Green Chemistry

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Journal:	*Green Chemistry*
Manuscript ID	GC-CRV-09-2019-003265.R2
Article Type:	Critical Review
Date Submitted by the Author:	19-Dec-2019
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Ecotoxicological assessment of pharmaceuticals and personal care products using predictive toxicology approaches

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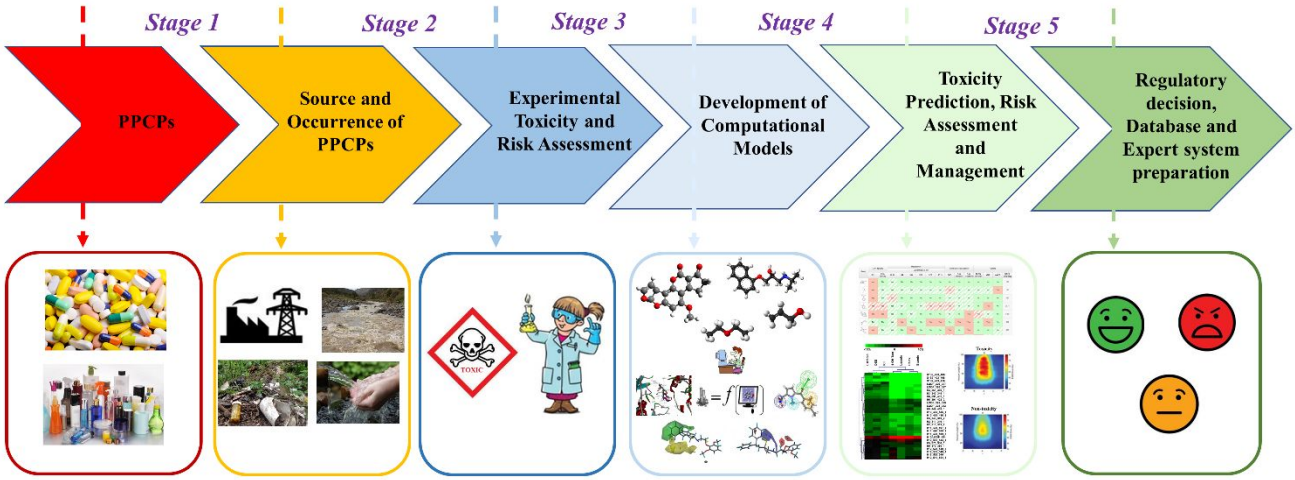
Abstract

The use of active pharmaceutical ingredients (APIs) and personal care products (PCPs) is growing day by day in all over the world. Thus, these materials have appeared as the contaminants of emerging concern (CEC) responsible for hazards and toxicity towards aquatic and terrestrial living systems as well as to humans. Regulatory agencies from all over the world have formulated multiple rules, guidelines and regulations for the risk assessment of pharmaceuticals and PCPs (PPCPs) to the ecosystem. As the generation of huge amount of experimental data is time consuming, costly, and also requires sacrifice of a large number of animals, computational modeling or *in silico* approaches are proving an efficient technique for not only risk assessment but also for risk management and data gap filling. The present review deals with critical assessment of hazardous potential of PPCPs in the environment. The importance of *in silico* modeling approaches of the environmental toxicity endpoints to diverse organisms covering all compartments of taxonomy, details of the most commonly employed endpoints, ecotoxicity databases and expert systems as rapid screening tools are discussed meticulously with completele mechanistic interpretations of *in silico* models over the years.



Graphical Abstract

1. Introduction

Active pharmaceutical ingredients (APIs) and personal care products (PCPs) have appeared as contaminants of emerging concern (CECs). It is due to their accelerating usage, perpetual disposal resulting in a pseudo-persistent existence in the environment and potentially excess toxicity towards non-target organisms due to their intrinsic mechanism of action (MoA) they have on living organisms.[1,2] Often APIs are inherently much more bioactive than PCPs, which are typically more inert, *e.g.,* PCPs such as are detergents have a nonspecific narcotic MoA. Although they are completely different from each other in respect to MoA to specific organism/species, fate and transformation in ecosystem, for ease of the discussion we use the recognized term both forms of chemicals have received as so-called 'Pharmaceuticals and Personal Care Products (PPCPs)"[1-3] throughout this review. A series of serious adverse effects on living species and ecotoxicological effects of PPCPs and their metabolites are reported[3] over the years along with their occurrence at concentrations of ng/l to µg/l in wastewater treatment plants (WWTPs)[4,5], surface water[6,7], ground water[8,9], sewage treatment plants (STPs)[10,11], marine biota[12,13], river[14,15] and lakes[16,17]. The aquatic environment is highly affected due to the intrinsic toxic effects of PPCPs and therefore the United Nations has announced the 2030 Agenda for Sustainable Development and formulated Sustainable Development Goal number 6 "*to ensure availability and sustainable management of water and sanitation for all*".[18] Regulatory agencies like European Parliament and US Environmental Protection Agency (US EPA) endorsed multiple rules and regulation to identify potential contaminants under PPCPs to include them in 'priority list'[19] and 'contaminant candidate list (CCL-3)'[20], respectively. Although in a regulatory context, for WWTPs with sound technologies, the effluent is rarely causing environmental risks due to APIs only, there can be risks for sure when there is little wastewater treatment and large batches are operational.

The fact of PPCP occurrence and environmental toxicity (the present manuscript deals with environmental toxicity only, so adverse drug reactions (ADRs) or toxicity related to humans is not discussed in the present review) is quite known and studied over the years but to understand the real scenario, one has to understand that majority of PPCPs exist as a complex mixture of individual constituents which exert toxicity either synergistically or through antagonism.[21] Another significant point is consideration of toxicity of active pharmaceutical ingredient (API) only by neglecting the effects of all possible transformed products (TPs) or metabolites of specific API in its life cycle which is offering wrong evaluation of risk associated with PPCPs.[22] There are hundreds of evidences that metabolites are more persistent, bioaccumulative, and toxic than the parent molecules, which suggests the importance of identification of all possible metabolites followed by their toxicity assessment like APIs.[23] For instance, about thirty active metabolites of carbamazepine with genotoxic effect have been detected in WWTPs[24] and thirteen TPs of diclofenac in freshwater generated by photolysis are toxic in nature.[25] One of the major metabolites of ibuprofen is 4-isobutylacetophenone (4-IBAP) which showed toxicity towards cultured erythrocytes and fibroblasts at a concentration of 1mM under in vitro study.[26]

Analysis of diverse aquatic environment leads to identification of around 600 PPCPs and their TPs in the surface water, ground water, STPs, WWTPs and soil sample covering 71 countries[27,28] where majority of them cover antibiotics,[29] analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs),[30] anticancer,[31] cardiovascular,[32] CNS acting drugs[24] and hormones[33] under pharmaceuticals; and disinfectants,[34] fragrances,[35] preservatives[36] and UV filters[37] under PCPs. Pharmaceuticals like diclofenac (1.2 μg/l),[38] metoprolol (1.54 μg/l),[39] 17β-estradiol (0.013 μg/l),[39] carbamazepine (2.1 μg/l),[38] clofibric acid (0.2 μg/l),[40] erythromycin (1.7 μg/l)[39] *etc.* were detected in major river waters over the years. Norfloxacin and ciprofloxacin were reported with a median concentration of 0.12 μg/l and 0.02 μg/l in 139 surface stream water samples of the USA[41] whereas

ciprofloxacin was detected in wastewater of Swiss hospitals with a concentration of 0.7–124.5 μg/l.[42] Even in drinking water, diclofenac, propylphenazone, and clofibric acid were detected in Berlin, Germany;[43] carbamazepine, paracetamol, and diclofenac were found in southern France;[44] diazepams and clofibric acid were identified in Milan, Italy[45] in ng/l concentration. Ibuprofen and its metabolic product ibuprofen methyl ester were detected and quantified with a concentration of 0.93 μg/l and 4.95 μg/l, respectively in drinking water.[46] One of the common APIs in contraceptive pills is 17α-ethinylestradiol (EE2) detected in samples of tap water and ground water.[47] Antimicrobial agents like triclosan (TCS), triclocarban (TCC) were detected in WWTPs samples ranging from 50 to 200 ng/l[48] whereas TCS reported in biosolids, surface water, and WWTPs with concentration of 0.09-16.79 mg/kg, 75 ng/l and 23 to 434 ng/l, respectively in Australia.[49] Insect repellant N, N-Diethyl-meta-toluamide (DEET) was detected in a WWTP of North Carolina, and the observed concentration in the ground water of the adjacent site was 540 to 1010 ng/l.[50] Although the existence of different PPCPs in diverse samples have been evaluated and quantified over the years, still the risk associated data is quite low. Therefore, to evaluate the toxicity of PPCPs to diverse species of environment, improved analytical detection techniques are very much necessary.

Continuous detection of PPCPs in different environment compartments lead to the introduction of guidelines for risk assessment associated with the PPCPs by the United States Food and Drug Administration (US FDA) and The European Medicines Agency (EMA) (previously known as European agency for the evaluation of medicinal products (EMEA)). The EMA guideline was introduced in 2006 which is a marketing authorization application for any medicinal product for human usage.[51] The US FDA guidance suggested that any API with a possible concentration of 1 μg/l in the aquatic environment required a complete risk assessment report before market approval.[52] The European Union (EU) Directive 2015/495/EU amended[53] the previous watch list of contaminants of emerging concern prepared under Directive 2013/39/EU.[54] The final watchlist

consists of pharmaceuticals like diclofenac, 17-alpha-ethynilestradiol (EE2), clarithromycin, azithromycin, erythromycin, estrone E1; and PCPs like UV filter octinoxate and food additive butylated hydroxytoluene.[55]

Unavailability of sufficient experimental risk assessment data and restriction of animal studies encourage *in silico* or computational approaches to fill the risk assessment data gaps followed by prediction of possible risk hazards much before chemical's physical synthesis and/or market approval.[56] Most importantly, *in silico* approaches represent economical and time-saving techniques with respect to orthodox experimental approaches. Regulatory bodies like US EPA, European Union Commission's Scientific Committee on Toxicity, Ecotoxicity and Environment (CSTEE) and regulation like Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) under EU had endorsed in silico approaches for toxicity and fate prediction of PPCPs.[57] Among the *in silico* approaches, quantitative structure-activity relationship (QSAR) is one of the most commonly practiced techniques for ecotoxicity prediction. Few QSAR models have been developed to model toxicity endpoints of PPCPs to diverse species over the years.[58-64] But to address the complex issue of prediction for mixtures, metabolites and a large number of untested compounds, knowledge-based expert systems (KBES) [Assessment Tools for the Evaluation of Risk (ASTER), Computer Assisted Evaluation of industrial chemical Substances According to Regulations (CAESAR), DEREK, Ecological Structure Activity Relationships (ECOSAR), OECD Tool box, TOPKAT] have a huge role to play in the present scenario.[65, 66] Integration of available ecotoxicity databases (ACToR, ChEMBL, ECOTOX, eTOX, Integrated Risk Information System (IRIS), OECD HPV, TOXNET) with expert system requires the usage of artificial intelligence.[65, 66]

The aim of the present review is to provide guidance concerning improved and reliable application of computational models for ecotoxicity prediction. These can be used both in risk assessments as well as in risk management through designing of environmentally safer PPCPs. Existing ecotoxicity

QSAR models helps us to understand the major features and structural fragments related to intrinsic chemical reactivity followed by chemical's environmental fate, transformation, and toxicity. Therefore, a good number of existing computational ecotoxicity models are thoroughly interpreted along with the models dealing with removal of hazardous PPCPs from ecosystem through advanced materials. The responsibility of regulatory authorities related to environmental safety and their role in implementing computational models in environmental risk assessment and management purpose is illustrated. Along with the importance of prediction models for the risk assessment, the green chemistry (GC) has a vital role to play in the risk management by reducing the intrinsic risk associated with PPCPs from the beginning of the risk cycle. A combination of *in silico* technique with GC is capable of designing safer chemicals (here, APIs of PPCPs) by reducing ADRs as well as ecotoxicity (the present review deals with this aspect) taking into consideration of all possible physicochemical properties, structural fragments responsible for toxicity, reactive metabolites, toxicity pathway and pharmacokinetic and pharmacodynamics (PK/PD) nature of individual PPCP. We have provided inclusive lists of environmental toxicity endpoints, databases and test species to have idea about reasonable sources to construct computational models for risk assessment and management. A thorough introspection of expert systems are also discussed for readily available ecotoxicity prediction models both for experts and novice users. The real challenges of mixture toxicity and risk associated with the metabolites or TPs of PPCPs for ecotoxicity prediction are clarified in details.

2. OCCURRENCE AND ECOTOXICITY OF PHARMACEUTICALS

Occurrence and concentration of pharmaceuticals and their TPs in various compartments of the environment are directly related to the sources.[1] Thus, a clear and precise idea about major sources and life cycle of any pharmaceutical is utmost necessary. Most common sources and route to aquatic, terrestrial and soil environments for any pharmaceutical is portrayed in **Figure 1**. Existence of

pharmaceuticals in the environment needs to be monitored continuously along with their acute and chronic toxicity evaluation by standard test methods employing regulatory guidelines depending on the nature of marketing approval and country laws.

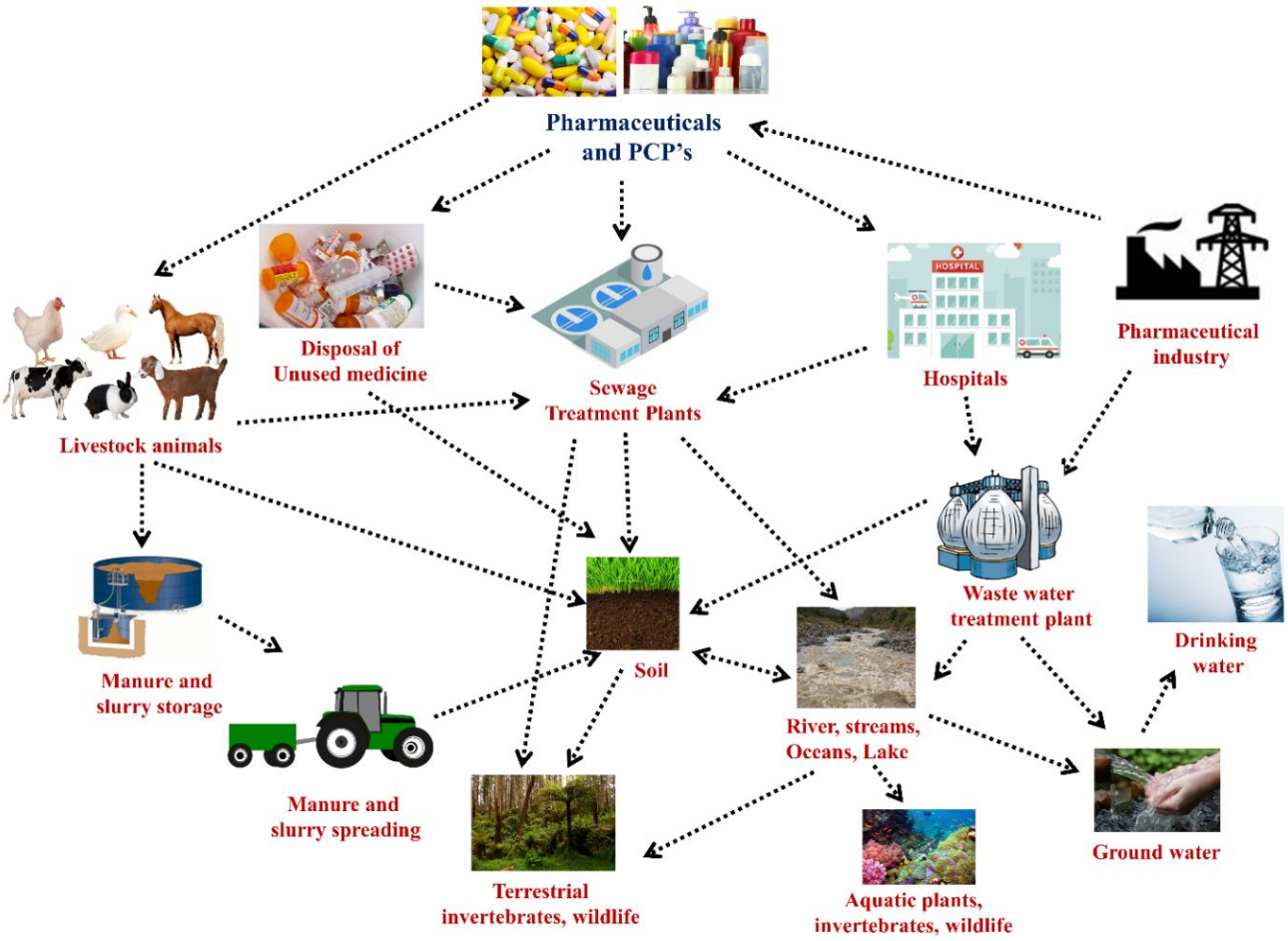


Figure 1 Source and complete life cycle of PPCPs in the diverse environmental compartments.

2.1 Active Pharmaceutical Ingredients (APIs)

2.1.1 Antibiotics: The maximum detected concentration of ciprofloxacin in wastewater were 1.4 µg/l in Holland,[67] 3.7 µg/l in Italy,[68] 0.6 µg/l in Canada[69] and 6.9 µg/l in Australia[70] while average concentration of sulfamethoxazole in same WWTPs were 1.8 µg/l in Canada[69] and 1.7 µg/l in Europe.[71] Out of 12 soil samples, chlortetracycline and tetracycline found in 10 samples with

average concentration of tetracycline in three different layers of soil were 86.2 μg/kg in 0-10 cm layer, 198.7 μg/kg in 10-20 cm layer and 171.7 μg/kg in 20-30 cm layer while chlortetracycline was detected with average concentration of 4.6-7.3 μg/kg in all three sublayers.[72] Amoxicillin was detected in WWTP of Delhi, India with a concentration of 172.6 ng/l in influent and 62.5 ng/l in effluent,[73] while much higher concentration (100–2000 ng/l) was found in the activated sludge of a WWTP in Japan.[74] Cefuroxime (0.6 μg/l), ampicillin (17.7 μg/l), sparfloxacin (0.5 μg/l) and gatifloxacin (3.7 μg/l) were identified in effluent of WWTP in Delhi, India.[75] Twelve sulfonamides were identified in bacteria, non-target plants and algae in aquatic environment where inhibition assays reported EC_{50} values ranged from >250 mg/l for all sulfonamides whereas sulfadimethoxine exhibits growth inhibition of duckweed with concentration of 0.02 mg/l.[76] Ciprofloxacin was found to be active at a concentration of 5 mg/l to *Allivibrio fischeri*.[77]

2.1.2 β-blockers: In hospital effluents, propranolol and metoprolol were detected with concentrations of 6.5 mg/l and 25.1 mg/l, respectively.[78] Propranolol was detected with 100% frequency and median concentration of 76 ng/l in the STP effluent sample in UK.[79] Martin *et al.*[80] found propranolol at concentration of 3.37 mg/kg in the sediment collected from Guadiamar River in Spain. Propranolol affects the reproduction of *C. dubia* with the no-observed-effect-concentration (NOEC) and lowest-observed-effect concentrations (LOEC) of 125 and 250 μg/l, respectively whereas in case of *H. Azteca*, the concentration was 100 μg/l after exposure for 27 days.[81] Metoprolol exhibited a negative chronotropic effect on the heart of *D. magna* at high concentration (10^{-4} M) exposure and positive chronotropy at low concentration (ranges from 10^{-8}-10^{-6} M).[82] The NOEC and LOEC values for embryo-larval growth rate of 3.2 mg/l and 10 mg/l, respectively were obtained for fathead minnows under atenolol exposure.[83]

2.1.3 Analgesics and Nonsteroidal Anti-inflammatory Drugs (NSAIDs): NSAIDs like paracetamol, ibuprofen and diclofenac were reported in higher concentration between 0.4 ng/l and 15 µg/l in surface water.[84] Paracetamol was present with a concentration of 78.17 µg/l in surface water and in STP effluents the concentration can reach around 20 ng/l to 4.3 µg/l while most of the values were higher than the predicted no-effect concentration (PNEC) of 9.2 µg/l.[83] Ibuprofen was found in a German WWTPs with maximum concentrations of 3.5 and 0.3 mg/l in influent and effluent.[85] Ashton et al.[79] investigated STP effluent samples from East Hyde, Great Billing, Corby, Harpenden and Ryemeads in the UK and found ibuprofen (frequency 84%, at concentration of 3086 ng/l), dextropropoxyphene (frequency 74%, at concentration of 195 ng/l), diclofenac (frequency 86%, at concentration of 424 ng/l) and mefenamic acid (frequency 81%, at concentration of 133 ng/l) at reasonably high concentrations. In Guadiamar River water of Spain, salicylic acid and naproxen were detected at concentrations of 9.49 and 11.2 mg/kg, respectively.[80] Sediment samples analysis from Llobregat, Iberian River basins, Jucar, Ebro, and Guadalquivir in Spain reported ibuprofen at a high concentration of 13 ng/g.[86] Naproxen was detected in the STP effluent and Mississippi River in Louisiana at concentrations 81-106 ng/l and at 22-107 ng/l, respectively.[87] Most commonly detected and toxic analgesic is diclofenac with reported EC_{50} below 100 mg/l,[88] whereas phytoplanktons are highly sensitive to it in acute and high-level exposure with EC_{50} of 14.5 mg/l at 96 hours.[89] Renal lesions and gill alterations in trout fish (concentration of 5 mg/l with 28days exposure) are also reported for diclofenac.[90] An analysis suggested that naproxen exerts protein and lipid oxidation followed by oxidative DNA damage in the *H. Azteca*.[91] Diclofenac causes inner organs damage in rainbow trout[92] and its detected concentration in surface water sample in 12 countries exceed the PNEC of 0.1mg/l.[93] Reproduction of *D. longispina* and *D. magna* was affected with acetylsalicylic acid at a concentration of 1.8 mg/l.[90] Chronic toxicity of ibuprofen was observed towards water flea and *D. magna* with concentrations ranging from 0 to 80 mg/l. In case of naproxen, the EC_{50} values were 30.1 or 50 mg/l for *D. magna* which is the most sensitive species towards it.[83]

2.1.4 Antineoplasic/Anticancers: Tamoxifen has been found at alarming concentrations in surface water and WWTP samples with maximum concentrations of 25 ng/l and 102 ng/l, respectively.[94] Anastrozole, used in hormone-based chemotherapy, has been measured with a high frequency with maximum concentrations of 0.3-0.4 ng/l in WWTP effluent and 2.38-3.70 ng/l in hospital effluent.[95] Tamoxifen has been detected with a frequency of 4% and at a concentration of <10 ng/L in STP effluent samples of UK.[79] 5-fluorouracil and cisplatin showed growth inhibition of cyanobacteria *Synechococcus leopoliensis* (EC_{50} 1.20 and 0.67 mg/l, respectively) and algae *Pseudokirchneriella subcapitata* (EC_{50} 0.13 and 1.52 mg/l, respectively).[96] Zounkova et al.[97] reported cytarabine and 5-fluorouracil exerted reproduction inhibition of *D. magna* (EC_{50} 10 and 0.1 mg/l, respectively) and growth inhibition of *P. putida* (EC_{50} 17 and 0.044 mg/l, respectively). Methotrexate exhibited acute toxicity to *Tetrahymena pyriformis* and teratogenicity to fish embryos with EC_{50} of 45 mg/l for 48h[98] and 85 mg/l after 48h[99], respectively.

2.1.5 Blood lipid lowering agents: The lipid regulator bezafibrate was noticed in river water of Germany at concentration of 3.5 mg/l.[38] Based on the sediment sample analysis from different rivers (Iberian River basins, Jucar, Llobregat, Ebro and Guadalquivir) of Spain, gemfibrozil was detected as one of the most frequently found pharmaceuticals at a concentration of 6 ng/g.[86] In Canadian STP samples, carbamazepine was found at a concentration of 2.3 mg/l.[100] Fibrates was found to be toxic in toxicity tests towards *C. dubia*, *B. calyciflorus* and zebrafish with reported NOEC values of 640 µg/l (7 days), 246 µg/l (2 days) and 70 mg/l (10 days), respectively.[92] Clofibrate is specifically toxic to aquatic species with reported LC_{50} value of 7.7-39.7 mg/l while fish *Gambusia holbrooki* is the most sensitive one with LC_{50} (96 h) of 7.7 mg/l.[101] Bezafibrate and gemfibrozil are toxic towards nano-target organism with EC_{50} values of 10 to 100 mg/l and 1 to 10

mg/l, respectively.[102] Exposure of gemfibrozil for 14 days on *Carssius auratus* exhibited 50% reduction on plasma testosterone.[103]

2.1.6 CNS Acting Drugs: Thioridazine (antipsychotic) and carbamazepine (antiepileptic) were detected in Medway River, UK in upstream sewage effluent samples at concentration of 6-22 ng/l and 53-265 ng/l, respectively.[104] According to a study performed in Cape Cod, Massachusetts, most frequently found antiepileptic drugs are carbamazepine and phenytoin detected in well water samples at maximum concentrations of 72 and 66 ng/l, respectively.[105] Paraxanthine detected in agriculture land as well as in western Lake Erie basin in Ohio at maximum concentration of 1.8 mg/l.[106] Carbamazepine exhibits carcinogenic effect to rats while does not have mutagenic effect to mammals.[107] Chronic toxicity analysis suggested NOEC values of carbamazepine are 377 μg/l (2 days), 25 μg/l (7 days) and 25 mg/l (10 days) toward *B. calyciflorus*, *C. dubia* and zebrafish, respectively.[92] Carbamazepine showed acute toxicity through growth inhibition of *D. magna* at concentration of 17.2 mg/l.[107] Experimental studies suggested that the *in vitro* growth of *T. gondii* is inhibited by mood stabilizer valproic acid and antipsychotic drug haloperidol.[108] Sertraline demonstrates high toxicity towards rainbow trout with LC_{50} of 0.38 mg/l at a 96-h exposure.[109]

2.1.7 Antiviral and antiparasitic drugs: Extremely high concentrations (2-12 mg/l) of oseltamivir and its bioactive metabolite oseltamivir carboxylate (OC) were detected in WWTPs in the time of pandemic suggesting almost 80% of the active drug is excreted from the source.[110] In a farm of UK, higher concentration of antiparasitic pharmaceuticals were found in dung (doramectin 0.112 mg/kg and ivermectin 1.85 mg/kg) and soil (around 0.046 mg/kg) while the detected concentration of the studied drugs in soil was relatively low than the dung.[111] In river waters of the UK, the most frequently (59%) detected drug is clotrimazole at a maximum concentration of 22 ng/l,

and a mean concentration of 7 ng/l.[112] Fenbendazole and ivermectin affect the survival of *Pristionchus maupasi* with concentration of 10-20 mg dung/kg and 3 mg dung/kg, respectively.[113]

2.1.8 Hormones: 17α-estradiol and estriol are found in high concentration (about 180 and 590 ng/l, respectively) in WWTPs samples in USA.[114] EE2, an estrogenic hormone, was detected in surface waters of each UN regions. Based on the analysis data collected from North America, Europe and southeast Asian countries, the identified concentration range were from 0.001 to 0.040 mg/l.[115] In an experiment in a Canadian lake, feminization of male fish (*Pimephales promelas*) occurred at concentrations of 5 ng/l to 6 ng/l of EE2 which is capable to hamper complete population collapse of the studied species.[116] Egg fertilization reduction in flathead minnow fish reported with exposure of EE2 with concentration of 320 pg/l for 150 days post hatching.[117] Hydrocortisone is capable of intensifying the ectoparasitic infections in fish while estradiol enhanced the vulnerability of cyprinids to hemoflagellates through the suppression of lymphocyte proliferation.[118]

2.2 Metabolites

In spite of good amount of research regarding occurrence and toxicity of pharmaceuticals to the environment, comparatively a handful of data is accessible regarding likely biotic and abiotic TPs of parent APIs, namely, degradation products, metabolites and/or conjugates.[119] The EMA[120] and the Veterinary International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH)[121] have released guiding principles related to which toxic and stable TPs need to be evaluated and included in the risk assessment study. The EU REACH regulation also suggested the assessment of toxicity and bioaccumulation of TPs are necessary for any marketed APIs.[122] As major orthodox analytical methods are unable to identify the trace levels of TPs in the environment, three strategies are considered for future screening:[123-124] (a) Target analysis

with reference standards, (b) Suspect screening with suspected substances without reference standards, and (c) Nontarget screening with no reference standards and no prior information.

As erythromycin (ERY) is not stable in aquatic media, thus it is immediately converted to its TP erythromycin-H_2O (ERY-H_2O), which is one of the most studied TP in the environment among antibiotics and used as marker to detect ERY in any sample. The identified concentrations of ERY-H_2O in WWTPs are 1978[125] and 6000 ng/l[126] in China and Germany, respectively. ERY-H_2O detected in concerning concentration in drug manufacturing effluents (7840 ng/l) and hospital sewage (6110 ng/l).[127] Four hydrolysis TPs of amoxicillin (AMX) antibiotic [(5S)-AMXO, (5R)-AMXO, (5R)-AMX- diketopiperazine-2′,5′ and (5S)-AMX- diketopiperazine-2′,5′] are detected in the influent and effluent samples of WWTPs in Spain.[128] Active metabolite of metronidazole antibiotic is hydroxylated metronidazole (METR-OH) identified in WWTPs and in hospital swage in Portugal where the concentration in hospital effluents reach upto 11 µg/l.[129] Among statins, o-hydroxy-atorvastatin and p-hydroxy atorvastatin are two major metabolites of atorvastatin are identified in WWTPs influents with a concentration of 196 and 280 ng/l, respectively whereas much lower concentration (10 ng/l) of simvastatin hydroxyl acid, which is a TP of simvastatin detected in influents of WWTPs.[130] Antiviral drug oseltamivir (OSL) and its carboxylated TP oseltamivir carboxylate (OSLCAR) are detected in wastewater (42.7 ng/l in influents and 17.3 ng/l in effluents) which may cause serious hazards as OSL-CAR resistance in wildfowl, and birds are capable hosts of influenza viruses.[131] Three metabolites (2-hydroxy-estradiol (2-OHE2), 2-hydroxy estrone (2-OHE1) and 4-hydroxyestrone (4-OHE1)) of the estradiol hormone are detected at a concentration level of 14 ng/l in WWTP influents in UK.[132]

Metabolites of NSAIDS and analgesics are frequently detected in STPs and WWTPs. The most common TPs are salicylic acid (SA), carboxy-ibuprofen (CX-IBU), 1-hydroxy-ibuprofen (1′-OH-

IBU), 2-hydroxy-ibuprofen (2′-OH-IBU), 4-hydroxy-diclofenac (4′-OH-DCF), 4-hydroxydiclofenac-dehydrate (4′-OH-DCF-H_2O), 5-hydroxydiclofenac (5′-OH-DCF), 4-hydroxyacelofenac (4′-OH-ACF), 1,5-dimethyl-1,2-dehydro-3-pyrazolone (DP), 1-acetyl-1-methyl-2-phenylhydrazide (AMPH) and1-acetyl-1-methyl-2-dimethyloxamoyl-2-phenylhydrazide (AMDOPH).[119] SA is the active metabolite of acetylsalicylic acid which is detected with a frequency of 100% in WWTPs and surface water.[133] CX-IBU and OH-IBU are detected at very high concentration in WWTP influents (38.4 and 6840 ng/l, respectively) and effluents (10.6 and 1130 ng/l, respectively).[134] Clofibric acid is the hydrolyzed metabolic product of clofibrate and etofibrate recurrently identified in industrial, municipal and hospital waste water up to 41.4 μg/l level.[135] Fenofibric acid, a TP of fenofibrate, was detected at concentrations of 349 ng/l in WWTPs in Spain.[136] Among antidepressant drugs, hydroxyl (3-hydroxy-diazepam,10-hydroxy-amitriptyline, hydroxy-bupropion) and desmethyl (N-desmethyl venlafaxine, desmethyl sertraline, desmethyl citalopram, O-desmethyl venlafaxine, didesmethyl citalopram, nortriptyline) metabolites are most regularly detected ones in waste waters with concentrations of 5500 and 2000 ng/l for O-desmethyl-venlafaxine and hydroxy-bupropion, respectively.[137] Hydroxy-tamoxifen (OH-TMX) and 4,4-dihydroxy desmethyltamoxifen (endoxifen) are major metabolites of tamoxifen (TMX) showed higher estrogenicity than the TMX detected in waste water sample of hospitals.[138]Around 30 metabolites are formed for carbamazepine (CBZ), but major reported metabolites in the ecosystems are CBZ-10,11-epoxide (CBZ-Ep) formed through oxidation (pharmacologicallyactive with anticonvulsant properties), hydration product 10,11-dihydro-10,11-trans-dihydroxy-carbamazepine (DiOH-CBZ), followed hydroxylated TPs 2-hydroxy-CBZand 3-hydroxy-CBZ.[24] The metabolite DiOH-CBZ wasthe predominant analyte in the aqueous phase with concentrations higher than the parent compound, reaching few μg/l in influents and effluents as well (up to 4000 and 3400 ng/l, respectively). On the otherhand, CBZ-Ep, which is present in human plasma at 3- to 4-fold lower concentrations than DiOH-CBZ, was found in some cases at concentrationsat least 50-fold lower than those of DiOH-CBZ in sewage.[24]

Di-desmethyl, N-desmethyl and N-oxide metabolic products of amitriptyline and imipramine showed toxic effects towards *Spirostomum ambiguum* and *Thamnocephalus platyurus* in experiemntal studies.[139] Norfluoxetine, a major metabolite of fluoxetine is known to bioaccumulate in fish tissues and exert 50% higher toxicity than the parent chemical, whereas another metabolite trifluoromethylphenol exhibits a lower toxicity.[140] Metabolites of aspirin or acetylsalicylic acid showed toxicity to embryos of zebrafish with EC50 of 37 mg/l by SA;[99] and acute and chronic toxicity to *Daphnia longispina* and *Daphnia magna* by gentisic acid.[141] Based on multiple fish reproduction studies, the recommended PNEC for EE2 is 0.35 ng/l in surface water.[142] Almost similar level of toxicity was experienced for freshwater and marine species through exposure of CBZ-Ep compare to CBZ while the toxicity concern was higher in case of 2-OH-CBZ, 3-OH-CBZ and DiOH-CBZ.[143] *V. fisheri* exhibits higher toxicity under the exposure of 2-OH-CBZ and 3-OH-CBZ compare to parent compound CBZ.[143]

In **Table S1 (See Supporting Information)**, we have reported the concentrations of pharmaceuticals from diverse therapeutic classes in different samples covering countries from all continents along with ecotoxicity data to definite toxicological endpoints.[29-33,83,133-134,144-167] **Figure 2** portrayed the existence of a number of pharmaceuticals identified in surface water, ground water and drinking water all over the world.[1] The world Health Organization (WHO) had provided recommendations and practical guidance for managing the concern about pharmaceuticals in drinking-water giving uttermost emphasize in prioritizing the water safety management including microorganism present in the aquatic environment.[168] **Figure 3** represents chemical structures of top 50 pharmaceuticals including metabolites based on detection frequency as well as concerning concentrations considering available literatures.[67-168]

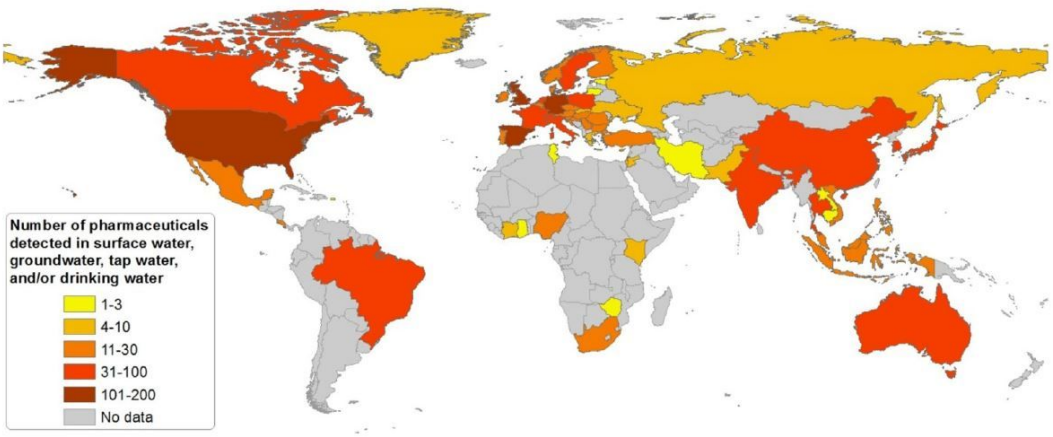


Figure 2 Number of pharmaceuticals detected in groundwater, surface waters and drinking water covering all countries. [Reprinted by taking permission from Reference[1]]

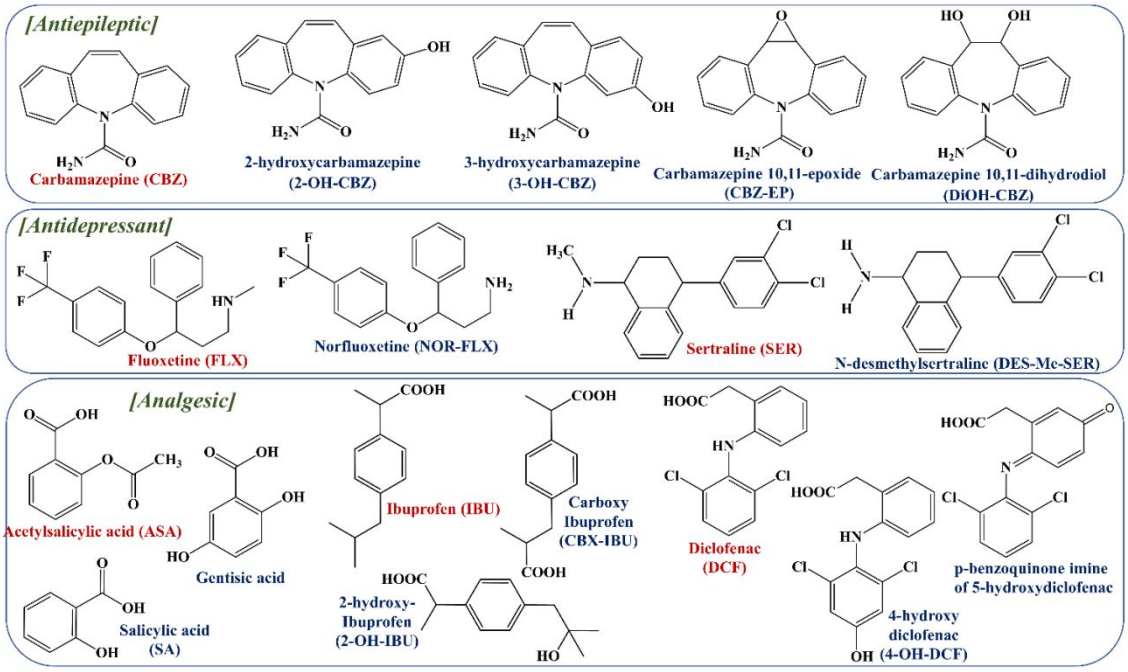


Figure 3 Chemical structures of top fifty most frequently detected pharmaceuticals in alarming higher concentrations including metabolites (Compound's name in red denotes parent compound and in blue suggests metabolite or TPs).

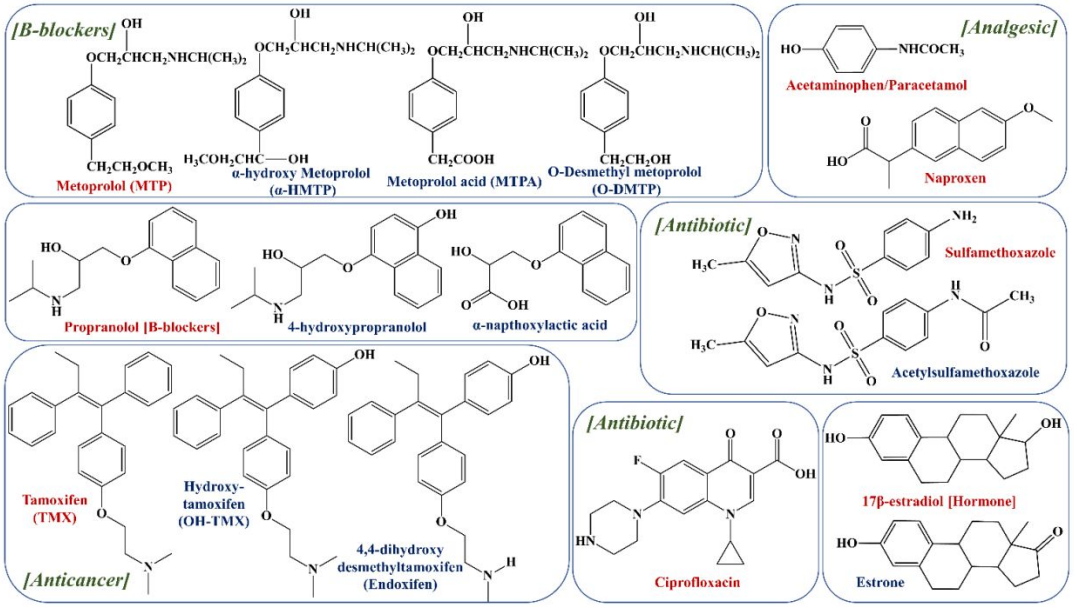


Figure 3 (Continued) Chemical structures of top fifty most frequently detected pharmaceuticals in alarming higher concentrations including metabolites (Compound's name in red denotes parent compound and in blue suggests metabolite or TPs).

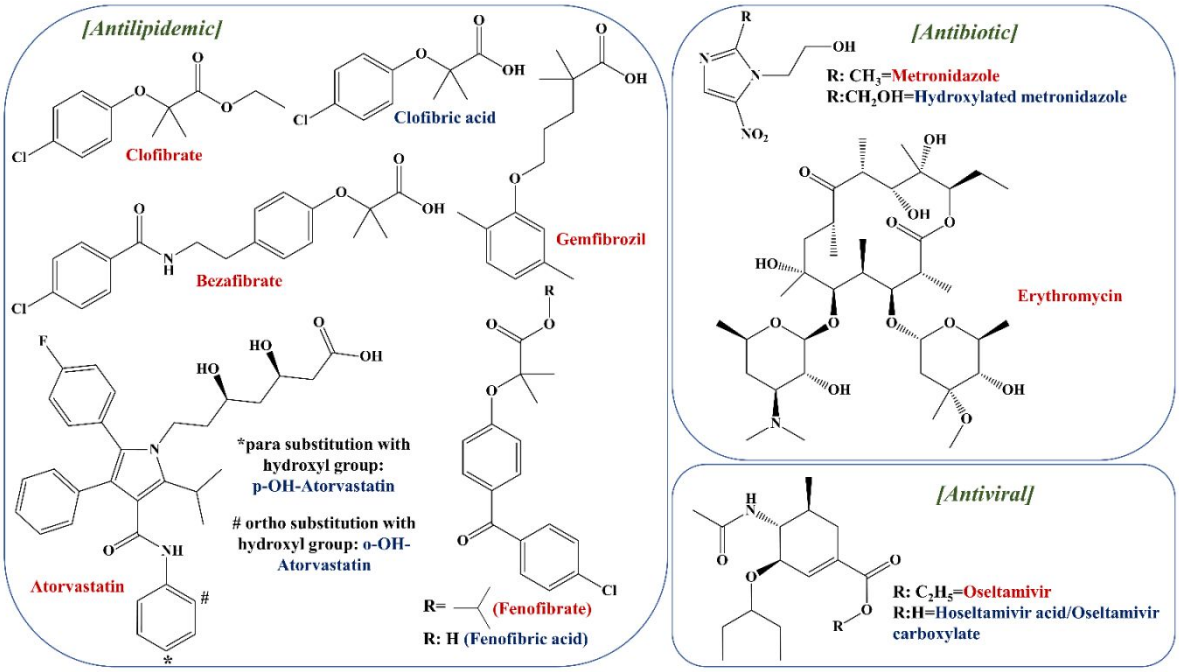
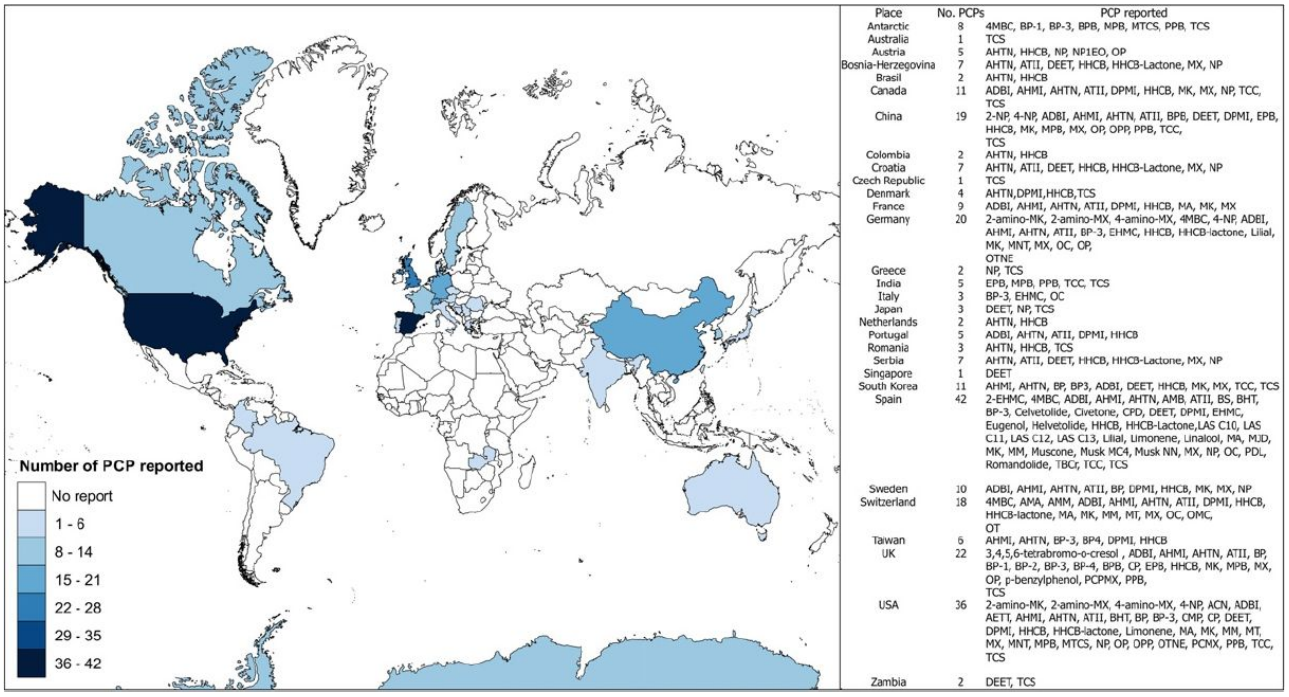


Figure 3 (Continued) Chemical structures of top fifty most frequently detected pharmaceuticals in alarming higher concentrations including metabolites (Compound's name in red denotes parent compound and in blue suggests metabolite or TPs).

3. OCCURRENCE AND ECOTOXICITY OF PERSONAL CARE PRODUCTS (PCPS)

The PCPs are one of the major classes of emerging pollutants apart from pharmaceuticals found in the environment at a significant level of concentration worldwide which majorly contribute to the toxicity to aquatic biota and soil.[169-170] The major sources and routes to ecotoxicity of PCPs are illustrated in **Figure 1**. However, very few ecotoxicity data exist for PCPs in the public domain. PCPs include diverse group of synthetic organic chemicals used in daily life like cosmetics, soaps, toothpaste, lotions, preservatives (Methylparaben (MPB, Ethylparaben (EPB), Propylparaben (PPB)), fragrance/musk (Galaxolide (HHCB), Tonalide (AHTN), Celestolide (ADBI)), sunscreens/UV filters (2-Ethyl-hexyl-4-trimethoxycinnamate (EHMC), 4-Methyl-benzylidene-camphor (4-MBC), Octyl-methoxycinnamate (OMC), Octyl-triazone (OC)), disinfectants (Methyltriclosan, triclosan (TCS), Triclocarbon (TCC)), insect repellents (N,N-diethyl-m-toluamide (DEET), 1,4-dichlorobenzene).[170,171] Most of the PCPs are intended for external usage and therefore not subjected to metabolic changes inside the body unlike pharmaceuticals; thus, an enormous number of unaltered PCPs are washed and/or excreted into the surface water of waterbodies,[170] waste water treatment plants (WWTPs)[172] along with rivers and oceans.[13] Extensive usage, inappropriate disposal followed by ineffective treatment in WWTPs contribute as the major sources of aquatic toxicity due to PCPs and their transformed products in the ecosystem.[5,173-174] Studies have confirmed that many of them are environmentally bioactive, persistent, and display high bioconcentration and bioaccumulation in aquatic organisms.[13,170,171] A number of evidences supported endocrine disruption effect in aquatic species along with acute and chronic toxicity towards them by PCPs.[175] Considering spread of PCP usage, a little portion of PCPs are tested experimentally in different samples of environment. Thus, evaluation of toxicity data of major PCPs requires fast and precise analytical techniques for monitoring, followed by continuous investigation of their fate and transformation typically at low levels of concentration in ng/l as

majority of them exist as mixture at trace levels in aquatic environment.[176] PCPs identified in water matrices throughout different countries covering all continents are illustrated in **Figure 4**.[171]



Place	No. PCPs	PCP reported
Antarctic	8	4MBC, BP-1, BP-3, BPB, MPB, MTCS, PPB, TCS
Australia	1	TCS
Austria	5	AHTN, HHCB, NP, NP1EO, OP
Bosnia-Herzegovina	7	AHTN, ATII, DEET, HHCB, HHCB-Lactone, MX, NP
Brasil	2	AHTN, HHCB
Canada	11	ADBI, AHMI, AHTN, ATII, DPMI, HHCB, MK, MX, NP, TCC, TCS
China	19	2-NP, 4-NP, ADBI, AHMI, AHTN, ATII, BPB, DEET, DPMI, EPB, HHCB, MK, MPB, MX, OP, OPP, PPB, TCC, TCS
Colombia	2	AHTN, HHCB
Croatia	7	AHTN, ATII, DEET, HHCB, HHCB-Lactone, MX, NP
Czech Republic	1	TCS
Denmark	4	AHTN,DPMI,HHCB,TCS
France	9	ADBI, AHMI, AHTN, ATII, DPMI, HHCB, MA, MK, MX
Germany	20	2-amino-MK, 2-amino-MX, 4-amino-MX, 4MBC, 4-NP, ADBI, AHMI, AHTN, ATII, BP-3, EHMC, HHCB, HHCB-lactone, Lilial, MK, MNT, MX, OC, OP, OTNE
Greece	2	NP, TCS
India	5	EPB, MPB, PPB, TCC, TCS
Italy	3	BP-3, EHMC, OC
Japan	3	DEET, NP, TCS
Netherlands	2	AHTN, HHCB
Portugal	5	ADBI, AHTN, ATII, DPMI, HHCB
Romania	3	AHTN, HHCB, TCS
Serbia	7	AHTN, ATII, DEET, HHCB, HHCB-Lactone, MX, NP
Singapore	1	DEET
South Korea	11	AHMI, AHTN, BP, BP3, ADBI, DEET, HHCB, MK, MX, TCC, TCS
Spain	42	2-EHMC, 4MBC, ADBI, AHMI, AHTN, AMB, ATII, BS, BHT, BP-3, Celvetolide, Civetone, CPD, DEET, DPMI, EHMC, Eugenol, Helvetolide, HHCB, HHCB-Lactone,LAS C10, LAS C11, LAS C12, LAS C13, Lilial, Limonene, Linalool, MA, MJD, MK, MM, Muscone, Musk MC4, Musk NN, MX, NP, OC, PDL, Romandolide, TBCP, TCC, TCS
Sweden	10	ADBI, AHMI, AHTN, ATII, BP, DPMI, HHCB, MK, MX, NP
Switzerland	18	4MBC, AMA, AMM, ADBI, AHMI, AHTN, ATII, DPMI, HHCB, HHCB-lactone, MA, MK, MM, MT, MX, OC, OMC, OT
Taiwan	6	AHMI, AHTN, BP-3, BP4, DPMI, HHCB
UK	22	3,4,5,6-tetrabromo-o-cresol , ADBI, AHMI, AHTN, ATII, BP, BP-1, BP-2, BP-3, BP-4, BPB, CP, EPB, HHCB, MK, MPB, MX, OP, p-benzylphenol, PCPMX, PPB, TCS
USA	36	2-amino-MK, 2-amino-MX, 4-amino-MX, 4-NP, ACN, ADBI, AETT, AHMI, AHTN, ATII, BHT, BP, BP-3, CMP, CP, DEET, DPMI, HHCB, HHCB-lactone, Limonene, MA, MK, MM, MT, MX, MNT, MPB, MTCS, NP, OP, OPP, OTNE, PCMX, PPB, TCC, TCS
Zambia	2	DEET, TCS

Figure 4 Number of PCPs detected in diverse water matrices covering all countries. [Reprinted by taking permission from Reference[171]]

3.1 Disinfectants and bactericides: Biphenyl ethers like TCC and TCS are frequently detected in waste water which are generally used as antimicrobials in deodorants, soaps, lotions toothpaste and plastics.[34] TCS has been detected in surface water, ground water, STPI, marine biota worldwide[170] with a multiple study characterizing presence of its methyl derivative methyl triclosan (M-TCS) in WWTP effluent (up to 650 ng/l TCS and 11 ng/l M-TCS), STP influents (0.2–16.6 µg/l of TCS) and effluents (0.08–2.7 µg/l of TCS),[5,177] surface water (74 ng/l of TCS)[7] and fish tissue (2100 ng/g of lipid of M-TCS).[178] Due to lipophilic nature, M-TCS is stable and tends to bioaccumulate with high concentration in fish.[7] In surface water, TCC, TCS and chloroxylenol (PCMX) were detected with concentrations up to 478,[14,16] 24,000[16,179] and 358,000 ng/l,[179] respectively. TCS exerts toxicity to biofilm algae and aquatic bacteria by enhancing mortality rate with a no effect concentration (NEC) of 210 ng/l[15], inhibition of the growth[180] and photosynthetic efficiency (NEC:

420 ng/l).[9,15] Among all species, algae growth was the most sensitive towards TCS and was affected at concentrations less than 1 μg/l.[181] Considering behavior pattern change, TCS is found to alter swimming performance of *Danio rerio, Oncorhynchus mykiss,* and *Oryzias latipes* at concentrations as low as 71 μg/l.[182] In surface water, TCC is found in alarming concentration in species like fishes (*Danio rerio, Oryzias latipes*), crustacean (*D. magna, Mysidopsis bahia*), planktonic copepod (*Acartia tonsa*), water flea (*Ceriodaphnia dubia, P. subcapitata*.[183,184] Recent studies showed toxicity of TCC is on the higher side to fish and aquatic invertebrate for both short- and long-term exposures than TCS.[185]

3.2 Fragrances: Commercialized synthetic fragrances are either polycyclic musks (HHCB, AHTN, ADBI) or nitro musks (musk ketone (MK), musk moskene (MM), musk tibetene (MT), musk ambrette (MA)) majorly used in deodorants, detergents and soap industry.[170] Due to high octanol–water partition coefficients (5.4 to 5.9 for polycyclic musks and 3.8 for nitro musk), they are bioaccumulating in aquatic species and benthic invertebrates with potential to accumulate in humans, and have been alleged to be endocrine disruptors.[186] In the present time, nitro musks are largely substituted with polycyclic musks due to their higher environmental persistence and aquatic toxicity.[187] HHCB and AHTN have been placed by the USEPA in the High Production Volume (HPV) list due to production over 1 million pounds per year.[188] HHCB and AHTN were frequently detected in STP influents with concentration of 0.043–13.7 μg/l all over the world whereas MX and MK found in 83 to 90% of STP effluents but at low concentrations.[170] The mean concentrations of AHTN and HHCB were 0.18 μg/l (0.05–0.44 μg/l) and 1.86 μg/l (0.45–4.79 μg/l), respectively based on the samples collected from 40 STPs.[11] In surface water also, most commonly found musk is HHCB with a concentration of 13,920 ng/l.[189] Among 33 documented fragrances in WWTPs, AHTN (influents: 0.41–68,120 ng/l, effluents: 0.05–7555 ng/l)[35] and HHCB (influents: 1.44–595,480 ng/l, effluents: 0.14–108,000 ng/l)[190] were frequently detected ones in 16 countries.

Although studies found that the amount of HHCB is under the EC_{50} for *O. latipes, Danio rerio, Mysidopsis bahia, D. magna, Acartia tonsa* and *P. subcapitata*,[183,184] but few studies also suggested it could exert toxicity due to its bioaccumulation, changes on fecundity, growth and development of exposed species.[3] The detected concentrations of HHCB in WWTPs effluents are above the threshold of chronic toxicity for species like *Neopachyloidesspinipes* (LOEC: 20,000 ng/l), *A. tonsa* (EC50: 59,000 ng/l) and *D. magna* (NOEC: 10,000 ng/l).[191] In case of species like *Potamopyrgus antipodarum* and *Capitella,* juveniles were more sensitive than the adult ones to HHCB.[192]

3.3 Insect repellants

DEET and 1,4-dichlorobenzene are commonly used as insect repellants routinely detected in surface waters[193,194] as well as in WWTP effluents throughout the United States with concentrations of 0.2 μg/l and 0.28 μg/l, respectively.[193,195] DEET is detected in 8 countries in WWTP samples with concentrations of 15.1–6900 ng/l in influents and 6.4–2110 ng/l in effluents.[196-197] Unlike other PCPs, DEET showed lower bioconcentration and bioaccumulation in aquatic organisms.[193] The detected concentration of DEET is drastically reduced in winter due to less usage.[198] The risk assessment report of moth repellant 1,4-dichlorobenzene suggested that it is sensitive to fish in long-term exposure while *D. magna* appears to be sensitive to short-term exposure[199].

3.4 Preservatives

Parabens, esters of para-hydroxybenzoic acid, are used as preservatives in cosmetics, pharmaceuticals, toiletries and food.[200] Most commonly employed parabens are substituted with alkyl or benzyl groups (benzyl paraben (BnPB), ethyl paraben (EPB), methyl (MPB), propyl (PPB), butyl paraben (BuPB)).[200] BnPB appears to be the toxic one where as MPB and EPB are least toxic with lower LC_{50} values which are 3 times less than BnPB.[201] MPB is recorded in WWTPs samples

of 4 countries with concentration of 1193.9–30,688,000 ng/l for influents and ND–155,000 ng/l for effluents.[179] In case of STP influents, the PPB and MPB are detected with concentrations of up to 20 μg/l and 30 μg/l, respectively.[202] Concerning amounts of parabens are detected in surface water with concentrations ranging from 15 to 400 ng/l.[179] Interestingly, MPB was detected in mineral water of Spain with a concentration of 40 ng/l.[202] Dobbins et al.[203] revealed that MPB and EPB are least toxic towards fish and invertebrates whereas most toxic ones are BnPB and BuPB which is supported with the following theory: toxicity increases with the increased chain length of parabens, and chlorination also noticeably increases toxicity.[204] The acute toxicity of parabens also increases with hydrophobicity and alkyl chain length increases along with increased octanol-water partition coefficient[204]. MPB was detected in fish species, marine mammals, sharks from Washington, Alaska, Florida coast with high concentration and frequency at ng/g and ng/l levels.[36,205] In few instances, EPB and PPB were found in seawater and EPB found in sediments at ng/g and ng/l level.[36] In all fish samples of Philippines, EPB, MPB and PPB were detected at concentration levels of 0.01–1.4 ng/g, 0.2–4.5 ng/g and 0.02–1.5 ng/g[206,207] whereas MPB is detected in pelagic fishes and demersal in Florida coasts with concentration of 2.1–92.9 ng/g[208] and 1.0–6.1 ng/g were found in Antarctic fishes.[209] 4-hydroxybenzoic acid (4-HB) is the only paraben metabolite detected at significant concentrations in fishes (6.4 μg/g), molluscs (68.1 μg/g), marine plants (15.7 μg/g)[208] and mammals (32.6 μg/g)[36]. A good number of studies have demonstrated elicit estrogenic responses of parabens at low concentration levels.[210]

3.5 UV filters/Sunscreen agents

UV filters (UVF) and UV stabilizers (UVS) are employed in sunscreens, cosmetics and lotions to protect skin against UV radiation. More than 10,000 tons of UV filters are used annually and released to water bodies resulting in growing concerns of adverse health effects to human as well as aquatic species due to their high hydrophobicity followed by higher bioconcentration factor.[186]

Twenty-six organic compounds are allowed as UVF according to the EU regulations,[211] and most significant ones are 3-benzylidene camphor (3-BC), benzophenone-3 (BP-3), benzophenone-4 (BP-4), 4-Methyl-benzilidine-camphor (4MBC), ethylhexyl methoxy cinnamate (EHMC), octocrylene (OC) and Octyl dimethyl-p-aminobenzoic acid (ODPABA). Due to high bioconcentration and bioaccumulation of UVF in aquatic organism, especially in fish, they are potentially toxic in nature.[170] In WWTPs, BP-4 was detected with concentrations of 6,325,000 ng/l[179] which exceeded the LOEC for *Oncorhynchus mykiss* (4,897,000 ng/l),[212] whereas BP-3 was detected in six countries with concentrations of 7–3,975,000 ng/l in influents and 1.1-2,196,000 ng/l in effluents of WWTPs.[213] BP-4 was also detected in surface water with concentration of 323,000 ng/l[179] which exceeds the predicted no effect concentration (PNEC) of 50,000 ng/l for *D. magna*.[214] Experimental studies supported that UVFs have potential endocrine disruption, estrogenic effects as well as affect on reproduction and fecundity to fishes like *O. mykiss* and *P. promelas*.[170,215] Four UV filters (EHMC, BP3, 4MBC, and OC) were found in surface water, WWTPs and in fish tissue in Switzerland where 4MBC was detected at highest concentration in all samples (2.7 μg/l in WWTP, 35 ng/l in surface water and 123 ng/g lipid tissue).[17] Again, BP-3 was detected with concentrations of 5–125 ng/l in Swiss lakes.[216] Based on worldwide sample data, OC and 4MBC were detected in WWTP effluents (77% and 95%, respectively) and surface water (14% and 86%, respectively).[170] OD-PABA was found in fishes in Hong Kong with concentrations of 6.4–10.3 ng/g[217] and in *Mytilus galloprovincialis* in Portugal with concentrations up to 800 ng/g.[218] Occurrence of OC in mammals, especially in Franciscana dolphins was detected by Gago-Ferrero et al.[219]

Table S2 (See Supporting Information) gives a broader overview of occurrence of PCPs from diverse classes in different sample types along with the ecotoxicity for specified endpoints and species.[5,8,22-23,37,160,177,181,189,197,220-257] **Figure 5** represents chemical structures of top 20 PCPs

including metabolites based on detection frequency as well as concerning concentrations considering available literatures.[169-257]

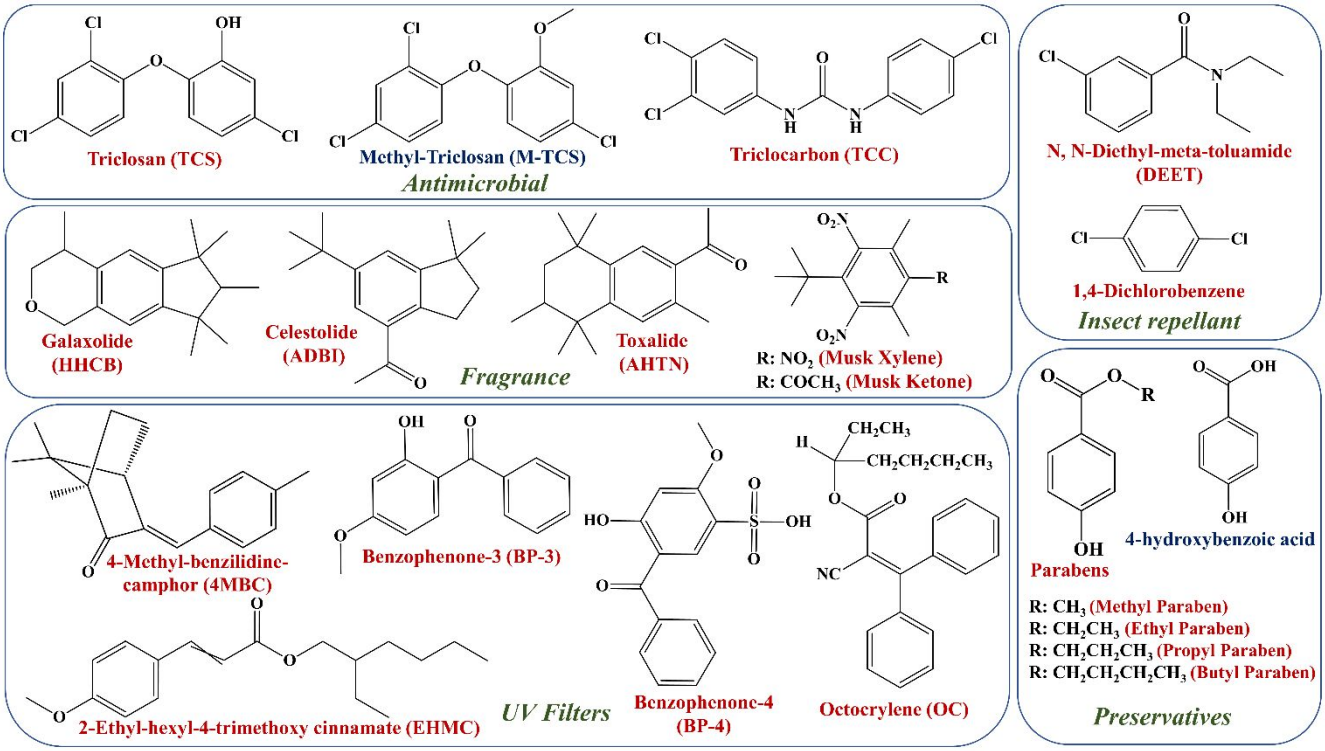


Figure 5 Chemical structures of top twenty most frequently detected PCPs in alarming higher concentrations including metabolites (Compound's name in red denotes parent compound and in blue suggests metabolite or TPs).

4. ENVIRONMENTAL RISK ASSESSMENT (ERA)

The ERA is a procedure of evaluation of the concentration, occurrence, frequency, and level of environment and human exposure of hazardous chemicals, here PPCPs. The primary objectives of ERA are risk mitigation and risk management.[258] To prepare an effective environmental policy, dependable and appropriate risk assessment is necessary. Thus, the ERA process must be prepared with updated techniques and intradisciplinary science. The purpose of majority of risk assessment data is to set risk threshold and acceptable toxicity limits for individual products before their approval for markets by regulatory agencies. Interestingly, the ERA data for pharmaceuticals projected for human use was not considered a reason for denying market approval few decades ago; even many products are already in the market without enough ecotoxicity data. In the present situation, most of the regulatory guidelines suggested that the ERA should be prepared by industries and evaluated by regulators.[51] A risk assessment has to be done for the entire life cycle of a chemical including its TPs and metabolites followed by reporting of all hazardous characteristics to different species and media along with complete environmental exposure, fate and effects (**Figure 6**).

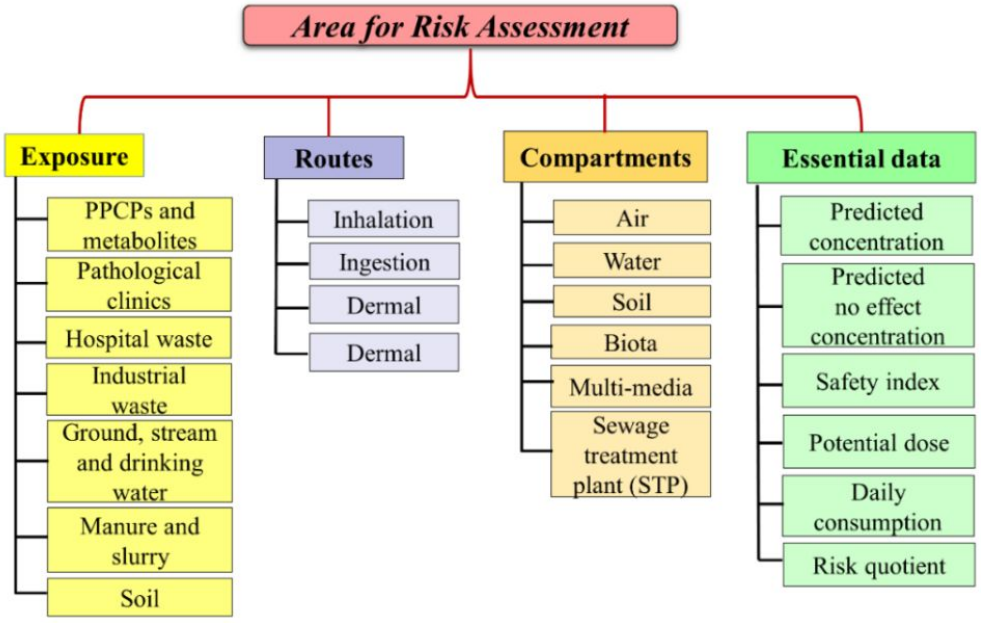


Figure 6 Area to focus for risk assessment.

4.1 ERA approaches

The risk assessment of the potential risks of PPCPs to the environment is a stepwise multi-phased procedure which depends on the regulatory authorities, and the guidelines may vary country wise. Most frequently used ERA approaches of single PPCPs along with their mixtures and metabolites are reported in **Table 1**.[259-267]

Table 1 Fundamental ERA approaches implemented by major regulatory agencies.

Method	Role
Hazard Identification	The first step is identification of source and occurrence of hazards along with the intensity of risk for a compound. In case of lack of enough *in vitro* data to explicit species and specific environment compartment, the researcher needs to rely on the *in vivo* data obtained from the sacrifice of large number of animals. Thus, a greater attention needs to be given for the proper and efficient use of *in vitro* assays in human cells along with *in silico* modeling studies to generate good number of data for hazard identification.[259]
Dose-Response Assessment	Identification of threshold dose of the toxicity is essential for scientific risk assessment of any hazards. Dose-response information over a wide range of test concentrations should be evaluated employing quantitative high throughput screening (q-HTS) technique. There should be accessibility of sensitive assays proficient of detecting toxicity at very low doses or below environmental levels experienced by living organisms. If required, statistical approaches can be used to assess critical concentrations data and extrapolate adversarial responses and to assess critical concentrations. Most importantly the extrapolation techniques will be essential to interpret *in vitro* test data in terms of *in vivo* data employing a

	suitable internal tissue dose metric.[260]
Dose and Species Extrapolation	Evaluation of low-dose toxicity and extrapolation of interspecies data are two major drawbacks. Regulatory authorities and government administrations have supported such extrapolations, including linear and threshold models for low-dose extrapolation and body weight or surface area alterations for interspecies extrapolation implementing *in silico* models and expert systems as alternatives. *In vitro* to *in vivo* extrapolation and physiologically based pharmacokinetic (PBPK) models are agreeable to sensitivity, variability, and uncertainty analysis using conventional tools.[261]
Exposure Assessment	The human exposure assessment is assessed primarily on the measured levels of environmental hazards. For few instances, internal dose measurement performed employing biomonitoring and/or pharmacokinetic modeling. For precise exposure assessment, the emphasis should be on direct measures of critical toxicity pathway agitations in humans and other significant species by employing advanced biomonitoring techniques coupled with new high throughput approaches.[262]
Risk Characterization	The final phase is risk characterization which integrates the analyses from the exposure and ecological effects characterization along with the doubts, hypothesis, strengths and limitations of the analyses. The risk characterization has two major components: (a) risk estimation and (b) risk description. Again, risk estimation compares integrated exposure and effects data in the context of Levels of Concern (LOCs) and states the potential for risk.[263]
Deterministic Approach and Calculation	The US EPA recommends the deterministic approach and the risk quotient (RQ) calculation to assess the toxicity to environment exposure. The RQ can be calculatedaccording to EMA guidelines:[264]

of Risk Quotients	$RQ = {Estimated\ esposure}/{Estimated\ toxicity}$ $= \frac{\text{Measured Environmental Concentration (MEC) in water/sedim}}{Predicted\ No-Effect\ Concentration\ (PNEC) in\ LC_{50}\ or\ EC_{50}\ c}$ Where NOEC is No observed effect concentration; $PNEC_{Acute} = {EC_{50}\ or\ LC_{50}}/{1000}$ $PNEC_{Chronic} = {NOEC}/{Assessment\ Factor\ (AF)}$ MEC corresponds to the highest measured concentration detected in samples and PNEC is estimated using the lowest values of acute EC_{50} or LC_{50} or the chronic NOEC.[265] According to Water Framework Directive (WFD), for each pharmaceutical compound, two estimations need to be made with the toxicity data obtained from the literature for three different representative trophic levels of the ecosystem, such as fish, invertebrates and algae. The first was the PNEC estimated from the acute toxicity test results and the second was the PNEC estimated from the chronic toxicity test results.[266] Thresholds[267] are following: High risk (**RQ ≥ 1**), medium risk (**0.1 < RQ < 1**) and Low risk (**0.01 < RQ < 0.1**). The computation of RQ depends upon following factors: a) ecological effects data, b) hazards use data, c) fate and transport data, and d) estimates of exposure to the hazards.
Probabilistic risk assessment	The goal of probabilistic environmental risk assessment (PERA)[268] is to estimate the likelihood and the extent of adverse effects occurring to ecological systems due to exposure(s) to substances. It is based on the comparison of an exposure concentration distribution (ECD) with a species sensitivity distribution (SSD) derived from toxicity data. So, where the deterministic risk assessment only uses single value the probabilistic uses a distribution of all the values to predict risk.

Although the steps for risk assessment by regulatory authorities are different from each other, but the basic idea is same, which is characterization and quantification of the risk associated with a specific product to definite species and environment. The major regulatory agencies role and functioning methods are discussed in detail in section 7. But, for basic understanding, how EMA under EU functions in the three-phase risk assessment process is reported in **Figure 7** as an example.

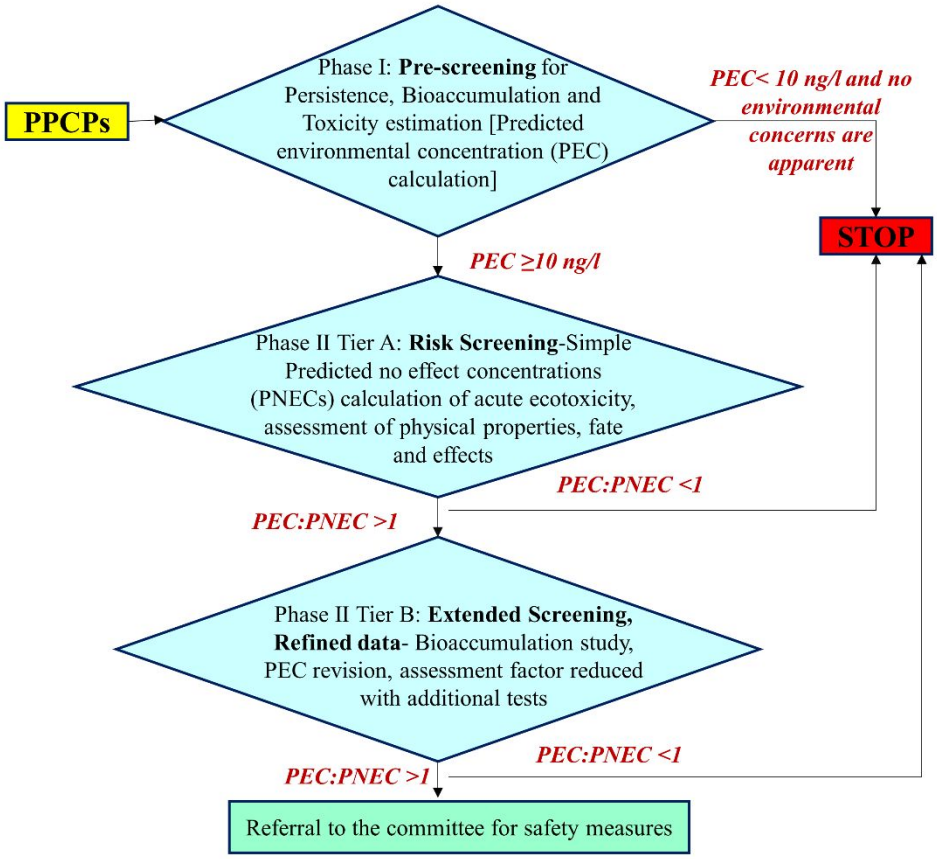


Figure 7 Three phase risk assessment process by EMA.

4.2 ERA modeling

The ERA model comprises both risk assessment and risk management processes to understand the safety issues in a quantitative manner like concentrations, dosages, and risk quotients of each PPCPs. The ERA model considers the safety issues and RQ of each chemical carefully to reflect the associated risk of it to specific species and environmental compartment. To estimate the concentration of individual products in different compartments, the guidelines developed by the

EMA and US FDA need to be followed. But, before preparation of an ERA model, one needs to evaluate the exposure of any PPCPs employing following points:[65,66]

- The exposure of a specific product needs to be evaluated in form of environmental concentration to which the system is affected along with the time period, intensity and frequency, not using concentrations to which any specific species of individual is exposed. Again, the exposure relies upon multiple parameters like the sorption effects, metabolism, fate, and transformation rate of the product.
- Species, which is affected by PPCP's toxicity, needs to be monitored for a definite period and throughout its life cycle to replicate the behavioral pattern in modeling.
- Dose-response study and PK/PD data of individual chemical are important as they are directly related to absorption, distribution, metabolism, excretion, and toxicity (ADMET) pattern.
- The toxicokinetic and comprehensive bioavailability study is required for each product.
- Knowledge of toxicity pathways and target sites in the biological system need to be understood for individual PPCP.
- The MOA is different from species to species for each PPCP; thus species wise understanding of MOA is important to portray its molecular and functional effects.
- The risk occurred from intrinsic toxicity of the pharmaceuticals due to its chemical properties is desired to be studied.

5. ENVIRONMENTAL RISK MANAGEMENT (ERM)

The risk management is a process of protecting public health by recognizing, appraising, and executing actions to decrease the risk to human health and to ecosystems associated with any hazardous/toxic product. The objective of the ERM is integrated actions to reduce or prevent the

risk effects considering cost-effective and risk benefit analysis taking into considerations of social, ethical, cultural, political, and legal aspects.[269] Examples of risk management activities comprise following:

- How much and where active substances and treated residuals will be discharged by industry,
- Decision to make which product may be stored at a hazardous waste disposal facility and how they will be treated before release to environment,
- Deciding to what level a perilous waste site must be cleaned up,
- Establishing permit levels for discharge, storage, or transport,
- Setting national ambient air and water quality standards,
- Determining permissible levels of contamination of PPCPs in drinking water.[270]

Risk assessment delivers knowledge on likely health or ecological risks, and risk management is the action taken based on the information. Thus, ERA and ERM are complementary to each other scientifically. The principle steps for ERA and ERM are portrayed in **Figure 8**. The major factors and commonly employed risk management approaches are provided in **Table 2**.

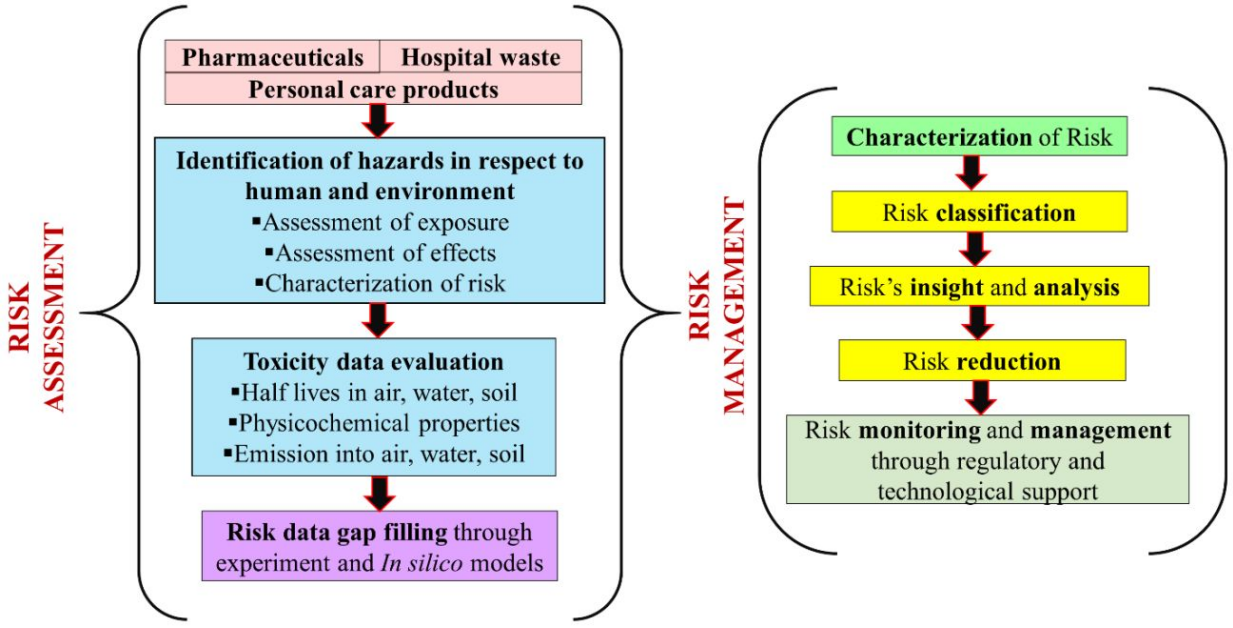


Figure 8 Fundamental steps under ERA and ERM.

Table 2 Factors and methods associated with ERM.

ERM	Type	Explanation
F A C T O R S	Scientific	Offers fundamental understanding about the risk assessment, counting information drawn from chemistry, biology, toxicology, epidemiology, ecology, and statistics.
	Economic	Calculation of the risk cost and the paybacks of reducing them, the costs of risk mitigation or remediation options and the distributional effects are important factor before choosing right management option.
	Social	Although social factors have no direct involvement, but it has an indirect role to play in ERM. Ethnic background, income level, land use, community values, availability of health care, psychological condition and life style of the suffered populations, may affect the vulnerability of an individual or a specific group to risks from a particular stressor.
	Laws and legal decisions	Outline the basis for the Agency's ERA and ERM decisions, and, in some instances, the schedule, level or methods for risk reduction.
	Technological	Includes the impacts, feasibility, and range of risk management options.
	Political	The cooperation among Federal, state, and local government authorities, and even with foreign governments are important. Most importantly, regulatory agencies and industries need to work parallelly.
	People	Peoples requirement and public values replicate the far-reaching attitudes of society about environmental risk management.
A P P	Preventive measures	A series of guidelines has been proposed by the EMA as safety measures for risk management: 1. Early assessment of risk for each marketed product,

R O A C H		2. Packaging should have apposite product labeling and summary product characteristics (SPC) for proper recycle of unused and expired product, 3. Educated patients about the possible toxicity toward humans as well as environment through Package leaflet (PL), 4. Safe storage and disposal of pharmaceutical products.
	High-end and Advanced Sewage Treatment	Majority of the risk management can be controlled with advanced waste water and sewage treatment which can treat most of the products before releasing to the environment removing or neutralizing the toxic products up to manifolds. Among the treatment, most common ones are oxidation, adsorption, photochemical and filtration.[271]
	Training and Awareness of stakeholders	Awareness and training about occurrence and effect of individual PPCPs along with their corresponding effects toward environment is important along with the facts about disposal process. The awareness needs to be spread among all the stakeholders. In this specific approach, industry has a huge role to play to generate material safety data sheets (MSDSs) for each raw materials, APIs and formulations.[272]
	Green and Sustainable Pharmacy	The approach for future which demands for environmentally benign compound. Though this method is less practiced, in terms of sustainability, it seems to be the most reassuring one in the long run. Implication of green chemistry has immense role to play in designing followed by synthesis of easy and fast degradable PPCPs to reduce. Regarding sustainability issue, the understanding of life cycle, fate, transformation and related pathway is very much important for implementation of green pharmacy in required phase.[272]

6. GLOBAL REGULATORY AGENCIES RELATED TO ECOTOXICITY OF PPCPs

The idea of ecotoxicity is a global affair which cannot be confined in certain boundaries of countries. Ecotoxicity due to PPCPs is not only related to environmental hazards but also directly connected with existence of living system on earth. Therefore, a mutual harmony and collective efforts are required from all regulatory bodies to come up with policies, guidelines and strict rules regarding safety issues and one of the burning topics of the present time, i.e., ecotoxicity due to uncontrollable usage of PPCPs. Regulatory agenciesare in chargethroughout the world for the risk characterization, risk assessment and management of PPCPs ecotoxicity andthe major ones are:

- Australian Environment Agency (AEA)[273]
- Center for drug evaluation and research (CDER) and USFDA[274]
- European Medicines Agency (EMA) for the evaluation of medicinal products[275]
- European Union Commission's scientific committee on toxicity, ecotoxicity and environment (EU-CSTEE)[276]
- The Ministry of Health, Labor and Welfare of Japan (MHLW)[277]
- National Industrial Chemicals Notification and Assessment Scheme (NICNAS)[278]
- Registration, Evaluation, Authorization and Restriction of Chemicals (REACH)[279]
- Swedish Environmental Classification and Information System (SECIS)[280]
- The UBA – Umweltbundesamt (UBA) of Germany[281]
- United States Environmental Protection Agency (US EPA)[282]
- Canadian Environmental Protection Act (CEPA)[284]

The role and responsibility of most of the agencies are slightly different from each other based on the requirement of countries environmental and industrial rules and regulations but the basic idea behind all of them are same *i.e.* the ERA and ERM of environmentally hazardous chemicals and PPCPs. If we summarize the responsibility of these agencies together then they can be following:

1. Environmental exposure/emission estimation assessment including higher tier/probabilistic modelling for aquatic exposure. [273-283]

2. Guidance on the environmental behaviour and/or fate of chemicals on the following issues: Biodegradation (persistence) in different environmental media, bioaccumulation, physical and chemical properties, interpretation and summaries of laboratory and field studies, multimedia (environmental distribution) modelling, estimation of exposure concentrations, higher tier ground and aerial spray drift modelling.[273-275]

3. Environmental hazards assessment for the aquatic environment, terrestrial environment and estimation of acceptable or "safe threshold" exposure concentrations.[273-280]

4. Environmental risk characterisation including probabilistic risk assessment and ERM advice.[273]

5. Preparation of environmental risk assessment reports within National frameworks.[273]

6. US FDA implemented a Note for Guidance paper in which all drugs entering the aquatic compartment at levels below 1$\mu g l^{-1}$ Predicted Environmental Concentration ($PEC_{EFFLUENT}$) were exempted from a detailed risk assessment.[274]

7. Pre-screening and estimation of exposure for the API, screening and initial prediction of risk, Extended and compartment-specific risk assessment are the three tier ERA of EMA.[275]

8. The risk assessment is evaluated by the PEC/PNEC ratio or $\Sigma PEC_i/PNEC_i$[277,280].

9. Circulation of safety information of substances and their effects on the human health and environment.[278]

10. Identification, evaluation and regulating “Persistent, Bioaccumulating and Toxic substances (PBT)” effectively. In addition, the REACH regulation endorses the use of valid QSARs for predicting the environmental and toxicological properties of chemicals.[279,280]

11. UBA evaluated more than 240 human pharmaceuticals and around 180 veterinary drugs. Cytostatic medicines, contrast agents and hormones dominated the human pharmaceutical dossiers measured by UBA.[281]

12. The US EPA ensures clean air, land and water and provide efforts to decrease environmental risks generated from diverse set of industrial chemicals and PPCPs. Several statute and citations were enabled by US EPA like Toxic Substances Control Act/15 USC § 2603, Safe Drinking Water Act/42 USC § $300g^{-1}$, Federal Water Pollution Control Act (Clean Water Act)/33 USC §§ 1312-1333, Food Quality Protection Acta/21 USC § 346a(b), Federal Insecticide, Fungicide, and Rodenticide Act/7 USC §§ 136a, 136w, Clean Air Act/42 USC §§ 7408(a), 7412(f).[282]

13. In Danish EPA, the QSAR models are employed for identification of PBT substances of around 166000 chemicals, and these data can be used for self-classification of around 20000 chemicals based on QSAR. [283]

14. The CEPA applies SAR for the prediction of biodegradation, toxicity and fate of Domestic Substance List (DSL) chemicals and to support in the categorization process. Environment Canada also evaluated six modelling packages (TOPKAT, ECOSAR, CNN, PNN, ASTER and OASIS) to predict acute toxicity, with application to prioritizing chemicals within the Canadian DSL.[283]

As the number of PPCPs are too huge, laboratory experiments and animal tests are not the ultimate solution. Again, considering time and economy, there is no doubt about the widespread use of computational models (especially QSAR models) by regulatory authorities to predict toxicity, fate and risk associated with the used substances along with the risk management. Although India and China are among the top 10 producers of APIs, till now these computer models is mainly employed in the US, but also increasingly in Canada and the EU. So, global regulatory agencies need to come

together and focus more on those countries which are lacking to provide sufficient information regarding the deleterious effects of PPCPs to the environment and human health along with structured implication of fast and economical *in silico* approaches for risk assessment and risk management.

7. WHY IS *IN SILICO* MODELING IN ECOTOXICOLOGICAL ASSESSMENT OF PPCPS?

The number of results obtained by Google search with the terms "*In silico* and Environmental toxicity" is around 1,810,000 in November 2018. The term '*In silico*', coming from latin in silicon, is an expression suggesting "computer simulation" in reference to biological problems and/or experiments. A good number of *in silico* tools have been developed to predict and/or model diverse responses of chemicals and materials successfully in the last five decades. Regression- and classification-based QSAR, machine learning, toxicophore, read-across, interspecies, docking and a good number of expert systems can be considered under *in silico* modeling which have been employed to model a huge number of environmental toxicants including PPCPs. The purpose of *in silico* modeling is to provide a fast analysis of untested and/or new potential chemical to cause adverse effects to environmental species, as well as being capable to predict a range of physico-chemical parameters and fate properties along with some extent of mechanistic interpretations. The models have been employed for the ERA/ERM by different regulatory authorities across the globe as well as to support the design of greener PPCPs with reduced or no animal testing. The reasons to use in silico models in ecotoxicity assessment are following:

- *The prohibition of animal experiment:* Council Directive 86/609/EEC on the approximation of Laws, Regulations and Administrative (EU) restricted animal experimentation. The testing ban on the active ingredients or combined products applied on 11th March 2009 and on finished

cosmetic products applied since 11th September 2004. Thus, regulatory agencies around the world introduced molecular modelling approach for risk assessment.[285,286]

- *The 3Rs concept:* The principle of 3Rs implies "Reduction", "Replacement" and "Refinement" of regarding animals' usage in scientific experiments. 'Reduction' signifies less number of animal usages, 'Replacement' links to the usage of non-living resources to replace higher taxonomical animals, and 'Refinement' advises muffle the harshness or brutality to the experimental animals.[287] *In silico* models are the answer for all three principles of the 3Rs approach.

- *Regulatory decision: In silico* models help regulatory and government bodies for risk assessment and management, predicting toxicity of new and untested compounds, evaluation of physicochemical parameters and fate properties evaluation.

- *Data gaps filling:* The toxicity assessment of APIs and PCPs was not necessary before introduction to the market up to 2006 according to EMA guidelines. Thus, a huge number of PPCPs are already in the market which has no toxicity data available for even single species. The available ecotoxicity data of PPCPs is less than 5%.[288] Thus, filling out this huge data gaps, *in silico* models are fast and economical approach.

- *Mechanistic interpretation:* In many cases, the generated mathematical equation from *in silico* model is capable to identify the responsible structural as well as physicochemical properties for toxicity to a specific organism or animal system. Generally, it assumed that compounds fitting the similar *in silico* (especially QSAR model) models are acting by the same MOA.[289]

- *Cost and time saving: In silico* models can save huge monetary cost along with fast risk assessment and prediction of toxicity for diverse species in diverse compartments. Toxicity predictions of PPCPs are possible even before the product synthesis which can help for earlier toxicity study.[290] A graphical depiction is reported in **Figure 9** where importance of *in silico* models are illustrated evaluating pharmaceuticals ecotoxicity.

Figure 9 Significance of *in silico* modeling evaluating the influence of PPCPs ecotoxicity.

It is true that environmental toxicity is not a significant determinant for approving APIs on to the market as efficacy is a more important criterion. But, here we make a point that *in silico* approaches can offer a fast check of ecotoxicity before the final approval of a drug. The knowledge related to probable ecotoxicity of API can be helpful for environmental risk assessment and risk management along with safe handling and disposal of these chemicals.

8. *IN SILICO* TOOLS

Although each phrase has a different meaning, many times researchers use *in silico* approaches as synonymous with computational modeling and/or molecular modeling methods. *In silico* techniques constitute an integral part of the high throughput screening (HTS) procedure for the virtual screening of toxicity of new and/or untested chemical entities. *In silico* methods are capable of providing information about the physicochemical properties of chemicals and the necessary structural fragments influencing the biological response (here, toxicity).[56,57] The need of *in silico*

techniques in predicting toxicological and hazardous properties of PPCPs are taking the central stage of attention day by day among the scientific community, regulatory bodies and the public in general.[290-293] The quantitative structure-activity/property/toxicity relationship (QSAR/QSPR/QSTR) is one of the most commonly used techniques among different *in silico* approaches. Advanced QSTR predictive models are being developed and tested by different international industries of different countries for the final approval from governing regulatory agencies to assess physical, chemical, and biological properties of individual chemical entities using applications that are specific for decision-making frameworks in safety assessments.[290] It is important to mention that when the toxicity endpoints are modeled and predicted, the QSAR term is denoted as QSTR. As this present review is dealing with ecotoxicity modeling, the term QSAR will be expressed here as QSTR. The most employed *in silico* tools for ecotoxicity prediction are illustrated in **Figure 10**.

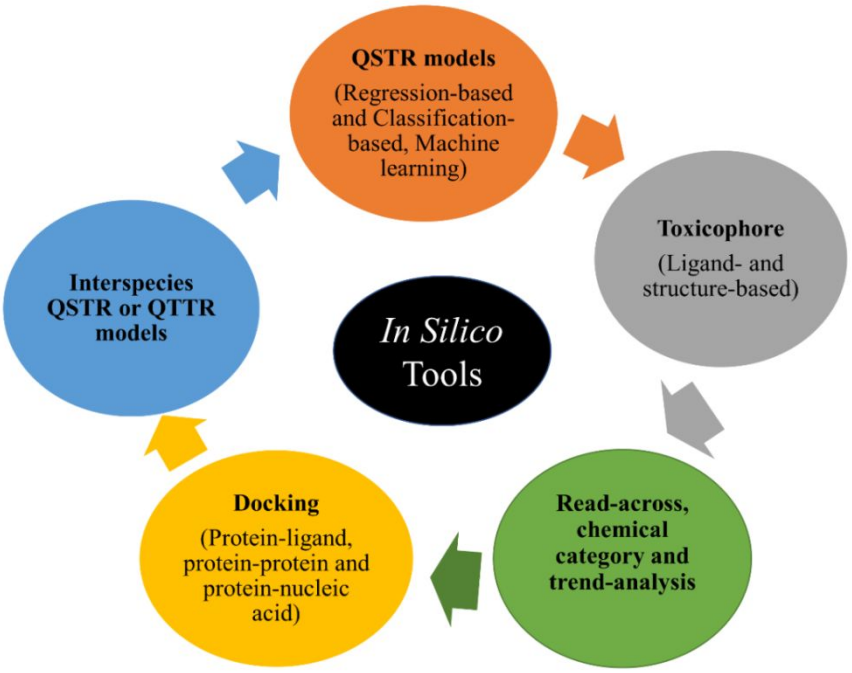


Figure 10 Most commonly used *in silico* tools for ecotoxicity modeling and prediction of PPCPs.

8.1 Quantitative Structure-Toxicity Relationship (QSTR) modeling

8.1.1 Definition and hypothesis of QSTR

QSTR is a statistical model which can be developed based on a similarity principle to correlate the changes in the toxicity of chemicals with changes in their structural features or other physicochemical properties making it possible to develop quantitative mathematical models for structure-activity correlations.[56,57] The QSTR models are extensively used for regulatory purposes in the chemical industries of the EU in view of the REACH regulations and other EU regulations.[291-293] The basic formalism of QSTR approach can be mathematically defined with following expressions:

$$Toxicity\ (Y) = f(Chemical\ attributes) = f(Information\ about\ chemical\ structure\ and\ physicochemical\ properties)$$

$$= f(Descriptors) = f(X_1 + X_2 + \cdots + X_N) \quad (1)$$

Chemical attributes are the essential information of the compounds which regulate its specific response that are often defined in terms of the information generated straight from the chemical structure and the physicochemical properties. The obtained information in form of numerical values are labeled as descriptors which help to find the best possible correlation with the toxicity response. The QSTR equation can be mathematically stated as follows:

$$Y = a_0 + a_1X_1 + \cdots + a_nX_n \quad (2)$$

Here, a_1, a_2, …, a_n are the coefficients suggest contributions of specific descriptors to the toxicity, with a_0 being a constant.

In the QSTR model, the toxicity response acts as the dependent variable and descriptors play the role of predictor variables or independent variables. In some cases, the response parameter like

toxicity may act as a predictor variable for the modeling of another toxicity endpoint. This specific model is termed as quantitative toxicity–toxicity relationship (QTTR) or interspecies-QSTR (i-QSTR). The hypothesis is explained schematically in **Figure 11**. The details about i-QSTR will be discussed later.

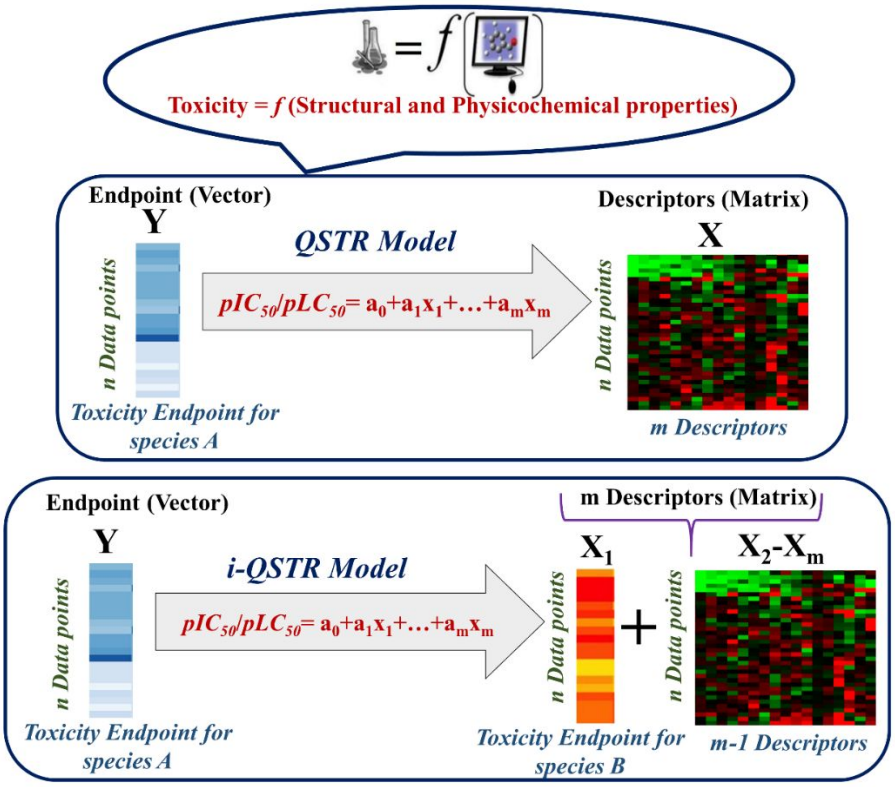


Figure 11 Hypothesis underlying QSTR and i-QSTR models.

8.1.2 Principles of QSTR

The regulatory QSTR models should be developed based on five principles proposed by Organization for Economic Co-operation and Development (OECD) for QSAR model development and validation.[294] These guidelines recommend a defined endpoint for modeling ensuring similar experimental protocol for the endpoint values (Principle 1), an unambiguous algorithm for model development which ascertains reproducibility (Principle 2), a defined chemical applicability

domain (AD) of the model which ensures that the query chemicals are sufficiently similar to the compounds used for model development (Principle 3), appropriate use of statistical measures for checking fitness and predictive ability of the developed model which decides the acceptability of a model (Principle 4) and finally, mechanistic interpretability of the model, if possible (Principle 5). It is necessary to apply a variety of statistical methods and metrics depending on the regression-based or classification-based modeling methods being used to examine the statistical quality of the developed models. The OECD principles, the fundamental steps for a QSTR study and how OECD principles are related to each step are reported in **Figure 12**.

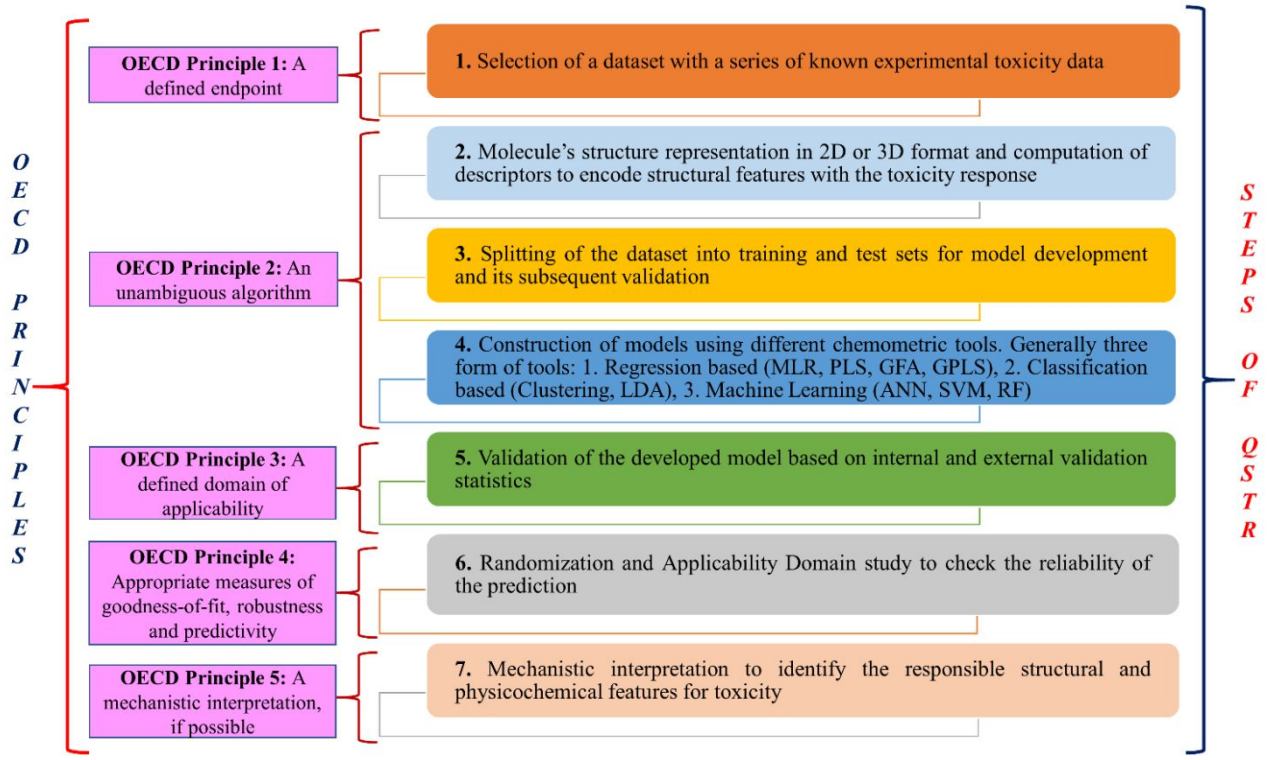


Figure 12 OECD principles and fundamental steps for QSTR formalism.

8.1.3 Classification of QSTR models

QSTR models are most commonly classified based on the linearity and non-linearity techniques for the development of the models where linear models are obtained by simple correlation between the toxicity response (Y variable) and the descriptors (X variables). On the

contrary, the non-linear models might be generated within the linear modeling framework employing quadratic terms, spline functions, and other higher order polynomials. Again, the models may also be classified into regression-, classification-based approach and machine learning tools. A comprehensive and commonly employed representative chemometric tools like multiple linear regression (MLR),[295] stepwise regression,[296] partial least squares (PLS),[297] genetic function approximation (GFA),[298-299] genetic partial least square analysis (G/PLS)[297,299] *etc.* under regression-based methods; principal component analysis (PCA),[300] factor analysis (FA),[301] factor analysis followed by MLR (FA-MLR),[301] factor analysis followed by PLS (FA-PLS),[297] linear discriminant analysis (LDA)[302] *etc.* under classification-based methods; and artificial neural network (ANN),[303] support vector machine (SVM),[304] random forest (RF)[305] etc. under machine learning techniques to build the QSAR/QSTR model is discussed elsewhere in details.[295-305] Again, based on geometric dimension of descriptors employed for model development, QSTR models can be categorized into multiple methods which are illustrated in **Figure 13**. For elaborate discussion and examples for individual methods, please refer to literatures.[56,57]

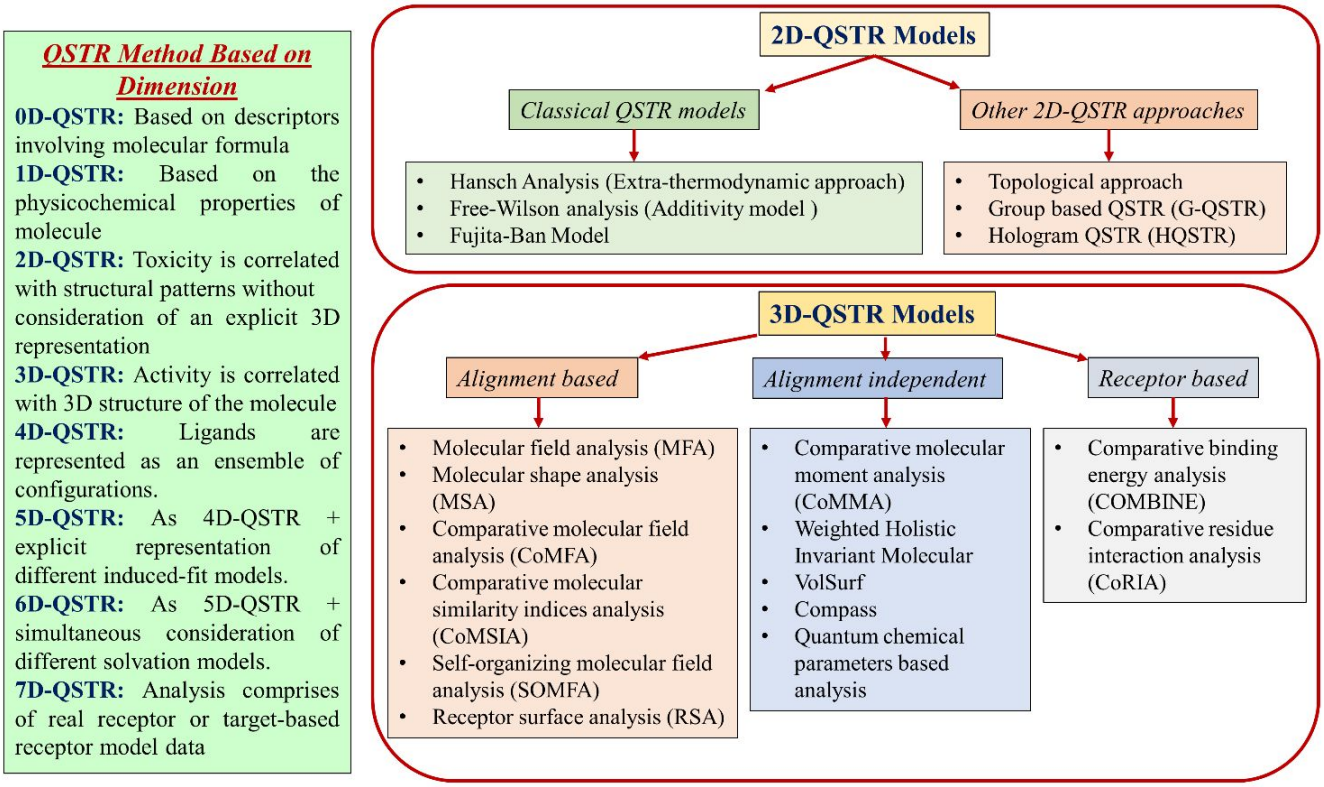


Figure 13 Classification of QSTR models based on dimensional geometry.

8.2 Interspecies quantitative structure-toxicity relationship (i-QSTR) modeling

8.2.1 Hypothesis and expression

The interspecies correlation estimation (ICE) model is a simple correlation between biological response (here, toxicity) of two species which is majorly employed to extrapolate toxicity data of a set of chemicals from one species to another species.[306] An ICE model can be calculated according to the below mentioned mathematical expression:

$$\log_{10}\left({}^{1}/_{Y[Predicted\ species]}\right) = a + a_1 \times \log_{10}\left({}^{1}/_{Y[Surrogate\ species]}\right) \quad (3)$$

Here, the toxicity response Y can be expressed as EC_{50}, ED_{50}, IC_{50} or LD_{50} values, a and $a_1$are the intercept and slope of the line, respectively.

An interspecies-QSTR (i-QSTR) model is a combination of ICE and simple QSTR models where the experiemental toxicity data for a specific species acts as a predictor variable along with other

descriptors to establish a correlation against another species for similar endpoint.[307] The toxicity endpoint which acts as a predictor variable can highlight the MOA of a series of molecules to some extent as it is produced by an experimental bioassay, while other descriptors are obtained purely from chemical structure and/or physicochemical experiments. The mathematical expression of i-QSTR model is:[59,308]

$$\log_{10}\left({}^{1}/_{Y[\text{Predicted species}]}\right) = a + a_1 \times \log_{10}\left({}^{1}/_{Y[\text{Surrogate species}]}\right) + a_2 \times X \quad (4)$$

Here, a_1 and a_2 are the coefficients of predictor descriptors, *i.e.*, surrogate species toxicity and descriptor X, respectively. Although we have shown here only one physicochemical/structural descriptor X, but based on the complexity of the model, the descriptor number may vary from 2 to *n*.

Zhang et al.[309] proposed four rules to distinguish the importance of physicochemical descriptors which need to be considered in i-QSTR modeling as reported in **Figure 14**.

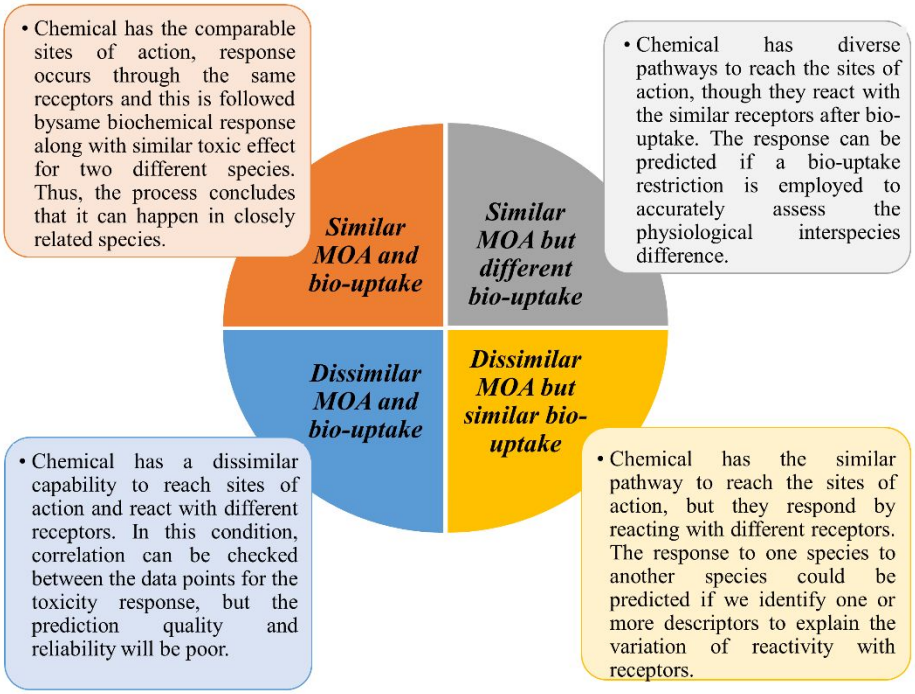


Figure 14 Four conditions for making of ideal i-QSTR models.

Following four conditions, Zhang et al.[309] modified equation 4 by including features signifying difference in bio-uptake and variance in toxic MOA between two species for an explicit endpoint:

$$\log_{10}\left(^{1}/_{Y[Predicted\ species]}\right) = a + a_1 \times \log_{10}\left(^{1}/_{Y[Surrogate\ species]}\right) + F_B + F_M \quad (5)$$

Here, F_B is a physicochemical parameter to invalidate the difference of bio-uptake, F_M is a physicochemical parameter to correct the variation of toxic MOA between two species. The difference between ICE and i-QSTR models is only consideration of bio-uptake factors and physicochemical parameters to recognize the probable MOA of toxicity in an explicit species.

8.2.2 Significance of i-QSTR/QTTR models

- *Extrapolation of toxicity data:* i-QSTR models are capable of extrapolating toxicity data from one species to another species for a specific toxicity endpoint when the experimental data for the second species are unavailable. Thus, this approach is very much important for data gap filling.
- *Identification of toxicity MOA:* The i-QSTR models can help to understand the MOA of studied chemicals for diverse species and definite endpoints through correlations between two species. As i-QSTR model employs the toxicity of one species as a predictor variable, it is capable of identifying the MOA to some extent as it is derived by a standard experimental bioassay.
- *Species-specific toxicities*: A good correlation specifies that the chemicals studied may share the similar toxic MOA between two species. On the contrary, a poor interspecies correlation may specify that the chemicals have different MOA for the studied species.
- *Reduction of animal usage*: A complete replacement of animal experiment is not possible, thus the i-QSTR models can be the right choice for toxicity prediction purpose by encouraging

the decrease in the use of higher class of animals/organisms for toxicity testing. Extrapolation of toxicity data from lower class species to higher class species is possible with i-QSTR models.

- *Data gap filling*: In addition, it can also extrapolate toxicological features and helps in filling data-gaps while dealing with the absolute assessment of chemical hazards.

8.3 Read-across (RA)

RA can be defined as a method capable of interpolating response data for a target compound from the corresponding experiemental data of closely related chemicals.[310] The idea of interpolation and/or extrapolation for target or query chemicals is reported in **Figure 15 (Left)**. The RA can be considered as a vital data-gap filling approach in ecotoxicity prediction of PPCPs. Regarding the subject of chemicals similarity, they can be structurally similar, the MOA can be similar for a specific system, they may share similar ADMET profile*etc*.[310,311] Depending on the nature of endpoint data, RA can be quantitative or qualitative. RA is generally performed in four ways to fill data gaps as demonstrated in **Figure 15 (Right)**.

Based on the employed methods, the RA can be categorized into two approaches:[312]

a) Analog approach (AN): Consider one-to one approach which uses one or few analogs for similarity measure. This particular method is sensitive to outliers as two analogs may have unrelated response profiles.

b) Category approach (CA): This approach employs many-to-one criteria and uses multiple analogs. The CA is a better approach than the AN, as it notices trends within a category and is helpful in toxicity predictions within confidence limit. Chemicals with similar properties or with a regular structural pattern can be considered as a group, or ‘category’ of substances. These similarities can be any factor: common functional group, constant pattern in changing potency, common precursor or breakdown products, common constituents or chemical class.

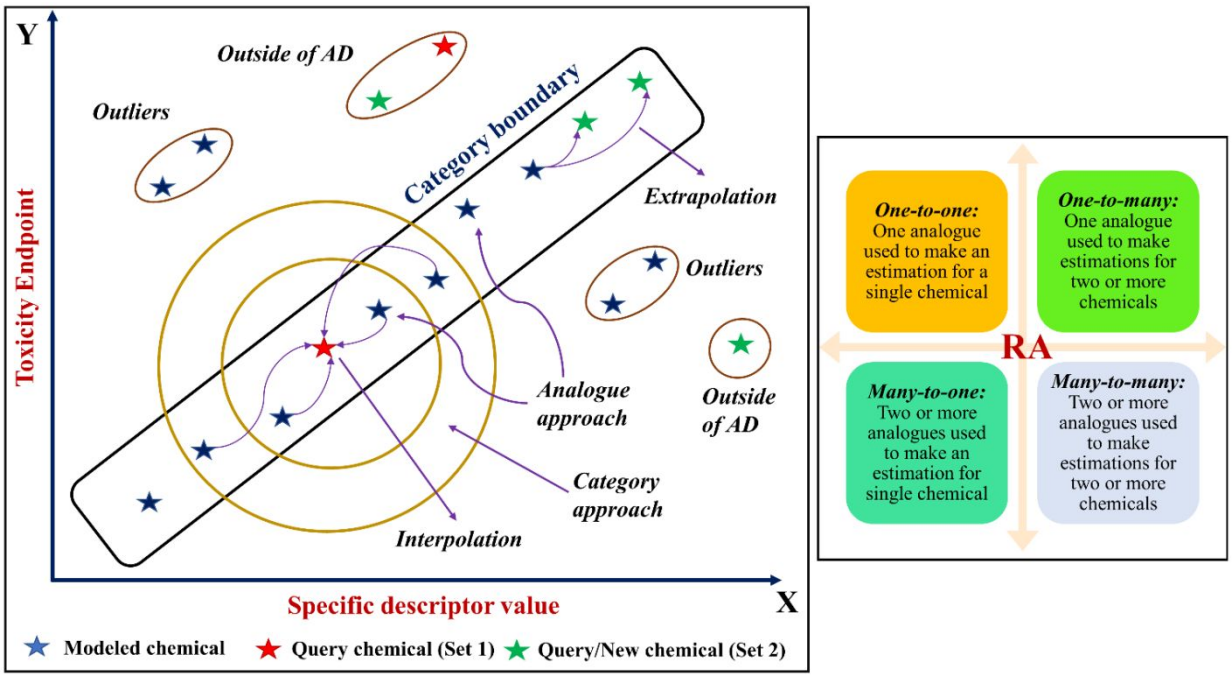


Figure 15 (Left) Hypothesis and classification of different approach of RA; (**Right**) ways to perform the RA approach.

The similarity among chemicals can be performed by identifying chemicals through feature vectors of chemical properties followed by the calculation of similarity percentage. The first step is applied employing either holographic fingerprints or binary. A holographic fingerprint utilizes the frequency of features (example: number of specific functional group). But a binary fingerprint is a feature vector of binary bits representing absence (0) or presence (1) of a property (example: specific functional group present or not). Then, the identified categories are divided employing another feature to create subcategories and so on. The hierarchy is helpful for inspecting the importance of individual features and can ease the model interpretation. Statistical similarity among the compounds can be checked through distances measuring approach in 2D or 3D spaces using Euclidean, Mahalanobis, Tanimoto distance, Hamming, or linear or nonlinear relationships of the features.[56,57] Tools executing the RA approach are Toxmatch,[313] The OECD QSAR Toolbox,[314] AMBIT,[315] ToxTree[316] *etc*.

8.4 Pharmacophore (Toxicophore)

A pharmacophore is the ensemble of steric and electronic features of a molecule that are necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response.[317] The pharmacophore does not represent a real molecule or a real association of functional groups, but a purely abstract concept that accounts for the common molecular interaction capacities of a group of compounds towards their target structure.[318] A pharmacophore can also be thought of as a template, a partial description of a molecule where certain blanks need to be filled. It starts with the selection of ligands from which the pharmacophore model is to be constructed. Hypothesis and common features of pharmacophore along with example are portrayed in **Figure 16**. Conformational expansion is the most critical step, since the goal is not only to have the most representative coverage of the conformational space of a molecule, but also to have either the bioactive conformation as part of the set of generated conformations or at least a cluster of conformations that are close enough to the bioactive conformation. This conformational search can be divided into following categories: systematic search in the torsional space, optionally followed by clustering, stochastic methods, *e.g.*, Monte Carlo, sampling, Poling, and molecular dynamics (MD).[319,320] The next step is 3D pharmacophore generation which is a formalized description of the hared features found in the previous step.[321] The derived pharmacophore model can be used to search compound databases and screening purpose. In case of toxicity response, pharmacophores can be employed to understand the structural template which is responsible for toxicity. In the case of toxicity response modeling, the pharmacophore is defined as a toxicophore. Thus, the hypothesis is completely the same for pharmacophore and toxicophore, just the modeled responses are biological activity and toxicity, respectively. Again, toxicophore/toxic fragments are denoted as structural alerts (SAs)[312,322] in a chemical structure that indicate or associate to toxicity. Toxicophore models can be built in four steps process and they are following:

a) Diverse conformation generation: In the first step, the conformational analysis of the compounds is done which eradicates much of the redundancy in conformation generation followed by improve the coverage of conformational space.

b) Generation of 3D toxicophore models: Thetoxicophores are created in three stages. The first stage is the constructive phase which generates toxicophores considering active molecules of the training set. The next stage is the subtractive phase which deals with the toxicophores created in the constructive phase and eliminates less important or useless toxicophores from the data structure. Finally, the optimization is done using the simulated annealing algorithm followed by models developed with different toxicophore features: (i) Hydrogen-bond donor (HBD), (ii) Hydrogen-bond acceptor (HBA), (iii) Hydrophobic (HYD) [HYDROPHOBIC (aromatic) and HYDROPHOBIC (aliphatic)], (iv) Positive charge (POS CHARGE), (v) Negative charge (NEG CHARGE), (vi) Negative ionizable (NEG IONIZABLE), (vii) Positive ionizable (POS IONIZABLE) and (viii) Ring aromatic (RA). The model's quality is analyzed in terms of their correlation coefficients and the cost function values.

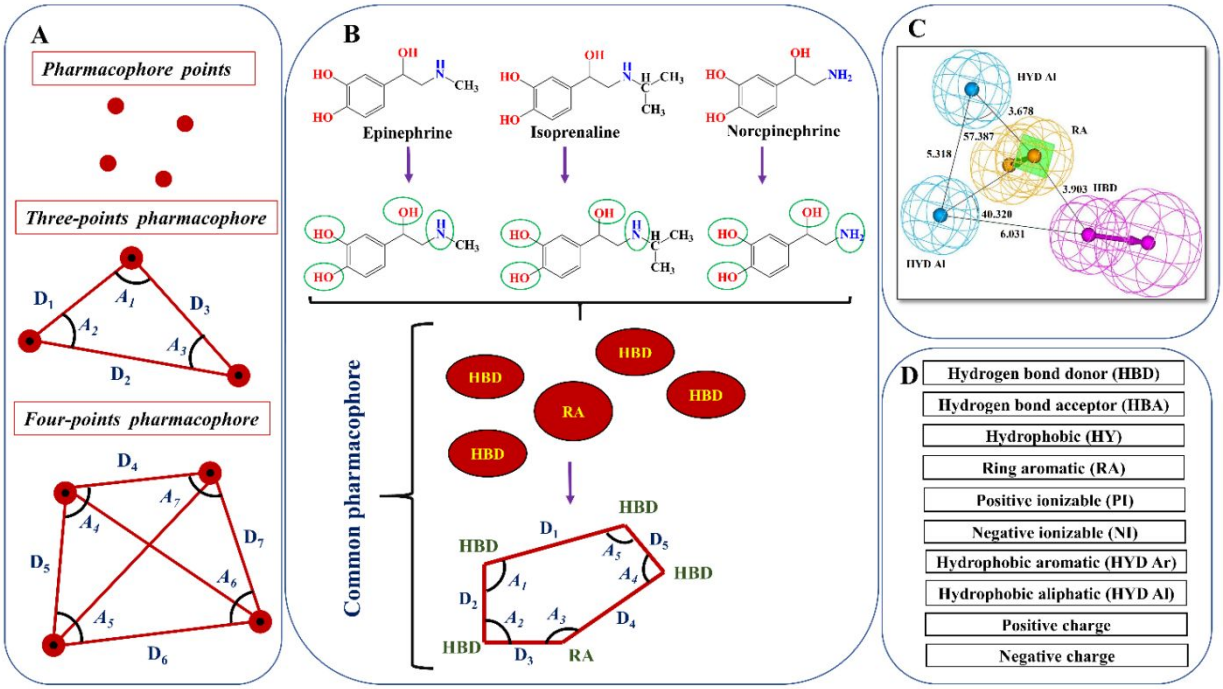


Figure 16 (A) Points/Atoms/Features based Pharmacophore (Toxicophore) hypothesis; (B) Representation of common pharmacophore features identification from three chemicals; (C) Example of a common pharmacophore model; (D) Common pharmacophore features.

c) Assessment of the quality of toxicophore hypotheses: To measure the quality of toxicophore hypothesis, a subsequent cost calculation needs to be done (**Table 3**).

Table 3 Cost hypothesis for quality measure of toxicophore models.

Parameter	Definition	Equation
Total cost	A small range of the total hypothesis cost obtained for each of the hypothesis indicates homogeneity of the corresponding hypothesis, and the training set selected for the purpose of toxicophore generation is adequate	$cost = eE + wW + cC$ Here, e, w, and c are the coefficients associated with the error (E), weight (W), and configuration (C) components, respectively.
Fixed cost	A fixed cost calculation which represents the simple model that fits all the data of the dataset	$fixed\ cost = eE(x = 0) + wW(x = 0) + cC$ Here, x is the deviation from the expected values of weight and error and other signs are the same as above.
Null cost	A null cost calculation that assumes that there is no relationship in the dataset and that the experimental activities are normally distributed about their average value and the toxicophore has no features	$null\ cost = eE(\chi_{est} = \overline{\chi})$ Here, χ_{est} is the averaged scaled toxicity of the training set molecules.

Criteria for acceptance of toxicophore hypothesis:[323]

- Total cost values should be close to the fixed costs, suggesting that the hypotheses generated are statistically robust.
- The variances between the generated hypothesis cost and the null hypothesis cost should be in the higher side (40-60 bits difference) which specifies that it has a 75-90% chance of suggesting a true correlation for the modeled dataset.
- The total cost of any hypothesis should be close to the value of fixed cost for any acceptable predictive model.
- Another important criterion is configuration cost value which should be lower than 17 for the acceptability of the developed model. If it is more than 17, then the model is developed by chance.
- The error cost rises as the value of the root mean square (rms) increases, which shows the quality of the correlation between the experimental and predicted data.

d) Validation of toxicophore model: Validation of a toxicophore model is performed in order to determine whether the developed model can identify active structures and forecast their activity precisely. Validation of the obtained models can be done using two procedures, viz. Fischer's validation and external validation using the test set prediction method.

8.5 Docking: Molecular docking is an application, wherein molecular modeling techniques are used to predict how a protein (enzyme) interacts with small molecules (ligands).[324] The ability of a protein/enzyme to interact with small molecules (example: pharmaceuticals) plays a major role in the dynamics of the protein which may enhance/inhibit its biological function. The capability to bind large molecules, such as other proteins and nucleic acids to form a supra-molecular complex plays an important role in controlling biological activity. The behavior of small molecules in the

binding pockets of target proteins can be described by molecular docking. The method aims to identify correct binding poses of ligands in the binding pocket (known as active sites) of a protein and to predict the affinity between ligand and the protein. The basic principle of docking, protein-ligand docked structure, binding dispositions with amino acid residues and 2D interaction maps solved for a docked ligand is provided as an example in **Figure 17**. Molecular docking can be classified as: (i) protein-small molecule docking, (ii) protein-nucleic acid docking, and (iii) protein-protein docking.

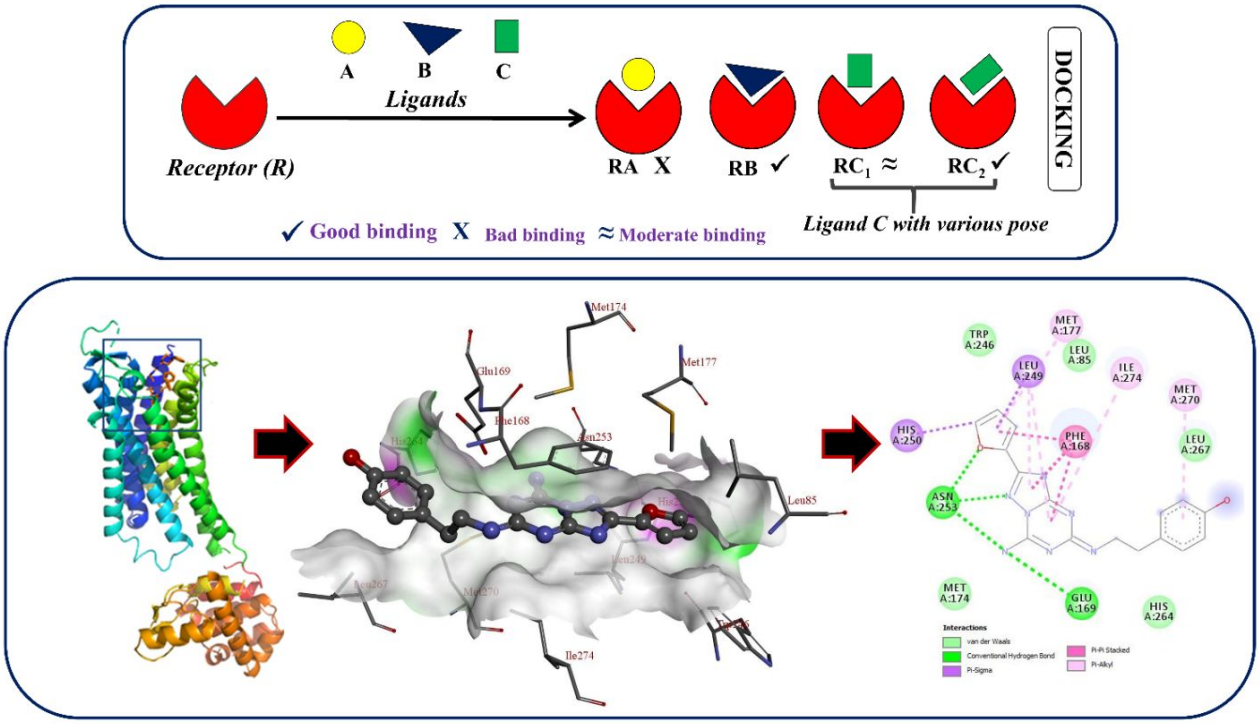


Figure 17 Fundamental hypothesisof the docking (Top); Docked complex of ligand-protein, binding dispositions with amino acid residues and 2D interaction maps solved for docked complex (below).

Protein-small molecule/ligand docking represents a simpler end of the complexity spectrum, and there are many available programs that perform particularly well in predicting molecules that may potentially inhibit proteins. Protein-protein docking is typically much more complex. The reason is that proteins are flexible, and their conformational space is quite vast. Docking can be performed

by placing rigid molecules or fragments into the protein's active site using different approaches like the clique-search, geometric hashing, or pose clustering. The performance of a docking depends on the search algorithm like Monte Carlo methods, Genetic algorithms, Fragment-based methods, Tabusearches, Distance geometry methods etc. and the scoring functions like Force-field methods, Empirical free energy scoring functions etc. The first thing is the composition of all possible conformations and orientations of the protein paired with the ligand. The scoring function takes input and returns a number which indicates favorable interaction.[325] Dockingis primarily a three-step process regardless of software and docking algorithms.[326,327] The steps are following: a) Ligand preparation, b) Protein preparation and c) Ligand-protein docking. Once the ligand is docked into a protein, one can check the binding interactions with amino acid residues, binding energy along with RMSD difference with the co-crystalized ligand. The overall steps of the docking formalism are illustrated in **Figure 18**.

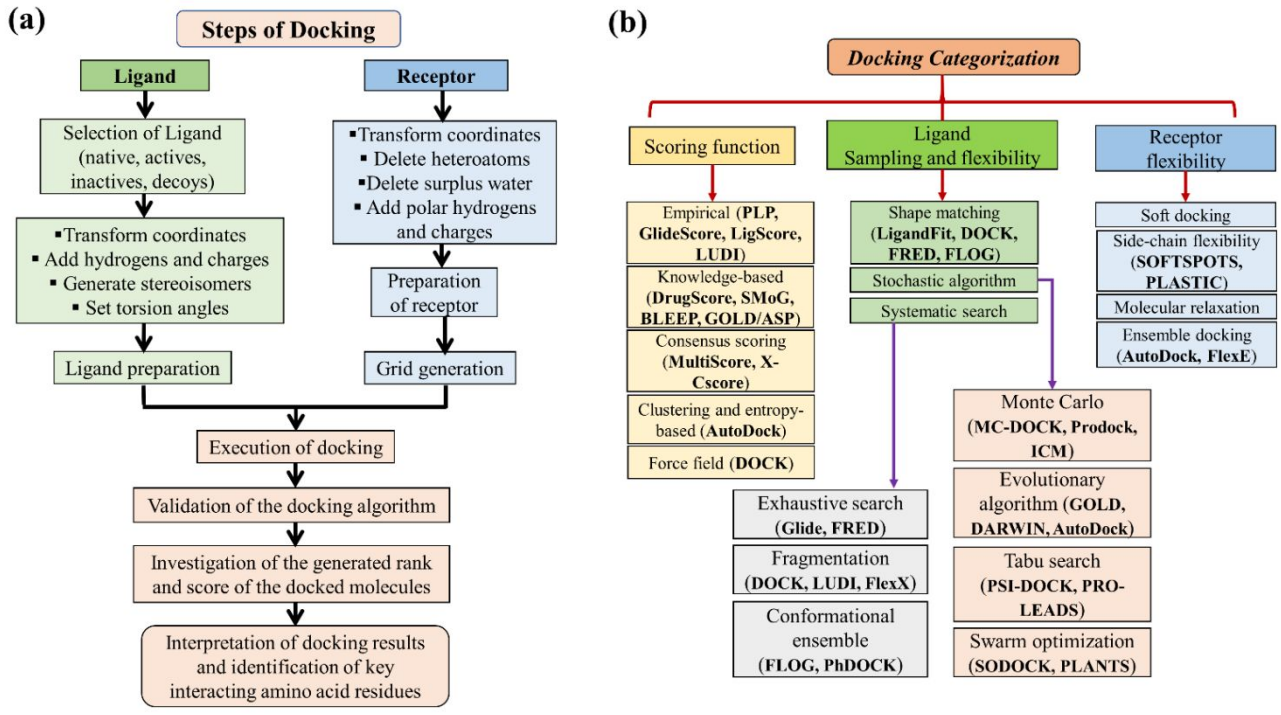


Figure 18. (a) Fundamental steps of docking method; (b) Classification of docking (Software tools are mentioned in bold and under bracket).

In case of toxicity evaluation, the docking study can identify the important structural fragments present in a small molecule as well as amino acid residues in specific a protein which are creating toxic effects by interacting with each other. The protein-ligand docking can be classified based on three vital criteria: (i) ligand sampling, (ii) protein flexibility, and (iii) scoring function, as demonstrated in **Figure 18**. As the present review deals with a different topic, to understand the docking technique in depth, please refer to a more extensive literature.[56]

9. EXPERT SYSTEMS FOR ECOTOXICITY PREDICTION OF PPCPs

An expert system is any formalized system, not necessarily computer-based, which enables a user to obtain rational predictions about the toxicities of chemicals. All expert systems for the prediction of chemical toxicities are built upon experimental data representing one or more effects of chemicals in biological systems (the database), and/or rules derived from such data (the rule base). Accordingly, the treatment by an expert system tool can be indicated as 'automated rule-indication system' where model statistics gets priority while another type of tools includes 'knowledge-based systems (KBS)' that provide mechanistic information. Expert systems are a convenient option for toxicity prediction over the traditional QSTR models as most of the time they require only the input of structure. The complete prediction can be performed even with a single click in no time and easy to recompute as per the requirement and modification of endpoints and species for a definite molecule. Majority of regulatory authorities, industries and academic people are employing expert systems for toxicity prediction, risk assessment and characterization along with identification of toxic or non-toxic molecules for the diverse compartment of environment and species. Manifold mechanisms can show comparable toxic effects which require precise and effective predictive tools, and which can distinguish manifold regions in the activity space. Expert systems can handle a wide spectrum structural and mechanistic complexity region in comparison to the local (single)

QSTR models. Open access, as well as commercially available expert systems capable to deal with PPCPs ecotoxicity, are illustrated in **Table 4**.[328-345]

10. TEST BATTERIES FOR ECOTOXICOLOGICAL ASSESSMENT

Specific ecotoxicity of PPCPs should be assayed on a definite species/organism or test batteries maintaining similar experimental protocols and environment. The hypothesis behind it is quite clear, as one can thoroughly check the toxic effects of all studied PPCPs to a specific species and can monitor changes from one compound to another. Thus, in-depth understanding about the assessed ecotoxicity and experimental species are important to develop precisely, statistically and mechanistically interpretable *in silico* models. Selection of appropriate endpoint species is very much significant to replicate the mode of toxicity to a definite environment. There is a specific list of species available to model the toxicity according to regulatory agencies and diverse environment conserve programs like Office of Prevention, Pesticides and Toxic Substances (OPPTS), Office of Pesticide Programs (OPP), Office of Technology Solutions (OTS), OECD, USEPA, REACH. Again, all the experimental PPCPs employed for modeling to a specific toxicity endpoint hypothetically work via similar MOA for the studied species. An exhaustive literature search has been done to enlist all possible test species for ecotoxicity assessment in **Table 5.**[65,66,285,290,346]

11. ENDPOINTS FOR IN SILICO MODELING OF ECOTOXICITY

The response which needs to be modeled in computational modeling is known as ‘Endpoint’. To understand and quantify risk related to the environment, one needs to categorize the endpoints carefully. Once the endpoints are identified, it is easy to model them for future screening and toxicity prediction of new compounds. Ecotoxicity modeling can be performed not only for toxicity endpoints but also for physicochemical and fate properties which are indirectly related to

ecotoxicity. Endpoints for ecotoxicity modeling according to OECD's guidelines where *in silico* models can be employed for risk prediction is illustrated in **Figure 19**.

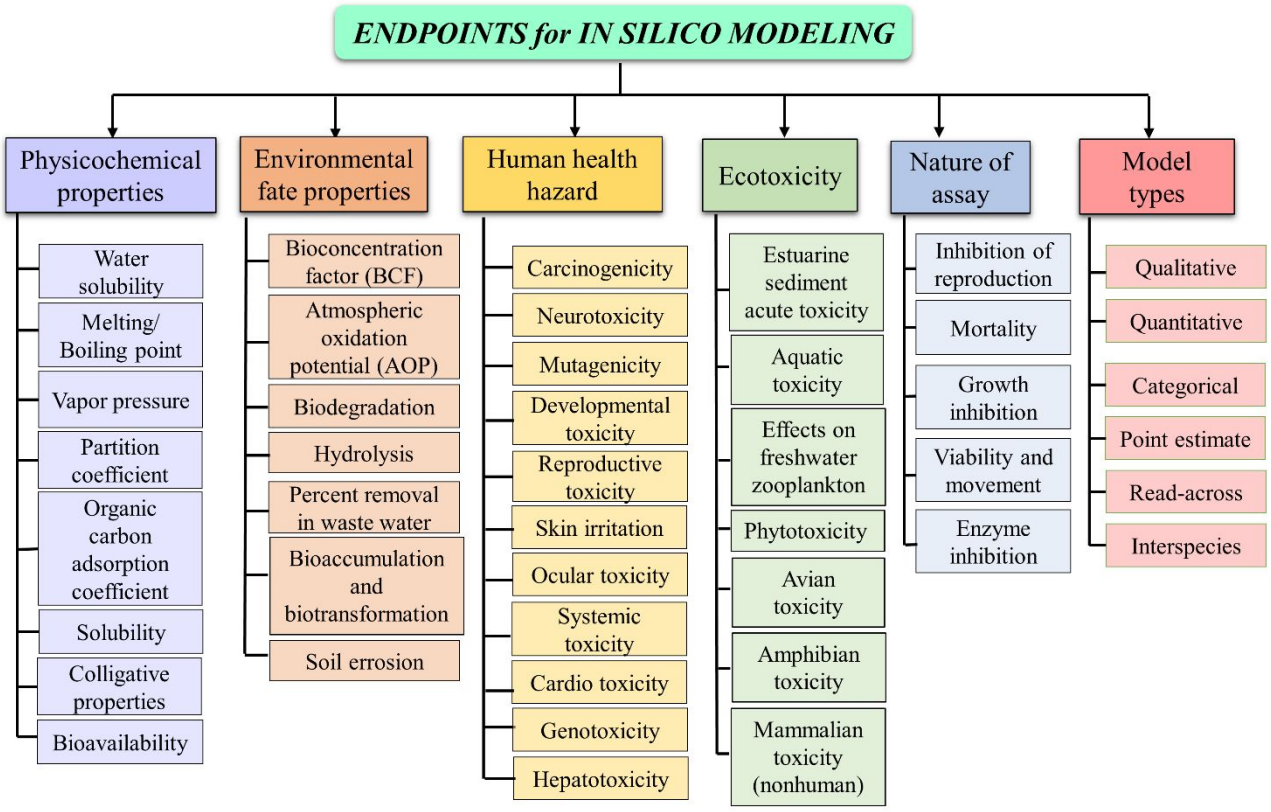


Figure 19 Most frequently modeled endpoints for ecotoxicity according to the OECD guidelines.

12. ECOTOXICITY DATABASES IN RELATION TO PPCPS

An ecotoxicity database is a comprehensive source of information on adverse and/or toxic effects of multiple chemicals to ecologically relevant aquatic and terrestrial species as well as environment compartments. The ecotoxicity databases consist of information in the form of qualitative or quantitative or sometimesa combination of both along with detailed experimental protocols followed by test species and endpoints. The ecotoxicity databases are rich sources of evidence for not only modeling purpose but also to validate different *in silico* models in respect to definite endpoints and species. To develop acceptable toxicity prediction computational models, good quality experimental ecotoxicity data is important with minimizing the experimental errors and the

procedural similarity. Although significant numbers of toxicity databases related to drug discovery and development are available, the number of databases related to ecotoxicity due to PPCPs are reasonably less. The number of such databases should be based on the present demands with detail and to the point toxicity information along with open accessibility which is an utmost requirement for transparent development of *in silico* models. An inclusive list of ecotoxicity databases is represented in **Table 6** which can be employed for *in silico* modeling, toxicity screening and validation for extensiverisk assessment, management, safety evaluation followed by hazard characterization of PPCPs.[347-400]

Table 4 Illustrative list of freely available and commercial expert systems to predict environmental toxicity due to PPCPs.[328-345]

Expert system	Company/ Organization	Description
ASTER[328]	US EPA, NHEERL	Assessment Tools for the Evaluation of Risk (ASTER) is an integration of ECOTOX database and a structure-activity based expert system which is freely available to provide high quality data for discrete chemicals.
CAESAR[329]	EC funded project (Project no. 022674 SSPI)	Computer Assisted Evaluation of industrial chemical Substances According to Regulations (CAESAR) is dedicated to develop QSTR models for the REACH legislation, specifically generates reproducible toxicity models. Five endpoints considered are bioconcentration factor, skin sensitization, carcinogenicity, Mutagenicity, developmental toxicity.
CATALOGIC[330]	LMC in "Prof. Dr. AsenZlatarov" University BURGAS, Bulgaria	Platform for models and databases related to the environment fate of chemicals such as abiotic and biotic degradation, bioaccumulation and acute aquatic toxicity.
DEREK[331]	Harvard University Office of Technology Development	DEREK, a KBES, developed in collaboration with industrial partners, which makes predictions based on SA, reasoning rules and examples contained within its knowledge base. Currently, 21 structural alerts for teratogenicity or teratogenic endpoints are considered under this expert system.

DfW[332]	Lhasa Limited	Derek for Windows (DFW), a KBES, covers 361 toxicological endpoints alerts with toxicophore. The skin sensitization knowledge base was developed in collaboration with Unilever in 1993 using its database of GPMT data for 294 chemicals. Version 9.0.0 contains 64 alerts for skin sensitization.
ECOSAR[333]	US EPA	ECOSAR is freely available from the US EPA which utilizes a number of class-specific log K_{ow}-based QSTRs to predict the toxicity (both short-term and long term) of chemicals. Hazard assessment of environmentally occurring pharmaceuticals to fish, daphnids and green algae can be performed.
HazardExpert Pro[334]	CompuDrug Inc.	Teratogenicity and reproductive toxicity predicted based on the structural fragments.
MCASE/ MC4PC[335]	MultiCASE Inc.	A commercial KBES which develops QSTR models and evaluates the structural features for non-congeneric molecules and identifies the substructures responsible for the response. Predictive models for blue gill, FHM, rainbow trout, red killifish are available. 180 modules covering various areas of toxicology and pharmacology endpoints including skin sensitization, retinoids, developmental toxicity under FDA/TERIS and developmental toxicants in FDA teratogenicity are available.
OASIS & TIMES[336]	Laboratory of Mathematical Chemistry, University “As Zlatarov”,	OASIS is commercial software uses the response-surface approach for modelling acute toxicity for two types of toxico-chemical domains: reversible acting chemicals and irreversible bioreactive chemicals. Interspecies correlations for acute toxicity to 17 aquatic species, such as fish, snail, tadpole, hydrozoan,

	Bourgas, Bulgaria	crustacean, insect larvae and bacteria have been developed. The Tissue MEtabolism Simulator (TIMES) platform is used to predict the individual and interspecies models for acute aquatic toxicity.
OECD (Q)SAR Toolbox[337]	OECD	A platform allows the user to develop categories and perform read-across, QSTR and trend analyses. A platform that will allow chemical information management, similarity searches and toxicological profiling.
OncoLogic[338]	US FDA/CDER	A desktop computer program that evaluates the probability that a chemical may induce cancer. OncoLogic predicts cancer-causing potential by applying the rules of structure-activity relationship (SAR) analysis, mimicking the decision logic of human experts, and incorporating knowledge of how chemicals cause cancer.
OSIRIS property explorer[339]	Actelion Pharmaceuticals Ltd., Allschwil, Switzerland	OSIRIS is an on-line system, which predicts reproductive effects on the basis of structural fragments which developed from the analysis of 3570 compounds with reproductive effects listed in Registry of Toxic Effects of Chemical Substances (RTECS).
PASS[340]	Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences, Moscow	PASS assesses the similarity of molecules to those with known activity and predicts over 30 endpoints relevant to reproductive toxicity. The employed endpoints are abortion inducer, alkylator, carcinogenic, DNA intercalator, DNA repair enzyme inhibitor, DNA synthesis inhibitor, DNA topoisomerase ATP hydrolyzing Inhibitor, DNA topoisomerase inhibitor, DOPA decarboxylase inhibitor, embryotoxic,

		oestradiol 17β-dehydrogenase stimulant, ER modulator, oestronesulphatase inhibitor, oestronesulphotransferase stimulant, fertility enhancer, menopausal disorders treatment, mutagenic *etc*.
SARET[341]	MRC "MEDTOXECO", Department of General Hygiene, Russia and IBMC RAMS, Russia	SARET base and SARET model are used as computer programs for the computation of descriptors (properties). SARET base includes the information on more than 190 characteristics for 8500 substances: chemical structure, physicochemical properties (density, boiling and melting points, $\log K_{ow}$ *etc.*), adverse effect doses and concentrations for acute and chronic exposure. The SARET model is prepared for statistical analysis of data and calculation of unknown parameters of substances on the basis of (Q)SARs. The application of SARET provides the information essential to assess the hazard of chemicals and to approximate their unknown characteristics.
TerraQSTR-FHM[342]	TerraBase Inc., Hamilton, Ontario, Canada	Commercial software and a standalone neural network-based program to compute the acute toxicity of organic chemicals to the FHM using a proprietary neural network algorithm.
TIMES-SS[343]	LMC University "As Zlatarov", Bourgas, Bulgaria	TIMES-SS is hybrid expert system, can encode structure-toxicity and structure metabolism relationships through a number of transformations simulating skin metabolism (mimics metabolism using 2D structural information) and interaction of the generated reactive metabolites with skin proteins. The covalent reactions with proteins are described by 47 alerting groups.

TOPKAT[344]	BIOVIA	TOPKAT is a statistical commercial expert system in which QSTR models developed from a huge number of heterogeneous databases of toxicological information using sub-structural fragments and (electro)-topological indices. Developmental toxicity potential is taken from FDA/TERIS. The program uses a range (Q)SAR models for assessing acute toxicity to FHM and Daphnia. The TOPKAT LD_{50} (acute oral toxicity) modelling approach has been used by the Danish EPA in their project to develop QSTR models for evaluation of dangerous properties of around 47,000 organic substances on the EINECS list.
Toxmatch[345]	EU Reference Laboratory for alternatives to animal testing	The open-source computer program of Joint Research Centre (EC) that encodes several chemical similarity indices in order to facilitate the grouping of chemicals, thereby supporting the development of chemicals and the application of read-across between analogues.

Table 5 Representative list of test species for the modeling of PPCPs ecotoxicity.

Test batteries	**Species**	**Description**	**Test Guidelines**
Algae	*Chlorella vulgaris* *Chlorella pyrenoidosa*	Unicellular fresh-water green micro algae comprising a major part of phytoplankton to study the toxic action of organic compounds.	OECD 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test;

	Pseudokirchneriella subcapitata, Selenastrumcapricornutum	Perfect test organisms for ecotoxicity evaluation through their growth rate inhibition.	OPPTS 850.4500: Algal Toxicity; OPPTS 850.4550: Cyanobacteria Toxicity.
	Scenedesmus obliquus	A type of Chlorophyta and a common cosmopolitan green alga, occurring as almost a pure culture in fresh water plankton. It can grow in industrial wastewaters of different origins showing good adaptation ability and very versatile microalgae as a test endpoint.	
	Scenedesmus vacuolatus	Green algaof the Chlorophyceae family, which is colonial and non-motile in nature; has been used in the prediction of photoinduced toxicity of polycyclic aromatic hydrocarbons and the ecotoxicity of ionic liquids (ILs).	
Bacterium	*Escherichia coli*	A Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* is used as a model organism in ecotoxicity. A good number of studies are performed to evaluate metal oxide nanoparticles cytotoxicity.	OECD 471: Bacterial Reverse Mutation Assay; OECD 472: *E. coli*, Reverse Mutation Assay; EU Method B.13/14: Mutagenicity, Reverse Mutation Test

	A. fischeri, Vibrio natriegens	Gram-negative rod-shaped bacterium having bioluminescent properties and found principally in symbiosis with different marine species. Majorly employed in the research of microbial bioluminescence, quorum sensing along with ecotoxicity testing.	Using Bacteria; EPA OPPTS 870.5100: Bacterial Reverse Mutation Test; EPA OPPTS 870.5265: Salmonella typhimurium Bacterial Reverse Mutation Test; EPA OPPTS 870.5500: Bacterial DNA Damage or Repair Tests; EPA OTS 798.5100: Escherichia coli WP2 and UVRA Reverse Mutation Test; EPA OTS 798.5500: Bacterial DNA Damage or Repair Tests.
	Bacillus	A genus of gram-positive, rod-shaped bacteria and member of the phylum Firmicutes. Chlorophenols toxicity is tested on bacillus species.	
	Pseudomonas fluorescens	*P. fluorescens* has a versatile metabolism. Generally found in the soil and water. According to the literature, it is employed for modeling of antibiotics toxicity and resistance studies.	
Crustaceans	*Daphnia ambigua, Daphnia magna, Daphnia melanica Daphnia pulex*	Small aquatic crustaceans commonly called water flea. As an invertebrate species in aquatic food webs, *D. magna* has been used as a representative test species for ecotoxicological evaluation of organic chemicals using immobilization test.	EU Method C.2: Acute Toxicity for Daphnia; EPA OPP 72-2: Aquatic Invertebrate Acute Toxicity Test; OPPTS 850.1010: Aquatic invertebrate acute toxicity, test, freshwater daphnids; OECD 211: Daphnia magna Reproduction Test;
	Thamnocephalusplaturus	A family of crustaceans with a wide distribution including Western Australia and Southern Africa. The 24 hours	

		toxicity test is employed for screening of pure compounds, effluents, sediments, surface and ground waters, wastewaters, and biotoxins.	OPPTS 850.1300 Daphnid chronic toxicity test; OPPTS 850.1790: Chironomid sediment toxicity test; OPPTS 850.1020: Gammarid acute toxicity test; OPPTS 850.1025: Oyster acute toxicity test (shell deposition); OPPTS 850.1035: Mysid acute toxicity test; OPPTS 850.1350: Mysid chronic toxicity test; OECD 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment (OECD TG 219); EPA OPPTS 850.1020/ EPA OTS 795.1200: Gammarid Acute Toxicity Test.
	Chironomus sp (*C. riparius*, *C. dilutus* and *C. yoshimatsui*)	Used to assess the effects of prolonged exposure of chemicals to the sediment-dwelling larvae of the freshwater. In assay, Chironomid emergence and development rate is measured at the end of the test.	
	Gammarus fasciatus, *G. pseudolimnaeus*, and *G. lacustris*	Gammarids can be cultured in the laboratory or collected from natural sources. If collected, they must be held in the laboratory for at least 14 days prior to testing.	
	Mysidopsisbahia	*Mysidopsisbahia* is the organism specified for aquatic toxicity test. Juvenile mysids, ≤ 24–h old, are to be used to start the test.	
Duckweed/Plant	*Lemna minor* *Lemna gibba*	One form of aquatic vascular plant floats on the surface of the water. *Lemna minor* is mostly employed in modeling of phytotoxicity of ILs and growth inhibition test of duckweeds	OECD 221: Lemna sp. Growth Inhibition Test; OPPTS 850.4400: Aquatic Plant Toxicity Test Using Lemna sp.; OPPTS

		where *Lemnagibba* is used in testing the phytotoxicity of pesticides and other environmental chemicals to higher plants.	850.4450: Aquatic Plants Field Study.
Enzyme	Acetylcholinesterase	Acetylcholinesterase plays the most important role in autonomic nervous system function which catalyses the hydrolysis of acetylcholinesters with a relative specificity for acetylcholine. Responses like (a) enzyme inhibition data of the acetylcholinesterase from electric eel (*Electrophorus electricus*), (b) the AMP deaminase and (c) the antioxidant enzyme system of mouse liver are important for toxicity prediction andmodeling.	OECD 419: Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study.
Fish	Channel Catfish Ovary (CCO)	CCO is the cell line of choice for the propagation and diagnosis of Channel Catfish Virus (CCV) and is the standard for diagnosing Channel Catfish Virus Disease (CCVD) in farm reared Channel Catfish. Prediction of ILs has been performed by using this endpoint according to many literatures.	OECD 210, OPPTS 850.1400: Fish Early-life Stage Toxicity test; EPA OPP 72-3: Estuarine/Marine Fish, Mollusk, Acute Toxicity Test; OECD 236: Fish Embryo Acute Toxicity test; OECD 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages; OECD 215: Fish Juvenile
	Fathead minnow *(Pimephales promelas)*	EPA recommended vertebrate species for freshwater chronic toxicity tests (test of survival and weight of the larvae). It is	

		studied to investigate the effects of these waste materials on the aquatic life and effects induced by progestins.	Growth Test; OPPTS 850.1075 Fish acute toxicity test, freshwater and marine; OPPTS 850.1085 Fish acute toxicity mitigated by humic acid; OECD 204: Fish Prolonged Toxicity Test, 14-Day Study; OECD 230: 21-day Fish Assay; OECD 229: Fish Short Term Reproduction Assay; OECD 234: Fish Sexual Development Test; OPPTS 850.1500 Fish life cycle toxicity.
	Rainbow trout (Oncorhynchus mykiss)	Rainbow trout is a streamlined, salmonid form fish. *Oncorhynchus mykiss* is one of the important endpoints for study aquatic toxicity as well as an alternative model for studying the inhibition of aromatase (CYP 19).	
	Zebrafish *(Danio rerio)*	A tropical freshwater fish belonging to the family Cyprinidae. It plays an important role in ecotoxicology as a prominent model vertebrate. It is standardized under the OECD and is employed to test chemicals, pharmaceuticals as well as industrial effluents.	
Mammalian cells	Human keratinocyte cell line (HaCaT)	Naturally immortalized human keratinocyte line is utilized for studies of skin biology and cytotoxicity assessment of metal oxide.	OPPTS 870.5300: In Vitro Mammalian Cell Gene Mutation Test; OPPTS 870.5550 Unscheduled DNA Synthesis in Mammalian Cells in Culture; OECD 473:In Vitro Mammalian Chromosomal Aberration Test; OECD 476: In Vitro
	CaCo-2	Heterogeneous human epithelial colorectal adenocarcinoma cells. Permeability coefficients across the cellular membranes of Caco-2 cells are generally employed for modeling.	

	HeLa	A prototypical cell of the human epithelium derived from cervical cancer cells and mostly employed for anticancer activity.	Mammalian Cell Gene Mutation Test using the Hprt and xprt genes; OECD Guideline 479: Genetic Toxicology, In Vitro Sister Chromatid Exchange Assay in Mammalian Cells; OECD 487: In vitro Mammalian Cell Micronucleus Test; OECD 490: In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene.
	Prostate cancer cell line (PC3)	Human prostate cancer cell lines employed in modeling of prostate cancer inhibitors.	
	Human malignant melanoma (Fem-X)	Derived from a lymph node metastasis of a melanoma patient and used for modeling of anticancer drugs.	
	HT-29	A human colorectal adenocarcinoma cell line with epithelial morphology and mostly sensitive to the chemotherapeutic drugs used in colorectal cancer modeling.	
	Rat cell line - IPC-81	Promyelotic leukemia rat cell line IPC-81 is employed in cytotoxicity assays of ILs.	
Protozoa	*Tetrahymena thermophila* *Tetrahymena pyriformis*	Free-living unicellular ciliated protozoa and one of the most popular endpoints for environmental toxicity assessment.	OECD 244: Protozoan Activated Sludge Inhibition Test.
Tadpoles	American bullfrog	One of the largest frog species in North America, this can grow to	OPPTS 850.1800: Tadpole/sediment

	(Lithobatescatesbeianus or Rana catesbeiana	a length of 8 inches (Tadpoles 6.75) or more and weigh up to 1.5 pounds. Due to its size and fast growth considered for aquatic ecotoxicity.	subchronic toxicity test; OECD 231: Amphibian Metamorphosis Assay.
	Bufo vulgaris formosus, Rana japonica	A common and sensitive species; the larva of the frogs, are typical amphibious bridging the gap between aquatic and terrestrial animals. Recurrently used for toxicity testing purposes and risk assessments and have been recommended by the EU-TGD.	
Yeast	*Saccharomyces cerevisiae*	One form of budding yeasts and one of the most popular studied eukaryotic model organisms in molecular and cell biology. Small in size, accessible, reproduction time quick and potentially economic. Considered as important species for ecotoxicity prediction.	OECD 480: Gene Mutation Assay; OECD 481: Mitotic Recombination Assay; EU Method B.16: Mitotic Recombination test; EPA OPPTS 870.5575/798.5575: Mitotic Gene Conversion assay

Table 6 A comprehensive list of publicly available databases comprises of information of the ecotoxicity due to PPCPs.

Database	Description
ACToR[347]	A database by US EPA National Center for Computational Toxicology (NCCT), consisting of chemical structure, physicochemical values, and provides *in vitro* and *in vivo* toxicology data for over 500,000 environmental chemicals.
BDSM[348]	Birth Defects Systems Manager (BDSM) database dealing with developmental toxicity and developed by University of Louisville.
CCRIS[349]	Chemical carcinogenesis research information system (CCRIS) created by the National Cancer Institute (NCI). It contains carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition test results for over 8,000 chemicals.
ChEMBL[350]	Containing data for over 12 million activities and 1 million assays for over 1.36 million chemicals.
ChemSpider[351]	Database of more than 30 million unique structures, providing physico-chemical information and toxicological data from various species and different routes of administration.
COSMOSdb[352]	It consists of two datasets (US FDA PAFA and RepeatToxDB) that hold information for 12,538 toxicological studies across 27 endpoints for 1,660 compounds.
CPDB[353]	The Carcinogenic potency database, developed by the University of California, Berkeley and the Lawrence Berkeley National Laboratory, analyses animal cancer tests used in support of cancer risk assessments for human. It includes 6,540 chronic, long-term animal cancer tests from the literature as well as from the NCI and the National Toxicology

	Program (NTP).
CTD[354]	Comparative Toxicogenomics Database (CTD) contains manually curated data describing cross-species chemical-gene/protein interactions and chemical- and gene-disease relationships. The results provide insight into the molecular mechanisms underlying variable susceptibility and environmentally influenced diseases.
Danish (Q)SAR[355]	Database includes estimates for more than 200 (Q)SARs from free and commercial platforms and related to physicochemical properties, ecotoxicity, environmental fate, ADME and toxicity. Developed by the National Food Institute, Technical University of Denmark, with support from the Danish EPA, the Nordic Council of Ministers and the European Chemicals Agency.
DART[356]	DART provides more than 400,000 journal references covering teratology and other aspects of developmental and reproductive toxicology.
DevTox[357]	Developmental toxicity data and control database for various strains of common laboratory animals.
Drugs@FDA[358]	Complete information related to US FDA-approved drugs are available.
DSSTox[359]	Distributed Structure-Searchable Toxicity (DSSTox) database developed by NCCT, US EPA. It provides downloadable, structure-searchable, standardized chemical structure files associated with chemical inventories or toxicity data sets of environmental relevance.
ECOTOX[360]	Database for single chemical toxicity information for aquatic and terrestrial life, developed by US EPA.
ESIS[361]	European Chemical Substances Information system (ESIS) provides information on

	chemicals related to risk and safety.
eTox[362]	A drug safety database from pharmaceutical industry consists of toxicology reports and public toxicology data.
Fraunhofer RepDose[363]	A database containing more than 3100 studies on subacute to chronic toxicity within a variety of routes of administration for about 930 chemicals.
GAC[364]	Genetic Alterations in Cancer (GAC) is a database that quantifies specific mutations found in cancers induced by environmental chemicals developed by US National Institutes of Health (NIH) and National Institute of Environmental Health Sciences (NIEHS).
GAP[365]	Genetic Activity Profile (GAP) database under US EPA and International Agency for Research on Cancer Monograph (IARC); provides quantitative genotoxicity results of ≈ 500 chemicals to support hazard classification of human carcinogens.
Gene-Tox[366]	GENE-TOX provides genetic toxicology (mutagenicity) data for more than 3,000 chemicals from the US EPA.
HESS[367]	The Hazard Evaluation Support System (HESS) database supports the evaluation of repeated dose toxicity and has two databases. One is a toxicity knowledge database which contains information on repeated dose toxicity and toxicity mechanisms. The other is a metabolism knowledge database containing rat metabolism maps and information on ADME in rats and humans.
HSDB[368]	Hazardous Substances Data Bank (HSDB) focuses on the toxicology of potentially hazardous chemicals. It provides information on human exposure, industrial hygiene, emergency handling procedures, environmental fate, regulatory requirements, nanomaterials, and related areas.

IARC Monograph[369]	IARC is developed by World Health Organization (WHO) which identifies environmental factors (include chemicals, complex mixtures, occupational exposures, physical agents, biological agents) that can increase the risk of human cancer.
IRIS[370]	Integrated Risk Information System (IRIS) is under National Center for Environmental Assessment (NCEA), US EPA. It is a compilation of reports on 540 environmental chemical substances and their potential to cause human health effects.
ISSCAN[371]	ISSCAN is a database on chemical carcinogens (long-term carcinogenicity bioassay on rodents) created by IstitutoSuperiore di Sanità, Italy.
ITER[372]	International Toxicity Estimates for Risk (ITER) is developed by TERA (Toxicity Excellence for Risk Assessment), consists of human health risk values and cancer classifications for over 680 chemicals of environmental concern.
IUCLID[373]	International Uniform ChemicaL Information Database (IUCLID) is a software application to capture, store, maintain and exchange data on intrinsic and hazard properties of chemical substances.
JECDB[374]	A Toxicity Database by Japanese Ministry of Health, Labour and Welfare which contains toxicity test reports of environmental chemicals.
JRC QSTR[375]	European Commission, Joint Research Centre's database of REACH relevant to QSARs.
KATE[376]	KAshinhou Tool for Ecotoxicity (KATE) is created by Japanese National Institute for Environmental Studies (NIES) which uses structural domain named *C*-judgement and performs categorization of chemicals as potential hazards.
KemI[377]	This database is prepared by the Swedish Chemicals Inspectorate which consists of risk associated data for environment and health contaminants.

LAZAR[378]	It is a Structure-Activity Relationships database that provides QSTR predictions for liver toxicity, mutagenicity, and carcinogenicity.
Leadscope[379]	It is a commercial database containing over 400,000 data covering acute, (sub-) chronic, carcinogenicity, genotoxicity, and reproductive toxicity for around 180,000 chemicals.
MDL[380]	Commercially available structure-searchable database containing data from both *in vitro* and *in vivo* studies covering acute, carcinogenicity, mutagenicity and reproductive toxicity studies for over 150,000 chemicals along with information from RTECS.
NTP[381]	National Toxicology Program initiated by US NIH/NIEHS. NTP testing status and information of agents registered in the US of public health interest.
OECD eChemPortal[382]	Access to information on physicochemical properties, environmental fate and toxicity of hazardous chemicals for the environment.
OECD HPV[383]	Data includes acute aquatic toxicity necessary to determine a potential hazard.
OEHHA[384]	Toxicity Criteria Database of chronic reference exposure levels for State of California.
OSIRIS[385]	Data on aquatic toxicity, carcinogenicity, mutagenicity and repeat dose toxicity.
RAIS[386]	Risk Assessment Information System (RAIS) deals with chemical-specific toxicity values sponsored by the U.S. Department of Energy (DOE), Office of Environmental Management, Oak Ridge Operations (ORO) Office through a contract between Bechtel Jacobs Company LLC and the University of Tennessee.
RITA[387]	Registry of Industrial Toxicology Animal-data (RITA) is generated by Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM) Hannover for comparing and

	interpreting rodent carcinogenicity studies and tumor data.
Tox21[388]	US EPA Tox21 is currently screening over 10,000 chemicals at the NIH using the ToxCast HTS assays to provide risk assessors with data for use when making decisions about protecting human health and the environment.
ToxCast[389]	US EPA is using various HTS assays to measure changes in biological activity. Currently ToxCast has evaluated over 2,000 chemicals within over 700 high throughput assay covering roughly 300 signaling pathways.
TOXLINE[390]	TOXLINE is a bibliographic database provides references covering the biochemical, pharmacological, physiological, and toxicological effects of drugs and chemicals.
TOXNET[391]	US National Library of Medicine Toxicology Data Network is a group of databases covering chemicals and drug, environmental health, occupational safety, risk assessment and regulations, and toxicology.
TOXMAP[392]	Environmental Health Maps provides searchable, interactive maps of EPA Toxics Release Inventory (TRI) and Superfund data, plus US Census and NCI health data.
ToxRefDB[393]	Contains information of *in vivo* study results including acute, (sub-)chronic, developmental and reproductive endpoints for 474 chemicals ToxRefDB also links with both ACToR and ToxCast databases.
Toxtree[394]	Open-source application that places chemicals into categories and predicts various kinds of toxic effects by applying decision tree approaches.
TRI[395]	Toxics Release Inventory with information about annual environmental releases of over 600 toxic chemicals by U.S. facilities.
TSCATS[396]	Toxic Substances Control Act Test Submissions (TSCATS) is an online database of

	chemical testing results and adverse effects of chemicals on health and ecological systems constructed by U.S. Department of Commerce National Technical Information Service Alexandria, Virginia. The collection currently exceeds 25,000 titles of studies that are submitted to the US EPA by U.S. Industry under several section of the TSCA.
US FDA CERES[397]	US FDA Chemical Estimation Risk Evaluation System (CERES) is a centralised, sustainable data management, and storage system that will provide support in decision making for both pre- and post-market safety assessment for food ingredients.
USGS[398]	US Geological Survey (USGS) is developed by the Columbia Environmental Research Center for the aquatic acute toxicity tests.
VITIC Nexus[399]	VITIC Nexus is a database provides information for a variety of toxicological endpoints including carcinogenicity, mutagenicity, and hepatotoxicity.
WikiPharma[400]	Database of effects caused by pharmaceuticals on non-target organisms developed within the Swedish research program MistraPharma (www.mistrapharma.se). Itcontains basic information for 831 APIs representing 35 different drug classes. Effect data have been identified and included for 116 of these substances and ecotoxicity test data have been extracted from 156 different sources.

13. CRITICAL ANALYSIS OF SAR AND QSAR STUDIES FOR ECOTOXICOLOGICAL ASSESSMENT OF PPCPs

It is estimated that up to 30 animal studies are needed for characterizing one substance.[401,402] Hence, environmental regulatory use of (Q)SAR including both QSARs and SARs (structure-activity relationships without quantified predictions) is goal-driven support for decision making and policy development in accordance with obligations in the 3R's strategy to reduce the use of

animals in toxicity testing most OECD countries have signed. (Q)SARs are used in the lower tiers of the risk assessment process and have traditionally been used for priority setting rather than actual risk assessment.[403] (Q)SARs may be developed to have either high sensitivity and low specificity thus yielding an elevated number of false positives - or with low sensitivity and high specificity, thus yielding an elevated number of false negatives. This represents the general optimization dilemma for development of (Q)SARs. Another general dilemma is the risk of over-fitting models and decreasing transparency of the model with increased sophistication e.g. in neural network models – it is important to avoid QSARs used as a "black-box" and ensure their proper use in a regulatory setting.[404] However, currently most of the regulatory environmental toxicity (Q)SAR used in, e.g., the OECD QSAR tool box, identifies substructures of the target compound that appear mostly in active molecules and may therefore be responsible for toxicity. They generally initially start by identifying possible linear relation between octanol-water partition coefficient (K_{OW}) and observed toxicity (baseline narcosis), explained by its lipophilicity (narcosis effect). Bradbury[405] found that approximated 70% of all industrial organic chemicals are estimated to act via baseline and polar narcosis modes of action in acute exposures (1-14 days). Octanol is not an optimal surrogate for biological membranes, hence models are being developed with other descriptors. The ideal (Q)SAR should have a well-defined and measurable endpoint based on a diverse data set, and a statistical method that needs to be transparent and appropriate to the toxicity endpoint data. It should consider an adequate number of chemicals for sufficient statistical representation and reasonable distribution of active and inactive chemicals. A wide range of quantified toxic potency should be present in the training set, and the model should provide a mechanistic toxicity explanation. The data sets should be

curated to a high quality and must meet the basic requirements underlying the statistical procedure used to develop the (Q)SAR model.[404]

Specifically, for PCPs, there is a concern that baseline toxicity might not be appropriate due to the pharmacodynamic nature of the compounds and the known conservation of receptor targets across species.[406] Moreover, the concern regarding receptor-mediated toxicity is typically more relevant for chronic effects rather than acute ditto. Sanderson and Thomsen[407] have shown that for the majority of PCPs, narcosis based QSAR results with the accepted extrapolation factor could accurately predict PCP toxicity relative to the GHS classification system, and that their acute toxicity for the most part (70%) were narcotic. This shows that novel models and approaches are needed to demonstrate more accurately the toxicity PCPs may represent.[408] He et al.[409] provide an example of QSAR development for prediction of endocrine disruption in fish, similar developments are needed to demonstrate the role of QSARs in an Adverse Outcome Pathway (AOP) context for PCPs.[408] The previously mentioned regulatory development and use criteria by Walker et al.[404] still apply to the novel QSARs and tools, that need to be developed to support assessment and decision-making regarding PCPs in the environment. The most prominent limitation is lack of access to good chronic data to provide sound statistical models as well as appropriate elucidation of key molecular interactions between the compound and the receptors and the relevance of this interaction for the organism.

13.1 Models for PPCPs

13.1.1 Ecotoxicity models of PPCPs for diverse test species

Sanderson et al.[58] ranked 2986 PPCPs into 51 classes relative to their hazards toward daphnia, algae, and fish using the EPIWIN software, especially the ECOSAR program, available from the US EPA site (http://www.uoguelph.ca/~hsander/) for assessing toxicity to the aquatic environment and to provide a baseline to fill the screening data regarding the environmental toxicity of APIs. The study suggested that modifying additives were the most toxic classes whereas gastrointestinal drugs, cardiovascular, anxiolytics, hypnotics, sedatives, antivirals, antipsychotics, thyroid, and corticosteroid pharmaceuticals were predicted under most perilous therapeutic classes. The global relative order of vulnerability was assessed to be daphnia > fish > algae. The authors ranked $\log K_{ow}$ data for all 51 classes which is an important indicator for the potential to bioaccumulate in the ecosystem. The authors evaluated overall hazard ranking for all 51 classes based on the mean predicted toxicity as a function of the frequency of the predicted toxicity within each class. The overall hazard ranking defined as mean HQ*percent HQ > 1; where HQ is a hazard quotient and expressed as the ratio of PEC and PNEC. The relative ranking of top 20 therapeutic classes based on $\log K_{ow}$ and overall hazard ranking of top 15 therapeutic classes to algae, daphnia and fish are reported in **Figure 20**. Considering the combined effects of all results, the authors found that 16% of the classes would exceed a HQ of 1 even without the assessment factor of 1000. The cardiovascular drugs, sedatives, anxiolytics, antipsychotics and hypnotics, and gastrointestinal drugs were predicted to be the most hazardous therapeutics for all three species. Sex-hormones, sunscreen agents, antimalarials and antifungals are predicted to be the most frequent hazardous therapeutic pharmaceuticals (% HQ < 1). Among the personal PCPs, modifying additives (paraffins and surfactants) were the most toxic, followed by pesticides and repellants, nutritional agents and vitamins as per the study. The obtained hazardous effect trend of all PPCPs is quite similar with the relative ranking of $\log K_{ow}$.

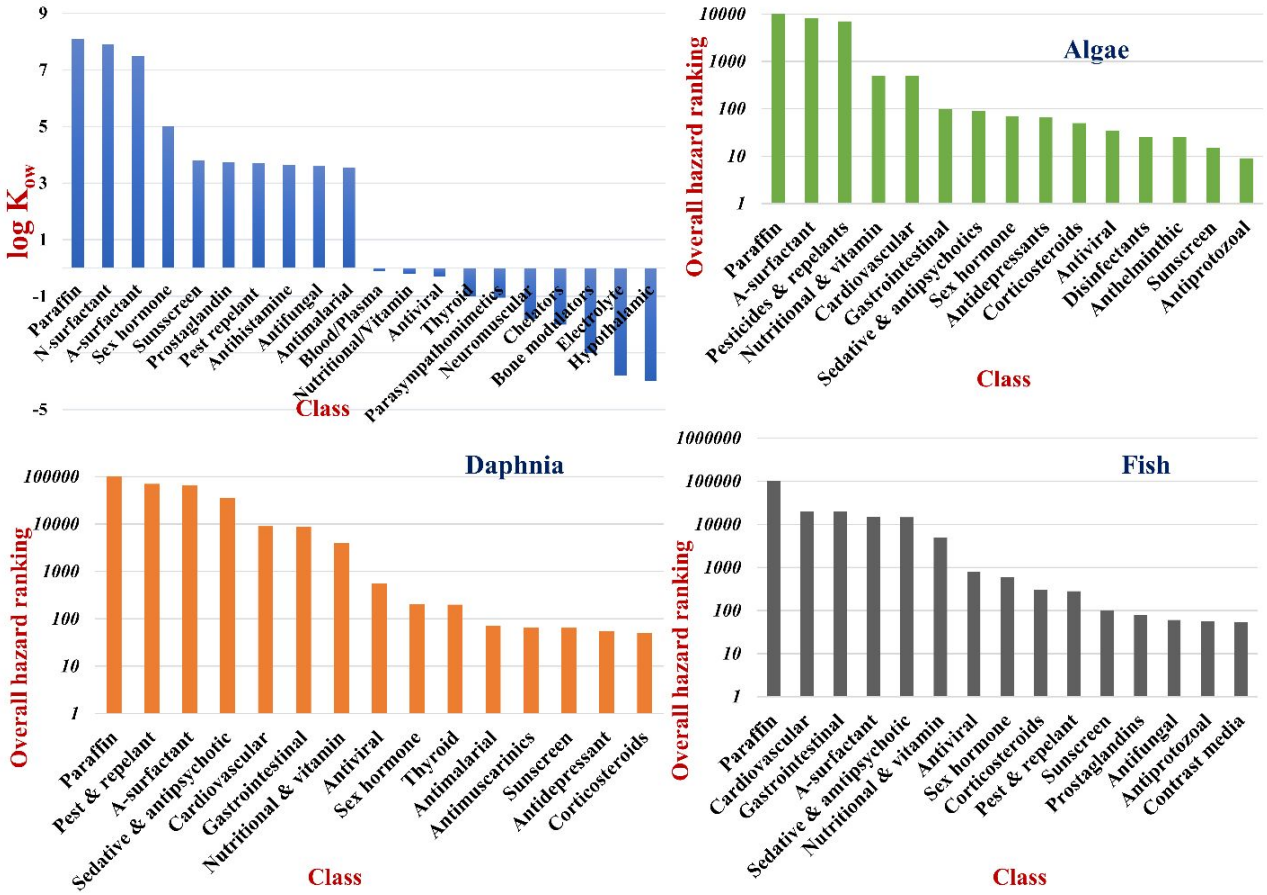


Figure 20 Top left: The relative ranked log K_{ow} for top 20 classes of PPCPs; The overall hazard raking for top 15 classes of PPCPs to algae (**top right**), daphnia (**bottom left**), fish (**bottom right**).

Kar and Roy[59] developed one of the first interspecies QSAR models to correlate the ecotoxicity of structurally diverse 77 pharmaceuticals to *Daphnia magna* and fish. The  (aasC) fragment and keto group are predominantly accountable for higher toxicity of pharmaceuticals to *D. magna* (**Figure 21**). Again, along with the keto group, structural fragments like X=C=X, R–C(=X)–X, and R–C≡X are significant features for the high toxicity values to fish (**Figure 22**). The interspecies QSAR models were further implemented to predict fish toxicity of 59

pharmaceuticals (where experimental Daphnia toxicity present) and Daphnia toxicity of 30 pharmaceuticals (where experimental fish toxicity present). The models demonstrated an enhanced and comprehensive risk assessment of pharmaceuticals where toxicity data is missing for a specific species.

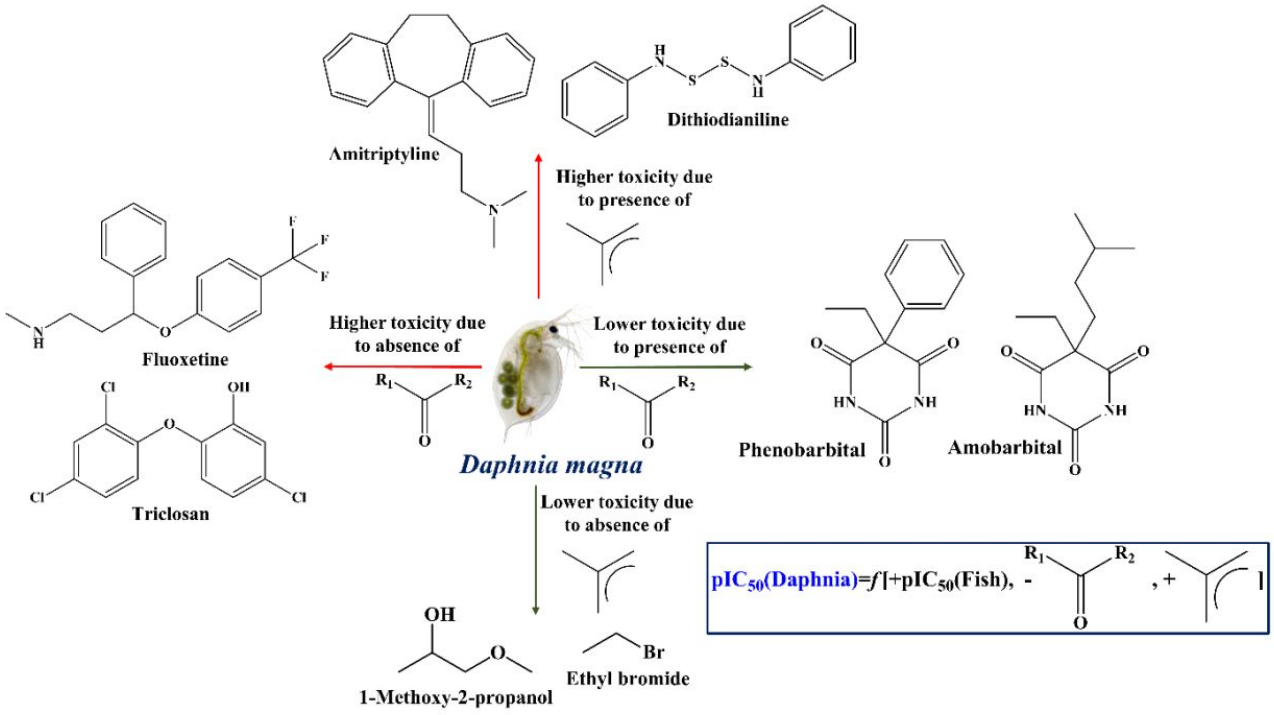


Figure 21 Mechanistic interpretation of pharmaceuticals toxicity to *D. magna*.

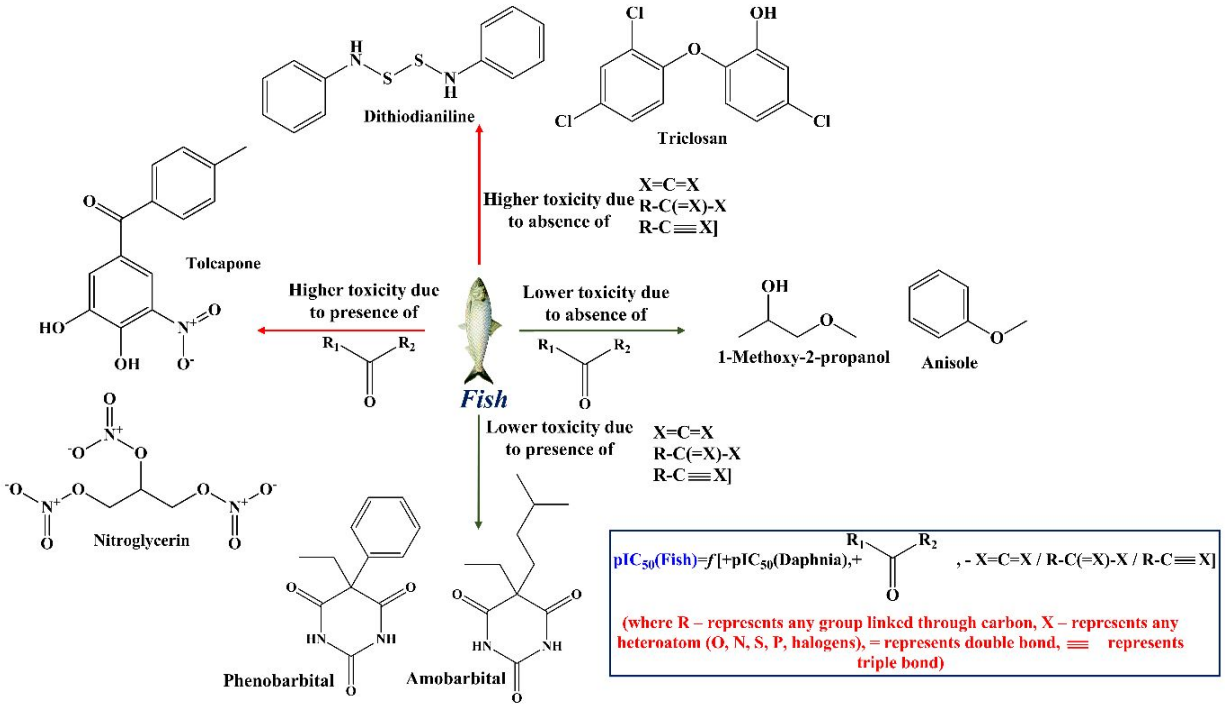


Figure 22 Mechanistic interpretation of pharmaceuticals toxicity to fish.

Sangion and Gramatica[60] developed quantitative activity–activity relationship (QAAR) models with a good correlation between toxicity of PPCPs towards invertebrate *Daphnia magna*and towards two fish species namely *Pimephales promelas*and *Oncorhynchus mykiss* with single theoretical molecular descriptor which helped to explore the relationship between toxicities in invertebrate-fish species. The authors developed interspecies models employing three datasets: *D. magna-O. mykiss* (51 PPCPs, case 1), *D. magna–P. promelas* (44 PPCPs, case 2) and *P. promelas–O. mykiss* (36 PPCPs, case 3 and 4). MLR by the ordinary least squares (MLR-OLS) technique was applied by using the QSARINS software.[61] The study demonstrated importance of autocorrelation descriptors in interspecies correlations. The developed QAAR models can fill the data gap and are helpful tools for the prioritization of the hazardous PPCPs. The models are able to decrease the requirement of more complex experimental tests on upper trophic organisms also

saving animal lives. The most significant conclusion provided the by authors are the followings: a) daphnia toxicity could serve as a surrogate for fish toxicity and b) the fish–fish intercorrelations could be applied for assessing toxicity data when experimental information is unavailable. Major obtained mechanistic interpretation of toxicity towards each studied species is illustrated in **Figure 23**.

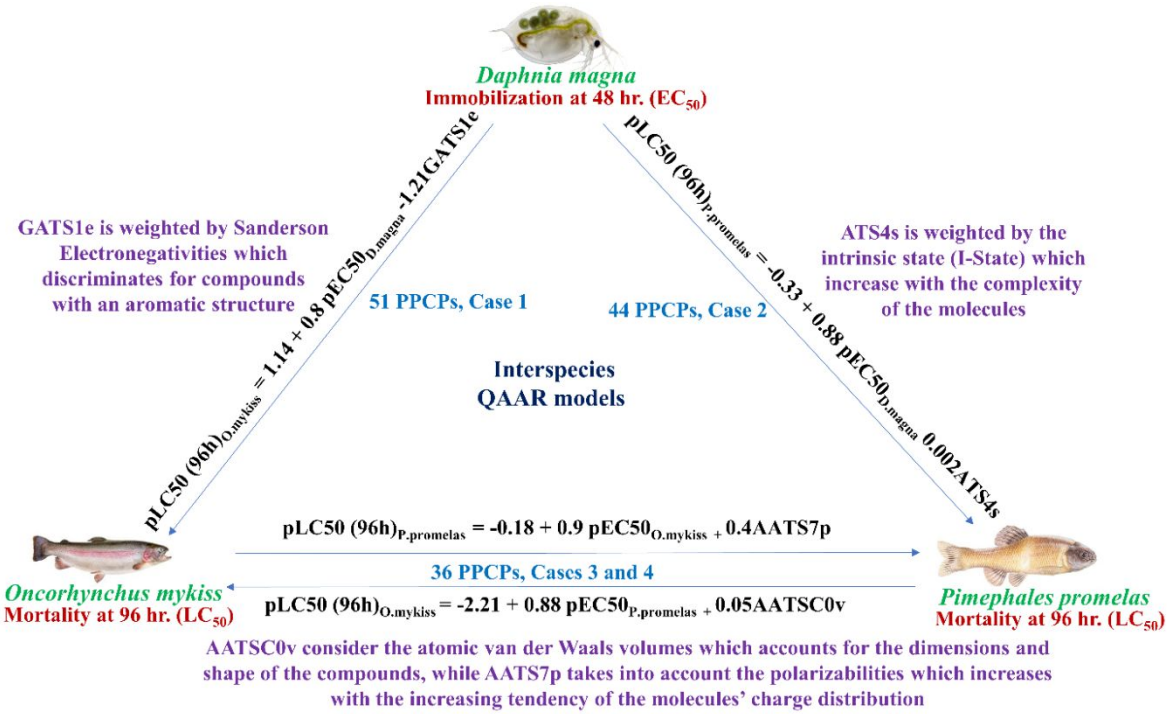


Figure 23 Mechanistic interpretation of toxicity towards *D. magna*, *O. mykiss* and *P. promelas*.

Acute toxicity (48-h concentration causing 50% mortality) of 55 PPCPs towards the freshwater planarian *Dugesia japonica* were modeled with the QSTR tool by Önlü and Saçan.[410] Like majority toxicity modeling studies, the authors found hydrophobicity as one of the important parameters to model the aquatic toxicity for the mentioned species and found a correlation coefficient value of 0.58. To improve the model's quality, the authors computed DRAGON descriptors and generated the final QSTR model employing MLR-OLS. The five descriptor

QSTR equation including hydrophobicity explained more than 80% variance of the toxicity. The positive coefficients of all descriptors appearing in the QSTR equation contribute to *D. japonica* toxicity positively (**Figure 24**). Log K_{ow} or hydrophobicity seemed to be the most imperative feature for the toxicity because of simple perturbation of membrane function. The second important identified feature is GATS7p, which signifies higher atomic polarizability in a chemical resulting in the toxicity. SpMaxA_G/D defines the folding degree of a molecule whose value leads to 1 for linear chemical and decreases along with the branching, suggesting the changes in molecular size and shape whichhas a positive contribution towards the toxicity. The reason behind this is quite clear as chemicals with higher number of flexibile fragments can fit better within the interacting proteins and show stronger binding afinity with the toxicity causing amino acid residues. The descriptor Mor31s signifies molecule depiction of structures based on electron diffraction calculated upon the scattering para-meter and weighted by the intrinsic state (I-state). The I-state of an atom can be interpreted the probable partitioning of the effect of non-σ electrons throughout the σ bonds. Thus, the less partitioning of the electron impact can be attributed to the valance electrons followed by the intermolecular interactions, resulting in higher toxicity to the studied species. The fifth and the last identified descriptor is CATS2D_08_DL, which designates the presence of a hydrogen bond donor and a lipophilic center at 8-bond topological distance, and it increases the toxicity. To apply a QSTR equation for data gap filling of untested compounds' toxicity for the *D. japonica*, the authors employed 792 industrial chemicals including 317 designated HPV chemicals according to OECD. The AD study suggested reliable prediction of 85% of the total number of chemicals. The authors also developed the i-QSTR to predict the toxicity to *D. japonica* for 266 chemicals which have

experimental data for *D. magna*. The QTTR model reliably predicted 259 chemicals within the AD which is 97% of the total number of modeled compounds.

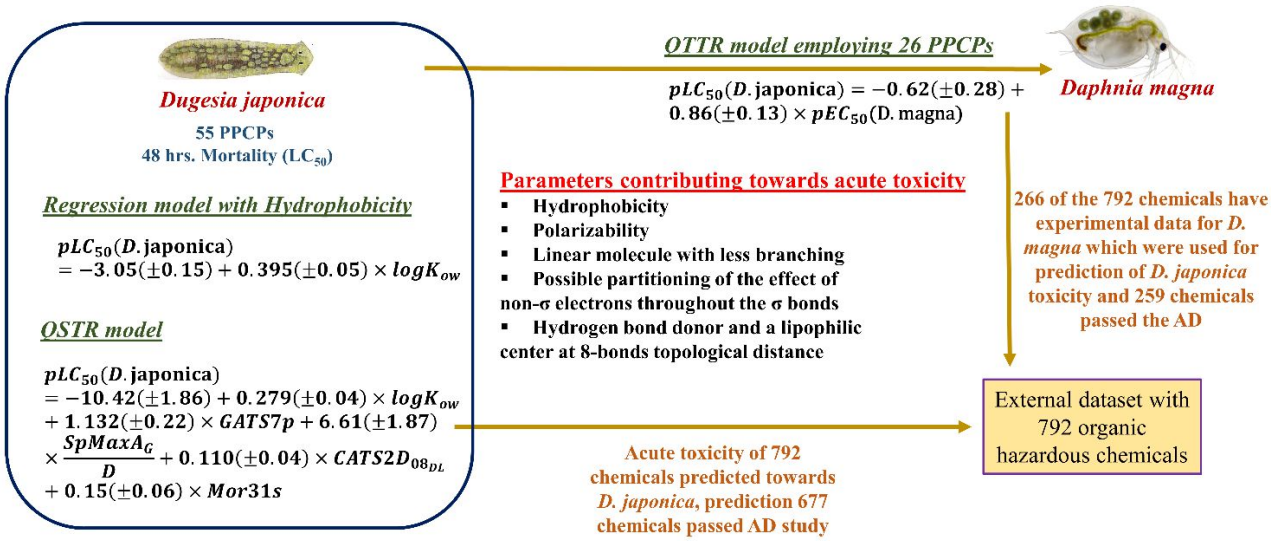


Figure 24 Mechanistic interpretation of QSTR and QTTR models for *D. japonica* and *D. magna*.

Khan et al.[411] developed ecotoxicological QSTR models for 260 pharmaceuticals spanning over diverse therapeutic classes on three trophic level species like green algae *Scenedesmus subspicatus* (134), crustacean *Daphnia magna* (209) and fish *Brachydanio rerio* (192) employing the PLS approach using simple 2D descriptors. Followed by the development of QSTR models, the authors reported i-QSTR models using GA-MLR statistical tool to identify relationships among the toxicity values across the hierarchy of genetics in different taxonomical classes. Utilizing the respective i-QSTR models, the toxicity data of 103 pharmaceuticals were predicted for fish and algae where daphnia data were present, 86 pharmaceuticals were predicted for daphnia and algae where fish data were present, and 28 pharmaceuticals were predicted for fish and daphnia where algae data were present. Most importantly, the authors successfully utilized all i-QSTR models to fill the data gaps for 260 pharmaceuticals, where experimental data were

missing for at least one of the endpoints. The authors testified hydrophobic property to play the maximum contribution towards the toxicity of the considered pharmaceuticals irrespective of species followed by contributions of structural fragments like tertiary amines, carboxamide, carboxylate, allyl functionalities etc. which are also responsible to the toxicity of pharmaceuticals towards diverse aquatic species. A complete summary and findings of the study are demonstrated in **Figure 25**. Khan et al.[411] suggested that if pharmacophoric prerequisite demands a hydrophobic group for pharmacodynamic effects, then a substitution with higher polarity should be preferred while such developed molecules may be less active systemically. Further, individual QSTR models were utilized for prioritizing 7106 pharmaceuticals without having experimental data using predicted response values, and ranking of pharmaceuticals was reported to provide the toxicity threshold for each species. The authors projected top 30, 13 and 25 top toxic pharmaceuticals based on their study from the modeled dataset, drug-like molecule dataset, and Interbioscreen dataset, respectively.

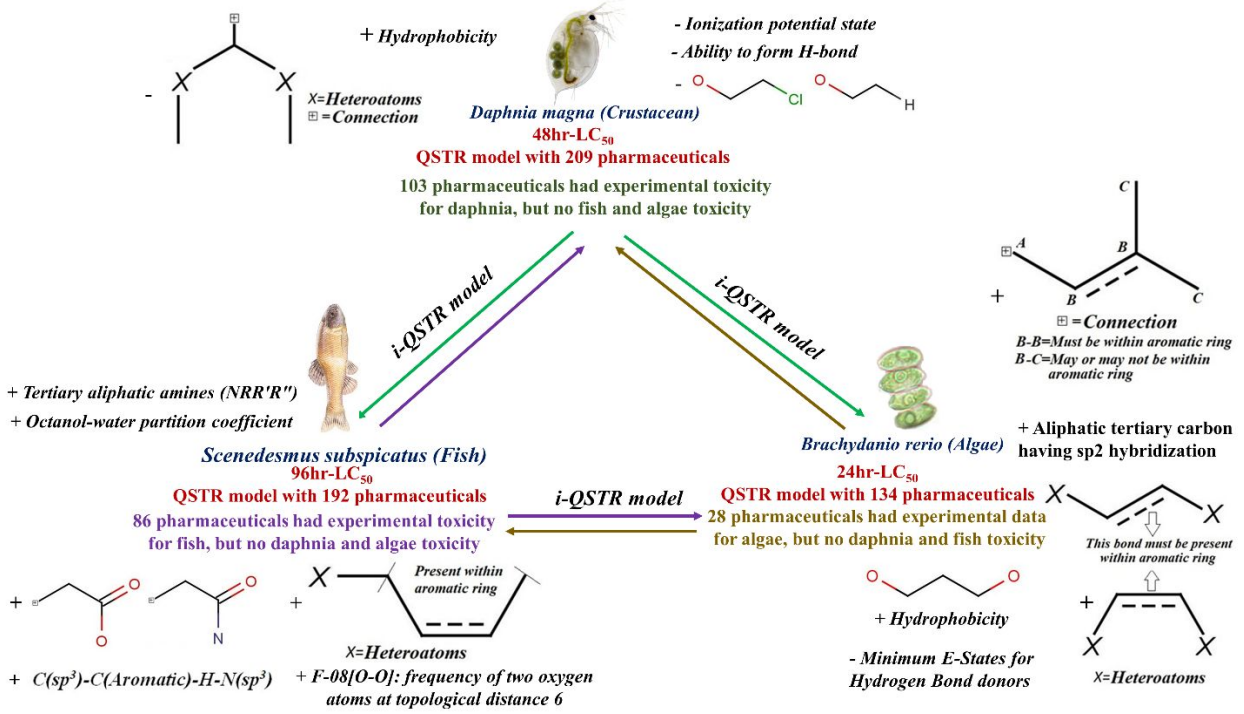


Figure 25 Mechanistic interpretation from obtained QSTR and i-QSTR models for *D. magna*, *S. subspicatus* and *B. rerio* (+ and – before the features showed the contribution of the features).

Önlü and Saçan[62] developed one of the first QSTR models to predict the cytotoxicity of PPCPs on the rainbow trout (*Oncorhynchus mykiss*) liver cell line RTL-W1. The models were developed by employing cytotoxicity data obtained from the 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) and Alamar Blue (AB) assays. The authors found a strong correlation (R = 0.986; $p < 0.01$) between the two cytotoxicity endpoints (pEC_{50}, CFDA-AM and pEC_{50}, AB). For both endpoints, two common properties encoded the relationship between structure and cytotoxicity which measure the metabolic activity and membrane integrity, respectively. The first feature is nRCOOH, a simple, one-dimensional functional molecular descriptor representing the number of aliphatic carboxylic acid present in the molecule of interest. The negative contribution of nRCOOH signifies that cytotoxicity decreases along with the increase in a number of aliphatic carboxylic acid functional groups in a compound. The authors claimed that the hydrophilic and polar nature, as well as the hydrogen bond forming capability, make compounds with the carboxylic group more water-soluble followed by increasing the metabolism and eventually preferring their elimination. The second important descriptor is E_{HOMO}, the highest energy level containing electrons in the compound which gives information about reactivity/stability of specific fragment of compounds and capable to measure the nucleophilicity of a molecule. Molecules with a high E_{HOMO} value can donate their electrons more easily compared to molecules with a low E_{HOMO} value, and hence are more reactive. The positive influence of this feature suggests that cytotoxicity and E_{HOMO} are directly proportional. The authors suggested that cytotoxicity increases with increasing nucleophilicity and reactivity of the

studied compounds. The authors applied the QSTR model to predict the cytotoxicity of 101 chemicals including PPCPs and industrial chemicals on RTL-W1 cell line with 91% structural coverage. Based on the external set prediction (pEC_{50}, AB values), they concluded that antibacterial chemicals are relatively the least cytotoxic, whereas antipsychotic pharmaceuticals are relatively the most cytotoxic. Further, the authors explored a good correlation between *in vivo* fish acute toxicity (pLC_{50} for 96 h) and *in vitro* cytotoxicity (pEC_{50}, AB).

Khan et al.[412] developed QSAR models for ecotoxicity of pharmaceuticals collected from the ECOTOX database[413] along with other literature on four aquatic species *Pseudokirchneriella subcapitata*, *Daphnia magna*, *Oncorhynchus mykiss* and *Pimephales promelas* employing G/PLS statistical tool and 2D descriptors. The authors employed hydrophobicity parameters for modeling purpose due to the known dependence of toxicity on logP terms. The major toxicity contributing features were the size and bulk of molecules, polarity, hydrophobicity. The authors mentioned that molecules having highly polar groups with complex and rigid core structures showed higher toxicity against algae suggesting the idea of polar narcosis. Organometallic compounds and molecules with macrocyclic ring tend to show more toxicity towards aquatic species. The features contributing positively and negatively for each species are described in detail in **Figure 26**. Further, the developed consensus models were employed to predict acute toxicity of 9188 pharmaceuticals and drug-like compounds from the DrugBank which have no experimental toxicity data for all four species. Additionally, the ECOSAR software[413] was used for parallel prediction for comparison and check the reliability of predictions from consensus models followed by prioritization of toxic pharmaceuticals for each species considering

predictions from the developed models and ECOSAR. Finally, a prioritized list of 500 most toxic pharmaceuticals and drug-like compounds had been reported.[412]

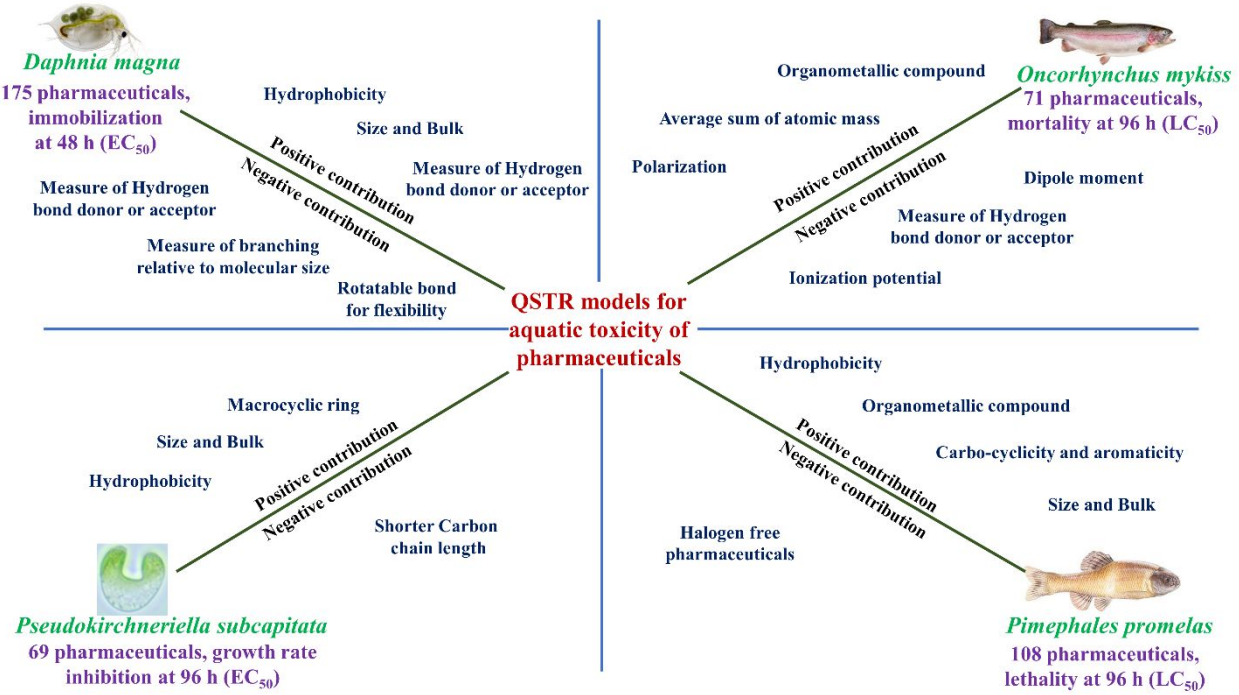


Figure 26 Critical features responsible for toxicity towards *D. magna*, *O mykiss*, *P. subcapitata* and *P. promelas*.

Sangion and Gramatica[414] developed MLR-OLS based QSTR models employing 1267 human and veterinary pharmaceuticals collected from the ECOTOX database[360] to predict acute toxicity in four species (*D. magna*, *P. subcapitata*, *P. promelas*, *O. mykiss*) spanning from three main aquatic trophic levels. In case of *P. subcapitata*, electrotopological state of the H atom bonded to sp^2 hybridized carbons accounting for electronic perturbations of the near substituents and the availability of a bond to be attacked by atoms in intermolecular interactions features contributed in decreasing the toxicity. On the other hand, the presence of multiple bonds in relation to their length helps in increasing the toxicity. For *D. magna,* lipophilicity contributes significantly as discussed in earlier literature. The models for *O. mykiss* suggested that the molecular size, more

branching and ring closure influence the toxicity positively whereas higher possibility of forming hydrogen bonds may decrease the toxicity. The hybridization ratio *i.e.* the fraction of sp^3 C to sp^2 C discriminates the aromatic structures from the non-aromatic ones which are inversely related to the toxicity. This clearly suggested that the presence of double bonds or aromatic rings in chemical structure has a positive impact on toxicity. In case of *P. promelas*, the spatial density of atoms in a molecule and van der Waals volume have positive effects on the toxicity. A higher value of these features increases the toxicity. On the contrary, the number of hydrogen bond acceptor atoms have a negative impact on the toxicity (**Figure 27**). Thereafter, the constructed models were applied to predict acute toxicity of huge number of APIs without having experimental data employing PCA approach. Further, individual APIs were ranked based on toxicity and "Aquatic Toxicity Index (ATI)" was generated which will be highly helpful for toxicity data gap filling followed by ERA.

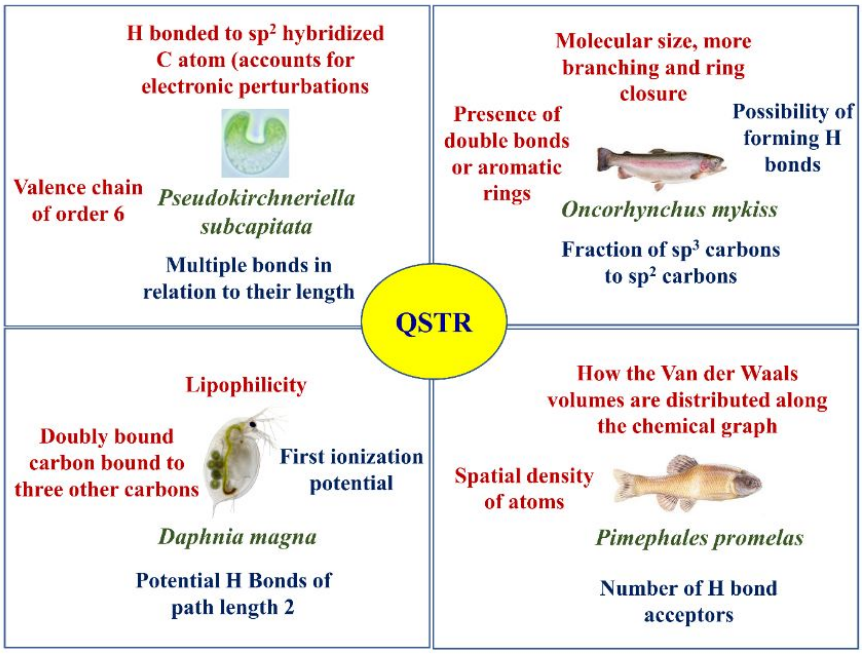


Figure 27 Mechanistic interpretation from the obtained QSTR models for all four species. (Features colored in red text and blue text denote positive and negative contribution to toxicity, respectively.)

Hossain and Roy[415] reported QSTR models employing 75 CECs including pharmaceuticals, surfactants, UV filters, hormones, preservatives and organophosphates to predict aquatic ecotoxicity towards freshwater planarian (*D. japonica*) employing the PLS statistical tool. The most significant features and fragments responsible for higher and lower toxicity are illustrated in **Figure 28** as identified from all five cumulative PLS models. Out of 75 CECs in the *D. japonica* data set, 47 had their reported toxicity values against *D. magna* and 19 for fish (*P. promelas*), and that is why the author's developed i-QSTR models for both species with *D. japonica*. The i-QSTR model between *D. japonica* and *D. magna* explored two important features for better correlations, and they are B08[C-O] and B09[N-O]. B08[C-O] represents the topological distance 8 between C and O atoms in a specific molecule and has a positive contribution suggesting that the presence of this fragment in a compound will increase the toxicity. Among the studied molecules 17-α-ethinylestradiol, 17-β-estradiol, diethyl-stilbesterol possess this fragment and comparatively showed more aquatic toxicity than others. The second significant fragment is B09[N-O] which indicates the topological distance (the number of consecutive bonds) 9 between atoms N and O which has a negative contribution on the toxicity as it has H-bond donating capability which makes the molecules hydrophilic and offers resistance to penetrate through a biological membrane. Antibiotics like chlortetracycline, tetracycline, ofloxacin, trimethoprim comprising this fragment exhibit less toxicity. Again, the interspecies model between *D. japonica* and *fish* explored two imperative features for improved correlations, and they are C-006 and H-052. The C-006 descriptor defines the number of CH_2RX

fragments, where X is a hetero atom (O, N, S, P, Se or halogens) and R is any group linked through carbon, having a positive contribution to the toxicity, and thus signifying that the molecule with this specific fragment will be more toxic. Compounds like propranolol, sodium dodecyl sulfate, chlorpyrifos, glyphosate *etc*. comprising of this fragment showed higher aquatic toxicity. The authors proposed that CH_2RX influences the size of a molecule due to the number of CH_2 groups and electronegativity due to the number of X atoms which could be the reason for toxicity. The second atom-centered fragment H-052 encrypts the number of hydrogens attached to sp^3 hybridized C

with one hetero atom at the β position. The negative contribution of this fragment suggests that it makes molecules less toxic to aquatic species. The developed QSTR and i-QSTR models were utilized to predict acute toxicity of ECOTOX database consisting of 99 CECs (daphnia toxicity present) and 51 CECs (with fish toxicity present) for the toxicity against *D. japonica*. The interspecies models for fish and daphnia showed 80% and 91% prediction coverage, respectively.

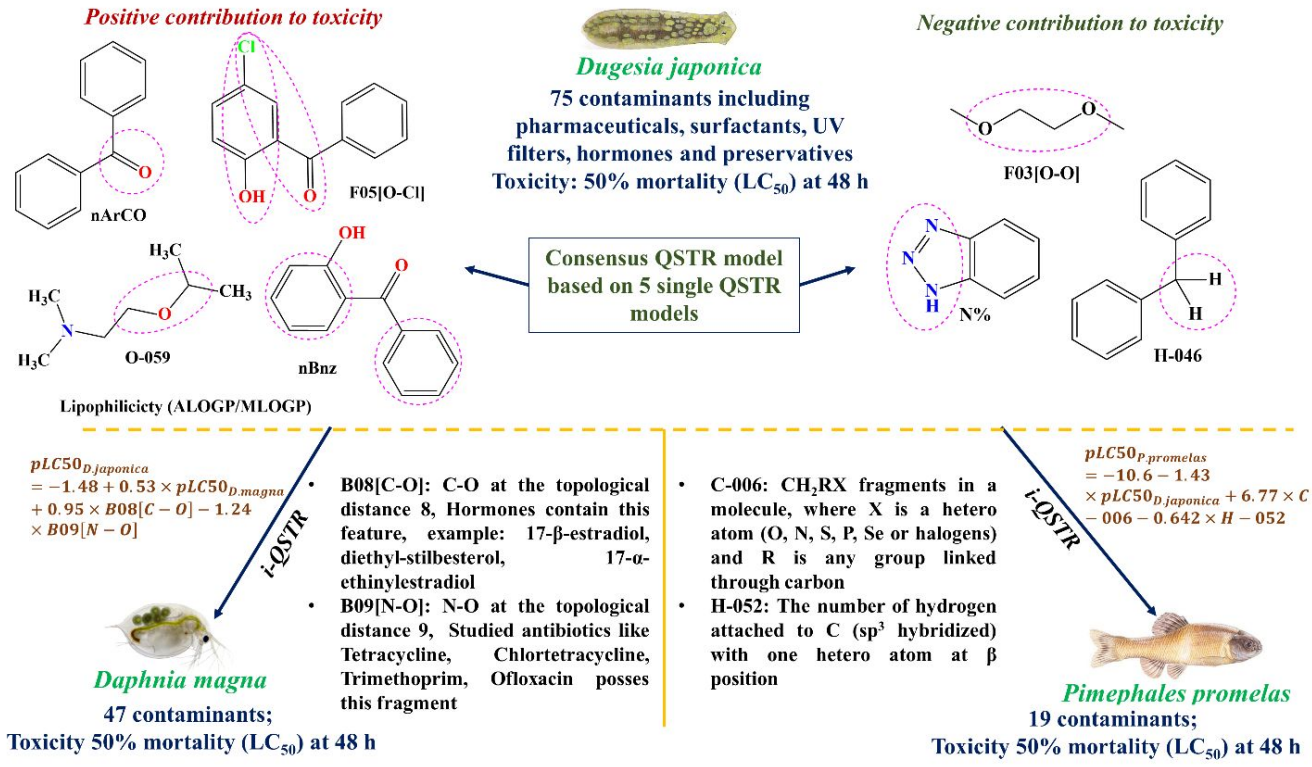


Figure 28 Mechanistic interpretation of toxicity to *D. japonica*, *D. magna* and *P. promelas* from developed QSTR and i-QSTR models.

Khan and Roy[416] reported ecotoxicological QSTR model for PCPs on three aquatic organisms namely *Daphnia magna* (134 PCPs), *Pseudokirchneriella subcapitata* (30 PCPs), and *Pimephales promelas* (74 PCPs) employing the PLS statistical tool following the OECD guidelines. The obtained models highlight the structural requirements and molecular properties essential to design safer cosmetics. The obtained models suggested that with an increase in log P, molecular size, polarizability, a higher number of branching and rotatable bonds as well as the presence of sulphuratoms, the toxicity of PCPs increases. The individual model-specific features can be checked in **Figure 29** with a complete workflow employed by authors. The authors then compared their predicted results with the ECOSAR software outcome which is generally employed for risk assessment approach by regulatory agencies. Predictions obtained from the

QSTR models and ECOSAR tools were used to rank the PCPs based on their average scaled aquatic toxicity values. Further, for ranking of an entire external set of 596 compounds, all three obtained QSTR models as well as ECOSAR software were applied by authors to predict the toxicity values of the entire dataset against respective endpoints. Interestingly, the ranking of PCPs was done purely based on the average scaled scores, without taking into consideration any structural class of chemicals or functional groups. Khan and Roy[418] listed top 100 chemicals comparing the model and ECOSAR based predictions where Phthalate, UV-filter, fragrance, and antimicrobials are in the top 20 toxic PCPs.

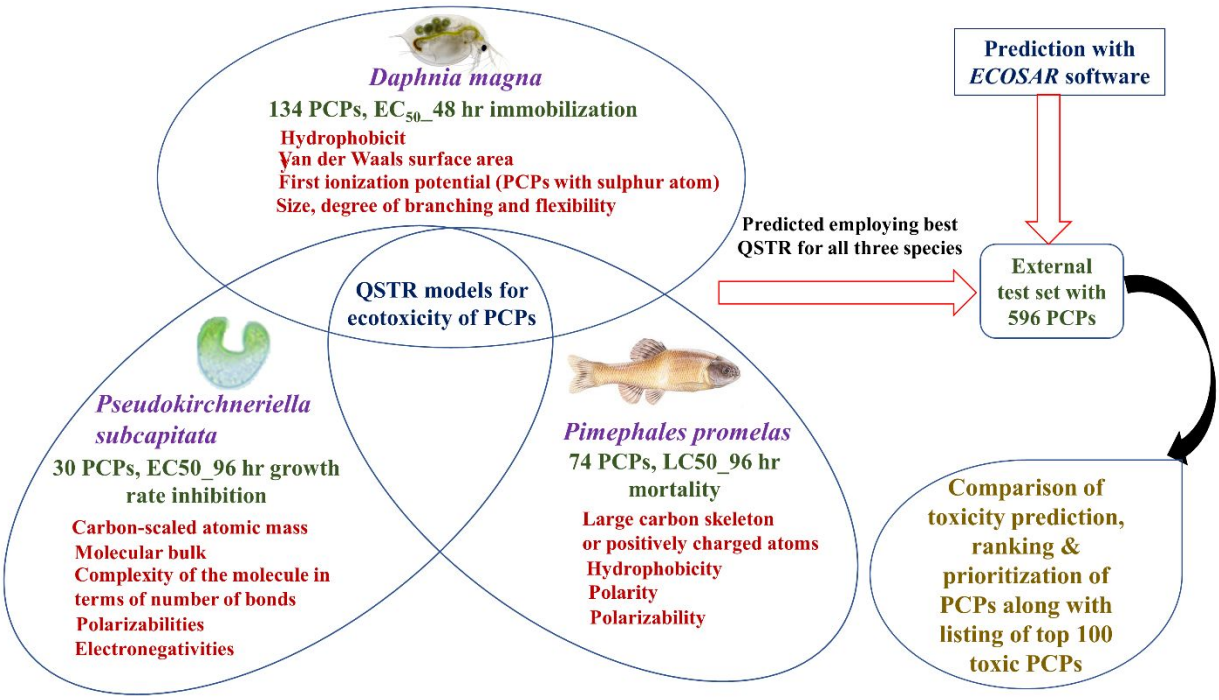


Figure 29 Mechanistic interpretation of toxicity of PCPs towards *D. magna*, *P. subcapitata* and *P. promelas*.

13.1.2 Models for miscellaneous toxicity due to PPCPs

The environmental behavior of PCPs needs to be examined; data on persistence, bioaccumulation, and toxicity (PBT) are very rare for the majority of PCPs. Cassani and Gramatica[63] investigated the possible cumulative PBT behavior of 534 PCPs consisting of 393 fragrance and flavoring agents, 66 UV filters/sunscreen agents, 38 phthalates, 27 hair-dye ingredients, 8 parabens, and 2 antimicrobial agents employing two modeling tools: the Insubria PBT Index, a QSAR model under the QSARINS software, and the USEPA PBT Profiler. The screening allowed to identify the most hazardous PCPs, which are predicted as potential PBTs by both methods, in a consensus approach. The PBT Index prediction allows classifying PCPs into non-PBT, "medium" PBT and PBT chemicals considering a preset arbitrary threshold. The authors considered the threshold at PBT Index ≥1.5, to highlight the PBT and very persistent very bioaccumulative (vPvB) chemicals while remaining PCPs that are predicted with a PBT Index <1.5 are considered non-PBT. A priority list of the potentially most hazardous PCPs was reported in agreement by both the modeling tools. Only eight PCPs (7 of them are UV-filters) were prioritized by consensus as for the most hazardous considering the PBT behavior, while the majority (472 out of 534 studied PCPs) are predicted as potential non-PBTs (**Figure 30**). The linear OLS model consists of four molecular descriptors which are independent of the molecular conformation. The descriptor nX defines a number of halogen atoms, and nBondsM suggests the number of multiple bonds or the unsaturation degree, which counts the total number of bonds that have bond order greater than one. Due to the positive sign, both features are helping to increase the cumulative PBT behavior. The third feature nHBDon_Lipinski defines a number of hydrogen bond donors using Lipinski's definition which characterizes the prospect to form hydrogen bonds with water, increasing its solubility, while MAXDP2, maximal electro-topological positive variation encrypts for the distribution of electronic features and polarity.

Interestingly both features have negative signs as soluble and more polar PCPs have, in general, lower cumulative PBT behavior. This study strongly suggested that the PBT Index could be an effective tool to identify instantly from the molecular structure safer and more environmentally sustainable chemicals even before synthesis to avoid high failure rate and monetary expense.

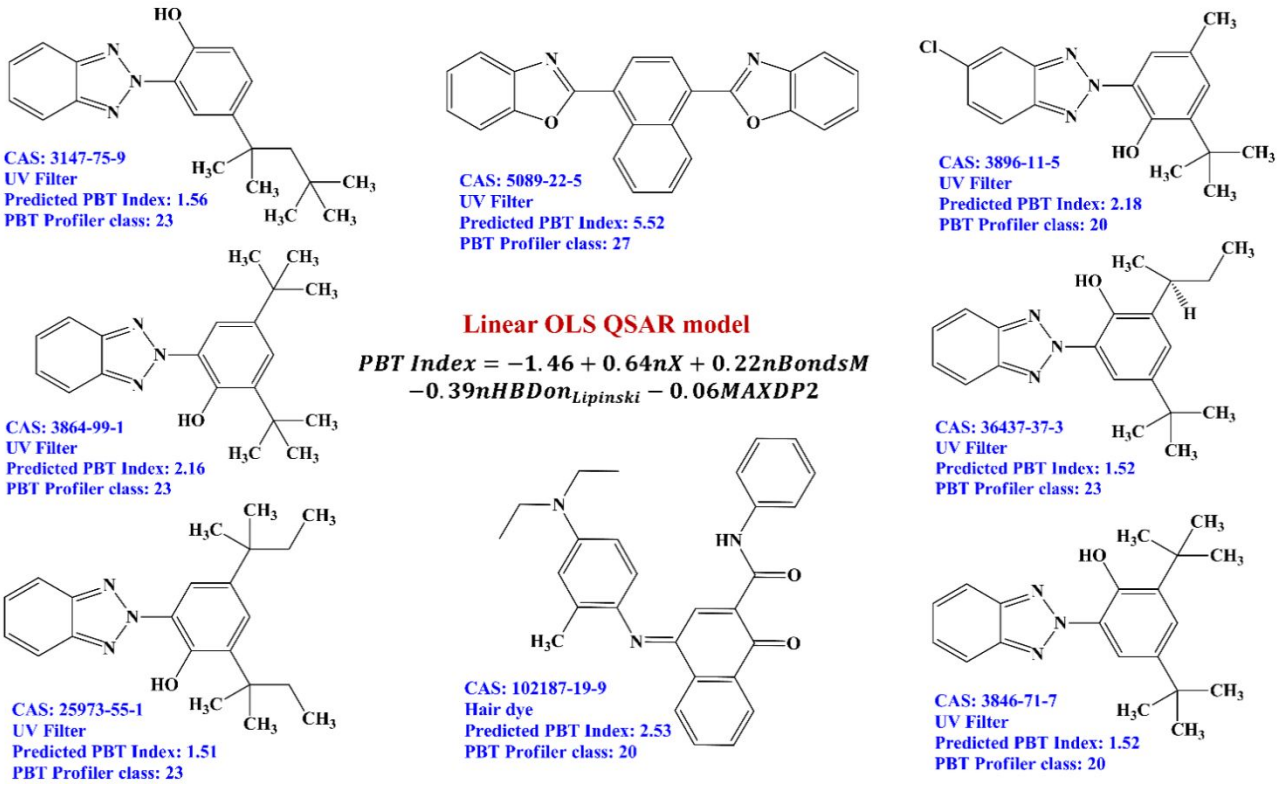


Figure 30 Eight potential PCPs as per reported PBT Priority list by the consensus approach along with the developed QSTR model.

The endocrine disrupting activity of perfluoroalkyl substances (PFASs) is modeled employing regression and classification based QSTR models followed by docking studies to understand important structural fragments responsible for higher and lower toxicity profiles by Kar et al.[64] A combination of ligand and structure-based modeling conclude that carbon chain length has a major role to play in determining the toxicity potency. The following significant observations (**Figure 31**) are reported by authors:

- The studied PFASs containing acid functional groups are highly toxic with the carbon chain length between 6 and 10 (example: 7H-Perfluoroheptanoic acid, Perfluorohexanoic acid). On the contrary, lower toxicity is observed when PFASs consist of C chain length greater than 10 or below 6 (examples: Perfluorotetradecanoic acid, Perfluorododecanoic acid).
- PFASs consisting of sulfonate or sulfinate functional groups are toxic ones (examples: Perfluorohexane sulfonate, Perfluorooctane sulfinate). Compounds will be of lower toxicity if the carbon chain length is over 8 (Example: perfluorodecane sulfonate).
- PFASs substituted with an alcohol functional group are lower or non-toxic, regardless of their carbon chain length (Example: 2-Perfluorohexyl ethanol, 2-Perfluorooctyl ethanol).
- PFASs consisting of sulfonamide functional groups are toxic when C chain length equal to 8 (Perfluorooctane sulfonamide). On the other hand, substitution of sulfonamide groups with alkyl or alcohol group leads to lower or non-toxic regardless of their carbon chain length (N-methyl perfluorooctane sulfonamide, N-ethyl perfluorooctane sulfonamide).

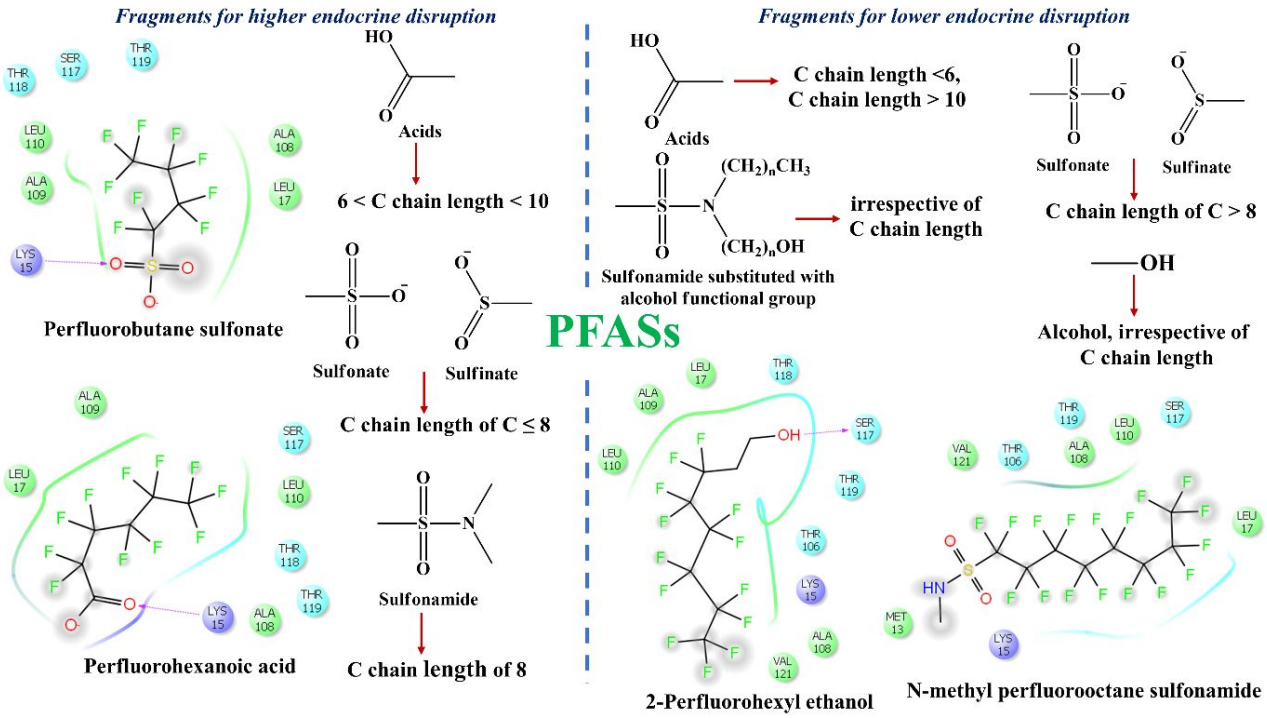
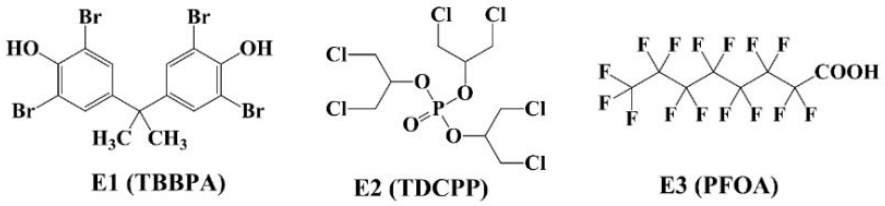


Figure 31 Critical features related to endocrine disruptions of PFASs.

Kar et al.[417] developed statistically robust QSTR models employing single and mixture halogenated molecules using a weighted descriptors approach for developmental toxicity on zebrafish (*Danio rerio*) embryos. The developed model further implemented to predict two external test sets of halogenated compounds (16) and PFASs (2324) after checking the AD of the studied molecules. The first external test set consists of binary and tertiary mixtures used to check their possible threshold and mode of toxicity for future risk assessment; and the PFAS dataset consists of single (24), binary (276) and tertiary (2024) mixtures of PFASs. Based on a complete study, the authors concluded that chemicals in mixtures exhibited concentration addition (dose addition) of a specific chemical signifying a similar mode of toxic action and non-interaction. Additionally, mixtures of halogenated compounds including PFASs showed less toxicity than their single counterparts, and the observed toxicity trend is Single >Binary

mixture> Tertiary mixture. The predicted values of a huge external set of mixtures can be useful as a toxicity profile repository due to the huge scarcity of mixture toxicity data of PFASs. How the toxicity values are changing from single to binary and tertiary mixtures is demonstrated in **Figure 32** taking results for three studied chemicals as examples.

Studied chemicals in mixtures displayed *concentration addition* of individual chemical suggesting *a similar mode of toxic action and non-interaction* of chemicals and the models can be identified as non-interaction or null models with *effect summation*



E1 (TBBPA) E2 (TDCPP) E3 (PFOA)

Single/Mixture	Toxicity as pIC50 in molar scale
E1	5.62
E2	5.36
E3	2.94
E1+E2	4.88
E1+E3	3.86
E2+E3	3.55
E1+E2+E3	3.57

Figure 32 How toxicity trend is changing from single molecule to mixture.

Jean et al.[418] generated a statistically significant and predictive QSAR model for 67 environmental chemicals including good number of PCPs [alcohols, polychlorinated dibenzodioxins (PCDDs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs)] employing the experimental data of adipose/blood partition coefficient for mammals. The reported model identifies that chemicals with higher octanol-water partition coefficients displayed higher adipose/blood partition coefficients. On the contrary, molecules with lipophilic or hydrophobic feature showed higher adipose/blood partition coefficients (**Figure 33**). Followed by the AD check, the best QSAR model was employed by the authors to predict adipose/blood partition coefficient of 513 PCBs,

PCDDs, PBDEs, and PAHs from the US EPA website for environmental risk assessment analysis and data gap filling which would be helpful for pharmacokinetic as well as toxicokinetic profiling of these chemicals in near future. Analyzing the results, it can be confirmed that presence of higher number of halogens (~ 6 to 10) in the studied chemicals resulted in high octanol-water partition coefficient followed by the high value of adipose/blood partition coefficient

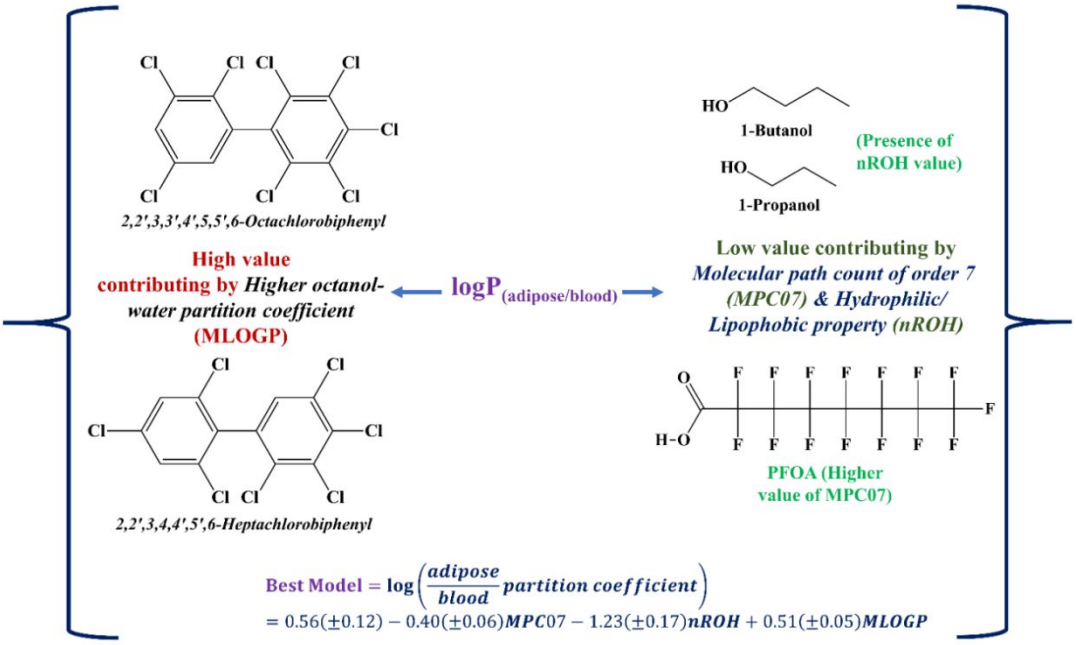


Figure 33 Features responsible for the high and low value of logP(adipose/Blood) for the studied alcohols, PCBs, PBDEs, PCDDs, and PAHs.

Endocrine disruption toxicity was modeled for 144 chemicals including various PPCPs employing QSTR and i-QSTR approaches for 14 species covering four trophic levels across all spheres of environmental compartments by Khan *et al.*[419] to explore crucial features responsible for toxicity to individual species. The obtained GA-PLS models summarized following common

conclusion for all species: chemicals consisting of sulphur, phosphorus and halogens highly impact the toxicity along with hydrophobicity as suggested by logP terms like XlogP and ALOGP2. On the contrary, hydrophilic moieties like aliphatic ethers, esters, branching in molecule and increased O atom contents reduce the toxicity. The major features for each species are depicted in **Figure 34**.

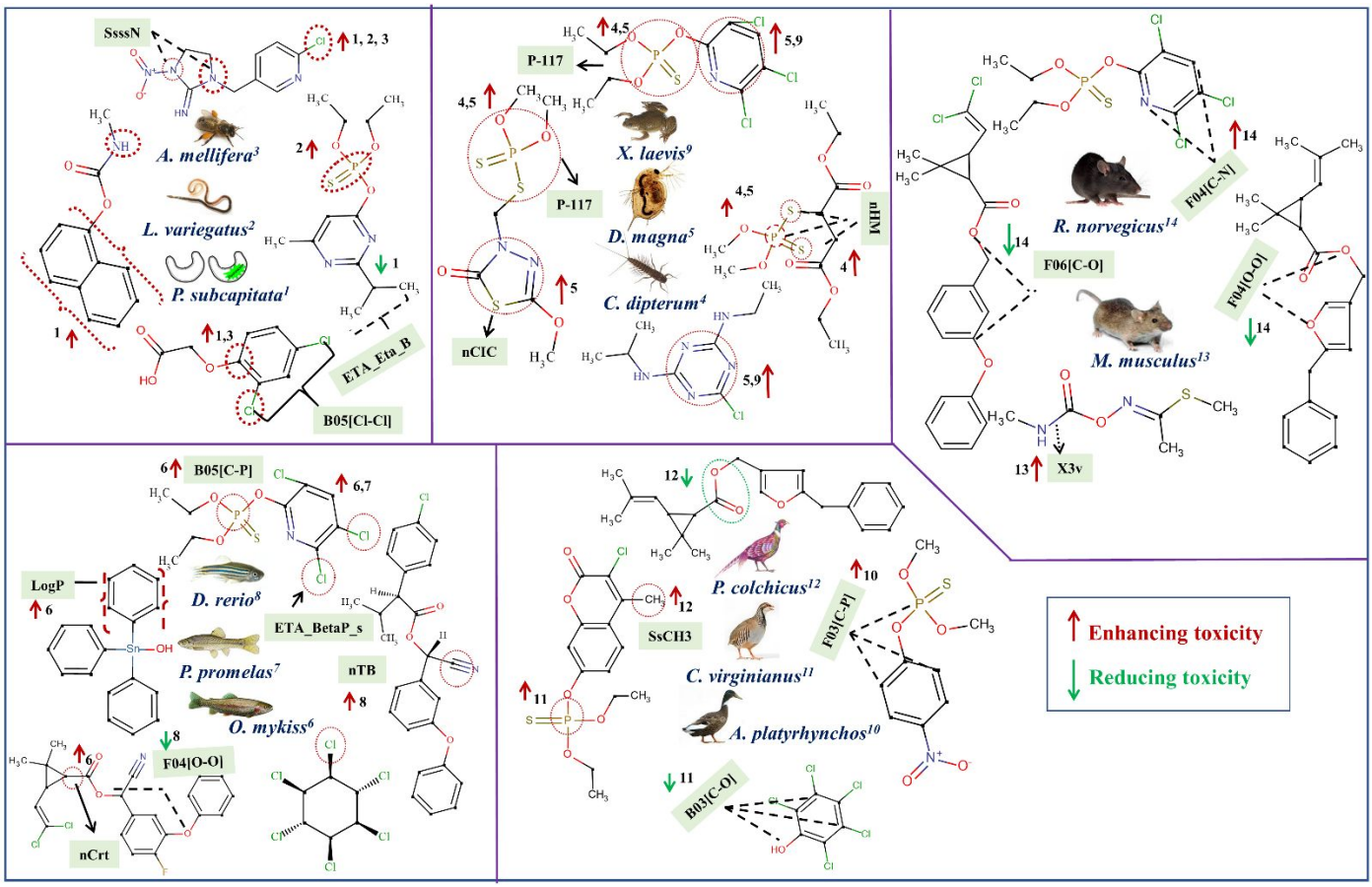


Figure 34 Critical structural fragments accountable for endocrine disruption toxicity against various species.

13.2 Imperative features responsible for ecotoxicity and fate

Comprehensive introspection of the discussed models as well as other literatures[58-64,410-434] help us to understand the major physicochemical properties and structural fragments related to intrinsic chemical reactivity followed by chemical's environmental fate, transformation and toxicity as demonstrated in **Table 7**.[58-64,410-434] Here aquatic, biota, terrestrial and air toxicity are summarized in a single term *i.e.* 'ecotoxicity'; and fate and biotransformation are classified into biodegradation, bioaccumulation and bioconcentration for the simplification of discussion under **Table 7**. Identifying these important features will help chemists for designing of PPCPs with reduced ecotoxicity, better biodegradability followed by reduced bioconcentration and bioaccumulation.

Table 7. Major intrinsic properties and structural fragments related to PPCPs toxicity, fate and transformation.

Property	**Role/How it works**
Physical Properties for ecotoxicity	Freezing point, boiling point, melting point, molecular weight, viscosity, and density are directly associated with environmental fate and health effects. Molecular size and weight increases, bioavailability and aquatic toxicity decrease. At MW > 1000 Da, bioavailability is negligible. Caution must be taken, however, to consider possible breakdown products that may have MW < 1000 Da and exert toxicity.[424]
Solvation properties for ecotoxicity	*Phase partitioning/Partition coefficient*: • LogP/logK_{ow} is the ratio of concentrations of a given compound across two mixed, immiscible phases at equilibrium where one solvent is water or an aqueous phase and the second is organic and hydrophobic, such as 1-octanol (i.e., octanol/water

	partition coefficient [K_{ow}] represented by *P*). Molecular hydrophobicity or lipophilicity (often referred as logP) is one of the most significant parameters for toxicity of PPCPs towards different species of environment. For nonionic organic chemicals that operate though narcosis, acute and chronic toxicity increases exponentially with increases in logP up to a value of ~5. For those whose logP> 5, bioavailability decreases along with acute toxicity, but bioaccumulation also increases. Minimal toxicity is likely with logP < 1.[425] Considering LogP/logK_{ow}, cardiovascular, sedatives anxiolytic antipsychotics and hypnotics, and gastrointestinal were predicted to be the most hazardous therapeutics for *Daphnia magna*, fish and algae.[59,60,410-412,414] • Log*D* is defined as the ratio of the concentration of compound in the lipid phase to the concentration of all species (ionized and un-ionized) in an aqueous phase at a given pH. This ratio is directly affected by the pH of the system; thus, noted as logD_{pH}. Ionizable compounds with log$D_{7.4}$ < 1.7 have been shown to have increased probability of being safe to freshwater fish than those with log$D_{7.4}$ > 1.7.[426] • Log*D* for acids/bases can be readily calculated from log*P* when pK_avalues are known. pK_a values provide insights into the lipophilicity and solubility of ionizable compounds which can be used to better anticipate and predict the compound's toxicokinetic behavior for processes such as membrane permeability, protein binding, gastrointestinal absorption, and metabolic transformations.[427]
	Solubility: Refers to the ability of the solute to dissolve in a solvent. The primary

	measurement of interest in chemical alternatives assessment is solubility in water. In case of aquatic toxicity solubility of a chemical play huge role to toxicity.[428]
	Aqueous solubility: It is a direct measure of the hydrophobicity of a substance. The solubility equation developed by Ran and Yalkowsky[429] can be used to estimate intrinsic water solubility at 25°C (logS) for structurally diverse organic substances. This equation uses regression-derived correlation with logP and melting point (MP) for solids: $$logS = 0.8 - logP - 0.01(MP - 25)$$ Compounds with higher logP have lower water solubility. Very poorly water-soluble chemicals (<1 ppb) generally have low bioavailability and are less toxic.[428] Aqueous solubility also dependent on temperature and pressure which are not considered here. Salinity or salting-out indicates that above equation is not suitable to use for high-melting, non-ionic solids.[427]
	Colligative properties: Colligative properties are properties of solutions that are not dependent on the chemical but instead on the ratio of the number of solute particles to the number of solvent molecules in a solution. Examples of colligative properties include lowering of vapor pressure, elevation of boiling point, and depression of freezing point which play important role in transformation and biodegradation of chemicals.
Molecular Attributes for	*Molecular attribute* is used to describe properties related to molecular shape and size. • Electronic parameters like frontier orbital energies (Highest Occupied Molecular

ecotoxicity	Orbital [HOMO], Lowest Unoccupied Molecular Orbital [LUMO), and the energy gap [ΔE] between the HOMO and LUMO orbitals) dipole moments (μ), polarizabilities (α) of molecules that affect chemical reactivity with biological targets. In many instances, electronic properties have been shown to be helpful in identifying chemicals of high toxicity.[427] • E_{HOMO} is the energy of the highest energy level molecular orbital containing electrons capable of measuring the nucleophilicity of a molecule. Molecules with a high E_{HOMO} value can donate their electrons more easily compared to molecules with a low E_{HOMO} value, and hence are more reactive. The positive influence of this feature suggests that cytotoxicity and E_{HOMO} are directly proportional.[62] • LUMO energies > 2 eV have shown to be associated with chemicals that are not toxic to *Pimephales promelas*. This is rationalized by the reduced electrophilicity of chemicals in this groups; the higher the LUMO energy of a chemical, the less likely it is to be a strong electrophile.[430] • The HOMO–LUMO gap (ΔE), which is a known measure of kinetic stability and responsible for high acute aquatic toxicity.[426] • Atomic polarizability in a chemical might cause an interaction resulting in toxicity, especially for daphnids.[410,418] • Properties that describe molecular size and shape include solvent accessible surface area, molecular volume, globularity, and ovality, and they can be related to bioavailability and reactivity.
Structural	• Keto group is predominantly accountable for higher toxicity of pharmaceuticals to

Attributes for ecotoxicity	*D. magna* and fish.[59] • Structural fragments like X=C=X, R–C(=X)–X, and R–C≡X are significant features for the high toxicity value to fish[59] • Organometallic compounds are toxic to fish like *Oncorhynchus mykiss* and *Pimephales promelas*[412] • Molecules with macrocyclic rings showed higher toxicity to *Pseudokirchneriella subcapitata*[412] • Molecules having highly polar groups with complex and rigid core structures showed higher toxicity against algae suggesting the idea of polar narcosis[62,411] • Large size molecules and too much branching with hydrogen bond donor and acceptor tend be to higher toxic[62,410-412] • Molecules with sulphur atom tends to show high first ionization potential to show high toxicity[418] • Increase steric hindrance lowers the aquatic toxicity[418]
Structural Attributes that Enhance Biodegradation	Factors are combined from literatures:[431-433] • Minimal number of strong electrons withdrawing substituents, like F and Cl. The biodegradability highly hampered if more than three Cl/F present • Minimal chemical branching is good, but avoid quaternary carbons. Exception: Vitamin A, Cholesterol • Avoid heterocyclic residues. For example, aliphatic ether, imidazole etc. except ethoxylates • Minimal number of tertiary amines, nitro, nitroso, azo, and arylamino groups.

	• Minimal number of polycyclic residues (especially more than three fused rings). • Avoid chlorine atom on phenyl ring as it become less susceptible to attack by oxygenase enzymes • Presence of esters (including phosphonates) which are susceptible to enzymatic hydrolysis • Presence of oxygen atoms in form of hydroxyl, aldehyde, carboxylic acid, ketone groups. • Presence of short linear alkyl chains (< 4 C) or phenyl rings that can act as sites for oxygenase enzyme activity. • MW should be <1000 • Decrease steric hindrance at active site increases availability of biodegradation enzymes • Avoid bulky ortho substitutions help in accessibility of biodegradation enzymes
Structural Attributes that minimizing Bioaccumulation/ Bioconcentration	Factors are combined from literatures:[428,430,434] • logP/logK_{ow} (for aquatic environment), logK_d (phase partition coefficient in soil and water, more likely to absorb in soil), log$K_{w/g}$ (phase partition coefficient in water and air) provide insight into environmental partitioning of the molecule and the potential for bioaccumulation in specific environment. Bioaccumulation directly proportional with the partition coefficient and that's why its value should be low. • Bioaccumulation generally is considered very high when logPexceeds 5 to 6 and generally considered low when the logP< 2. It should be noted, however, that a

	compound with a high log*P* value may be rapidly metabolized or degraded, and in these cases, would not bioaccumulate. • Poorly lipid-soluble chemicals, those that are highly lipophillic (logP > 8), or chemicals with a molecular weight > 700 Da will generally not bioconcentrate. • Chemical's physical state is very important to check in which compartment (air, water, sediment, biota, soil) the chemical will partition. Physical state can be predicted employing boiling point, melting point, and vapor pressure • Highly volatile chemicals will escape from soil or water and primarily be present in the air. Conversely, chemicals with a high propensity to sorb onto organic carbon or move into lipid phases are likely to remain in soils or sediments or move into biota, respectively.

14. CHALLENGES

14.1 Ecotoxicity due to PPCP mixtures

The real challenge in risk assessment for the environment is mixtures of chemicals belonging to different chemical classes acting through diverse MOA in a specific species/organism/system. Most of the time, researchers deal with single chemical toxicity for ERA. Not only that, industries, regulatory authorities provide chemical toxicity data for a definite species and environmental compartment. On the contrary, the real scenario is completely different, as risk exposure occurs through mixtures rather than single chemicals. Thus, assessment of single chemical toxicity may not display the real toxicity information.[435] Another important point is that similar chemical mixtures with different ratios may show changed toxicity responses. So, evaluation of the toxicity of mixtures is quite complex experimentally as well as

computationally. In case of computational modeling of mixtures, the challenge is daunting one, as rational mathematical relationship between the experimental toxicity and the molecular descriptors of the structure is dependent on multiple activity assessment hypothesis, which is in most cases proficiently accomplished for single chemicals.[436] The reasons are the followings:

a) toxicity response varies with diverse combination/ratio of similar chemical mixture,

b) interaction among chemicals is the reason for multifaceted and noteworthy changes in the apparent response of the components,

c) the form of exposure is also important along with nature of the environment compartment,

d) assessment of the composition of each chemical in a mixture is also difficult and sometime present in NOEC

To formulate the toxicity data for computational modeling purpose, one has to follow the steps mentioned below:[437]

(i) Evaluation of dosage response curves for mixture: A dosage response (DR) curve needs to be prepared for each chemical employing the model organism with the variation of concentrations.

(ii) Checking the effect of the chemical mixture: The effect of a chemical mixture to the model organism requires to be checked in presence and absence of the chemical mixture experimentally. It is important to mention that one should quantity a dilution series of the mixture which will permit to achieve wide-ranging dose response curve of the mixture.

(iii) Hypothesis identification for modeling: The components in a mixture can observe additive behavior of effects or may show either amplified (synergistic) or reduced (antagonistic) effects. Thus, identification of MOA hypothesis is very much vital before modeling to attain a practical

mathematical relationship through computational approach (See **Table 8**). The most commonly acceptable hypothesis recognition of all present chemicals and their concentration in a mixture is also important for this step.

Table 8 The MOA assessment hypothesis for mixtures.

Hypothesis	Description
Concentration addition (CA)	The hypothesis assumes that chemicals act *via* similar MOA to produce an effect, thus one chemical acts as a dilution of the other and can be replaced at a persistent quantity for the other. The CA model can be explained by Loewe additivity equation. For instance, the equation for binary mixture of compounds A and B: $$\frac{C_A}{EC_{xA}} + \frac{C_B}{EC_{xB}} = 1$$ where C_A and C_B are the specific concentrations of the compounds A and B creating the mixture, which results an effect x, and EC_{xA} and EC_{xB} signify the corresponding effect concentrations of the individual compounds A and B that alone would generate the same response x as the mixture. The combined effect or sum of c_A and c_B is x. The sum of equation is always equal to 1 for the CA modeling.
Independent action (IA)	Chemicals act independently, and they have different MOA. The collective effect is computed employing the effects of components and their interactions in the mixture. The IA modeling can be explained through following formula: $$E = 1 - ((1 - e_A)(1 - e_B)(\cdots))$$ E is the outcome of the mixture at an explicit concentration; e_A is the effect of

	compound A at that definite concentration and same for chemical B *i.e.* e_B. The equation can be expanded from binary mixtures to more component's mixtures.
Synergistic and antagonistic actions	Toxicity of synergistic action is superior to the individual response of components, while the antagonistic act has lower effects than the response of individual components. Considering the Loewe additivity equation, when the sum is higher than 1 (>1), it suggests that higher total concentration is required to produce the same effect which assumes an antagonistic effect (infra-additive). If the value is lower than 1 (<1), then it is a synergistic effect (supra-additive).
Generalized concentration addition (GCA) models	The CA and IA models are not valid for chemicals that have high potency but low efficacy. Thus, a generalized concentration addition (GCA) model was created by Howard and Webster to eliminate these limitations. The GCA considers the cumulative effect of a mixture by means of the efficacy and potency of the mixture's constituents. The GCA model can be explained by following equation: $$E = \frac{\frac{\max effect\ level_X[X]}{EC_{50X}} + \frac{\max effect\ level_Y[Y]}{EC_{50Y}} + \cdots}{1 + \frac{[X]}{EC_{50X}} + \frac{[Y]}{EC_{50Y}} + \cdots}$$ E is the effect of the mixture at a definite concentration. Here, 'max effect level X' is the maximal effect level of chemical X, [X] is the concentration of X in the mixture at an explicit mixture concentration, EC_{50X} is the EC_{50} value of X. Similarly, meaning for all notations related to chemical Y etc can be interpreted.

(iv) Modeling for toxicity prediction and mechanistic interpretation: A good number of *in silico* methods exist but most successful and fruitful tool in mixture toxicity is QSTR.[437] Boeijeaet

al.[438] developed QSTR models of alcohol ethoxylate mixtures with a correlation coefficient of 95% for three ecotoxicological species, *i.e., Pimephales promelas*, *Daphnia magna*, and mesocosms. Wang *et al.*[439] generated highly predictive QSTR models employing forward stepwise-MLR and nonlinear radial basis function neural networks (RBFNNs). Kar *et al.*[416] performed QSTR modeling using mixture toxicity data of halogenated chemicals mixtures on zebrafish embryos and identified that the studied chemicals in mixtures exhibited dose addition or concentration addition of each chemical which explain for similar MOA and non-interaction among chemicals. The important factors of these modeling works are identification of best possible hypothesis for MOA followed by calculation of descriptors for QSTR modeling. The details about descriptor calculation and the validation protocol exclusive for mixtures are discussed below.

(a) Descriptors for mixtures: Mixture descriptors can be computed primarily based on two approaches.[440,441] The first one is unweighted descriptor method where the mixture consists of one descriptor vector and has only one property value. In a simple term, this is the general average of the numerical value for each component in a mixture for respective descriptors. The second approach is weighted descriptors where descriptor vectors and property depend on the composition of mixtures' components. **Figure 40** demonstrates how descriptors are calculated for mixtures. Here for better understanding, binary mixture is considered.[442]

(b) Validation protocol: Traditional external validation procedure where the compounds are arbitrarily placed in the external set is undesirable in case of mixture models due to the overestimation of the prediction exclusively when mixtures of the similar chemicals with diverse ratios exist multiple times in the modeled dataset.[442] Certainly, if both training and external/test sets contain compounds corresponding to the similar mixture, then true prediction of a model

will not be assessed. Three acceptable strategies are illustrated in **Table 9** for rigorous external validation:

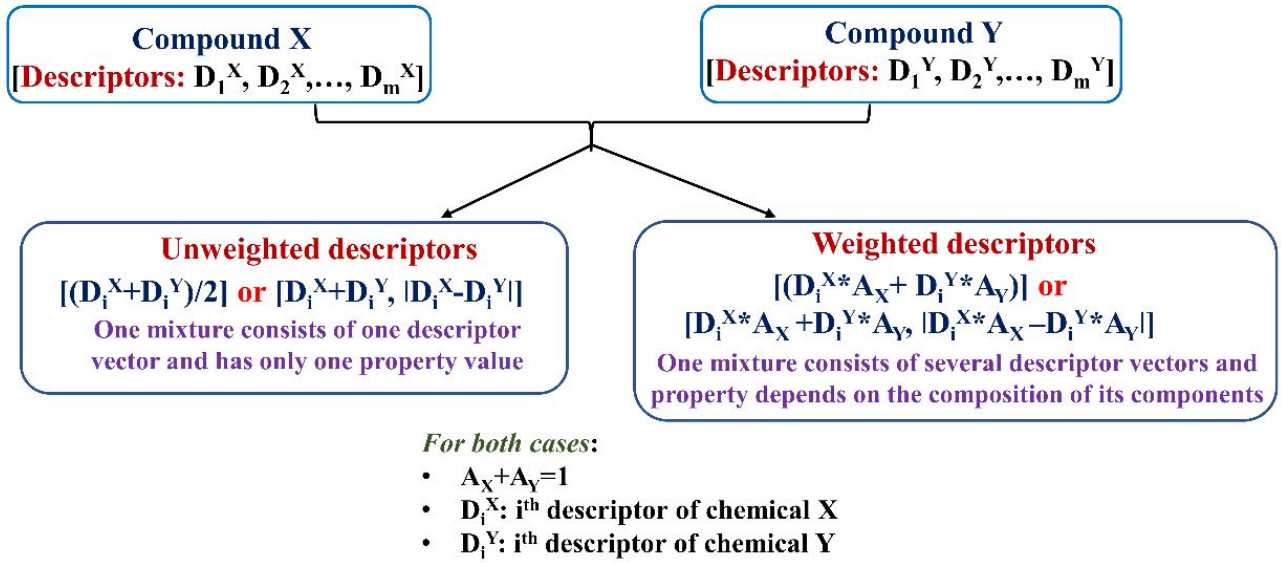


Figure 35 Hypothesis to calculate descriptors of mixtures for *in silico* modeling.

Table 9 External validation strategies for QSTR modeling of mixtures.

Strategy	Description	Interpretation
Points out	Chemicals are arbitrarily placed in each fold of the external cross-validation set. Each mixture is present simultaneously in both the training and the external sets. But, among three strategies, this is the weakest validation protocol	Reflects the capability of models to predict present mixtures with original compositions
Mixtures out	All data points corresponding to mixtures are composed of the identical constituents, but in dissimilar ratios, are simultaneously removed and placed in the same external fold. Thus, every mixture is present either in the training or in the external set, but never in both sets	Assesses the prediction quality of a model for new mixtures

Compounds out	Pure compounds and their mixtures are simultaneously placed in the same external fold. Thus, every mixture in the external set contains at least one compound that is absent from the training set. This is the most rigorous strategy	Protocol does so for new compounds

Although the concept of mixture toxicity is quite old, the necessity of evaluation of mixtures toxicity evolved much later than the apprehensions for single chemical toxicity assessment. There is no doubt among toxicologists and regulatory authorities that to get a crystal-clear idea, one has to acquire comprehensive toxicity data for mixtures in different compartment of environment. Considering critical aspect of mixtures ecotoxicity assessment followed by management, we are suggesting following points for unproblematic future efforts:

- Standardized protocol to identify the exposures, generation of biomarkers, and assessment of relevant mixtures hazardous to environment.
- Need to evaluate the mixture toxicity upto No-observed-adverse-effect level (NOAEL).
- Development of new techniques with a combination of experimental and computational methods as there is no single method which can address the multifaceted issue of mixture toxicity. Thus, more collaboration is expected between experimentalists and computational communities.
- Computational modeling is not possible without sufficient experimental data. The real problem for mixture's ecotoxicity modeling is lack of experimental data. Thus, preparation of ecotoxicity database spanning over different compartment, multiple species and diverse experimental condition is the need of the hour.

- Expert systems are advantageous for mixtures assessment, for predicting dose-dependent interactive effects along with prediction of untested and new mixtures in no time.
- Hausdorff-like similarity (Hs) measure may be beneficial in modeling of mixtures.[443] Hausdorff similarity able to equally weigh both existing components in a mixture. To compute the diversity association between the two sets M and N, the Hausdorff formula can be expressed as following:

$$dHaus_{MN} = max\left\{\underset{m \in M}{sup}\left[\underset{n \in N}{inf}(d_{mn})\right], \underset{n \in N}{sup}\left[\underset{m \in M}{inf}(d_{mn})\right]\right\} \quad (7)$$

from which, the equivalent similarity measure can be calculated as:

$$sHaus_{mn} = min\left\{\underset{m \in M}{sup}\left[\underset{n \in N}{inf}(s_{mn})\right], \underset{n \in N}{sup}\left[\underset{m \in M}{inf}(s_{nm})\right]\right\} \quad (8)$$

Where, the signs *s* and*d* denote to the similarity and the distance measures, correspondingly.

In case two sets M and N, the Hausdorff-like similarity can be expressed as following:

$$Hs_{MN} = \frac{\sum_{m \in M} \underset{n \in N}{max} [s_{mn}] + \sum_{n \in N} \underset{m \in M}{max} [s_{nm}]}{x_M + x_N} \quad (9)$$

Here,s_{mn} and s_{nm} are pair-wise similarity measures between the *p*-dimensional elements m and nof the sets M and N, correspondingly. x_M and x_N are number of components for both sets. The signs under numerator indicates the maximum similarity between the separate components for both sets.

14.2 Transformation, metabolism and toxicity pathways

PPCPs are used as single chemicals or a combination of multiple chemicals. After occurrence in the different compartments of the environment by any means, they go through a series of transformations by metabolic pathways. As a chemical can experience manifold of metabolism,

thus every metabolic step can generate a new form of hazardous substance leading to diverse forms of toxicity. Interestingly, majority of risk assessment work oriented towards single chemical toxicity which offers only one directional toxicity measurement. Rather, the scenario is much more complex than what researchers observe. Multiple transformations through different metabolic pathways can exist for single chemicals.[444] Subsequently, transformation rate data for chemicals for prioritization of competing pathways is necessary for the toxicity evaluation. The prioritization procedure necessitates the integration of consistent and precise transformation rate data. Thus, chemical fates, transformation, metabolism followed by respective toxicity due to metabolites are the real challenge in the risk assessment of PPCPs towards environment.[445]

The first and foremost step to fight the challenge is to create databases with metabolic and transformation rate constants, as the real issue is the absence of sufficient amount of data.[444] According to US EPA, following steps need to be followed for the acquiring of data:

(1) Generation of metabolic rate constants data by means of in vivo and in vitro experiment using advanced analytical tools. Once sufficient amount of experimental data are available, reliable and predictive computational model can be prepared and used.

(2) When there is no data at all, it requires to use mechanistic QSTR models and rate constants derived from SPARC computer model from US EPA.

(3) Data mining from the literature and Program Offices of regulatory agencies.

(4) Implication of exposure genomics (assessing gene expression profiles) can provide early indications of chemical exposure due to modification in gene expression which will be utilized to direct chemical fate and metabolism studies. The exposure genomics will offer

following information: (a) the minimum concentrations at which biological action observe; and (b) the recognition of toxicity expressive chemicals in mixtures.

Along with the development of resourceful databases, there are few more areas where researchers need to focus and they are following:

- Combination of databases and computational tools for the concurrent environmental fate and metabolism evaluation;
- Accretion of crystal-clear idea about metabolic simulator to understand the complete metabolic pathway and rate of transformation;
- Buildup of KBES to offer expansion and application of transformation and/or metabolic simulators.
- The study metabolomics is helpful to identify toxicity pathways and the measurement of metabolites in presence of certain physiological stimuli and/or genetic modification in a living system. To understand the changes in metabolic pattern of a chemical in association with certain modification (biofluids, different species) followed by toxicity response, application of metabolomics is imperative. Metabolic profiles can deliver a degree of the actual outcome of possible changes as the outcome of xenobiotic exposure.
- To determine the level of exposure of a chemical to specific organisms, the modeling of the fate and transport need to be done after its release into a definite environment.
- The developed metabolism model of the chemical should be prepared inside the target organisms as metabolite of the original stressor induces a biological response. In other cases, it may show false outcome.

Organizations like US EPA, Office of research and development (ORD), National exposure research laboratory (NERL), Ecosystems Research Division and Processes & Modeling branch are working on these challenging issues.[444,446] ORD's and EPA's Computational Toxicology research (CompTox) Program is working efficiently in classifying chemicals and their metabolites in respect to toxicity pathway. But much more efforts are required not only from regulatory agencies but also from the producers of those compounds, i.e., industries which can provide sufficient information about the probably fate, transformation and metabolic pathway of individual PPCPs.

15. OVERVIEW AND CONCLUSION

This review has dealt with the present status of computational modeling of ecotoxicity endpoints of PPCPs along with the evolving areas of molecular designing approach for toxicity and risk management. The integration of *in silico* techniques with the GC principles are needed not only for generating precise predictive models but also for designing as well as synthesizing less hazardous and possibly non-toxic PPCPs to prevent risksin the first place. The KBES is the most commonly employed predictive tool not only for toxicity prediction purpose but also for deriving design rulesfor less hazardous PPCPs nowadays. Without any doubt, no KBS can be considered as a universal system for prediction purpose, as all have their advantages and drawbacks, with varying specificity, sensitivity, and accuracy. Thus, the choice of KBS should depend on multifaced criteria. They include experimental condition, species, biological and toxicological pathway, metabolism of the studied compounds evaluated using mechanistically interpretable properties or descriptors in modeling. Accessing expert guidelines would be useful for chemists and environmentalists to have *a priori* knowledge of what the most apparent red flag

physicochemical properties and structural fragments are, so that they can integratethis knowledge into molecular design. Thus, a comprehensive analysis of imperative features responsible for ecotoxicity as well as strategies to reduce those toxicities were discussed.

The future directions for novel PPCP specific predictive models will rely on more receptor specific descriptors of chronic toxicity to elucidate the receptor mediate pharmacodynamics MoA of the compounds.[447] The chronic effects can be related to a multitude of sublethal endpoints not only *e.g.* reproduction but also more subtle changes such as altered heart rate; behavior; metabolism, *etc*. Hence, to demonstrate these types of effects one requires application and development of AOP analysis in depth.[448,449] The more widespread and systemic development of AOPs requires integration withHigh-Throughput Big Data generation *e.g.*, as outlined in the ToxCast programme.[450] This is in our view necessary to move forward. The Comparative Toxicogenomics Database (CTD)[451] is an example of how Big Data can be combined and integrated in novel ways between databases and across geographies to allow prediction of toxicogenomic effects. Therefore, the present review provides vast collections of ecotoxicity databases and expert KBS to users so that one can integrate the required components basedon their analysis requirement. These tools can be combined in a Machine-Learning or Artificial Intelligence (A.I.) setting to allow more detailed assessments of PPCP receptor mediated chronic environmental toxicity prediction. The reader might ask is this still a QSAR? And the answer is no. This approach includes more information about the compound than structure-based descriptors. As such these are second-generation computational predictive tools of toxicity. The structural information is the initial information in establishing the Key initiating Event (KiE) of the AOP, but the computation power and analysis lays in the subsequent analysis

of AOP where different source of information and databases are connected as in e.g. CTD to deliver decision relevant toxicity outputs. This might still seem a bit like science-fiction to some - but the chemical and biological information is available in the terabits of data and the tools to access and combine the information to answer relevant questions about toxicity are being developed based on A.I. as the information is too big for humans to grasp. The question is how comfortable we as a society are with less-transparent high complex predictive tools and involvement of A.I. in decision making, and how well the models and outputs will be integrated in our regulatory frameworks and legislation – stay tuned time will tell.

Acknowledgements

SK and JL are thankful to the National Science Foundation (NSF/CREST HRD-1547754 and NSF/RISE HRD-1547836) for financial support.KR thanks the European Commission for financial assistance under the project VERMEER [LIFE16 ENV/IT/000167].

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