia	5	16.1	0.024
Octylphenol monoethoxylate	Kallikrateia	5	8.1	0.012
Triclosan	Athens, Mytilene, Chalkida, Nafplion, Herakleion and Kallikrateia	64	1831	2.7
	Perfluorinated compounds			

Perfluoropentanoic acid	Athens and Mytilene	26	3.2	0.005
Perfluorohexanoic acid	Athens and Mytilene	26	2.3	0.003
Perfluoroheptanoic acid	Athens and Mytilene	26	1.9	0.003
Perfluorooctanoic acid	Athens and Mytilene	26	4.3	0.006
Perfluorononanoic acid	Athens and Mytilene	26	2.0	0.003
Perfluorodecanoic acid	Athens and Mytilene	26	2.0	0.003
Perfluoroundecanoic acid	Athens and Mytilene	26	134	0.20
Perfluorododecanoic acid	Athens and Mytilene	26	0.78	0.001
Perfluorotridecanoic acid	Athens and Mytilene	26	2.3	0.003
Perfluorotetradecanoic acid	Athens and Mytilene	26	0.47	0.0007
Perfluorohexanesulfonate	Athens and Mytilene	26	1.3	0.002
Perfluoroheptanesulfonate	Athens and Mytilene	26	1.5	0.002
Perfluorooctanesulfonate	Athens and Mytilene	26	5.3	0.008
Perfluorooctane sulfonamide	Athens and Mytilene	26	0.71	0.001
	Benzotriazoles			
1H-benzotriazole	Athens	16	93	0.14
Xylytriazole	Athens	16	4	0.006
Tolytriazole	Athens	16	123	0.18
	Benzothiazoles			

2-(methylthio)benzothiazole	Athens	16	57	0.083
2-hydroxybenzothiazole	Athens	16	99	0.15
Benzothiazole	Athens	16	116	0.17
	Siloxanes			
Hexamethylcyclotrisiloxane (D3)	Athens	7	9	0.013
Octamethylcyclotetrasiloxane (D4)	Athens	7	110	0.16
Decamethylcyclopentasiloxane (D5)	Athens	7	15100	22.2
Dodecamethylcyclohexasiloxane (D6)	Athens	7	5030	7.4
Tetradecamethylcycloheptasiloxane (D7)	Athens	7	800	1.18
Octamethyl trisiloxane (L3)	Athens	7	220	0.32
Decamethyl tetrasiloxane (L4)	Athens	7	56	0.082
Dodecamethylpentasiloxane (L5)	Athens	7	220	0.32
Tetradecamethylhexasiloxane (L6)	Athens	7	3630	5.3
L7[1]	Athens	7	6520	9.6
L8[1]	Athens	7	8510	12.5
L9[1]	Athens	7	10700	15.7
L10[1]	Athens	7	11300	16.6
L11[1]	Athens	7	7870	11.6

L12[1]	Athens	7	3380	5.0
L13[1]	Athens	7	1100	1.6
L14[1]	Athens	7	450	0.66

[1] Polydimethylsiloxanes

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Table S12

Reported concentrations of triclosan in treated wastewater of European Sewage Treatment Plants (STPs). Information is also given for the type and the number of samples and the type of treatment.

Country	Type of treatment before sampling	Number of samples	Type of samples	Treated wastewater concentration ($\mu g\ L^{-1}$)				Reference
				Min	Max	Mean	Median	
Spain	Secondary biological treatment (activated sludge) and tertiary treatment (coagulation, flocculation, filtration and disinfection by UV)	7	Composite	0.037	0.064	0.048	0.044	Carmona et al., 2014
	Secondary biological treatment (nitrogen removal) and tertiary treatment (coagulation, flocculation, filtration and disinfection by UV)	7	Composite	0.036	0.071	0.054	0.057	
	Secondary biological treatment (activated sludge with phosphorus removal) and tertiary treatment (coagulation, flocculation, filtration and disinfection by UV)	7	Composite	0.009	0.071	0.036	0.041	
Spain	Secondary biological treatment (activated sludge)	8	Grab	N.D.[1]				Nallanthigal et al., 2014
Spain	Secondary biological treatment and tertiary treatment	10	Grab	*	*	0.093	*	Matamoros and

	(coagulation, flocculation, lamellar settlement, filtration and disinfection by UV and chlorination)							Salvadó, 2013
	Secondary biological treatment and tertiary treatment (coagulation, flocculation, lamellar settlement, filtration and disinfection by UV and chlorination)	10	Grab	*	*	0.041	*	
Spain	5 STPs: Secondary biological treatment (activated sludge)	8	Composite	*	*	0.126	*	Martínez Bueno et al., 2012
		9	Composite	*	*	0.159	*	
		22	Composite	*	*	0.594	*	
		12	Composite	*	*	0.343	*	
		15	Composite	*	*	0.281	*	
Spain	3 STPs: 2 with secondary biological treatment (activated sludge) and 1 with primary treatment	*	Composite	*	*	*	0.016	Rodil et al., 2012
Spain	3 STPs: Secondary biological treatment (1 with upflow anaerobic sludge blanket reactor and 2 constructed wetlands, surface and horizontal subsurface flow)	48	Composite	*	*	*	*	Reyes-Contreras et al., 2011
Spain	Secondary biological treatment	2	Composite	0.075	0.215	0.145	0.145	Rodríguez et al., 2011
Spain	Secondary biological treatment	2	*	*	*	0.071	0.071	Ricart et al., 2010

	Secondary biological treatment and tertiary treatment (microfiltration system)	2	*	*	*	0.066	0.066	
	Secondary biological treatment and tertiary treatment (reverse osmosis)	2	*	*	*	0.029	0.029	
Spain	*	2	*	0.141	0.178	0.160	0.160	Villaverde-de-Sáa et al., 2010
Spain	*	3	*	<LOQ[2]				Pedrouzo et al., 2010
Spain	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	*	*	<LOQ[2]	0.512	0.219	*	Rosal et al., 2010
Spain	*	3	*	<LOD[3]				Pedrouzo et al., 2009
Spain	*	3	*	*	*	0.028	*	Regueiro et al., 2009a
Spain	*	*	*	<LOD[3]				Regueiro et al., 2009b
Spain	5 STPs: Secondary biological treatment (activated sludge)	20	Composite	0.060	0.719	0.209	*	Gómez et al., 2009
Spain	Secondary biological treatment	4	Grab	*	*	0.059	*	González-Mariño et al., 2009
Spain	Secondary biological treatment (activated sludge)	2	Grab	0.074	0.104	0.089	0.089	Montes et al., 2009
Spain	Secondary biological treatment (activated sludge with carbonaceous organic matter and nitrogen removal) and tertiary	*	*	0.024	1.10	0.31	*	Muñoz et al., 2009

	treatment (membrane treatment)							
	Secondary biological treatment (activated sludge with phosphorous and nitrogen removal)	*	*	0.052	2.50	0.34	*	
Spain	5 STPs:	3	*	*	*	0.317	*	Brun et al., 2008
	Primary treatment	3	*	*	*	0.081	*	
		3	*	*	*	0.097	*	
		3	*	*	*	0.608	*	
		3	*	*	*	0.584	*	
Spain	Secondary biological treatment (activated sludge) and tertiary treatment (membrane bioreactors)	16	Composite	0.085	0.554	0.159	0.144	Kantiani et al., 2008
	Secondary biological treatment (activated sludge)	7	Composite	0.112	0.586	0.266	0.217	
	Secondary biological treatment (activated sludge)	2	Composite	0.225	0.471	0.348	0.348	
	Secondary biological treatment (activated sludge)	2	Composite	0.020	0.176	0.098	0.098	
	Secondary biological treatment (activated sludge)	2	Composite	0.099	0.188	0.144	0.144	
	Secondary biological treatment (activated sludge)	1	Composite	0.40				
	Secondary biological treatment (activated sludge)	2	Composite	0.083	0.090	0.087	0.087	
	Secondary biological treatment (activated sludge)	3	Composite	0.375	1.283	0.790	0.712	
	Secondary biological treatment (activated sludge)	1	Composite	0.402				
Spain	Secondary biological treatment (activated sludge)	10	*	*	0.045	0.045	*	Kuster et al., 2008

Spain	Secondary biological treatment	7	Composite	0.20	0.70	*	*	Farré at al., 2008
	Secondary biological treatment and tertiary treatment (membrane bioreactor)	8	Composite	0.10	0.60	*	*	
	Secondary biological treatment and tertiary treatment (membrane bioreactor)	8	Composite	0.10	0.20	*	*	
Spain	Secondary biological treatment (activated sludge)	*	Composite and grab	0.08	0.40	0.20	*	Gomez et al., 2007a
Spain	*	3	Grab	0.115	0.268	0.198	0.212	Gomez et al., 2007b
Spain	Secondary biological treatment	2	Composite	0.209	0.321	0.265	0.265	Canosa et al., 2005
Spain	Primary treatment	9	*	0.1	269	47.8	2.8	Mezcua et al., 2004
Spain	Primary treatment	4	*	0.4	22.1	10.7	10.2	Agüera et al., 2003
Greece	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	3	Composite	N.D.[1]	0.131	<LOQ[2]	*	Kosma et al., 2014
	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	3	Composite	N.D.[1]	0.288	0.134	*	
	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	3	Composite	N.D.[1]	<LOQ[2]	*	*	
	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	3	Composite	N.D.[1]	<LOQ[2]	*	*	

	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	3	Composite	N.D.[1]	*	*	*	
	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	3	Composite	N.D.[1]	<LOQ[2]	*	*	
	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	3	Composite	N.D.[1]	*	*	*	
	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	3	Composite	N.D.[1]	0.452	0.139	*	
Greece	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	9	Grab	0.07	0.15	0.11	*	Samaras et al., 2013
	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	9	Grab	0.04	0.24	0.13	*	
Greece	Secondary biological treatment (activated sludge) and tertiary treatment (sand filtration and chrorination)	3	Grab	0.025	0.087	0.056	*	Stamatis and Konstantinou, 2013
Greece	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	14	Composite	0.031	0.211	0.067	0.058	Stasinakis et al., 2013
Greece	Secondary biological treatment (activated sludge)	6	Grab	0.075	0.120	0.101	*	Stasinakis et al., 2012
Greece	Secondary biological treatment (activated sludge)	1	Composite	0.078				Samaras et al., 2011
Greece	Secondary biological treatment (activated sludge with nitrogen	*	*	N.D.[1]	<LOQ[2]	*	*	Kosma et al., 2010

	and phosphorus removal)							
	Secondary biological treatment (activated sludge with nitrogen and phosphorus removal)	*	*	N.D.[1]		*	*	
Greece	Secondary biological treatment (activated sludge)	*	Grab	<LOD[3]	<LOQ[2]	*	*	Antoniou et al., 2009
Greece	Secondary biological treatment (activated sludge)	5	Grab	*	*	0.076	*	Pothitou & Voutsa, 2008
Greece	3 STPs: Secondary biological treatment (activated sludge)	30	Composite and grab	<LOD[3]	6.88	1.10	0.43	Stasinakis et al., 2008
Greece	Secondary biological treatment (activated sludge)	3	Grab	0.230	1.12	0.593	0.43	Gatidou et al., 2007
Greece	Secondary biological treatment (activated sludge)	*	Grab	*	*	0.19	*	Paxéus, 2004
	Secondary biological treatment (activated sludge)	*	Composite	*	*	0.13	*	
United Kingdom	Secondary biological treatment (activated sludge)	3	Grab	*	*	0.170	*	Petrie et al., 2014
	Secondary biological treatment (full-scale trickling filter)	3	Grab	*	*	0.264	*	
United Kingdom	162 STPs: 98 with secondary biological treatment and 64 with tertiary treatment	*	Grab	*	*	*	0.2	Gardner et al., 2012
United Kingdom	Secondary biological treatment (activated sludge) and tertiary treatment	1	*	0.011				Price et al., 2010

	Secondary biological treatment and tertiary treatment	1	*	0.128				
	Secondary biological treatment (activated sludge)	5	*	0.053	0.157	0.107	*	
	Secondary biological treatment (activated sludge)	1	*	0.044				
	Secondary biological treatment (activated sludge)	3	*	0.086	0.109	0.099	*	
	Secondary biological treatment	8	*	0.137	0.341	0.223	*	
	Secondary biological treatment	9	*	0.153	0.461	0.33	*	
	Secondary biological treatment and tertiary treatment	8	*	0.106	0.244	0.184	*	
	Secondary biological treatment and tertiary treatment	11	*	0.034	0.239	0.174	*	
	Secondary biological treatment and tertiary treatment	1	*	0.213				
	Secondary biological treatment and tertiary treatment	1	*	0.216				
	Secondary biological treatment (activated sludge)	1	*	0.043				
	Secondary biological treatment (activated sludge)	2	*	0.020	0.028	0.024	0.024	
	Secondary biological treatment (activated sludge)	1	*	0.018				
	Secondary biological treatment (activated sludge) and tertiary treatment	4	*	0.017	0.025	0.021	*	
	Secondary biological treatment (activated sludge) and tertiary treatment	1	*	0.248				
	Secondary biological treatment	3	*	0.203	0.220	0.213	*	
	Secondary biological treatment and tertiary treatment	2	*	0.117	0.482	0.30	0.30	

	Secondary biological treatment (activated sludge) and tertiary treatment	1	*	0.042				
	Secondary biological treatment and tertiary treatment	1	*	0.138				
	Secondary biological treatment and tertiary treatment	1	*	0.160				
United Kingdom	*	*	*	*	*	0.328	*	Guitart and Readman, 2010
United Kingdom	Secondary biological treatment (trickling filter beds)	*	Grab	<LOQ[2]	0.052	0.025	*	Kasprzyk-Hordern et al., 2009
	Secondary biological treatment (activated sludgewith BOD/COD and nitrogen removal)	*	Composite and grab	0.013	0.082	0.057	*	
United Kingdom	Secondary biological treatment (rotating biological contactors and reed beds)	3	Grab	0.145	1.117	0.510	0.267	Thompson et al., 2005
	Secondary biological treatment (oxidation ditches)	3	Grab	0.004	0.104	0.055	0.056	
	Secondary biological treatment (biofilters and polishing lagoon)	2	Grab	0.040	0.29	0.165	0.165	
United Kingdom	Secondary biological treatment (rotating biological contactor and reed beds	*	Composite	*	*	0.069	*	Kanda et al., 2003
	Secondary biological treatment (submerged aerated filter)	*	Composite	*	*		*	
	Secondary biological treatment (oxidation ditch)	*	Composite	*	*		*	
	Secondary biological treatment (two biological filter beds	*	Composite	*	*		*	

	system)							
	Secondary biological treatment (activated sludge, non nitrifying and nitrifying)	*	Composite	*	*		*	
	Secondary biological treatment (activated sludgewith trickling filters)	*	Composite	*	*		*	
United Kingdom	Secondary biological treatment (activated sludge)	*	Composite and grab	*	*	1.1	*	Sabaliunas et al., 2003
	Secondary biological treatment (trickling filter)	*	Composite and grab	*	*	0.34	*	
Germany	Secondary biological treatment (planted sand-based unsaturated pilot-scale vertical flow wetland)	10	Grab	*	*	0.06	*	Ávila et al., 2014
	Secondary biological treatment (planted sand-based unsaturated pilot-scale vertical flow wetland)	10	Grab	*	*	0.05	*	
	Secondary biological treatment (planted gravel-based unsaturated pilot-scale vertical flow wetland)	10	Grab	*	*	0.12	*	
	Secondary biological treatment (planted saturated pilot-scale vertical flow wetland with active aeration)	10	Grab	*	*	0.06	*	
Germany	Secondary biological treatment (unplanted pilot-scale horizontal flow constructed wetland)	19	Grab	0.32	3.25	*	1.06	Carranza-Diaz et al., 2014

	Secondary biological treatment (planted pilot-scale horizontal flow constructed wetland)	19	Grab	0.40	5.12	*	1.05	
Germany	Secondary biological treatment (activated sludgewith nutrient removal)	*	Grab	*	*	0.397	*	Strittmatter et al., 2012
Germany	Secondary biological treatment (activated sludge)	*	Composite	0.01	0.6	*	*	Bester, 2005
	Secondary biological treatment (combination of physical and activated sludge process)	*	Composite			*	*	
Germany	Secondary biological treatment	1	*	0.18				Weigel et al., 2004
Germany	Secondary biological treatment	5	Composite	0.043	0.059	0.051	0.050	Bester, 2003
France	Secondary biological treatment and tertiary treatment (powdered activated carbon)	3	Composite	0.086	0.119	0.103	*	Mailler et al., 2015
France	Secondary biological treatment and tertiary treatment (phosphorus precipitation)	12	Composite	*				Pasquini et al, 2014
France	8 STPs: Secondary biological treatment (7 with activated sludge and 1 with membrane bioreactor)	*	Composite	<LOQ[2]				Martin Ruel et al., 2010
France	Secondary biological treatment (activated sludge)	3	Composite	*	*	0.17	*	Paxéus, 2004
	Secondary biological treatment (activated sludge)	*	Composite	*	*	0.43	*	
Sweden	Secondary biological treatment	2	Composite	*	*	0.087	*	Lundström et al.,

	Secondary biological treatment and tertiary treatment (sand filter)	2	Composite	*	*	0.089	*	2010
	Secondary biological treatment and tertiary treatment (sand filter and moving bed biofilm reactor)	2	Composite	*	*	0.065	*	
	Secondary biological treatment and tertiary treatment (sand filter and ozonation)	2	Composite	*	*	0.0035	*	
	Secondary biological treatment and tertiary treatment (sand filter and moving bed biofilm reactor combined with ozonation)	2	Composite	*	*	0.0022	*	
	Secondary biological treatment (drum filter and membrane bio reactor)	2	Composite	*	*	0.016	*	
Sweden	Chemical treatment (flocculation of phosphorus with ferrus sulfate) and secondary biological treatment	2	Grab	*	*	0.09	*	Olofsson et al., 2010
Sweden	Secondary biological treatment (activated sludge with chemical phosphorous removal)	*	Composite	*	*	0.16	*	Bendz et al., 2005
Italy	*	*	Composite	<LOQ[2]				Celano et al., 2014
Italy	Secondary biological treatment (activated sludge)	*	Grab	*	*	0.58	*	Paxéus, 2004
	Secondary biological treatment (activated sludge)	*	Grab	*	*	0.7	*	
	Secondary biological treatment (activated sludge)	4	Grab	*	*	0.37	*	

Poland	Secondary biological treatment (activated sludge)	3	Composite	N.D.[1]				Kotowska et al., 2014
	Secondary biological treatment (activated sludge)	20	Composite	N.D.[1]	0.82	0.54	*	
	Secondary biological treatment (activated sludge)	9	Composite	N.D.[1]	0.10	0.06	*	
	Secondary biological treatment (activated sludge)	6	Composite	N.D.[1]	0.91	0.91	*	
	Secondary biological treatment (activated sludge)	1	Composite	0.84				
	Secondary biological treatment (activated sludge)	1	Composite	N.Q.[4]				
	Secondary biological treatment (activated sludge)	1	Composite	0.10				
	Secondary biological treatment (activated sludge)	1	Composite	0.02				
	Secondary biological treatment (activated sludge)	1	Composite	N.D.[1]				
Poland	Secondary biological treatment (activated sludge)	*	Composite	*	*	0.430	*	Nosek et al., 2014
Switzerland	Secondary biological treatment and tertiary chemical treatment	3	Composite	0.07	0.136	0.102	0.1	Lindström et al., 2002
	Secondary biological treatment and tertiary chemical treatment	1	Composite	0.183				
	Secondary biological treatment and tertiary chemical treatment	1	Composite	0.110				
	Secondary biological treatment and tertiary chemical treatment	1	Composite	0.250				
	Secondary biological treatment and tertiary chemical treatment	1	Composite	0.650				
Switzerland	Secondary biological treatment (nitrification) and tertiary treatment (flocculation and filtration)	*	Composite	*	*	0.103	*	Singer et al., 2002
	Secondary biological treatment (nitrification and anoxic zone for denitrification) and tertiary treatment (flocculation and	*	Composite	*	*	0.213	*	

	filtration)							
	Secondary biological treatment (nitrification) and tertiary treatment (flocculation and filtration)	*	Composite	*	*	0.058	*	
	Secondary biological treatment (nitrification and anoxic zone for denitrification) and tertiary treatment (flocculation and filtration)	*	Composite	*	*	0.042	*	
	Secondary biological treatment (nitrification) and tertiary treatment (flocculation and filtration)	*	Composite	*	*	0.123	*	
	Secondary biological treatment (nitrification) and tertiary treatment (flocculation and filtration)	*	Composite	*	*	0.173	*	
	Secondary biological treatment (nitrification) and tertiary treatment (flocculation and filtration)	*	Composite	*	*	0.103	*	
Czech Republic	*	6	*	0.0095	0.023	0.0144	0.014	Grabic et al., 2010
Cyprus	Tertiary treatment	*	Grab	*	*	0.0057	*	Makris and Snyder, 2010
Denmark	Secondary biological treatment (activated sludge with biological nutrient removal)	*	Composite	*	*	0.09	*	Paxéus, 2004
Norway	Primary treatment (mechanical filtration)	4	*	0.16	0.48	0.39	0.46	Weigel et al., 2004

Portugal	Secondary biological treatment (activated sludge)	*	Composite	0.5	0.8	*	*	Novo et al., 2013
Romania	*	*	Grab	*	*	0.209	*	Moldovan et al., 2007
	*	*	Grab	*	*	0.284	*	
	*	*	Grab	*	*	0.353	*	
	*	*	Grab	*	*	0.299	*	
	*	*	Grab	*	*	0.253	*	

[*] The specific data was not reported; [1]Not detected; [2] Below limit of quantification; [3] Below limit of detection; [4]Not quantified

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Table S13

Acute (EC50/LC50) and chronic (NOEC) aquatic toxicity data of TCS on algae, *Daphnia magna* and fish.

Species	Test	Duration	Dose descriptor	Value ($\mu g\ L^{-1}$)	Reference
Algae					
Selenastrum capricortunum	Biomass	72 h	EC50	4.46	Orvos et al., 2002
Scenedesmus subspicatus	Biomass	72 h	EC50	0.7	
Scenedesmus subspicatus	Growth rate	72 h	EC50	2.8	
Scenedesmus subspicatus	Biomass	96 h	EC50	1.4	
Sceletonema costatum	Biomass	72 h	EC50	> 66.0	
Navicula pelliculosa	Biomass	96 h	EC50	19.1	
Synedra sp.	Biomass	≤13 d	NOEC	0.15	Wilson et al., 2003
Selenastrum capricortunum	Growth inhibition	72 h	EC50	4.7	Tatarazako et al., 2004
Dunaliella tertiolecta	Population cell density	96 h	EC50	3.55	De Lorenzo and Fleming, 2008
Scenedesmus vacuolatus	Cell density-reproduction	24 h	EC50	1.9	Franz et al., 2008
Scenedesmus vacuolatus	Inhibition of photosynthesis	24 h	EC50	3.7	
Nitzschia palea	Growth in suspension	24 h	EC50	390	
Nitzschia palea	Growth in biofilm	24 h	EC50	430	

Selenastrum capricortunum	Growth inhibition	96 h	EC50	12	Harada et al., 2008
Pseudokirchneriella subcapitata	Growth inhibition	72 h	EC50	0.53	Yang et al., 2008
Pseudokirchneriella subcapitata	Growth inhibition	72 h	EC50	37	Rosal et al., 2010
Pseudokirchneriella subcapitata	Growth inhibition	72 h	EC50	5.1	Tamura et al., 2013
Scenedesmus subspicatus	Biomass (pH 7.0)	72 h	EC50	3.5	Roberts et al., 2014
Scenedesmus subspicatus	Biomass (pH 8.0)	72 h	EC50	9.1	
Scenedesmus subspicatus	Biomass (pH 8.5)	72 h	EC50	41.4	
Scenedesmus subspicatus	Growth rate (pH 7.0)	72 h	EC50	16.8	
Scenedesmus subspicatus	Growth rate (pH 8.0)	72 h	EC50	175.9	
Scenedesmus subspicatus	Growth rate (pH 8.5)	72 h	EC50	175.1	
Scenedesmus subspicatus	Growth rate	72 h	EC50	5.48	
Scenedesmus subspicatus	Biomass	72 h	EC50	1.62	
Crustacean					
Daphnia magna	Mortality	48 h	EC50	390	Orvos et al., 2002
Daphnia magna	Survival	21 d	NOEC	200	
Daphnia magna	Mobility inhibition (Daphtoxkit FTM)	48 h	EC50	260	Harada et al., 2008
Daphnia magna	Immobilisation	24 h	EC50	73	Lopez-Rondal et al., 2012
Daphnia magna	Immobilisation	48 h	EC50	52	
Daphnia magna	Immobilisation	48 h	EC50	180	Tamura et al., 2013

Daphnia magna	Mortality	48 h	LC50	330	Peng et al., 2013
Daphnia magna	Mortality	48 h	LC50	338	Wang et al., 2013
Daphnia magna	Immobilisation	48 h	LC50	856.8	Silva et al., 2015
Daphnia magna	Feeding inhibition	48 h	EC50	549.3	
Daphnia magna	Feeding inhibition	48 h	EC50	478.0	
Daphnia magna	Reproduction	48 h	EC50	206.2	
Daphnia magna	Mortality	24 h	LC50	350	Rozas et al., 2016
Daphnia magna	Mortality	48 h	LC50	190	
Fish					
Pimephales promelas	Mortality	24 h	LC50	500	Mayer and Ellersieck, 1986
Pimephales promelas	Mortality	96 h	LC50	360	
Oryzias latipes	Mortality post hatch	48 h	LC50	352	Foran et al., 2000
Oncorhynchus mykiss	*	*	EC50	350	Lindström et al., 2002
Pimephales promelas	Static test	24 h	LC50	360	Orvos et al., 2002
Pimephales promelas	Static test	48 h	LC50	270	
Pimephales promelas	Static test	72 h	LC50	270	
Pimephales promelas	Static test	96 h	LC50	260	
Lepomis macrochirus	Survival static test	24 h	LC50	440	
Lepomis macrochirus	Survival static test	48 h	LC50	410	

Lepomis macrochirus	Survival static test	96 h	LC50	370	
Oncorhynchus mykiss	Early life-stage toxicity test	61 d	NOEC	34.1	
Oncorhynchus mykiss	Early life-stage toxicity test, growth	61 d	NOEC	15.1	
Pimephales promelas	Survival test	7 d	NOEC	100	
Pimephales promelas	Larval growth assay	7 d	NOEC	50	
Oryzias latipes	Embryos mortality	96 h	LC50	399	Ishibashi et al., 2004
Oryzias latipes	Larvae mortality	96 h	LC50	602	
Oryzias latipes	Mortality hatch	14 d	NOEC	156	
Oryzias latipes	Hepatic vitellogenin	21 d	NOEC	162	
Oryzias latipes	Adult female morphology length	21 d	NOEC	17	
Oryzias latipes	Larvae mortality	96 h	LC50	600	Kim et al., 2009
Danio rerio	Embryos assay	96 h	LC50	420	Oliveira et al., 2009
Danio rerio	Adult assay	96 h	LC50	340	
Oryzias latipes	Mortality	96 h	LC50	210	Tamura et al., 2013
Danio rerio	Larvae hatching and survival	9 d	NOEC	26	
Xiphophorus helleri	Mortality	96 h	LC50	1,470	Liang et al., 2013
Misgurnus anguillicaudatus	Mortality	96 h	LC50	45	Wang et al., 2013
Paracanthopoma parva	Mortality	96 h	LC50	71	

Carassius auratus	Mortality	96 h	LC50	1,839	
Tanichthys albonubes	Mortality	96 h	LC50	889	
Poecilia vivipara	Mortality	96 h	LC50	513	Escarrone et al., 2016
Poecilia vivipara	Mortality	96 h	LC50	676	

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