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Inhibition of human drug transporter activities by succinate dehydrogenase inhibitors

Marie Kerhoas¹, Marc Le Vée¹, Jennifer Carteret¹, Elodie Jouan¹, Valentin Tastet¹, Arnaud Bruyère¹, Laurence Huc^{1,2} and Olivier Fardel³

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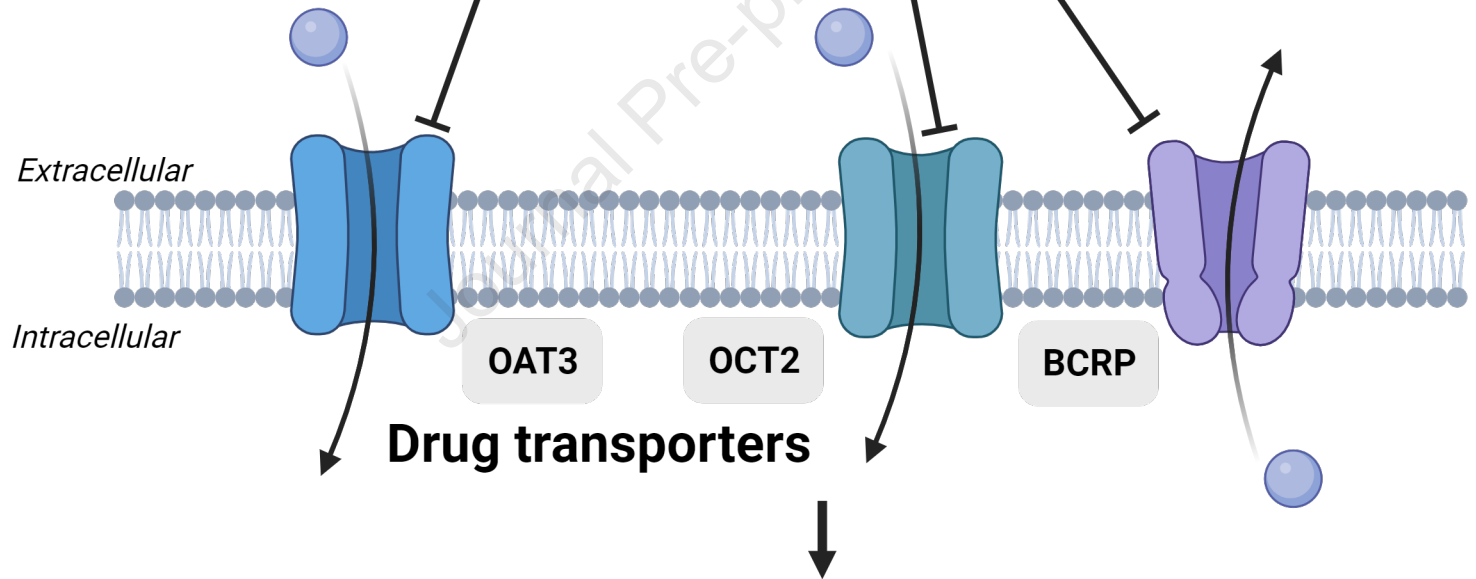
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SDHIs fungicides

Endogenous substrates
(*e.g* hormones, metabolites)
Exogenous substrates
(*e.g* drugs, pesticides)

Benzovindiflupyr
Bixafen
Fluxapyroxad
Pydiflumetofen
Sedaxane



Drug transporters



**Human health risks ?
Toxicity ?**

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27 Abstract

28 Succinate dehydrogenase inhibitors (SDHIs) are widely-used fungicides, to which humans are
29 exposed and for which putative health risks are of concern. In order to identify human molecular
30 targets for these environmental chemicals, the interactions of 15 SDHIs with activities of main
31 human drug transporters implicated in pharmacokinetics were investigated *in vitro*. 5/15
32 SDHIs, *i.e.*, benzovindiflupyr, bixafen, fluxapyroxad, pydiflumetofen and sedaxane, were
33 found to strongly reduce activity of the renal organic anion transporter (OAT) 3, in a
34 concentration-dependent manner (with IC₅₀ values in the 1.0-3.9 μM range), without however
35 being substrates for OAT3. Moreover, these 5/15 SDHIs decreased the membrane transport of
36 estrone-3 sulfate, an endogenous substrate for OAT3, and sedaxane was predicted to inhibit *in*
37 *vivo* OAT3 activity in response to exposure to the acceptable daily intake (ADI) dose. In
38 addition, pydiflumetofen strongly inhibited the renal organic cation transporter (OCT) 2
39 (IC₅₀=2.0 μM) and benzovindiflupyr the efflux pump breast cancer resistance protein (BCRP)
40 (IC₅₀=3.9 μM). Other human transporters, including organic anion transporting polypeptide
41 (OATP) 1B1 and OATP1B3 as well as multidrug and toxin extrusion protein (MATE) 1 and
42 MATE2-K were moderately or weakly inhibited by SDHIs, whereas P-glycoprotein, multidrug
43 resistance-associated protein (MRP), OCT1 and OAT1 activities were not or only marginally
44 impacted. Then, some human drug transporters, especially OAT3, constitute molecular targets
45 for SDHIs. This could have toxic consequences, notably with respect to levels of endogenous
46 compounds and metabolites substrates for the considered transporters or to potential SDHI-drug
47 interactions. This could therefore contribute to putative health risk of these fungicides.

48

49 Keywords

50 Drug transporters; fungicides; health risks; organic anion transporter 3; succinate
51 dehydrogenase inhibitors; toxicokinetics

52 1. Introduction

53 Succinate dehydrogenase inhibitors (SDHIs) constitute an important family of chemical
54 fungicides, primarily targeting the mitochondrial complex II of the respiratory chain (RCII),
55 also known as succinate dehydrogenase (SDH) (Li et al., 2021; Luo and Ning, 2022). SDH
56 transfers electrons to the quinone pool directly in the mitochondrial respiratory chain (through
57 ubiquinone reductase activity); it also catalyzes the oxidation of succinate to fumarate in the
58 tricarboxylic acid/Krebs cycle (through SDH activity). Consequently, SDHIs affect both
59 oxidative phosphorylation and Krebs cycle in fungi, thus inhibiting the growth of these
60 pathogens (Li et al., 2021). These chemicals are increasingly used on cereal crops, fruits,
61 vegetables, and seeds (Umetsu and Shirai, 2020). Humans are exposed to them *via* the
62 respiratory and/or dermal way in the case of occupational or residential exposure, but also *via*
63 the food chain, through the ingestion of contaminated food and drink (Béranger et al., 2020;
64 Ottenbros et al., 2023; Polledri et al., 2019). SDHIs are regulated substances, which have not
65 been deemed to be a health concern. Nevertheless, these fungicides can interact with SDH from
66 various species, including mammals, worm and bee (Mowery et al., 1977, 1976). They exert
67 toxic effects towards various non-target organisms like earthworms (Ji et al., 2023), zebrafish
68 (Chen et al., 2022; Lin et al., 2021) and bees (Simon-Delso et al., 2018), and some interrogations
69 towards possible deleterious effects of SDHIs towards humans have recently emerged, but
70 remain to be clearly documented (Bénil et al., 2022; Bouillaud, 2023; Yanicostas and Soussi-
71 Yanicostas, 2021). In this context, interactions of SDHIs with human cellular and/or molecular
72 targets may be particularly informative through providing clues to predict toxicity according to
73 a mechanistic/adverse outcome pathway approach. Some SDHIs have thus been recently
74 demonstrated to inhibit *in vitro* activity of human SDH, but also that of human RCIII, also
75 termed Q-cytochrome c oxidoreductase, with possible consequences in term of cell death for
76 cultured cells exposed to these fungicides (Bénil et al., 2019; d'Hose et al., 2021). Mechanistic

77 studies on the molecular interactions of SDHIs with human cellular and molecular targets are
78 however lacking, particularly at real exposure levels.

79 Drug transporters are membrane proteins acting as uptake or efflux proteins across
80 membranes, especially the plasma membrane, for drugs and other xenobiotics (Giacomini et
81 al., 2010). These transporters belong to either the solute carrier (SLC) or the ATP-binding
82 cassette (ABC) transporter families (Roberts, 2021). They are expressed in key cells implicated
83 in drug disposition, including enterocytes, hepatocytes, endothelial cells of the blood-brain
84 barrier and renal proximal tubular cells (Giacomini et al., 2010). Therefore, they play major
85 roles in different steps of pharmacokinetics, such as intestinal absorption, drug distribution
86 across blood-tissue barriers, and liver and kidney excretion (Liu, 2019). Importantly, inhibition
87 of their activity by drugs acting as “perpetrators” can result in (i) drug-drug interactions, by
88 reducing membrane passage of “victims” drugs (Müller and Fromm, 2011), or (ii) drug toxicity,
89 by blocking the transport of endogenous substrates (Hafey et al., 2022).

90 There is growing evidence that chemical pollutants interact with drug transporters
91 (Clerbaux et al., 2019; Michel et al., 2023; Nicklisch and Hamdoun, 2020; Tastet et al., 2023a).
92 Environmental contaminants belonging to various chemical classes thus inhibit transporters
93 and/or are substrates for them, as notably demonstrated by *in vitro* assays (Clerbaux et al., 2018;
94 Guéniche et al., 2020a). Then, drug transporters can be considered as possible human molecular
95 targets for pollutants, including pesticides, with consequences in term of pollutant
96 toxicokinetics and toxicity (Darney et al., 2020; Fardel et al., 2012; Michel et al., 2023). The
97 status of most of pesticides with respect to potential modulation of drug transporter activities
98 remains however to be established, notably for SDHIs. The present study was consequently
99 designed to determine whether SDHIs can impair human drug transporter activities, in order to
100 get original information about (i) the human molecular targets of SDHIs and (ii) the interactions
101 of pollutants such as SDHIs with drug transporters. Fifteen fungicides SDHIs, whose chemical

102 structures are shown in Fig. S1, were investigated: ten SDHIs currently authorised in the
103 European Union (benzovindiflupyr, bixafen, boscalid, fluopyram, flutolanil, fluxapyroxad,
104 isofetamid, penflufen, penthiopyrad and sedaxane), two recently withdrawn from the market
105 (carboxin and isopyrazam, withdrawn in 2020 and 2021, respectively), one pending marketing
106 authorization (pydiflumetofen) and two not authorised at European level (furametpyr and
107 thifluzamide). The studied human transporters were mainly those of concern for drug agencies
108 (Lee et al., 2017): the ABC efflux pumps P-glycoprotein (P-gp/*ABCB1*), breast cancer
109 resistance protein (BCRP/*ABCG2*) and multidrug resistance-associated proteins
110 (MRPs/*ABCCs*), SLC transporters for organic cations (organic cation transporter (OCT)
111 1/*SLC22A1*, OCT2/*SLC22A2*, multidrug and toxin extrusion protein (MATE) 1/*SLC47A1* and
112 MATE2-K/*SLC47A2*) and SLC transporters for organic anions (organic anion transporting
113 polypeptide (OATP) 1B1/*SLCO1B1*, OATP1B3/*SLCO1B3*, organic anion transporter (OAT)
114 1/*SLC22A6* and OAT3/*SLC22A8*). Results showed a SDHI- and transporter-dependent
115 regulation of drug transporter activities by these fungicides. This demonstrates that SDHIs
116 interact with human SDH-unrelated molecular targets such as transporters, especially OAT3,
117 and, beyond, re-enforces the conclusion that transporters represent common targets for
118 pollutants.

119

120 2. Materials and Methods

121 2.1. Chemicals

122 SDHIs fungicides were provided by LGC group (Teddington, Middlesex, UK).
123 Amitriptyline, cyclosporin A, 2',7'-dichlorofluorescein (DCF), fumitremorgin C, glutaric acid,
124 probenecid, rhodamine 123, rifamycin SV, sulfobromophthalein (BSP), and 6-
125 carboxyfluorescein (6-CF) were supplied by Sigma-Aldrich (Saint-Quentin-Fallavier, France).

126 Carboxy-2',7'-dichlorofluorescein (CDCF) diacetate and Hoechst 33342 were from Life
127 Technologies (Villebon-sur-Yvette, France). 8- (2- [Fluoresceinyl]aminoethylthio) adenosine-
128 3', 5'- cyclic monophosphate (8-FcA) and 4-(4-(dimethylamino)styryl)-N-methylpyridinium
129 iodide (ASP⁺) were provided by Biolog Life Institute (Bremen, Germany) and Thermo Fisher
130 Scientific (Waltham, MA, USA), respectively. Radiolabeled 3,4-[ring-2,5,6-³H]
131 dihydroxyphenylethylamine (Dopamine) (specific activity = 37.8 Ci/mmol), [6,7-³H(N)]-
132 estrone-3-sulfate (E3S) (specific activity = 51.8 Ci/mmol), and N-[methyl-³H]-methyl-4-
133 phenylpyridinium acetate (MPP) (specific activity = 82.9 Ci/mmol) were from PerkinElmer
134 (Villepinte, France). Stock solutions of SDHIs and chemicals were prepared in dimethyl
135 sulfoxide (DMSO), except for probenecid diluted in NaOH and rhodamine 123 prepared in
136 ethanol. Control cells were treated with 0.1% (vol/vol) DMSO.

137 2.2. Cell culture

138 Human HEK-293 cells stably overexpressing human SLC transporters, *i.e.*, OCT1,
139 OCT2, MATE1, MATE2-K, OATP1B1, OATP1B3, OAT1 or OAT3, as well as control HEK-
140 MOCK cells, have been previously described and characterized (Chedik et al., 2017; Sayyed et
141 al., 2019). They were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented
142 with 10% (vol/vol) fetal calf serum (FCS), 1% (vol/vol) MEM non-essential amino acids
143 solution (Life Technologies), 1 µg/mL bovine insulin, 20 IU/mL penicillin, and 20 µg/mL
144 streptomycin (Sigma Aldrich).

145 Mammary MCF7R cells, overexpressing human P-gp (Jouan et al., 2016b), and
146 hepatoma HuH-7 cells, expressing MRP2/*ABCC2* and MRP4/*ABCC4* (Jouan et al., 2016a),
147 were cultured in DMEM containing 4.5 g/L D-glucose (Life Technologies), supplemented with
148 10% (vol/vol) FCS, 20 IU/mL penicillin and 20 µg/mL streptomycin, as already reported
149 (Chedik et al., 2017). Human BCRP-transfected HEK-293 cells (HEK-BCRP cells), donated
150 by Pr X. Decleves (Faculty of Pharmacy, Université Paris Cité, Paris, France), were cultured in

151 DMEM supplemented with 10% (vol/vol) FCS, 100 µg/mL erythromycin, 100 IU/mL
152 amoxicillin, and 2000 µg/mL G418.

153 2.3. *Transporter inhibition assays*

154 Fluorescent or radio-labeled reference substrates and reference inhibitors were used to
155 evaluate the inhibitory effects of SDHIs on SLC and ABC transporter activities, through
156 measuring cellular accumulation or retention of the reference substrates in the absence (control
157 condition) or presence of SDHIs or reference inhibitors, in transporter-overexpressing cells, as
158 previously described (Chedik et al., 2017; Tastet et al., 2023b). The incubation times, analytical
159 methods, transporter-overexpressing cells and reference substrates and inhibitors used for these
160 transport assays have been previously described and validated (Bruyère et al., 2021; Guéniche
161 et al., 2020b) and are summarized in Table S1. These transport assays were performed in a
162 dedicated assay medium composed of 136 mM NaCl, 1.1 mM KH₂PO₄, 5.3 mM KCl, 1.8 mM
163 CaCl₂, 0.8 mM MgSO₄, 10 mM HEPES, 11 mM D-glucose and adjusted to pH 7.4 (excepted
164 for MATE transporter assays, for which pH was adjusted to 8.4) (Chedik et al., 2017). Cellular
165 accumulation or retention of fluorescent reference substrates was determined by
166 spectrofluorimetry using an EnSpire[®] spectrofluorometer (PerkinElmer), whereas cellular
167 accumulation of radiolabeled substrates was analyzed by scintillation counting using a Tri-
168 Carb[®] 2910TR analyzer (PerkinElmer). The results were finally normalized to cellular protein
169 content, determined by the Bradford method (Bradford, 1976).

170 Data were expressed as percentages of substrate accumulation in control cells not exposed
171 to reference inhibitors or SDHIs, arbitrarily set at 100%, or as transporter activity percentages,
172 as described in the following equations (Tastet et al., 2023b):

173 For SLC transporters:

174 Transporter activity (%)

$$175 = \frac{([Substrate_{SDHI}] - [Substrate_{Reference\ inhibitor}]) \times 100}{[Substrate_{Control} - Substrate_{Reference\ inhibitor}]} \quad (A)$$

176 For ABC transporters:

$$177 \text{ Transporter activity (\%)} = \frac{([Substrate_{SDHI}] - [Substrate_{Control}]) \times 100}{[Substrate_{Reference\ inhibitor} - Substrate_{Control}]} \quad (B)$$

178

179 with $[Substrate_{SDHI}]$ = cellular concentration of the reference substrate incubated in the
 180 presence of one SDHI, $[Substrate_{Reference\ inhibitor}]$ = cellular concentration of the reference
 181 substrate incubated with the reference inhibitor and $[Substrate_{Control}]$ = cellular concentration
 182 of the reference substrate in control cells not incubated with SDHIs or the reference inhibitor.

183 The percentage of inhibition of transporter activity by SDHI was as following:

$$184 \text{ Transporter activity inhibition (\%)} = 100\% - \text{transporter activity (\%)} \quad (C)$$

185 with transporter activity (%) determined as described in equation (A) (for SLC transporters) or
 186 equation (B) (for ABC transporters).

187 The half maximal inhibitory concentration (IC_{50}) values of SDHIs towards transporter
 188 activities were determined from nonlinear regression of concentration-response data based on
 189 the four-parameter logistic function. They were calculated using the Prism 8.3 software
 190 (GraphPad, San Diego, CA, USA), through the following equation:

$$191 A = \frac{100}{1 + 10^{([I] - \text{Log}(IC_{50})) \times \text{Hill slope}}} \quad (D)$$

192 with A = percentage of transporter activity for a given concentration of SDHI, determined as
 193 described in equation (A) or equation (B), [I] = the SDHI concentration in the medium, and Hill
 194 slope = a coefficient describing the steepness of the curve.

195 The inhibitory constant (K_i) value for OAT3 inhibition by SDHIs was estimated using the
 196 Cheng-Prusoff equation (E), as previously reported (Zou et al., 2021):

$$197 \quad K_i = \frac{IC_{50}}{1 + \frac{S}{K_m}} \quad (E)$$

198 with SDHI IC_{50} value experimentally determined as described in equation (D), $S = 6\text{-CF}$
 199 concentration in the transport assay medium ($10 \mu\text{M}$) and $K_m =$ Michaelis constant for transport
 200 of 6-CF by OAT3 ($K_m = 10.7 \mu\text{M}$) (Zou et al., 2021).

201 *2.4. Chemical combination assays*

202 The fungicide SDHI bixafen and the fungicide prothioconazole (belonging to the
 203 chemical class of triazolinthiones) are frequently used together in commercial formulations, as
 204 notably reported by the French Agency for Food, Environmental and Occupational Health &
 205 Safety (ANSES) (<https://ephy.anses.fr/>). Their combined effects towards OAT3 activity were
 206 therefore investigated through median drug effect analysis, as previously described (Chou,
 207 2006). For this purpose, combinations of bixafen and prothioconazole were tested on OAT3-
 208 mediated transport of 6-CF, using serial dilutions with several concentration points below and
 209 above the IC_{50} values of each fungicide used as a single chemical. The ratio of the two chemicals
 210 in these combinations was chosen based on their respective IC_{50} values (Chou, 2010) and was
 211 fixed at 3:1 (Bixafen:Prothioconazole). Experimental data from combined and individual
 212 chemical effects towards OAT3 activity were finally processed using the CompuSyn program
 213 (ComboSyn, Inc., Paramus, New Jersey, USA), enabling calculation of the combination index
 214 (CI) values according to the following equation (Zhao et al., 2004):

$$215 \quad CI = \frac{C_{Bixafen/mixture}}{C_{Bixafen}} + \frac{C_{Prothioconazole/mixture}}{C_{Prothioconazole}} \quad (F)$$

216 where $C_{Bixafen/mixture}$ and $C_{Prothioconazole/mixture}$ are the concentrations of bixafen and
 217 prothioconazole used in combination to achieve x % percentage of OAT3 activity inhibition

218 and C_{Bixafen} and $C_{\text{Prothioconazole}}$ are the concentrations of individual substances achieving the same
219 efficacy. Combination effects were considered as synergistic when $CI < 0.8$, as additive when
220 $0.8 \leq CI \leq 1.2$ and as antagonistic when $CI > 1.2$ (Bijnsdorp et al., 2011).

221 2.5. *Transporter trans-stimulation assays*

222 *Trans*-stimulation assays were performed in HEK-OAT3 cells using the OAT3 substrate
223 6-CF as a reference tracer substrate and glutaric acid as a reference *trans*-stimulating agent
224 (Apiwattanakul et al., 1999; Sayyed et al., 2017). For these *trans*-stimulation assays, HEK-
225 OAT3 cells were first incubated with 1 mM glutaric acid or various concentrations of SDHIs
226 for 15 min at 37 °C. After washing with phosphate-buffered saline (PBS), cells were next re-
227 incubated with 10 µM 6-CF for 5 min at 37 °C in the transport assay medium described above.
228 Intracellular accumulation of the fluorescent dye was finally determined by spectrofluorimetric,
229 using excitation and emission wavelengths shown in Table S1. Data were expressed as
230 percentages of 6-CF accumulation found in control cells not exposed to glutaric acid or SDHIs.

231 2.6. *SDHI accumulation assays*

232 HEK-MOCK and HEK-OAT3 cells were incubated for 5 min at 37°C with 10 µM SDHI
233 or the reference OAT3 substrate 6-CF, in the transporter assay medium described above, and in
234 the absence (control) or presence of 2 mM probenecid used here as a reference OAT3 inhibitor.
235 Cells were next washed twice with ice-cold PBS and lysed in distilled water. Cellular proteins
236 were next precipitated using 50 % (vol/vol) acetonitrile. After 2 h centrifugation at 4500 rcf and
237 4°C, supernatants were collected, diluted (1:1) in an aqueous solution of the internal standard
238 (caffeine) with 0.1% formic acid, and analysed using a liquid chromatography-tandem mass
239 spectroscopy (LC-MS/MS) system. This system corresponds to a high-performance LC Aria
240 system (Agilent, Les Ulis, France), equipped with a Kromasyl C18 (4.6 × 150 mm) column
241 (Interchim, Montluçon, France) and coupled to a tandem mass spectrometry TSQ Quantum

242 Ultra (Thermo Fisher Scientific, Villebon sur Yvette, France), fitted with an electrospray
243 ionization source (ESI+). Monitored ion transitions for SDHIs and caffeine are indicated in
244 Table S2. Cellular accumulation of 6-CF was investigated by spectrofluorimetry. Cellular
245 accumulations of SDHIs and 6-CF were finally normalized to protein content.

246 *2.7. Molecular descriptor generation*

247 274 molecular descriptors belonging to the blocks “constitutional indices” (n = 47),
248 “geometrical descriptors” (n = 38), “functional group counts” (n = 154), “charge descriptors”
249 (n = 15) and “molecular properties” (n = 20) were determined for SDHIs using the Dragon 7.0
250 software (Kode Chemoinformatics, Pisa, Italy) (See [https://chm.kode-solutions.net/
251 products_dragon_descriptors.php](https://chm.kode-solutions.net/products_dragon_descriptors.php) for a complete list of these descriptors). SDHIs, initially
252 expressed in SMILES format using the PubChem website (<https://pubchem.ncbi.nlm.nih.gov/>),
253 were converted to 3D format using the MarvinView software (ChemAxon, Budapest, Hungary)
254 before processing by the Dragon 7.0 software to obtain molecular descriptors.

255 *2.8. Prediction of in vivo modulation of transporter activity by SDHIs*

256 The *in vivo* inhibition of transport activities by SDHIs was predicted from *in vitro* generated
257 data using the criteria defined by the International Transporter Consortium (ITC) (Giacomini
258 et al., 2010). Briefly, drug transporters present in the liver, kidney or blood-tissue barriers can
259 be inhibited *in vivo* by plasma concentrations of xenobiotics if the ratio maximum unbound
260 plasma concentration of chemicals ($I_{\max,u}$)/ IC_{50} towards activity ≥ 0.1 . Because data about
261 human pharmacokinetics of SDHIs are currently not available, $I_{\max,u}$ values for SDHIs were
262 evaluated through considering the acceptable daily intake (ADI) values for SDHIs provided by
263 the European Food Safety Authority (EFSA, <https://www.efsa.europa.eu/fr>), as previously
264 described (Bénit et al., 2019; d’Hose et al., 2021). Briefly, ADIs permitted to calculate the
265 maximal theoretical doses of SDHIs ingested at once by a 70 kg-weighting subject for 24 h.

266 These ingested doses were considered to be fully absorbed and distributed into the standard
267 volume (5 L) of blood for the subjects, allowing thus to determine the maximal total blood
268 concentrations of SDHI (I_{max}), as the ratio “ingested dose/blood volume”. The $I_{max,u}$ values are
269 next calculated after taking into account the unbound plasma fractions of SDHIs, predicted *in*
270 *silico* using the online tool Admetlab2.0 (<https://admetmesh.scbdd.com/>). With respect to the
271 P-gp and BCRP expressed at the brush-border membrane of enterocytes, it can be predicted that
272 they are inhibited *in vivo* by luminal gut concentrations of xenobiotics if the ratio luminal gut
273 concentration ($I_{gut}/IC_{50} \geq 10$). I_{gut} is defined by the ratio “orally ingested dose/250 mL”
274 according to the ITC (Giacomini et al., 2010), with the orally ingested dose calculated as
275 described above.

276 2.9. Statistical analysis

277 Data were usually expressed as means \pm SEM from at least three independent
278 experiments, each being performed in technical triplicate. They were statistically analyzed
279 using Prism 8.3 software through analysis of variance (ANOVA) followed by Dunnett's or
280 Tukey's *post-hoc* test. Correlation between molecular descriptor indexes and percentages of
281 transporter activity inhibition by SDHIs was done through Spearman rank correlation, whereas
282 comparison between SDHI and drug K_i values was performed using the Student's *t*-test. The
283 criterion of significance for statistical tests was $p < 0.05$.

284

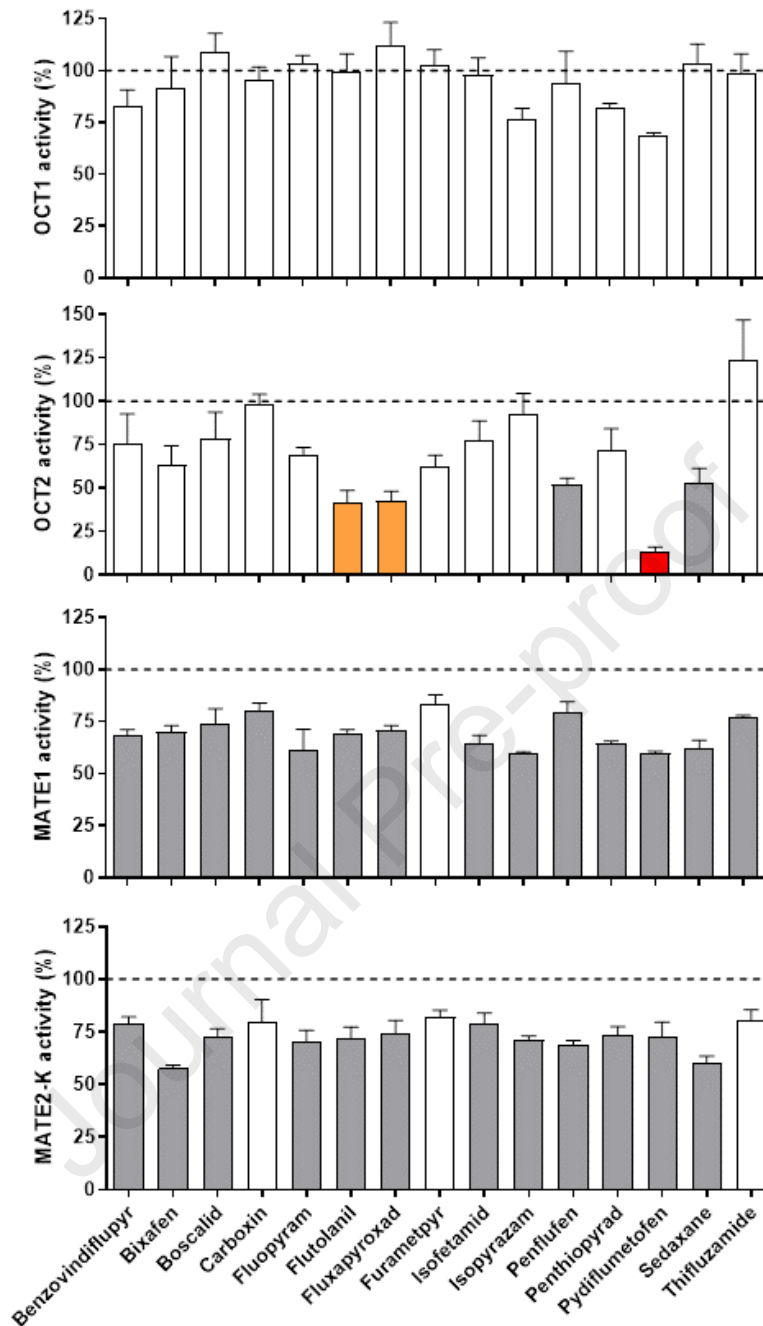
285 3. Results

286 3.1. Effects of SDHIs towards drug transporter activities

287 The concentration of SDHIs initially tested towards transporter activities was 10 μ M, in
288 the range of those previously used in SDHI studies with cultured cells (Bénil et al., 2019;
289 d'Hose et al., 2021). Data related to inhibition of SLC and ABC transporters by this 10 μ M

290 SDHI concentration are shown in Fig. 1, Fig. 2 and Fig. 3 and are summarized in Table S3.
291 SDHIs were considered as inhibitors when they significantly reduced transporter activities
292 ($p < 0.05$); inhibitions were ranked as strong, moderate or weak when transporter inhibition
293 percentage $\geq 66.6\%$ (red histograms on the Fig. 1, Fig. 2 and Fig. 3), $50\% \leq$ transporter
294 inhibition percentage $< 66.6\%$ (orange histograms), or transporter inhibition percentage $< 50\%$
295 (grey histograms), respectively.

296 With respect to SLCs handling organic cations (Fig. 1), OCT1 was not inhibited by any
297 SDHI, whereas MATE1 and MATE2-K were weakly inhibited by all SDHIs (except furametpyr
298 for MATE1 and MATE2-K and carboxin and thifluzamide for MATE2-K, without significant
299 effects). OCT2 activity was reduced by 5/15 SDHIs: Penflufen and sedaxane behaved as weak
300 inhibitors, whereas fluxapyroxad and flutolanil were moderate inhibitors and pydiflumetofen a
301 strong inhibitor (Fig. 1).



302

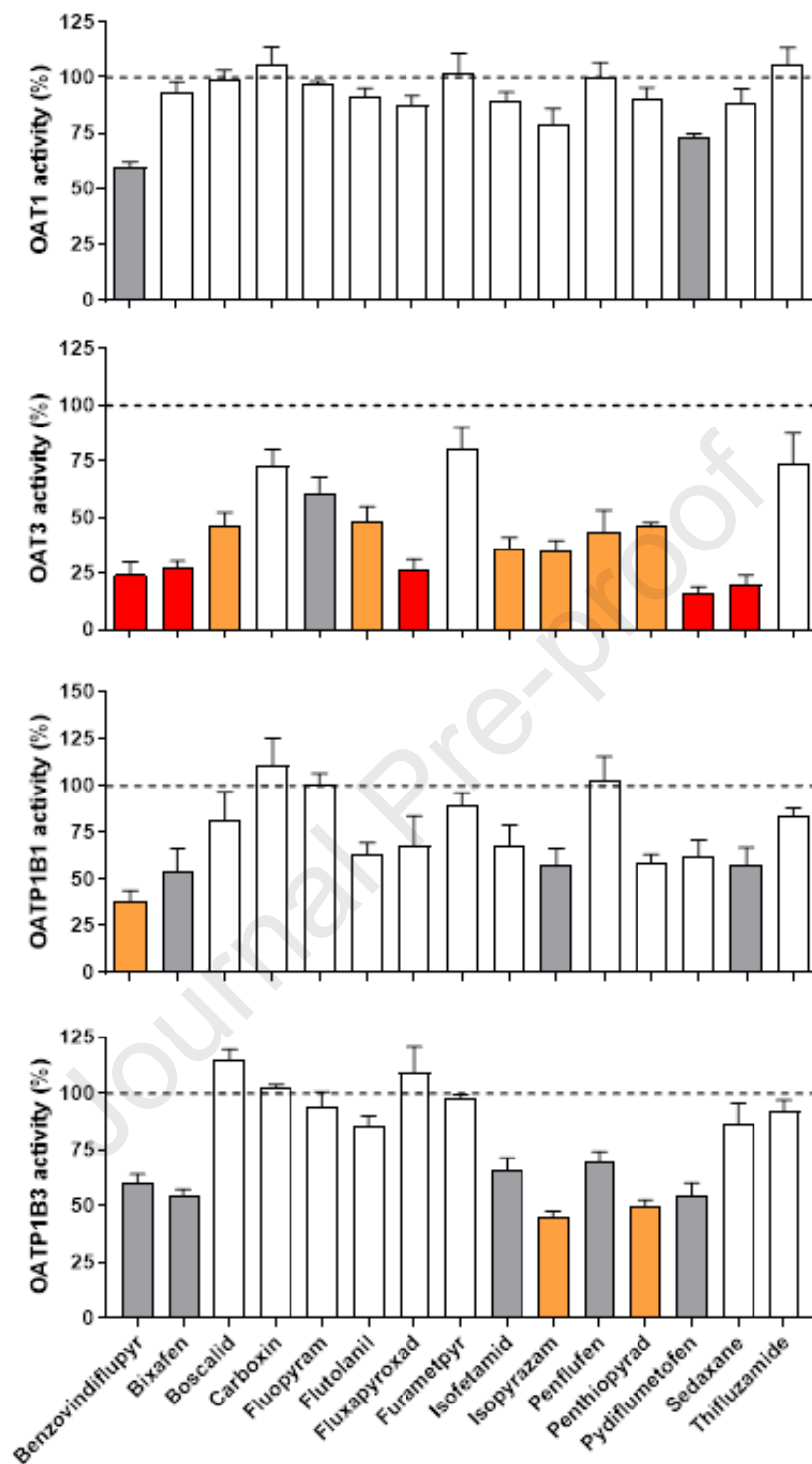
303 *Fig. 1. Effects of 10 μM SDHIs on activities of SLC transporters handling organic cations*
 304 *(OCTs and MATEs). Transporter substrates are ASP⁺(for OCTs) and [³H]-MPP (For MATEs).*
 305 *Data are expressed as % of transporter activity found in control cells not exposed to SDHIs,*
 306 *arbitrarily set at 100 % and indicated by dashed lines on the graphs. SDHIs inhibiting*
 307 *transporter activity (p < 0.05) are defined as strong inhibitors (transporter inhibition % ≥*
 308 *66.6%, represented by red histograms), moderate inhibitors (50 % ≤ transporter inhibition %*
 309 *< 66.6%, represented by orange histograms) or weak inhibitors (transporter inhibition % <*
 310 *50%, represented by grey histograms); SDHIs not inhibiting transporter activity (p ≥ 0.05)*

311 correspond to white histograms. Data are the means \pm SEM of at least three independent
312 experiments.

313

314 For SLCs handling organic anions (Fig. 2), OATP1B1 activity was inhibited by 4/15
315 SDHIs, *i.e.*, bixafen, isopyrazam and sedaxane, acting as weak inhibitors, and benzovindiflupyr,
316 as a moderate inhibitor. OATP1B3 activity was reduced by 7/15 SDHIs: 5 SDHIs
317 (benzovindiflupyr, bixafen, isofetamid, penflufen and pydiflumetofen) were weak inhibitors
318 whereas 2 SDHIs (isopyrazam and penthiopyrad) were moderate inhibitors (Fig. 2). Only 2/15
319 SDHIs decreased OAT1 activity, *i.e.*, benzovindiflupyr and pydiflumetofen, behaving as weak
320 inhibitors (Fig. 2). By contrast, OAT3 was inhibited by 12/15 SDHIs; one (fluopyram) was a
321 weak inhibitor, six (boscalid, flutolanil, isofetamid, isopyrazam, penflufen and penthiopyrad)
322 were moderate inhibitors and five (benzovindiflupyr, bixafen, fluxapyroxad, pydiflumetofen,
323 and sedaxane) were strong inhibitors (Fig. 2).

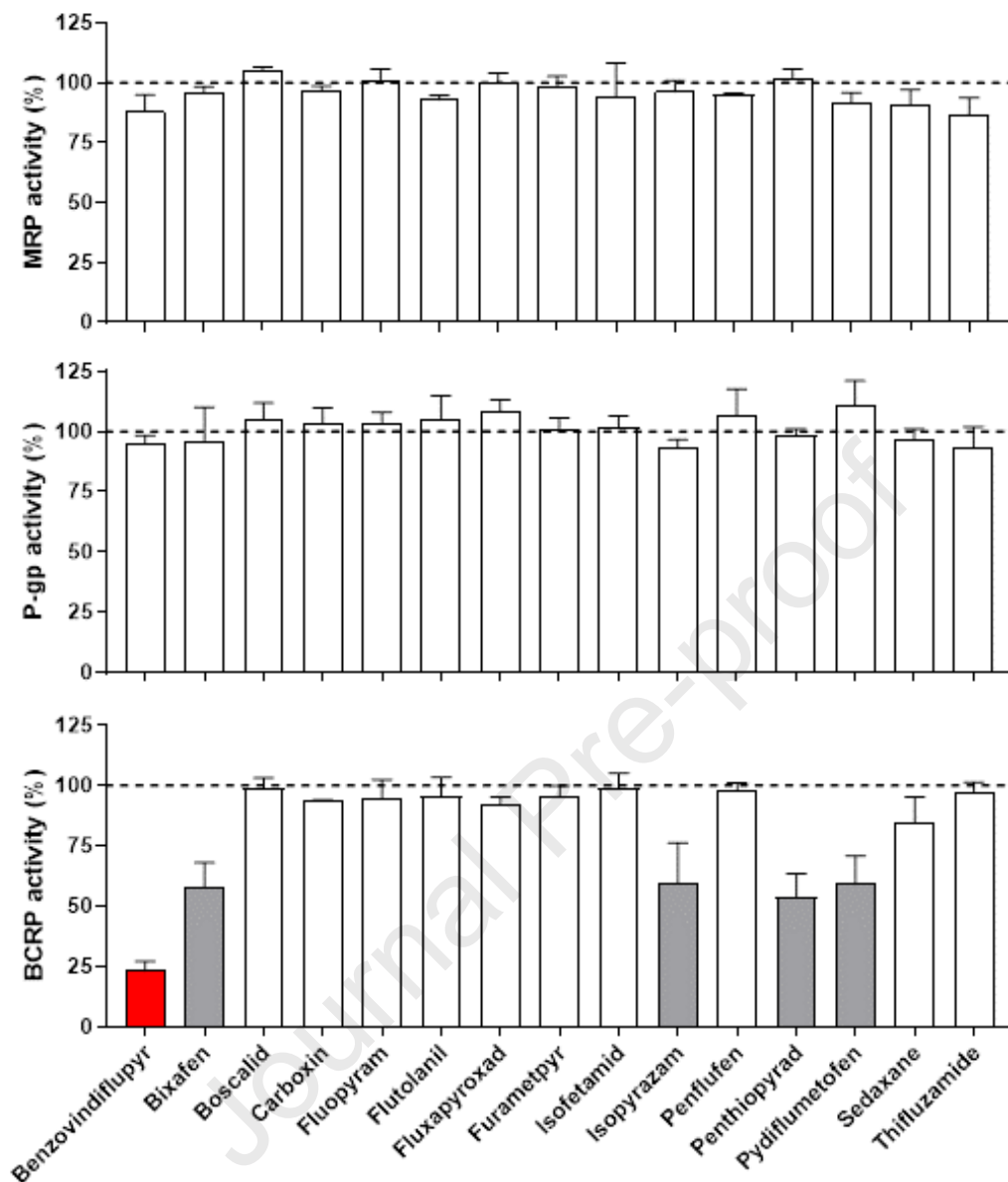
324 For ABC transporters (Fig. 3), SDHIs inhibited neither P-gp nor MRP activity. By
325 contrast, BCRP activity was inhibited by 5/15 SDHIs, with four (bixafen, isopyrazam,
326 penthiopyrad and pydiflumetofen) acting as weak inhibitors and one (benzovindiflupyr) as a
327 strong inhibitor.



328

329 *Fig. 2. Effects of 10 μM SDHIs on activities of SLC transporters handling organic anions*
 330 *(OATPs and OATs). Legend is the same as in Fig. 1, except for transporter substrates which*
 331 *are 6-CF (for OATs), DCF (for OATP1B1) and 8-FcA (for OATP1B3).*

332



333

334 *Fig. 3. Effects of 10 μ M SDHIs on activities of ABC transporters (MRPs, P-gp and BCRP).*
 335 *Legend is the same as in Fig. 1, except for transporter substrates which are CDCF (for MRPs),*
 336 *rhodamine 123 (for P-gp) and Hoechst 33342 (for BCRP).*

337

338 3.2. Features of SDHIs-mediated OAT3 activity inhibition

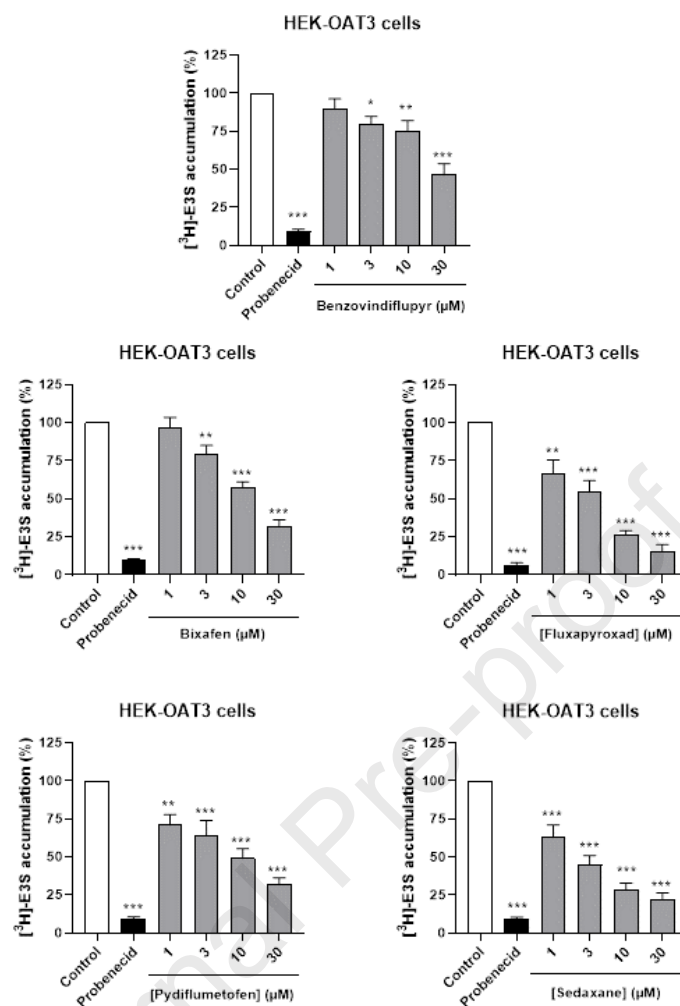
339 Because OAT3 was the transporter most impacted by SDHIs, *i.e.*, it was strongly inhibited
 340 by 5/15 SDHIs (Fig. 2), we next focussed on it. As indicated in Fig. S2, the five SDHIs behaving
 341 as strong OAT3 inhibitors were found to act in a concentration-dependent manner, with IC₅₀

342 values ranging from 1.0 μM (for sedaxane) to 3.9 μM (for benzovindiflupyr) and K_i values
 343 from 0.5 μM (for sedaxane) to 2.0 μM (for benzovindiflupyr) (Table 1). These K_i values for
 344 SDHIs did not statistically differ from those reported for a set of 12 drugs inhibiting OAT3-
 345 mediated transporter of 6-CF with high affinity (Zou et al., 2021) (Fig. S3). The OAT3
 346 inhibitors pydiflumetofen and benzovindiflupyr also decreased activity of OCT2 and BCRP,
 347 respectively, in a concentration-dependent manner (IC_{50} values of 2.0 and 3.9 μM for OCT2
 348 and BCRP inhibition) (Fig. S4).

349 Table 1. IC_{50} and K_i values for SDHI-mediated inhibition of OAT3-mediated 6-CF transport.

SDHI	IC_{50} (μM)	K_i (μM)
Benzovindiflupyr	3.9	2.0
Bixafen	2.6	1.3
Fluxapyroxad	2.9	1.5
Pydiflumetofen	3.5	1.8
Sedaxane	1.0	0.5

350
 351 Effects of SDHIs towards transport of the endogenous OAT3 substrate E3S were next
 352 analyzed (Fig. 4). Benzovindiflupyr, bixafen, fluxapyroxad, pydiflumetofen, and sedaxane
 353 were found to inhibit OAT3-mediated transport of E3S, in a concentration-dependent manner.
 354 The low concentration of 1 μM fluxapyroxad, pydiflumetofen and sedaxane was notably
 355 sufficient to decrease significantly E3S uptake in HEK-OAT3 cells (Fig. 4). Pydiflumetofen
 356 additionally inhibited transport of the endogenous OCT2 substrate dopamine (Fig. S5).



357

358 *Fig. 4. Effects of SDHIs towards OAT3-mediated transport of the endogenous substrate E3S.*
 359 *HEK-OAT3 cells were incubated with 4.09 nM [³H]-E3S for 5 min at 37°C in the absence*
 360 *(control) or presence of various concentrations of benzovindiflupyr, bixafen, fluxapyroxad,*
 361 *pydiflumetofen or sedaxane or of the reference OAT3 inhibitor probenecid. Intracellular*
 362 *accumulation of E3S was next determined by scintillation counting. Data are expressed as %*
 363 *of E3S accumulation in control cells, arbitrarily set at 100%, and are the means ± SEM of at*
 364 *least three independent experiments. *, p < 0.05, **, p < 0.01 and ***, p < 0.001 when compared*
 365 *to E3S accumulation in control cells.*

366

367 In order to investigate the physico-chemical structural requirements for OAT3 inhibition
 368 by SDHIs, the percentages of OAT3 activity inhibition by the 15 SDHIs were next confronted
 369 to the values of 274 SDHI molecular descriptors, through Spearman correlation analysis. 17
 370 /274 molecular descriptors were found to be significantly either positively (n = 13) or negatively

371 (n = 4) correlated with OAT3 inhibition (Table S4). Among these correlated descriptors, the
372 most significant ones, *i.e.*, those for which $p < 0.005$ and Spearman rank correlation coefficient
373 (r_s) ≥ 0.7 , belong to the blocks “functional group counts” (number of CHR_{X2}/nCHR_{X2}),
374 “geometrical descriptors” (quadrupole x-component value/weighted by Sanderson
375 electronegativity/QXXe or ionization potential/QXXi) and “constitutional indices” (sum of
376 atomic van der Waals volumes (scaled on Carbon atom)/Sv (Table S4). Lipophilicity
377 (Moriguchi octanol-water partition coeff. (logP)/MLOGP) is also positively correlated with
378 OAT3 inhibition (Table S4). By contrast, the molecular weight/MW, the number of donor
379 atoms for H-bonds (N and O)/nHDon, the number of acceptor atoms for H-bonds
380 (N,O,F)/nHAcc, the number of rotatable bonds/RBN and the topological polar surface area
381 using N,O,S,P polar contributions/TPSA(Tot) were not correlated to OAT3 inhibition (data not
382 shown).

383 We next investigated whether inhibitions of OAT3 and human SDH by SDHIs may be
384 correlated. This was performed with 8 SDHIs for which IC₅₀ values towards human SDH are
385 available (Bénil et al., 2019). As shown in Fig. S6, IC₅₀ values for human SDH were found to
386 be significantly negatively correlated to the percentages of OAT3 inhibition ($r_s = -0.85$,
387 $p=0.011$). This suggests that the most active SDHIs towards human SDH, *i.e.*, those with the
388 lower IC₅₀ values, were also those with the highest percentages of OAT3 activity inhibition,
389 with however the limit of the relative low number of considered SDHIs (n=8).

390 SDHIs are commonly used in association with fungicides belonging to other chemical
391 class. It is notably the case for bixafen, associated with prothioconazole in commercial
392 formulations. The effect of the mixture bixafen/prothioconazole on OAT3 activity is
393 consequently likely important to consider, especially since prothioconazole acts by itself as an
394 OAT3 inhibitor (Fig. S4). For this purpose, we have characterized the effects of the mixture
395 towards OAT3 activity through calculating CI using the CompuSyn software. As indicated in

396 Table S5, for most of tested concentrations, the combination bixafen/prothioconazole exerted
397 synergistic inhibition of OAT3 activity (CI<0.8).

398 3.3. Transport of SDHIs by OAT3

399 To determine whether SDHIs that strongly interact with OAT3, *i.e.*, benzovindiflupyr,
400 bixafen, fluxapyroxad, pydiflumetofen, and sedaxane, may be substrates for this SLC, *trans*-
401 stimulation assays were first performed because substrates are presumed to *trans*-stimulate
402 transporter activity (Lefèvre et al., 2021; Sayyed et al., 2017). The SDHIs, used at various
403 concentrations (1-50 µM), however failed to *trans*-stimulate OAT3 activity, but rather *trans*-
404 inhibited it, notably at the 50 µM concentration (Fig. S7). By contrast, the OAT3 substrate
405 glutaric acid (Bakhiya et al., 2003) was able to *trans*-stimulate OAT3 activity, *i.e.*, to enhance
406 uptake of the reference OAT3 substrate 6-CF (Fig. S7).

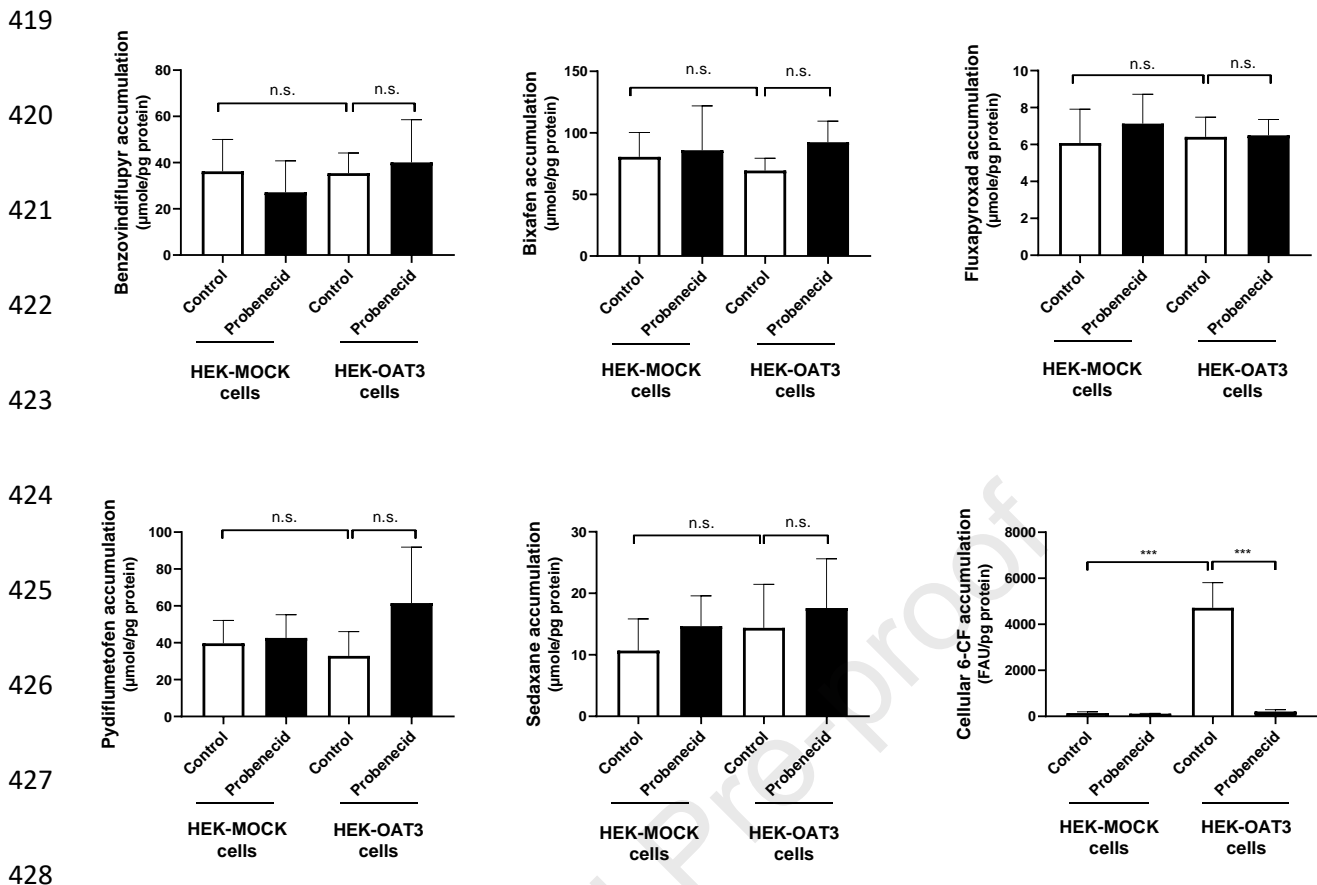
407 SDHI accumulation assays were next performed in HEK-OAT3 cells and control HEK-
408 MOCK cells, in the absence or presence of the reference OAT3 inhibitor probenecid. HEK-
409 OAT3 and HEK-MOCK cells similarly accumulated benzovindiflupyr, bixafen, fluxapyroxad,
410 pydiflumetofen, and sedaxane, without any significant effect of probenecid (Fig. 5), thus ruling
411 out the hypothesis of an OAT3 contribution to their cellular uptake. By contrast, HEK-OAT3
412 cells displayed accumulation of the OAT3 substrate 6-CF much higher than that found in HEK-
413 MOCK cells; probenecid was moreover able to prevent 6-CF uptake in HEK-OAT3 cells (Fig.
414 5). This demonstrated that OAT3 was fully functional in HEK-OAT3 cells.

415

416

417

418



429 *Fig. 5. Accumulation of SDHIs in HEK-OAT3 and HEK-MOCK cells. HEK-MOCK and HEK-*
 430 *OAT3 cells were incubated for 5 min at 37°C with 10 µM benzovindiflupyr, bixafen,*
 431 *fluxapyroxad, pydiflumetofen or sedaxane or 10 µM 6-CF, in the absence (control) or presence*
 432 *of 2 mM probenecid. Intracellular accumulation of SDHIs and 6-CF were next determined by*
 433 *LC-MS/MS or spectrofluorimetry. Data are expressed as µmole/µg protein (SDHIs) or as*
 434 *fluorescence arbitrary units (FAU)/µg protein (6-CF) and correspond to the means ± SD of*
 435 *three independent experiments. n.s., not statistically significant, ***, p<0.001.*

436

437 3.4. Prediction of *in vivo* OAT3 inhibition by SDHIs

438 *In vivo* inhibition of OAT3 activity by blood concentrations of benzovindiflupyr,
 439 bixafen, fluxapyroxad, pydiflumetofen, and sedaxane (at the ADI dose) was predicted from
 440 their *in vitro* IC₅₀ data reported above, using (i) the criteria defined by the ITC, (ii) the calculated
 441 blood SDHIs levels for the ADI values and (iii) the predicted unbound plasma fraction of SDHIs
 442 in humans. As shown in Table 2, only sedaxane was predicted to *in vivo* inhibit OAT3 activity
 443 at the ADI dose. Pydiflumetofen was additionally predicted to not *in vivo* inhibit OCT2 activity

444 (Table S6); similarly, benzovindiflupyr is presumed to not block BCRP activity at both non-
 445 intestinal (Table S6) and intestinal (Table S7) level when applying criteria from the ITC.

446 Table 2: Prediction of *in vivo* inhibition of OAT3 activity by SDHIs^a

447 .

SDHI	ADI (mg/kg/day)	Approximated I_{max}^b	F_u^c	$I_{max, u}^d$	IC ₅₀ for OAT3	Ratio $I_{max, u}/IC_{50}$	Predicted <i>in vivo</i> inhibition
Benzovindiflupyr	0.05	1.76 μ M	0.015	0.03	3.9 μ M	0.0077	No inhibition
Bixafen	0.02	0.68 μ M	0.015	0.01	2.6 μ M	0.0038	No inhibition
Fluxapyroxad	0.02	0.73 μ M	0.018	0.01	2.9 μ M	0.0034	No inhibition
Pydiflumetofen	0.09	2.95 μ M	0.016	0.05	3.5 μ M	0.014	No inhibition
Sedaxane	0.11	4.88 μ M	0.028	0.13	1.0 μ M	0.137	Inhibition

448 ^a *In vivo* inhibition of drug transporter activity can be predicted if the ratio $I_{max, u}/IC_{50} \geq 0.1$ (Giacomini et al., 2010).

449 ^b defined as the maximum plasma concentration.

450 ^c defined as the unbound plasma fraction and *in silico* predicted using ADMETlab 2.0.

451 ^d defined as the maximum unbound plasma concentration.

452

453 4. Discussion

454 The data reported in the present study indicate that the fungicides SDHIs can inhibit
 455 human drug transporters, thus adding these pesticides to the growing list of environmental
 456 pollutants reducing human drug transporter activities. This fully supports the hypothesis that
 457 SDHIs can interact with human targets, apart from SDH and RCIH (Bénit et al., 2019). This
 458 should have to take into account when considering putative toxic effects of these chemicals for
 459 human health.

460 SDHIs were able to inhibit activities of various important drug transporters, including
 461 especially OAT3 (strongly or moderately inhibited by 11/15 SDHIs) and OCT2 (strongly or
 462 moderately inhibited by 3/15 SDHIs) (Table S3). MATE1 and MATE2-K were impacted by
 463 almost all SDHIs, although in a weak manner (inhibition of activity by less than 50% in
 464 response to 10 μ M SDHIs). The ABC transporter BCRP was strongly inhibited by 1/15 SDHIs
 465 and weakly by 4/15 SDHIs. The hepatic transporters OATP1B1 and OATP1B3 displayed
 466 moderate repression of their activity in response to 1/15 and 2/15 SDHIs respectively, whereas

467 3/15 and 5/15 SDHIs weakly inhibited them, respectively (Table S3). However, it is noteworthy
468 that some transporters such as P-gp, MRPs, OCT1 were not affected at all by SDHIs, or only
469 marginally (OAT1, weakly inhibited by 2/15 SDHIs). This indicates that drug transporters are
470 not general targets for SDHIs, as already demonstrated for other environmental pollutants like
471 carbamate pesticides and organophosphate flame retardants (Guéniche et al., 2020b; Tastet et
472 al., 2023b). This rules out the hypothesis that the inhibitions of transporter activities in response
473 to SDHIs result from unspecific toxic effects of these chemicals.

474 Among SDHIs, most of them (12/15) inhibited at least 3 transporters, even weakly (Table
475 S3). The most “active” SDHIs, *i.e.*, those inhibiting the greater number of transporters, were
476 benzovindiflupyr and pydiflumetofen (each inhibiting 7/11 transporters) and bixafen and
477 isopyrazam (each inhibiting 6/11 transporters). By contrast, thifluzamide and carboxin poorly
478 interact with drug transporters, with only 1/11 transporters, *i.e.*, MATE1, impacted in a weak
479 manner (Table S3). This indicates that interactions with transporters are not shared by all the
480 members of a chemical or pharmacological class of xenobiotics, as already described for
481 organophosphate flame retardants and bisphenols (Bruyere et al., 2017; Tastet et al., 2023b).

482 The potential toxic consequences of the inhibition of drug transporter activities by SDHIs
483 remain to be identified. Alteration of the handling and pharmacokinetics of drugs substrates for
484 the transporters targeted by SDHIs, acting here as possible “perpetrators” of pollutant-drug
485 interactions, could be suspected. This could at first concern “victims” drugs substrates for
486 OAT3, or also for OCT2 or BCRP, which are strongly inhibited by some SDHIs, with IC_{50}
487 values in the 1.0-3.9 μ M range. In this context, a wide range of structurally- and
488 pharmacologically-diverse compounds may have to be considered. This includes, for OAT3,
489 antibiotics, especially β -lactams and cephalosporins, histamine H₂ receptor antagonists,
490 diuretics, non-steroidal anti-inflammatory drugs (NSAIDs), statins, uricosurics and
491 methotrexate (Burckhardt, 2012), for OCT2, metformin and cisplatin (Koepsell, 2021), and for

492 BCRP, anticancer drugs and statins (Mao and Unadkat, 2015). Renal secretion of drugs may be
493 particularly impacted due to the fact that some SDHIs inhibit various transporters expressed in
494 the kidney. It is notably the case for pydiflumetofen, targeting diverse renal transporters,
495 especially OAT3 and OCT2, but also OAT1, MATE1, MATE2-K and BCRP (Table S3).
496 However, these putative transporter-based pollutant-drug interactions have not been clinically
497 reported, according to the best of our knowledge. In addition to impairing transport of drugs,
498 that of endogenous substrates could also be impacted, which could contribute to potential toxic
499 effects of SDHIs. The fact that several SDHIs markedly reduced the uptake of the OAT3
500 substrate E3S, as well as the inhibition of OCT2-mediated transport of dopamine by
501 pydiflumetofen, fully support this hypothesis. The strong inhibition of renal OAT3 activity by
502 benzovindiflupyr, pydiflumetofen, sedaxane, fluxapyroxad and bixafen likely deserves special
503 attention, due to the important physiological roles of this transporter in the kidney and beyond
504 the kidney (Jamshidi and Nigam, 2022). OAT3 transports hormones, like E3S, and also many
505 metabolites, components of distinct biochemical and signaling pathways, including those
506 involving amino acids, lipids, bile acids, uremic toxins and gut microbiome-derived products
507 (Nigam et al., 2020). Inhibition of OAT3 activity is therefore susceptible to have various
508 metabolic consequences, including at systemic level and at the gut-liver-kidney axis, to which
509 OAT3 contributes (Bush et al., 2017). Importantly, OAT3 handles some metabolites of the
510 Krebs cycle; α -ketoglutarate is notably a high affinity substrate for OAT3, whereas succinate
511 and fumarate exhibit much lower affinities (Kaufhold et al., 2011). Inhibition of SDH as well
512 as that of OAT3 activity may consequently be hypothesized to both contribute to possible
513 alterations of Krebs cycle metabolite levels in response to SDHIs. The fact that inhibition of
514 OAT3 activity by SDHIs was correlated to their SDH inhibition potential (Fig. S6) further
515 strengthens this hypothesis. Mammalian metabolic networks are nevertheless considered as

516 robust and able to compensate different types of perturbation, including possibly those related
517 to OAT3 or SDH inhibition (Kamp et al., 2021).

518 The possible deleterious effects of SDHIs-mediated inhibitions of transporters, especially
519 OAT3, depend however on the *in vivo* relevance of such inhibitions, experimentally described
520 *in vitro* in the present study. For predicting such potential *in vivo* inhibitions, we have used
521 criteria from the ITC (Giacomini et al., 2010). Because SDHI pharmacokinetics data are
522 currently lacking in humans, we have estimated maximal blood and intestinal levels of SDHIs
523 from their ADI values. OAT3 was predicted to be potentially *in vivo* inhibited by sedaxane in
524 response to its ADI value. This suggests that OAT3 could represent an effective target for
525 humans exposed to sedaxane and that special interest to this SDHI could be warranted. In this
526 context, it is noteworthy that the K_i value for OAT3 inhibition by sedaxane is low ($K_i = 1.0 \mu\text{M}$)
527 and similar to that reported for probenecid ($K_i = 1.25 \mu\text{M}$) (Zou et al., 2021). This indicates
528 that sedaxane may be as effective as probenecid for inhibiting OAT3. Because probenecid is
529 well known to *in vivo* impair OAT3-mediated renal secretion of benzylpenicillin or furosemide
530 (Gessner et al., 2019), sedaxane may be hypothesized to similarly cause clinical drug-pollutant
531 interactions. Further studies, including direct measurement of *in vitro* inhibition of OAT3-
532 mediated transport of benzylpenicillin or furosemide by sedaxane and *in vivo* assays, are
533 however likely required to confirm this hypothesis. By contrast, sedaxane is unlikely to target
534 the renal elimination of antivirals because these drugs are primarily transported rather by OAT1
535 than by OAT3 (Burckhardt and Burckhardt, 2011). Unlike sedaxane, other SDHIs behaving as
536 strong *in vitro* inhibitors of OAT3, OCT2 or BCRP were not predicted to block *in vivo* these
537 transporters. This conclusion may nevertheless be challenged by the fact that humans are
538 probably not exposed to SDHIs as single agents, but rather to mixtures of SDHIs and other
539 pollutants. The combined effects of these mixed pollutants towards transporters may be additive
540 or synergistic, as demonstrated for marine pollutants inhibiting P-gp activity (Nicklisch et al.,

541 2016) and for the combination bixafen/prothioconazole analyzed in the present study towards
542 OAT3 activity (Table S5).

543 Although strongly interacting with OAT3, benzovindiflupyr, bixafen, fluxapyroxad,
544 pydiflumetofen, and sedaxane were not substrates for this transporter. This is demonstrated by
545 their similar accumulation in HEK-OAT3 and HEK-MOCK cells, the absence of effect of
546 probenecid on their cellular uptake in HEK-OAT3 cells and their lack of *trans*-stimulating
547 effects towards OAT3 activity. Human membrane transporters that could handle SDHIs have
548 therefore to be identified, which would likely deserve further studies. Transporters of
549 nucleobases such as sodium-dependent nucleobase transporter (SNBT) 1 (*SLC23A4*),
550 equilibrative nucleoside transporter (ENT) 1 (*SLC29A1*), ENT2 (*SLC29A2*) and equilibrative
551 nucleobase transporter (ENBT) 1 (*SLC43A3*) (Inoue, 2017) may represent promising
552 candidates, because multiple nucleobase transporters contribute to boscalid uptake and
553 sensitivity in *Aspergillus nidulans* (Kalampokis et al., 2018). The possible contribution of
554 passive transport of SDHIs across membranes has also to be considered, notably in relationship
555 with the rather hydrophobic nature of SDHIs (Bouillaud, 2023).

556 The mechanism by which SDHIs block activities of transporters, notably that of OAT3,
557 remains to be characterized. Direct physical interactions of SDHIs with transporters is likely,
558 as for drugs inhibiting transporters (Wigler and Patterson, 1993). The estimated K_i values for
559 SDHIs-mediated inhibition of OAT3 activity are rather low and in the range of those reported
560 for 12 drugs acting as potent inhibitors of OAT3 (Zou et al., 2021). This suggests that SDHIs
561 also behave as high-affinity inhibitors for OAT3. Because benzovindiflupyr, bixafen,
562 fluxapyroxad, pydiflumetofen, and sedaxane are not substrates for OAT3, they probably
563 interact with the transporter outside of its drug binding site or may alternatively bind to it
564 without being transported. In this context, it is noteworthy that these SDHIs not only *cis*-
565 inhibited OAT3 activity (Fig. 2), but also *trans*-inhibited it (Fig. S7). This indicates that these

566 agrochemicals block OAT3 activity extracellularly (*cis*-inhibition) and intracellularly (*trans*-
567 inhibition). Similar *cis*-inhibition and *trans*-inhibition have also been reported for other SLC
568 inhibitions, including cyclosporin A-mediated inhibition of OATP1B1 (Izumi et al., 2022) and
569 tauro lithocholic acid-mediated inhibition of the sodium-taurocholate co-transporting
570 polypeptide (NTCP/*SLC10A1*) (Lowjaga et al., 2021). With respect to molecular characteristics
571 supporting OAT3 inhibition by SDHIs, lipophilicity (MLOGP) as well as the volume (Sv) or
572 the number of CHRX2 group (nCHRX2) appear to be significant ones. Lipophilicity, but not
573 volume, has already been shown to represent a molecular descriptor allowing to discriminate
574 between OAT3 inhibitors and non-inhibitors from a clinical drug library (Duan et al., 2012).
575 Finally, it is noteworthy that, although OAT1 and OAT3 exhibit nearby functional properties
576 (Burckhardt, 2012; Emami Riedmaier et al., 2012), SDHIs appear to be strong inhibitors of
577 OAT3, but not of OAT1. Such a difference with respect to OAT1/OAT3 inhibition profiles has
578 also been reported for drugs (Duan et al., 2012). This justifies to consider separately OAT1 and
579 OAT3 with respect to inhibition studies.

580 **5. Conclusions**

581 The current work demonstrates that various SDHIs inhibit activities of important human
582 drug transporters. These widely-used fungicides can therefore be added to the growing list of
583 pollutants interacting with drug transporters. Among the human transporters targeted by SDHIs,
584 the renal transporter OAT3 is likely a major one, strongly inhibited *in vitro* by 5/15 SDHIs, *i.e.*,
585 benzovindiflupyr, bixafen, fluxapyroxad, pydiflumetofen, and sedaxane, which, however, were
586 not substrates. Moreover, OAT3 inhibition by sedaxane was predicted to occur *in vivo*, in
587 response to exposure to its ADI value. These inhibitions of transporter activities are susceptible
588 to have consequences, notably with respect to SDHI-drug interactions or to levels of
589 endogenous compounds and/or metabolites substrates for the incriminated transporters. This
590 could contribute to putative toxic effects of these fungicides towards human health, which,

591 however, remain to be firmly established or ruled out. This initial study will serve as the basis
592 for further studies, including epidemiological, exposure and toxicokinetics analyses, in order to
593 fully deciphering the potential effects of SDHIs towards human health.

594 **CRedit authorship contribution statement**

595 Marie Kerhoas: Conceptualization, Formal analysis, Investigation, Validation, Writing -
596 original draft. Marc Le Vée: Conceptualization, Investigation, Methodology, Supervision,
597 Validation. Jennifer Carteret: Investigation; Elodie Jouan: Investigation, Resources; Valentin
598 Tastet: Investigation; Arnaud Bruyère: Investigation, Methodology. Laurence Huc:
599 Conceptualization, Funding acquisition, Supervision, Validation, Writing - review & editing;
600 Olivier Fardel: Conceptualization, Funding acquisition, Project administration, Supervision,
601 Validation, Writing - original draft, Writing - review & editing.

602 **Declaration of Competing Interest**

603 The authors declare that they have no known competing financial interests or personal
604 relationships that could have appeared to influence the work reported in this paper.

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614 **References**

- 615 Apiwattanakul, N., Sekine, T., Chairoungdua, A., Kanai, Y., Nakajima, N., Sophasan, S.,
616 Endou, H., 1999. Transport properties of nonsteroidal anti-inflammatory drugs by
617 organic anion transporter 1 expressed in *Xenopus laevis* oocytes. *Mol. Pharmacol.* 55,
618 847–854.
- 619 Bakhiya, A., Bahn, A., Burckhardt, G., Wolff, N., 2003. Human organic anion transporter 3
620 (hOAT3) can operate as an exchanger and mediate secretory urate flux. *Cell. Physiol.*
621 *Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol.* 13, 249–256.
622 <https://doi.org/10.1159/000074539>
- 623 B nit, P., Goncalves, J., El Khoury, R., Rak, M., Favier, J., Gimenez-Roqueplo, A.-P., Rustin,
624 P., 2022. Succinate Dehydrogenase, Succinate, and Superoxides: A Genetic, Epigenetic,
625 Metabolic, Environmental Explosive Crossroad. *Biomedicines* 10, 1788.
626 <https://doi.org/10.3390/biomedicines10081788>
- 627 B nit, P., Kahn, A., Chretien, D., Bortoli, S., Huc, L., Schiff, M., Gimenez-Roqueplo, A.-P.,
628 Favier, J., Gressens, P., Rak, M., Rustin, P., 2019. Evolutionarily conserved
629 susceptibility of the mitochondrial respiratory chain to SDHI pesticides and its
630 consequence on the impact of SDHIs on human cultured cells. *PLoS One* 14, e0224132.
631 <https://doi.org/10.1371/journal.pone.0224132>
- 632 B ranger, R., Hardy, E.M., Binter, A.-C., Charles, M.-A., Zaros, C., Appenzeller, B.M.R.,
633 Chevrier, C., 2020. Multiple pesticides in mothers' hair samples and children's
634 measurements at birth: Results from the French national birth cohort (ELFE). *Int. J.*
635 *Hyg. Environ. Health* 223, 22–33. <https://doi.org/10.1016/j.ijheh.2019.10.010>
- 636 Bijnsdorp, I.V., Giovannetti, E., Peters, G.J., 2011. Analysis of drug interactions. *Methods Mol.*
637 *Biol.* Clifton NJ 731, 421–434. https://doi.org/10.1007/978-1-61779-080-5_34
- 638 Bouillaud, F., 2023. Inhibition of Succinate Dehydrogenase by Pesticides (SDHIs) and Energy
639 Metabolism. *Int. J. Mol. Sci.* 24, 4045. <https://doi.org/10.3390/ijms24044045>
- 640 Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram
641 quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72,
642 248–254. <https://doi.org/10.1006/abio.1976.9999>
- 643 Bruyere, A., Hubert, C., Le Vee, M., Chedik, L., Sayyed, K., Stieger, B., Denizot, C.,
644 Parmentier, Y., Fardel, O., 2017. Inhibition of SLC drug transporter activities by
645 environmental bisphenols. *Toxicol. Vitro Int. J. Publ. Assoc. BIBRA* 40, 34–44.
646 <https://doi.org/10.1016/j.tiv.2016.12.009>

- 647 Bruyère, A., Le Vée, M., Jouan, E., Molez, S., Nies, A.T., Fardel, O., 2021. Differential in vitro
648 interactions of the Janus kinase inhibitor ruxolitinib with human SLC drug transporters.
649 *Xenobiotica Fate Foreign Compd. Biol. Syst.* 51, 467–478.
650 <https://doi.org/10.1080/00498254.2021.1875516>
- 651 Burckhardt, G., 2012. Drug transport by Organic Anion Transporters (OATs). *Pharmacol. Ther.*
652 136, 106–130. <https://doi.org/10.1016/j.pharmthera.2012.07.010>
- 653 Burckhardt, G., Burckhardt, B.C., 2011. In vitro and in vivo evidence of the importance of
654 organic anion transporters (OATs) in drug therapy. *Handb. Exp. Pharmacol.* 29–104.
655 https://doi.org/10.1007/978-3-642-14541-4_2
- 656 Bush, K.T., Wu, W., Lun, C., Nigam, S.K., 2017. The drug transporter OAT3 (SLC22A8) and
657 endogenous metabolite communication via the gut-liver-kidney axis. *J. Biol. Chem.*
658 292, 15789–15803. <https://doi.org/10.1074/jbc.M117.796516>
- 659 Chedik, L., Bruyere, A., Le Vee, M., Stieger, B., Denizot, C., Parmentier, Y., Potin, S., Fardel,
660 O., 2017. Inhibition of Human Drug Transporter Activities by the Pyrethroid Pesticides
661 Allethrin and Tetramethrin. *PloS One* 12, e0169480.
662 <https://doi.org/10.1371/journal.pone.0169480>
- 663 Chen, X., Qiu, T., Pan, M., Xiao, P., Li, W., 2022. Fluxapyroxad disrupt erythropoiesis in
664 zebrafish (*Danio rerio*) embryos. *Ecotoxicol. Environ. Saf.* 247, 114259.
665 <https://doi.org/10.1016/j.ecoenv.2022.114259>
- 666 Chou, T.-C., 2010. Drug combination studies and their synergy quantification using the Chou-
667 Talalay method. *Cancer Res.* 70, 440–446. <https://doi.org/10.1158/0008-5472.CAN-09-1947>
- 668
- 669 Chou, T.-C., 2006. Theoretical basis, experimental design, and computerized simulation of
670 synergism and antagonism in drug combination studies. *Pharmacol. Rev.* 58, 621–681.
671 <https://doi.org/10.1124/pr.58.3.10>
- 672 Clerbaux, L.-A., Coecke, S., Lumen, A., Kliment, T., Worth, A.P., Paini, A., 2018. Capturing
673 the applicability of in vitro-in silico membrane transporter data in chemical risk
674 assessment and biomedical research. *Sci. Total Environ.* 645, 97–108.
675 <https://doi.org/10.1016/j.scitotenv.2018.07.122>
- 676 Clerbaux, L.-A., Paini, A., Lumen, A., Osman-Ponchet, H., Worth, A.P., Fardel, O., 2019.
677 Membrane transporter data to support kinetically-informed chemical risk assessment
678 using non-animal methods: Scientific and regulatory perspectives. *Environ. Int.* 126,
679 659–671. <https://doi.org/10.1016/j.envint.2019.03.003>

- 680 d'Hose, D., Isenborghs, P., Brusa, D., Jordan, B.F., Gallez, B., 2021. The Short-Term Exposure
681 to SDHI Fungicides Boscalid and Bixafen Induces a Mitochondrial Dysfunction in
682 Selective Human Cell Lines. *Mol. Basel Switz.* 26, 5842.
683 <https://doi.org/10.3390/molecules26195842>
- 684 Darney, K., Turco, L., Buratti, F.M., Di Consiglio, E., Vichi, S., Roudot, A.C., Béchaux, C.,
685 Testai, E., Dorne, J.L.C.M., Lautz, L.S., 2020. Human variability in influx and efflux
686 transporters in relation to uncertainty factors for chemical risk assessment. *Food Chem.*
687 *Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* 140, 111305.
688 <https://doi.org/10.1016/j.fct.2020.111305>
- 689 Duan, P., Li, S., Ai, N., Hu, L., Welsh, W.J., You, G., 2012. Potent inhibitors of human organic
690 anion transporters 1 and 3 from clinical drug libraries: discovery and molecular
691 characterization. *Mol. Pharm.* 9, 3340–3346. <https://doi.org/10.1021/mp300365t>
- 692 Emami Riedmaier, A., Nies, A.T., Schaeffeler, E., Schwab, M., 2012. Organic anion
693 transporters and their implications in pharmacotherapy. *Pharmacol. Rev.* 64, 421–449.
694 <https://doi.org/10.1124/pr.111.004614>
- 695 Fardel, O., Kolasa, E., Le Vee, M., 2012. Environmental chemicals as substrates, inhibitors or
696 inducers of drug transporters: implication for toxicokinetics, toxicity and
697 pharmacokinetics. *Expert Opin. Drug Metab. Toxicol.* 8, 29–46.
698 <https://doi.org/10.1517/17425255.2012.637918>
- 699 Gessner, A., König, J., Fromm, M.F., 2019. Clinical Aspects of Transporter-Mediated Drug-
700 Drug Interactions. *Clin. Pharmacol. Ther.* 105, 1386–1394.
701 <https://doi.org/10.1002/cpt.1360>
- 702 Giacomini, K.M., Huang, S.-M., Tweedie, D.J., Benet, L.Z., Brouwer, K.L.R., Chu, X., Dahlin,
703 A., Evers, R., Fischer, V., Hillgren, K.M., Hoffmaster, K.A., Ishikawa, T., Keppler, D.,
704 Kim, R.B., Lee, C.A., Niemi, M., Polli, J.W., Sugiyama, Y., Swaan, P.W., Ware, J.A.,
705 Wright, S.H., Wah Yee, S., Zamek-Gliszczynski, M.J., Zhang, L., The International
706 Transporter Consortium, 2010. Membrane transporters in drug development. *Nat. Rev.*
707 *Drug Discov.* 9, 215–236. <https://doi.org/10.1038/nrd3028>
- 708 Guéniche, N., Bruyere, A., Le Vée, M., Fardel, O., 2020a. Implication of human drug
709 transporters to toxicokinetics and toxicity of pesticides. *Pest Manag. Sci.* 76, 18–25.
710 <https://doi.org/10.1002/ps.5577>
- 711 Guéniche, N., Bruyere, A., Ringeval, M., Jouan, E., Huguet, A., Le Hégarat, L., Fardel, O.,
712 2020b. Differential interactions of carbamate pesticides with drug transporters.
713 *Xenobiotica* 50, 1380–1392. <https://doi.org/10.1080/00498254.2020.1771473>

- 714 Hafey, M.J., Aleksunes, L.M., Bridges, C.C., Brouwer, K.R., Chien, H.-C., Leslie, E.M., Hu,
715 S., Li, Y., Shen, J., Sparreboom, A., Sprowl, J., Tweedie, D., Lai, Y., 2022. Transporters
716 and Toxicity: Insights From the International Transporter Consortium Workshop 4.
717 Clin. Pharmacol. Ther. 112, 527–539. <https://doi.org/10.1002/cpt.2638>
- 718 Inoue, K., 2017. Molecular Basis of Nucleobase Transport Systems in Mammals. Biol. Pharm.
719 Bull. 40, 1130–1138. <https://doi.org/10.1248/bpb.b17-00374>
- 720 International Transporter Consortium, Giacomini, K.M., Huang, S.-M., Tweedie, D.J., Benet,
721 L.Z., Brouwer, K.L.R., Chu, X., Dahlin, A., Evers, R., Fischer, V., Hillgren, K.M.,
722 Hoffmaster, K.A., Ishikawa, T., Keppler, D., Kim, R.B., Lee, C.A., Niemi, M., Polli,
723 J.W., Sugiyama, Y., Swaan, P.W., Ware, J.A., Wright, S.H., Yee, S.W., Zamek-
724 Gliszczynski, M.J., Zhang, L., 2010. Membrane transporters in drug development. Nat.
725 Rev. Drug Discov. 9, 215–236. <https://doi.org/10.1038/nrd3028>
- 726 Izumi, S., Nozaki, Y., Lee, W., Sugiyama, Y., 2022. Experimental and Modeling Evidence
727 Supporting the Trans-Inhibition Mechanism for Preincubation Time-Dependent, Long-
728 Lasting Inhibition of Organic Anion Transporting Polypeptide 1B1 by Cyclosporine A.
729 Drug Metab. Dispos. 50, 541–551. <https://doi.org/10.1124/dmd.121.000783>
- 730 Jamshidi, N., Nigam, S.K., 2022. Drug transporters OAT1 and OAT3 have specific effects on
731 multiple organs and gut microbiome as revealed by contextualized metabolic network
732 reconstructions. Sci. Rep. 12, 18308. <https://doi.org/10.1038/s41598-022-21091-w>
- 733 Ji, C., Miao, J., Xia, B., Dai, Y., Yang, J., Zhang, G., Zhang, Q., Wang, F., Tang, T., Zhao, M.,
734 2023. Evaluation of the toxic effects of fluindapyr, a novel SDHI fungicide, to the
735 earthworms *Eisenia fetida*. Sci. Total Environ. 899, 165697.
736 <https://doi.org/10.1016/j.scitotenv.2023.165697>
- 737 Jouan, E., Le Vée, M., Denizot, C., Parmentier, Y., Fardel, O., 2016a. Drug Transporter
738 Expression and Activity in Human Hepatoma HuH-7 Cells. Pharmaceutics 9, 3.
739 <https://doi.org/10.3390/pharmaceutics9010003>
- 740 Jouan, E., Le Vée, M., Mayati, A., Denizot, C., Parmentier, Y., Fardel, O., 2016b. Evaluation
741 of P-Glycoprotein Inhibitory Potential Using a Rhodamine 123 Accumulation Assay.
742 Pharmaceutics 8, 12. <https://doi.org/10.3390/pharmaceutics8020012>
- 743 Kalampokis, I.F., Kapetanakis, G.C., Aliferis, K.A., Diallinas, G., 2018. Multiple nucleobase
744 transporters contribute to boscalid sensitivity in *Aspergillus nidulans*. Fungal Genet.
745 Biol. FG B 115, 52–63. <https://doi.org/10.1016/j.fgb.2018.02.004>
- 746 Kamp, H., Wahrheit, J., Stinchcombe, S., Walk, T., Stauber, F., Ravenzwaay, B.V., 2021.
747 Succinate dehydrogenase inhibitors: in silico flux analysis and in vivo metabolomics

- 748 investigations show no severe metabolic consequences for rats and humans. *Food*
749 *Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* 150, 112085.
750 <https://doi.org/10.1016/j.fct.2021.112085>
- 751 Kaufhold, M., Schulz, K., Breljak, D., Gupta, S., Henjakovic, M., Krick, W., Hagos, Y.,
752 Sabolic, I., Burckhardt, B.C., Burckhardt, G., 2011. Differential interaction of
753 dicarboxylates with human sodium-dicarboxylate cotransporter 3 and organic anion
754 transporters 1 and 3. *Am. J. Physiol. Renal Physiol.* 301, F1026-1034.
755 <https://doi.org/10.1152/ajprenal.00169.2011>
- 756 Koepsell, H., 2021. Update on drug-drug interaction at organic cation transporters:
757 mechanisms, clinical impact, and proposal for advanced in vitro testing. *Expert Opin.*
758 *Drug Metab. Toxicol.* 17, 635–653. <https://doi.org/10.1080/17425255.2021.1915284>
- 759 Lee, S.-C., Arya, V., Yang, X., Volpe, D.A., Zhang, L., 2017. Evaluation of transporters in drug
760 development: Current status and contemporary issues. *Adv. Drug Deliv. Rev.* 116, 100–
761 118. <https://doi.org/10.1016/j.addr.2017.07.020>
- 762 Lefèvre, C.R., Le Vée, M., Gaubert, S., Jouan, E., Bruyere, A., Moreau, C., Fardel, O., 2021.
763 Substrate-Dependent Trans-Stimulation of Organic Cation Transporter 2 Activity. *Int.*
764 *J. Mol. Sci.* 22, 12926. <https://doi.org/10.3390/ijms222312926>
- 765 Li, S., Li, X., Zhang, H., Wang, Z., Xu, H., 2021. The research progress in and perspective of
766 potential fungicides: Succinate dehydrogenase inhibitors. *Bioorg. Med. Chem.* 50,
767 116476. <https://doi.org/10.1016/j.bmc.2021.116476>
- 768 Lin, H., Lin, F., Yuan, J., Cui, F., Chen, J., 2021. Toxic effects and potential mechanisms of
769 Fluxapyroxad to zebrafish (*Danio rerio*) embryos. *Sci. Total Environ.* 769, 144519.
770 <https://doi.org/10.1016/j.scitotenv.2020.144519>
- 771 Liu, X., 2019. Overview: Role of Drug Transporters in Drug Disposition and Its Clinical
772 Significance. *Adv. Exp. Med. Biol.* 1141, 1–12. https://doi.org/10.1007/978-981-13-7647-4_1
- 774 Lowjaga, K.A.A.T., Kirstgen, M., Müller, S.F., Goldmann, N., Lehmann, F., Glebe, D., Geyer,
775 J., 2021. Long-term trans-inhibition of the hepatitis B and D virus receptor NTCP by
776 tauro lithocholic acid. *Am. J. Physiol. Gastrointest. Liver Physiol.* 320, G66–G80.
777 <https://doi.org/10.1152/ajpgi.00263.2020>
- 778 Luo, B., Ning, Y., 2022. Comprehensive Overview of Carboxamide Derivatives as Succinate
779 Dehydrogenase Inhibitors. *J. Agric. Food Chem.* 70, 957–975.
780 <https://doi.org/10.1021/acs.jafc.1c06654>

- 781 Mao, Q., Unadkat, J.D., 2015. Role of the breast cancer resistance protein (BCRP/ABCG2) in
782 drug transport--an update. *AAPS J.* 17, 65–82. [https://doi.org/10.1208/s12248-014-](https://doi.org/10.1208/s12248-014-9668-6)
783 9668-6
- 784 Michel, M.E., Wen, C.C., Yee, S.W., Giacomini, K.M., Hamdoun, A., Nicklisch, S.C.T., 2023.
785 TICBase: Integrated Resource for Data on Drug and Environmental Chemical
786 Interactions with Mammalian Drug Transporters. *Clin. Pharmacol. Ther.*
787 <https://doi.org/10.1002/cpt.3036>
- 788 Mowery, P.C., Ackrell, B.A.C., Singer, T.P., White, G.A., Thorn, G.D., 1976. Carboxins:
789 Powerful selective inhibitors of succinate oxidation in animal tissues. *Biochem.*
790 *Biophys. Res. Commun.* 71, 354–361. [https://doi.org/10.1016/0006-291X\(76\)90290-4](https://doi.org/10.1016/0006-291X(76)90290-4)
- 791 Mowery, P.C., Steenkamp, D.J., Ackrell, B.A.C., Singer, T.P., White, G.A., 1977. Inhibition of
792 mammalian succinate dehydrogenase by carboxins. *Arch. Biochem. Biophys.* 178, 495–
793 506. [https://doi.org/10.1016/0003-9861\(77\)90220-X](https://doi.org/10.1016/0003-9861(77)90220-X)
- 794 Müller, F., Fromm, M.F., 2011. Transporter-mediated drug-drug interactions.
795 *Pharmacogenomics* 12, 1017–1037. <https://doi.org/10.2217/pgs.11.44>
- 796 Nicklisch, S.C.T., Hamdoun, A., 2020. Disruption of small molecule transporter systems by
797 Transporter-Interfering Chemicals (TICs). *FEBS Lett.* 594, 4158–4185.
798 <https://doi.org/10.1002/1873-3468.14005>
- 799 Nicklisch, S.C.T., Rees, S.D., McGrath, A.P., Gökirmak, T., Bonito, L.T., Vermeer, L.M.,
800 Cregger, C., Loewen, G., Sandin, S., Chang, G., Hamdoun, A., 2016. Global marine
801 pollutants inhibit P-glycoprotein: Environmental levels, inhibitory effects, and cocrystal
802 structure. *Sci. Adv.* 2, e1600001. <https://doi.org/10.1126/sciadv.1600001>
- 803 Nigam, A.K., Li, J.G., Lall, K., Shi, D., Bush, K.T., Bhatnagar, V., Abagyan, R., Nigam, S.K.,
804 2020. Unique metabolite preferences of the drug transporters OAT1 and OAT3
805 analyzed by machine learning. *J. Biol. Chem.* 295, 1829–1842.
806 <https://doi.org/10.1074/jbc.RA119.010729>
- 807 Ottenbros, I., Lebet, E., Huber, C., Lommen, A., Antignac, J.-P., Čupr, P., Šulc, L., Mikeš, O.,
808 Szigeti, T., Középesy, S., Martinsone, I., Martinsone, Z., Akulova, L., Pardo, O.,
809 Fernández, S.F., Coscollá, C., Pedraza-Díaz, S., Krauss, M., Debrauwer, L., Wagner,
810 K., Nijssen, R., Mol, H., Vitale, C.M., Klanova, J., Molina, B.G., León, N., Vermeulen,
811 R., Luijten, M., Vlaanderen, J., 2023. Assessment of exposure to pesticide mixtures in
812 five European countries by a harmonized urinary suspect screening approach. *Int. J.*
813 *Hyg. Environ. Health* 248, 114105. <https://doi.org/10.1016/j.ijheh.2022.114105>

- 814 Polledri, E., Mercadante, R., Nijssen, R., Consonni, D., Mol, H., Fustinoni, S., 2019. Hair as a
815 matrix to evaluate cumulative and aggregate exposure to pesticides in winegrowers. *Sci.*
816 *Total Environ.* 687, 808–816. <https://doi.org/10.1016/j.scitotenv.2019.06.061>
- 817 Roberts, A.G., 2021. The Structure and Mechanism of Drug Transporters. *Methods Mol. Biol.*
818 Clifton NJ 2342, 193–234. https://doi.org/10.1007/978-1-0716-1554-6_8
- 819 Sayyed, K., Camillerapp, C., Le Vée, M., Bruyère, A., Nies, A.T., Abdel-Razzak, Z., Fardel,
820 O., 2019. Inhibition of organic cation transporter (OCT) activities by carcinogenic
821 heterocyclic aromatic amines. *Toxicol. Vitro Int. J. Publ. Assoc. BIBRA* 54, 10–22.
822 <https://doi.org/10.1016/j.tiv.2018.08.015>
- 823 Sayyed, K., Le Vee, M., Abdel-Razzak, Z., Fardel, O., 2017. Inhibition of organic anion
824 transporter (OAT) activity by cigarette smoke condensate. *Toxicol. Vitro Int. J. Publ.*
825 *Assoc. BIBRA* 44, 27–35. <https://doi.org/10.1016/j.tiv.2017.06.014>
- 826 Simon-Delso, N., Martin, G.S., Bruneau, E., Hautier, L., 2018. Time-to-death approach to
827 reveal chronic and cumulative toxicity of a fungicide for honeybees not revealed with
828 the standard ten-day test. *Sci. Rep.* 8, 7241. [https://doi.org/10.1038/s41598-018-24746-](https://doi.org/10.1038/s41598-018-24746-9)
829 9
- 830 Tastet, V., Le Vée, M., Bruyère, A., Fardel, O., 2023a. Interactions of human drug transporters
831 with chemical additives present in plastics: Potential consequences for toxicokinetics
832 and health. *Environ. Pollut.* 121882. <https://doi.org/10.1016/j.envpol.2023.121882>
- 833 Tastet, V., Le Vée, M., Kerhoas, M., Zerdoug, A., Jouan, E., Bruyère, A., Fardel, O., 2023b.
834 Interactions of organophosphate flame retardants with human drug transporters.
835 *Ecotoxicol. Environ. Saf.* 263, 115348. <https://doi.org/10.1016/j.ecoenv.2023.115348>
- 836 Umetsu, N., Shirai, Y., 2020. Development of novel pesticides in the 21st century. *J. Pestic.*
837 *Sci.* 45, 54–74. <https://doi.org/10.1584/jpestics.D20-201>
- 838 Wigler, P.W., Patterson, F.K., 1993. Inhibition of the multidrug resistance efflux pump.
839 *Biochim. Biophys. Acta* 1154, 173–181. [https://doi.org/10.1016/0304-4157\(93\)90010-](https://doi.org/10.1016/0304-4157(93)90010-1)
840 1
- 841 Yanicostas, C., Soussi-Yanicostas, N., 2021. SDHI Fungicide Toxicity and Associated Adverse
842 Outcome Pathways: What Can Zebrafish Tell Us? *Int. J. Mol. Sci.* 22, 12362.
843 <https://doi.org/10.3390/ijms222212362>
- 844 Zhao, L., Wientjes, M.G., Au, J.L.-S., 2004. Evaluation of combination chemotherapy:
845 integration of nonlinear regression, curve shift, isobologram, and combination index
846 analyses. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 10, 7994–8004.
847 <https://doi.org/10.1158/1078-0432.CCR-04-1087>

848 Zou, L., Matsson, P., Stecula, A., Ngo, H.X., Zur, A.A., Giacomini, K.M., 2021. Drug
849 Metabolites Potently Inhibit Renal Organic Anion Transporters, OAT1 and OAT3. J.
850 Pharm. Sci. 110, 347–353. <https://doi.org/10.1016/j.xphs.2020.09.004>

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Highlights

- SDHIs inhibit *in vitro* activities of various drug transporters, especially of OAT3
- SDHIs reduce OAT3-mediated transport of the endogenous molecule estrone-3-sulfate
- SDHIs are not substrates for OAT3
- The SDHI sedaxane was predicted to *in vivo* inhibit human OAT3 activity
- Drug transporters, especially OAT3, appear as new molecular human targets for SDHIs

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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