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Ecotoxicological assessment of pharmaceuticals and personal care products using predictive toxicology approaches†

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The use of active pharmaceutical ingredients (APIs) and personal care products (PCPs) is growing day by day all over the world. Thus, these materials have appeared as 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 a huge amount of experimental data is time consuming and costly, and also requires sacrifice of a large number of animals, computational modeling or *in silico* approaches are proving to be an efficient technique for not only risk assessment, but also for risk management and data gap filling. The present review deals with the critical assessment of the hazardous potential of PPCPs in the environment. The importance of *in silico* modeling approaches for 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 complete mechanistic interpretations of *in silico* models reported over the years.

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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 the intrinsic mechanism of action (MoA) they have on living organisms.^{1,2} Often APIs are inherently much more bioactive than PCPs, which are typically relatively mild, *e.g.*, detergents have a nonspecific narcotic MoA. Although they are completely different from each other with

respect to MoA to a specific organism/species, fate and transformation in the ecosystem, for ease of the discussion we use the recognized term both forms of chemicals have received, that is ‘pharmaceuticals and personal care products (PPCPs)’^{1–3} throughout this review. A series of serious adverse effects on living species and the ecotoxicological effects of PPCPs and their metabolites have been reported³ over the years along with their occurrence at concentrations of ng l⁻¹ to µg l⁻¹ in wastewater treatment plants (WWTPs),^{4,5} surface water,^{6,7} groundwater,^{8,9} sewage treatment plants (STPs),^{10,11} marine biota,^{12,13} rivers^{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 the European Parliament and US Environmental Protection Agency (US EPA) endorsed multiple rules and regulation to identify potential contaminants under PPCPs to include them in the ‘priority list’¹⁹ and the ‘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.

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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 has been studied over the years but to understand the real scenario, one has to understand that the majority of PPCPs exist as a complex mixture of individual constituents which exert toxicity either synergistically or through antagonism.²¹ Another significant point is the consideration of the toxicity of the 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 is substantial evidence that metabolites are often 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 effects have been detected in WWTPs²⁴ 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 1 mM under *in vitro* study.²⁶

The analysis of diverse aquatic environments has led to identification of around 600 PPCPs and their TPs in the surface water, groundwater, STP, WWTP and soil samples covering 71 countries^{27,28} where the majority of them cover antibiotics,²⁹ analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs),³⁰ anticancer,³¹ cardiovascular,³² CNS acting drugs²⁴

and hormones³³ under pharmaceuticals, and disinfectants,³⁴ fragrances,³⁵ preservatives³⁶ and UV filters³⁷ under PCPs. Pharmaceuticals like diclofenac ($1.2 \mu\text{g l}^{-1}$),³⁸ metoprolol ($1.54 \mu\text{g l}^{-1}$),³⁹ 17β -estradiol ($0.013 \mu\text{g l}^{-1}$),³⁹ carbamazepine ($2.1 \mu\text{g l}^{-1}$),³⁸ clofibric acid ($0.2 \mu\text{g l}^{-1}$),⁴⁰ erythromycin ($1.7 \mu\text{g l}^{-1}$)³⁹ etc. were detected in major river waters over the years. Norfloxacin and ciprofloxacin were reported with a median concentration of $0.12 \mu\text{g l}^{-1}$ and $0.02 \mu\text{g l}^{-1}$ in 139 surface stream water samples of the USA⁴¹ whereas ciprofloxacin was detected in the wastewater of Swiss hospitals with a concentration of $0.7\text{--}124.5 \mu\text{g l}^{-1}$.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, Italy⁴⁵ in ng l^{-1} concentration. Ibuprofen and its metabolic product ibuprofen methyl ester were detected and quantified with a concentration of $0.93 \mu\text{g l}^{-1}$ and $4.95 \mu\text{g l}^{-1}$, respectively in drinking water.⁴⁶ One of the common APIs in contraceptive pills is 17α -ethynodiol (EE2) detected in samples of tap water and groundwater.⁴⁷ Antimicrobial agents like triclosan (TCS) and triclocarban (TCC) were detected in WWTP samples ranging from 50 to 200 ng l^{-1} (ref. 48) whereas TCS was reported in biosolids, surface water, and WWTPs with a concentration of $0.09\text{--}16.79 \text{ mg kg}^{-1}$, 75 ng l^{-1} and 23 to 434 ng l^{-1} , respectively in Australia.⁴⁹ The insect repellent *N,N*-diethyl-meta-toluamide (DEET) was detected in a WWTP of North Carolina, and the observed concentration in the groundwater of the adjacent site was 540 to 1010 ng l^{-1} .⁵⁰ Although the existence of different PPCPs in diverse samples has been evaluated and quantified over the



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Hans Sanderson

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years, still the risk associated data are quite low. Therefore, to evaluate the toxicity of PPCPs to diverse species in the environment, improved analytical detection techniques are very much necessary.

Continuous detection of PPCPs in different environment compartments led to the introduction of guidelines for risk assessment associated with PPCPs by the United States Food and Drug Administration (US FDA) and The European Medicines Agency (EMA) (previously known as the 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 $1 \mu\text{g l}^{-1}$ in the aquatic environment required a complete risk assessment report before market approval.⁵² The European Union (EU) Directive 2015/495/EU amended⁵³ 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-ethynodiol, clarithromycin, azithromycin, erythromycin, and estrone E1, and PCPs like the UV filter octinoxate and the food additive butylated hydroxytoluene.⁵⁵

The 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 a 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 the 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 the EU have endorsed *in silico* approaches for toxicity and fate prediction of PPCPs.⁵⁷ Among the *in silico* approaches, the quantitative structure-activity relationship (QSAR) is one of the most commonly practiced techniques for ecotoxicity prediction. A 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 systems 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 and in risk management through the design



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Emilio Benfenati

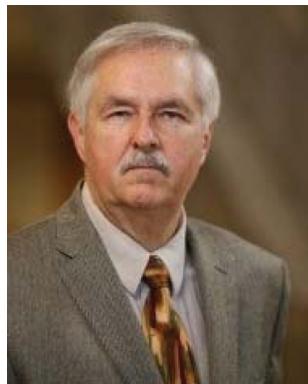
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of environmentally safer PPCPs. Existing ecotoxicity QSAR models help us to understand the major features and structural fragments related to the intrinsic chemical reactivity followed by a chemical's environmental fate, transformation, and toxicity. Therefore, a good number of existing computational ecotoxicity models are thoroughly interpreted along with the models dealing with the removal of hazardous PPCPs from the ecosystem through advanced materials. The responsibility of regulatory authorities related to environmental safety and their role in implementing computational models for environmental risk assessment and management purposes are illustrated. Along with the importance of prediction models for the risk assessment, 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 an *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 pharmacodynamic (PK/PD) nature of individual PPCPs. We have provided inclusive lists of environmental toxicity endpoints, databases and test species to have an idea about reasonable sources to construct computational models for risk assessment and management. A thorough introspection of expert systems is 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 detail.



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2. Occurrence and ecotoxicity of pharmaceuticals

The 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 the major sources and life cycle of any pharmaceutical is of utmost necessity. The most common sources and routes to aquatic, terrestrial and soil environments for any pharmaceutical are portrayed in Fig. 1. The 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.

2.1 Active pharmaceutical ingredients (APIs)

2.1.1 Antibiotics. The maximum detected concentration of ciprofloxacin in wastewater was $1.4 \mu\text{g l}^{-1}$ in Holland,⁶⁷ $3.7 \mu\text{g l}^{-1}$ in Italy,⁶⁸ $0.6 \mu\text{g l}^{-1}$ in Canada⁶⁹ and $6.9 \mu\text{g l}^{-1}$ in Australia⁷⁰ while the average concentration of sulfamethoxazole in the same WWTPs was $1.8 \mu\text{g l}^{-1}$ in Canada⁶⁹ and $1.7 \mu\text{g l}^{-1}$ in Europe.⁷¹ Out of 12 soil samples, chlortetracycline and tetracycline were found in 10 samples and the average concentration of tetracycline in three different layers of soil was $86.2 \mu\text{g kg}^{-1}$ in the 0–10 cm layer, $198.7 \mu\text{g kg}^{-1}$ in the 10–20 cm layer and $171.7 \mu\text{g kg}^{-1}$ in the 20–30 cm layer while chlortetracycline was detected with the average concentration of $4.6\text{--}7.3 \mu\text{g kg}^{-1}$ in all three sublayers.⁷² Amoxicillin was detected in the WWTP of Delhi, India with a concentration of 172.6 ng l^{-1} in the influent and 62.5 ng l^{-1} in the effluent,⁷³ while a much higher concentration ($100\text{--}2000 \text{ ng l}^{-1}$) was found in the activated sludge of a WWTP in Japan.⁷⁴ Cefuroxime ($0.6 \mu\text{g l}^{-1}$), ampicillin ($17.7 \mu\text{g l}^{-1}$), sparfloxacin ($0.5 \mu\text{g l}^{-1}$) and gatifloxacin ($3.7 \mu\text{g l}^{-1}$) were identified in the effluent of WWTP in Delhi, India.⁷⁵ Twelve sulfonamides were identified in bacteria, non-target plants and algae in the aquatic environment where inhibition assays reported EC₅₀ values ranging from $>250 \text{ mg l}^{-1}$ for all sulfonamides whereas sulfadimethoxine exhibits growth inhibition of duckweed with a concentration of 0.02 mg l^{-1} .⁷⁶ Ciprofloxacin was found to be active at a concentration of 5 mg l^{-1} towards *Allivibrio fischeri*.⁷⁷

2.1.2 β -Blockers. In hospital effluents, propranolol and metoprolol were detected with concentrations of 6.5 mg l^{-1} and 25.1 mg l^{-1} , respectively.⁷⁸ Propranolol was detected with 100% frequency and a median concentration of 76 ng l^{-1} in the STP effluent sample in UK.⁷⁹ Martin *et al.*⁸⁰ found propranolol at a concentration of 3.37 mg kg^{-1} in the sediment collected from the Guadiana 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 $250 \mu\text{g l}^{-1}$, respectively, whereas in the case of *H. Azteca*, the concentration was $100 \mu\text{g l}^{-1}$ after exposure for 27 days.⁸¹ Metoprolol exhibited a negative chronotropic effect on the heart of *D. magna* at a high concentration (10^{-4} M)

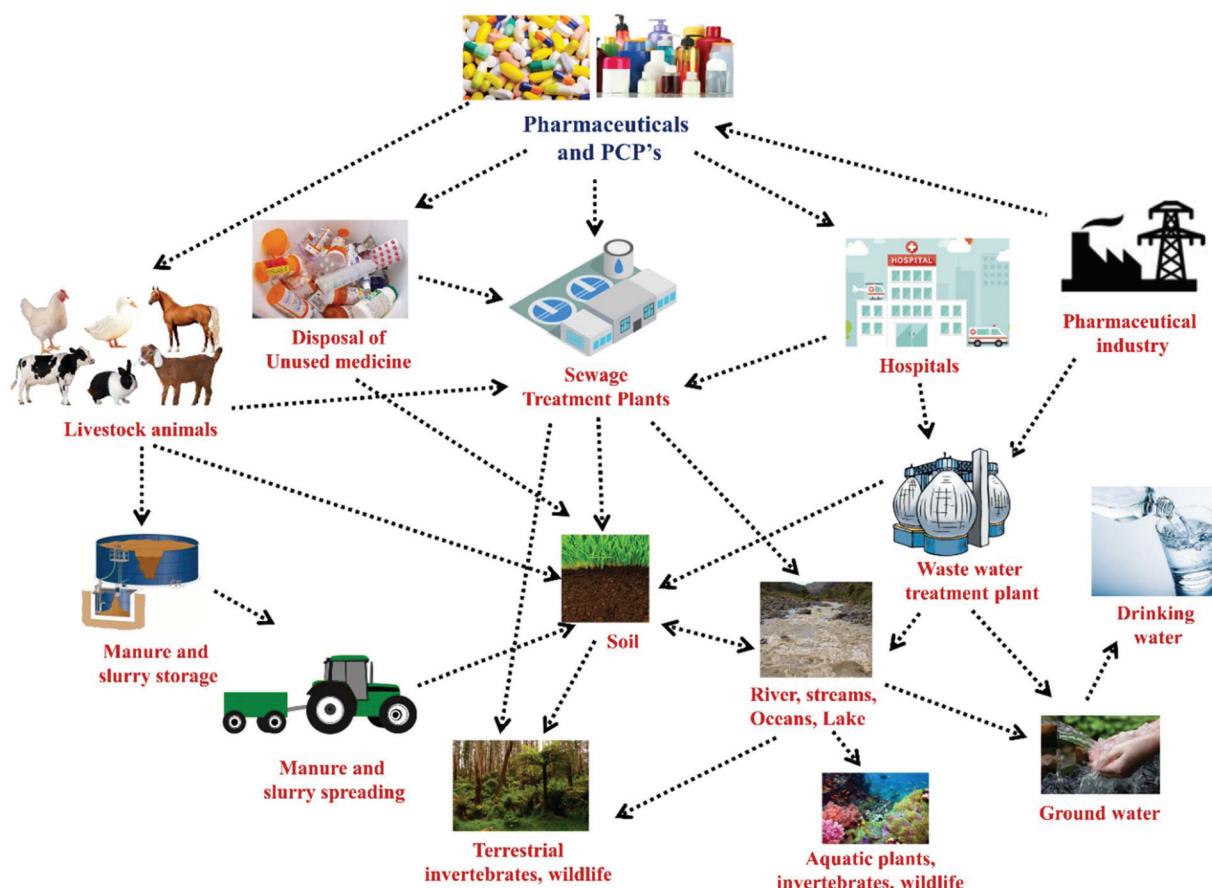


Fig. 1 Source and complete life cycle of PPCPs in the diverse environmental compartments.

and positive chronotropy at low concentration (ranges from 10^{-8} – 10^{-6} M).⁸² The NOEC and LOEC values for the embryolarval growth rate of 3.2 mg l^{-1} and 10 mg l^{-1} , 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 at a higher concentration between 0.4 ng l^{-1} and $15 \mu\text{g l}^{-1}$ in surface water.⁸⁴ Paracetamol was present with a concentration of $78.17 \mu\text{g l}^{-1}$ in surface water and in STP effluents the concentration can reach around 20 ng l^{-1} to $4.3 \mu\text{g l}^{-1}$ while most of the values were higher than the predicted no-effect concentration (PNEC) of $9.2 \mu\text{g l}^{-1}$.⁸³ Ibuprofen was found in German WWTPs with maximum concentrations of 3.5 and 0.3 mg l^{-1} in the influent and effluent, respectively.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 a concentration of 3086 ng l^{-1}), dextropropoxyphene (frequency 74%, at a concentration of 195 ng l^{-1}), diclofenac (frequency 86%, at a concentration of 424 ng l^{-1}) and mefenamic acid (frequency 81%, at a concentration of 133 ng l^{-1}) at reasonably high concentrations. In the Guadiamar River water of Spain, salicylic acid and naproxen were detected at concentrations of 9.49 and 11.2 mg kg^{-1} , respectively.⁸⁰ Sediment sample ana-

lysis from Llobregat, Iberian River basins, Jucar, Ebro, and Guadalquivir in Spain reported ibuprofen at a high concentration of 13 ng g^{-1} .⁸⁶ Naproxen was detected in the STP effluent and Mississippi River in Louisiana at concentrations of 81 – 106 ng l^{-1} and at 22 – 107 ng l^{-1} , respectively.⁸⁷ The most commonly detected and toxic analgesic is diclofenac with a reported EC₅₀ below 100 mg l^{-1} ,⁸⁸ whereas phytoplankton are highly sensitive to it under acute and high-level exposure with an EC₅₀ of 14.5 mg l^{-1} at 96 hours.⁸⁹ Renal lesions and gill alterations in trout fish (a concentration of 5 mg l^{-1} with 28 days exposure) are also reported for diclofenac.⁹⁰ An analysis suggested that naproxen causes protein and lipid oxidation followed by oxidative DNA damage in *H. Azteca*.⁹¹ Diclofenac causes inner organ damage in rainbow trout⁹² and its detected concentration in the surface water sample in 12 countries exceeds the PNEC of 0.1 mg l^{-1} .⁹³ Reproduction of *D. longispina* and *D. magna* was affected by acetylsalicylic acid at a concentration of 1.8 mg l^{-1} .⁹⁰ Chronic toxicity of ibuprofen was observed towards the water flea and *D. magna* with concentrations ranging from 0 to 80 mg l^{-1} . In the case of naproxen, the EC₅₀ values were 30.1 or 50 mg l^{-1} for *D. magna* which is the most sensitive species towards it.⁸³

2.1.4 Antineoplastic/anticancers. Tamoxifen has been found at alarming concentrations in surface water and WWTP

samples with the maximum concentrations of 25 ng l⁻¹ and 102 ng l⁻¹, respectively.⁹⁴ Anastrozole, used in hormone-based chemotherapy, has been measured with a high frequency with the maximum concentrations of 0.3–0.4 ng l⁻¹ in WWTP effluent and 2.38–3.70 ng l⁻¹ in hospital effluent.⁹⁵ Tamoxifen has been detected with a frequency of 4% and at a concentration of <10 ng L⁻¹ in STP effluent samples of the UK.⁷⁹ 5-Fluorouracil and cisplatin showed growth inhibition of the cyanobacteria *Synechococcus leopoliensis* (EC₅₀ of 1.20 and 0.67 mg l⁻¹, respectively) and the algae *Pseudokirchneriella subcapitata* (EC₅₀ of 0.13 and 1.52 mg l⁻¹, respectively).⁹⁶ Zounkova *et al.*⁹⁷ reported that cytarabine and 5-fluorouracil caused reproduction inhibition of *D. magna* (EC₅₀ 10 and 0.1 mg l⁻¹, respectively) and growth inhibition of *P. putida* (EC₅₀ 17 and 0.044 mg l⁻¹, respectively). Methotrexate exhibited acute toxicity to *Tetrahymena pyriformis* and teratogenicity to fish embryos with an EC₅₀ of 45 mg l⁻¹ for 48 h (ref. 98) and 85 mg l⁻¹ after 48 h,⁹⁹ respectively.

2.1.5 Blood lipid lowering agents. The lipid regulator bezafibrate was noticed in the river water of Germany at a concentration of 3.5 mg l⁻¹.³⁸ 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⁻¹.⁸⁶ In Canadian STP samples, carbamazepine was found at a concentration of 2.3 mg l⁻¹.¹⁰⁰ Fibrates were found to be toxic in toxicity tests towards *C. dubia*, *B. calyciflorus* and zebrafish with reported NOEC values of 640 µg l⁻¹ (7 days), 246 µg l⁻¹ (2 days) and 70 mg l⁻¹ (10 days), respectively.⁹² Clofibrate is specifically toxic to aquatic species with a reported LC₅₀ value of 7.7–39.7 mg l⁻¹ while fish *Gambusia holbrookii* is the most sensitive one with an LC₅₀ (96 h) of 7.7 mg l⁻¹.¹⁰¹ Bezafibrate and gemfibrozil are toxic towards nano-target organisms with EC₅₀ values of 10 to 100 mg l⁻¹ and 1 to 10 mg l⁻¹, respectively.¹⁰² Exposure to gemfibrozil for 14 days of *Carsius auratus* exhibited 50% reduction in 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 a concentration of 6–22 ng l⁻¹ and 53–265 ng l⁻¹, 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 the maximum concentrations of 72 and 66 ng l⁻¹, respectively.¹⁰⁵ Paraxanthine was detected in agriculture land as well as in the western Lake Erie basin in Ohio at a maximum concentration of 1.8 mg l⁻¹.¹⁰⁶ Carbamazepine exhibits a carcinogenic effect to rats while it does not have a mutagenic effect on mammals.¹⁰⁷ Chronic toxicity analysis suggested that NOEC values of carbamazepine are 377 µg l⁻¹ (2 days), 25 µg l⁻¹ (7 days) and 25 mg l⁻¹ (10 days) toward *B. calyciflorus*, *C. dubia* and zebrafish, respectively.⁹² Carbamazepine showed acute toxicity through growth inhibition of *D. magna* at a concentration of 17.2 mg l⁻¹.¹⁰⁷ Experimental studies suggested that the *in vitro* growth of *T. gondii* is inhibited by the mood stabilizer valproic acid and the antipsychotic drug haloperidol.¹⁰⁸ Sertraline demonstrates

high toxicity towards rainbow trout with an LC₅₀ of 0.38 mg l⁻¹ under 96 h exposure.¹⁰⁹

2.1.7 Antiviral and antiparasitic drugs. Extremely high concentrations (2–12 mg l⁻¹) of oseltamivir and its bioactive metabolite oseltamivir carboxylate (OC) were detected in WWTPs in the time of pandemic suggesting that almost 80% of the active drug is excreted from the source.¹¹⁰ In a UK farm, higher concentrations of antiparasitic pharmaceuticals were found in dung (doramectin 0.112 mg kg⁻¹ and ivermectin 1.85 mg kg⁻¹) and soil (around 0.046 mg kg⁻¹) while the detected concentration of the studied drugs in soil was relatively lower than in 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⁻¹, and a mean concentration of 7 ng l⁻¹.¹¹² Fenbendazole and ivermectin affect the survival of *Pristionchus maupasi* with a concentration of 10–20 mg dung per kg and 3 mg dung per kg, respectively.¹¹³

2.1.8 Hormones. 17 α -Estradiol and estriol are found in high concentrations (about 180 and 590 ng l⁻¹, respectively) in WWTP samples in the USA.¹¹⁴ EE2, an estrogenic hormone, was detected in surface waters of each UN region. Based on the analysis data collected from North America, Europe and south-east Asian countries, the identified concentration range was from 0.001 to 0.040 mg l⁻¹.¹¹⁵ In an experiment in a Canadian lake, feminization of male fish (*Pimephales promelas*) occurred at concentrations of 5 ng l⁻¹ to 6 ng l⁻¹ of EE2 which is capable of hampering complete population collapse of the studied species.¹¹⁶ Egg fertilization reduction in flathead minnow fish was reported with exposure of EE2 with a concentration of 320 pg l⁻¹ 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 a good amount of research regarding the 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 EMA¹²⁰ 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 risk assessment studies. The EU REACH regulations also suggested that the assessment of toxicity and the bioaccumulation of TPs are necessary for any marketed APIs.¹²² As the 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, it is immediately converted to its TP erythromycin-H₂O (ERY-H₂O),

which is one of the most studied TPs in the environment among antibiotics and used as a marker to detect ERY in any sample. The identified concentrations of ERY-H₂O in WWTPs are 1978¹²⁵ and 6000 ng l⁻¹ (ref. 126) in China and Germany, respectively. ERY-H₂O was detected in a concerning concentration in drug manufacturing effluents (7840 ng l⁻¹) and hospital sewage (6110 ng l⁻¹).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 The active metabolite of metronidazole antibiotic is hydroxylated metronidazole (METR-OH) identified in WWTPs and in hospital sewage in Portugal where the concentration in hospital effluents reaches up to 11 µg l⁻¹.¹²⁹ Among statins, *o*-hydroxy-atorvastatin and *p*-hydroxy atorvastatin are two major metabolites of atorvastatin and are identified in WWTP influents with a concentration of 196 and 280 ng l⁻¹, respectively whereas a much lower concentration (10 ng l⁻¹) of simvastatin hydroxyl acid was observed, which is a TP of simvastatin detected in the influents of WWTPs.¹³⁰ The antiviral drug oseltamivir (OSL) and its carboxylated TP oseltamivir carboxylate (OSLCAR) are detected in wastewater (42.7 ng l⁻¹ in influents and 17.3 ng l⁻¹ in effluents) which may cause serious hazards such as OSL-CAR resistance in wildfowl, and birds are capable hosts of influenza viruses.¹³¹ Three metabolites (2-hydroxyestradiol (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⁻¹ 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-H₂O), 5-hydroxydiclofenac (5'-OH-DCF), 4-hydroxyacetofenac (4'-OH-ACF), 1,5-dimethyl-1,2-dehydro-3-pyrazolone (DP), 1-acetyl-1-methyl-2-phenylhydrazide (AMPH) and 1-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 a very high concentration in WWTP influents (38.4 and 6840 ng l⁻¹, respectively) and effluents (10.6 and 1130 ng l⁻¹, respectively).¹³⁴ Clofibric acid is the hydrolyzed metabolic product of clofibrate and etofibrate recurrently identified in industrial, municipal and hospital wastewater up to the 41.4 µg l⁻¹ level.¹³⁵ Fenofibric acid, a TP of fenofibrate, was detected at concentrations of 349 ng l⁻¹ in WWTPs in Spain.¹³⁶ Among anti-depressant 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 wastewaters with concentrations of 5500 and 2000 ng l⁻¹ for *O*-desmethyl-venlafaxine and hydroxy-bupropion, respectively.¹³⁷ Hydroxy-tamoxifen (OH-TMX) and 4,4-dihydroxy desmethyltamoxifen (endoxifen) are the major metabolites of

tamoxifen (TMX) which showed higher estrogenicity than the TMX detected in the wastewater sample of hospitals.¹³⁸ Around 30 metabolites are formed for carbamazepine (CBZ), but the major reported metabolites in the ecosystems are CBZ-10,11-epoxide (CBZ-Ep) formed through oxidation (pharmacologically active with anticonvulsant properties), the hydration product 10,11-dihydro-10,11-*trans*-dihydroxy-carbamazepine (DiOH-CBZ), followed by hydroxylated TPs 2-hydroxy-CBZ and 3-hydroxy-CBZ.²⁴ The metabolite DiOH-CBZ was the predominant analyte in the aqueous phase with concentrations higher than the parent compound, reaching a few µg l⁻¹ in influents and effluents as well (up to 4000 and 3400 ng l⁻¹, respectively). On the other hand, CBZ-Ep, which is present in human plasma at 3- to 4-fold lower concentrations than DiOH-CBZ, was found in some cases at concentrations at 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 experimental 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 the embryos of zebrafish with an EC50 of 37 mg l⁻¹ 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⁻¹ in surface water.¹⁴² An almost similar level of toxicity was experienced for freshwater and marine species through exposure of CBZ-Ep compared to CBZ while the toxicity concern was higher in the case of 2-OH-CBZ, 3-OH-CBZ and DiOH-CBZ.¹⁴³ *V. fischeri* exhibits higher toxicity under the exposure of 2-OH-CBZ and 3-OH-CBZ compared to the parent compound CBZ.¹⁴³

In Table S1 (see the ES†), 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} Fig. 2 shows the existence of a number of pharmaceuticals identified in surface water, groundwater and drinking water all over the world.¹ The World Health Organization (WHO) has provided recommendations and practical guidance for managing the concern about pharmaceuticals in drinking water placing uttermost emphasis on prioritizing the water safety management including microorganisms present in the aquatic environment.¹⁶⁸ Fig. 3 presents the chemical structures of the top 50 pharmaceuticals including metabolites based on the detection frequency as well as concerning concentrations considering available literature.^{67–168}

3. Occurrence and ecotoxicity of personal care products (PCPs)

PCPs are one of the major classes of emerging pollutants apart from pharmaceuticals found in the environment at a signifi-

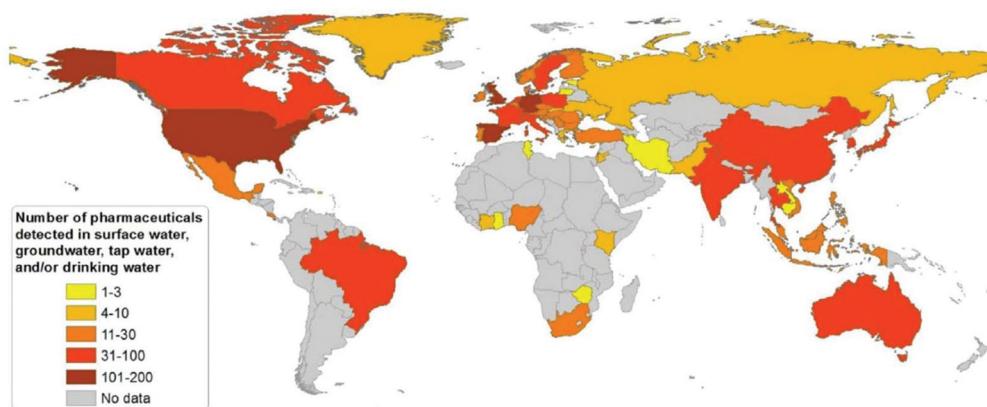


Fig. 2 Number of pharmaceuticals detected in groundwater, surface waters and drinking water covering all countries. [Reprinted by taking permission from ref. 1.]

cant level of concentration worldwide which majorly contribute to the toxicity to aquatic biota and soil.^{169,170} The major sources and routes to the ecotoxicity of PCPs are illustrated in Fig. 1. However, very few ecotoxicity data exist for PCPs in the public domain. PCPs include a 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 (methyl-triclosan, triclosan (TCS), triclocarbon (TCC)), and 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 and wastewater 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 and persistent, and display high bioconcentration and bioaccumulation in aquatic organisms.^{13,170,171} Some evidences supported the endocrine disruption effect in aquatic species along with acute and chronic toxicity towards them by PCPs.¹⁷⁵ Considering the spread of PCP usage, small portions of PCPs are tested experimentally in different samples from the environment. Thus, evaluation of toxicity data of the 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⁻¹ as the majority of them exist as a mixture at trace levels in the aquatic environment.¹⁷⁶ PCPs identified in water matrices throughout different countries covering all continents are illustrated in Fig. 4.¹⁷¹

3.1 Disinfectants and bactericides

Biphenyl ethers like TCC and TCS are frequently detected in wastewater and are generally used as antimicrobials in deodorants, soaps, lotions, toothpaste and plastics.³⁴ TCS has been detected in surface water, groundwater, STPI, and marine biota worldwide¹⁷⁰ with multiple studies characterizing the presence of its methyl derivative methyl triclosan (M-TCS) in the WWTP effluent (up to 650 ng l⁻¹ TCS and 11 ng l⁻¹ M-TCS), STP influents (0.2–16.6 µg l⁻¹ of TCS) and effluents (0.08–2.7 µg l⁻¹ of TCS),^{5,177} surface water (74 ng l⁻¹ of TCS)⁷ and fish tissue (2100 ng g⁻¹ of lipid of M-TCS).¹⁷⁸ Due to the lipophilic nature, M-TCS is stable and tends to bioaccumulate with a 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⁻¹,¹⁷⁹ respectively. TCS exerts toxicity to biofilm algae and aquatic bacteria by enhancing the mortality rate with a no effect concentration (NEC) of 210 ng l⁻¹,¹⁵ inhibition of the growth¹⁸⁰ and photosynthetic efficiency (NEC: 420 ng l⁻¹).^{9,15} Among all species, algae growth was the most sensitive towards TCS and was affected at concentrations less than 1 µg l⁻¹.¹⁸¹ Considering the behavior pattern change, TCS is found to alter the swimming performance of *Danio rerio*, *Oncorhynchus mykiss*, and *Oryzias latipes* at concentrations as low as 71 µg l⁻¹.¹⁸² In surface water, TCC is found in alarming concentrations in species like fishes (*Danio rerio*, *Oryzias latipes*), crustaceans (*D. magna*, *Mysidopsis bahia*), planktonic copepods (*Acartia tonsa*), and water fleas (*Ceriodaphnia dubia*, *P. subcapitata*).^{183,184} Recent studies showed that the toxicity of TCC is on the higher side towards fish and aquatic invertebrates for both short- and long-term exposure 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

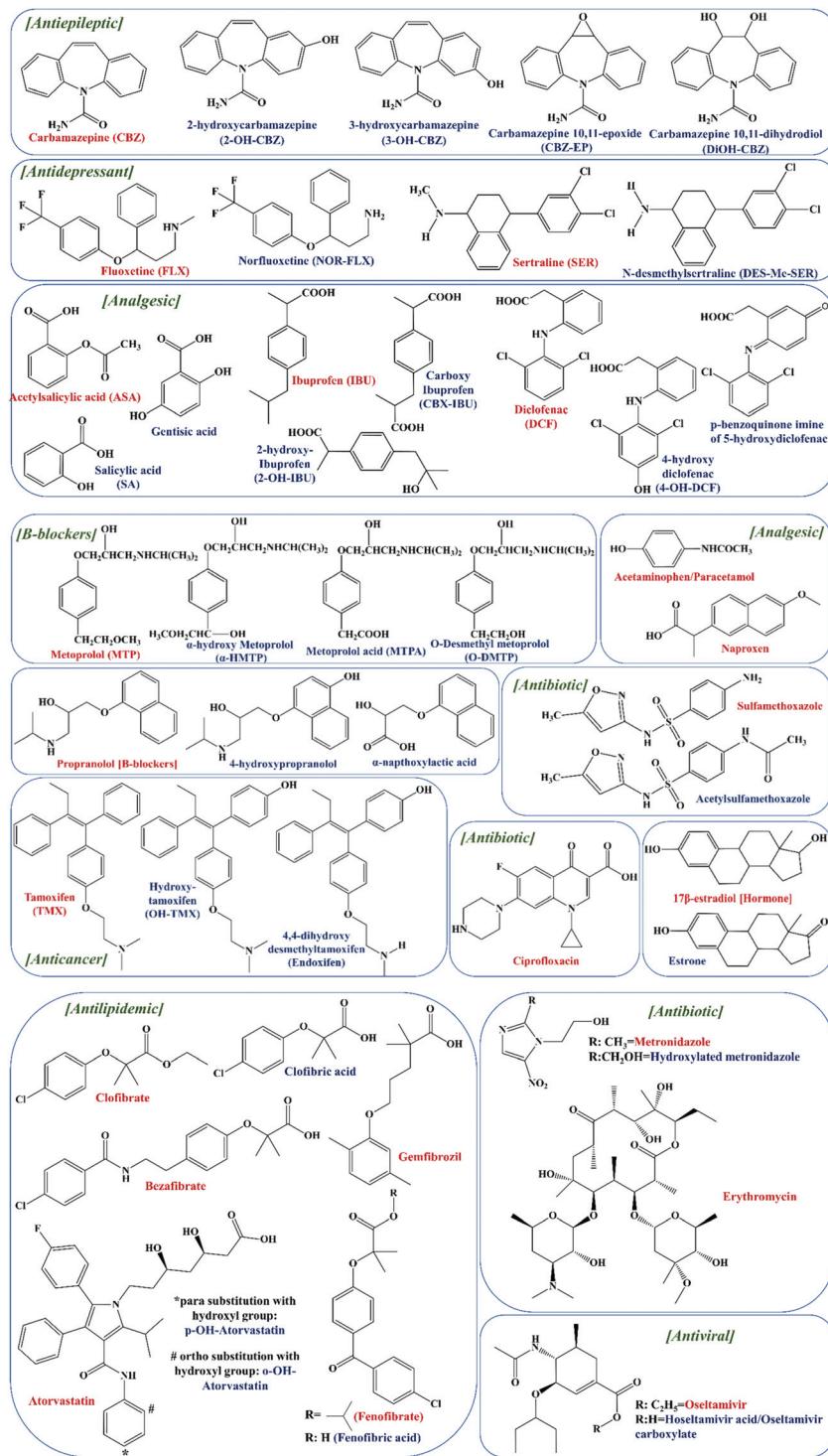


Fig. 3 Chemical structures of top fifty most frequently detected pharmaceuticals in alarming higher concentrations including metabolites (compound's name in red denotes the parent compound and in blue suggests the metabolite or TPs).

the soap industry.¹⁷⁰ Due to high octanol–water partition coefficients (5.4 to 5.9 for polycyclic musks and 3.8 for nitro musks), they are bioaccumulating in aquatic species and benthic invertebrates with potential to accumulate in humans, and have been known 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 US EPA in the High Production Volume (HPV) list due to their production of over 1 million pounds per year.¹⁸⁸ HHCB

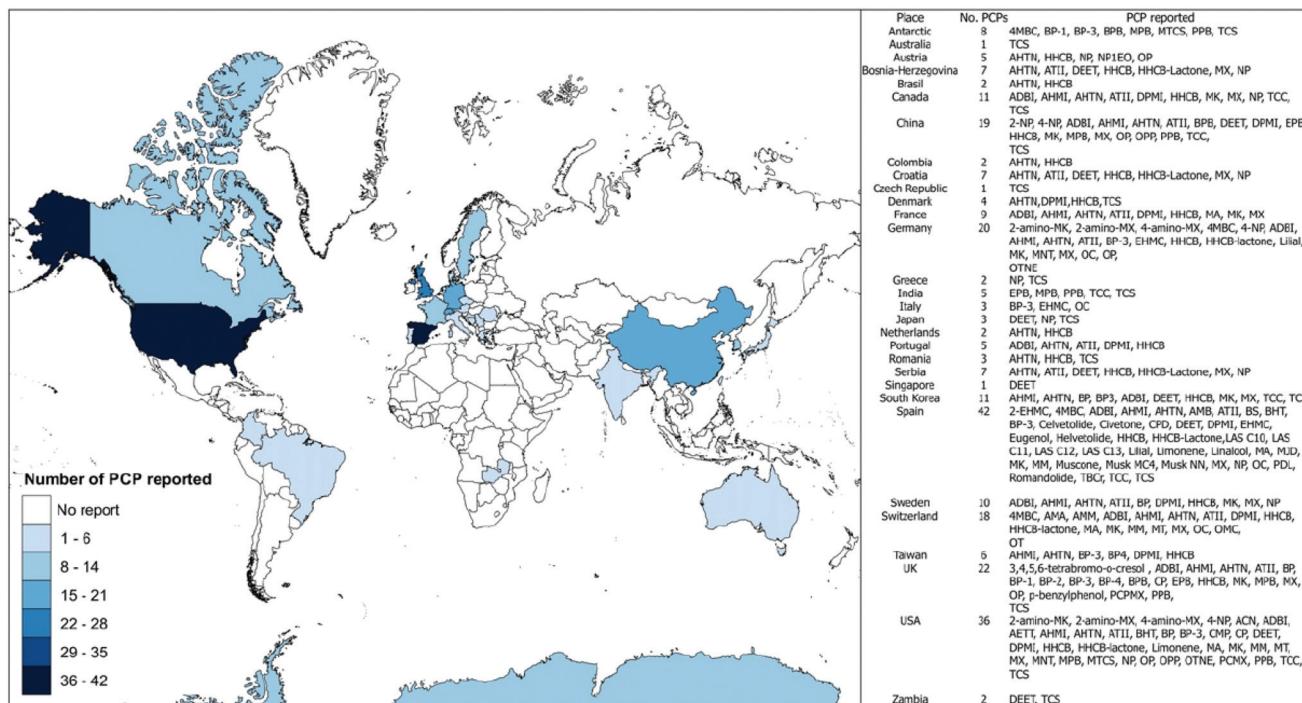


Fig. 4 Number of PCPs detected in diverse water matrices covering all countries. [Reprinted by taking permission from ref. 171.]

and AHTN were frequently detected in STP influents with a concentration of $0.043\text{--}13.7 \mu\text{g l}^{-1}$ all over the world whereas MX and MK were found in 83 to 90% of STP effluents but at low concentrations.¹⁷⁰ The mean concentrations of AHTN and HHCB were $0.18 \mu\text{g l}^{-1}$ ($0.05\text{--}0.44 \mu\text{g l}^{-1}$) and $1.86 \mu\text{g l}^{-1}$ ($0.45\text{--}4.79 \mu\text{g l}^{-1}$), respectively based on the samples collected from 40 STPs.¹¹ In surface water also, the most commonly found musk is HHCB with a concentration of $13\,920 \text{ ng l}^{-1}$.¹⁸⁹ Among 33 documented fragrances in WWTPs, AHTN (influents: $0.41\text{--}68\,120 \text{ ng l}^{-1}$, effluents: $0.05\text{--}7555 \text{ ng l}^{-1}$)³⁵ and HHCB (influents: $1.44\text{--}595\,480 \text{ ng l}^{-1}$, effluents: $0.14\text{--}108\,000 \text{ ng l}^{-1}$)¹⁹⁰ were the frequently detected ones in 16 countries. Although studies found that the amount of HHCB is under the EC₅₀ for *O. latipes*, *Danio rerio*, *Mysidopsis bahia*, *D. magna*, *Acartia tonsa* and *P. subcapitata*,^{183,184} a few studies also suggested that it could exert toxicity due to its bioaccumulation, and changes in fecundity, growth and development of exposed species.³ The detected concentrations of HHCB in WWTP effluents are above the threshold of chronic toxicity for species like *Neopachyloides spinipes* (LOEC: $20\,000 \text{ ng l}^{-1}$), *A. tonsa* (EC₅₀: $59\,000 \text{ ng l}^{-1}$) and *D. magna* (NOEC: $10\,000 \text{ ng l}^{-1}$).¹⁹¹ In the 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 the commonly used insect repellants routinely detected in surface waters^{193,194} as well as in WWTP effluents throughout the United States with concentrations of $0.2 \mu\text{g l}^{-1}$ and $0.28 \mu\text{g l}^{-1}$, respectively.^{193,195} DEET

is detected in 8 countries in WWTP samples with concentrations of $15.1\text{--}6900 \text{ ng l}^{-1}$ in influents and $6.4\text{--}2110 \text{ ng l}^{-1}$ 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 the moth repellent 1,4-dichlorobenzene suggested that fish is sensitive to the compound 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 whereas MPB and EPB are least toxic with lower LC₅₀ values which are 3 times less than BnPB.²⁰¹ MPB is recorded in the WWTP samples of 4 countries with a concentration of $1193.9\text{--}30\,688\,000 \text{ ng l}^{-1}$ for influents and ND – $155\,000 \text{ ng l}^{-1}$ for effluents.¹⁷⁹ In the case of STP influents, PPB and MPB are detected with concentrations of up to $20 \mu\text{g l}^{-1}$ and $30 \mu\text{g l}^{-1}$, respectively.²⁰² Concerning amounts of parabens are detected in surface water with concentrations ranging from 15 to 400 ng l^{-1} .¹⁷⁹ Interestingly, MPB was detected in mineral water of Spain with a concentration of 40 ng l^{-1} .²⁰² Dobbins *et al.*²⁰³ revealed that MPB and EPB are least toxic towards fish and invertebrates whereas the 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 the alkyl chain length increases along with increased octanol–water partition coefficient.²⁰⁴ MPB was detected in fish species, marine mammals, and sharks from Washington, Alaska, and the Florida coast with a high concentration and frequency at ng g⁻¹ and ng l⁻¹ levels.^{36,205} In a few instances, EPB and PPB were found in sea-water and EPB was found in sediments at ng g⁻¹ and ng l⁻¹ levels.³⁶ In all fish samples of the Philippines, EPB, MPB and PPB were detected at concentration levels of 0.01–1.4 ng g⁻¹, 0.2–4.5 ng g⁻¹ and 0.02–1.5 ng g⁻¹ (ref. 206 and 207) whereas MPB is detected in pelagic fishes and demersal in Florida coasts with concentrations of 2.1–92.9 ng g⁻¹ (ref. 208) and 1.0–6.1 ng g⁻¹ 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⁻¹), molluscs (68.1 µg g⁻¹), marine plants (15.7 µg g⁻¹)²⁰⁸ and mammals (32.6 µg g⁻¹).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 humans as well as aquatic species due to their high hydrophobicity followed by a 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 organisms, especially in fish, they are potentially toxic in nature.¹⁷⁰ In WWTPs, BP-4 was detected with concentrations of 6 325 000 ng l⁻¹ (ref. 179) which exceeded the LOEC for *Oncorhynchus mykiss* (4 897 000 ng l⁻¹),²¹² whereas BP-3 was detected in six countries with concentrations of 7–3 975 000 ng l⁻¹ in influents and 1.1–2 196 000 ng l⁻¹ in the effluents of WWTPs.²¹³ BP-4 was also detected in surface water with a concentration of 323 000 ng l⁻¹ (ref. 179) which exceeds the predicted no effect concentration (PNEC) of 50 000 ng l⁻¹ for *D. magna*.²¹⁴ Experimental studies supported that UVFs cause potential endocrine disruption and estrogenic effects as well as affect the 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 the highest concentration in all samples (2.7 µg l⁻¹ in WWTP, 35 ng l⁻¹ in surface water and 123 ng g⁻¹ lipid tissue).¹⁷ Again, BP-3 was detected with concentrations of 5–125 ng l⁻¹ 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⁻¹ (ref. 217) and in *Mytilus galloprovincialis* in Portugal with concentrations up to 800 ng g⁻¹.²¹⁸ The occurrence of OC in mammals, especially in Franciscana dolphins, was detected by Gago-Ferrero *et al.*²¹⁹

Table S2 (see the ESI†) gives a broader overview of the 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} Fig. 5 presents the chemical structures of the top 20 PCPs including metabolites based on the detection frequency as well as concerning concentrations considering the available literature.^{169–257}

4. Environmental risk assessment (ERA)

The ERA is a procedure of evaluation of the concentration, occurrence, frequency, the level in the 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 the majority of risk assessment data is to set the 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 were not considered a reason for denying market approval a few decades ago; many products are even already on 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 (Fig. 6).

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}

Although the steps for risk assessment by regulatory authorities are different from each other, the basic idea is the 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 Fig. 7 as an example.

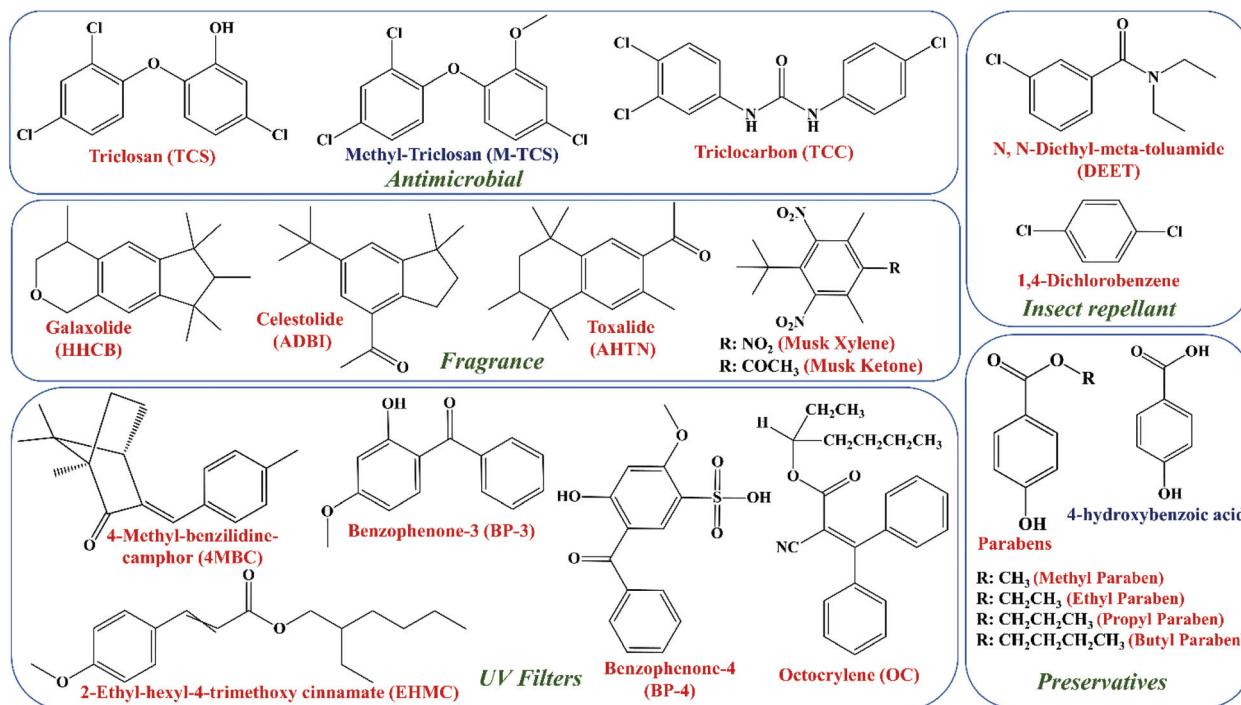


Fig. 5 Chemical structures of top twenty most frequently detected PPCPs in alarming higher concentrations including metabolites (compound's name in red denotes the parent compound and in blue suggests the metabolite or TPs).

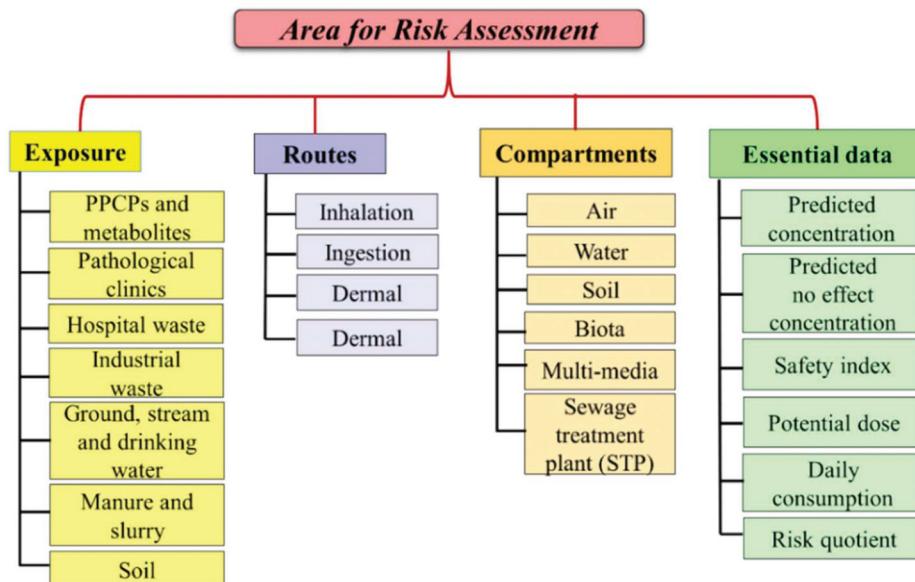


Fig. 6 Area of focus for risk assessment.

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 PPCP. The ERA model considers the safety issues and RQ of each chemical carefully to reflect the associated risk of it to specific

species and the environment. 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 the preparation of an ERA model, one needs to evaluate the exposure of any PPCP employing the following points:^{65,66}

- The exposure of a specific product needs to be evaluated in the form of environmental concentration with which the

Table 1 Fundamental ERA approaches implemented by the major regulatory agencies

Method	Role
Hazard identification	The first step is the identification of source and the occurrence of hazards along with the intensity of risk for a compound. In case of lack of enough <i>in vitro</i> data to explicit species and specific environment compartment, the researcher needs to rely on the <i>in vivo</i> data obtained from the sacrifice of a large number of animals. Thus, greater attention needs to be given for the proper and efficient use of <i>in vitro</i> assays in human cells along with <i>in silico</i> modeling studies to generate a good number of data for hazard identification. ²⁵⁹
Dose-response assessment	Identification of the threshold dose of the toxicity is essential for scientific risk assessment of any hazards. The dose-response information over a wide range of test concentrations should be evaluated by employing the 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 <i>in vitro</i> test data in terms of <i>in vivo</i> 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 <i>in silico</i> models and expert systems as alternatives. <i>In vitro</i> to <i>in vivo</i> 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. In a few instances, internal dose measurement was performed by 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 of risk quotients	The US EPA recommends the deterministic approach and the risk quotient (RQ) calculation to assess the toxicity to environment exposure. The RQ can be calculated according to EMA guidelines: ²⁶⁴
$RQ = \frac{\text{estimated exposure}}{\text{estimated toxicity}} = \frac{\text{measured environmental concentration (MEC) in water/sediment/air}}{\text{predicted no-effect concentration (PNEC) in LC}_{50} \text{ or EC}_{50} \text{ or NOEC}}$	
<p>where NOEC is the no observed effect concentration; $PNEC_{\text{acute}} = (\text{EC}_{50} \text{ or LC}_{50})/1000$ $PNEC_{\text{chronic}} = \text{NOEC}/\text{assessment factor (AF)}$ MEC corresponds to the highest measured concentration detected in samples and PNEC is estimated using the lowest values of acute EC₅₀ or LC₅₀ or the chronic NOEC.²⁶⁵ According to the 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 Thresholds²⁶⁷ are the following: high risk ($RQ \geq 1$), 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.</p>	
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.

system is affected along with the time period, intensity and frequency, not using concentrations to which any specific species is exposed. Again, the exposure relies upon multiple parameters like the sorption effects, metabolism, fate, and transformation rate of the product.

- A species, which is affected by PPCP's toxicity, needs to be monitored for a definite period of time and throughout its life cycle to replicate the behavioral pattern in modeling.
- Dose-response studies and PK/PD data of individual chemicals are important as they are directly related to the absorption, distribution, metabolism, excretion, and toxicity (ADMET) pattern.
- A toxicokinetic and comprehensive bioavailability study is required for each product.

- Knowledge of toxicity pathways and target sites in the biological system needs to be understood for individual PPCPs.
- The MoA is different from species to species for each PPCP; thus a species wise understanding of MoA is important to portray its molecular and functional effects.
- The risk occurring from the intrinsic toxicity of pharmaceuticals due to their chemical properties is desired to be studied.

5. Environmental risk management (ERM)

Risk management is a process of protecting public health by recognizing, appraising, and executing actions to decrease the

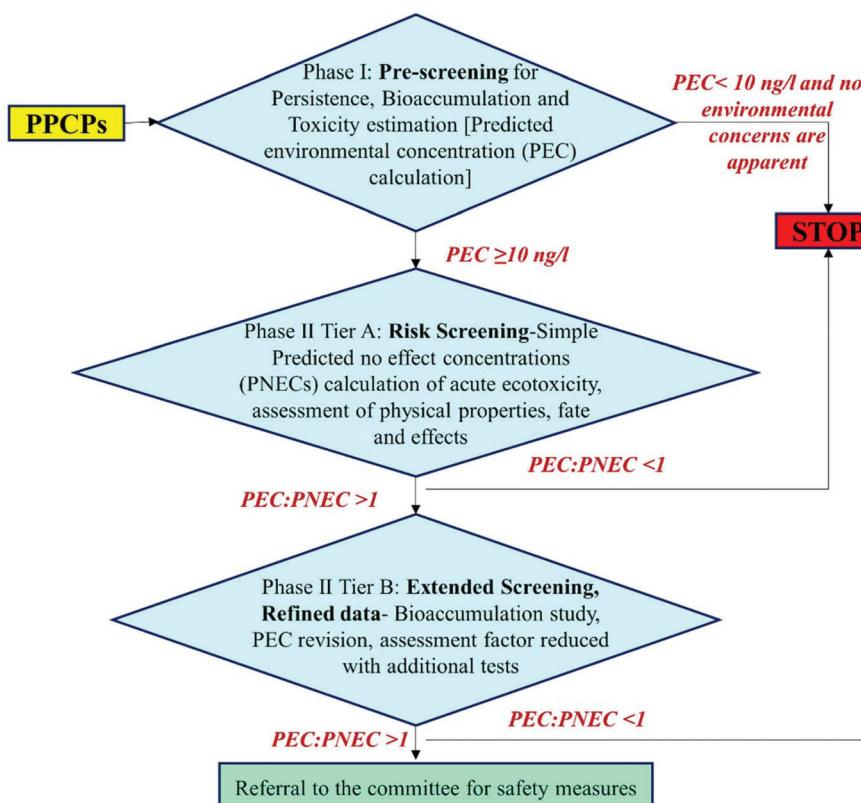


Fig. 7 Three phase risk assessment process by EMA.

risk to human health and to ecosystems associated with any hazardous/toxic product. The objective of the ERM is to take integrated actions to reduce or prevent the risk effects considering cost-effective and risk benefit analysis by taking into account the social, ethical, cultural, political, and legal aspects.²⁶⁹ Examples of risk management activities comprise the following:

- How much and where active substances and treated residuals will be discharged by industries,
- Deciding which product may be stored at a hazardous waste disposal facility and how they will be treated before release to the 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 the permissible levels of contamination of PPCPs in drinking water.²⁷⁰

Risk assessment delivers the 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 Fig. 8. The major factors and commonly employed risk management approaches are provided in Table 2.

6. Global regulatory agencies related to the 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 the existence of living systems 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 the uncontrollable usage of PPCPs. Regulatory agencies are in charge throughout the world for the risk characterization, risk assessment and management of PPCPs' ecotoxicity and the major ones are:

- Australian Environment Agency (AEA)²⁷³
- Center for drug evaluation and research (CDER) and US FDA²⁷⁴
- European Medicines Agency (EMA) for the evaluation of medicinal products²⁷⁵
- 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

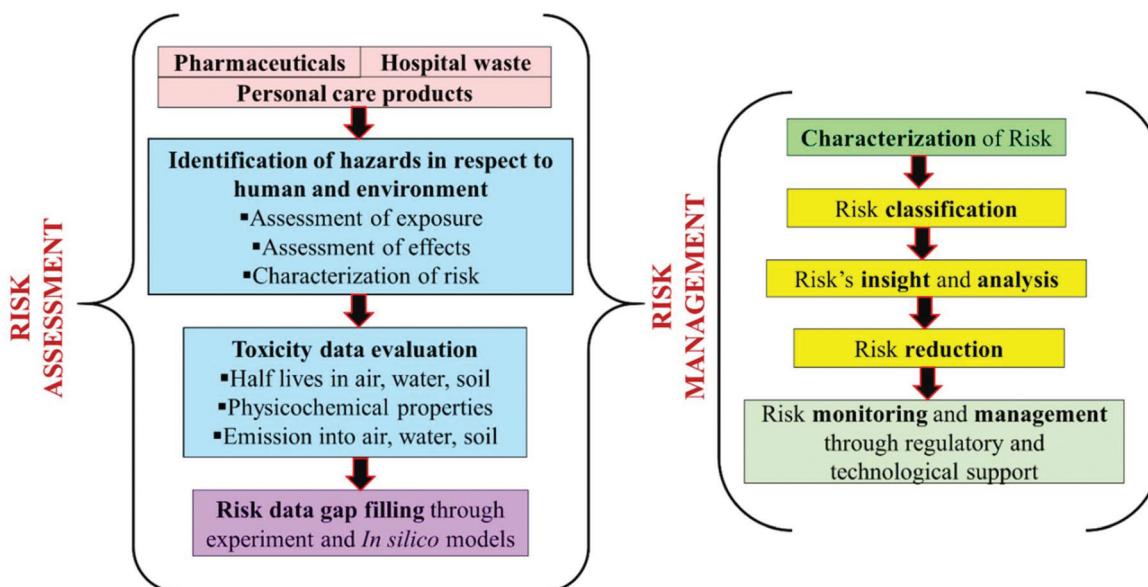


Fig. 8 Fundamental steps under ERA and ERM.

Table 2 Factors and methods associated with ERM

ERM	Type	Explanation
Factors	Scientific	Offers a 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 the right management option.
	Social	Although social factors have no direct involvement, they have 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 suffering 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 is important. Most importantly, regulatory agencies and industries need to work in parallel.
	People	People's requirement and public values replicate the far-reaching attitudes of society about environmental risk management.
Approach	Preventive measures	A series of guidelines has been proposed by the EMA as safety measures for risk management: <ol style="list-style-type: none"> 1. Early assessment of risk for each marketed product, 2. Packaging should have apposite product labeling and summary product characteristics (SPC) for proper recycling of unused and expired product, 3. Educate 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 wastewater and sewage treatment which can treat most of the products before releasing to the environment removing or neutralizing the toxic products up to manifold. Among the treatments, the most common ones are oxidation, adsorption, photochemical and filtration. ²⁷¹
	Training and awareness of stakeholders	Awareness and training about the occurrence and effect of individual PPCPs along with their corresponding effects toward the environment is important along with the facts about the disposal process. The awareness needs to be spread among all the stakeholders. In this specific approach, industry has a huge role to play in generating material safety data sheets (MSDSs) for each raw material, API and formulation. ²⁷²
	Green and sustainable pharmacy	The approach for the future which demands environmentally benign compounds. 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 an immense role to play in designing followed by synthesis of easy and fast degradable PPCPs to reduce. Regarding sustainability issues, the understanding of the life cycle, fate, transformation and related pathway is very much important for implementation of green pharmacy in the required phase. ²⁷²

- Registration, Evaluation, Authorization and Restriction of Chemicals (REACH)²⁷⁹
- Swedish Environmental Classification and Information System (SECIS)²⁸⁰
 - The UBA – Umweltbundesamt (UBA) of Germany²⁸¹
 - 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 is the 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 the 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 $1 \mu\text{g l}^{-1}$ Predicted Environmental Concentration ($\text{PEC}_{\text{EFFLUENT}}$) were exempted from a detailed risk assessment.²⁷⁴

7. Pre-screening and estimation of exposure for the API, screening and initial prediction of risk, and 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 $\sum \text{PEC}_i / \text{PNEC}_i$.^{277,280}

9. Circulation of the 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 provides efforts to decrease environmental risks generated from a

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 § 300 g⁻¹, Federal Water Pollution Control Act (Clean Water Act)/33 USC §§ 1312–1333, Food Quality Protection Act/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, QSAR models are employed for identification of PBT substances of around 166 000 chemicals, and these data can be used for self-classification of around 20 000 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 is too large, 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 are mainly employed not only in the USA, 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 in sufficient information regarding the deleterious effects of PPCPs on 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 used in the ecotoxicological assessment of PPCPs?

The number of results obtained by a Google search with the terms “*in silico* and environmental toxicity” was around 1 810 000 in November 2018. The term ‘*in silico*’, coming from the Latin word silicon, is an expression suggesting “computer simulation” with 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 in *in silico* modeling and 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 chemicals able to cause adverse effects on environ-

mental species, as well as to predict a range of physicochemical 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 to support the design of greener PPCPs with reduced or no animal testing. The reasons to use *in silico* models in ecotoxicity assessment are the following:

- *The prohibition of animal experiments:* 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 was applied on 11th March 2009 and on finished cosmetic products it has been applied since 11th September 2004. Thus, regulatory agencies around the world introduced the molecular modelling approach for risk assessment.^{285,286}

- *The 3Rs concept:* The principle of 3Rs implies “reduction”, “replacement” and “refinement” of animal usage in scientific experiments. ‘Reduction’ signifies the use of a smaller number of animals, ‘replacement’ relates to the usage of non-living resources to replace higher taxonomical animals, and ‘refinement’ signifies the prevention of harshness or brutality to 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 in risk assessment and management, predicting the toxicity of new and untested compounds, and evaluation of physicochemical parameters and fate properties evaluation.

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

- *Mechanistic interpretation:* In many cases, the generated mathematical equation from *in silico* models is capable of identifying the responsible structural and physicochemical properties for toxicity to a specific organism or animal system. Generally, it is assumed that compounds fitting 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 in earlier toxicity study.²⁹⁰ A graphical depiction is reported in Fig. 9 where the importance of *in silico* models is illustrated by evaluating pharmaceutical ecotoxicity.

It is true that environmental toxicity is not a significant determinant for approving APIs onto 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 the probable ecotoxicity of API can be helpful for environmental



Fig. 9 Significance of *in silico* modeling evaluating the influence of PPCP ecotoxicity.

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 the 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 for *in silico* techniques in predicting the toxicological and hazardous properties of PPCPs is 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 the 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

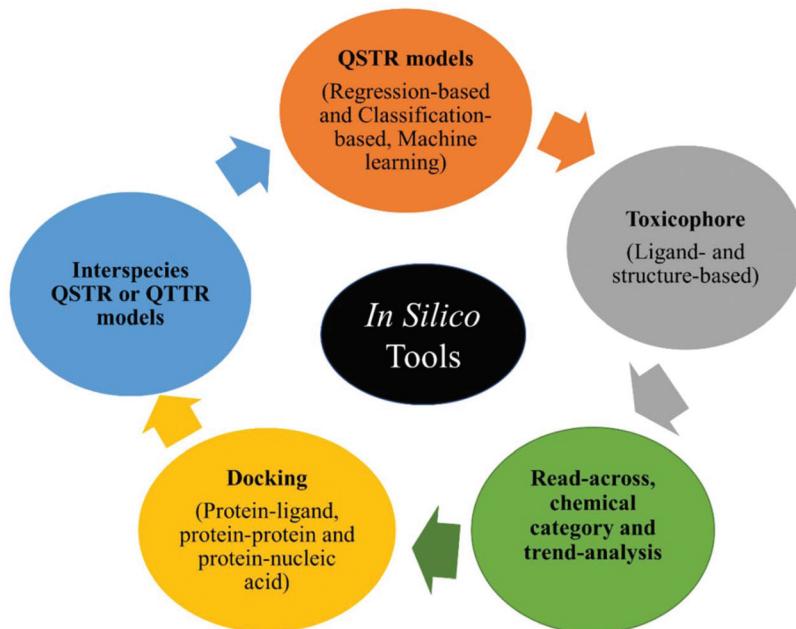


Fig. 10 Most commonly used *in silico* tools for ecotoxicity modeling and prediction of PPCPs.

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 Fig. 10.

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 the QSTR approach can be mathematically defined with the following expressions:

$$\begin{aligned} \text{Toxicity}(Y) &= f(\text{chemical attributes}) \\ &= f(\text{information about chemical structure and physicochemical properties}) \quad (1) \\ &= f(\text{descriptors}) = f(X_1 + X_2 + \dots + X_n) \end{aligned}$$

Chemical attributes are the essential information of compounds which regulate their specific response that is often defined in terms of the information generated straight from the chemical structure and the physicochemical properties. The obtained information in the form of numerical values is labeled as descriptors which help to find the best possible correlation with the toxicity

response. The QSTR equation can be mathematically stated as follows:

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

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

In the QSTR model, the toxicity response acts as a 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 the quantitative toxicity–toxicity relationship (QTTR) or interspecies-QSTR (i-QSTR). The hypothesis is explained schematically in Fig. 11. The details about i-QSTR will be discussed later.

8.1.2 Principles of QSTR. The regulatory QSTR models should be developed based on five principles proposed by the Organization for Economic Co-operation and Development (OECD) for QSAR model development and validation.²⁹⁴ These guidelines recommend a defined endpoint for modeling ensuring a 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 the 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

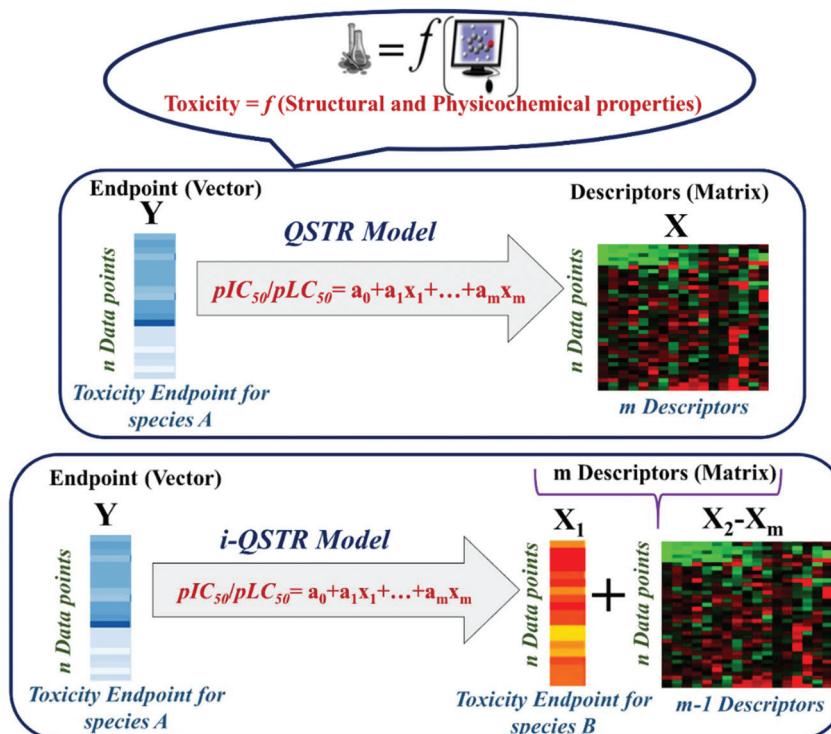


Fig. 11 Hypothesis underlying QSTR and i-QSTR models.

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 Fig. 12.

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). In contrast, 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-, and classification-based approaches and machine learning tools. Comprehensive and commonly employed representative chemometric tools like multiple linear regression (MLR),²⁹⁵ step-wise regression,²⁹⁶ partial least squares (PLS),²⁹⁷ genetic function approximation (GFA),^{298,299} genetic partial least squares 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 are discussed elsewhere in detail.^{295–305} Again, based on the geometric dimension of descriptors employed for model development, QSTR models

can be categorized into multiple methods which are illustrated in Fig. 13. For elaborate discussion and examples of individual methods, please refer to the literature.^{56,57}

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 the biological response (here, toxicity) of two species which is majorly employed to extrapolate the 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:

$$\log_{10} \left(\frac{1}{Y} [\text{predicted species}] \right) = a + a_1 \times \log_{10} \left(\frac{1}{Y} [\text{surrogate species}] \right) \quad (3)$$

Here, the toxicity response Y can be expressed as EC₅₀, ED₅₀, IC₅₀ or LD₅₀ values, and 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 experimental toxicity data for a specific species act as a predictor variable along with other descriptors to establish a correlation against another species for similar endpoints.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

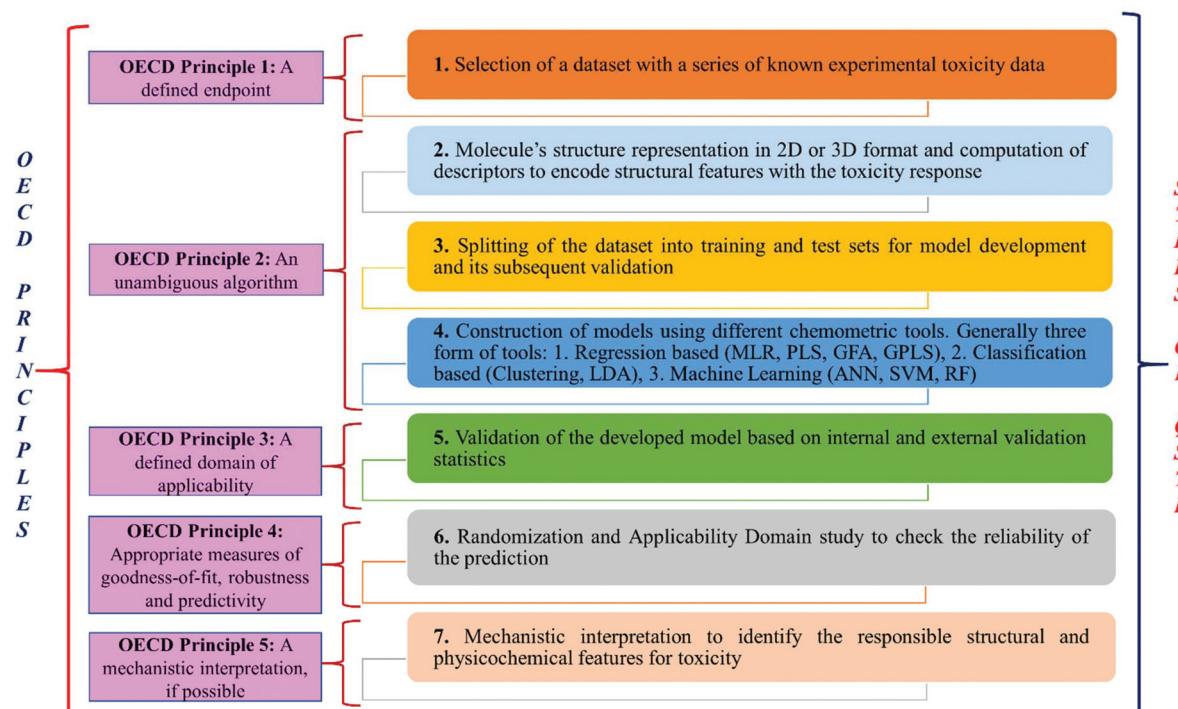


Fig. 12 OECD principles and fundamental steps for the QSTR formalism.

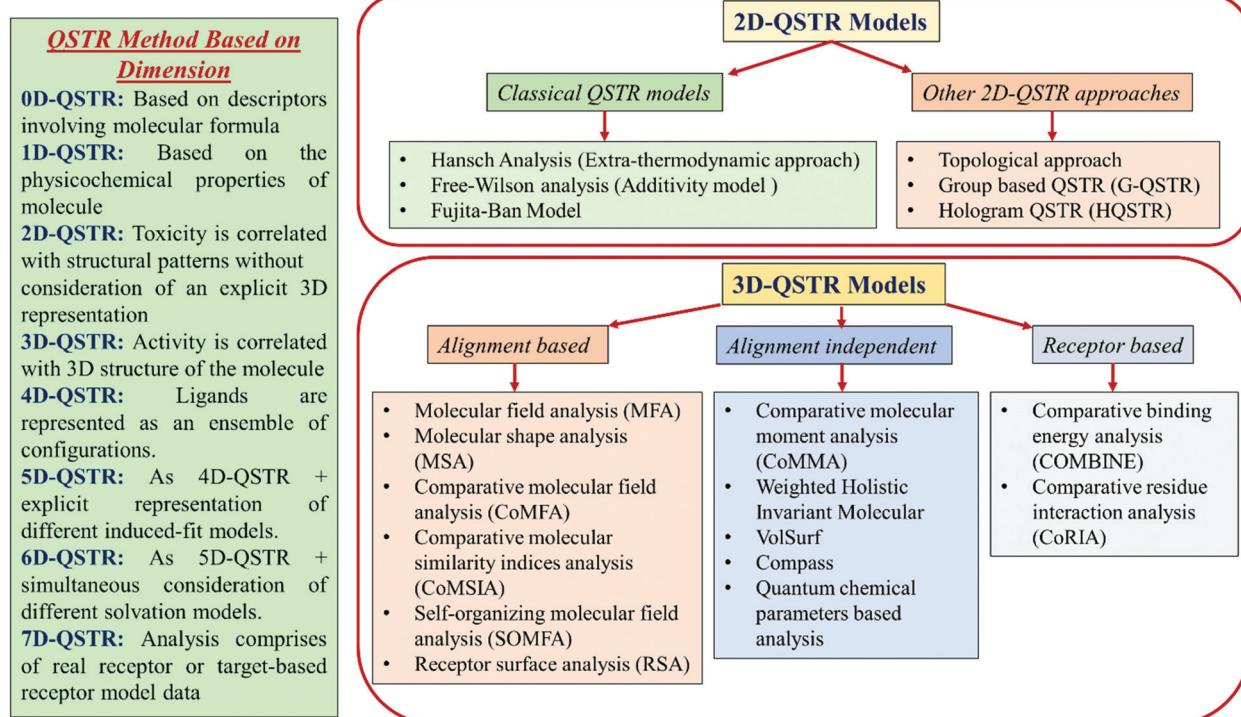


Fig. 13 Classification of QSTR models based on the dimensional geometry.

chemical structures and/or physicochemical experiments. The mathematical expression of the i-QSTR model is:^{59,308}

$$\log_{10} \left(\frac{1}{Y} [\text{predicted species}] \right) = a + a_1 \times \log_{10} \left(\frac{1}{Y} [\text{surrogate species}] \right) + a_2 \times X \quad (4)$$

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 , 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 Fig. 14.

By following four conditions, Zhang *et al.*³⁰⁹ modified eqn (4) by including features signifying the difference in bio-uptake and variance in toxic MoA between two species for an explicit endpoint:

$$\log_{10} \left(\frac{1}{Y} [\text{predicted species}] \right) = a + a_1 \times \log_{10} \left(\frac{1}{Y} [\text{surrogate species}] \right) + F_B + F_M \quad (5)$$

Here, F_B is a physicochemical parameter to invalidate the difference of bio-uptake, and F_M is a physicochemical parameter to correct the variation of MoA of toxicity between two species. The difference between ICE and i-QSTR models is only the 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 MoA of toxicity:* i-QSTR models can help to understand the MoA of the studied chemicals for diverse species and definite endpoints through correlations between two species. As i-QSTR models employ the toxicity of one species as a predictor variable, they are 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 similar MoA of toxicity between two species. In contrast, 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 experimentation is not possible, thus i-QSTR models can be the right choice for toxicity prediction purpose by encouraging the decrease in the use of a 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 help 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

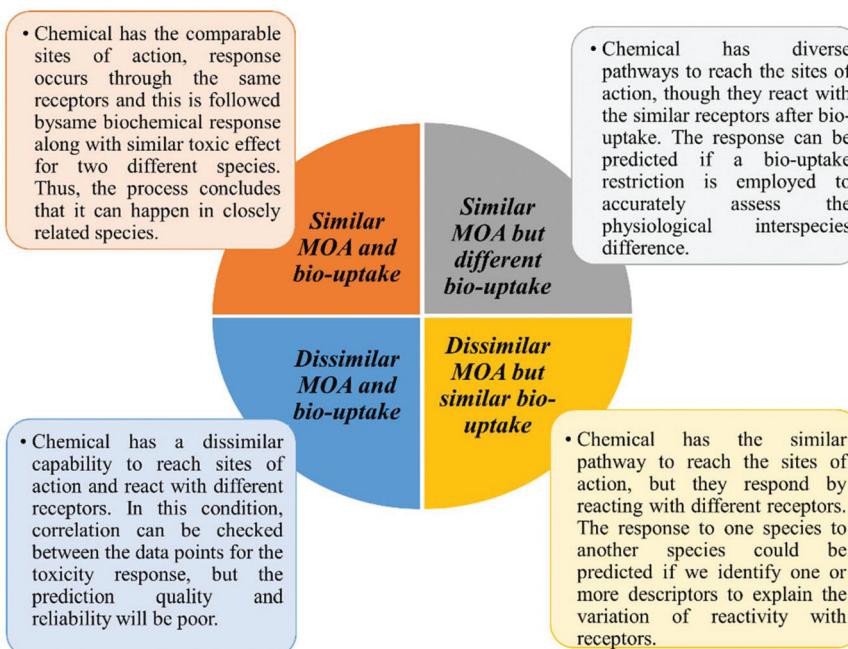


Fig. 14 Four conditions for making ideal i-QSTR models.

experimental data of closely related chemicals.³¹⁰ The idea of interpolation and/or extrapolation for target or query chemicals is reported in Fig. 15 (left). RA can be considered as a vital data-gap filling approach in the 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 profiles 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 Fig. 15 (right).

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

(a) *Analog approach (AN)*: An one-to-one approach is considered which uses one or a few analogs for similarity measurement. 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 the 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: a common functional group, a constant pattern in changing potency, a common precursor or breakdown product, common constituents or chemical class.

The similarity measurement 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 by employing either holographic fingerprints or binary. A holographic fingerprint utilizes the frequency of features (example: the number of specific

functional groups). But a binary fingerprint is a feature vector of binary bits representing the absence (0) or presence (1) of a property (example: a specific functional group present or not). Then, the identified categories are divided by 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 the distance 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 ToxTree³¹⁶ 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 A 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 pharmacophores along with examples are portrayed in Fig. 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

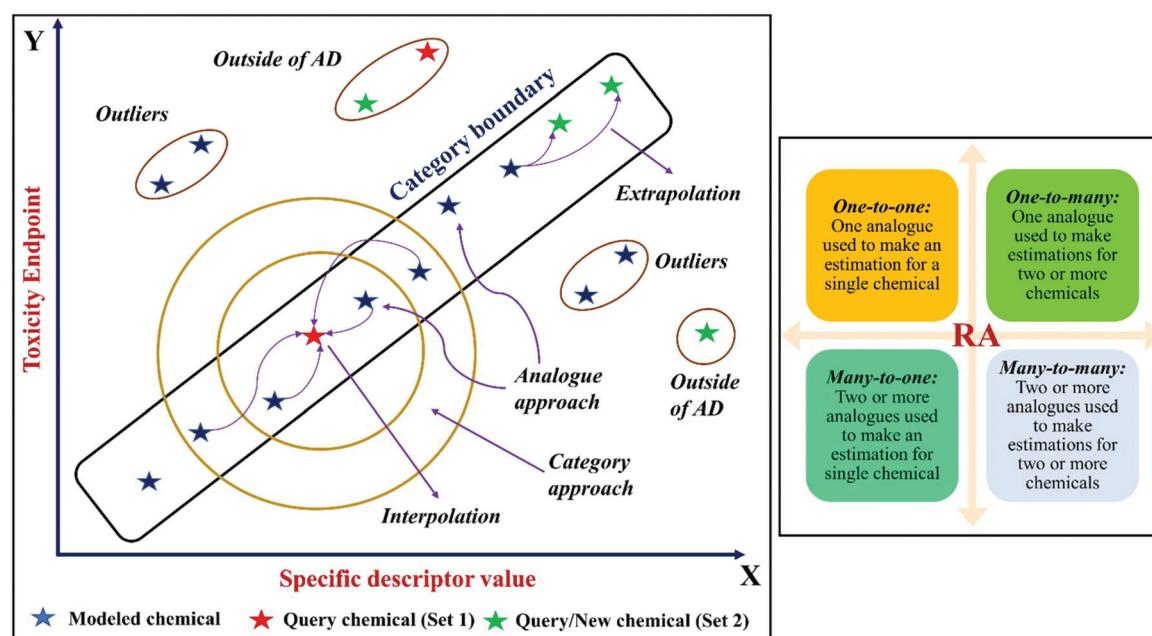


Fig. 15 (Left) Hypothesis and classification of different approaches of RA; (right) ways to perform the RA approach.

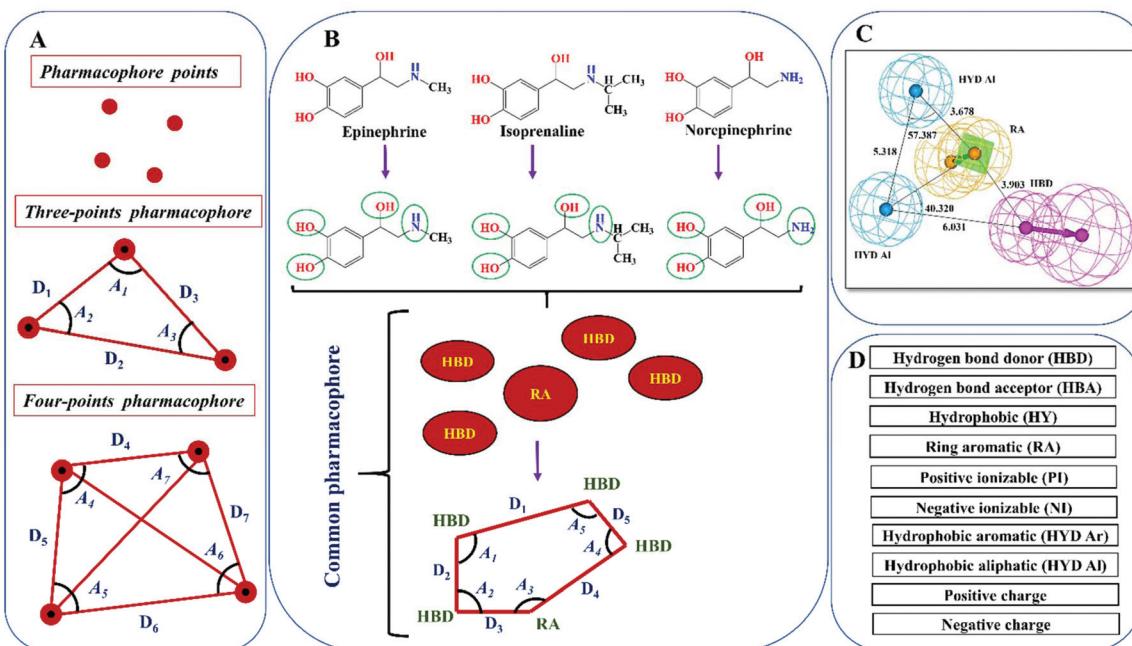


Fig. 16 (A) Points/atoms/features based pharmacophore (toxicophore) hypothesis; (B) representation of the common pharmacophore features identification from three chemicals; (C) example of a common pharmacophore model; (D) common pharmacophore features.

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 the 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 shared features found in the previous step.³²¹ The derived pharmacophore model can be used to search compound databases and for screening purposes. In the 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 are associated with toxicity. Toxicophore models can be built in four steps and they are the 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 improvement of the coverage of conformational space.

(b) *Generation of 3D toxicophore models*: Toxicophores 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.

(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).

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 the 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.

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 = $eE(\chi_{\text{est}} - \bar{\chi})$ Here, χ_{est} is the averaged scaled toxicity of the training set molecules.

(d) *Validation of a 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 the 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 the correct binding poses of ligands in the binding pocket (known as active sites) of a protein and to predict the affinity between the ligand and the protein. The basic principles of docking, protein-ligand docked structure, binding dispositions with amino acid residues and 2D interaction maps solved for a docked ligand are provided as an example in Fig. 17. Molecular docking can be classified as: (i) protein-small molecule docking, (ii) protein-nucleic acid docking, and (iii) protein-protein docking.

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–

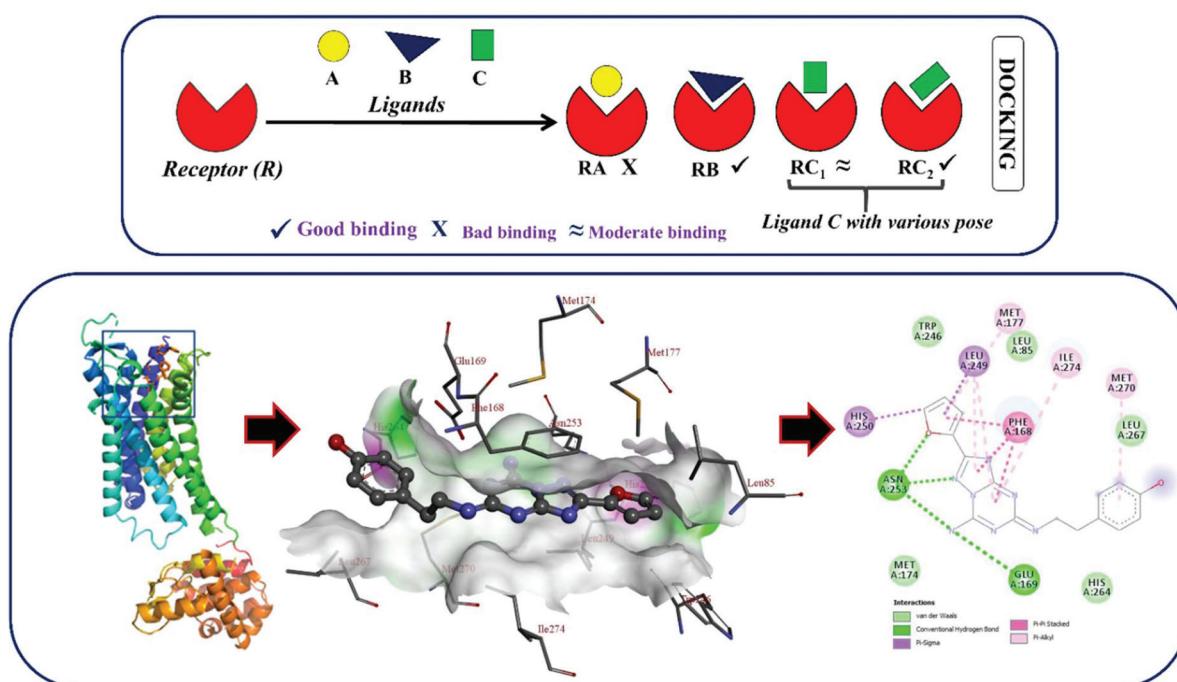


Fig. 17 Fundamental hypothesis of the docking (top); docked complex of ligand–protein, binding dispositions with amino acid residues and 2D interaction maps solved for the docked complex (below).

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 docking depends on the search algorithm like Monte Carlo methods, genetic algorithms, fragment-based methods, Tabu searches, 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 the favorable interaction.³²⁵ Docking is primarily a three-step process regardless of software and docking algorithms.^{326,327} The steps are the 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, and the binding energy along with the RMSD difference with the co-crystallized ligand. The overall steps of the docking formalism are illustrated in Fig. 18.

In the 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 a specific protein which are creating toxic effects by interacting with each other. Protein–ligand docking can be classified based on three vital criteria: (i) ligand sampling, (ii) protein flexibility, and (iii) scoring function, as demonstrated in Fig. 18. As the present review deals with a different topic, to understand the docking technique in depth, please refer to a more extensive study.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 the 'automated rule-indication system' where model statistics has priority while another type of tool 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 is easy to recompute as per the requirement and modification of endpoints and species for a definite molecule. The 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 of dealing with PPCP ecotoxicity are illustrated in Table 4.^{328–345}

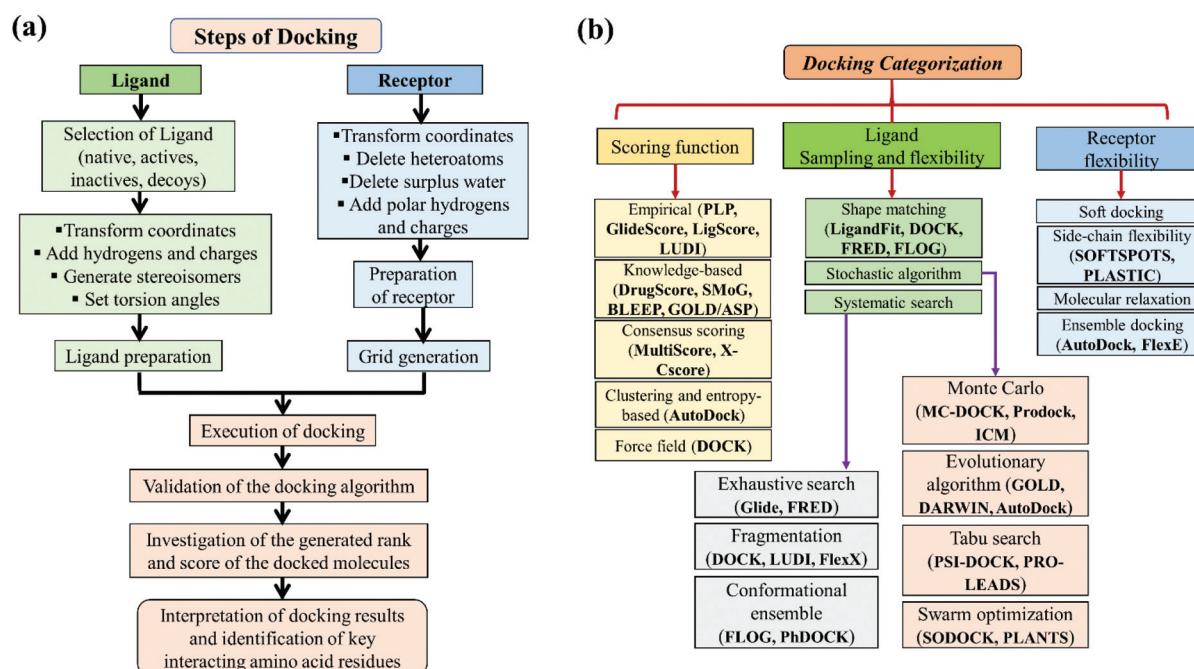


Fig. 18 (a) Fundamental steps of the docking method; (b) classification of docking (software tools are mentioned in bold and under bracket).

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 the bioconcentration factor, skin sensitization, carcinogenicity, mutagenicity, and 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 endpoint alerts with a 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”, Bourgas, Bulgaria	OASIS is commercial software and uses the response-surface approach for modelling acute toxicity for two types of toxicico-chemical domains: reversible acting chemicals and irreversible bioreactive chemicals. Interspecies correlations for acute toxicity to 17 aquatic species, such as fish, snail, tadpole, hydrozoan, 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 the cancer-causing potential by applying the rules of structure–activity relationship (SAR) analysis, mimicking the decision logic of human experts, and incorporating the knowledge of how chemicals cause cancer.
OSIRIS property explorer ³³⁹	Actelion Pharmaceuticals Ltd., Allschwil, Switzerland	OSIRIS is an on-line system, which predicts the reproductive effects on the basis of structural fragments which are developed from the analysis of 3570 compounds with reproductive effects listed in the 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 17 β -dehydrogenase stimulant, ER modulator, oestronesulphatase inhibitor, oestronesulphotransferase stimulant, fertility enhancer, menopausal disorders treatment, mutagenic etc.

Table 4 (Contd.)

Expert system	Company/organization	Description
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 a hybrid expert system, and can encode structure-toxicity and structure metabolism relationships through a number of transformations simulating skin metabolism (mimics metabolism using 2D structural information) and the 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 are 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 of (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.

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 environments. 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, an in-depth understanding about the assessed ecotoxicity and experimental species is 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 conservation programs like the Office of Prevention, Pesticides and Toxic Substances (OPPTS), office of pesticide programs (OPP), Office of Technology Solutions (OTS), OECD, USEPA, and 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 the 'endpoint'. To understand and quantify risks 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 physico-chemical 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 are illustrated in Fig. 19.

12. Ecotoxicity databases in relation to PPCPs

An ecotoxicity database is a comprehensive source of information on the adverse and/or toxic effects of multiple chemicals on 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 sometimes a 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 purposes but also to validate different *in silico* models with respect to definite endpoints and species. To develop acceptable toxicity prediction computational models, good quality experimental ecotoxicity data are important with minimum experimental errors and 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 is reasonably less. The number of such databases should be based on the present demands with detailed 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 presented in Table 6 which can be employed for *in silico* modeling, toxicity screening and validation for extensive risk assessment, management, safety evaluation followed by hazard characterization of PPCPs.^{347–400}

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, the 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)SARs used in, e.g., the OECD QSAR tool box, identify the substructures of the target compound that appear mostly in active molecules and may therefore be responsible for toxicity. They generally initially start by identifying the possible linear relationship between the octanol–water partition coefficient (K_{ow}) and observed toxicity (baseline narcosis), explained by its lipophilicity (narcosis effect). Bradbury⁴⁰⁵ found that approximately 70% of all industrial organic chemicals are estimated to act *via* baseline and polar narcosis modes of action in acute exposure (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 dataset, 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 datasets 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 effects. Sanderson and Thomsen⁴⁰⁷ 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%) was narcotic. This shows that novel models and approaches are needed to demonstrate more accurately the toxicity PCPs may present.⁴⁰⁸ He *et al.*⁴⁰⁹ provided 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 the lack of access to good chronic data to provide sound statistical models as well as appropriate elucidation of the 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 the

Table 5 Representative list of test species for the modeling of PPCP ecotoxicity

Test batteries	Species	Description	Test guidelines
Algae	<i>Chlorella vulgaris</i> , <i>Chlorella pyrenoidosa</i> , <i>Pseudokirchneriella subcapitata</i> , <i>Selenastrum capricornutum</i> , <i>Scenedesmus obliquus</i>	Unicellular fresh-water green micro algae comprising a major part of phytoplankton to study the toxic action of organic compounds. Perfect test organisms for ecotoxicity evaluation through their growth rate inhibition. 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 wastewater of different origins showing good adaptation ability and very versatile microalgae as a test endpoint. Green alga of 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). A Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus <i>Escherichia</i> is used as a model organism in ecotoxicity. A good number of studies are performed to evaluate metal oxide nanoparticle cytotoxicity.	OECD 201: freshwater alga and cyanobacteria, growth inhibition test; OPPTS 850.4500: algal toxicity; OPPTS 850.4550: cyanobacteria toxicity.
Bacterium	<i>Escherichia coli</i>	Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus <i>Escherichia</i> is used as a model organism in ecotoxicity. A good number of studies are performed to evaluate metal oxide nanoparticle cytotoxicity.	OECD 471: bacterial reverse mutation assay; OECD 472: <i>E. coli</i> , EU method B.13/14; mutagenicity, reverse mutation test 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 test; EPA OTS 798.5100: <i>Escherichia coli</i> WP2 and UVRA reverse mutation test; EPA OTS 798.5500: bacterial DNA damage or repair test.
A. fischeri, <i>Vibrio natriegens</i>		Gram-negative rod-shaped bacterium having bioluminescence properties and found principally in symbiosis with different marine species. Majorly employed in the research of microbial bioluminescence, quorum sensing along with ecotoxicity testing.	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: <i>Daphnia magna</i> reproduction test; OPPTS 850.1330 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.
Bacillus		A genus of Gram-positive, rod-shaped bacteria and a member of the phylum Firmicutes. Chlorophenols toxicity is tested on bacillus species. <i>B. fluorescens</i> has a versatile metabolism. Generally found in the soil and water. According to the literature, it is employed for modeling of antibiotic toxicity and resistance studies.	
<i>Pseudomonas fluorescens</i>		Small aquatic crustaceans commonly called water flea. As an invertebrate species in aquatic food webs, <i>D. magna</i> has been used as a representative test species for ecotoxicological evaluation of organic chemicals using the immobilization test.	
Crustaceans	<i>Daphnia ambigua</i> , <i>Daphnia magna</i> , <i>Daphnia melanica</i> , <i>Daphnia pulex</i>	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, wastewater, and biotoxins. Used to assess the effects of prolonged exposure of chemicals to the sediment-dwelling larvae of the freshwater. In the assay, the chironomid emergence and development rate is measured at the end of the test.	
<i>Thamnocephalus platyurus</i>		Gammarids can be cultured in the laboratory or collected from natural sources. If collected, they must be held in the laboratory for at least 1-4 days prior to testing.	
<i>Chironomus</i> sp. (<i>C. riparius</i> , <i>C. dilutus</i> and <i>C. yoshimatsui</i>)		<i>Mysidopsis bahia</i> is the organism specified for aquatic toxicity tests. Juvenile mysids, ≥ 24 h old, are to be used to start the test. One form of aquatic vascular plant floats on the surface of the water. <i>Lemna minor</i> is mostly employed in the modeling of phytotoxicity of ILs and the growth inhibition test of duckweeds where <i>Lemna gibba</i> is used in testing the phytotoxicity of pesticides and other environmental chemicals to higher plants.	OECD 221: <i>Lemna</i> sp. growth inhibition test; OPPTS 850.4400: aquatic plant toxicity test using <i>Lemna</i> sp.; OPPTS 850.4450: aquatic plants field study.
<i>Gamma marus fasciatus</i> , <i>G. pseudolimnaeus</i> , and <i>G. lacustris</i>		Acetylcholinesterase plays the most important role in autonomic nervous system function which catalyses the hydrolysis of acetylcholine esters with a relative specificity for acetylcholine.	OECD 419: delayed neurotoxicity of organophosphorus substances: 28-day repeated dose study.
<i>Mysidopsis bahia</i>		Responses like (a) enzyme inhibition data of the acetylcholinesterase from electric eel (<i>Electrophorus electricus</i>), (b) the AMP deaminase and (c) the antioxidant enzyme system of mouse liver are important for toxicity prediction and modeling.	
Duckweed/ plant	<i>Lemna minor</i> <i>Lemna gibba</i>		
Enzyme	Acetylcholinesterase		

Table 5 (Contd.)

Test batteries	Species	Description	Test guidelines
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 studies. 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.	OECD 210; OPPTS 850.1400; fish early-life stage toxicity test; EPA OPP 722-3: 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 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.
Fathead minnow (<i>Pimephales promelas</i>)			
Rainbow trout (<i>Oncorhynchus mykiss</i>)		Rainbow trout is a streamlined, salmonid form fish. <i>Oncorhynchus mykiss</i> is one of the important endpoints for studying aquatic toxicity as well as an alternative model for studying the inhibition of aromatase (CYP 19).	
Zebrafish (<i>Danio rerio</i>)		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 and industrial effluents.	
Mammalian cells	Human keratinocyte cell line (HaCat) CaCo-2	The naturally immortalized human keratinocyte line is utilized for studies of skin biology and cytotoxicity assessment of metal oxides. Heterogeneous human epithelial colorectal adenocarcinoma cells. Permeability coefficients across the cellular membranes of Caco-2 cells are generally employed for modeling. A prototypical cell of the human epithelium derived from cervical cancer cells and mostly employed for anticancer activity.	OPPTS 870.5300: <i>in vitro</i> mammalian cell gene mutation test; OPPTS 870.5550 unscheduled DNA synthesis in mammalian cells in culture; OECD 473: <i>in vitro</i> mammalian chromosomal aberration test; OECD 476: <i>in vitro</i> mammalian cell gene mutation test using the Hprt and xprt genes; OECD guideline 479: genetic toxicology, <i>in vitro</i> sister chromatid exchange assay in mammalian Cells; OECD 487: <i>in vitro</i> mammalian cell micronucleus test; OECD 490: <i>in vitro</i> mammalian cell gene mutation tests using the thymidine kinase gene.
HeLa		Human prostate cancer cell line (PC3)	
Prostate		Human malignant melanoma (Female X) HT-29	Derived from a lymph node metastasis of a melanoma patient and used for modeling of anticancer drugs.
		A human colorectal adenocarcinoma cell line with epithelial morphology and mostly sensitive to the chemotherapeutic drugs used in colorectal cancer modeling.	
		Promyelotic leukemia rat cell line IPC-81 is employed in cytotoxicity assays of ILs.	
Rat cell line – IPC-81		Free-living unicellular ciliated protozoa and one of the most popular endpoints for environmental toxicity assessment.	OECD 244: protozoan activated sludge inhibition test.
		One of the largest frog species in North America, this can grow to 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.	OPPTS 850.1800: tadpole/sediment subchronic toxicity test; OECD 231: amphibian metamorphosis assay.
Protozoa	<i>Tetrahymena thermophila</i> <i>Tetrahymena pyriformis</i>	A common and sensitive species; the larva of the frogs, are typical amphibious bridging the gap between aquatic and terrestrial animals.	OECD 480: gene mutation assay; OECD 481: mitotic recombination assay; EU method B.16: mitotic recombination test; EPA OPPTS
Tadpoles	American bullfrog (<i>Lithobates catesbeianus</i>) or <i>Rana catesbeiana</i>)	Recurrently used for toxicity testing purposes and risk assessments and have been recommended by the EU-TGD.	
		One form of budding yeast 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.	
Yeast	<i>Bufo vulgaris formosus</i> , <i>Rana japonica</i> <i>Saccharomyces cerevisiae</i>		

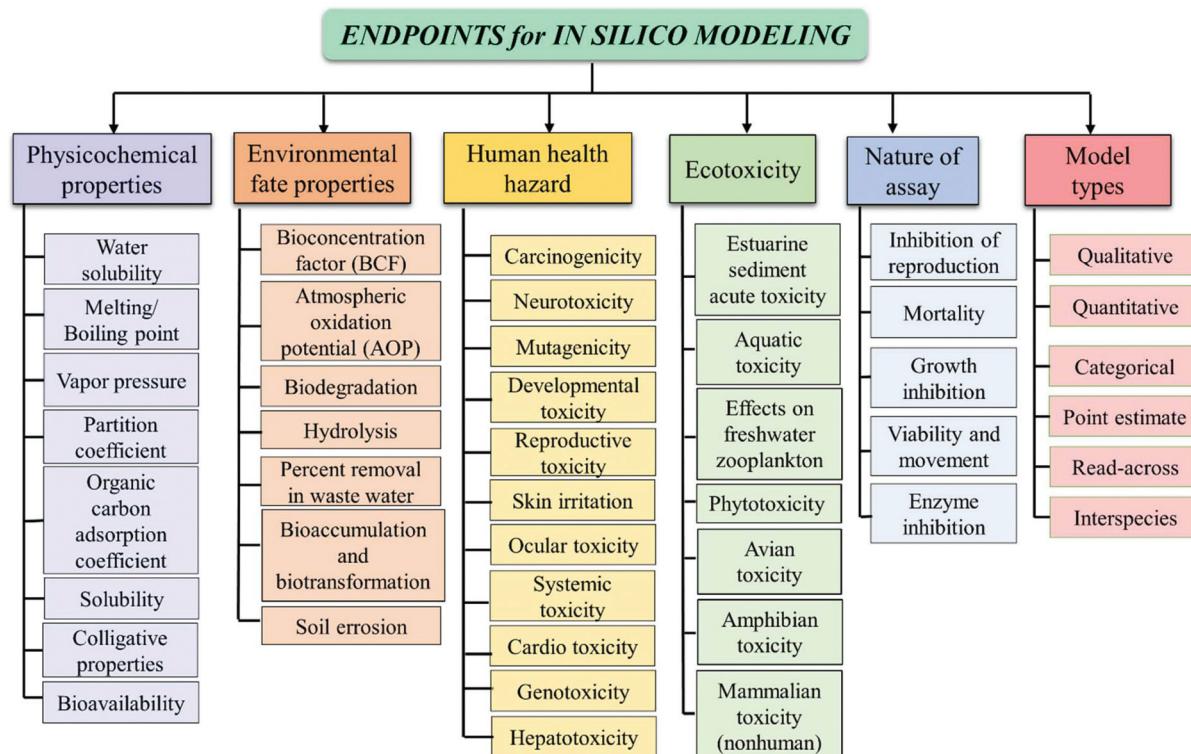


Fig. 19 Most frequently modeled endpoints for ecotoxicity according to the OECD guidelines.

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 the top 20 therapeutic classes based on $\log K_{ow}$ and the overall hazard ranking of the top 15 therapeutic classes to algae, daphnia and fish are reported in Fig. 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. 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 repellents, nutritional agents and vitamins as per the study. The obtained hazardous effect trend of all PPCPs is quite similar with the relative ranking of $\log K_{ow}$.

Kar and Roy⁵⁹ developed one of the first interspecies QSAR models to correlate the ecotoxicity of structurally diverse 77 pharmaceuticals with *Daphnia magna* and fish. The $\text{Y}\zeta$ (aasC) fragment and keto group are predominantly accountable for higher toxicity of pharmaceuticals to *D. magna* (Fig. 21). Again, along with the keto group, structural fragments like $\text{X}=\text{C}=\text{X}$, $\text{R}-\text{C}(=\text{X})-\text{X}$, and $\text{R}-\text{C}\equiv\text{X}$ are significant features for the high

toxicity values to fish (Fig. 22). The interspecies QSAR models were further implemented to predict fish toxicity of 59 pharmaceuticals (where experimental daphnia toxicity is present) and daphnia toxicity of 30 pharmaceuticals (where experimental fish toxicity is present). The models demonstrated an enhanced and comprehensive risk assessment of pharmaceuticals where toxicity data are missing for a specific species.

Sangion and Gramatica⁶⁰ developed quantitative activity-activity relationship (QAAR) models with a good correlation between the toxicity of PPCPs towards the invertebrate *Daphnia magna* and towards two fish species namely *Pimephales promelas* and *Oncorhynchus mykiss* with a single theoretical molecular descriptor which helped the exploration of 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 the 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 for more complex experimental tests on upper trophic organisms, also saving animal lives. The most significant conclusions provided by the authors are the following: (a) daphnia toxicity could serve as a surrogate for fish toxicity and (b) the fish-fish intercorrelations could be applied for asses-

Table 6 A comprehensive list of publicly available databases comprising 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 <i>in vitro</i> and <i>in vivo</i> toxicology data for over 500 000 environmental chemicals.
BDSM ³⁴⁸	Birth defects systems manager (BDSM) database dealing with developmental toxicity and developed by the University of Louisville.
CCCRIS ³⁴⁹	Chemical carcinogenesis research information system (CCCRIS) created by the National Cancer Institute (NCI). It contains carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition test results for over 8000 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 physicochemical 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 1660 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 humans. It includes 6540 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	A database containing more than 3100 studies on subacute to chronic toxicity within a variety of routes of administration for about 930 chemicals.
RepDose ³⁶³	
GAC ³⁶⁴	Genetic Alterations in Cancer (GAC) is a database that quantifies specific mutations found in cancers induced by environmental chemicals developed by the 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 3000 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 the World Health Organization (WHO) which identifies environmental factors (including 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 the 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 Istituto Superiore di Sanità, Italy.
ITER ³⁷²	International Toxicity Estimates for Risk (ITER) is developed by TERA (Toxicity Excellence for Risk Assessment), and 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 the 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 the Japanese National Institute for Environmental Studies (NIES) which uses structural domain named <i>C</i> -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.

Table 6 (Contd.)

Database	Description
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 <i>in vitro</i> and <i>in vivo</i> 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 the 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 2000 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 <i>in vivo</i> 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 US 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 the US 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 US 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 that 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 (http://www.mistrapharma.se). It contains 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.

sing toxicity data when experimental information is unavailable. The major obtained mechanistic interpretation of toxicity towards each studied species is illustrated in Fig. 23.

Acute toxicity (48 h concentration causing 50% mortality) of 55 PPCPs towards the freshwater planarian *Dugesia japonica* was modeled with the QSTR tool by Önlü and Saçan.⁴¹⁰ Like majority toxicity modeling studies, the authors found hydrophobicity was 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 (Fig. 24). $\log K_{ow}$ or hydrophobicity seemed to be the most imperative feature for the toxicity because of simple perturbation of the membrane function. The second important identified feature is GATS7p, which signifies higher atomic polarizability in a chemical resulting in toxicity. SpMaxA_G/D defines the folding degree of a molecule whose value leads to 1 for linear chemicals and decreases along with the branching, suggesting the changes in molecular size and shape which has a positive contribution towards toxicity. The reason behind this is quite clear as chemicals with a higher number of flexible fragments can fit better within the interacting proteins and show stronger binding affinity with the toxicity causing amino acid residues. The descriptor Mor31s signifies the molecular depiction of structures based on electron diffraction calculated upon the scattering para-

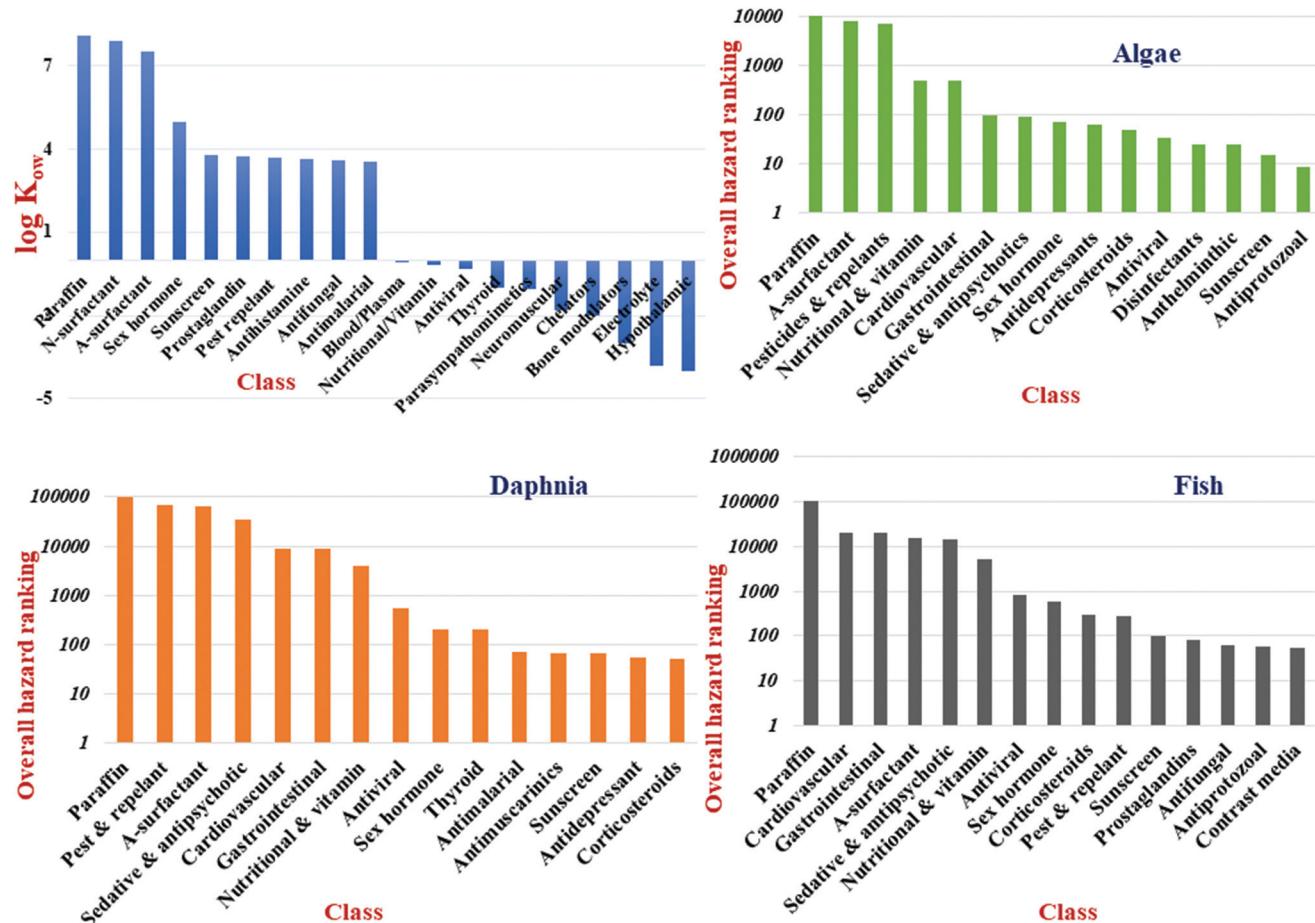


Fig. 20 Top left: The relative ranked $\log K_{ow}$ for top 20 classes of PPCPs; the overall hazard ranking for top 15 classes of PPCPs to algae (top right), daphnia (bottom left), and fish (bottom right).

meter and weighted by the intrinsic state (I-state). The I-state of an atom can be interpreted as 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 the 8-bond topological distance, and it increases the toxicity. To apply a QSTR equation for data gap filling of untested compounds' toxicity for *D. japonica*, the authors employed 792 industrial chemicals including 317 designated HPV chemicals according to OECD. The AD study suggested the reliable prediction of 85% of the total number of chemicals. The authors also developed 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.

Khan *et al.*⁴¹¹ developed ecotoxicological QSTR models for 260 pharmaceuticals spanning over diverse therapeutic classes on three trophic level species like the green algae *Scenedesmus*

subspicatus (134), the crustacean *Daphnia magna* (209) and the 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 the 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 confirmed the hydrophobic property to make the maximum contribution towards the toxicity of the considered pharmaceuticals irrespective of species followed by the contributions of structural fragments like tertiary amines, carboxamide, carboxylate, allyl functionalities *etc.* which are also responsible for the toxicity of pharmaceuticals towards diverse aquatic species. A complete summary and findings of the study are

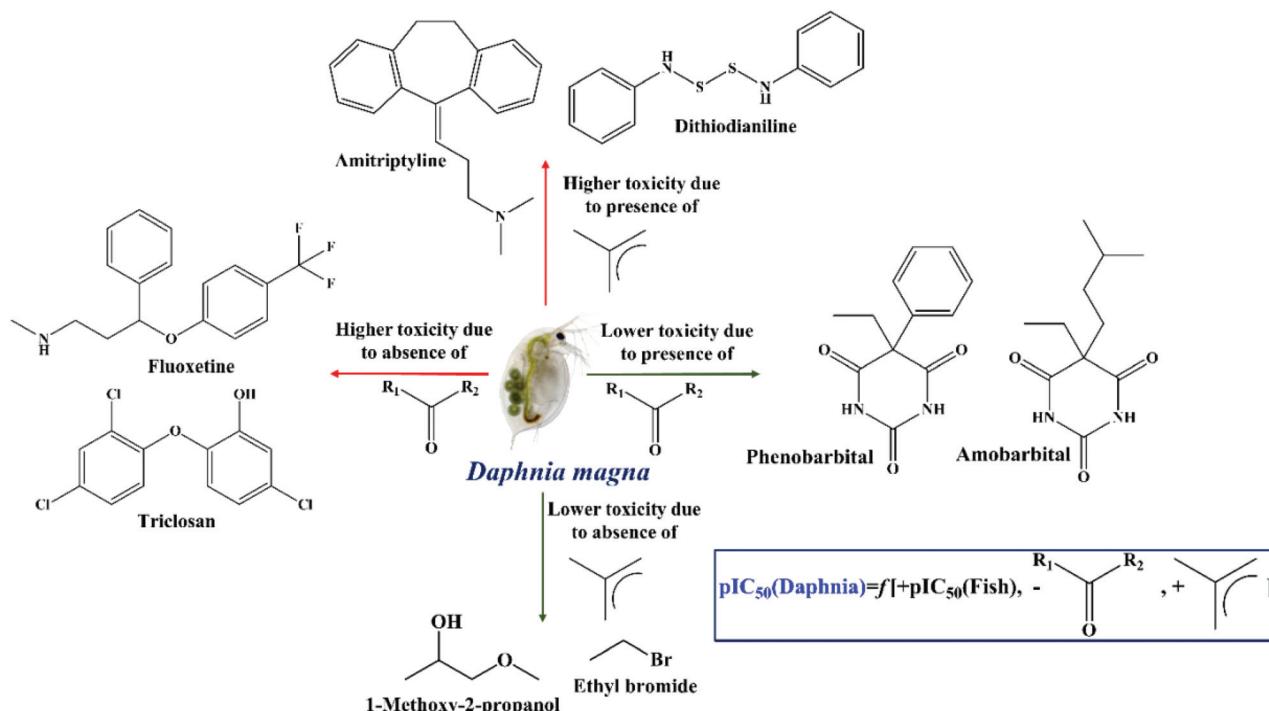


Fig. 21 Mechanistic interpretation of pharmaceutical toxicity to *D. magna*.

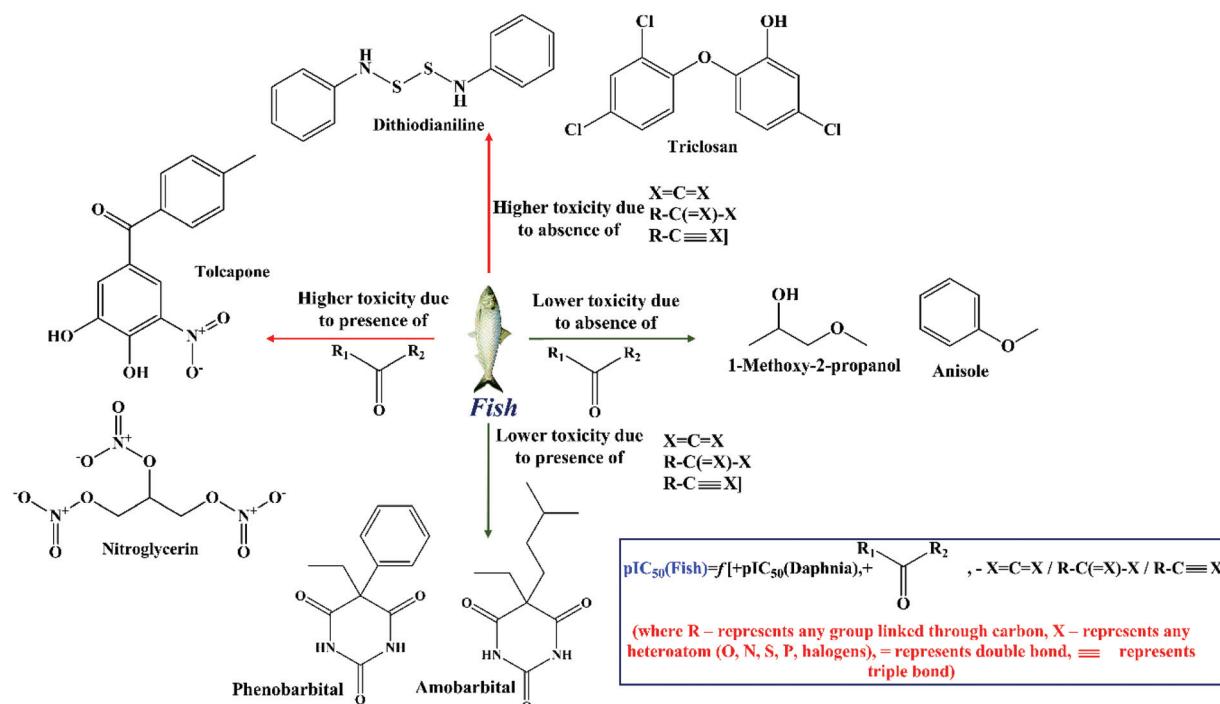
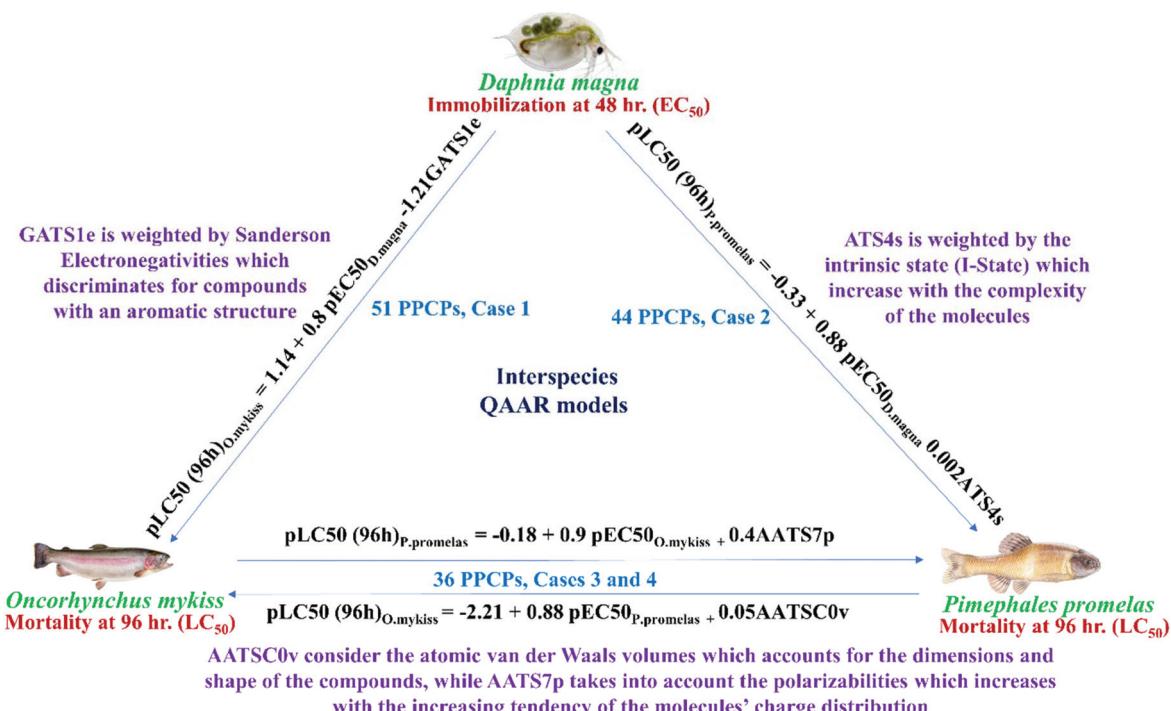
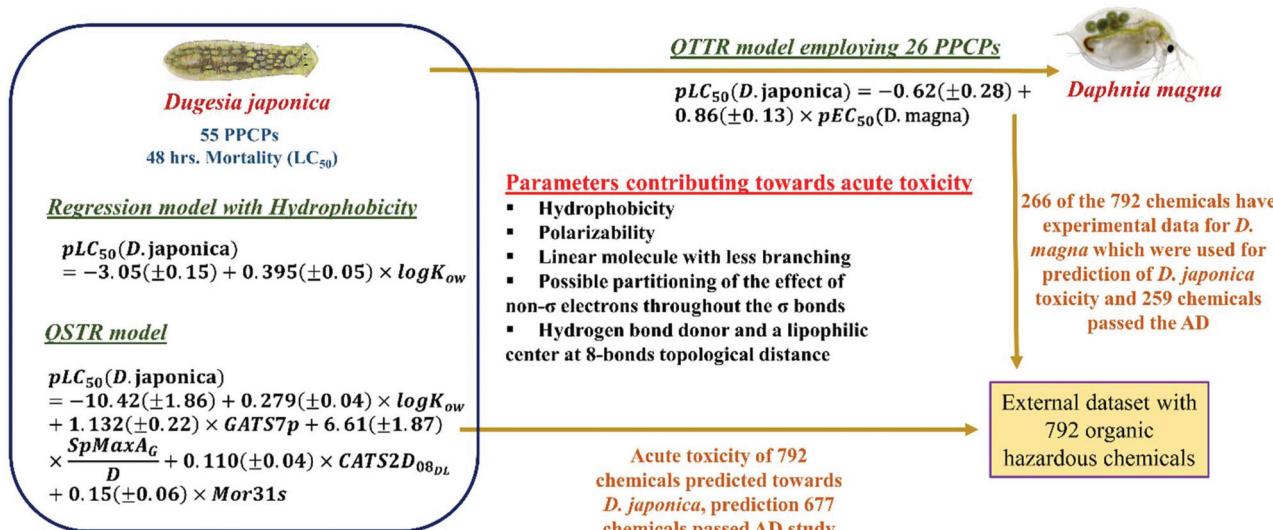


Fig. 22 Mechanistic interpretation of pharmaceutical toxicity to fish.

demonstrated in Fig. 25. Khan *et al.*⁴¹¹ suggested that if a 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. Furthermore, individual QSTR models were utilized for prioritizing 7106 pharmaceuticals without having experimental data using predicted response values, and ranking of pharmaceuticals was reported to

Fig. 23 Mechanistic interpretation of toxicity towards *D. magna*, *O. mykiss* and *P. promelas*.Fig. 24 Mechanistic interpretation of QSTR and QTTR models for *D. japonica* and *D. magna*.

provide the toxicity threshold for each species. The authors projected top 30, 13 and 25 toxic pharmaceuticals based on their study from the modeled dataset, drug-like molecule dataset, and Interbioscreen dataset, respectively.

Önlü and Saçan⁶² 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 acetoxyethyl 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 measures 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 acids present in the molecule of interest. The negative contri-

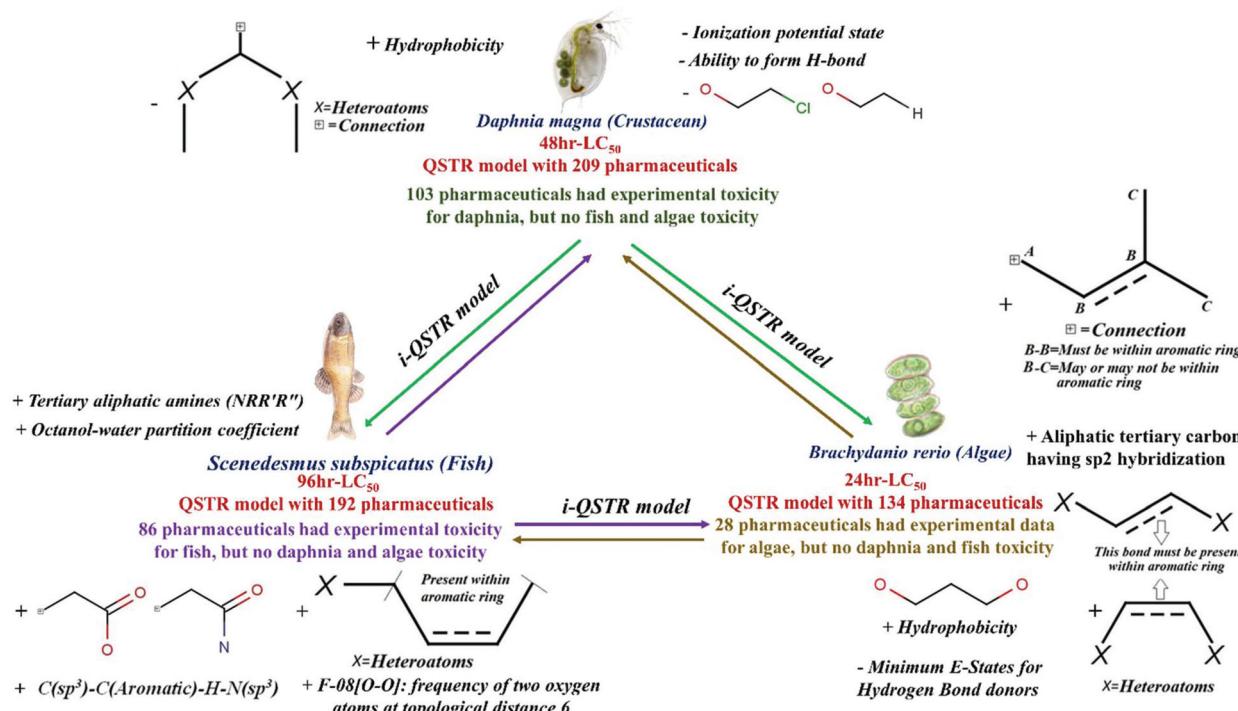


Fig. 25 Mechanistic interpretation from the obtained QSTR and i-QSTR models for *D. magna*, *S. subspicatus* and *B. rerio* (+ and – before the features showed the contribution of the features).

bution of nRCOOH signifies that cytotoxicity decreases along with the increase in the 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 the reactivity/stability of a specific fragment of compounds and is 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. 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 the RTL-W1 cell line with 91% structural coverage. Based on the external set prediction (pEC₅₀, AB values), they concluded that antibacterial chemicals are relatively the least cytotoxic, whereas antipsychotic pharmaceuticals are relatively the most cytotoxic. Furthermore, the authors explored a good correlation between *in vivo* fish acute toxicity (pLC₅₀ for 96 h) and *in vitro* cytotoxicity (pEC₅₀, AB).

Khan *et al.*⁴¹² developed QSAR models for the ecotoxicity of pharmaceuticals collected from the ECOTOX database⁴¹³ along with other studies on four aquatic species *Pseudokirchneriella*

subcapitata, *Daphnia magna*, *Oncorhynchus mykiss* and *Pimephales promelas* employing the G/PLS statistical tool and 2D descriptors. The authors employed hydrophobicity parameters for modeling purpose due to the known dependence of toxicity on log P terms. The major toxicity contributing features were the size and bulk of molecules, polarity, and 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 a macrocyclic ring tend to show more toxicity towards aquatic species. The features contributing positively and negatively for each species are described in detail in Fig. 26. Furthermore, the developed consensus models were employed to predict the 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 software⁴¹³ was used for parallel prediction for comparison and for checking 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 was reported.⁴¹²

Sangion and Gramatica⁴¹⁴ developed MLR-OLS based QSTR models employing 1267 human and veterinary pharmaceuticals collected from the ECOTOX database³⁶⁰ to predict acute toxicity in four species (*D. magna*, *P. subcapitata*, *P. promelas*, *O. mykiss*) spanning from three main aquatic trophic levels. In

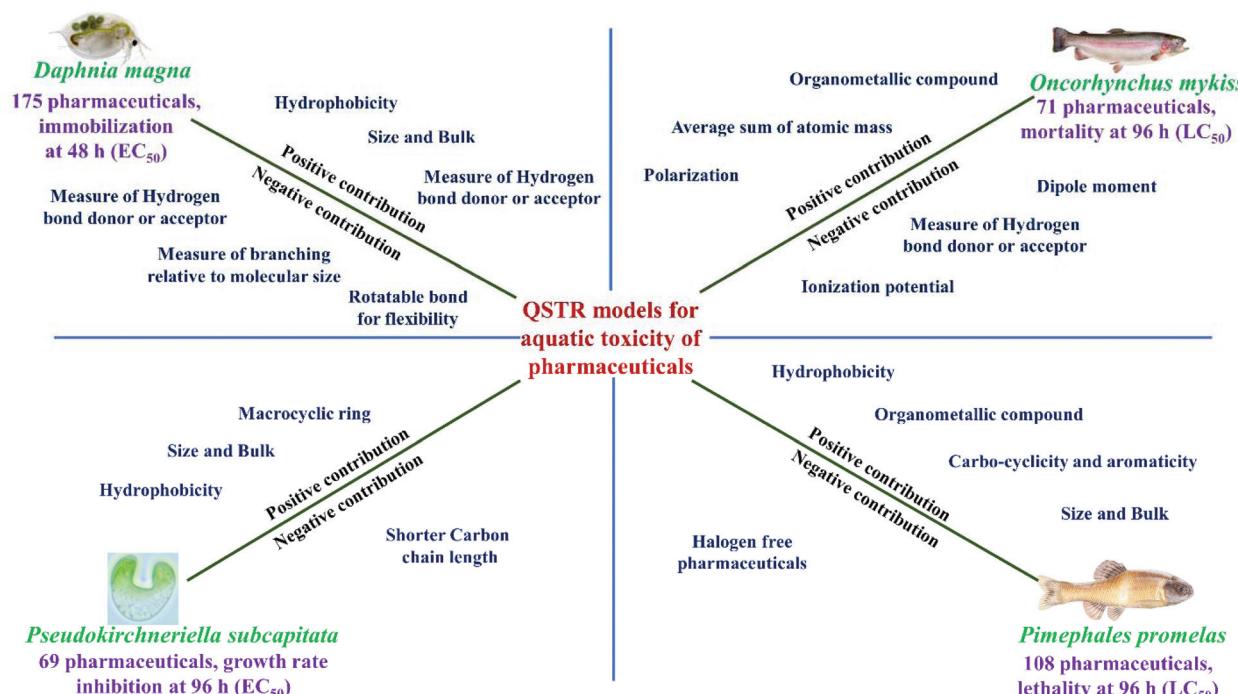


Fig. 26 Critical features responsible for toxicity towards *D. magna*, *O. mykiss*, *P. subcapitata* and *P. promelas*.

the case of *P. subcapitata*, the 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 contributed to the decrease of 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 studies. 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 the chemical structure has a positive impact on toxicity. In the 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. In contrast, the number of hydrogen bond acceptor atoms has a negative impact on the toxicity (Fig. 27). Thereafter, the constructed models were applied to predict the acute toxicity of a huge number of APIs without having experimental data employing the PCA approach. Furthermore, individual APIs were ranked based on toxicity and the “aquatic toxicity index (ATI)” was generated which will be highly helpful for toxicity data gap filling followed by ERA.

Hossain and Roy⁴¹⁵ 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 Fig. 28 as identified from all five cumulative PLS models. Out of 75 CECs in the *D. japonica* dataset, 47 had their reported toxicity values against *D. magna* and 19 for fish (*P. promelas*), and that is why the authors 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, and diethyl-stilbestrol 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 to the toxicity as it has a H-bond donating capability which makes the molecules hydrophilic and offers resistance to penetrate through a biological membrane. Antibiotics like chlortetracycline, tetracycline, ofloxacin, and 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, thus sig-

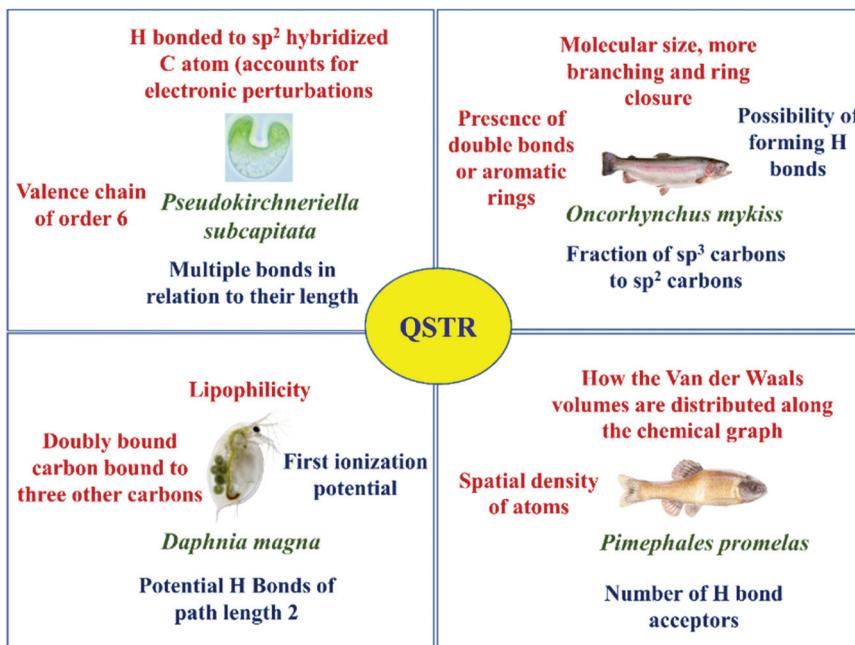


Fig. 27 Mechanistic interpretation from the obtained QSTR models for all four species. (Features colored in red text and blue text denote positive and negative contributions to toxicity, respectively.)

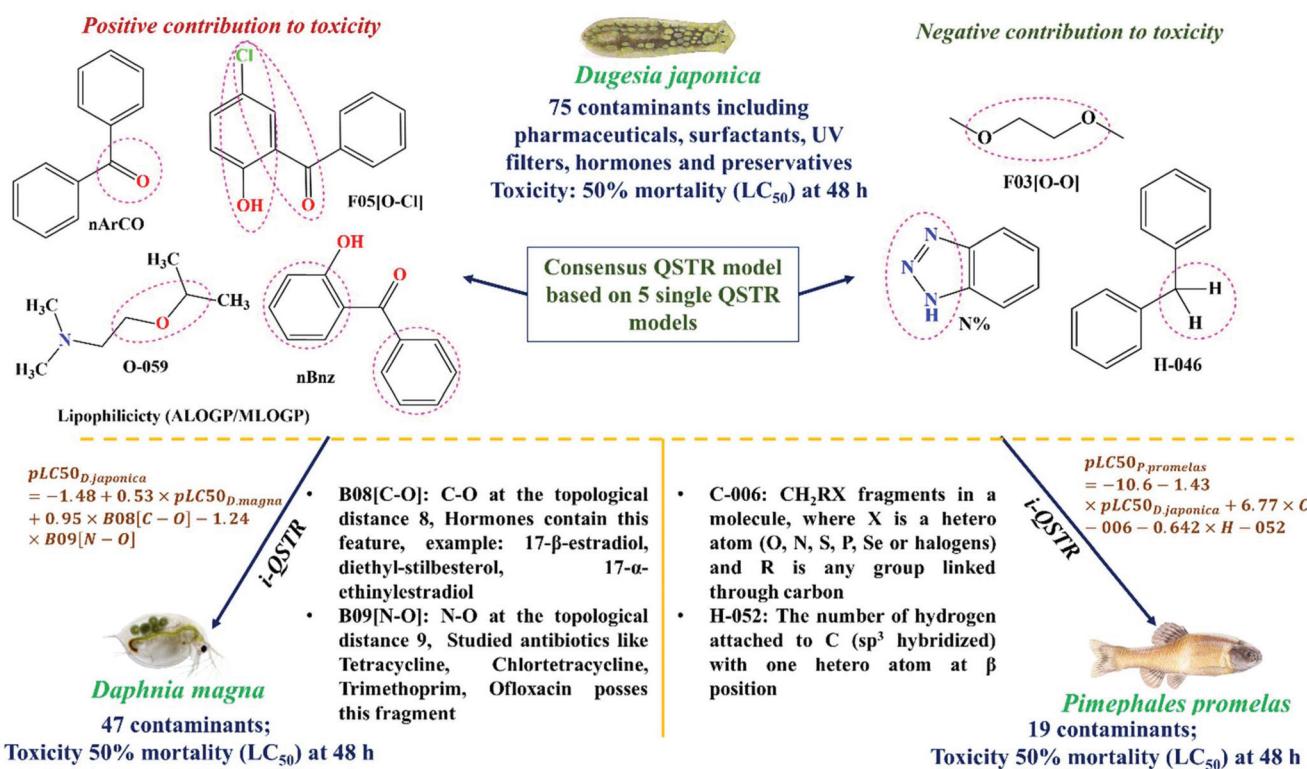


Fig. 28 Mechanistic interpretation of toxicity to *D. japonica*, *D. magna* and *P. promelas* from developed QSTR and i-QSTR models.

nifying that the molecule with this specific fragment will be more toxic. Compounds like propranolol, sodium dodecyl sulfate, chlorpyrifos, glyphosate etc. comprising 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 the acute toxicity of the ECOTOX database consisting of 99 CECs (daphnia toxicity present) and 51 CECs (with fish toxicity present) for the toxicity against *D. magna*. The interspecies models for fish and daphnia showed 80% and 91% prediction coverage, respectively.

Khan and Roy⁴¹⁶ reported ecotoxicological QSTR models 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 and the presence of sulphur atoms, the toxicity of PCPs increases. The individual model-specific features can be checked in Fig. 29 with a complete workflow employed by the authors. The authors then compared their predicted results with the ECOSAR software outcome which is generally employed for the 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. Furthermore, for ranking of an entire external set of 596 compounds, all three

obtained QSTR models as well as ECOSAR software were applied by the authors to predict the toxicity values of the entire dataset against the 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 Roy⁴¹⁶ 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.

13.1.2 Models for miscellaneous toxicity due to PPCPs.

The environmental behavior of PCPs needs to be examined; the data on persistence, bioaccumulation, and toxicity (PBT) are very rare for the majority of PCPs. Cassani and Gramatica⁶³ 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 the identification of the most hazardous PCPs, which are predicted as potential PBTs by both methods, in a consensus approach. The PBT index prediction allows the classification of PCPs into non-PBT, "medium" PBT and PBT chemicals considering a preset arbitrary threshold. The authors considered the threshold at a 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 prior-

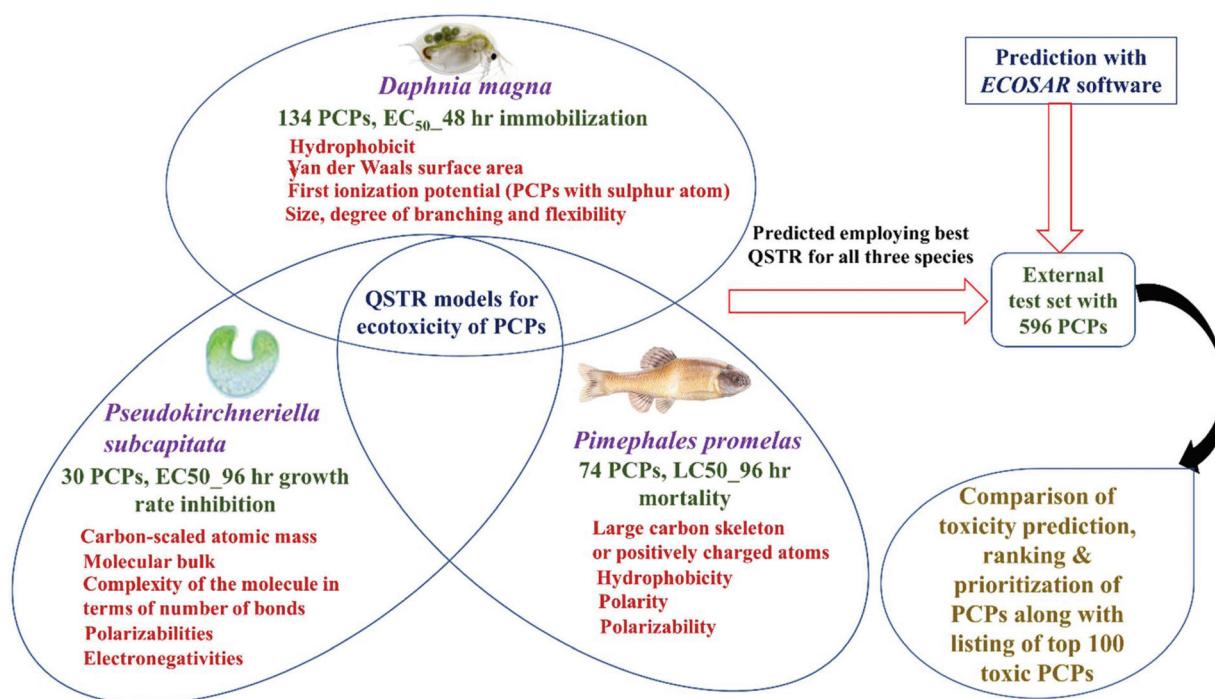


Fig. 29 Mechanistic interpretation of toxicity of PCPs towards *D. magna*, *P. subcapitata* and *P. promelas*.

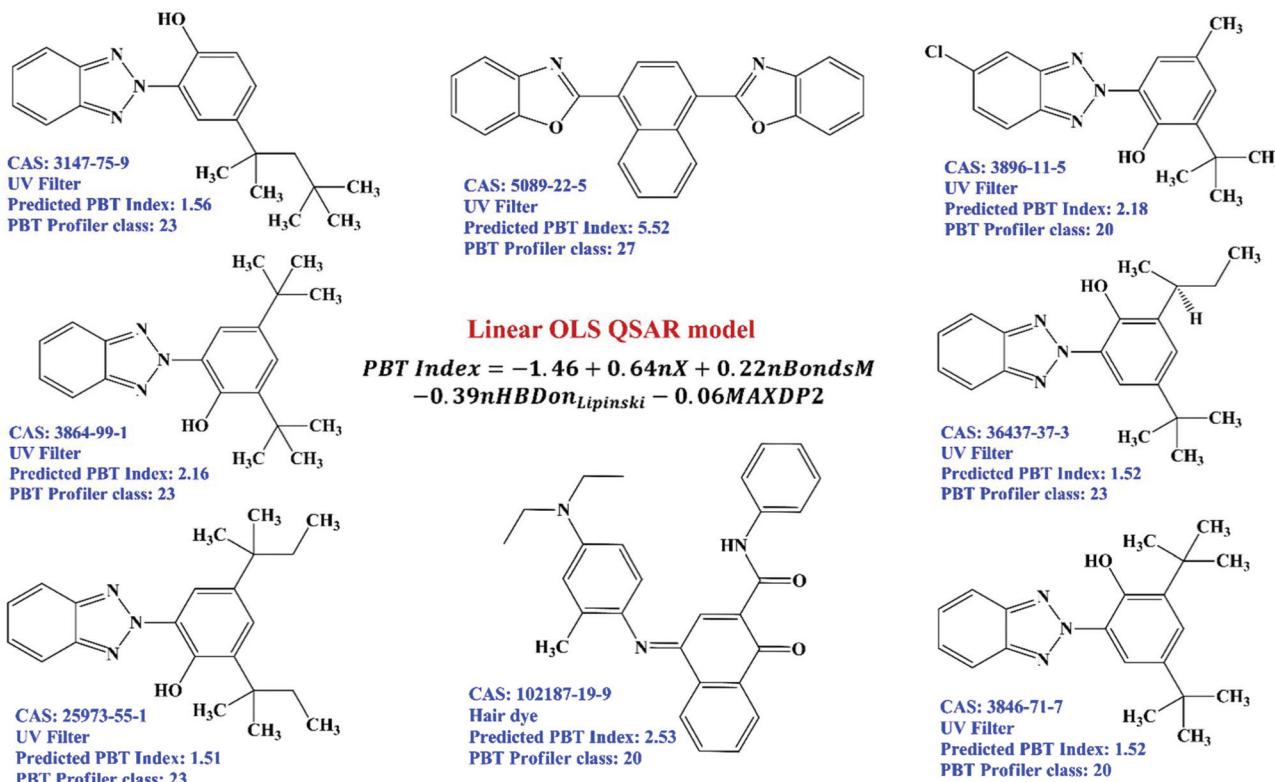


Fig. 30 Eight potential PCPs as per the reported PBT priority list by the consensus approach along with the developed QSTR model.

itized 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 (Fig. 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 the 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.

The endocrine disrupting activity of perfluoroalkyl substances (PFASs) is modeled by 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 concludes that the

carbon chain length has a major role to play in determining the toxicity potency. The following significant observations (Fig. 31) are reported by the authors:

- The studied PFASs containing acid functional groups are highly toxic with the carbon chain length between 6 and 10 (example: 7*H*-perfluoroheptanoic acid, perfluorohexanoic acid). In contrast, lower toxicity is observed when PFASs consist of a 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-perfluoroctyl ethanol).

- PFASs consisting of sulfonamide functional groups are toxic when the C chain length is equal to 8 (perfluorooctane sulfonamide). On the other hand, substitution of sulfonamide groups with an alkyl or alcohol group leads to lower toxicity or non-toxicity regardless of their carbon chain length (*N*-methyl perfluorooctane sulfonamide, *N*-ethyl perfluorooctane sulfonamide).

Kar *et al.*⁴¹⁷ developed statistically robust QSTR models employing single and mixed halogenated molecules using a weighted descriptors approach for developmental toxicity on

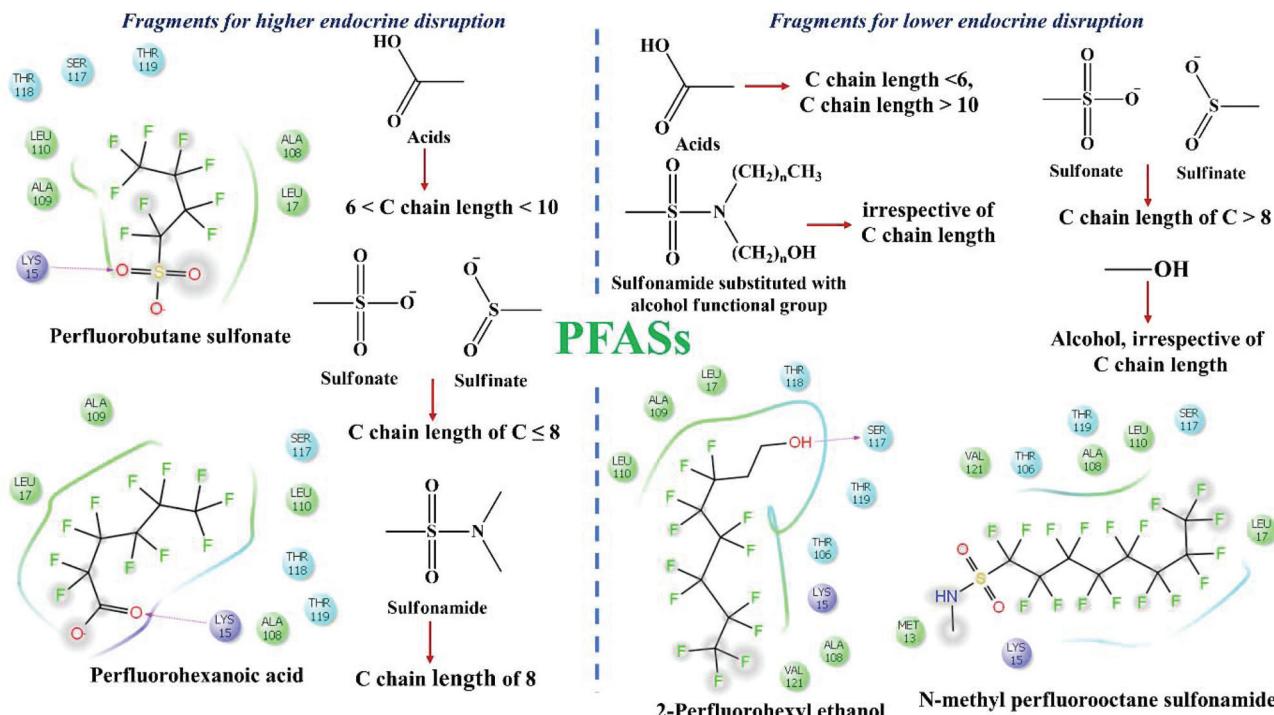


Fig. 31 Critical features related to endocrine disruption of PFASs.

zebrafish (*Danio rerio*) embryos. The developed model was 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 Fig. 32 taking the results for three studied chemicals as examples.

Jean *et al.*⁴¹⁸ generated a statistically significant and predictive QSAR model for 67 environmental chemicals including a good number of PPCPs [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. In contrast, molecules with a lipophilic or hydrophobic

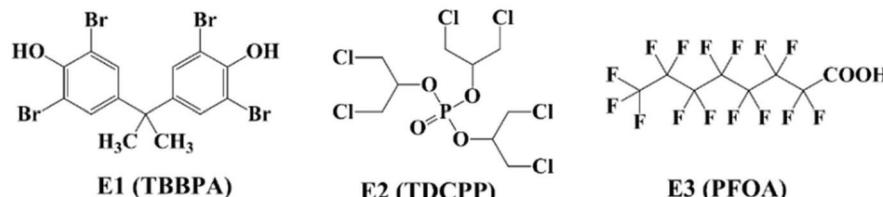
feature showed higher adipose/blood partition coefficients (Fig. 33). Followed by the AD check, the best QSAR model was employed by the authors to predict the adipose/blood partition coefficient of 513 PCBs, PCDDs, PBDEs, and PAHs from the US EPA website for environmental risk assessment analysis and data gap filling which would be helpful for pharmacokinetic as well as toxicokinetic profiling of these chemicals in the near future. Analyzing the results, it can be confirmed that the presence of a higher number of halogens (~6 to 10) in the studied chemicals resulted in a high octanol–water partition coefficient followed by a high value of adipose/blood partition coefficient.

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 the following common conclusion for all species: chemicals consisting of sulphur, phosphorus and halogens highly impact the toxicity along with hydrophobicity as suggested by $\log P$ terms like $X \log P$ and ALOGP2. In contrast, 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 Fig. 34.

13.2 Imperative features responsible for ecotoxicity and fate

Comprehensive introspection of the discussed models and other studies^{58–64,410–430} help us to understand the major physicochemical properties and structural fragments related to

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*



Single/Mixture	Toxicity as pIC ₅₀ 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

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

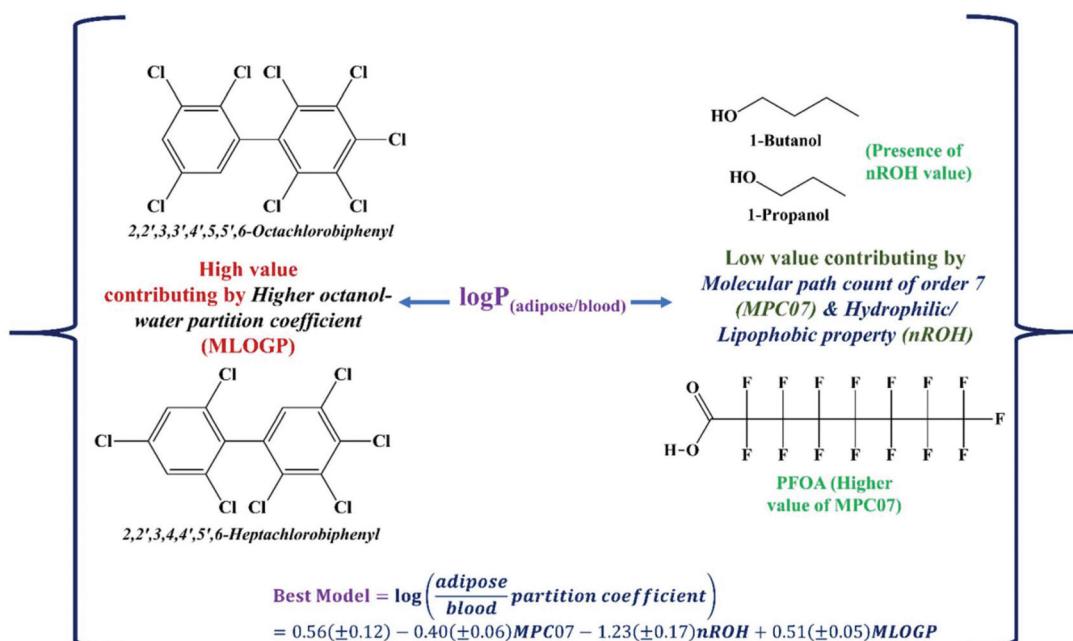


Fig. 33 Features responsible for the high and low value of $\log P(\text{adipose}/\text{blood})$ for the studied alcohols, PCBs, PBDEs, PCDDs, and PAHs.

the intrinsic chemical reactivity followed by chemical's environmental fate, transformation and toxicity as demonstrated in Table 7.^{58–64,410–430} 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 in Table 7. Identifying these important features will help chemists to design PPCPs with reduced ecotoxicity, better biodegradability followed by reduced bioconcentration and bioaccumulation.

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 and regulatory authorities provide chemical toxicity data for a definite species and environmental compartment. In contrast, the

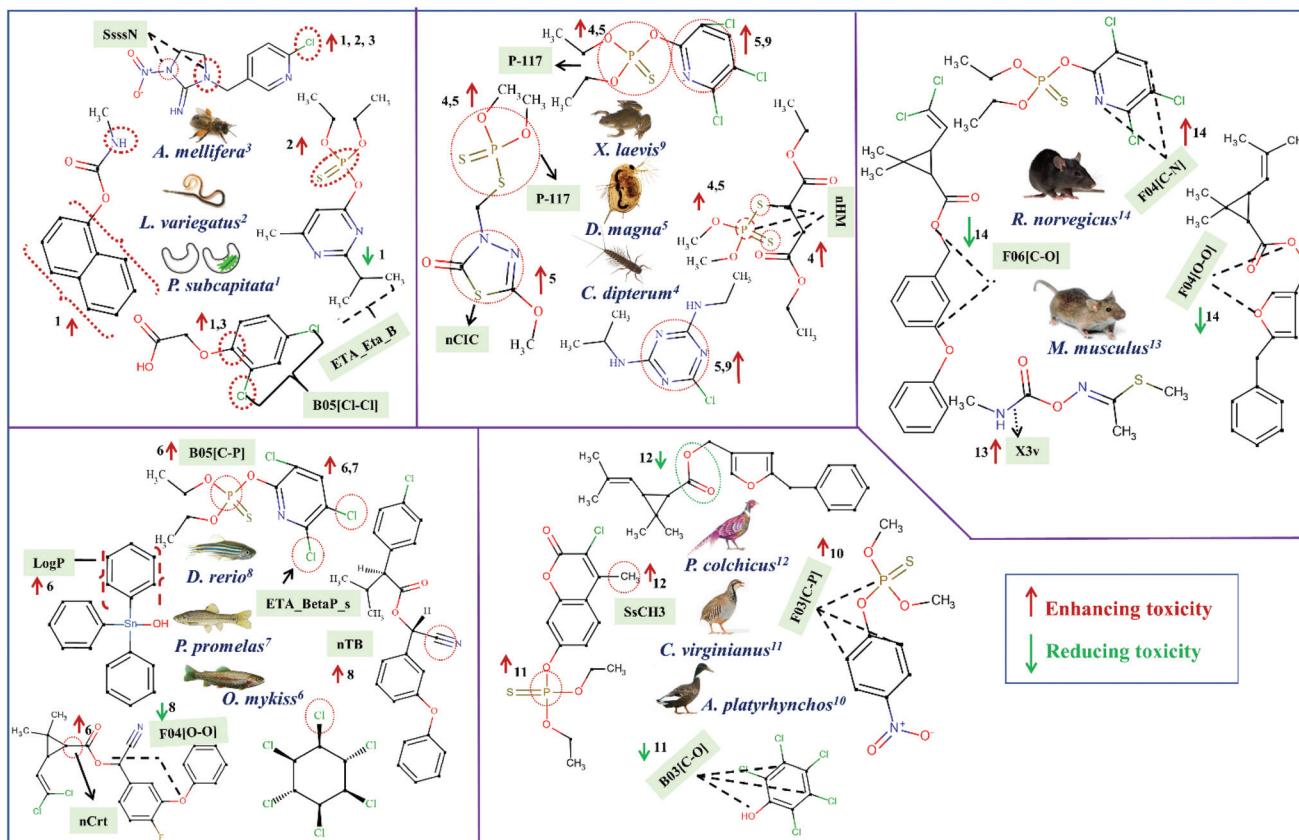


Fig. 34 Critical structural fragments accountable for endocrine disruption toxicity against various species.

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.⁴³¹ 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 the case of computational modeling of mixtures, the challenge is a daunting one, as the 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.⁴³² The reasons are the following:

- the toxicity response varies with diverse combinations/ratios of similar chemical mixtures,
- the interaction among chemicals is the reason for multi-faceted and noteworthy changes in the apparent response of the components,
- the form of exposure is also important along with the nature of the environment compartment,
- the assessment of the composition of each chemical in a mixture is also difficult and sometimes present in NOEC.

To formulate the toxicity data for computational modeling purpose, one has to follow the steps mentioned below:⁴³³

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

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

(iv) *Modeling for toxicity prediction and mechanistic interpretation:* A good number of *in silico* methods exist but the most successful and useful tool for measuring the mixture toxicity is QSTR.⁴³³ Boejea *et al.*⁴³⁴ developed QSTR models of alcohol ethoxylate mixtures with a correlation coefficient of 95% for three ecotoxicological species, *i.e.*, *Pimephales promelas*,

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

Property	Role/how it works
Physical properties for ecotoxicity	<p>Freezing point, boiling point, melting point, molecular weight, viscosity, and density are directly associated with environmental fate and health effects.</p> <p>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.⁴²⁰</p>
Solvation properties for ecotoxicity	<p><i>Phase partitioning/partition coefficient:</i></p> <ul style="list-style-type: none"> • $\log P/\log K_{ow}$ 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>i.e.</i>, octanol/water partition coefficient [K_{ow}] represented by P). Molecular hydrophobicity or lipophilicity (often referred as $\log P$) is one of the most significant parameters for toxicity of PPCPs towards different species of environment. For nonionic organic chemicals that operate through narcosis, acute and chronic toxicity increases exponentially with increases in $\log P$ up to a value of ~5. For those whose $\log P > 5$, bioavailability decreases along with acute toxicity, but bioaccumulation also increases. Minimal toxicity is likely with $\log P < 1$.⁴²¹ Considering $\log P/\log K_{ow}$, cardiovascular, sedatives anxiolytic antipsychotics and hypnotics, and gastrointestinal were predicted to be the most hazardous therapeutics for <i>Daphnia magna</i>, 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 $\log D_{pH}$. 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 $\log D_{7,4} > 1.7$.⁴²² • $\log D$ for acids/bases can be readily calculated from $\log P$ when pK_a values 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.⁴²³ <p><i>Solubility:</i> 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 plays a huge role in toxicity.⁴²⁴</p> <p><i>Aqueous solubility:</i> It is a direct measure of the hydrophobicity of a substance. The solubility equation developed by Ran and Yalkowsky⁴²⁵ can be used to estimate intrinsic water solubility at 25 °C ($\log S$) for structurally diverse organic substances. This equation uses regression-derived correlation with $\log P$ and melting point (MP) for solids:</p> $\log S = 0.8 - \log P - 0.01(MP - 25)$ <p>Compounds with higher $\log P$ have lower water solubility. Very poorly water-soluble chemicals (<1 ppb) generally have low bioavailability and are less toxic.⁴²⁴</p> <p>Aqueous solubility also dependent on temperature and pressure which are not considered here.</p> <p>Salinity or salting-out indicates that the above equation is not suitable to use for high-melting, non-ionic solids.⁴²³</p> <p><i>Colligative properties:</i> 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 roles in the transformation and biodegradation of chemicals.</p>
Molecular attributes for ecotoxicity	<p><i>Molecular attribute</i> is used to describe properties related to molecular shape and size.</p> <ul style="list-style-type: none"> • Electronic parameters like frontier orbital energies (highest occupied molecular 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.⁴²³ • 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 been shown to be associated with chemicals that are not toxic to <i>Pimephales promelas</i>. This is rationalized by the reduced electrophilicity of chemicals in this group; the higher the LUMO energy of a chemical, the less likely it is to be a strong electrophile.⁴²⁶ • The HOMO-LUMO gap (ΔE), which is a known measure of kinetic stability and responsible for high acute aquatic toxicity.⁴²² • 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.

Table 7 (Contd.)

Property	Role/how it works
Structural attributes for ecotoxicity	<ul style="list-style-type: none"> Keto group is predominantly accountable for higher toxicity of pharmaceuticals to <i>D. magna</i> 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 fish⁵⁹ Organometallic compounds are toxic to fish like <i>Oncorhynchus mykiss</i> and <i>Pimephales promelas</i>.⁴¹² Molecules with macrocyclic rings showed higher toxicity to <i>Pseudokirchneriella subcapitata</i>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 donors and acceptors tend to be of higher toxicity^{62,410–412} Molecules with sulphur atoms tend to show high first ionization potential to show high toxicity⁴¹⁸ Increase steric hindrance lowers the aquatic toxicity⁴¹⁸ <p>Factors are combined from the literature:^{427–429}</p> <ul style="list-style-type: none"> Minimal number of strong electron withdrawing substituents, like F and Cl. The biodegradability is 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 <i>etc.</i> 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 atoms on phenyl rings as they become less susceptible to attack by oxygenase enzymes Presence of esters (including phosphonates) which are susceptible to enzymatic hydrolysis Presence of oxygen atoms in the 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 Avoiding bulky <i>ortho</i> substitutions helps in accessibility of biodegradation enzymes <p>Factors are combined from the literature:^{424,426,430}</p> <ul style="list-style-type: none"> $\log P/\log K_{ow}$ (for aquatic environment), $\log K_d$ (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 a 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 $\log P$ exceeds 5 to 6 and generally considered low when $\log P < 2$. 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 lipophilic ($\log P > 8$), or chemicals with a molecular weight >700 Da will generally not bioconcentrate. A 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.
Structural attributes that enhance biodegradation	
Structural attributes that minimize bioaccumulation/bioconcentration	

Daphnia magna, and mesocosms. Wang *et al.*⁴³⁵ generated highly predictive QSTR models employing forward stepwise-MLR and nonlinear radial basis function neural networks (RBFNNs). Kar *et al.*⁴¹⁷ performed QSTR modeling using the mixture toxicity data of halogenated chemical mixtures on zebrafish embryos and identified that the studied chemicals in mixtures exhibited dose addition or concentration addition of each chemical which explains similar MoA and non-interaction among chemicals. The important factors of these modeling studies are identification of the 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.^{436,437} The first one is the unweighted descriptor method where the mixture consists of one descriptor vector and has only one property value. In simple terms, this is the general average of the numerical value for each component in a mixture for the respective descriptors. The second approach is weighted descriptors where descriptor vectors and properties depend on the composition of mixtures' components. Fig. 35 demonstrates how descriptors are calculated for mixtures. Here for a better understanding, a binary mixture is considered.⁴³⁸

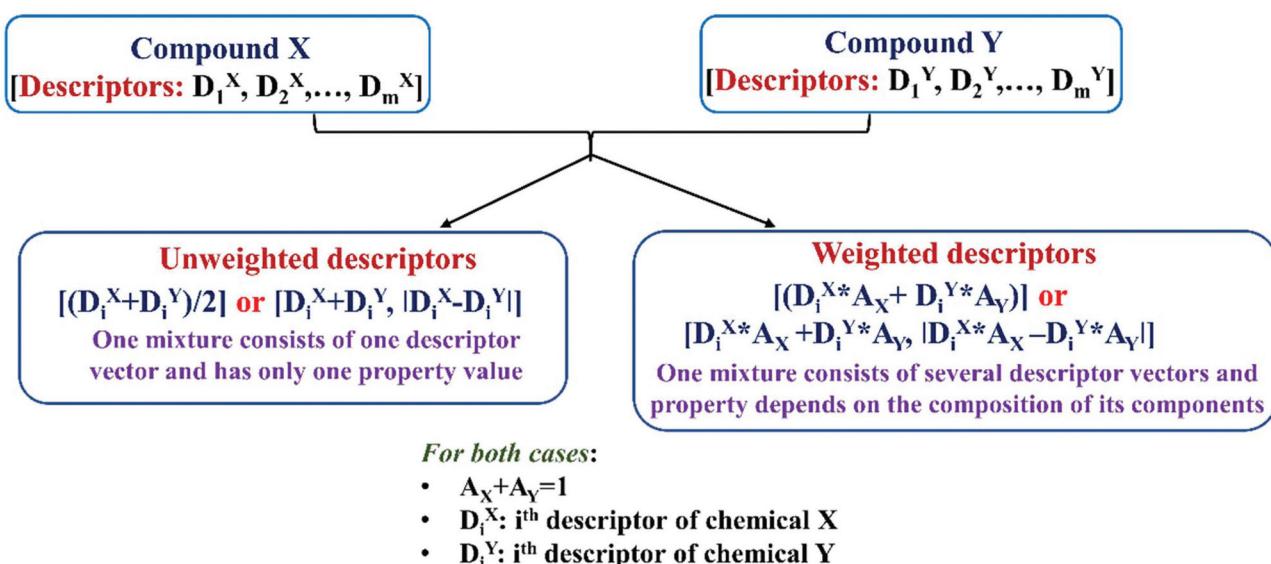
(b) *Validation protocol:* The traditional external validation procedure where the compounds are arbitrarily placed in the

Table 8 The MoA assessment hypothesis for mixtures

Hypothesis	Description
Concentration addition (CA)	The hypothesis assumes that chemicals act <i>via</i> 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 the Loewe additivity equation. For instance, the equation for a binary mixture of compounds A and B: $\frac{C_A}{EC_{xA}} + \frac{C_B}{EC_{xB}} = 1$ <p>where C_A and C_B are the specific concentrations of the compounds A and B creating the mixture, which results in 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 the equation is always equal to 1 for the CA modeling.</p>
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: $E = 1 - ((1 - e_A)(1 - e_B)(\dots))$ <p>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>i.e.</i> e_B. The equation can be expanded from binary mixtures to more components' mixtures.</p>
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:

$$E = \frac{\max \text{effect level}_X[X] + \max \text{effect level}_Y[Y] + \dots}{1 + \frac{[X]}{EC_{50X}} + \frac{[Y]}{EC_{50Y}} + \dots}$$

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.

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

external set is undesirable in the case of mixture models due to the overestimation of the prediction exclusively when the mixtures of the similar chemicals with diverse ratios exist multiple times in the modeled dataset.⁴³⁸ Certainly, if both training and external/test sets contain compounds corresponding to a similar mixture, then true prediction of a model will not

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 external sets. But, among three strategies, this is the weakest validation protocol	Reflects the capability of models to predict the presented mixtures with original compositions
Mixtures out	All data points corresponding to mixtures are composed of the identical constituents, but in dissimilar ratios, they 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	The protocol does so for new compounds

be assessed. Three acceptable strategies are illustrated in Table 9 for rigorous external validation:

Although the concept of mixture toxicity is quite old, the necessity of evaluation of mixture 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 compartments of the environment. Considering the critical aspect of mixtures' ecotoxicity assessment followed by management, we are suggesting the following points for unproblematic future efforts:

- A standardized protocol to identify the exposure, generation of biomarkers, and assessment of relevant mixtures hazardous to the environment.
- Need to evaluate the mixture toxicity up to the 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 ecotoxicity modeling is the lack of experimental data. Thus, preparation of an ecotoxicity database spanning over different compartments, multiple species and diverse experimental conditions is the need of the hour.
- Expert systems are advantageous for mixture assessment, for predicting dose-dependent interactive effects along with prediction of untested and new mixtures in no time.
- A Hausdorff-like similarity (H_s) measure may be beneficial in the modeling of mixtures.⁴³⁹ Hausdorff-like similarity is 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 follows:

$$dHaus_{MN} = \max \left\{ \sup_{m \in M} \left[\inf_{n \in N} (d_{mn}) \right], \sup_{n \in N} \left[\inf_{m \in M} (d_{mn}) \right] \right\} \quad (6)$$

from which the equivalent similarity measure can be calculated as:

$$sHaus_{mn} = \min \left\{ \sup_{m \in M} \left[\inf_{n \in N} (s_{mn}) \right], \sup_{n \in N} \left[\inf_{m \in M} (s_{nm}) \right] \right\} \quad (7)$$

where the signs s and d denote the similarity and the distance measures, correspondingly.

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

$$Hs_{MN} = \frac{\sum_{m \in M} \max_{n \in N} [s_{mn}] + \sum_{n \in N} \max_{m \in M} [s_{nm}]}{x_M + x_N} \quad (8)$$

Here, s_{mn} and s_{nm} are pair-wise similarity measures between the p -dimensional elements m and n of the sets M and N , correspondingly. x_M and x_N are the number of components for both sets. The signs in the numerator indicate 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 the 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, the majority of risk assessment work is 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.⁴⁴⁰ 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, and metabolism followed by respective toxicity due to metabolites are the real challenge in the risk assessment of PPCPs towards the environment.⁴⁴¹

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.⁴⁴⁰ According to US EPA, the following steps need to be followed for acquiring data:

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

(2) When there is no data at all, it is necessary to use mechanistic QSTR models and rate constants derived from the 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 the following information: (a) the minimum concentrations at which the biological action is observed; and (b) the recognition of toxicity expressive chemicals in mixtures.

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

- Combination of databases and computational tools for the concurrent environmental fate and metabolism evaluation;
- Accretion of a crystal-clear idea about a 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.

Metabolomics is helpful to identify toxicity pathways and the measurement of metabolites in the presence of certain physiological stimuli and/or genetic modification in a living system. To understand the changes in the metabolic pattern of a chemical in association with certain modification (biofluids, different species) followed by toxicity response, the 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 the metabolite of the original stressor induces a biological response. In other cases, it may show a false outcome.

Organizations like the 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.^{440,442} ORD's and EPA's computational toxicology research (CompTox) program is working efficiently in classifying chemicals and their metabolites with respect to the toxicity pathway. But

much more effort is required not only from regulatory agencies but also from the producers of those compounds, *i.e.*, industries which can provide sufficient information about the probable fate, transformation and metabolic pathway of individual PPCPs.

15. Overview and conclusion

This review has dealt with the present status of computational modeling of the ecotoxicity endpoints of PPCPs along with the evolving areas of the molecular designing approach for toxicity and risk management. The integration of *in silico* techniques with the GC principles is needed not only for generating precise predictive models but also for designing and synthesizing less hazardous and possibly non-toxic PPCPs to prevent risks in the first place. KBES is the most commonly employed predictive tool not only for toxicity prediction purposes but also for deriving design rules for less hazardous PPCPs nowadays. Without any doubt, no KBS can be considered as a universal system for prediction purposes, as all have their advantages and drawbacks, with varying specificity, sensitivity, and accuracy. Thus, the choice of KBS should depend on multi-faceted criteria. They include experimental conditions, species, biological and toxicological pathways, and 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 integrate this knowledge into molecular design. Thus, a comprehensive analysis of imperative features responsible for ecotoxicity as well as strategies to reduce these toxicities was discussed.

The future directions for novel PPCP specific predictive models will rely on more receptor specific descriptors of chronic toxicity to elucidate the receptor mediated pharmacodynamic MoA of the compounds.⁴⁴³ The chronic effects can not only be related to a multitude of sublethal endpoints *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 the application and development of AOP analysis in depth.^{444,445} More widespread and systematic development of AOPs requires integration with high-throughput big data generation *e.g.*, as outlined in the ToxCast programme.⁴⁴⁶ This is in our view necessary to move forward. The Comparative Toxicogenomics Database (CTD)⁴⁴⁷ is an example of how big data can be combined and integrated in novel ways between databases and across geographies to allow the 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 based on 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 lie in the subsequent analysis of AOP where different sources 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.

Conflicts of interest

There are no conflicts to declare.

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