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Induction of drug metabolizing enzyme and drug transporter expression by antifungal triazole pesticides in human HepaSH hepatocytes

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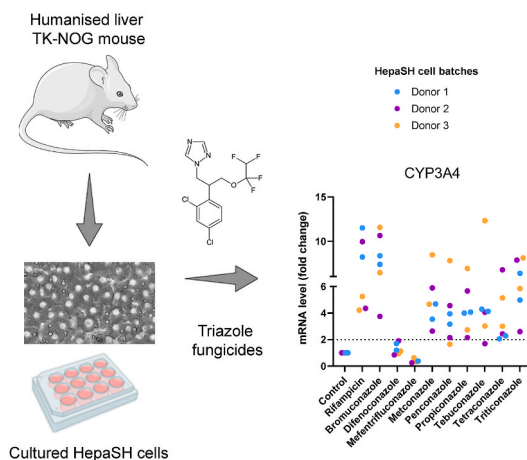
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HIGHLIGHTS

- Triazoles induce CYP and transporter mRNA expression in hepatic HepaSH cells.
- Triazoles directly inhibit activities of some CYPs.
- CYP induction predominates over direct CYP activity inhibition for some triazoles.
- Bromuconazole is predicted to *in vivo* enhance CYP2B6 and CYP3A4 expression.
- Hepatic drug detoxifying proteins appear as major human targets for triazoles.

GRAPHICAL ABSTRACT



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ABSTRACT

Triazole pesticides are widely used fungicides, to which humans are rather highly exposed. They are known to activate drug-sensing receptors regulating expression of hepatic drug metabolizing enzymes and drug transporters, thus suggesting that the hepatic drug detoxification system is modified by these agrochemicals. To investigate this hypothesis, the effects of 9 triazole fungicides towards expression of drug metabolizing enzymes and transporters were characterized in cultured human HepaSH cells, that are human hepatocytes deriving from chimeric humanized liver TK-NOG mice. Most of triazoles used at 10 μ M were found to act as inducers of cytochrome P-450 (CYP) 1A1, CYP1A2, CYP2B6, CYP3A4 and UDP-glucuronosyltransferase 1A1 mRNA levels and of CYP3A4 protein; some triazoles also enhanced mRNA expression of the canalicular transporters P-

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glycoprotein/*MDR1*, multidrug resistance-associated protein 2 and breast cancer resistance protein. Triazoles however concomitantly inhibited CYP2B6 and CYP3A4 activities and thus appeared as dual regulators of these CYPs, being both inducers of their expression and inhibitors of their activity. The inducing effect however predominated, at least for bromoconazole, propiconazole and tebuconazole. Bromoconazole was moreover predicted to enhance CYP2B6 and CYP3A4 expression in humans exposed to this fungicide in a chronic, acute or occupational context. These data demonstrate that key-actors of the human hepatic detoxification system are impacted by triazole pesticides, which may have to be considered for the risk assessment of these agrochemicals. They additionally highlight that the use of human HepaSH cells as surrogates to primary human hepatocytes represents an attractive and promising way for studying hepatic effects of environmental chemicals.

1. Introduction

Triazoles constitute a major class of azole antifungal agents (Martínez-Matías and Rodríguez-Medina, 2018; Zubrod et al., 2019), structurally defined by the presence of a five-membered ring of two carbon atoms and three nitrogen atoms. These azole chemicals suppress the synthesis of ergosterol, a key component of fungal membrane, through competitively inhibiting 14α -lanosterol demethylase, also known as cytochrome P-450 (CYP) 51 (Price et al., 2015). Triazole fungicides are commonly used in veterinary and human medicine and also in agriculture (Toda et al., 2021). They notably represent the second largest class of agrochemical fungicides in the world by market value (21%) (Marciano et al., 2024) and more than 30 commercial triazole agrochemicals have been described. Their wide use for agricultural purpose has led to a contamination of soils, water and food by these antifungals (Zubrod et al., 2019), owing to their chemical and photochemical stability, low biodegradability and easy transport through the environment (Bellot et al., 2022). It results in a notable exposure of human populations to these chemicals (Béranger et al., 2020; Marciano et al., 2024), especially through the diet (Boon et al., 2015). In addition, agricultural workers as well as people living around farmland are rather highly exposed to these agrochemicals, especially during the spreading season, through inhalation or dermal contact (Mercadante et al., 2019; Xiao et al., 2023).

Environmental, dietary or occupational exposure to triazole pesticides is of particular concern for public health (Dong et al., 2023). Indeed, these agrochemicals are thought to exert pleiotropic deleterious effects for human health, including oxidative stress, endocrine disruption, neurodevelopmental alteration, lipid metabolism perturbation and reproductive and placental toxicity (Ku et al., 2023; Rjiba-Touati et al., 2022; Serra et al., 2023; Wu et al., 2021; Zhou et al., 2016). In addition, triazole pesticides may be hypothesized to interfere with the hepatic drug detoxification system, which may alter the capacities of the human organism to detoxify drugs and environmental chemicals. Indeed, some triazole agrochemicals activate nuclear receptors acting as drug sensors such as pregnane X receptor (PXR), constitutive androstane receptor (CAR) or aryl hydrocarbon receptor (AhR) (Knebel et al., 2019b; Lichtenstein et al., 2020; Marx-Stoelting et al., 2020). By this way, triazole pesticides may modify the expression of drug metabolizing enzymes targets for these drug receptors, especially the phase I enzymes CYP3A4 and CYP2B6 (Knebel et al., 2018; Nagai et al., 2016; Wang et al., 2022), as well as that of hepatic drug transporters (Hammer et al., 2020). Triazole agrochemicals can also directly inhibit CYP activity (Habenschus et al., 2019; Lv et al., 2017), as azoles used in human medicine such as the imidazole ketoconazole and the triazoles itraconazole or voriconazole (Czyrski et al., 2021; Venkatakrishnan et al., 2000).

It is however noteworthy that most of the present data about interactions of triazole agrochemicals with hepatic drug sensing receptors and drug metabolizing enzymes have been collected in a rather scattered way, through studies performed in rodents, acellular systems, like liver microsomes, or human liver cell lines (Knebel et al., 2018, 2019a; Lichtenstein et al., 2020; Marx-Stoelting et al., 2017; Wang et al., 2022), that do not recapitulate all the aspects of the detoxifying potential of normal human hepatocytes (Gómez-Lechón et al., 2003; Kyffin et al.,

2018). There is a need to carefully and extensively investigate the effects of triazole pesticides towards expression of hepatic drug metabolizing enzymes and drug transporters in cultured human hepatocytes, that represent the gold standard for hepatic toxicological studies (Gómez-Lechón et al., 2014; Ruoß et al., 2020). The present study was designed to get insight about this point for a set of nine authorized and rather widely-used triazole fungicides, *i.e.*, bromoconazole difenoconazole, metconazole, mefentrifluconazole, penconazole, propiconazole, tebuconazole, tetraconazole and triticonazole. For this purpose and owing to the expensive cost of primary human hepatocytes isolated from human donors (Gómez-Lechón et al., 2004) and their rather limited and scarce availability, even if there are now multiple suppliers worldwide, we used human hepatocytes deriving from chimeric humanized liver TK-NOG mice (Hasegawa et al., 2011; Uehara et al., 2023). Indeed, these human hepatocytes, called HepaSH cells, have been previously shown to represent convenient surrogates to human hepatocytes freshly isolated from donors for investigating drug metabolizing enzyme and transporter regulatory pathways (Uehara et al., 2019, 2023; Zerdoug et al., 2022). Our data indicate that triazole pesticides commonly interact with various drug metabolizing enzymes and transporters in human HepaSH hepatocytes, most of them acting as inducers of CYP and transporter expression. This fully supports the conclusion that the human hepatic detoxification system is impacted by triazole pesticides, which should be taken into account for risk assessment of these agrochemicals.

2. Material and methods

2.1. Chemicals

The following chemicals were provided by Sigma-Aldrich (Saint-Quentin-Fallavier, France): bupropion, bromoconazole, difenoconazole, metconazole, midazolam, omeprazole, penconazole, phenacetin, phenobarbital, rifampicin, tebuconazole, tetraconazole and triticonazole. Mefentrifluconazole, propiconazole and the PXR antagonist SPA70 were obtained from MedChem Tronica (Sollentuna, Sweden) and ketoconazole from Santa Cruz Biotechnology (Dallas, TX, USA). Stock solutions of chemicals, including triazole pesticides whose chemical structures are depicted in Fig. S1, were prepared in dimethyl sulfoxide (DMSO).

2.2. Cell culture and treatment

HepaSH cells were prepared at Central Institute for Experimental Medicine and Life Science (CIEM) (Kawasaki, Japan), as previously described (Uehara et al., 2023). They were sent to Research Institute for Environmental and Occupational Health (Irset) (Rennes, France) for 48–72 h as fresh cells in cold University of Wisconsin solution, a hyperkalemic, hyperosmolar solution used here for preserving isolated hepatocytes during their cold storage, as previously described (Sorrentino et al., 1991). At reception, cells were washed twice with phosphate-buffered saline (PBS) 1X (Invitrogen, Cergy-Pontoise, France) and centrifuged at $200\times g$ for 2 min. Cells were next placed into OneStep PHep thawing medium (Biopredic International, Saint Grégoire, France) and centrifuged at $200\times g$ for 6 min. After washing

with PBS 1X, HepaSH cells were collected for re-suspension in Williams' E medium (Invitrogen), supplemented with 10% (vol/vol) foetal calf serum (FCS) (Cytiva, Velizy-Villacoublay, France), 5 µg/ml bovine insulin (Sigma-Aldrich), 100 IU/ml penicillin, 100 µg/ml streptomycin (Invitrogen), and 2 mM glutamine (Invitrogen). Cells were next seeded on plastic plates at density of 2.1×10^5 cells/cm² for culturing them under a monolayer 2-D configuration. After a 4 to 16 h-seeding period, medium was discarded, and HepaSH cells were cultured in maintenance medium composed of Williams' E medium, supplemented with 1% (vol/vol) insulin-transferrin-selenium (ITS) 100X (Thermo Fisher Scientific, Waltham, MA, USA), 10^{-7} M dexamethasone (Santa Cruz Biotechnology), 100 IU/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine. It is noteworthy that the used submicromolar concentration of dexamethasone is thought to activate the classical glucocorticoid receptor, but not PXR (Pascucci et al., 2000), and is required to get full responsiveness to CYP inducers in primary human hepatocytes (Donato et al., 1990). The culture medium was routinely renewed every 2–3 days.

A total of seven batches of fresh HepaSH cells, prepared from TK-NOG mice engrafted with primary human hepatocytes from three human donors, was used in the study; each batch originated from one or pooled TK-NOG mice engrafted with the same human donor. Information about the human donors and the batches per donor used in the study are indicated in Table S1.

For cell treatment, 5 days-cultured fresh HepaSH cells were exposed for 48 h to 0.1% (vol/vol) DMSO, as vehicle control, to 10 µM triazole pesticides or to reference CYP and transporter inducers (50 µM omeprazole for induction of CYP1A1, CYP1A2, UDP-glucuronosyltransferase (UGT) 1A1 and breast cancer resistance protein (BCRP/ABCG2), 1 mM phenobarbital for induction of CYP2B6 and multidrug resistance-associated protein (MRP) 2/ABCC2, and 10 µM rifampicin for induction of CYP3A4 and P-glycoprotein (P-gp), encoded by multidrug resistance gene 1 (MDR1/ABCB1).

2.3. Cellular viability assays

Cellular viability of HepaSH cells exposed to 10 µM triazoles for 48 h was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay or the neutral red uptake assay, as previously described (Chesné et al., 1991). Data were expressed as percentages of viability of control cells not treated by triazoles, set at 100%.

2.4. RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays

Total RNAs were extracted from cells using the TRIzol reagent (Thermo Fisher Scientific) and were quantified using the NanoDrop 8000 Spectrophotometer™ (Thermo Fisher Scientific). RT-qPCR assays were next performed as previously described (Zerdoug et al., 2023), using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and the fluorescent dye Power SYBR™ Green PCR Master Mix from Applied Biosystems. The used gene primer sequences are listed in Table S2. Amplification curves were analysed with the CFX Maestro software (Bio-Rad, Hercules, CA, USA), using the comparative cycle threshold method. Relative quantification of target mRNA levels was calculated after normalizing the total amount of cDNA tested against the internal reference (18S rRNA), using the $2^{(-\Delta\Delta Ct)}$ method, resulting in the normalized expression of the target gene. Data were next expressed as fold change in comparison with 0.1% (vol/vol) DMSO-treated cell control, according to equation (1):

$$\text{Fold change} = \frac{\text{Normalized expression}_{\text{Treated sample}}}{\text{Normalized expression}_{\text{Vehicle control}}} \quad (1)$$

where Normalized expression_{Treated sample} = normalized expression of

the target gene in cells exposed to triazole pesticides or to reference inducers (rifampicin, phenobarbital or omeprazole) and Normalized expression_{Vehicle Control} = normalized expression of the target gene in control cells exposed to 0.1% (vol/vol) DMSO.

Data were also expressed as percentages of the positive control, i.e., as percentages of mRNA expression in reference inducer-treated cells, according to equation (2):

$$\% \text{ positive control} = \frac{(\text{Mean Fold change}_{\text{Treated sample}} - 1)}{(\text{Mean Fold change}_{\text{Reference inducer}} - 1)} \times 100 \quad (2)$$

The half-maximal effect concentration (EC₅₀) values of bromuconazole towards CYP3A4 and CYP2B6 mRNA induction were determined from nonlinear regression of dose-response data in fresh HepaSH cells, based on the four-parameter logistic function. They were calculated using the Prism 8.3 software (GraphPad, San Diego, CA, USA), through the following equation (3):

$$A = \frac{100}{1 + 10^{(\text{Log}(\text{EC}_{50}) - [I]) \times \text{Hill slope}}} \quad (3)$$

where A = percentage of CYP3A4 or CYP2B6 mRNA expression in HepaSH cells for a given concentration of bromuconazole determined as described in equation (2), [I] = the bromuconazole pesticide concentration in the medium, and Hill slope = a coefficient describing the steepness of the curve.

2.5. Western blot analysis

Cell lysis and protein extraction were performed through exposing cells to RIPA buffer, supplemented with 2 mM dithiothreitol, 1 mM orthovanadate sodium, 1 mM phenylmethanesulfonyl fluoride, 1 mM sodium fluoride, and 1X complete Protease Inhibitor Cocktail™ (Roche, Basel, Switzerland), for 30 min. After cell lysate sonication, protein extracts were separated on polyacrylamide gel and electrophoretically transferred onto nitrocellulose membranes. After blocking with Tris-buffered saline containing 3% (vol/vol) bovine serum albumin for 60 min, membranes were incubated overnight at 4 °C with primary antibodies against CYP1A2 (Proteintech, Rosemont, IL, USA), CYP3A4 (clone D9U6N, Cell Signaling Technology, Danvers, MA, USA), or HSC70 (Santa Cruz Biotechnology), used here as a loading control. After washing, membranes were next re-incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (Agilent-Dako, Santa Clara, CA, USA) for 1 h. Immuno-labelled proteins were finally visualized by chemiluminescence and quantified by densitometry analysis using the Image Lab software (Bio-Rad). Quantitative data were expressed as fold change comparatively to CYP levels in control cells.

2.6. CYP activity assay

HepaSH cells cultured in 96 well plates were first incubated with specific CYP substrates, i.e., phenacetin (200 µM, 5 h) for CYP1A2 activity, bupropion (100 µM, 4 h) for CYP2B6 activity and midazolam (50 µM, 1 h) for CYP3A4 activity. CYP activities were stopped by adding ice-cold acetonitrile and supernatants were collected after centrifugation and stored at -20 °C until analysis. CYP activities-related metabolites, i.e., acetaminophen (for CYP1A2 activity), hydroxybupropion (for CYP2B6 activity) and 1'-hydroxymidazolam (for CYP3A4 activity) were next quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an ExionLC™ AC system coupled to a triple quadrupole mass spectrometer AB Sciex Triple Quad 5500+ (Sciex, Framingham, MA, USA), equipped with an electrospray ion source in positive mode. Compounds were separated on a Xselect HSS T3 column (2.1 × 75 mm, 2.5 µm, Waters, Milford, MA, USA) maintained at 35 °C. The mobile phase was composed of 2 mM ammonium formate containing 0.1% formic acid (phase A) and acetonitrile (phase B).

Acquisition was performed using the multiple reaction monitoring (MRM). Data were processed using Analyst 1.7.2 software (Sciex). The maximum reaction rate (V_{\max}) for each CYP activity was calculated using equation (4):

$$V_{\max} = [\text{Metabolite}] \times \frac{\text{volume of incubation}}{\text{incubation time} \times \text{amount of protein}} \quad (4)$$

with [Metabolite] = measured concentration of metabolite, volume of incubation = volume of incubated substrate, incubation time = incubation time with substrate and amount of protein = quantity of protein per incubated well, determined by the Bradford method.

Data were finally expressed as percentages of V_{\max} /CYP activity found in control cells not exposed to triazole pesticides set at 100% (for judging direct inhibitory effects of triazoles on CYP activity, *i.e.*, CYP activities were measured in the presence of triazoles in HepaSH cells previously not exposed to the agrochemicals) or as fold changes of CYP activity in triazoles-treated HepaSH cells when compared to control cells (for evaluating CYP activity after a 48 h-treatment by triazoles).

2.7. PXR activation assay

PXR activation by various concentrations of bromoconazole was analysed using the all-inclusive cell-based luciferase reporter assay kit targeting PXR, provided by Indigo Biosciences (State College, PA, USA), as previously described (Reddy and Nyunoya, 2021). Data were expressed as percentages of PXR activation in response to the reference PXR agonist rifampicin (used at 10 μM), arbitrarily set at 100%.

2.8. Prediction of *in vivo* induction of CYP3A4 and CYP2B6 expression by bromoconazole

In vivo predictions of hepatic CYP2B6 and CYP3A4 induction by bromoconazole were performed according to the correlation approach (Fahmi and Ripp, 2010). The ratio maximum unbound plasma concentration of bromoconazole ($I_{\max,u}$) versus their EC_{50} towards CYP2B6 or CYP3A4 mRNA expression induction were calculated and considered for the prediction: ratio ≤ 0.001 , $0.001 < \text{ratio} \leq 0.01$ and ratio > 0.01 would predict bromoconazole to be a weak, moderate and strong CYP3A4 or CYP2B6 inducer, respectively (Fahmi and Ripp, 2010). These weak, moderate and strong CYP inductions correspond to a decrease of less than 30%, between 30 and 60% or more than 60% of total exposure to prototypical CYP substrates, exemplified by substrate area under the curve (AUC) "substrate plasma concentration versus time" (Fahmi and Ripp, 2010). As data on the human pharmacokinetics of bromoconazole are not currently available, $I_{\max,u}$ values for bromoconazole were evaluated using acceptable daily intake (ADI) value (for chronic exposure), acute reference dose (ARfD) (for acute exposure) and acceptable operator exposure level (AOEL) value (for occupational exposure), provided by the European Food Safety Authority (EFSA, <https://www.efsa.europa.eu/fr>), as described previously (Kerhoas et al., 2024). Briefly, ADI, ARfD and AOEL permitted to calculate the maximal theoretical doses of bromoconazole absorbed at once by a 70 kg-weighting subject for 24 h in a chronic, acute or occupational context, even if the fact that ADI, ArfD and AOEL values likely correspond to the worst situations of high exposures to triazoles has to be kept in mind. These absorbed doses were considered to be fully absorbed and distributed into the standard volume (5 L) of blood for the subjects, allowing thus to determine the maximal total blood concentrations of bromoconazole (I_{\max}), as the ratio "ingested dose/blood volume". Bromoconazole $I_{\max,u}$ values were next calculated after taking into account the unbound plasma fraction of bromoconazole (f_u), predicted *in silico* using the online tool Admetlab2.0 (<https://admetmesh.scbdd.com/>) (Xiong et al., 2021).

2.9. Analysis of data

Data relative to drug metabolizing enzyme and drug transporter expression and activity induction by triazoles are shown for each population/batch of HepaSH cells, owing to the fact that variations between independent human hepatocyte populations commonly occur with respect to hepatic CYP and transporter regulation in response to xenobiotics (Bulutoglu et al., 2020; Jigorel et al., 2006; Kerhoas et al., 2024). The criteria previously proposed for classifying a compound as an *in vitro* inducer of CYP in primary human hepatocytes (Kenny et al., 2018), *i.e.*, a 2.0-fold induction as a threshold, observed in more than 33.3% of the analysed hepatocyte populations, was applied for mRNA and activity CYP regulation by triazoles. For regulation of CYP protein levels, owing to the fact that amplitudes of protein level induction for hepatic CYPs are commonly lower than those for mRNA levels (Halladay et al., 2012; Savaryn et al., 2021), the threshold for being considered as inducer was arbitrarily reduced at 1.5-fold change in more than 33% of the batches. The same threshold, *i.e.*, a 1.5-fold change, was applied for transporter mRNA regulation by triazoles, as previously described for xenobiotic-mediated-relation of hepatic drug transporters and for taking into account the relative low inducibility of transporter expression (Jigorel et al., 2006).

Data related to cell viability assays and direct inhibition of CYP activity assays are the means \pm SEM from at least three independent experiments, each being performed in triplicate or quadruplicate. They were statistically analysed through analysis of variance (ANOVA) followed by Dunnett's post-hoc test. The criterion of significance was $p < 0.05$.

The Prism 10.3.0 software (GraphPad software, Boston, MA, USA) was used to perform statistical analyses and to draw the graphs shown in figures of the manuscript.

3. Results

3.1. Effect of triazole pesticides on HepaSH cell viability

For investigating the effects of triazoles towards drug metabolizing enzyme and transporter expression, fresh HepaSH cells were exposed for 48 h to 10 μM triazole pesticides. This 10 μM triazole concentration has been previously successfully used for characterizing *in vitro* the effects of triazole agrochemicals (Ku et al., 2023; Leite et al., 2024) and the 48 h treatment time is recognized as the optimal one for studying mRNA expression regulation of hepatic CYPs such as CYP3A4 (Wong et al., 2021). These treatment conditions did not result in any significant alteration of HepaSH cell viability by triazole pesticides, as demonstrated by the MTT and neutral red uptake assays (Fig. S2).

3.2. Effect of triazole pesticides on CYP mRNA expression

Expression of CYP1A2, CYP2B6 and CYP3A4 mRNAs in control and triazole-treated HepaSH cells from 6 batches (all triazoles, except mefentrifluconazole) or 4 batches (mefentrifluconazole) was investigated by RT-qPCR. Data are expressed as fold change of CYP expression comparatively to controls and are shown for each batch of HepaSH cells (Fig. 1A). The previously proposed threshold of 2.0-fold induction, observed in more than 33.3% of the analysed hepatocyte populations (Kenny et al., 2018), was applied. With such criteria, triazole agrochemicals (excepted mefentrifluconazole) enhancing CYP mRNA levels by at least a 2.0-fold factor in at least 3/6 batches of HepaSH cells (that necessarily correspond to batches from at least two donors because two batches per donor were analysed in the study) were considered as CYP inducers. For mefentrifluconazole, the threshold for being inducer was to induce CYP mRNA levels in at least 2/4 batches. Proven reference inducers of CYP expression, *i.e.*, the AhR model inducer omeprazole, the CAR activator phenobarbital and the PXR agonist rifampicin, enhanced mRNA expression of CYP1A2, CYP2B6 and CYP3A4, respectively, in

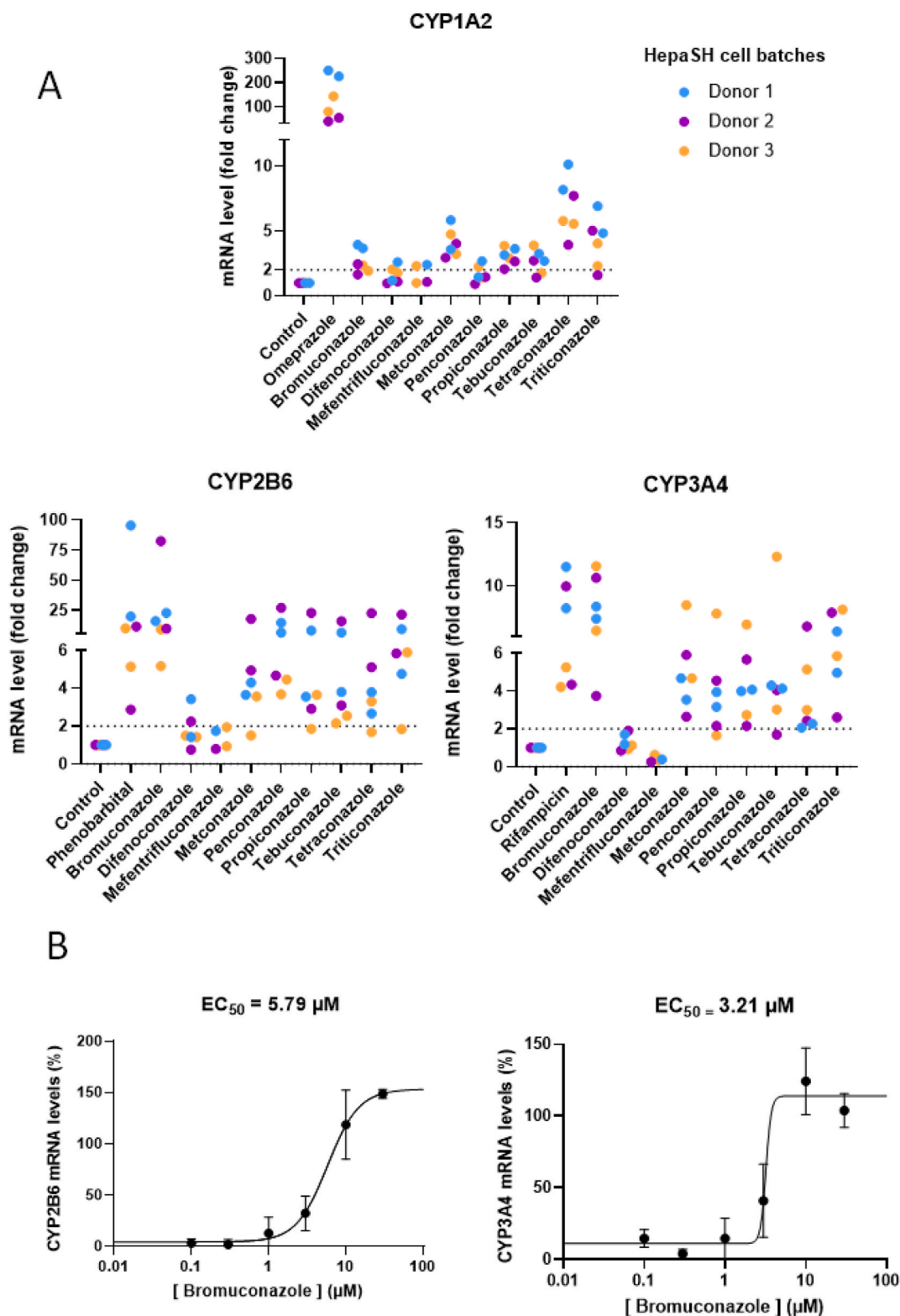


Fig. 1. Regulation of CYP1A2, CYP2B6 and CYP3A4 mRNA expression by a 48-h exposure to triazoles in human HepaSH cells. (A) HepaSH cells from 4 to 6 batches generated from three human liver donors (one or two batches/donor) were either untreated (control) or exposed to 50 μM omeprazole, 1 mM phenobarbital, 10 μM rifampicin or 10 μM triazoles for 48 h. CYP mRNA expressions were next determined by RT-qPCR. Data are expressed as fold changes of CYP mRNA levels comparatively to control. Values for each HepaSH cell batch are shown by dots, differently coloured according to the human liver donors from which HepaSH cell batches derive. An identical colour therefore applies to HepaSH cell batches deriving from chimeric mice engrafted with human hepatocytes from the same human liver donor. Dashed lines on the graphs indicate the 2.0-fold induction factor, used as a threshold for being considered as CYP mRNA inducer. (B) HepaSH cells were exposed to various concentrations of bromuconazole for 48 h CYP2B6 and CYP3A4 mRNA expressions were then determined by RT-qPCR. Data are expressed as percentages of CYP mRNA increase and are the means ± SEM of values from two HepaSH cell batches (from two donors). HepaSH cells exposed to 1 mM phenobarbital or 10 μM rifampicin were used to determine the 100% induction. Bromuconazole EC₅₀ values are indicated on the tops of the graphs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cultured HepaSH cells from 6/6 batches (Fig. 1A–Table S3), thus indicating that each batch of HepaSH cells was responsive to CYP inducer. All triazoles, except difenoconazole and penconazole, acted as CYP1A2 mRNA inducers (Fig. 1A). The fold change of CYP1A2 mRNA levels triggered by triazoles inducers are however rather low, *i.e.*, they range from 1.7 (for mefentrifluconazole) to 6.9 (for tetraconazole), whereas that for omeprazole is much higher (131.9) (Table S3). Inductions of CYP1A2 mRNAs by triazoles are consequently rather weak, not exceeding 4.5% of that due to omeprazole (Table S3). Triazoles, except difenoconazole and mefentrifluconazole, behaved as CYP2B6 and CYP3A4 mRNA inducers (Fig. 1A). For CYP2B6 and CYP3A4, induction levels caused by triazoles were at least 19.8% (for metconazole) or 41.3% (for tetraconazole) of those due to phenobarbital or rifampicin, respectively (Table S3). Among triazoles, bromuconazole was the most active, enhancing CYP2B6 and CYP3A4 mRNA levels by 24.4-fold and 8.0-fold, respectively, which corresponds to induction factors similar to those observed with phenobarbital (24.2-fold change for CYP2B6) and rifampicin (7.2-fold change for CYP3A4) (Table S3). Bromuconazole

was next shown to regulate CYP2B6 and CYP3A4 in a concentration-dependent manner, with EC_{50} values of 5.79 μ M and 3.21 μ M, respectively (Fig. 1B). PXR, a nuclear receptor implicated in CYP3A4 induction, was additionally demonstrated to be activated by bromuconazole in a concentration-dependent manner, with an EC_{50} value of 2.6 μ M (Fig. S3). The PXR antagonist SPA70 was moreover able to prevent CYP3A4 mRNA up-regulation by bromuconazole (Fig. S4).

Besides CYP1A2, CYP2B6 and CYP3A4 mRNA expression, that of other drug metabolizing enzymes was also impacted by triazoles in HepaSH cells. These agrochemicals, excepted difenoconazole, thus induced CYP1A1 mRNA expression, with fold changes ranging from 2.7 (for penconazole) to 55.9 (for tetraconazole), much lower than the 621.1-fold change due to omeprazole (Fig. S5, Table S4). Triazoles, excepted difenoconazole and mefentrifluconazole, as well as the reference inducers omeprazole, phenobarbital and rifampicin, also enhanced mRNA expression of UDP-glucuronosyltransferase (UGT) 1A1 (Fig. S5, Table S4).

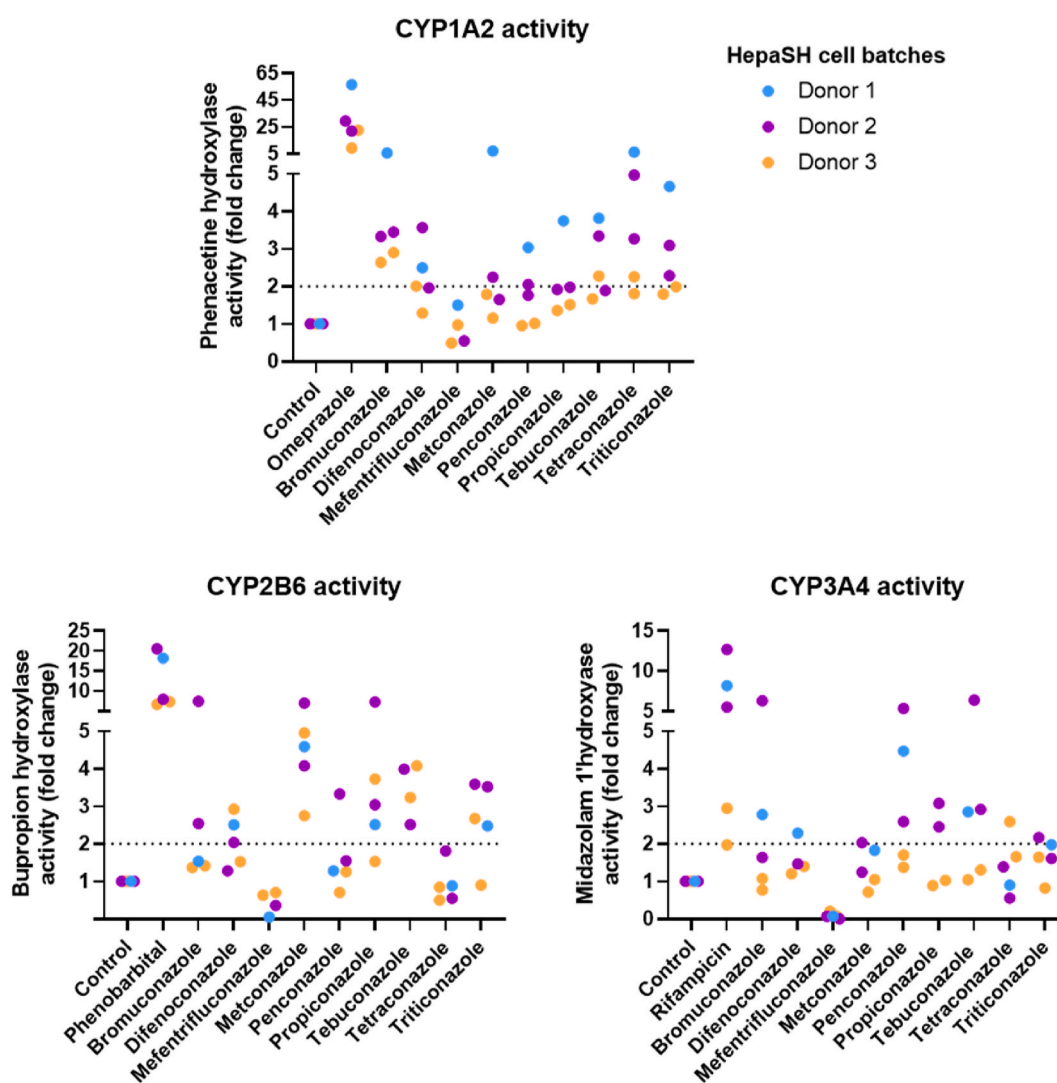


Fig. 2. Regulation of CYP1A2, CYP2B6 and CYP3A4 activity by a 48-h exposure to triazoles in human HepaSH cells. HepaSH cells from 4 to 5 batches generated from three human liver donors (one or two batches/donor) were either untreated (control) or exposed to 50 μ M omeprazole, 1 mM phenobarbital, 10 μ M rifampicin or 10 μ M triazoles for 48 h. CYP activities, *i.e.*, phenacetine hydroxylase (CYP1A2), bupropion hydroxylase (CYP2B6) and midazolam 1'hydroxylase (CYP3A4) activities, were next determined by LC-MS/MS. Data are expressed as fold changes of CYP activity comparatively to control. Values for each HepaSH cell batch are shown by dots, differently coloured according to the human liver donors from which HepaSH cell batches derive. An identical colour therefore applies to HepaSH cell batches deriving from chimeric mice engrafted with human hepatocytes from the same human liver donor. Dashed lines on the graphs indicate the 2.0-fold induction factor, used as a threshold for being considered as CYP activity inducer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Effect of triazole pesticides on CYP protein expression and activity

Effects of 10 μM triazole pesticides on CYP1A2 and CYP3A4 protein expression in HepaSH cells were investigated by Western-blotting (Fig. S6). With the threshold for being considered as an inducer arbitrarily set at 1.5-fold change in more than 33% of the batches, only tetraconazole induced CYP1A2 protein, with however a rather very modest 1.3-fold induction factor, weaker than that (5.4-fold change) observed for omeprazole (Fig. S6A, Table S5). CYP3A4 protein was induced by all triazoles, excepted difenoconazole and mefentri-fluconazole (that also failed to up-regulate CYP3A4 mRNA levels, as reported above), with fold changes ranging from 1.4 (for triticonazole) to 1.9 (for bromuconazole), similar to that (1.6-fold change) observed for rifampicin (Fig. S6B, Table S5).

With respect to CYP activities, using a 2.0-fold change in at least 33.3% of the HepaSH cell populations as a threshold, all triazoles (excepted mefentrifluconazole) used at 10 μM , were found to be CYP1A2 activity inducers. The fold change means of CYP1A2 activity induction by triazoles, ranging from 1.8 (for penconazole) to 3.7 (for tetraconazole), were however low when compared to the 28.0-fold increase of CYP1A2 activity caused by omeprazole (Fig. 2, Table S6). Difenoconazole, although not inducing CYP1A2 mRNA expression (Fig. 1A), was able to enhance CYP1A2 activity (Fig. 2). CYP2B6 activity was enhanced by 6/9 triazoles, i.e., bromuconazole, difenoconazole, metconazole, propiconazole, tebuconazole and triticonazole, with however a rather low level of induction when compared to that triggered by phenobarbital (Fig. 2, Table S6). With respect to CYP3A4 activity, it was induced by 5/9 triazoles, i.e., bromuconazole, penconazole, propiconazole, tebuconazole and triticonazole (Fig. 2, Table S6). The lack of induction of CYP3A4 activity by difenoconazole and mefentrifluconazole as well as that of CYP2B6 activity by mefentrifluconazole (Fig. 2, Table S6) are expected because these compounds failed to induce CYP3A4 and/or CYP2B6 mRNA expression (Fig. 1) as well as CYP3A4 protein expression (Fig. S6B). By contrast, the lack of induction of CYP2B6 activity by tetraconazole and of CYP3A4 activity by metconazole and tetraconazole are more surprising because these agrochemicals induced CYP2B6 and/or CYP3A4 expression (Fig. 1 and Fig. S6B). Such apparent contradictory results may be due to the direct inhibition of CYP activities by the chemicals, as already reported for drugs such as the antiviral agent ritonavir or for some pesticides belonging to the succinate dehydrogenase inhibitor (SDHI) class (Halladay et al., 2012; Kerhoas et al., 2024). To test this hypothesis, CYP2B6 and CYP3A4 activities were measured in the presence of 10 μM triazoles in HepaSH cells previously not exposed to the fungicides. As shown in Fig. 3, all triazoles directly and significantly inhibited CYP2B6 and CYP3A4 activities, with the exception of CYP2B6 that was not inhibited by triticonazole. In particular, tetraconazole, but also bromuconazole, mefentrifluconazole, penconazole and propiconazole inhibited CYP2B6 activity by more than 75%, being more active than the CYP2B6 inhibitor ketoconazole (O'Donnell et al., 2007), which reduced CYP2B6 activity by 65%. In the same way, even if all triazoles, including metconazole, tetraconazole and triticonazole, acted as CYP3A4 inhibitors, bromuconazole, difenoconazole, mefentri-fluconazole, metconazole and propiconazole appeared to be potent ones, inhibiting CYP3A4 activity by more than 80%, as ketoconazole used here as a reference potent CYP3A4 inhibitor (O'Donnell et al., 2007) (Fig. 3).

3.4. Effect of triazole pesticides on drug transporter mRNA expression

Regulations of mRNA levels of the ATP-binding cassette (ABC) canalicular transporters P-gp/MDR1, MRP2 and BCRP by triazoles are shown in Fig. 4; phenobarbital and rifampicin are concomitantly used here as reference inducers of human canalicular transporters (Jigorel et al., 2006). Using a 1.5-fold change in at least 33.3% of the HepaSH cell populations as a threshold (Jigorel et al., 2006), P-gp/MDR1 mRNA levels were induced by 3/9 azoles, i.e., bromuconazole, tetraconazole

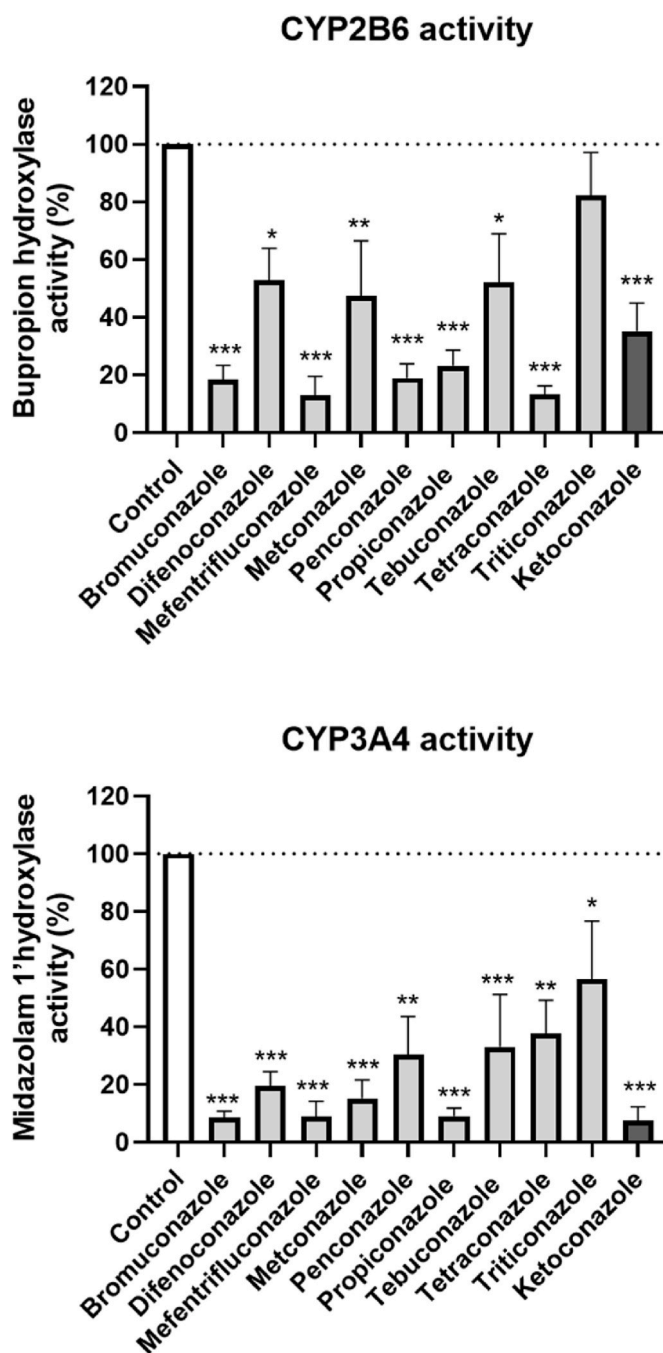


Fig. 3. Direct inhibitory effects of triazoles on CYP2B6 and CYP3A4 activities. HepaSH cells were exposed to 100 μM bupropion for 4 h or to 50 μM midazolam for 1 h, in the absence (control) or presence of 10 μM triazoles or 10 μM ketoconazole (used here as a reference CYP inhibitor). CYP2B6 and CYP3A4 activities, i.e., bupropion hydroxylase (CYP2B6 activity) and midazolam 1'-hydroxylase activity (CYP3A4 activity), were next measured by LC-MS/MS. Data are expressed as percentages of CYP activity found in control cells, set at 100%, and are the means \pm SEM of values from 4 to 6 HepaSH cell batches. The dashed lines represent 100% CYP activity. *, $p < 0.005$, **, $p < 0.01$ and ***, $p < 0.001$ when compared to control.

and triticonazole, whereas those of MRP2 were increased by 4/9 azoles, i.e., bromuconazole, mefentrifluconazole, tetraconazole and triticonazole, and those of BCRP by 6/9 azoles, i.e., mefentrifluconazole, metconazole, penconazole, propiconazole, tetraconazole and triticonazole (Table S7). The reference inducers, i.e., omeprazole, phenobarbital and rifampicin similarly induced P-gp/MDR1, MRP2 and BCRP mRNA

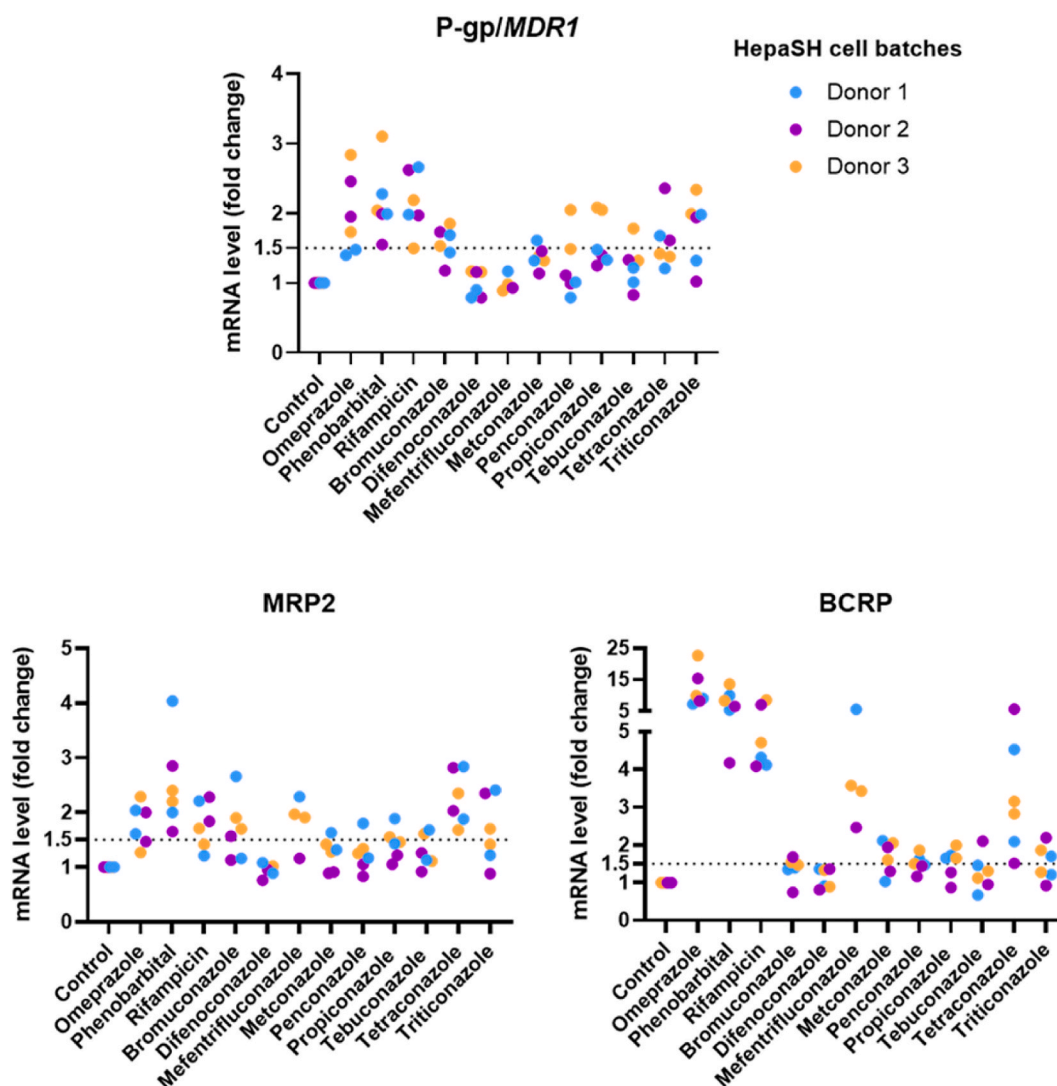


Fig. 4. Regulation of P-gp/MDR1, MRP2 and BCRP mRNA expression by a 48-h exposure to triazoles in human HepaSH cells. (A) HepaSH cells from 4 to 6 batches generated from three human liver donors (one or two batches/donor) were either untreated (control) or exposed to 50 μM omeprazole, 1 mM phenobarbital, 10 μM rifampicin or 10 μM triazoles for 48 h. Transporter mRNA expressions were next determined by RT-qPCR. Data are expressed as fold changes of transporter mRNA levels comparatively to control. Values for each HepaSH cell batch are shown by dots, differently coloured according to the human liver donors from which HepaSH cell batches derive. An identical colour therefore applies to HepaSH cell batches deriving from chimeric mice engrafted with human hepatocytes from the same human liver donor. Dashed lines on the graphs indicate the 1.5 fold induction factor, used as a threshold for being considered as transporter mRNA inducer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

expression (Fig. 4). The fold changes for P-gp/MDR1 or MRP2 mRNA induction by reference inducers were however rather moderate, i.e., they ranged from 1.8 to 2.5; by contrast, BCRP mRNA levels were induced in a more robust manner by reference inducers, notably by omeprazole (induction by a 12.1-fold factor) (Table S7).

For the sinusoidal solute carrier (SLC) transporters organic anion transporter polypeptide (OATP) 1B1/SLCO1B1, OATP1B3/SLCO1B3, OATP2B1/SLCO2B1 and organic cation transporter (OCT) 1/SLC22A1, they were not induced by triazole pesticide in HepaSH cells with respect to their mRNA expression, excepted OATP1B3 induced by mefentrifluconazole (by a 2.2-fold factor) and tetraconazole (by a 1.9-fold factor) (Fig. S7). In the same way, omeprazole, rifampicin and phenobarbital failed to impair mRNA expression of these hepatic SLC transporters (Fig. S7).

3.5. Prediction of *in vivo* induction of CYP2B6 and CYP3A4 by bromuconazole

Predictions of *in vivo* induction of hepatic CYP2B6 and CYP3A4 in humans chronically, acutely or occupationally exposed to bromuconazole, which represents the most potent one among tested triazoles for *in vitro* inducing mRNA expression of these CYPs (Fig. 1A), was performed as previously described (Fahmi and Ripp, 2010; Kerhoas et al., 2024). These predictions were based on EC_{50} values of the triazole pesticide towards CYP2B6 and CYP3A4 mRNA expression (Fig. 1B) and bromuconazole $I_{\text{max}, \text{u}}$ calculated from its ADI (chronic exposure), its ARfD (acute exposure) or its AOEL (occupational exposure for workers). As indicated in Table 1, bromuconazole was predicted to be a strong (acute exposure) or moderate (chronic or occupational exposure) *in vivo* inducer of CYP2B6 and CYP3A4 mRNA expression.

Table 1
Prediction of *in vivo* CYP2B6 and CYP3A4 mRNA induction by bromuconazole.

Exposure type	Bromuconazole					Predicted <i>in vivo</i> induction ^d		
	Toxicological reference values	f_u ^a	$I_{max, u}$ ^b (μM)	EC ₅₀ (μM) (CYP mRNA induction) ^c	Ratio $I_{max, u}/EC_{50}$	CYP2B6	CYP3A4	CYP2B6
Chronic exposure	ADI = 0.01 mg/kg bw/day	0.032	0.012	5.79 (CYP2B6) 3.21 (CYP3A4)	0.002	0.004	Moderate induction	Moderate induction
Acute exposure	ARfD = 0.1 mg/kg bw/day		0.119		0.021	0.037	Strong induction	Strong induction
Occupational exposure	AOEL = 0.025 mg/kg bw/day		0.030		0.005	0.009	Moderate induction	Moderate induction

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

^b defined as the maximum unbound plasma concentration.

^c according to Fig. 1B.

^d *In vivo* induction of CYP2B6 and CYP3A4 mRNA expression was predicted according to the ratio $I_{max, u}/EC_{50}$: ratio ≤ 0.001 , $0.001 < \text{ratio} \leq 0.01$ and ratio > 0.01 would predict bromuconazole to be a weak, moderate and strong CYP3A4 or CYP2B6 inducer, respectively (Fahmi and Ripp, 2010), as described in the Material and methods section. bw, body weight.

4. Discussion

The data reported in the present study demonstrate that most of triazole agrochemicals used at a non-toxic 10 μM concentration can regulate expression of various drug detoxifying enzymes and drug transporters in human hepatic HepaSH cells, thus highlighting that the human hepatic detoxication system is impacted by these fungicides in a notable way, as already suggested (Knebel et al., 2018; Marx-Stoelting et al., 2020). This conclusion is fully supported by the fact that various triazoles not investigated in the present study, such as myclobutanil, triadimefon, cyproconazole and epoxiconazole, also induced mRNA expression of various CYPs in primary human hepatocytes or differentiated human hepatoma HepaRG cells (Goetz and Dix, 2009; Seeger et al., 2019). The profile of drug metabolizing enzyme and drug transporter mRNA regulation by 10 μM triazoles, summarized in Table 2, however depends of the nature of the fungicide. Indeed, bromuconazole, tetraconazole and triticonazole induced mRNA expression of 7–9/12 enzymes and transporters, whereas mefenfentrifluconazole, penconazole and tebuconazole that of only 4 or 5/12 enzymes and transporters and difenoconazole none (Table 2). In addition, enzyme and transporter mRNA expressions were differentially impacted by triazoles. CYPs as well as UGT1A1 were thus induced by at least 7/9 triazoles, whereas ABC transporters like P-gp/*MDR1*, MRP2 and BCRP were up-regulated by 3–5/9 triazoles and the SLC transporter OATP1B3 by 2/9 triazoles (Table 2). None of triazoles induced expression of OCT1, OATP1B1 and OATP2B1 mRNAs. Moreover, the quantitative levels of drug metabolizing enzyme and transporter mRNA inductions by triazoles, comparatively to those triggered by reference inducers, vary according to the considered enzyme or transporter. CYP1A1 and CYP1A2 mRNA levels were thus rather poorly induced by 10 μM triazoles when compared to 50 μM omeprazole (Tables S3 and S4), whereas, by contrast, 10 μM bromuconazole was as effective as 1 mM phenobarbital and 10 μM rifampicin for inducing expression of CYP2B6, CYP3A4, UGT1A1 or MRP2 (Tables S3, S4 and S7). The nature of the enzyme or transporter as well as that of the triazole fungicide are thus key parameters to consider for apprehending the regulation of drug metabolizing enzyme or drug transporter mRNA expression by these agrochemicals.

Induction of CYP3A4 mRNA expression by triazoles paralleled that of CYP3A4 protein, thus fully confirming that CYP3A4 is a target for triazoles. By contrast, CYP1A2 protein was not induced by triazoles, excepted by tetraconazole. This may be due to the relative low level of CYP1A2 mRNA induction by triazoles, as already discussed above, and to the fact that Western-blotting is rather a semi-quantitative technique (Heidebrecht et al., 2009), probably not optimal for detecting low levels of protein induction. CYP1A2 induction by triazoles is nevertheless confirmed by the increased CYP1A2 activity in HepaSH cells exposed to various triazoles, even if the increase is rather modest when compared to that due to the reference inducer omeprazole, but agrees with the low

Table 2

Summary of inducing effects of 10 μM triazole fungicides towards drug metabolizing enzyme and transporter mRNA expression in HepaSH cells.

Triazole agrochemical	Induced genes (mRNA expression)		
	Drug metabolizing enzyme	Drug transporter	Total number of regulated genes/ total number of investigated genes ^a
Bromuconazole	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1	P-gp/ <i>MDR1</i> , MRP2	7/12
Difenoconazole	–	–	0/12
Mefentrifluconazole	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1	MRP2, OATP1B3	4/12
Metconazole	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1	BCRP	6/12
Penconazole	CYP1A1, CYP2B6, CYP3A4, UGT1A1	BCRP	5/12
Propiconazole	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1	BCRP	6/12
Tebuconazole	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1	–	5/12
Tetraconazole	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1	P-gp/ <i>MDR1</i> , MRP2, BCRP, OATP1B3	9/12
Triticonazole	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1	P-gp/ <i>MDR1</i> , MRP2, BCRP	8/12

^a The investigated genes were CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1, P-gp/*MDR1*, MRP2, BCRP, OATP1B1, OATP1B3, OATP2B1 and OCT1.

induction of CYP1A2 mRNA levels. With respect to CYP2B6 and CYP3A4 activities, they were directly inhibited by triazoles, excepted triticonazole that failed to decrease CYP2B6 activity. Most of triazoles appear therefore as dual regulators of these CYPs, *i.e.*, they enhance their expression but directly inhibit their activity, as already described for

drugs and other fungicides like SDHs (Kerhoas et al., 2024). For CYP2B6, the resulting effect is however a global increase of its activity after a 48-h treatment by bromuconazole, difenoconazole, metconazole, propiconazole, tebuconazole and triticonazole, suggesting that the inducing effect towards CYP2B6 expression predominates over the CYP2B6 activity inhibition for these triazoles. In the same way, for CYP3A4, the inducing effect predominates for bromuconazole, penconazole, propiconazole and tebuconazole and triticonazole.

The mechanism by which triazoles induce expression of drug metabolizing enzymes and drug transporters in HepaSH cells likely involves drug-sensing nuclear receptors. Indeed, some triazoles, such as propiconazole and tebuconazole, are well known to activate PXR, CAR and AhR in reporter assays (Knebel et al., 2018, 2019b). These nuclear receptors control expression of various enzymes and transporters, such as CYP1A1 and CYP1A2 (regulated by AhR) (Nebert et al., 2004), CYP2B6, CYP3A4, P-gp/*MDR1* and MRP2 (regulated by CAR and PXR) (Burk et al., 2005; Geick et al., 2001; Goodwin et al., 2001; Kast et al., 2002; Willson and Kliewer, 2002), and UGT1A1 and BCRP (regulated by AhR, PXR and CAR) (Sugatani et al., 2005; Tompkins et al., 2010; Wang et al., 2010; Whyte-Allman et al., 2017; Yueh et al., 2003). The fact that these genes targeted by AhR, PXR and/or CAR are impacted by triazoles in HepaSH cells fully supports the implication of these nuclear receptors. Involvement of PXR in CYP3A4 mRNA induction in bromuconazole-treated HepaSH cells is moreover supported by the findings that (i) bromuconazole was reported to activate PXR in the present study, with an EC₅₀ value (2.6 μM) close to that (3.21 μM) observed for the induction of the reference PXR target gene CYP3A4 in HepaSH cells and (ii) chemical inhibition of PXR using SPA70 hindered CYP3A4 induction by bromuconazole in HepaSH cells.

The *in vivo* relevance of our *in vitro* findings, *i.e.*, the regulation of hepatic drug metabolizing enzymes and drug transporters by triazole fungicides in HepaSH cells, remains to be determined. In this context, the fact that at least bromuconazole was predicted to *in vivo* induce CYP3A4 and CYP2B6 expression in response to chronic, acute or occupational exposure is worthy of interest, even if the induction is predicted to be strong only for the acute situation. Indeed, this suggests that the hepatic detoxifying system may interact with triazoles in humans exposed to these agrochemicals, at least in the worst situations of high exposures, corresponding to the triazole ADI, ArfD and AOEL values, and with the limitation that weak levels of exposure to the fungicides may be ineffective. Through increasing CYP expression and activity (notably for bromuconazole, propiconazole and tebuconazole), and also expression of canalicular transporters such as P-gp/*MDR1*, MRP2 and BCRP, implicated in biliary excretion of xenobiotics, notable exposure to triazoles fungicides may be susceptible to enhance hepatic clearance of drugs, which may cause a decrease in their plasma concentrations, leading to drug inefficacy. Such putative triazole-drug interactions have however not been yet clinically reported, to the best of our knowledge, but they have likely to deserve attention, as potential interactions of other environmental chemicals with biotransformation pathways (Alvares, 1978; Hodgson and Rose, 2008). The fact that tebuconazole may change pharmacokinetics of drugs through impairing their intestinal absorption (Xu et al., 2023) as well as the inhibition of human renal transporter activities by triazoles (Nie et al., 2023) also support the interest for drug-triazole interactions. The enhanced expression of enzymes and transporters in response to triazoles may also result in increased hepatic metabolism of their endogenous substrates, including steroid and thyroid hormones. This may lead to altered half-life of these endogenous compounds, which may have toxic consequences. Increased metabolism of thyroid hormones due to hepatic UGT induction is thus thought to contribute to endocrine disrupting effects of triazoles in rats (Kadic et al., 2024); the induction of UGT1A1 expression by triazoles in human HepaSH cells supports the hypothesis that the hepatic metabolism, and consequently the physiological functions, of thyroid hormones may be similarly impacted in humans exposed to these agrochemicals. The mechanism by which triazoles up-regulate drug

metabolizing enzyme and transporter levels, *i.e.*, the activation of nuclear receptors, is also important to consider. Indeed, these receptors play various important physiological roles in addition to drug metabolizing enzyme and drug transporter regulation, notably in inflammatory response, cell proliferation, cell metabolism and cell migration (Oladimeji and Chen, 2018; Stern et al., 2022). Activation of these receptors by triazoles may therefore be hypothesized to trigger diverse potential deleterious effects for human health.

Finally, it is noteworthy that human hepatic HepaSH cells, cultured as fresh cells in the present study after their isolation from chimeric TK-NOG mice, were fully responsive to reference inducers of CYPs and transporters, such as omeprazole, phenobarbital and rifampicin. HepaSH cells have previously been shown to exhibit similar levels of drug metabolizing enzymes and transporters than primary human hepatocytes and support long-term culture conditions and drug metabolism activity (Uehara et al., 2023, 2024; Zerdoug et al., 2023). Taken together, these data fully support the interest of such cells as surrogates for primary human hepatocytes in *in vitro* xenobiotic metabolism and toxicity studies. In particular, their reinforced availability and sustainability, when compared to primary human hepatocytes, as well as the preservation of the genetic background of the liver donors from which HepaSH cells are derived (Jiang et al., 2024), make them attractive promising *in vitro* models for predicting and/or deciphering the human hepatic effects of environmental pollutants. Our present data, centred on triazole agrochemicals, fully support such a use of HepaSH cells for *in vitro* studying hepatotoxicity of environmental chemicals, which represents an important challenge to presently take into account.

5. Conclusion

Triazole fungicides were found to induce mRNA expression of various drug metabolizing enzymes, including CYPs and UGT1A1, and of drug transporters, especially ABC efflux transporters, in cultured human hepatic HepaSH cells. The profile of enzyme and transporter mRNA regulation however depends of the nature of the agrochemicals. Enhanced levels of CYP3A4 mRNAs in response to triazoles paralleled increased expression of CYP3A4 protein. Triazoles were additionally found to directly inhibit CYP2B6 and CYP3A4 activities, the inducing effects being however predominating for some of them such as bromuconazole, propiconazole and tebuconazole. Bromuconazole was moreover predicted to induce CYP2B6 and CYP3A4 mRNA expression in humans exposed to this fungicide in a chronic, acute or occupational context. These data thus demonstrate that the human hepatic detoxification system is impacted by triazole pesticides in a notable way, which may have to be taken into account for the risk assessment of these agrochemicals. They also highlight the interest of the use of human HepaSH cells as attractive and promising surrogates to primary human hepatocytes for studying hepatic effects of environmental chemicals.

CRedit authorship contribution statement

Anna Zerdoug: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marc Le Vée:** Writing – review & editing, Supervision, Formal analysis, Data curation, Conceptualization. **Hélène Le Mentec:** Investigation. **Jennifer Carteret:** Investigation. **Elodie Jouan:** Investigation. **Agnès Jamin:** Writing – review & editing, Methodology. **Béatrice Lopez:** Writing – review & editing, Methodology. **Shotaro Uehara:** Resources. **Yuichiro Higuchi:** Resources. **Nao Yoneda:** Resources. **Christophe Chesné:** Writing – review & editing, Supervision. **Hiroshi Suemizu:** Writing – review & editing, Resources. **Olivier Fardel:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

- Alvares, A.P., 1978. Research review. Interactions between environmental chemicals and drug biotransformation in man. *Clin. Pharmacokinet.* 3, 462–477. <https://doi.org/10.2165/00003088-197803060-00004>.
- Bellot, P., Brischoux, F., Fritsch, C., Goutte, A., Alliot, F., Rocchi, S., Angelier, F., 2022. Evidence of environmental transfer of tebuconazole to the eggs in the house sparrow (*Passer domesticus*): an experimental study. *Chemosphere* 308, 136469. <https://doi.org/10.1016/j.chemosphere.2022.136469>.
- Béranger, R., Hardy, E.M., Binter, A.-C., Charles, M.-A., Zaros, C., Appenzeller, B.M.R., Chevrier, C., 2020. Multiple pesticides in mothers' hair samples and children's measurements at birth: results from the French national birth cohort (ELFE). *Int. J. Hyg. Environ. Health* 223, 22–33. <https://doi.org/10.1016/j.ijheh.2019.10.010>.
- Boon, P.E., van Donkersgoed, G., Christodoulou, D., Crépet, A., D'Addezio, L., Desvignes, V., Ericsson, B.-G., Galimberti, F., Ioannou-Kakouri, E., Jensen, B.H., Rehrkova, I., Retz, J., Ruprich, J., Sand, S., Stephenson, C., Strömberg, A., Turrini, A., van der Voet, H., Ziegler, P., Hamey, P., van Klaveren, J.D., 2015. Cumulative dietary exposure to a selected group of pesticides of the triazole group in different European countries according to the EFSA guidance on probabilistic modelling. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* 79, 13–31. <https://doi.org/10.1016/j.fct.2014.08.004>.
- Bulutoglu, B., Rey-Bedón, C., Mert, S., Tian, L., Jang, Y.-Y., Yarmush, M.L., Usta, O.B., 2020. A comparison of hepato-cellular in vitro platforms to study CYP3A4 induction. *PLoS One* 15, e0229106. <https://doi.org/10.1371/journal.pone.0229106>.
- Burk, O., Arnold, K.A., Geick, A., Tegude, H., Eichelbaum, M., 2005. A role for constitutive androstane receptor in the regulation of human intestinal MDR1 expression. *Biol. Chem.* 386, 503–513. <https://doi.org/10.1515/BC.2005.060>.
- Chesné, C., Guyomard, C., Grislain, L., Clerc, C., Fautrel, A., Guillouz, A., 1991. Use of cryopreserved animal and human hepatocytes for cytotoxicity studies. *Toxicol. Vitro Int. J. Publ. Assoc. BIBRA* 5, 479–482. [https://doi.org/10.1016/0887-2333\(91\)90076](https://doi.org/10.1016/0887-2333(91)90076).
- Czyski, A., Reszta, M., Świdarski, P., Brylak, J., Główna, F.K., 2021. The overview on the pharmacokinetic and pharmacodynamic interactions of triazoles. *Pharmaceutics* 13, 1961. <https://doi.org/10.3390/pharmaceutics13111961>.
- Donato, M.T., Gómez-Lechón, M.J., Castell, J.V., 1990. Effect of xenobiotics on monooxygenase activities in cultured human hepatocytes. *Biochem. Pharmacol.* 39, 1321–1326. [https://doi.org/10.1016/0006-2952\(90\)90008-9](https://doi.org/10.1016/0006-2952(90)90008-9).
- Dong, Z., Cui, K., Liang, J., Guan, S., Fang, L., Ding, R., Wang, J., Li, T., Zhao, S., Wang, Z., 2023. The widespread presence of triazole fungicides in greenhouse soils in Shandong Province, China: a systematic study on human health and ecological risk assessments. *Environ. Pollut. Barking Essex* 328, 121637. <https://doi.org/10.1016/j.envpol.2023.121637>, 1987.
- Fahmi, O.A., Ripp, S.L., 2010. Evaluation of models for predicting drug-drug interactions due to induction. *Expert Opin. Drug Metab. Toxicol.* 6, 1399–1416. <https://doi.org/10.1517/17425255.2010.516251>.
- Geick, A., Eichelbaum, M., Burk, O., 2001. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J. Biol. Chem.* 276, 14581–14587. <https://doi.org/10.1074/jbc.M010173200>.
- Goetz, A.K., Dix, D.J., 2009. Toxicogenomic effects common to triazole antifungals and conserved between rats and humans. *Toxicol. Appl. Pharmacol.* 238, 80–89. <https://doi.org/10.1016/j.taap.2009.04.016>.
- Gómez-Lechón, M.J., Donato, M.T., Castell, J.V., Jover, R., 2004. Human hepatocytes in primary culture: the choice to investigate drug metabolism in man. *Curr. Drug Metab.* 5, 443–462. <https://doi.org/10.2174/1389200043335414>.
- Gómez-Lechón, M.J., Donato, T., Ponsoda, X., Castell, J.V., 2003. Human hepatic cell cultures: in vitro and in vivo drug metabolism. *Altern. Lab. Anim. ATLA* 31, 257–265. <https://doi.org/10.1177/026119290303100307>.
- Gómez-Lechón, M.J., Tolosa, L., Conde, I., Donato, M.T., 2014. Competency of different cell models to predict human hepatotoxic drugs. *Expert Opin. Drug Metab. Toxicol.* 10, 1553–1568. <https://doi.org/10.1517/17425255.2014.967680>.
- Goodwin, B., Moore, L.B., Stoltz, C.M., McKee, D.D., Kliever, S.A., 2001. Regulation of the human CYP2B6 gene by the nuclear pregnane X receptor. *Mol. Pharmacol.* 60, 427–431.
- Habenschus, M.D., Nardini, V., Dias, L.G., Rocha, B.A., Barbosa, F., de Oliveira, A.R.M., 2019. In vitro enantioselective study of the toxicokinetic effects of chiral fungicide tebuconazole in human liver microsomes. *Ecotoxicol. Environ. Saf.* 181, 96–105. <https://doi.org/10.1016/j.ecoenv.2019.05.071>.
- Halladay, J.S., Wong, S., Khojasteh, S.C., Grepper, S., 2012. An 'all-inclusive' 96-well cytochrome P450 induction method: measuring enzyme activity, mRNA levels, protein levels, and cytotoxicity from one well using cryopreserved human hepatocytes. *J. Pharmacol. Toxicol. Methods* 66, 270–275. <https://doi.org/10.1016/j.jvascn.2012.07.004>.
- Hammer, H., Schmidt, F., Heise, T., Knebel, C., Dabrowski, A., Planatscher, H., Kneuer, C., Marx-Stoelting, P., Pötter, O., 2020. Induction and repression effects on CYP and transporter protein abundance by azole mixture uptake in rat liver. *EXCLI J* 19, 904–916. <https://doi.org/10.17179/excli2020-2311>.
- Hasegawa, M., Kawai, K., Mitsui, T., Taniguchi, K., Monnai, M., Wakui, M., Ito, M., Suematsu, M., Peltz, G., Nakamura, M., Suemizu, H., 2011. The reconstituted "humanized liver" in TK-NOG mice is mature and functional. *Biochem. Biophys. Res. Commun.* 405, 405–410. <https://doi.org/10.1016/j.bbrc.2011.01.042>.
- Heidebrecht, F., Heidebrecht, A., Schulz, I., Behrens, S.-E., Bader, A., 2009. Improved semiquantitative Western blot technique with increased quantification range. *J. Immunol. Methods* 345, 40–48. <https://doi.org/10.1016/j.jim.2009.03.018>.
- Hodgson, E., Rose, R.L., 2008. Metabolic interactions of agrochemicals in humans. *Pest Manag. Sci.* 64, 617–621. <https://doi.org/10.1002/ps.1563>.
- Jiang, C., Li, P., Ma, Y., Yoneda, N., Kawai, K., Uehara, S., Ohnishi, Y., Suemizu, H., Cao, H., 2024. Comprehensive gene profiling of the metabolic landscape of humanized livers in mice. *J. Hepatol.* 80, 622–633. <https://doi.org/10.1016/j.jhep.2023.11.020>.
- Jigorel, E., Le Vee, M., Boursier-Neyret, C., Parmentier, Y., Fardel, O., 2006. Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by xenobiotics activating drug-sensing receptors in primary human hepatocytes. *Drug Metab. Dispos. Biol. Fate Chem.* 34, 1756–1763. <https://doi.org/10.1124/dmd.106.010033>.
- Kadic, A., Oles, P., Fischer, B.C., Retz, A.E., Sylla, B.S., Feiertag, K., Ritz, V., Heise, T., Marx-Stoelting, P., Tralau, T., Renko, K., Solano, M. de L.M., 2024. In vitro and in vivo investigation of a thyroid hormone system-specific interaction with triazoles. *Sci. Rep.* 14, 6503. <https://doi.org/10.1038/s41598-024-55019-3>.
- Kast, H.R., Goodwin, B., Tarr, P.T., Jones, S.A., Anisfeld, A.M., Stoltz, C.M., Tontonoz, P., Kliever, S., Willson, T.M., Edwards, P.A., 2002. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *J. Biol. Chem.* 277, 2908–2915. <https://doi.org/10.1074/jbc.M109326200>.
- Kenny, J.R., Ramsden, D., Buckley, D.B., Dallas, S., Fung, C., Mohutsky, M., Einolf, H.J., Chen, L., Dekeyser, J.G., Fitzgerald, M., Goosen, T.C., Siu, Y.A., Walsky, R.L., Zhang, G., Tweedie, D., Hariparsad, N., 2018. Considerations from the innovation and quality induction working group in response to drug-drug interaction guidances from regulatory agencies: focus on CYP3A4 mRNA in vitro response thresholds, variability, and clinical relevance. *Drug Metab. Dispos. Biol. Fate Chem.* 46, 1285–1303. <https://doi.org/10.1124/dmd.118.081927>.
- Kerhoas, M., Carteret, J., Huchet, L., Jouan, E., Huc, L., Vée, M.L., Fardel, O., 2024. Induction of human hepatic cytochrome P-450 3A4 expression by antifungal succinate dehydrogenase inhibitors. *Ecotoxicol. Environ. Saf.* 276, 116261. <https://doi.org/10.1016/j.ecoenv.2024.116261>.
- Knebel, C., Buhrke, T., Süßmuth, R., Lampen, A., Marx-Stoelting, P., Braeuning, A., 2019a. Pregnane X receptor mediates steatotic effects of propiconazole and tebuconazole in human liver cell lines. *Arch. Toxicol.* 93, 1311–1322. <https://doi.org/10.1007/s00204-019-02445-2>.
- Knebel, C., Heise, T., Zanger, U.M., Lampen, A., Marx-Stoelting, P., Braeuning, A., 2019b. The azole fungicide tebuconazole affects human CYP1A1 and CYP1A2 expression by an aryl hydrocarbon receptor-dependent pathway. *Food Chem. Toxicol.* 123, 481–491. <https://doi.org/10.1016/j.fct.2018.11.039>.
- Knebel, C., Neeb, J., Zahn, E., Schmidt, F., Carazo, A., Holas, O., Pavek, P., Püschel, G.P., Zanger, U.M., Süßmuth, R., Lampen, A., Marx-Stoelting, P., Braeuning, A., 2018. Unexpected effects of propiconazole, tebuconazole, and their mixture on the receptors CAR and PXR in human liver cells. *Toxicol. Sci.* 163, 170–181. <https://doi.org/10.1093/toxsci/kfy026>.
- Ku, T., Tan, X., Liu, Y., Wang, R., Fan, L., Ren, Z., Ning, X., Li, G., Sang, N., 2023. Triazole fungicides exert neural differentiation alteration through H3K27me3 modifications: in vitro and in silico study. *J. Hazard Mater.* 459, 132225. <https://doi.org/10.1016/j.jhazmat.2023.132225>.
- Kyffin, J.A., Sharma, P., Leedale, J., Colley, H.E., Murdoch, C., Mistry, P., Webb, S.D., 2018. Impact of cell types and culture methods on the functionality of *in vitro* liver systems – a review of cell systems for hepatotoxicity assessment. *Toxicol. Vitro* 48, 262–275. <https://doi.org/10.1016/j.tiv.2018.01.023>.
- Leite, F.G., Silva, C., de, P.M., Miranda, R.G., Dorta, D.J., 2024. Comparison of in vitro toxicity in HepG2 cells: toxicological role of Tebuconazole-tert-butyl-hydroxy in exposure to the fungicide Tebuconazole. *Pestic. Biochem. Physiol.* 202, 105954. <https://doi.org/10.1016/j.pestbp.2024.105954>.

- Lichtenstein, D., Luckert, C., Alarcán, J., de Sousa, G., Gioutlakis, M., Katsanou, E.S., Konstantinidou, P., Machera, K., Milani, E.S., Peijnenburg, A., Rahmani, R., Rijkers, D., Spyropoulou, A., Stamou, M., Stoop, G., Sturla, S.J., Wollschied, B., Zucchini-Pascal, N., Braeuning, A., Lampen, A., 2020. An adverse outcome pathway-based approach to assess steatotic mixture effects of hepatotoxic pesticides in vitro. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* 139, 111283. <https://doi.org/10.1016/j.fct.2020.111283>.
- Lv, X., Pan, L., Wang, J., Lu, L., Yan, W., Zhu, Y., Xu, Y., Guo, M., Zhuang, S., 2017. Effects of triazole fungicides on androgenic disruption and CYP3A4 enzyme activity. *Environ. Pollut. Barking Essex* 222, 504–512. <https://doi.org/10.1016/j.envpol.2016.11.051>, 1987.
- Marciano, L.P.A., Costa, L.F., Cardoso, N.S., Freire, J., Feltrim, F., Oliveira, G.S., Paula, F. B.A., Silvério, A.C.P., Martins, L., 2024. Biomonitoring and risk assessment of human exposure to triazole fungicides. *Regul. Toxicol. Pharmacol.* 147, 105565. <https://doi.org/10.1016/j.yrtph.2024.105565>.
- Martínez-Matías, N., Rodríguez-Medina, J.R., 2018. Fundamental concepts of azole compounds and triazole antifungals: a beginner's review. *P. R. Health Sci. J.* 37, 135–142.
- Marx-Stoelting, P., Ganzenberg, K., Knebel, C., Schmidt, Flavia, Rieke, S., Hammer, H., Schmidt, Felix, Pötz, O., Schwarz, M., Braeuning, A., 2017. Hepatotoxic effects of cyproconazole and prochloraz in wild-type and hCAR/hPXR mice. *Arch. Toxicol.* 91, 2895–2907. <https://doi.org/10.1007/s00204-016-1925-2>.
- Marx-Stoelting, P., Knebel, C., Braeuning, A., 2020. The connection of azole fungicides with xeno-sensing nuclear receptors, drug metabolism and hepatotoxicity. *Cells* 9, 1192. <https://doi.org/10.3390/cells9051192>.
- Mercadante, R., Polledri, E., Rubino, F.M., Mandic-Rajcevic, S., Vaiani, A., Colosio, C., Moretto, A., Fustinoni, S., 2019. Assessment of penconazole exposure in winegrowers using urinary biomarkers. *Environ. Res.* 168, 54–61. <https://doi.org/10.1016/j.envres.2018.09.013>.
- Nagai, M., Konno, Y., Satsukawa, M., Yamashita, S., Yoshinari, K., 2016. Establishment of in silico prediction models for CYP3A4 and CYP2B6 induction in human hepatocytes by multiple regression analysis using azole compounds. *Drug Metab. Dispos. Biol. Fate Chem.* 44, 1390–1398. <https://doi.org/10.1124/dmd.115.068619>.
- Nebert, D.W., Dalton, T.P., Okey, A.B., Gonzalez, F.J., 2004. Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J. Biol. Chem.* 279, 23847–23850. <https://doi.org/10.1074/jbc.R400004200>.
- Nie, J., Zhou, J., Shen, Y., Lin, R., Hu, H., Zeng, K., Bi, H., Huang, M., Yu, L., Zeng, S., Miao, J., 2023. Studies on the interaction of five triazole fungicides with human renal transporters in cells. *Toxicol. Vitro Int. J. Publ. Assoc. BIBRA* 88, 105555. <https://doi.org/10.1016/j.tiv.2023.105555>.
- O'Donnell, C.J., Grime, K., Courtney, P., Slee, D., Riley, R.J., 2007. The development of a cocktail CYP2B6, CYP2C8, and CYP3A5 inhibition assay and a preliminary assessment of utility in a drug discovery setting. *Drug Metab. Dispos. Biol. Fate Chem.* 35, 381–385. <https://doi.org/10.1124/dmd.106.012344>.
- Oladimeji, P.O., Chen, T., 2018. PXR: more than just a master xenobiotic receptor. *Mol. Pharmacol.* 93, 119–127. <https://doi.org/10.1124/mol.117.110155>.
- Pascucci, J.M., Drocourt, L., Fabre, J.M., Maurel, P., Vilarem, M.J., 2000. Dexamethasone induces pregnane X receptor and retinoid X receptor-alpha expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol. Pharmacol.* 58, 361–372. <https://doi.org/10.1124/mol.58.2.361>.
- Price, C.L., Parker, J.E., Warrilow, A.G., Kelly, D.E., Kelly, S.L., 2015. Azole fungicides – understanding resistance mechanisms in agricultural fungal pathogens. *Pest Manag. Sci.* 71, 1054–1058. <https://doi.org/10.1002/ps.4029>.
- Reddy, R.T., Nyunoya, T., 2021. Identification of novel pregnane X receptor (PXR) agonists by in silico and biological activity analyses and reversal of cigarette smoke-induced PXR downregulation. *Biochem. Biophys. Res. Commun.* 555, 1–6. <https://doi.org/10.1016/j.bbrc.2021.02.145>.
- Rjiba-Touati, K., Ayed-Boussema, I., Hamdi, H., Azzebi, A., Abid, S., 2022. Bromuconazole fungicide induces cell cycle arrest and apoptotic cell death in cultured human colon carcinoma cells (HCT116) via oxidative stress process. *Biomark. Biochem. Indic. Expo. Response Susceptibility Chem.* 27, 659–670. <https://doi.org/10.1080/1354750X.2022.2098378>.
- Ruoß, M., Vosough, M., Königsrainer, A., Nadalin, S., Wagner, S., Sajadian, S., Huber, D., Heydari, Z., Ehnert, S., Hengstler, J.G., Nussler, A.K., 2020. Towards improved hepatocyte cultures: progress and limitations. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* 138, 111188. <https://doi.org/10.1016/j.fct.2020.111188>.
- Savaryn, J.P., Sun, J., Ma, J., Jenkins, G.J., Stresser, D.M., 2021. Broad application of CYP3A4 LC-MS protein quantification in hepatocyte cytochrome P450 induction assays identifies nonuniformity in mRNA and protein induction responses. *Drug Metab. Dispos.* <https://doi.org/10.1124/dmd.121.000638>.
- Seeger, B., Mentz, A., Knebel, C., Schmidt, F., Bednarz, H., Niehaus, K., Albaum, S., Kalinowski, J., Noll, T., Steinberg, P., Marx-Stoelting, P., Heise, T., 2019. Assessment of mixture toxicity of (tri)azoles and their hepatotoxic effects in vitro by means of omics technologies. *Arch. Toxicol.* 93, 2321–2333. <https://doi.org/10.1007/s00204-019-02502-w>.
- Serra, L., Estienne, A., Caria, G., Ramé, C., Jolivet, C., Froger, C., Henriot, A., Amalric, L., Guérif, F., Froment, P., Dupont, J., 2023. In vitro exposure to triazoles used as fungicides impairs human granulosa cells steroidogenesis. *Environ. Toxicol. Pharmacol.* 104, 104295. <https://doi.org/10.1016/j.etap.2023.104295>.
- Sorrentino, D., Van Ness, K., Ribeiro, L., Miller, C.M., 1991. Functional and morphological features of isolated hepatocytes preserved in University of Wisconsin solution. *Hepatology* 14, 331–339.
- Stern, S., Kurian, R., Wang, H., 2022. Clinical relevance of the constitutive androstane receptor. *Drug Metab. Dispos. Biol. Fate Chem.* 50, 1010–1018. <https://doi.org/10.1124/dmd.121.000483>.
- Sugatani, J., Nishitani, S., Yamakawa, K., Yoshinari, K., Sueyoshi, T., Negishi, M., Miwa, M., 2005. Transcriptional regulation of human UGT1A1 gene expression: activated glucocorticoid receptor enhances constitutive androstane receptor/pregnane X receptor-mediated UDP-glucuronosyltransferase 1A1 regulation with glucocorticoid receptor-interacting protein 1. *Mol. Pharmacol.* 67, 845–855. <https://doi.org/10.1124/mol.104.007161>.
- Toda, M., Beer, K.D., Kuivila, K.M., Chiller, T.M., Jackson, B.R., 2021. Trends in agricultural triazole fungicide use in the United States, 1992–2016 and possible implications for antifungal-resistant fungi in human disease. *Environ. Health Perspect.* 129, 055001. <https://doi.org/10.1289/EHP7484>.
- Tompkins, L.M., Li, H., Li, L., Lynch, C., Xie, Y., Nakanishi, T., Ross, D.D., Wang, H., 2010. A novel xenobiotic responsive element regulated by aryl hydrocarbon receptor is involved in the induction of BCRP/ABCG2 in LS174T cells. *Biochem. Pharmacol.* 80, 1754–1761. <https://doi.org/10.1016/j.bcp.2010.08.016>.
- Uehara, S., Higuchi, Y., Yoneda, N., Ito, R., Takahashi, T., Murayama, N., Yamazaki, H., Murai, K., Hikita, H., Takehara, T., Suemizu, H., 2023. HepaSH cells: experimental human hepatocytes with lesser inter-individual variation and more sustainable availability than primary human hepatocytes. *Biochem. Biophys. Res. Commun.* 663, 132–141. <https://doi.org/10.1016/j.bbrc.2023.04.054>.
- Uehara, S., Higuchi, Y., Yoneda, N., Yamazaki, H., Suemizu, H., 2019. Expression and inducibility of cytochrome P450s in human hepatocytes isolated from chimeric mice with humanized livers. *Xenobiotica* 49, 678–687. <https://doi.org/10.1080/00498254.2018.1495346>.
- Uehara, S., Murayama, N., Higuchi, Y., Shimizu, M., Suemizu, H., Guengerich, F.P., Yamazaki, H., 2024. In vivo and in vitro induction of cytochrome P450 3A4 by thalidomide in humanized-liver mice and experimental human hepatocyte HepaSH cells. *Chem. Res. Toxicol.* 37, 671–674. <https://doi.org/10.1021/acs.chemrestox.4c00069>.
- Venkatakrishnan, K., von Moltke, L.L., Greenblatt, D.J., 2000. Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. *Clin. Pharmacokinet.* 38, 111–180. <https://doi.org/10.2165/00003088-200038020-00002>.
- Wang, X., Sykes, D.B., Miller, D.S., 2010. Constitutive androstane receptor-mediated up-regulation of ATP-driven xenobiotic efflux transporters at the blood-brain barrier. *Mol. Pharmacol.* 78, 376–383. <https://doi.org/10.1124/mol.110.063685>.
- Wang, Y., Ning, X., Li, G., Sang, N., 2022. New insights into potential estrogen agonistic activity of triazole fungicides and coupled metabolic disturbance. *J. Hazard Mater.* 424, 127479. <https://doi.org/10.1016/j.jhazmat.2021.127479>.
- Whyte-Allman, S.-K., Hoque, M.T., Jenabian, M.-A., Routy, J.-P., Bendayan, R., 2017. Xenobiotic nuclear receptors pregnane X receptor and constitutive androstane receptor regulate antiretroviral drug efflux transporters at the blood-testis barrier. *J. Pharmacol. Exp. Ther.* 363, 324–335. <https://doi.org/10.1124/jpet.117.243584>.
- Willson, T.M., Kiewer, S.A., 2002. PXR, CAR and drug metabolism. *Nat. Rev. Drug Discov.* 1, 259–266. <https://doi.org/10.1038/nrd753>.
- Wong, S.G., Ramsden, D., Dallas, S., Fung, C., Einolf, H.J., Palamanda, J., Chen, L., Goosen, T.C., Siu, Y.A., Zhang, G., Tweedie, D., Hariparsad, N., Jones, B., Yates, P.D., 2021. Considerations from the innovation and quality induction working group in response to drug-drug interaction guidance from regulatory agencies: guidelines on model fitting and recommendations on time course for in vitro cytochrome P450 induction studies including impact on drug interaction risk assessment. *Drug Metab. Dispos. Biol. Fate Chem.* 49, 94–110. <https://doi.org/10.1124/dmd.120.000055>.
- Wu, S., Ji, X., Wang, J., Wu, H., Han, J., Zhang, H., Xu, J., Qian, M., 2021. Fungicide bromuconazole has the potential to induce hepatotoxicity at the physiological, metabolic and transcriptomic levels in rats. *Environ. Pollut. Barking Essex* 280, 116940. <https://doi.org/10.1016/j.envpol.2021.116940>, 1987.
- Xiao, J., Fang, K., Zhang, S., Jiang, S., Liu, T., Lv, M., Liao, M., Cao, H., Shi, Y., 2023. Inhalation bioaccessibility of inhaled triazole fungicides and health risk assessment during spraying. *Pest Manag. Sci.* 79, 1768–1776. <https://doi.org/10.1002/ps.7354>.
- Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Yin, M., Zeng, X., Wu, C., Lu, A., Chen, X., Hou, T., Cao, D., 2021. ADMETLab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Res* 49, W5–W14. <https://doi.org/10.1093/nar/gkab255>.
- Xu, Y., Zhang, Y., Tao, Q., Sun, Q., Zheng, Y., Yin, D., Yang, Y., 2023. A possible but unrecognized risk of acceptable daily intake dose triazole pesticides exposure-bile acid disturbance induced pharmacokinetic changes of oral medication. *Chemosphere* 322, 138209. <https://doi.org/10.1016/j.chemosphere.2023.138209>.
- Yueh, M.-F., Huang, Y.-H., Hiller, A., Chen, S., Nguyen, N., Tukey, R.H., 2003. Involvement of the xenobiotic response element (XRE) in Ah receptor-mediated induction of human UDP-glucuronosyltransferase 1A1. *J. Biol. Chem.* 278, 15001–15006. <https://doi.org/10.1074/jbc.M300645200>.
- Zerdoug, A., Le Vée, M., Uehara, S., Jamin, A., Higuchi, Y., Yoneda, N., Lopez, B., Chesné, C., Suemizu, H., Fardel, O., 2023. Drug transporter expression and activity in cryopreserved human hepatocytes isolated from chimeric TK-NOG mice with humanized livers. *Toxicol. Vitro* 90, 105592. <https://doi.org/10.1016/j.tiv.2023.105592>.
- Zerdoug, A., Le Vée, M., Uehara, S., Lopez, B., Chesné, C., Suemizu, H., Fardel, O., 2022. Contribution of humanized liver chimeric mice to the study of human hepatic drug transporters: state of the art and perspectives. *Eur. J. Drug Metab. Pharmacokinet.* 47, 621–637. <https://doi.org/10.1007/s13318-022-00782-9>.
- Zhou, J., Zhang, J., Li, F., Liu, J., 2016. Triazole fungicide tebuconazole disrupts human placental trophoblast cell functions. *J. Hazard Mater.* 308, 294–302. <https://doi.org/10.1016/j.jhazmat.2016.01.055>.
- Zubrod, J.P., Bundschuh, M., Arts, G., Brühl, C.A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J.J., Rohr, J., Scharfmüller, A., Smalling, K., Stehle, S., Schulz, R., Schäfer, R.B., 2019. Fungicides: an overlooked pesticide class? *Environ. Sci. Technol.* 53, 3347–3365. <https://doi.org/10.1021/acs.est.8b04392>.