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Abstract

Introduction: Drug transporters are now recognized as major actors of pharmacokinetics. They are also likely implicated in toxicokinetics and toxicology of environmental pollutants, notably pesticides, to which humans are widely exposed and which are known to exert various deleterious effects towards health. Interactions of pesticides with drug transporters are therefore important to consider.

Areas covered: This review provides an overview of the interactions of pesticides with membrane drug transporters, *i.e.*, inhibition of their activity, regulation of their expression and handling of pesticides. Consequences for toxicokinetics and toxicity of pesticides are additionally summarized and discussed.

Expert opinion: Some pesticides belonging to several chemical classes, such as organochlorine, pyrethroid and organophosphorus pesticides, have been demonstrated to interact with various uptake and efflux drug transporters, including the efflux pump P-glycoprotein and the uptake organic cation transporters (OCTs). This provides the proof of the concept that pesticide-transporter relationships merit attention. More extensive and systematic characterization of pesticide-transporter relationships, possibly through the use of *in silico* methods, is however likely required. In addition, consideration of transporter polymorphisms, pesticide mixture effects and realistic pesticide concentrations reached in humans, may help to better define the *in vivo* relevance of pesticide-transporter interactions in terms of toxicokinetics and toxicity.

Key-words: Drug transporters; environmental exposure; pesticides; toxicity; toxicokinetics

Article highlights

- Interactions of organic pesticides with drug transporters are important to consider owing to the major contribution of transporters to toxicokinetics and the well-established toxic effects of pesticides, to which humans are widely exposed.

- Various pesticides belonging to diverse chemical classes, including organochlorines, pyrethroid and organophosphorus pesticides, can inhibit the activity of ATP-binding cassette or solute carrier drug transporters. Activities of the drug efflux pump P-glycoprotein and of the uptake organic cation transporter (OCT) 1 and OCT2 have notably been demonstrated to be impacted by pesticides.

- Concentrations of pesticides that block *in vitro* transporter activities (in the 1-100 μM range) are commonly much higher than those reached in humans in response to environmental exposure, making the *in vivo* relevance of transporter inhibition unlikely for most of pesticides.

- A limited number of pesticides has been shown to regulate expression of some drug transporters, notably in hepatic cells.

- Pesticides can additionally be substrates for drug transporters, which may contribute to their toxicokinetics and may also govern their toxicity.

- Overall, pesticides remain poorly characterized with respect to relationships with transporters. A more extensive and systematic characterization of the interactions of pesticides with drug transporters is consequently required. Polymorphisms in transporters,

pesticide mixture effects and the interplay between transporters and drug metabolizing enzymes constitute additional issues that warrant attention.

1. Introduction

Drug transporters are plasma membrane proteins, implicated in the cellular uptake or efflux of xenobiotics. They belong to the solute carrier (SLC) or ATP binding cassette (ABC) transporter superfamilies [1]. Human SLC transporters are usually implicated in the uptake of drugs, through mediating facilitated diffusion or secondary active transport across the plasma membrane [2], whereas ABC transporters are efflux pumps, responsible for the primary active export of drugs out of cells, through their intrinsic ATPase activity [3]. Both SLC and ABC transporters are expressed at anatomical/histological sites important for xenobiotic disposition, including intestine, blood-tissue barriers like the blood-brain barrier, liver and kidney [4]. By this way, drug transporters play a major role in the different steps of pharmacokinetics, including absorption, distribution and hepatic and renal elimination [5, 6]. They may consequently influence drug efficacy as well as drug toxicity. Moreover, drug-mediated inhibition of their activity can cause pharmacokinetic-based drug-drug interactions [7]. This has led drug regulatory agencies to edict guidances for the study of putative interactions of new molecular entities with clinically-relevant drug transporters [8].

In addition to drugs, environmental pollutants can interact with transporters, *i.e.*, they inhibit and/or are handled by them [9, 10, 11], which may have consequences in terms of the toxicokinetics and toxicity of pollutants. Environmental chemicals may additionally regulate levels of transporter expression, *i.e.*, they notably enhance transporter expression, which may in turn result in increased transport activity [12]. Among pollutants which have to be

considered for putative interactions with drug transporter activity and/or expression, chemical organic pesticides are likely major ones. Indeed, these compounds, defined as any chemical or mixture of chemical intended for preventing, destroying, repelling, or mitigating pests, and belonging to diverse chemical classes (See Table 1 for a schematic presentation of main classes of organic pesticides), are largely used for occupational (agriculture…) or domestic purposes, for notably their insecticide, herbicide, fungicide or rodenticide properties. They are consequently widely distributed in the environment and humans can be exposed to them in a major way, through the oral, dermal or pulmonary route. Such exposures are thought to promote the development of various pathologies, including cancers, neurologic diseases and endocrine disruption, owing to the diverse toxic effects of pesticides [13, 14]. In the present review, we have summarized the current knowledges about interactions of human drug transporters with organic pesticides, with special emphasis on possible consequences in terms of pesticide toxicokinetics and toxicity.

2. Interactions of pesticides with ABC transporter activities

2.1 Interactions with P-glycoprotein (P-gp) activity

P-gp, encoded by multidrug resistance gene 1 (*MDR1/ABCB1*), was historically characterized as an efflux pump for various structurally-unrelated anticancer drugs [15], thus conferring multidrug resistance to cancer cells (See Table 2 for a summary of main drug transporters). P-gp also handles many non-anticancer drugs like the cardiotonic drug digoxin. It is physiologically expressed at various blood-tissue barriers and in absorptive or excretory organs such as the gut, the liver and the kidney; by this way, P-gp plays a major role in pharmacokinetics [16].

Various pesticides, belonging to diverse classes, have been shown to inhibit human P-gp activity. This has been mainly demonstrated through analyzing their effects on cellular

accumulation or efflux of radiolabeled or fluorescent reference P-gp substrates in P-gp expressing cells [17, 18]. The threshold of at least 50% inhibition of P-gp activity by pesticides used at 100 µM or 250 µM concentration can be retained for considering a pesticide as a P-gp inhibitor. Using this criteria, the organochlorine insecticides chlordecone, heptachlor and heptachlor epoxide, the organophosphate insecticides azinphos-ethyl, chlorpyrifos, coumaphos, phosalone, chlorthiophos, dicapthon, parathion, diazinon and fenamiphos, the avermectins ivermectin, abamectin and emamectin benzoate and the fungicide clotrimazole have been identified as inhibitors of human P-gp [12, 17, 19, 20, 21, 22, 23, 24]. Tetrachlorohydroquinone, a major metabolite of pentachlorophenol, as well as the insecticide hydramethylnon and the fungicide propiconazole and its metabolites, also block P-gp-mediated transport [17, 25]. The dibenzoylhydrazines tebufenozide and methoxyfenozide, which exert their insecticide activity through permanent activation of the ecdysone receptor, constitute additional P-gp inhibitors; they decrease P-gp-mediated transport of the antiarrhythmic agent quinidine [26]. With respect to endosulfan, it was found to inhibit human P-gp in three studies [17, 21, 27], but not in two other studies [12, 20], which may reflect differences in the various P-gp activity assays used in these studies. The herbicides acetochlor, alachlor, metolachlor and metazachlor, unlike dimetachlor, propachlor and prynachlor, have also been described as P-gp inhibitors [28]. Half maximal inhibitory concentrations (IC_{50}) are available only for a few pesticides inhibiting P-gp. In the study of Bain and al. [19], they range from 7.3 µM (hydramethylnon) to 229.8 µM (parathion). IC_{50} values for P-gp inhibition by endosulfan, phosalone and propiconazole are however lower, *i.e.*, around 3 µM [21]; similarly, avermectins block P-gp-mediated efflux at low concentrations, around 0.2-0.6 µM [24]. Other pesticides such as the carbamates aldicarb, aldoxycarb, aminocarb, carbaryl and propoxur, the dithiocarbamate maneb, the organophosphate pesticides mevinphos, dialifos and phosmet, the phenoxy herbicide

fluazifop-butyl and various pyrethroids (allethrin, bifenthrin, β-cyfluthrin, λ-cyhalothrin, β-cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, fluvalinate, *cis*-permethrin, *trans*-permethrin, resmethrin, tefluthrin and tetramethrin), failed to inhibit human P-gp activity to a significant extent [12, 17, 18, 19]. It was also the case for atrazine, paraquat, propiconazole, vinclozolin and the organochlorine insecticides 2,4'- dichlorodiphenyltrichloroethane (DDT), chlordane and toxaphene [12, 17], as well as for cyperquat [29], a bipyridil compound also known as 1-methyl-4-phenylpyridinium (MPP+), which has been used as an herbicide in the past. Additional organochlorine pesticides such as 4,4'-DDT, dieldrin, lindane, methoxychlor and mirex did not block human P-gp-mediated transport [17], whereas they inhibited that of mouse P-gp [11], suggesting inter-species differences with respect to interaction of P-gp with pesticides. 2,4'-DDT and 4,4'-DDT as well as their metabolite dichlorodiphenyldichloroethane (DDE) nevertheless decreased human P-gp ATPase activity [30]. For major classes of pesticides, the relative percentage of chemicals inhibiting drug transporters, including P-gp, as well as the total number of compound tested against transporter activity, according to published data, are indicated in Table 3.

Transport of pesticides by human P-gp has been investigated through direct analysis of their cellular accumulation or efflux in P-gp-positive cells, and also by measuring the stimulation/modulation of P-gp ATPase activity, which constitutes an indirect argument for P-gp-mediated transport. According to experimental data, there is only limited evidence for pesticides as substrates for the efflux pump (See Table 4 for a summary of pesticides handled by drug transporters). P-gp has been demonstrated to handle paraquat and thus protects against cytotoxicity induced by this pesticide [31, 32, 33, 34, 35], but may fail to contribute to the systemic pharmacokinetics of this herbicide in mice [36]. The avermectins ivermectin and selamectin are substrates for P-gp [37, 38], notably in mouse where the pump limits the brain penetration of ivermectin [39]. Endosulfan may be a weak substrate for P-gp [17]. The

organophosphate pesticide diazinon as well as rotenone have also been suggested to be P-gp substrates, because they stimulated P-gp ATPase activity, unlike dieldrin, endosulfan, ivermectin and maneb, without however inhibiting P-gp-mediated transport of rhodamine 123 [20]. Dibenzoylhydrazine insecticides stimulated P-gp ATPase activity, but are in fact poorly transported by the pump [26]. P-gp ATPase activity is additionally stimulated by methylparathion, cypermethrin and fenvalerate [27], whereas P-gp seems to be not involved in resistance of intestinal Caco-2 cells to propoxur, thus likely discarding the hypothesis that the carbamate may be transported by the pump [40]. Propiconazole was also not handled by P-gp [25], as well as deltamethrin, *cis*-permethrin and *trans*-permethrin [41], the rodenticide anti-vitamin K warfarin [42] or the herbicide cyperquat/MPP+ [29, 43, 44].

2.2 Interactions with multidrug resistance-associated protein (MRP/ABCC) activities

MRPs constitute a group of ABC transporters, comprising nine members in humans. Seven of these nine MRPs, *i.e.*, MRP1 (*ABCC1*), MRP2 (*ABCC2*), MRP3 (*ABCC3*), MRP4 (*ABCC4*), MRP5 (ABCC5), MRP7 (*ABCC10*) and MRP8 (*ABCC11*), have been unambiguously demonstrated to transport drugs, especially anionic drugs [45]. MRP1 was historically the first identified MRP; it was initially characterized as an efflux transporter for anticancer drugs overexpressed in drug-resistant cancer cells. MRP1 was next demonstrated to exhibit a broad tissue distribution and to transport a wide range of xenobiotics, including anionic drugs and drug conjugates [46]. MRP2, like MRP1, primarily transports organic anions and is expressed in various tissues, especially in the liver, kidney and gastrointestinal tract [47]. MRP3 is present in several tissues, including the liver, where it is located at the sinusoidal pole of hepatocytes; it notably transports xenobiotics from the liver into the blood, for a secondary renal elimination. MRP4 is notably expressed in the kidney and the liver and at the blood-brain barrier. It has wide substrate specificity, including nucleoside analogues and antiviral drugs. MRP5 is almost ubiquitously expressed in human tissues. It effluxes a broad range of

natural and xenobiotic compounds such as cyclic GMP, antiviral compounds and cancer chemotherapeutic agents. MRP7 confers anticancer drug resistance [48], as well as MRP8, notably towards nucleoside analogs [49],

The avermectins abamectin, emamectin and ivermectin have been reported to inhibit MRP activity; the corresponding IC_{50} values are around 1.5-2.0 μM and have been determined through measuring MRP-mediated efflux of the dye glutathione methylfluorescein in human neuronal SH-SY5Y cells [24]. The fact that ivermectin inhibited MRP1, MRP2 and MRP3 ATPase activities after stimulation by their respective activators [38] fully supports this conclusion. Whether ivermectin may be a good substrate for MRP1 remains however to be established, because conflicting data about this point have been reported [37, 38]. The pyrethroids allethrin and tetramethrin, unlike bifenthrin, β-cyfluthrin, λ-cyhalothrin, β-cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, fluvalinate, *cis*-permethrin, *trans*-permethrin, resmethrin and tefluthrin, were found to inhibit MRP activity in hepatoma MRP2-expressing HuH-7 cells, with IC_{50} values around 40-50 μM [18]. The organochlorine pesticides endosulfan, chlordane, heptachlor and chordecone also blocked MRP-mediated efflux in HuH-7 cells, whereas 4,4'-DDT, 2,4'-DDT, dieldrin and lindane were without effect [12]. None of the chloroacetanilides acetochlor, alachlor, dimetachlor, metazachlor, metolachlor, propachlor and prynachlor interacts with MRP1 or MRP2; MRP1 was however demonstrated to transport an important intermediate of the acetochlor detoxification pathway [28]. MRP1 additionally handles the chloroacetanilide herbicide methoxychlor and confers resistance to the organophosphorus insecticide fenitrothion and the carbamate herbicide chlorpropham, suggesting that these two pesticides may also be substrates [50].

2.3 Interactions with breast cancer resistance protein (BCRP/ABCG2) activity

BCRP is an ABC transporter handling both anticancer drugs and non-anticancer drugs, like statins, as well as environmental chemicals like carcinogenic heterocyclic aromatic amines

[51] and endogenous compounds like urate [52]. It is expressed at blood-tissue barriers and in gut and excretory organs like liver and kidney. BCRP is also present in stem cells.

The organochlorine pesticides endosulfan, chlordane, heptachlor and chordecone inhibit BCRP efflux activity, whereas 4,4'-DDT, 2,4'-DDT, dieldrin and lindane are without effect [12]; 2,4'-DDT and 4,4'-DDT as well as their metabolite dichlorodiphenyldichloroethane (DDE) have nevertheless been reported to inhibit human BCRP ATPase activity [30]. The pyrethroids allethrin and tetramethrin, unlike bifenthrin, β-cyfluthrin, λ-cyhalothrin, β-cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, fluvalinate, *cis*-permethrin, *trans*-permethrin, resmethrin and tefluthrin, have been found to inhibit BCRP activity, *i.e.*, BCRP-mediated efflux of the fluorescent dye Hoechst 33342, with IC_{50} values around 40-70 μM [18]. The Hoechst H33342 accumulation assay also indicated that thirteen widely-used pesticidal active substances including the fungicides azoxystrobin, dimethomorph, dithianon and tolclofos-methyl, the benzidizamole carbendazim, the organophosphate pesticides chlorpyrifos and dimethoate, the herbicides chlormequat, diflufenican and ioxynil, the carbamates methiocarb and propamocarb and the sulfonylurea herbicide rimsulfuron are likely inhibitor of rabbit BCRP; no such evidence was obtained for chlorpyrifos-methyl, epoxiconazole, imazalil (also known as enilconazole), glyphosate and thiacloprid [53]. BCRP was additionally found to transport the rodenticide warfarin [54], whereas ivermectin is unlikely to be a substrate for the pump [55], even if it inhibits its activity, with a rather low IC_{50} value (2.2 μM) [56].

3. Interactions of pesticides with SLC drug transporter activities

3.1 Interactions with activity of SLC drug transporters handling organic cations

SLC drug transporters transporting organic cations mainly correspond to organic cation transporter (OCT) 1 (*SLC22A1*), OCT2 (*SLC22A2*) and OCT3 (*SLC22A3*), acting as

electrogenic and membrane potential-sensitive diffusional transporters, and to multidrug and toxin extrusion (MATE) protein 1 (*SLC47A1*) and MATE2-K (*SLC47A2*) (a functionally active isoform of MATE2), acting as electroneutral, sodium-independent and pH-dependent proton antiporters [57]. OCT1 and MATE1 are located at the sinusoidal and canalicular poles of hepatocytes, respectively, whereas OCT2 and MATE2-K are found at the basolateral and apical membranes of renal proximal tubular cells, respectively. OCT3 has a wide tissue distribution. OCT1, OCT2, MATE1 and MATE2-K handle cationic drugs, including metformin and oxaliplatin [58]. Endogenous compounds such as neurotransmitters are also transported by OCTs.

With a threshold of at least 50% inhibition of transport activity when used at 100 µM for being considered as a transporter inhibitor, the organochlorine pesticides endosulfan, chlordane, heptachlor, dieldrin and lindane, unlike 2-4'-DDT, 4'-DDT and chlordecone, were found to inhibit OCT1 activity in human hepatoma HepaRG cells, with IC_{50} values of 0.9 µM (dieldrin) and 1.5 µM (lindane) [12]. The pyrethroids allethrin, imiprothrin, prallethrin and tetramethrin and the organophosphorus pesticides fenamiphos, fenitrothion, malathion, methyl parathion, parathion, phosmet, profenofos and propetamphos also blocked OCT1 activity, as well as that of OCT2, in HEK293 cells overexpressing these transporters [59]; MATE1 activity was similarly inhibited by allethrin, tetramethrin, fenamiphos, phosmet and propetamphos [18, 59]. By contrast, bifenthrin, β-cyfluthrin, λ-cyhalothrin, β-cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, fluvalinate, *cis*-permethrin, *trans*-permethrin, resmethrin and tefluthrin failed to impair activities of OCT1, OCT2 and MATE1. Phenothrin also did not interfere with OCT1 and OCT2 activities. None of pyrethoids or organophosphorus pesticides impair MATE2-K activity [18, 59]. Allethrin and tetramethrin IC_{50} values towards OCT1 activity, *i.e.*, OCT1-mediated uptake of the OCT1 reference substrate 4',6-diamidino-2-phenylindole (DAPI), were around 2.6 µM and 4.9 µM,

respectively. These two pyrethroids, which also blocked OCT1-mediated uptake of the endogenous substrate dopamine, however failed to *trans*-stimulate OCT1 activity, indicating that they are unlikely to be substrates for OCT1 [18] (Table 4). Analysis of accumulation of these two pesticides into control (MOCK) HEK293 cells and OCT1-transduced HEK293 cells by liquid chromatography tandem-mass spectrometry (LC-MS/MS) fully supports this conclusion, through indicating similar levels of pesticides in control HEK293-MOCK cells and OCT1-positive counterparts (Fig. 1A). Allethrin and tetramethrin also similarly accumulated in MOCK- and OCT2-transduced HEK293 cells (Fig. 1A), thus discarding a transport of these two pesticides by OCT2. By contrast, OCT1-transduced cells displayed increased accumulation of the OCT1 reference substrate DAPI when compared to control MOCK-transduced HEK293 cells (Fig. 1B); in the same way, OCT2-transduced cells exhibited increased accumulation of the OCT2 reference substrate rhodamine 123 (Fig. 1B). Moreover, the OCT1 inhibitor verapamil failed to enhance allethrin and tetramethrin accumulation in OCT1-transduced HEK293 cells, whereas it markedly increased that of DAPI (Fig. 1). The OCT2 inhibitor amitriptyline also enhanced cellular level of rhodamine 123 in OCT2-transduced HEK293 cells, but not those of allethrin and tetramethrin (Fig. 1). Like allethrin and tetramethrin, fenamiphos and phosmet are not transported by OCT1 or OCT2 (Table 4) [59]. By contrast, cyperquat/MPP+ has been demonstrated to be transported by human OCT1, OCT2, OCT3, MATE1 and MATE2-K [60, 61, 62, 63]. MPP+ concomitantly inhibits the transport of the mutagenic vital dye ethidium by OCT1 and OCT2 [64]. OCT2 and MATE1, unlike OCT1 and OCT3, handle paraquat under its native N,N′-dimethyl-4,4′-bipyridinium dichloride form, which harbors two cationic charges [65]. The monocationic radical form of PQ, coming from redox cycling with cellular diaphorases such as NADPH oxidase and nitric oxide synthase, has nevertheless been shown to be a substrate for mouse Oct3 [66].

3.2 Interactions with activity of SLC drug transporters handling organic anions

There are two main families of SLC drug transporters handling organic anions. The first one corresponds to organic anion transporting polypeptides (OATPs/*SLCOs*), notably OATP1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3)* and OATP2B1 (*SLCO2B1*) [67]; the second one is that of organic anion transporters (OATs), comprizing OAT1 (SLC22A6) and OAT3 (*SLC22A8*) [68]. OATP1B1 and OATP1B3 are specifically located at the sinusoidal pole of hepatocytes; OAT1 and OAT3 are present at the basolateral pole of proximal tubular cells, whereas OATP2B1 is more widely distributed. Substrates for OATPs include anionic drugs and endogenous compounds, such as bile acids, bilirubin, prostaglandins and hormones, like thyroxine and steroid conjugates [69]. OATs transport anionic compounds, including drugs such as penicillins and cephalosporins, endogenous compounds such as estrone-3-sulfate and environmental contaminants such as perfluorooctanoic acid [70].

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is a common substrate for human renal OAT1 and OAT3, but its transport by OAT1 is markedly greater than that by OAT3 [71]. The organochlorine pesticides endosulfan, chlordane, heptachlor, dieldrin, lindane, 2-4'-DDT, 4-4'-DDT and chlordecone failed to inhibit OATP activity in human hepatoma HepaRG cells [12]. With respect to pyrethroids, allethrin and tetramethrin inhibited OATP1B1 and OAT3 activities, and *cis*-stimulated that of OATP2B1 [18]. Similarly, imiprothrin and prallethrin, unlike phenothrin, *cis*-stimulated OATP2B1 activity and inhibited that of OAT3. By contrast allethrin and tetramethrin failed to alter in a major way activities of OATP1B3 and OAT1. Similarly, bifenthrin, β-cyfluthrin, λ-cyhalothrin, β-cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, fluvalinate, *cis*-permethrin, *trans*-permethrin, resmethrin and tefluthrin did not impair OATP1B3 and OAT1 activities, as well as those of OATP1B1, OATP2B1 and OAT3 [18]. Regarding organophosphorus pesticides, OAT3 activity was inhibited by fenamiphos, malathion and profenofos, but *cis*-stimulated by

metasystox, whereas dichlorvos, fenitrothion, methamidophos, methyl parathion, monocrotophos, parathion, phosmet, propetamphos and temephos were without effect [59]. No organophosphorus pesticide blocked OAT1. Activity of OATP1B1 was inhibited by profenofos and temephos, whereas that of OATP2B1 was *cis*-stimulated by fenamiphos, malathion, parathion, phosmet and profenofos [59].

4. Regulation of drug transporter expression by pesticides

Pesticides have previously been shown to regulate expression of drug detoxifying proteins such as hepatic cytochromes P-450 (CYPs) [72, 73]. Indeed, pesticides are known activators of nuclear receptors like pregnane X receptor (PXR) and constitutive androstane receptor (CAR) [74], acting as xenobiotic-sensing receptors regulating expression of CYPs. By this way, several pesticides among organophosphate chemicals, pyrethroids, carbamates, organochlorines insecticides and phenylurea compounds up-regulated CYP3A4 and CYP2B6 expression in hepatocytes [73]. Drug transporters are also targets for nuclear receptors in human and/or rodent cells [75]. Indeed, PXR activation induces expression of P-gp in intestinal cells [76]. It also enhances levels of MRP2 in primary human hepatocytes [77], MRP3 in human hepatoma HuH-7 cells [78], Bcrp in mouse Sertoli cells [79] and OCT1 in chronic myeloid leukemia cells [80]. CAR additionally up-regulates P-gp in CAR-transfected hepatoma HepG2 cells [81] and intestinal LS174 cells [82]. CAR is also implicated in regulation of MRP2 in primary human hepatocytes [77], Bcrp in mouse and rat brain capillaries [83] and Mrp4 in mouse liver [84]. Another nuclear receptor, *i.e.*, the farnesoid X receptor (FXR), increases hepatic expression of MRP2 [77] and of bile salt export pump (BSEP/*ABCB11*) [85], a canalicular hepatic transporter involved in biliary acid elimination.

Pesticides activating drug sensing receptors are consequently susceptible to induce expression of drug transporters. In agreement with this hypothesis, the organochlorine

insecticides chlordane, heptachlor, dieldrin, lindane, 2,4'-DDT and chlordecone, which are known agonists of PXR [86], increased mRNA expression of P-gp, MRP2 and BCRP in hepatic HepaRG cells when used at 10 μM [12]. In the same way, chlorpyrifos, which notably activates PXR and CAR [86, 87], enhanced expression of P-gp and BCRP in human villous cytotrophoblast cells when used at concentration 10-100 μM [88]. The exact role played by PXR and/or CAR in this ABC transporter up-regulation in placental cells remains however unclear because the expression of PXR and CAR is very low in the human placenta [89]. PXR and CAR also contribute to the impact of fipronil on hepatic expression of transporters like MRP2 and MRP3 [90]. Nuclear receptors are additionally likely to participate to regulation of transporters (P-gp, BSEP, BCRP or OATP1B1) in primary human hepatocytes exposed to chemicals from the ToxCast320 chemical library, comprising many pesticide active ingredients [91]. Nuclear-receptor independent ways of drug transporter regulation by pesticides have also been described. Thus, ivermectin induced P-gp expression through mRNA stabilization in murine hepatocyte cell line [92]. The sinusoidal sodium-taurocholate cotransporting polypeptide (NTCP/*SLC10A1*), which is not known to be regulated by PXR or CAR, exhibited decreased mRNA expression in human hepatoma HepaRG cells exposed to organochlorines [12]. Finally, it is noteworthy that transporter induction may result from chronic exposure to pesticides. Thus, P-gp levels are increased in response to repeated exposure of intestinal Caco-2 cells to the organophosphophorus pesticide diazinon [93].

5. Pesticides as modulators of drug transporter activity or expression: implications for pesticide toxicity

5.1 Toxicity due to transporter activity inhibition by pesticides

Inhibition of ABC or SLC drug transporter activities by a chemical (called the perpetrator) is susceptible to cause alterations of pharmacokinetics of a drug (called the victim) substrate for

the incriminated transporter, finally leading to drug toxicity (in the case of increased drug plasma concentration) or drug inefficacy (in the case of decreased drug plasma concentration). In addition, transport of endogenous substrates may be impaired, which may lead to toxicity, like cholestasis due to inhibition of the bile salt transporter BSEP [94], or endocrine disruption linked to altered elimination of hormones. For such effects, a key point to take into account is whether the perpetrator, whose inhibitory effects towards transporter activity are commonly initially demonstrated in *in vitro* assays, reaches *in vivo* concentrations efficient against the considered transporter. When the perpetrator is a drug, its *in vitro* IC_{50} value has to be confronted to its maximum unbound plasma concentration (I_1) and to its maximal theoretical gastrointestinal concentration (I_2), calculated as the oral dose in a volume of 250 mL; according to 2012 FDA guidance for drug-drug interactions, transporter inhibition may be clinically achievable when $I_1/IC_{50} \geq 0.1$ and/or $I_2/IC_{50} \geq 10$ [95]. The application of such thresholds for pesticides may indicate that *in vivo* inhibition of drug transporter activity in response to environmental exposure to pesticides is very unlikely for most of them. Indeed, *in vitro* IC_{50} values of pesticides inhibiting transporter activity are usually in the 1-100 µM range (see above), whereas their plasma concentrations are often in the 1-100 nM range in humans [96, 97, 98]. The pesticide concentration may be even much lower when considering only the unbound free ones. This hypothesis is fully supported by the fact that at least some pesticides, such as dieldrin and atrazine, bind extensively to plasma proteins [99, 100]. Average oral daily intake of a pesticide in the food is additionally usually low, in the µg or ng range, making unlikely the fact that pesticide IC_{50} values against transporter activity may be reached in the gastrointestinal tract. Pesticide-drug interactions as well as inhibition of physiological substrate transport by pesticides may consequently be discarded, for most of pesticides found in the environment. It is however noteworthy that humans are often exposed to mixtures of pesticides or to pesticides and other environmental contaminants, whose inhibitory effects

towards transporters may be additive or synergic. This may result in *in vivo* inhibition of transporters in response to exposure to chemical mixtures, even if concentration of each single pesticide of the mixture is low. The fact that binary mixtures of pesticides, including diazinon, have been shown to exhibit synergistic inhibition of P-gp [21] likely supports this hypothesis. It is also noteworthy that pesticide metabolites may cause transporter inhibition. This may be hypothesized to add or synergize with transporter inhibition triggered by the parental pesticide. Examples of pesticide metabolites acting against transporters correspond to hydroxylated metabolites of propiconazole, active against P-gp [25], and to chlorpyrifos oxon, which inhibits drug labelling of P-gp and stimulates its ATPase activity, in contrast to parental chlorpyrifos [101].

5.2 Toxicity due to transporter expression changes caused by pesticides

Regulation of drug transporter expression by pesticides, *i.e.*, induction or repression, can theoretically result in enhanced or decreased transport of drugs or endogenous substrates, which may in turn alter pharmacokinetics and/or toxicity. One key point to consider is that environmental exposure to pesticides has to result in *in vivo* pesticide level sufficient for triggering transporter regulation, which may be unlikely owing to the relative low concentrations of pesticides reported in humans exposed to these chemicals, as already discussed above. Moreover, it is noteworthy that whether transporter expression modulation may result in clinically-significant alteration of drug transporter activity remains to be formally established. In fact most, if not all, clinical drug-drug interactions in relation with transporters are linked to transporter activity inhibition by the drug perpetrator, and not to altered transporter expression [7, 102]. The only example is perhaps that of therapeutic proteins like tocilizumab, a humanized monoclonal antibody against the interleukin-6 receptor. Tocilizumab is thought to restore normal expression of transporters, including P-gp,

repressed by IL-6 in inflammatory patients, which may be the source of drug-drug interactions [103].

6. Pesticides as substrates of drug transporters: implications for toxicokinetics and toxicity of pesticides

6.1 Implications for toxicokinetics

Handling of pesticides by ABC and/or SLC transporters is likely to contribute to the different steps of the disposition process, *i.e.*, intestinal absorption, drug disposition, notably across the blood-tissue barriers like the blood-brain barrier, and hepatic and renal elimination. Owing to the well-established drug transporter-drug metabolizing enzymes interplay [104], it may also influence pesticide metabolism. In this context, it is noteworthy that some pesticides such as organochlorine insecticides have historically been considered as lipophilic chemicals, which freely diffuses across the plasma membrane according to their concentration gradient, thus ruling out any major role for drug transporters. This assertion may now be questioned by the putative handling of some pesticides by ABC transporters, notably P-gp and BCRP. Indeed, these ABC transporters are present at the apical pole of intestinal cells, where they actively expel their substrates into the digestive lumen, thus preventing their absorption [105]. Handling of lipophilic pesticides by ABC transporters like P-gp and BCRP may therefore theoretically limits their intestinal absorption. Moreover, the concentration of pesticides in the gastrointestinal lumen is believed to be low, as already discussed above, which precludes any saturation of the ABC efflux pumps. Such lipophilic pesticides may therefore behave as class 2 compounds in the biopharmaceutics drug disposition classification system (BDDCS) [106]. Class 2 drugs are highly permeable, so they will generally be able to enter enterocytes by passive diffusion, unaided by uptake transporters. However, due to low solubility limiting luminal concentration, they are unlikely to saturate efflux transporters. Consequently, class 2

compounds can be pumped out of enterocytes, which can influence bioavailability and absorption rate. Pesticides exhibit limited luminal concentration, like class 2 drugs, even if this probably reflects the low dairy intake of pesticides, and not a poor solubility, as for class 2 drugs. Transport of pesticides by ABC pumps may additionally be implicated in reduced brain penetration of pesticides, through P-gp- and BCRP-mediated efflux into the lumen of brain capillary. The fact that silencing of P-gp led to a 100-fold increase of ivermectin distribution into the central nervous system of transgenic mice fully supports this assertion [39].

6.2 Implications for toxicity

Handling of pesticides by transporters may decrease or increase their toxicity, depending on the nature of transport. Thus, efflux by ABC transporters out of cells is expected to result in reduced intracellular accumulation of pesticides, and by this way, in decreased toxic effects. This is illustrated by the fact that P-gp expression confers resistance to paraquat, through stimulating its cellular export out of cells [32]. By this mechanism, P-gp notably protects against paraquat-induced toxicity in human and mouse proximal tubule cells [31]. Interestingly, the ABC transporter MRP1 may also confer resistance to paraquat; this may be due to inhibition of apoptosis caused by the pesticide [107]. MRP1 also decreased toxicity of fenitrothion, chlorpropham and methoxychlor in MRP1-transfected cells and protects seminiferous tubules from methoxychlor-induced damage [50]. Overexpression of ABC transporters has similarly been shown to confer resistance to pesticides in insect cells [108]. Transport of pesticides by ABC transporters at blood-tissue barriers, notably at the blood-brain barrier, also contributes to protect these tissues, notably the central nervous system, from pesticide toxicity, as already demonstrated for P-gp and neurologic toxicity of ivermectin [39]. In the same way, P-gp may play the same protective role towards pesticides at the placental barrier.

Handling of pesticides by drug transporters such as SLC transporters may also result in decreased toxicity of pesticides. Thus, mouse Oct3 mediates cellular entry of paraquat, but only under its reduced monovalent cation form [66]. Because Oct3 is mainly expressed by non-dopaminergic cells in the nigrostriatal region, this Oct3-mediated transport of paraquat contributes to reduce its accumulation in dopaminergic neurons; this highlights a buffering capacity by non-dopaminergic cells, which indirectly protects dopaminergic neurons from toxicity of paraquat [66]. Whether SLC transporters may, by contrast, favor toxicity of pesticides through increasing their intracellular accumulation remains yet unknown. Such a toxicity caused by SLC-transporter mediated uptake of chemicals has nevertheless already been demonstrated for other environmental contaminants such as the microcystin congeners. These compounds, produced by cyanobacteria, use OATP1B1 and OATP1B3 to enter into cells and, therefore, specifically target OATP1B1- or OATP1B3-expressing cells [109]. Such an OATP-mediated uptake is consequently responsible for the selective hepatic toxicity of microcystins [110].

7. Conclusion

Various reports have indicated that pesticides belonging to diverse pharmacological/chemical classes can interact with drug transporters. Most of these studies described inhibition of ABC or SLC drug transporter activities by pesticides; only a few of them concerns the handling of pesticides by transporters or the regulation of transporter expression by pesticides. Importantly, most, if not all, pesticides required concentrations in the µM range for inhibiting transporter activities; such concentrations are much higher than pesticide concentrations (in the nM range or less) commonly observed in humans exposed to environmental pesticides. Pesticides are therefore unlikely to cause pesticide-drug interaction based on drug transport alteration in humans. In the same way, putative pesticide-mediated alteration of endogenous

substrate transport and possible deleterious consequences in response to environmental exposure may probably be discarded. By contrast, for pesticides recognized as substrates, transporters may be implicated in their toxicokinetics and their toxicity towards human health.

8. Expert opinion

Drug transporters are now considered as major actors of pharmacokinetics [7], which fully justify the study of drug-transporter interactions during the development of new molecular entities by pharmaceutical companies. Data reported above unambiguously indicate that some pesticides also interact with transporters, *i.e.*, they can inhibit their activity, regulate their expression or be themselves substrates. This provides proof of concept that transporters should be considered when studying the toxicokinetics and toxicity of pesticides in humans. For transporter inhibition, the concentrations of pesticides *in vitro* blocking transporter activities have however to be confronted to the levels of unbound pesticides reached in humans in response to environmental exposure, in order to precise the *in vivo* relevance of such transporter inhibitions. Indeed, in most cases, the pesticide concentrations required to inhibit transporter may probably be much higher than pesticide blood concentrations reached in exposed humans. This likely discards any *in vivo* transporter inhibitions in response to environmental exposure to pesticides. In the same way, the *in vivo* relevance of the *cis*-stimulation of some transporter activities by certain pesticides remains to be clarified.

Chemical pesticides remain much less studied than drugs with respect to interactions with transporters, with no or only limited data available for most pesticides. Further studies are therefore required to characterize pesticide-transporter interactions in a more extensive and systematic manner and to determine their possible implications in deleterious effects of pesticides towards human health. To do this, the use of high-throughput assay panels for human drug transporters may be welcome. Such assays, mainly based on fluorescent probes

substrates for P-gp, MRPs, BCRP, OATPs, OATs and OCTs [111, 112], are fully applicable to large series of compounds tested at various concentrations and permit to reduce analysis costs. These functional assays may additionally provide structure-activity information, which may be useful for modeling quantitative structure-activity relationships (QSAR) with respect to interactions with transporters. Such QSAR studies may help to *in silico* predict inhibition of transporters by pesticides and/or handling of pesticides by transporters. QSAR approaches have already been applied with success to drug-transporter interactions, including those related to P-gp [113], MRP2 [114], BCRP [115], OCT1 [116], OATP1B1/1B3 [117] and OAT1/OAT3 [118]. Interestingly, molecular descriptors associated with OCT1 inhibition by pyrethroids have been determined and combining pairwise some of these descriptors allow to graphically and successfully predict interactions of imiprothrin, phenothrin and prallethrin with OCT1, OCT2, OATP2B1, OAT1 and OAT3 [18]. Besides, or together with QSAR studies, molecular docking analyses may constitute a valuable approach for predicting interaction of pesticides with transporters, as already established for drugs [119]. Overall, the application to pesticides of *in silico* tools developed for characterizing membrane permeability and transporter interactions with drugs, may represent a promising way, extending data and methods initially focused on drugs to the pesticide area. The graphical BOILED-Egg/SwissADME online method, based on lipophilicity and polarity and originally designed for drugs [120], has thus permitted to predict intestinal absorption and brain penetration of a large set of pesticides (n = 338) belonging to various chemical classes [121].

Genes encoding drug transporters are well-known to exhibit polymorphisms, which may have functional consequences, *i.e.*, some genetic variants may display increased or decreased transport activity [122]. Such transporter polymorphisms concern most, if not all, drug transporters, including P-gp, BCRP, OATPs, OATS and OCTs. They are thought to be responsible for population-specific differences in drug transport and considerable inter-

individual variation in physiology and pharmacotherapy [123]. In the context of environmental exposure to pesticides, such transporter polymorphisms may have to retain special attention. Indeed, they may result in inter-individual variation in toxicokinetics and toxicity of pesticides and may thus contribute in a notable way to individual susceptibility to these environmental pollutants. For example, subjects with genetic variants of *ABCB1* gene associated with low P-gp activity may have enhanced intestinal absorption and brain penetration of pesticides, by reduction of P-gp-mediated efflux of the chemicals at the intestinal and blood-brain barriers; such subjects may therefore be more susceptible to toxicity of pesticides. This hypothesis is fully supported by the fact that exposure to commonly used pesticides, specifically organochlorine and organophosphate insecticides, and the presence of variant *ABCB1* genotypes at two polymorphic sites, jointly increase the risk of Parkinson's disease [124].

The fact that pesticides are often used as mixtures of enantiomers has additionally to be taken into account when considering drug transporters and pesticides. Indeed, the stereoselectivity of chiral drug transport is well-established [125] and enantiomers of pesticides may therefore differentially interact with transporters. The handling of pesticides by SLC transporters distinct from main drug transporters, such as the L-type amino acid LAT1/2 (*SLC7A5/SLC7A8*) transporter or the dopamine transporter (DAT/*SLC6A3*), which handles glyphosate [126] or paraquat [66], respectively, also merits attention. In the same way, the molecular nature of the membrane transporters responsible for the established transepithelial transport of the herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA) [127] and of the pyrethroids deltamethrin, *cis*-permethrin and *trans*-permethrin [41] across intestinal Caco-2 cells remains to be determined. The functional interplay between drug-metabolizing enzymes and transporters in pesticide absorption and disposition has additionally to be considered, as already done for drugs [128]. This interplay may notably correspond to

preferential handling of pesticide metabolites by transporters, which suggests that pesticide metabolites may have to be investigated as potential transporter substrates and/or inhibitors. Additive or synergic effects towards transporters of pesticides in mixture with other pesticides or environmental contaminants constitute another important issue to apprehend. Finally, data about handling of pesticides by drug transporters may help to ameliorate the relevance and the accuracy of physiologically-based pharmacokinetic (PBPK) models, which represent promising approaches for pesticide risk assessment in humans [129, 130].

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Legend to figure

Figure 1. Accumulation of the pyrethroids allethrin and tetramethrin in OCT1- and OCT2-transfected HEK293 cells

(A) HEK293 cells transduced with MOCK (HEK-MOCK), OCT1 (HEK-OCT1) or OCT2 (HEK-OCT2) were incubated with 100 µM allethrin or 100 µM tetramethrin in the absence (untreated) or presence of the OCT1 inhibitor verapamil (50 µM) or the OCT2 inhibitor amitriptyline (100 µM) for 5 min at 37°C. Intracellular accumulations of the pyrethroids were next determined by LC-MS/MS analysis and normalized to total protein content. (B) Cellular accumulation of the OCT1 substrate DAPI and the OCT2 substrate rhodamine 123 was determined as previously described [18], in the absence (untreated) or presence of verapamil or amitriptyline. (A, B) Data are the means ± SEM of three independent experiments. F.A.U., fluorescence arbitrary unit. ***, $p<0.001$ (ANOVA followed by Newman-Keuls post-hoc test).

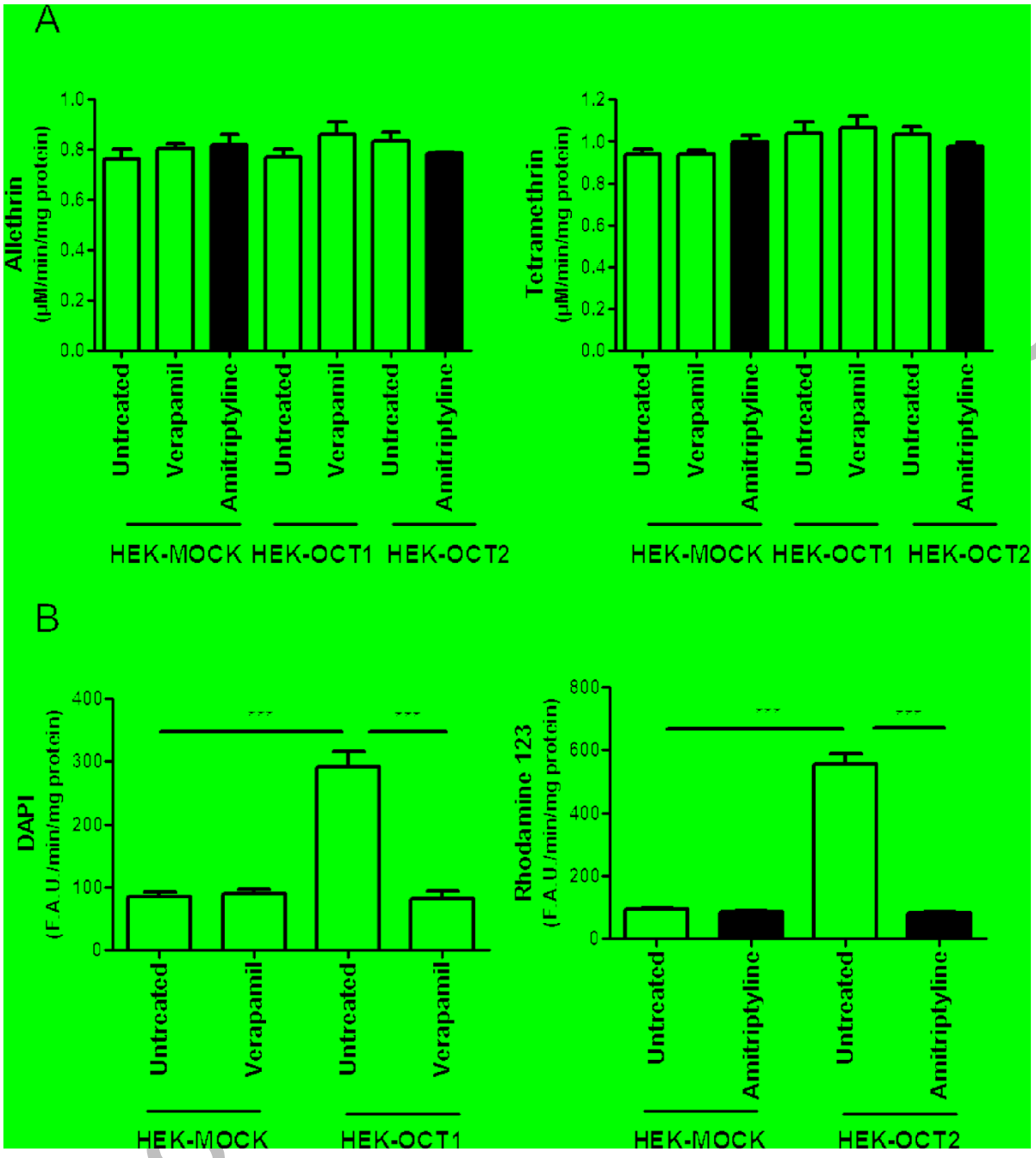
A
Allethrin
(µM/min/mg protein)
1.0
0.8
0.6
0.4
0.2
0.0
Untreated
Verapamil
Amitriptyline
Untreated
Verapamil
Untreated
Amitriptyline
HEK-MOCK
HEK-OCT1
HEK-OCT2
Tetramethrin
(µM/min/mg protein)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Untreated
Verapamil
Amitriptyline
Untreated
Verapamil
Untreated
Amitriptyline
HEK-MOCK
HEK-OCT1
HEK-OCT2
B
DAPI
(F.A.U./min/mg protein)
400
300
200
100
0
***
***
Untreated
Verapamil
Untreated
Verapamil
HEK-MOCK
HEK-OCT1
Rhodamine 123
(F.A.U./min/mg protein)
800
600
400
200
0
***
***
Untreated
Amitriptyline
Untreated
Amitriptyline
HEK-MOCK
HEK-OCT2

Table 1.Overview of the main classes of organic pesticide

Pesticide class	Pesticide examples	Main type of activity	Mechanism of action	Comments
Organophosphorus compounds	Chlorpyrifos, malathion, diazinon, dichlorvos	Insecticide	Irreversible acetylcholinesterase inhibition	Among the most used insecticides
Carbamates	Aldicarb, carbaryl, propoxur,	Insecticide	Reversible acetylcholinesterase inhibition	N-methylcarbamates for the majority
Pyrethroids	Allethrin, deltamethrin, resmethrin, cypermethrin	Insecticide	Voltage-sensitive sodium channel disruption	Among the most used insecticides
Organochlorine compounds	DDT, chlordane, aldrin, endrin, dieldrin, heptachlor, lindane, mirex, chlordecone	Insecticide	Alteration of the electrophysiological properties of cell membranes (particularly nerve axons)	Banned from most countries, but exposure continues due to high remanence of organochlorine pesticides
Rotenoids	Rotenone	Insecticide	mitochondrial respiratory chain inhibition	
Nicotine and neonicotinoids	Nicotine, imidacloprid, thiacloprid, acetamiprid, nitenpyram	Insecticide	Activation of the nicotinic acetylcholine receptor	
Formadinines	Chlordimeform, amitraz	Insecticide	Activation of octopamine receptor (insects) or α_2-adrenergic receptor (mammals)	
Phenylpyrazoles	Fipronil	Insecticide	Blockage of $GABA_A$-gated chloride channel	
Avermectins	Ivermectin	Insecticide/ antiparasitic	Activation of glutamate-dependent chloride channels	
Miscellaneous	N,N-diethyl-3-methylbenzamide (DEET)	Insect repellent	Inhibition of olfactory receptors of insecticides	30% of the USA population uses DEET every year
Phenoxy Compounds	2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-chloro-2-methylphenoxyacetic acid (MCPA), fluazifop-butyl	Herbicide	Chemical analogs of auxin	Among the most used herbicides
Bipyridil compounds	Paraquat, diquat	Herbicide	Photosynthesis inhibition	Among the most commonly used herbicides
Chloroacetanilides	Alachlor, acetochlor, metolachlor, methoxychlor	Herbicide	Inhibition of cyclisation enzymes, part of the gibberellin pathway	
Triazines	Atrazine, simazine, propazine	Herbicide	Inhibition of photosynthesis	
Phosphonomethyl amino acids	Glyphosate, glufosinate	Herbicide	Inhibition of amino acid synthesis	Most widely used herbicides worldwide
Phenylurea	Diuron	Herbicide	Inhibition of photosynthesis	
Chloroalkylthiol fungicides	Captan, folpet	Fungicide	Thiol reactant inhibiting respiration	
Dithiocarbamates	Maneb, ziram, zineb, mancozeb, thiram	Fungicide	Release of carbon disulfide	Often associated with metal cations
Halogenated benzonitrile	Chlorothalonil	Fungicide	Reduction of fungal intracellular glutathione molecules to alternate forms	Among the most used fungicide in the USA
Benzimidazoles	Benomyl, carbendazim	Fungicide	Inhibition of fungal growth by tubulin binding	
Azols	Clotrimazole, propiconazole, Epoxiconazole, enilconazole	Fungicide	Inhibition of lanosterol 14 α-demethylase	
Dicarboximides	Vinclozolin	Fungicide	Lipid synthesis inhibition	Banned in several countries
Coumarines/ indan-1,3 -dione derivatives	Warfarin, diphacinone	Rodenticide	Anticoagulant/ Anti-vitamin K	

Table 2. Classification of main drug transporters

Transporter family	Transporter	Main expression	Main type of substrates
ABCB	P-gp (*ABCB1*)	Intestine, liver, kidney, blood-brain barrier	Hydrophobic compounds
	BSEP (*ABCB11*)	Liver	Bile acids
ABCC	MRP1 (*ABCC1*)	Ubiquitous	Hydrophobic compounds, hydrophilic anions, conjugates
	MRP2 (*ABCC2*)	Intestine, liver, kidney	Hydrophilic anions, conjugates
	MRP3 (*ABCC4*)	Liver, kidney	Hydrophilic anions, conjugates
	MRP4 (*ABCC4*)	Liver, kidney, blood-brain barrier	Nucleotides
	MRP5 (*ABCC5*)	Ubiquitous	Nucleotides
ABCG	BCRP (*ABCG2*)	Intestine, liver, kidney, blood-brain barrier, stem cells	Hydrophobic compounds, hydrophilic anions, conjugates
SLCO	OATP1B1 (*SLCO1B1*)	Liver	Organic anions
	OATP1B3 (*SLCO1B3*)	Liver	Organic anions
	OATP2B1 (*SLCO2B1*)	Liver, intestine	Organic anions
SLC10A	NTCP (*SLC10A1*)	Liver	Bile acids
SLC22A	OCT1 (*SLC22A1*)	Liver	Organic cations
	OCT2 (*SLC22A2*)	Kidney	Organic cations
	OCT3 (*SLC22A3*)	Ubiquitous	Organic cations
	OAT1 (*SLC22A6*)	Kidney	Organic anions
	OAT2 (*SLC22A7*)	Liver	Organic anions
	OAT3 (*SLC22A8*)	Kidney	Organic anions
SLC47A	MATE1 (*SLC47A1*)	Liver, kidney	Organic cations
	MATE2-K (*SLC47A2*)	Kidney	Organic cations

Table 3. Inhibitory effects of pesticides towards drug transporter activities according to organic pesticide classes.

Transporter	Percentage of inhibitory pesticides[a] (n = total number of tested pesticides)				
	Organophosphorus pesticides	Organochlorine pesticides	Pyrethroids	Carbamates	Chloroacetanilides
P-gp	76.9 % (n=13) [17, 19]	30.8 % (n=13) [12, 17]	0.0 % (n=14) [18]	0.0 % (n=6) [17]	57.1 % (n=7) [28]
MRP1/MRP2	No data	50.0 % (n=8) [12]	14.3 % (n=14) [18]	No data	0.0 % (n=7) [28]
BCRP	66.7 % (n=3) [53]	50.0 % (n=8) [12]	14.3 % (n=14) [18]	100 % (n=2) [53]	0.0 % (n=7) [28]
OCT1	61.5 % (n=13) [59]	62.5 % (n=8) [12]	23.5 % (n=17) [18]	No data	No data
OCT2	61.5 % (n=13) [59]	No data	23.5 % (n=17) [18]	No data	No data
MATE1	23.1 % (n=13) [59]	No data	14.3 % (n=14) [18]	No data	No data
MATE2-K	0 % (n=13) [59]	No data	0 % (n=14) [18]	No data	No data
OATPs	15.4 % (OATP1B1) 0.0 % (OATP2B1) (n=13) [59]	0.0 % (Total OATP activity) (n=8) [12]	14.3 % (OATP1B1), 0.0 % (OATP1B3) (n=14) [18]	No data	No data
OAT1	0 % (n=13) [59]	No data	0.0 % (n=17) [18]	No data	No data
OAT3	23.1 % (n=13) [59]	No data	23.5 % (n=17) [18]	No data	No data

[a]Pesticide concentrations are usually set at 100-250 µM

Table 4: Pesticides substrates for drug transporters

Transporter	Substrate	Not substrate
P-gp	Paraquat [31], ivermectin [38, 39], endosulfan (weak substrate) [17]	Propiconazole [25], deltamethrin [41], *cis*-permethrin [41], *trans*-permethrin [41], warfarin [42], cyperquat/MPP+ [29]
MRP1	Methoxychlor [50], fenitrothion [50], chlorpropham [50], ivermectin [38]	
BCRP	Warfarin [54]	Ivermectin [55]
OCT1	Cyperquat/MPP+ [60]	Allethrin (present study), tetramethrin (present study), paraquat [65], fenamiphos [59], phosmet [59]
OCT2	Cyperquat/MPP+ [60], paraquat [65]	Allethrin (present study), tetramethrin (present study), fenamiphos [59], phosmet [59]
OCT3	Cyperquat/MPP+ [61]	Paraquat [65]
MATE1	Cyperquat/MPP+ [62], paraquat [65]	No data
MATE2-K	Cyperquat/MPP+ [63]	No data
OAT1	2,4-dichlorophenoxyacetic acid (2,4-D) [71]	No data
OAT3	2,4-dichlorophenoxyacetic acid (2,4-D) [71]	No data