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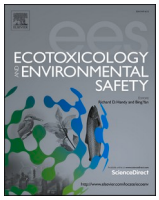

Endocrine disrupting potential of selected azole and organophosphorus pesticide products through suppressing the dimerization of human androgen receptor in genomic pathway

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ABSTRACT

Several pesticides widely used in agriculture have been considered to be endocrine disrupting chemicals through their binding affinities to estrogen or androgen receptors. This study was conducted to clarify the human androgen receptor (hAR)-mediated genomic endocrine disrupting mechanism of eight selected pesticide products by in vitro assay providing the Organization for Economic Co-operation and Development Test Guideline No. 458, 22Rv1/MMTV_GR-KO AR transcriptional activation assay and a homo-dimerization confirmation assay. None of the tested pesticide products showed an AR agonistic effect, whereas they were all determined to be AR antagonists at non-toxic concentrations. Also, the eight pesticide products were verified as true AR antagonists through a specificity control test. In the Bioluminescence Resonance Energy Transfer-based AR homo-dimerization confirmation assay, the eight pesticide products did not induce AR homo-dimerization. Additionally, western blotting revealed that none of the eight pesticide products induced AR translocation from the cytoplasm to the nucleus. In conclusion, we found for the first-time evidence to understand the AR-mediated endocrine disrupting mechanisms induced by selected azole and organophosphorus pesticide products.

1. Introduction

Pesticides belong to a category of chemicals used worldwide as herbicides, insecticides, and fungicides for the protection of agriculture and the treatment of disease in humans and animals (Draskau et al., 2019). The continuous and extensive use of pesticides has created residues and serious environmental pollution of the atmosphere, soil, and water (Chawla et al., 2018). Various pesticides used in agricultural fields have been frequently found in soil and wastewater (Kahle et al., 2008; Silva et al., 2019), and the exposure to pesticides could cause various diseases, such as cancer, hormone disruption, asthma, allergies, and hypersensitivity (Dang et al., 2016). In addition, pesticide exposure can lead to such adverse effects as birth defects, reduced birth weight, and fetal death (Wickerham et al., 2012; Baldi et al., 2010). Azole fungicides may have the ability to influence the endocrine system because they interact with several cytochrome P450 enzymes (Kahle et al., 2008). Organophosphorus and carbamate pesticides were shown to inhibit acetylcholinesterase, the enzyme that terminates the action of the neurotransmitter acetylcholine (Timchalk and Poet, 2008).

Following several cases in the 1990 s that suggested an association between chemicals and adverse effects on the reproductive and developmental system of humans and/or wildlife, endocrine disrupting chemicals (EDCs) have been considered a public and regulatory concern (Reif et al., 2010).

World Health Organization (WHO) defined an EDC as an exogenous chemical or mixture that interferes with the normal function(s) of the endocrine system and consequently brings on harmful influence in an intact organism, or its progeny, or (sub) populations" (Damstra, 2002).

To address this concern, the Organization for Economic Co-operation and Development (OECD) convened an international expert advisory group to decide how to test and assess the endocrine disrupting potential of chemicals, and they established a conceptional framework containing five levels for organizing and interpreting test data for EDCs in 2012 and updated in 2018 (OECD, 2018). Disruption of the endocrine system may cause through various inappropriate mechanisms: (1) endogenous hormones activity directly mediated via nuclear receptors binding; (2) endogenous hormones synthesis or degradation by steroidogenic enzymes; (3) regulation the metabolism of hormones; (4) distribution of

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hormones to target tissues; and (5) elimination of hormones from the body (OECD, 2018). With respect to (6), some endocrine active substances (EAS) act like endogenous hormone by mimicking the biological activity on action mechanism or interact with hormone signaling pathway factor of receptor activation, including the androgen receptor (AR), thus triggering downstream transactivation events.

Regarding the nuclear receptor-mediated endocrine disruption induced by pesticides, there have been increasing evidence that various pesticides have the ability to interact with the estrogen receptor (ER) and/or AR (Kjeldsen and Bonefeld-Jorgensen, 2013; Kojima et al., 2004; Li et al., 2008). Various publications have defined organochlorine pesticides, such as aldrin, atrazine, and p, p′-dichlorodiphenyltrichloroethane, as endocrine disruptors due to their adverse effects on the endocrine system, including ER agonists and AR antagonists (Cocco, 2002; Cooper et al., 2000; Lemaire et al., 2004). Regarding organophosphorus pesticides, diazinon induced the proliferation of the rat pituitary tumor cell line, MtT/Se, through an ER agonistic effect (Manabe et al., 2006). Furthermore, the pyrimidine fungicide, fenarimol, is active as an ER agonist and aromatase inhibitor (Vinggaard et al., 2000; Andersen et al., 2006).

The chemicals confirm to be nuclear receptor agonist and/or antagonist in in vitro test systems are decided to may be EDCs in vivo. The activity of chemicals can be prioritized through in vitro assay, which can save time and cost, by replacing in vivo assay. Also, these in vitro assays support the 3Rs principles (replace, reduce and refine) to reduce the study using animals testing due to unethical, unnecessary and unreliable reasons and helps demonstrate the underlying mechanisms.

The in vitro assays registered to OECD Test Guideline No. 458 were used to identify the potential AR agonistic and/or antagonistic ability of chemicals. The Androgen Receptor Transcriptional Activation (ARTA) assay using the AR-EcoScreen™ cell line was approved as the OECD TG No. 458 in 2016 (OECD, 2016). Afterward, ARTA assay using the 22Rv1/MMTV_GR-KO cell line was accepted as OECD TG No. 458 in 2020 (OECD, 2020).

In this study, we selected the eight pesticide products regulated in the Republic of Korea with established maximum residue limits (MRLs) (MFDS, 2020) and evaluated the AR agonistic/antagonistic activity using the 22Rv1/MMTV_GR-KO AR TA assay. Furthermore, we confirmed the induction of AR homo-dimerization in the cytosol using the Bioluminescence Resonance Energy Transfer (BRET) assay (Lee et al., 2021) and the transfer of AR to nucleus using the western blotting analysis to clarify their AR-mediated genomic endocrine disrupting pathways.

2. Materials and methods

2.1. Chemicals

All chemicals including reference substances for assays and test pesticide products were of analytical grade. 5α-dihydrotestosterone (DHT, ≥ 98.0%, CASRN 521–18–9), mestanolone (≥ 98.0%, CASRN 521–11–9), and bicalutamide (≥ 98.0%, CASRN 90357–06–5) were purchased from TCI (Tokyo, Japan). Bisphenol A (BPA, ≥ 98.0%, CASRN 80–05–7) and Di(2-ethylhexyl)phthalate (DEHP, ≥ 98.0%, CASRN 117–81–7) were purchased from Sigma (St. Louis, Mo, USA). The stock concentration and exposure range of each pesticide product were decided according to OECD TG No. 458 (Table 1). The details for the solubility test of each pesticide product are shown in Supplemental Materials and Methods 1.

2.2. Rv1/MMTV_GR-KO AR TA assay

The process for assessing the AR agonistic/antagonistic activities of eight pesticide products were accorded with the OECD TG No. 458. Protocol details are provided in Supplemental Materials and Methods 2. Each experiment was repeated at least 3 times on different days.

Table 1
List of eight tested pesticide products.

Class	Chemical	CAS No.	Stock Conc. (M)	Exposure range (M)
Azole	Epoxiconazole	106325–08–0	1	10^{-8}~10^{-3}
	Flusilazole	85509–19–9	0.1	10^{-9}~10^{-4}
	Tebuconazole	107534–96–3	1	10^{-8}~10^{-3}
	Triflumizole	68694–11–1	0.1	10^{-9}~10^{-4}
Organophosphorus	Chlorpyrifos	2921–88–2	1	10^{-8}~10^{-3}
	Chlorpyrifos-methyl	5598–13–0	1	10^{-8}~10^{-3}
	Diazinon	333–41–5	1	10^{-8}~10^{-3}
	Tolclofos-methyl	57018–04–9	1	10^{-8}~10^{-3}

2.3. BRET-based AR homo-dimerization assay

Suppression of AR homo-dimerization by AR antagonistic pesticide products was confirmed using BRET-based in vitro assay, which was developed in our previous study (Lee et al., 2021). The protocol details for the BRET-based AR homo-dimerization and cell viability assays are provided in Supplemental Materials and Methods 3. Each experiment was repeated at least 3 times on different days.

2.4. Confirmation of AR protein localization by pesticide products using western blotting

Briefly, proteins lysed from cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinyl difluoride membranes (Bio-Rad, Hercules, CA, USA) for western blotting. The membranes were incubated with primary antibodies (anti-AR and anti-β-actin) and horseradish peroxidase-conjugated secondary antibodies (Cell Signaling, Danvers, MA, USA). The localization of AR proteins was detected using a digital imaging system (Image Station 4000 MM Pro; Kodak, Rochester, NY, USA). Protocol details are provided in Supplemental Materials and Methods 4. Each experiment was repeated at least 3 times on different days.

3. Results

3.1. Proficiency test for the 22Rv1/MMTV_GR-KO AR TA assay

The stability of the cells and skill of the researcher were verified by a proficiency test according to OECD TG No. 458. The induction folds of 10 nM 5 α -dihydrotestosterone (DHT) were 89.5, 95.1, and 84.5, which satisfied the criteria for the AR agonist assay. Also, the responses of the reference standards were consistent with the expected responses, and the log PC_{10} and log PC_{50} values of the two positive reference standards (DHT and mestanolone) were acceptable according to the criteria (Table 2). In the AR antagonist assay, the induction folds of 800 pM DHT were 27.3, 22.6, and 27.8, which passed the criteria for the proficiency test. In addition, the responses of the reference standards were consistent with the expected responses, and the log IC_{30} and log IC_{50} values of the two positive reference standards (bicalutamide and bisphenol A [BPA]) were acceptable according to the criteria (Table 2).

3.2. AR agonistic/antagonistic effects of pesticides

The eight pesticide products that showed AR binding affinity in the competitive ligand binding assay were selected based on a report from the Korean Ministry of Food and Drug Safety (MFDS, 2016). The results showed that none of the eight pesticide products displayed an AR agonistic effect, whereas they were all determined to be AR antagonists with IC_{30} and IC_{50} values at non-toxic concentrations (Table 3 and Fig. 1). The relative transcriptional inhibition (RTI) induced by the eight pesticide products were expressed as a percentage in comparison with

Table 2
Acceptability criteria for proficiency testing in 22Rv1/MMTV_GR-KO AR transcriptional activation assay.

	Acceptable criteria		Results	
Fold induction of 10 nM DHT	≥ 13		89.5 95.1 84.5	
	$logPC_{10}$ (M)		$logPC_{50}$ (M)	
	Acceptable criteria	Results	Acceptable criteria	Results
5α-Dihydrotestosterone	-12.20 ~ – 9.70	-10.45 -10.42 -9.87	-10.60 ~ – 9.00	-9.36 -9.47 -9.03
Mestanolone	-12.30 ~ – 9.80	-9.94 -9.95 -9.81	-10.20 ~ – 8.60	-9.02 -9.03 -8.69
Di(2-ethylhexyl) phthalate	–	– – –	–	– – –
	Acceptable criteria		Results	
Fold induction of 800 pM DHT	≥ 10		27.3 22.6 27.8	
	$logIC_{30}$ (M)		$logIC_{50}$ (M)	
	Acceptable criteria	Results	Acceptable criteria	Results
Bicalutamide	-7.50 ~ – 6.20	-6.90 -6.62 -6.39	-7.00 ~ – 5.80	-6.56 -6.25 -5.91
Bisphenol A	-6.60 ~ – 5.40	-6.09 -5.85 -5.64	-6.20 ~ – 5.00	-5.63 -5.43 -5.17
Di(2-ethylhexyl) phthalate	–	– – –	–	– – –

Table 3
AR antagonist effects of eight pesticide products by the 22Rv1/MMTV_GR-KO AR transcriptional activation assay.

Chemical	IC_{30} (M)	IC_{50} (M)	RTI[a]
Bicalutamide (Positive control)	8.66×10^{-8}	2.90×10^{-7}	100
Epoxiconazole	2.46×10^{-6}	5.46×10^{-6}	5.31
Flusilazole	2.35×10^{-6}	6.70×10^{-6}	4.33
Tebuconazole	2.18×10^{-6}	5.21×10^{-6}	5.57
Triflumizole	1.26×10^{-6}	2.89×10^{-6}	10.03
Chlorpyrifos	1.81×10^{-6}	5.17×10^{-6}	5.61
Chlorpyrifos-methyl	3.37×10^{-7}	1.91×10^{-6}	15.18
Diazinon	2.97×10^{-6}	1.13×10^{-5}	2.57
Tolclofos-methyl	2.47×10^{-7}	1.58×10^{-6}	18.35

[a] Relative Transcriptional Inhibition = (IC_{50} of Bicalutamide/IC_{50} of test chemical) × 100.

the IC_{50} value of bicalutamide, which were 5.31, 4.33, 5.57, 10.03, 5.61, 15.18, 2.57, and 18.35, respectively.

3.3. Confirmation of competitive AR antagonist by the specificity control test

We conducted a specificity control test of eight AR antagonistic pesticide products using 800 pM and 100 nM DHT to exclude false antagonists. The competition of DHT with the pesticide products in binding to the AR was greatly induced with the higher DHT concentration (100 nM DHT), accompanying the delay in the dose response curve shift. In the case of a false antagonist, the two DHT concentrations showed overlapping dose response curves and this signal decrease was irrelevant to receptor binding (Milcamps et al., 2021). A quantification by the dose response curve shift was estimated via the square of the correlation coefficient (R^2), where the response similarity of the two DHT concentrations was compared (Milcamps et al., 2021). The R^2 of the eight pesticide products were calculated respectively as 0.73, 0.01, 0.67, 0.85, 0.84, 0.75, 0.88, and 0.60, with all pesticide products having R^2 values less than 0.9. These results indicated that the 8 pesticide products were true AR antagonists (Table 4 and Fig. 2).

3.4. Ligand-mediated homo-dimerization of AR antagonistic pesticide products

To investigate whether the AR antagonistic activity induced by the eight pesticide products mediated the response of AR in the cytosol, we conducted the BRET-based AR dimerization assay. The BRET unit induced by DHT, a well-known AR agonist, was a 100% response at a concentration of 10 nM DHT (Fig. 3). However, AR dimerization was not induced by all pesticide products at non-cytotoxic concentrations. These results identified that the AR antagonist activity induced by the eight pesticide products showed AR binding affinity in the competitive ligand binding assay but did not cause AR homo-dimerization in the BRET-based dimerization confirmation assay.

3.5. Localization of AR proteins by AR antagonistic pesticide products

The localization of AR proteins induced by AR antagonistic pesticide products in the cytoplasm and nucleus were conducted by western blotting and these results are shown in Fig. 4. All AR proteins were expressed in the cytoplasm when the cells were treated with the eight pesticide products. The levels of AR induced by the eight pesticide products in the nucleus were similar to that of 0.1% DMSO (vehicle control). Conversely, treatment with 1 μM DHT (AR agonist positive) induced the translocation of the AR protein from the cytoplasm into the nucleus. We confirmed by western blotting that the eight pesticide products could not translocate AR into the nucleus and these results were consistent with that of the BRET-based AR dimerization assay.

4. Discussion

In this study, our results indicated that selected azole (epoxiconazole, flusilazole, tebuconazole, and triflumizole), and organophosphorus (chlorpyrifos, chlorpyrifos-methyl, diazinon, and tolclofos-methyl) pesticide products were found to be AR antagonists through suppression of AR homo-dimerization in the cytosol based on AR binding affinity. To ensure the identification of the AR antagonist that is determined to be positive in the AR antagonist assay, OECD TG No. 458 suggested that a specificity control test should be conducted using AR agonist controls for the AR antagonist assay (800 pM DHT and high concentration 100 nM DHT). The inclusion of these two DHT concentrations in the antagonist assay is expected to result in a shift between the concentration-response curves of "true" AR antagonists and distinguish these chemicals from potential false positives. Regarding the four azole and four organophosphorus pesticide products, they were all determined to be true AR antagonists with R^2 values less than 0.9 in the specificity control test. Even if three pesticide products (triflumizole, chlorpyrifos, and diazinon) showed R^2 values close to 0.9 in the specificity control test, their mean R^2 value was less than 0.9, and they were determined to be true AR antagonists in accordance with the positive/negative decision criteria in OECD TG No. 458 (OECD, 2020). Regarding the false positive, as you know, OECD Validation Management Group for Non-Animal testing suggested specificity control assay to determine the complete AR antagonist, and they established the criteria for classifying false positive as more than R^2 value with 0.9 in OECD TG No.458. If the R^2 value induced by test substrate is more than 0.9, OECD TG No.458 decided suppressing the transcriptional activation signal of DHT induced by test substrate is not specific inhibition of the AR-luc mRNA translation.

Several pesticide containing organochlorides were shown to behave as EDCs via interference with endogenous hormone synthesis and degradation (Warner et al., 2020). For example, the estrogenic effect of DDT was first reported in 1952, and that of the insecticides, kepone and

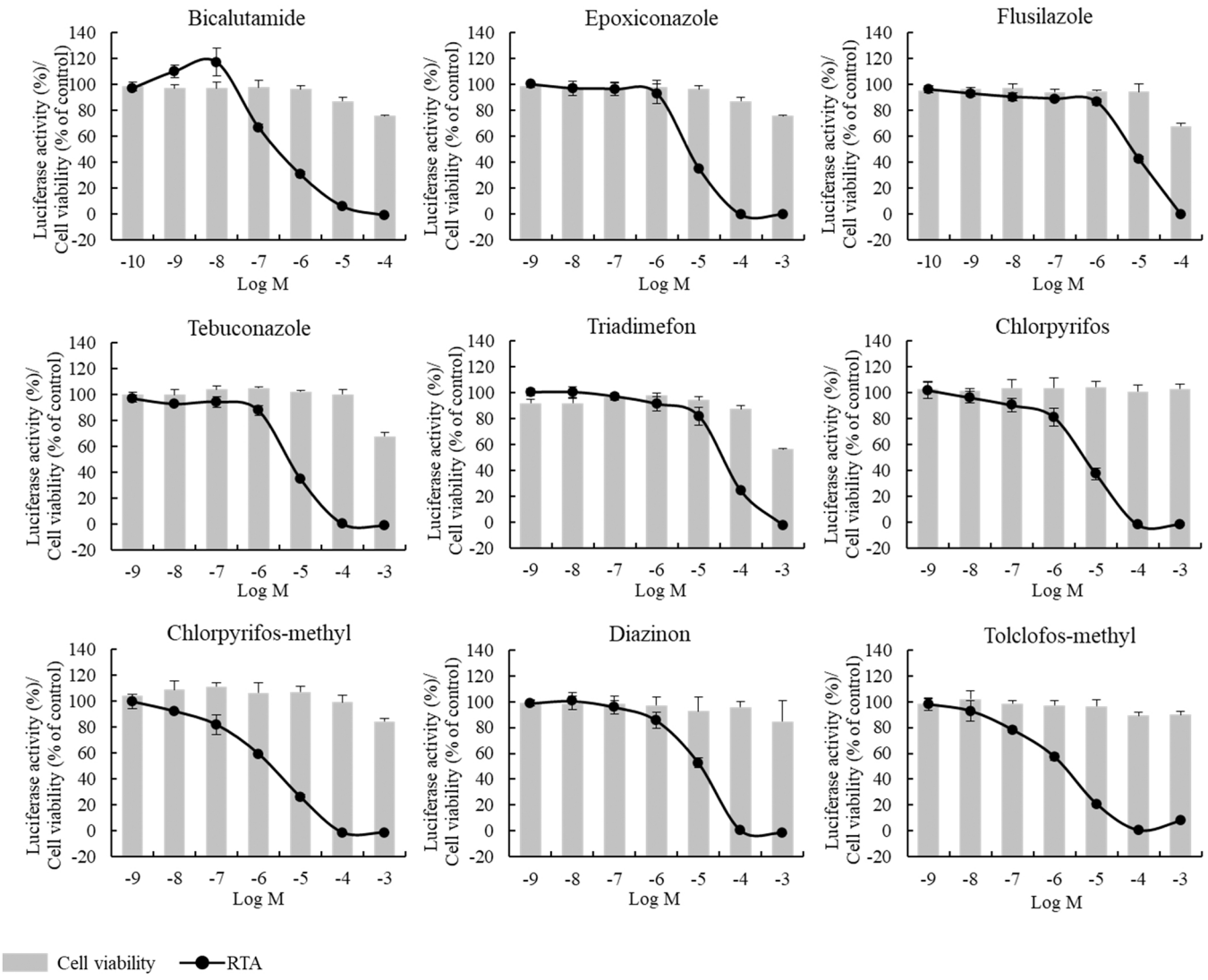

Fig. 1. AR antagonistic dose-response curves of eight pesticide products. Luciferase activities are expressed as the % of the activity for 800 pM DHT (± standard deviation). RTA: Related Transcriptional Activation.

Table 4
R^2 value of eight pesticide products in the specificity control test in the 22Rv1/MMTV_GR-KO AR transcriptional activation assay.

Chemical	Avg.	SD
Epoxiconazole	0.73	0.03
Flusilazole	0.01	0.02
Tebuconazole	0.67	0.05
Triflumizole	0.85	0.03
Chlorpyrifos	0.84	0.01
Chlorpyrifos-methyl	0.75	0.02
Diazinon	0.88	0.01
Tolclofos-methyl	0.60	0.05

methoxychlor, was reported in the 1970 s (Fisher et al., 1952; Gellert, 1978; Bulger et al., 1978). Also, Ostby et al. (1999) reported that linuron and vinclozolin showed anti-androgenic effects. Regarding the endocrine disrupting potential of azole pesticide, epoxiconazole and tebuconazole showed an AR antagonistic effect in the AR reporter gene assay (Kjaerstad et al., 2010). In addition, flusilazole displayed an AR antagonistic effect by disrupting steroid biosynthesis in vitro (Draskau et al., 2019). Flusilazole and tebuconazole dose-dependently inhibited testosterone-induced AR activation. The IC_{50} value and relative effect potency, as compared to flutamide were 3.61 and 0.22 in the AR reporter gene assay using T47D-ARE cells, respectively (Roelofs et al., 2014). The results of epoxiconazole, flusilazole, and tebuconazole from OECD TG No. 458 in this study coincided with studies where three pesticides were reported as AR antagonists.

Regarding organophosphorus pesticides, chlorpyrifos disrupted steroidogenesis through a significant decrease in testosterone biosynthesis in rat Leydig cells (Viswanath et al., 2010). Also, the chlorpyrifos analogue, chlorpyrifos-methyl, also exhibited anti-androgenic activity by suppressing testosterone propionate-stimulated accessory sex organ weight and increasing the adrenal gland weight in the Hershberger assay using castrated male rats (Kang et al., 2004). Additionally, Bisson and Hontela (2002) published that diazinon suppressed cortisol secretion in response to adrenocorticotropin in adrenocortical cells of rainbow trout. In addition, Kojima et al. (2004) reported that tolclofos-methyl had an inhibitory effect on AR agonistic effect of DHT in a reporter gene assay.

Even though reports concerning the AR antagonistic effects of various pesticides were published, their mechanism of action has not been clarified. According to the OECD, EAS mimic the endogenous hormones that, through a series of processes, directly bind to nuclear receptors, thus lead to downstream transactivation (OECD, 2018). The androgen-mediated genomic pathway induced by EAS is based on the binding of androgen to ARs in the cytoplasm, which acts as a

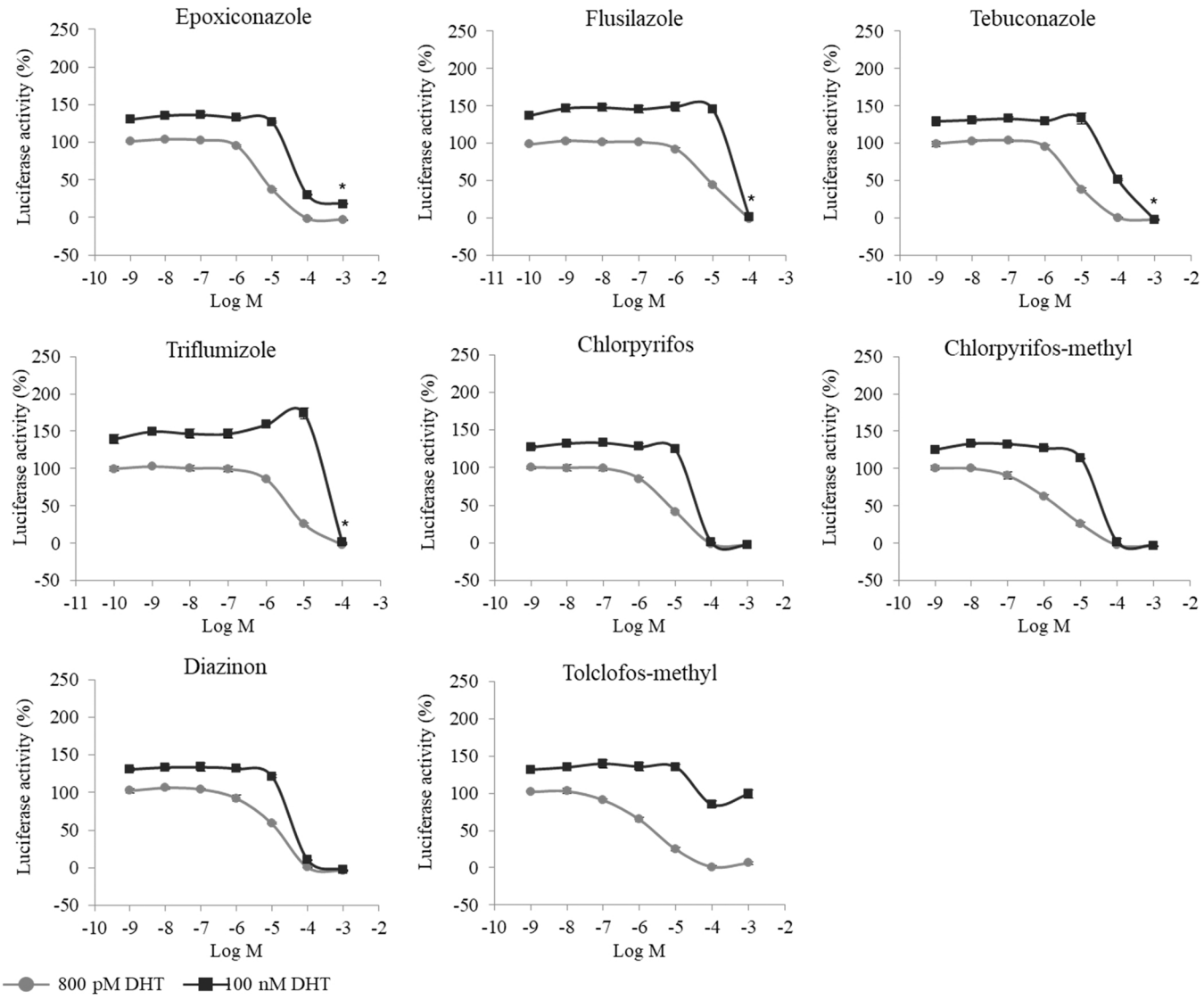

Fig. 2. Dose-response curves of eight pesticide products by the specificity control test in the the 22Rv1/MMTV_GR-KO AR transcriptional activation assay. The cytotoxic concentration of each pesticide product was presented as * , and the cytotoxic concentration data were excepted for calculating the square of the correlation coefficient (R^2).

transcription factor through binding several sequences. ARs act as transcription factors through processes such as cytoplasmic androgen binding, receptor dimerization based on conformational changes, AR dimer translocation to the nucleus, coactivator interactions, and direct interaction with androgen response elements (AREs) located on the promoter (Bennett et al., 2010). Regarding the genomic pathway mechanism, AR antagonists may compete with endogenous androgen for receptor binding, which may suppress cytosolic AR homo-dimerization or decrease nuclear AR-ARE binding stability, resulting in transcription activation inhibition (Kelce and Wilson, 2001). This study used the binding and transactivation assays to determine the AR agonist and/or AR antagonist. These assays may contribute to elucidating mechanisms for EDC binding to AR and changes in gene expression. Also, the evidence to confirm cytosolic AR homo-dimerization suppression is an important key event to clarify the AR antagonist mechanism.

Consequently, we tried to confirm whether AR antagonistic pesticide products inhibited cytosolic AR homo-dimerization using the BRET-based assay. Dimerization is one of the processes and occurs when EDCs directly bind to AR and AR is activated and undergo transcription. The BRET-based AR homo-dimerization assay is an in vitro test that can supply an exact mechanism of AR-mediated EDCs. In this respect, the BRET-based AR homo-dimerization assay could identify chemical-mediated AR homo-dimerization as one of the key events (KEs) in an adverse outcome pathway approach for accurate toxicological characterization of chemicals with AR antagonistic activity. Based on this systematic approach to clarify the AR antagonist mechanism in the genomic pathway, we that found azole and organophosphorus pesticide products behave as AR antagonists through AR homo-dimerization suppression at the cellular level. Also, eight pesticide products inhibited the translocation of AR proteins from the cytoplasm to the nucleus by DHT using western blotting. Additionally, we conducted western blotting for supplemental evidence that the expression of in cytoplasm and transfer of AR protein to the nucleus under the same conditions as for transcriptional activity, and the results were provided in Supplemental data 1. When 800 pM DHT alone was treated the level of AR was the highest in both cytoplasm and nucleus. On the other hand, level of AR was decreased in cytoplasm and translocation of the AR protein to the nucleus did not occur by 800 pM DHT in the presence of 8 pesticide products. The eight pesticide products did not occur AR dimerization and nuclear translocation, completely blocking AR-mediated transcriptional activity and accelerating proteasomal degradation. AR

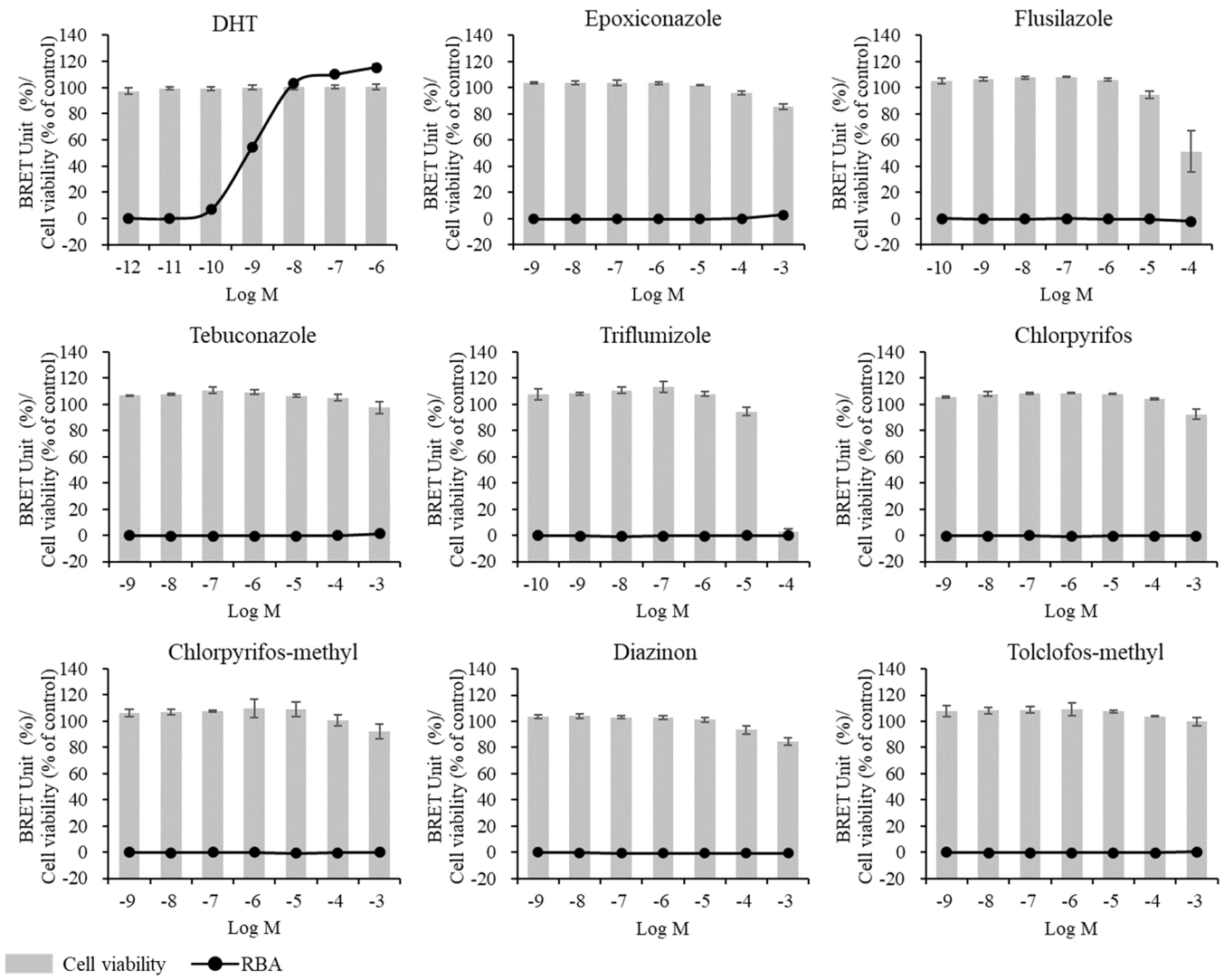

Fig. 3. Dose-response curves of eight pesticide products in the BRET-based AR dimerization assay. BRET units are expressed as the % of the activity for10 nM DHT (± standard deviation). RDA: Related Dimerization Activity; BRET: Bioluminescence Resonance Energy Transfer; AR: androgen receptor.

antagonistic mechanism of eight pesticide products could confirm as selective AR degraders (SARDs).

As we described in the Introduction, the eight pesticide products were regulated by MRLs, which was determined from acceptable daily intake (ADI). The quantitative values of AR-mediated endocrine disrupting effects by the in vitro OECD TG on epoxiconazole (ADI: 0.008 mg/kg body weight (bw) per day, EFSA, 2008a, 2008b), flusilazole (ADI: 0.002 mg/kg bw per day, EC, 2007), tebuconazole (ADI: 0.03 mg/kg bw per day, EFSA, 2008a, 2008b), triflumizole (ADI: 0.05 mg/kg bw per day, EFSA, 2009), chlorpyrifos (ADI: 0.001 mg/kg bw per day, EFSA, 2014), chlorpyrifos-methyl (ADI: 0.01 mg/kg bw per day, EC, 2005), diazinon (ADI: 0.0002 mg/kg bw per day, EFSA, 2006), and tolclofos-methyl (ADI: 0.064 mg/kg bw per day, EC, 2006) were lower than their ADI values. However, it is not correct to apply the interpretation of AR-mediated disruption values from in vitro models to HbGV of an animal model. That is the limitation that needs to be solved. Consequently, Studies using animal models should be continuously conducted in the future for clarifying the AR-mediated endocrine disrupting potential and HbGV.

5. Conclusions

We conducted these studies to clarify the genomic pathway signaling of AR antagonistic azole and organophosphorus pesticide products. Although our experiments were limited to in vitro assays using OECD TG, these results represented that four azole and four organophosphorus pesticide products have AR-mediated endocrine-disrupting potential. Furthermore, for the first time, we identified that these eight pesticide products have AR antagonistic effects based on their suppression of cytosolic AR homo-dimerization. These results suggested that azole and organophosphorus pesticide products could have endocrine-disrupting effects mediated by interactions with the AR and AR genomic mechanism has been identified as the SARDs.

However, as we described in the Discussion session, further studies including animal models are needed to prove the endocrine-disrupting effect of the eight pesticide products that showed the AR antagonist activity in this in vitro study.

CRediT authorship contribution statement

All authors accept the terms of the below Author Contributions Statement, **Da-Woon Jung:** Investigation, Writing – original draft., **Da-Hyun Jeong:** Validation, Writing – original draft, Review & editing. **Hee-Seok Lee**: Conceptualization, Data curation, Funding acquisition, Project administration, Investigation, Validation, Writing – original draft, Review & editing.

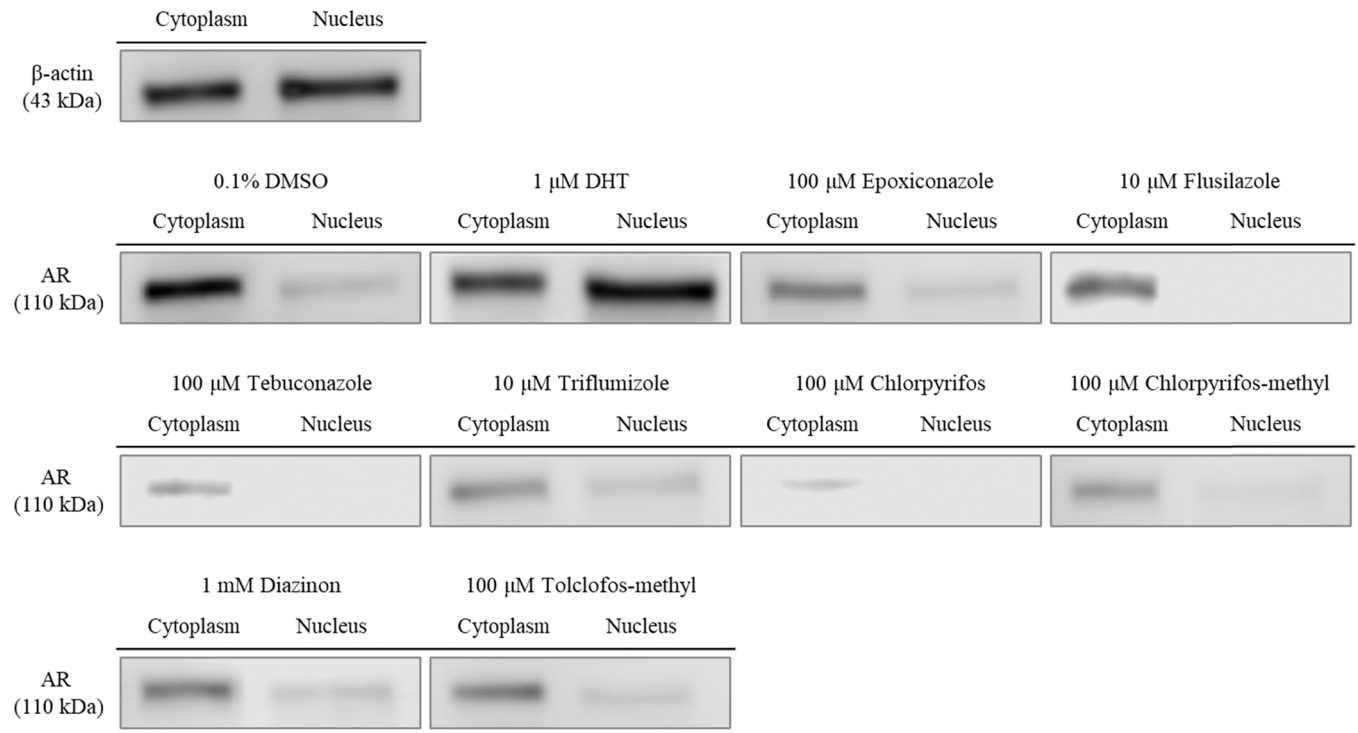

Fig. 4. Location of the AR protein induced by 0.1% DMSO (vehicle control), 1 μM DHT (AR agonist positive), and eight pesticide products in western blotting. AR: androgen receptor; DMSO: dimethyl sulfoxide; DHT: 5α-dihydrotestosterone.

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

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114246.

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