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Assessment of the endocrine-disrupting effects of organophosphorus pesticide triazophos and its metabolites on endocrine hormones biosynthesis, transport and receptor binding *in silico*

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ABSTRACT

Triazophos (TAP) was a widely used organophosphorus insecticide in developing countries. TAP could produce specific metabolites triazophos-oxon (TAPO) and 1-phenyl-3-hydroxy-1,2,4-triazole (PHT) and non-specific metabolites diethylthiophosphate (DETP) and diethylphosphate (DEP). The objective of this study involved computational approaches to discover potential mechanisms of molecular interaction of TAP and its major metabolites with endocrine hormone-related proteins using molecular docking *in silico*. We found that TAP, TAPO and DEP showed high binding affinity with more proteins and enzymes than PHT and DETP. TAP might interfere with the endocrine function of the adrenal gland, and TAP might also bind strongly with glucocorticoid receptors and thyroid hormone receptors. TAPO might disrupt the normal binding of androgen receptor, estrogen receptor, progesterone receptor and adrenergic receptor to their natural hormone ligands. DEP might affect biosynthesis of steroid hormones and thyroid hormones. Meanwhile, DEP might disrupt the binding and transport of thyroid hormones in the blood and the normal binding of thyroid hormones to their receptors. These results suggested that TAP and DEP might have endocrine disrupting activities and were potential endocrine disrupting chemicals. Our results provided further reference for the comprehensive evaluation of toxicity of organophosphorus chemicals and their metabolites.

1. Introduction

1.1. Chemical characteristics, residues and toxicity of triazophos

Triazophos/Triazofos/Hostathion/Phentriazophos (O,O-diethyl-O-(1-phenyl-1H-1,2,4-triazol-3-yl) phosphorothioate, CAS Registry No. 24017-47-8, chemical formula $C_{12}H_{16}N_3O_3PS$, molar mass 313.31 g mol^{-1}, log Kow 3.55, abbreviated as TAP) was an efficient and broad-spectrum organophosphorus pesticide (OP) used as insecticide, nematicide and acaricide, which was widely used in Asian countries, such as China, India, Pakistan, to protect various crops like cotton, rice, wheat, tea, fruits, oil seeds and vegetables (Bhandari et al., 2019; Chen et al., 2009; Duan et al., 2016; Fang et al., 2015; Hong et al., 2019; Kumari and John, 2019). However, the widespread application of TAP represented a risk to human health as well as the ecological system due to its high chemical and photochemical stability (http://sitem.herts.ac.uk/aeru/iupac/Reports/653.htm). According to the classification standard of World Health Organization (WHO), TAP was a Class Ib toxic organophosphorus insecticide (WHO, 2010). The neurotoxicity, hepatotoxicity, nephrotoxicity, reproductive toxicity and genotoxicity of TAP attracted considerable public attention over the last decade. It was reported that TAP had fairly high lethal toxicity to aquatic creatures and posed a threat to the health of aquatic ecosystems (Wang et al., 2010; Wu et al., 2018; Zhang et al., 2018a,b,c). Recent research also revealed that TAP induced oxidative stress and histomorphological changes in rats (Jain et al., 2011, 2013; Sharma and Sangha, 2014; Sharma et al., 2015a, 2015b). Zhang et al. (2011) found that TAP chronic dietary intake represented a significant risk to the elderly

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persons and an acute nutritional intake risk of TAP residues in apple, cabbage, rice and wheat meal reached an unacceptable range in China. Therefore, the wide application of TAP raised concerns on the environmental pollution and the potential risk to human health.

1.2. Major degradation metabolites of TAP

After reaching the liver, TAP would produce specific metabolites triazophos-oxon (TAPO), 1-phenyl-3-hydroxy-1,2,4-triazole (PHT), and non-specific metabolites diethylthiophosphate (DETP) and diethylphosphate (DEP) under the action of a series of metabolic detoxification enzymes (Bock and Their, 1976; Schwalbe-Fehl and Schmidt, 1986; Wang et al., 2015). Briefly, TAP desulfurized under the action of CYP450 enzymes to produce triazophos-oxon (TAPO). Because the inhibition of acetylcholinesterase by TAPO was stronger, this process was actually a "metabolic increased toxification" process, but TAPO was more conducive to the subsequent hydrolysis of paraoxonase 1, and TAPO was degraded into PHT and DEP. TAP could also be directly decomposed into PHT and DETP under the action of glutathione and glutathione S-transferase, and then DETP could be further desulfurized into DEP. It was worth noting that DETP and DEP were non-specific metabolites of many OPs, such as TAP, chlorpyrifos, diazinon, parathion, and phorate. More importantly, both DETP and DEP had high bioavailability, and the molar mass of human exposure to DETP and DEP was higher than that of OPs (Forsberg et al., 2011; Sudakin and Stone, 2011; Timchalk et al., 2007; Zhang et al., 2008). In fact, approximately 75% of the commonly used OPs could be metabolized and degraded into six dialkyl phosphates (DAPs) *in vivo* and in the environment, namely diethyldithiophosphate (DEDTP), DETP, DEP, dimethyldithiophosphate (DMDTP), dimethylthiophosphate (DMTP), and dimethylphosphate (DMP) (Shomar et al., 2014; Ueyama et al., 2015; Yusa et al., 2015). The concentrations of DAPs in human urine were often used as the biomarkers for the exposure of OPs to assess the correlation between OPs exposure and diseases (Bernieri et al., 2019; Omoike et al., 2015; Panuwet et al., 2018; Shrestha et al., 2018; Wang et al., 2017a,b). Interestingly, some organophosphorus flame retardants and plasticizers could also be degraded and metabolized to DEP (Chu and Letcher, 2018; Li et al., 2017; Reemtsma et al., 2011; Sun et al., 2018).

1.3. Endocrine disrupting effects of OPs

Many OPs have been reported to impact levels of the hormones involved in the hypothalamic-pituitary-adrenal (HPA) axis (Raees et al., 2012), hypothalamic-pituitary-thyroid (HPT) axis (Ahmad et al., 2018; Akande et al., 2016; Chebab et al., 2017; Mosbah et al., 2016), and hypothalamic-pituitary-gonadal (HPG) axis (Geng et al., 2015; Jallouli et al., 2016; Sharma et al., 2015a). Chlorpyrifos, a widely-studied OP, was found to upset the thyroid and adrenal gland homeostasis both in human and animal models (Chebab et al., 2017; John and Shaike, 2015) and exert an inhibitory effect on sex hormones (Adedara et al., 2018; Peiris and Dhanushka, 2017). Furthermore, there were studies reporting histopathological injury to the thyroid and parathyroid in mice induced by diazinon (Ahmad et al., 2018), as well as the inhibition effect of malathion in thyroid stimulating hormone-dependent pathway and transcription of thyroglobulin in FRTL-5 cell line (Xiong et al., 2017). The effects of TAP on thyroid have only been studied in zebrafish embryos by measuring the mRNA levels of thyroid hormones (Wu et al., 2018), lacking more comprehensive evidences. All these OPs were metabolized to DAPs in the environment and *in vivo*; however, it was unclear whether these effects were induced by the parent OPs or the metabolites, especially the specific and the non-specific metabolites.

1.4. Screening, identification and risk assessment of endocrine disrupting chemicals in silico

Exogenous chemicals, exposed through the environmental and dietary pathways, had potential endocrine disruption effects that harm human health and the ecosystem (Giulivo et al., 2016; He et al., 2015; Mimoto et al., 2017; Pande et al., 2019). Endocrine disrupting chemicals (EDCs) caused a variety of diseases, such as reproductive diseases, metabolic diseases and even cancers, by interfering with the endocrine system (The Lancet Diabetes & Endocrinology, 2019). If all the potential EDCs in the environment and foods were to be screened *via* traditional *in vitro* and *in vivo* methods, this would constitute an unbearable financial burden, would conflict with the "3R" principles of animal-use ethics, and the work would stretch over many years, making it difficult to meet the needs of toxicity and ecological risk assessment for EDCs (Burgdorf et al., 2019; Chen et al., 2018). In recent years, with the rapid development of computer technology and three-dimensional structure analysis methods of proteins, molecular docking technology has been gradually applied to toxicological evaluation field, and computational toxicology research was increasing, especially the study of EDCs (Browne et al., 2018; Selvaraj et al., 2018; Sun et al., 2019). Computational toxicology has been recommended as the screening and predicting method by the Organization for Economic Co-operation and Development (OECD) (OECD, 2018), the US Environmental Protection Agency (EPA) (Browne et al., 2017), and others. Meanwhile, the Nobel Prize in Chemistry 2013 was awarded to Martin Karplus, Michael Levitt, and Arieh Warshel for developing the multiscale models of complex chemical systems (Karplus et al., 2013), which demonstrated that in this era of exceptional computational power, *in silico* analyses could be as important as those conducted *in vitro*, and computer molecular simulation methods have made significant contributions to the development of life sciences and drug researches. Molecular docking provided another reasonable method for the evaluation of EDCs, especially for some compounds with missing experimental data, the application of molecular docking to explore the roles of EDCs and putative targets can supplement limited experimental data and improve the overall toxicity assessment of EDCs (Chen et al., 2018; Vuorinen et al., 2015; Yuriev et al., 2015). Molecular docking or molecular simulation *in silico* provided another effective way to evaluate the safety of EDCs more comprehensively by elucidating the interaction mechanism between exogenous small molecules and biological macromolecules at the molecular level (Hazarika et al., 2019; Jeong et al., 2019).

1.5. Biosynthesis, transport, and receptors binding of endocrine hormones

Corticosteroid and catecholamine hormones were secreted by the adrenal glands, androgens and estrogens were secreted by the gonads, and thyroid hormones were secreted by the thyroid gland (La Perle and Dintzis, 2018; Rosol et al., 2013; Wallig, 2018). It was precise because of the important physiological role of endocrine hormones that they were usually disturbed by EDCs such as exogenous environmental chemicals and dietary exposure pollutants, which affected the growth, development, reproduction, and survival of humans and animals (Evans, 2017). Biosynthesized cholesterol through the key enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), which could be transported into the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR), where it was cleaved by cholesterol side-chain cleavage enzyme (CYP11A1) to pregnenolone, the common precursor for other steroid hormones (Shen et al., 2016). The adrenal cortex and gonads were the sites of steroid hormone synthesis and different steroidogenic enzymes, such as steroid 17-alpha-hydroxylase/17,20 lyase (CYP17A1), steroid 21-hydroxylase (CYP21A2), aldosterone synthase (CYP11B2), aromatase (CYP19A1), and estradiol 17-beta-dehydrogenase 1 (17β-HSD1), which were all regulated by transcription factor steroidogenic factor 1 (SF-1), were expressed in the adrenal cortex and gonads (Shen et al., 2016). The enzymes tyrosine

Table 1
Information for selected receptors in PDB database.

Names	Abbreviation	PDB entry	Resolution (Å)	Residue count	Organism
3-hydroxy-3-methylglutaryl-CoA reductase	HMGR	1DQ9	2.8	1868	*Homo sapiens*
Steroidogenic factor 1	SF-1	4QJR	2.4	245	*Homo sapiens*
Steroidogenic acute regulatory protein	StAR	3P0L	3.4	884	*Homo sapiens*
Cholesterol side-chain cleavage enzyme	CYP11A1	3N9Z	2.17	974	*Homo sapiens*
Aldosterone synthase	CYP11B2	4DVQ	2.49	5796	*Homo sapiens*
Steroid 17-alpha-hydroxylase/17,20 lyase	CYP17A1	4NKX	2.794	1976	*Homo sapiens*
Aromatase	CYP19A1	3S79	2.75	503	*Homo sapiens*
Steroid 21-hydroxylase	CYP21A2	4Y8W	2.64	1446	*Homo sapiens*
Estradiol 17-beta-dehydrogenase 1	17β-HSD1	6CGC	2.1	656	*Homo sapiens*
Tyrosine hydroxylase	TH	2XSN	2.68	1372	*Homo sapiens*
Aromatic L-amino acid decarboxylase	AAAD	3RCH	2.8	480	*Homo sapiens*
Dopamine beta-hydroxylase	DBH	4ZEL	2.9	1156	*Homo sapiens*
Phenylethanolamine N-methyltransferase	PNMT	4MQ4	2.2042	578	*Homo sapiens*
Iodotyrosine deiodinase	IYD	4TTB	2.447	530	*Homo sapiens*
Human serum albumin	HSA	2BX8	2.7	1170	*Homo sapiens*
Corticosteroid binding globulin	CBG	2VDY	2.3	746	*Homo sapiens*
Sex hormone-binding globulin	SHBG	1D2S	1.55	170	*Homo sapiens*
Thyroxine-binding globulin	TBG	2CEO	2.8	758	*Homo sapiens*
Transthyretin	TTR	1ICT	3.0	1016	*Homo sapiens*
Glucocorticoid receptor	GR	4MDD	2.4	516	*Homo sapiens*
Mineralocorticoid receptor	MR	2AA2	1.95	275	*Homo sapiens*
Androgen receptor	AR	2AM9	1.64	266	*Homo sapiens*
Constitutive androstane receptor	CAR	1XVP	2.6	1016	*Homo sapiens*
Estrogen receptor alpha	ERα	2IOG	1.6	246	*Homo sapiens*
Estrogen receptor beta	ERβ	1QKM	1.8	255	*Homo sapiens*
Estrogen related receptor alpha	ERRα	3K6P	1.996	248	*Homo sapiens*
Estrogen related receptor gamma	ERRγ	2P7G	2.1	251	*Homo sapiens*
Progesterone receptor	PR	1SQN	1.451	522	*Homo sapiens*
Thyroid hormone receptor alpha	TRα	4LNW	1.9	267	*Homo sapiens*
Thyroid hormone receptor beta	TRβ	3GWS	2.2	259	*Homo sapiens*
Beta-1 adrenergic receptor	β1AR	4BVN	2.1	315	*Meleagris gallopavo*
Beta-2 adrenergic receptor	β2AR	3NY9	2.84	490	*Homo sapiens*
D2 dopamine receptor	D2DR	6CM4	2.87	430	*Homo sapiens*
D3 dopamine receptor	D3DR	3PBL	2.89	962	*Homo sapiens*
D4 dopamine receptor	D4DR	5WIU	1.96	422	*Homo sapiens*

hydroxylase (TH), aromatic L-amino acid decarboxylase (AAAD), dopamine beta-hydroxylase (DBH), and phenylethanolamine N-methyltransferase (PNMT), which were related to catecholamines biosynthesis in adrenal medulla (Kvetnansky et al., 2009, 2013). The iodotyrosine deiodinase (IYD) salvaged iodide from mono- and diiodotyrosine formed during the biosynthesis of the thyroid hormones, and the rescue and recycling of iodide by the action of IYD (Targovnik et al., 2017; Thomas et al., 2009).

Binding proteins in the peripheral circulation were important in regulating the transport, bioavailability, and metabolism of their cognate ligands, such as hormones, fatty acids, vitamins, and drugs (Goldman et al., 2017). The functions of human serum albumin (HSA) were the maintenance of colloid osmotic blood pressure, the transport of hormones, fatty acids, drugs, other compounds and buffering pH, and its binding also modulate acute and chronic toxicity (Grumetto et al., 2019). The major sex steroid hormones—testosterone, 5α-dihydrotestosterone, and 17β-estradiol—bound predominantly to sex hormone-binding globulin (SHBG) and to HSA and to a lesser extent to corticosteroid binding globulin (CBG) (Goldman et al., 2017). CBG was also the primary transporter for glucocorticoids (cortisol and corticosterone) and progestins (progesterone and 17-hydroxyprogesterone), and it regulated the partitioning of circulating cortisol into bound and unbound fractions (Bolton et al., 2014; Goldman et al., 2017). Thyroxine-binding globulin (TBG), transthyretin (TTR), HSA could bind and transport thyroid hormones (Benvenga and Guarneri, 2016), and TBG had the highest affinity and carries most of thyroxine and triiodothyronine in human serum, TTR and HSA bound only a small proportion of thyroxine and triiodothyronine despite large capacity (Janssen and Janssen, 2017). TBG homologues were α1-antitrypsin, α1-antichymotrypsin, antithrombin III, and CBG, while TBG nonhomologous carriers were α1-acid glycoprotein, which belonged to the lipocalin family, SHBG and apolipoproteins (Benvenga and Guarneri, 2016). The relationship between hormone molecules and receptor proteins also played an important role in signal transduction of endocrine systems. Hormone nuclear receptors, such as glucocorticoid receptor (GR), mineralocorticoid receptor (MR), androgen receptor (AR), constitutive androstane receptor (CAR), estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), estrogen related receptor alpha (ERRα), estrogen related receptor gamma (ERRγ), progesterone receptor (PR), thyroid hormone receptor alpha (TRα), thyroid hormone receptor beta (TRβ), belonged to a kind of superfamily hormone receptors with similar structure and function. After binding with ligands, they entered the nucleus and perform the function of transcription factors to activate the expression of downstream target genes (Gronemeyer et al., 2004; Ribeiro Filho et al., 2019; Weikum et al., 2018). Adrenergic receptors (α and β subtypes) were the basic targets of endogenous neurotransmitters noradrenaline and adrenaline, especially in mediating sympathetic activation to peripheral organs, but also in the central nervous system (Pytka et al., 2016). Dopamine belonged to catecholamines alongside noradrenaline and adrenaline. It was not only a precursor of the latter, but also exerted its physiological effect (Pytka et al., 2016). Dopamine receptors were G protein-coupled ones; they were expressed, inter alia, in the central nervous system, with two families amongst them were distinguished: D1-like comprising D1 and D5 receptors and D2-like consisting of D2, D3, and D4 (Beaulieu and Gainetdinov, 2011).

In this study, the potential endocrine disruption effects of TAP and its major metabolites were evaluated using the Discovery Studio 2019 *in silico*. Molecular docking *in silico* using Discovery Studio software has also been reported in the literatures (Cheurfa et al., 2019; da Silva Hage-Melim et al., 2019; Zengin et al., 2018). This study mainly assessed the disturbance of TAP and its main metabolites on the

physiological processes of the adrenal glands, gonads, thyroid gland related hormones biosynthesis key enzymes/proteins, blood circulation transport binding proteins, binding receptors and others. The crystal structures of all selected enzymes/proteins have been identified and their ligand binding domain active sites were known. This study provided more detailed and comprehensive parameters for the toxicity assessment of TAP or other OPs with similar chemical structures and their possible mechanisms of endocrine disruption.

2. Materials and methods

2.1. Receptors preparation

The structure files of proteins such as biosynthesis key enzymes/proteins, transport proteins, and receptors for hormones were derived from the Protein Data Bank (PDB) (http://www.rcsb.org/), and see Table 1 for details. Then the Protein Clean tool under Manual Preparation in the Macromolecules Tool in Discovery Studio 2019 (version 19.1.0.18287, Dassault Systèmes BIOVIA, Discovery Studio Modeling 178 Environment, Release 2018, San Diego, CA, USA) was used to correct the issues of non-standard named amino acids, inconsistent conformations, wrong bonds, number of hydrogen bonds, missing side chains and skeleton atoms in PDB file proteins. After cleaning the protein, the hydrogen bond on the protein was removed by default, so it was necessary to add hydrogen atoms manually and use the added Hydrogens option in the Chemistry tool to add polar hydrogen to the receptor protein. Each receptor protein itself already contained a small ligand molecule, which was located at the active sites of the protein, so the small molecule ligand was deleted.

2.2. Ligands preparation

The structure files and characteristic information of TAP and its major metabolites were referenced from the PubChem (https://pubchem.ncbi.nlm.nih.gov/); see Table 2 for details. Then the ligand molecules were further optimized using the Minimization in the Small Molecules tool in Discovery Studio 2019.

2.3. Molecular docking using CDOCKER

Discovery Studio 2019 provided several different molecular docking methods. Among them, the CDOCKER, a CHARMm-based docking engine (Wu et al., 2003), was a flexible molecular docking procedure, which could be applied to the study of receptor-ligand induced conjugation, interaction modes and related functional mechanisms, improving the accuracy of the docking results. With CDOCKER, initial ligand conformations were sampled *via* high temperature molecular dynamics and were also allowed to flex during the refinemen. Crucially, CDOCKER also provided a physics-based scoring function, *via* the CHARMm energy of the docked complex. CDOCKER has been shown to give highly accurate docked poses (Erickson et al., 2004).

Previously processed receptor proteins were defined as receptor molecules by Define and Edit Binding Site under the Receptor-Ligand Interactions tool. The location and size of the active sites were determined according to the position of the original ligand of the receptor protein. Using Docking Optimization under the Receptor-Ligand Interactions tool in Discovery Studio 2019, we opened the docking process of CDOCKER, set the corresponding parameters in turn, and added CHARMm force field to the ligand and receptor molecules respectively for molecular docking.

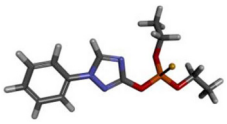
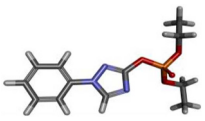
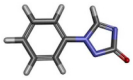
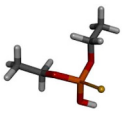
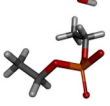
2.4. Analysis of molecular docking results

The Receptor-Ligand Interactions toolbar under Discovery Studio 2019 was used to view the interactions between ligands and receptor protein complexes, such as hydrogen bonds, Pi-Pi bonds, Pi-Sulfur bonds, salt bridges, and attraction charge. We could also use the Analyze Ligand Pose process in the Receptor-Ligand Interactions toolbar of Discovery Studio 2019 to further analyze the poses obtained by molecular docking. The docking analysis mainly included: the number of hydrogen bonds formed by the docked poses and the receptor protein complex, the number of hydrogen bonds formed with each amino acid residue, the near contact with the entire complex, and the near contact with each amino acid residue etc. At the same time, used the lowest CDOCKER_INTERACTION_ENERGY to evaluate conformations during docking simulations. The lower the binding energy, the stronger the interactions between the ligand and receptor protein.

2.5. Reliability verification of molecular docking

While the ligands such as TAP and its major metabolites were docked into receptor protein molecules, the accuracy and reliability of

Table 2
Structures and features of TAP and its major metabolites.

Compounds	Structure	Molecular Formula	CAS No.	PubChem CID
Triazophos (TAP)		$C_{12}H_{16}N_3O_3PS$	24017-47-8	32184
Triazophos-oxon (TAPO)		$C_{12}H_{16}N_3O_4P$	–	–
1-phenyl-3-hydroxy-1,2,4-triazole (PHT)		$C_8H_7N_3O$	4231-68-9	77910
Diethyl thiophosphate (DETP)		$C_4H_{11}O_3PS$	2465-65-8	655
Diethyl phosphate (DEP)		$C_4H_{11}O_4P$	598-02-7	654

molecular docking need to be investigated. All of the receptor proteins selected in this study contained the original ligands. The original ligands were deleted first, and then docked to the original active sites. The root mean squared deviation (RMSD) values of the conformations of the original ligands and TAP and its major metabolites were then compared. Since the resolution of the crystal structure in the PDB database was approximately 2 Å, we believed that there was no difference in the two molecular structures when the RMSD was within 2 Å. If the RMSD < 2.0 Å, the reliability of the docking method and the rationality of the docking parameters could be confirmed, and the strong interaction between TAP and its major metabolites and receptor proteins could be further demonstrated. The calculation of RMSD was carried out using Biopolymer RMSD Calculation dialog under Discovery Studio 2019 software. Original ligand in the crystal structure of receptor protein was chosen as a reference molecule from the reference molecule dropdown list. TAP and its major metabolites in docking complexes of receptor protein from the list were selected as the tested molecules for the calculation of RMSD. The RMSD of the original ligand and its corresponding receptor protein was 0.0000 Å.

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\delta_i^2} \quad (1)$$

In Equation (1), δ_i is the distance between the ith pair of atoms.

3. Results

There were several types of interactions that could be monitored between a ligand and a protein in Discovery Studio 2019 (Table 3). The categories of interactions were: Hydrogen Bonds, Electrostatic, Hydrophobic, Miscellaneous and Unfavorable. Meanwhile, the 2D interactions of the lowest binding energy poses for TAP, TAP's major metabolites with enzymes/proteins also could be observed in Discovery Studio 2019. The 2D interactions directly reflected the interactions between TAP, TAP's major metabolites and enzymes/proteins in graphical way, such as hydrogen bonding, Pi-Pi stacking effect, van der Waals force, hydrophilic and hydrophobic force, which provided theoretical basis for understanding the interaction mechanism between TAP, TAP's major metabolites and enzymes/proteins. In our study, 10 poses were generated for TAP and its four derivatives, TAPO, PHT, DETP and DEP, against the binding pockets of these enzymes/proteins and for each of the ligand. The best pose for each ligand was shown *via* the figures and tables.

Table 3
Interaction types between TAP, TAP's major metabolites and the active sites of enzymes/proteins in this study.

Category	Sub-Category	Type
Hydrogen Bonds	Classical	Conventional Hydrogen Bond
	Non-Classical	Carbon Hydrogen Bond
	Salt Bridge	Salt Bridge
Electrostatic	Charge	Attractive Charges
	Charge	Salt Bridge
	Pi-Charge	Pi-Cation
	Pi-Charge	Pi-Anion
Hydrophobic	Pi-Hydrophobic	Pi-Pi Stacked
	Pi-Hydrophobic	Pi-Pi T-Shaped
	Pi-Hydrophobic	Amide-Pi Stacked
	Alkyl Hydrophobic	Alkyl
	Mixed Pi/Alkyl Hydrophobic	Pi-Sigma
	Mixed Pi/Alkyl Hydrophobic	Pi-Alkyl
Miscellaneous	Sulfur	Pi-Sulfur
	Sulfur	Sulfur-X
	Lone Pair	Pi-Lone Pair
Unfavorable	Acceptor	Acceptor-Acceptor Clashes

Table 4
The lowest interaction energy (kcal/mol) between TAP, TAP's major metabolites and steroid hormones biosynthesis-related enzymes/proteins.

Receptors	TAP	TAPO	PHT	DETP	DEP
HMGR	−32.5477	−37.0916	−22.1141	−25.1874	−31.2638
SF-1	−28.8641	−28.9289	−17.8068	−18.2077	−32.8502
StAR	−34.1590	−37.7853	−23.6999	−23.7911	−23.8388
CYP11A1	−29.3898	−32.2015	−23.0100	−20.9167	−39.8313
CYP11B2	−31.5445	−37.5892	−18.9738	−16.5541	−49.5906
CYP17A1	−36.1763	−35.8308	−21.9371	−22.1311	−23.1907
CYP19A1	−34.5617	−38.8542	−24.3682	−19.9855	−43.6539
CYP21A2	−36.1355	−40.1772	−25.5294	−22.1745	−20.0655
17β-HSD1	−36.5765	−37.7614	−22.0903	−20.4887	−23.5693

3.1. Effects of TAP and its major metabolites on key enzymes/proteins of steroid hormones biosynthesis in adrenal cortex and gonads

We docked TAP and its major metabolites with the following proteins: HMGR, SF-1, StAR, CYP11A1, CYP111B2, CYP117A1, CYP19A1, CYP21A2, and 17β-HSD1. These proteins were transcription factors, transport proteins and catalytic enzymes in the biosynthesis of steroid hormones. StAR and CYP11A1 were considered the key enzymes/proteins in steroid hormone biosynthesis. From Table 4, molecular docking results showed that DEP and CYP11A1, CYP11B2, and CYP19A1 all had very low CDOCKER interaction energies of −39.8313, −49.5906, and −43.6539 kcal/mol, respectively. TAPO and CYP21A2 had very low CDOCKER interaction energy of −40.1772 kcal/mol. TAP and its major metabolites had higher CDOCKER interaction energies with other enzymes/proteins. Therefore, we mainly analyzed the interaction modes of TAP and its major metabolites with CYP11A1, CYP11B2, CYP19A1, and CYP21A2, respectively. The results were shown in Table 5 and Figs. S1, S2, S3, and S4, respectively.

These results suggested that DEP, the non-specific metabolite of OPs, could strongly interact with the enzymes CYP11A1, CYP11B2 and CYP19A1 related to steroid hormones synthesis, which might affect steroid hormones (adrenocortical steroid hormones and/or sex hormones) production. TAPO could strongly interact with the enzyme CYP21A2 related to adrenocortical steroid hormones synthesis, which might disturb the production of glucocorticoids and/or mineralocorticoids.

3.2. Effects of TAP and its major metabolites on key enzymes of catecholamines biosynthesis in adrenal medulla

We docked TAP and its major metabolites with adrenal medulla catecholamines biosynthesis enzymes TH, AAAD, DBH, and PNMT, respectively. From Table 6, the results of molecular docking showed that TAPO, DEP and AAAD had lower CDOCKER interaction energies of −38.0241 and −38.9285 kcal/mol, respectively. TAP, TAPO docked into PNMT have very low CDOCKER interaction energies of −42.7998 and −47.0563 kcal/mol, respectively. However, TAP and its major metabolites and other catecholamines biosynthetic enzymes had higher CDOCKER interaction energy. Therefore, we mainly discussed the interaction of TAP and its major metabolites with AAAD and PNMT, respectively. The results were shown in Table 7, Figs. S5 and S6.

These results indicated that TAP, TAPO, and DEP might disrupt the functions and activities of the enzymes AAAD and PNMT, which were related to catecholamines biosynthesis in the adrenal medulla, thereby blocking its production of adrenaline.

3.3. Effects of TAP and its major metabolites on the enzyme iodotyrosine deiodinase (IYD) of thyroid hormones biosynthesis in thyroid gland

Restricted by the crystal structure of enzymes related to thyroid hormone biosynthesis and metabolism that have been resolved in the

Table 5
Interaction sites, main types and distances between residues at the active sites of steroid hormones biosynthesis-related enzymes and TAP, TAP's major metabolites.

Receptors	Ligands	Interaction sites	Main types	Distances (Å)
CYP11A1	TAP	Gln 356	Conventional hydrogen bond	Lig – Gln 356 (2.48)
	TAPO	Phe 202, Thr 354, Gln 356	Pi-Pi T shaped, Conventional hydrogen bond	Lig – Phe 202 (5.98) Lig – Thr 354 (2.76) Lig – Gln 356 (2.57)
	PHT	Val 353, Thr 354, Gln 356	Conventional hydrogen bond	Lig – Val 353 (2.41) Lig – Thr 354 (2.17) Lig – Gln 356 (2.85)
	DETP	Arg 81, Ser 352, Thr 354, Gln 356	Conventional hydrogen bond	Lig – Arg 81 (2.90) Lig – Ser 352 (1.91) Lig – Thr 354 (2.82) Lig – Gln 356 (2.43)
	DEP	Arg 81, Arg 112	Conventional hydrogen bond, Attractive charge	Lig – Arg 81 (2.49) Lig – Arg 112 (5.52)
CYP11B2	TAP	Trp 116, Phe 130	Pi-Pi T-shaped, Pi-Pi Stacked	Lig – Trp 116 (4.95) Lig – Phe 130 (4.00)
	TAPO	Arg 110, Phe 130	Conventional hydrogen bond, Pi-Sigma, Pi-Pi Stacked	Lig – Arg 110 (2.18) Lig – Phe 130 (2.80)
	PHT	Trp 116, Phe 130, Phe 487	Pi-Pi T-shaped, Pi-Pi Stacked, Conventional hydrogen bond	Lig – Trp 116 (5.21) Lig – Phe 130 (5.46) Lig – Phe 487 (2.05)
	DETP	–	–	–
	DEP	Arg 110, Arg 384, Phe 445	Conventional hydrogen bond, Salt bridge, Attractive charge, Pi-Anion	Lig – Arg 110 (1.77) Lig – Arg 384 (1.79) Lig – Phe 445 (2.55)
CYP19A1	TAP	Arg 115	Conventional hydrogen bond	Lig – Arg 115 (2.17)
	TAPO	Arg 115, Trp 224, Cys 437	Conventional hydrogen bond, Pi-Pi Stacked,	Lig – Arg 115 (2.39) Lig – Trp 224 (5.40) Lig – Cys 437 (3.00)
	PHT	Arg 115, Met 374	Conventional hydrogen bond, Attractive charge	Lig – Arg 115 (2.66) Lig – Met 374 (2.00)
	DETP	–	–	–
	DEP	Arg 115, Arg 145, Arg 435, Ala 438	Attractive charge, Salt bridge, Conventional hydrogen bond	Lig – Arg 115 (2.56) Lig – Arg 145 (2.67) Lig – Arg 435 (4.94) Lig – Ala 438 (2.03)
CYP21A2	TAP	Trp 202	Pi-Sulfur	Lig – Trp 202 (4.46)
	TAPO	Trp 202	Pi-Pi Stacked	Lig – Trp 202 (4.24)
	PHT	Asp 107, Asp 288	Conventional hydrogen bond	Lig – Asp 107 (2.00) Lig – Asp 288 (2.13)
	DETP	–	–	–
	DEP	Trp 202	Pi-Anion	Lig – Trp 202 (4.91)

Table 6
The lowest interaction energy (kcal/mol) between TAP, TAP's major metabolites and catecholamines biosynthesis-related enzymes.

Receptors	TAP	TAPO	PHT	DETP	DEP
TH	−35.2452	−38.8217	−25.9069	−24.6320	−24.9642
AAAD	−31.6843	−38.0241	−21.5726	−24.0328	−38.9285
DBH	−22.0572	−27.4095	−14.3949	−16.2235	−28.5891
PNMT	−42.7998	−47.0563	−26.8873	−27.9821	−19.9956

PDB database, we could only dock TAP and its main metabolites with the thyroid hormones biosynthesis enzyme iodotyrosine deiodinase (IYD) in the thyroid gland. Table 8 showed that DEP and IYD had very low CDOCKER interaction energy of −45.7062 kcal/mol. The docking complexes of TAP, TAPO, PHT, DETP, DEP and IYD and the corresponding IYD interacting amino acid residues were shown in Table 9 and Fig. S7. These demonstrated the interaction of TAP and its major metabolites with amino acids of IYD protein, respectively. DEP could interact strongly with IYD, indicating that DEP might be a potential thyroid hormone endocrine disruptor.

3.4. Effects of TAP and its major metabolites on hormone transport proteins

We docked TAP and its major metabolites with hormone transport binding proteins in blood such as HSA, CBG, SHBG, TBG, and TTR, respectively. The results of Table 10 showed that TAP, TAPO, DEP docked into HSA could form very low CDOCKER interaction energies of −39.9807, −44.1985 and −40.1406 kcal/mol, respectively. TAPO formed a very low CDOCKER interaction energy of −40.2857 kcal/mol with SHBG. DEP formed very low CDOCKER interaction energy of −40.5303 kcal/mol with TBG. Therefore, we mainly listed the interaction modes of TAP and its major metabolites with HSA, SHBG, and TBG, respectively. These results were shown in Table 11 and Figures S8-S10 respectively.

Based on these results, we could speculate that TAP, TAPO, and DEP could affect the binding transport of HSA with adrenocortical hormones, sex hormones, and thyroid hormones through intermolecular interaction with HSA. Meanwhile, TAPO could disturb the circulation of sex hormones by binding with SHBG, and DEP might affect the transport of thyroid hormones in the blood by interacting with TBG.

3.5. Effects of TAP and its major metabolites on hormone receptors

We docked TAP and its major metabolites with hormone receptors, such as GR, MR, AR, CAR, ERα, ERβ, ERRα, ERRγ, PR, TRα, TRβ, tβ1AR, β2AR, D2DR, D3DR, and D4DR, respectively. The molecular docking results in Table 12 showed that TAP and GR, AR, TRα, and TRβ could form very low CDOCKER interaction energies of −36.8425, −37.9152, −40.2179, and −41.7089 kcal/mol, respectively. TAPO formed very low CDOCKER interaction energies with more hormone receptors. For example, TAPO formed low interaction energies of −38.0438, −38.6282, −40.0815, −39.2319, −38.6160, −40.2356,

Table 7
Interaction sites, main types and distances between residues at the active sites of catecholamine hormones biosynthesis-related enzymes and TAP, TAP's major metabolites.

Receptors	Ligands	Interaction sites	Main types	Distances (Å)
AAAD	TAP	Tyr 79, Lys 303, Arg 447	Pi-Pi T-shaped, Conventional hydrogen bond, Pi-Cation	Lig – Tyr 79 (5.64) Lig – Lys 303 (1.81) Lig – Arg 447 (3.17)
	TAPO	Tyr 79, Lys 303, Arg 447	Pi-Pi T-shaped, Conventional hydrogen bond, Pi-Cation	Lig – Tyr 79 (5.46) Lig – Lys 303 (1.71) Lig – Arg 447 (3.20)
	PHT	Lys 303	Pi-Cation	Lig – Lys 303 (3.09)
	DETP	Lys 303	Conventional hydrogen bond	Lig – Lys 303 (1.89)
	DEP	Thr 246, Lys 303	Conventional hydrogen bond, Attractive charge	Lig – Thr 246 (2.39) Lig – Lys 303 (2.35)
PNMT	TAP	Tyr 27, Gly 79, Asp 101, Phe 102, Asn 106	Pi-Pi Stacked, Unfavorable Acceptor-Acceptor, Pi-Anion, Pi-Pi T-shaped, Conventional hydrogen bond	Lig – Tyr 27 (5.80) Lig – Gly 79 (2.99) Lig – Asp 101 (3.54) Lig – Phe 102 (4.70) Lig – Asn 106 (2.59)
	TAPO	Tyr 27, Phe 30, Tyr 35, Asp 101, Phe 102, Cys 183	Pi-Pi Stacked, Pi-Pi T-shaped, Conventional hydrogen bond, Pi-Anion, Pi-Sulfur	Lig – Tyr 27 (5.21) Lig – Phe 30 (5.57) Lig – Tyr 35 (2.70) Lig – Asp 101 (3.42) Lig – Phe 102 (4.64) Lig – Cys 183 (5.30)
	PHT	Tyr 27, Tyr 35, Ser 80, Thr 83	Pi-Pi T-shaped, Conventional hydrogen bond, Pi-Pi Stacked	Lig – Tyr 27 (5.18) Lig – Tyr 35 (2.33) Lig – Ser 80 (2.04) Lig – Thr 83 (2.30)
	DETP	Tyr 35, Phe 182	Conventional hydrogen bond	Lig – Tyr 35 (2.71) Lig – Phe 182 (1.95)
	DEP	Tyr 35	Conventional hydrogen bond	Lig – Tyr 35 (2.81)

Table 8
The lowest interaction energy (kcal/mol) between TAP, TAP's major metabolites and the thyroid hormones biosynthesis-related enzyme IYD.

Receptor	TAP	TAPO	PHT	DETP	DEP
IYD	−21.4739	−31.4681	−15.7375	−16.2651	−45.7062

−40.5868, −42.7165, −41.6788 kcal/mol with GR, MR, AR, CAR, ERα, PR, TRα, TRβ, and β1AR, respectively. The interaction energies of PHT and DETP with these hormone receptors were higher. DEP formed a lower CDOCKER interaction energy of −37.7211 kcal/mol with TRα. Therefore, we mainly described the interaction patterns of TAP and its major metabolites with GR, MR, AR, CAR, ERα, PR, TRα, TRβ, and β1AR, respectively. These results were shown in Table 13 and Figs. S11, S12, S13, S14, S15, S16, S17, S18, and S19, respectively. In addition, the homology of β1AR amino acid sequences between humans (*Homo sapiens*) and turkeys (*Meleagris gallopavo*) was approximately 54.93%. The sequence alignment results showed that the amino acids that interacted with TAP and its major metabolites, in these two proteins were consistent and conservative (Fig. S20).

Table 10
The lowest interaction energy (kcal/mol) between TAP, TAP's major metabolites and hormone transport proteins.

Receptors	TAP	TAPO	PHT	DETP	DEP
HSA	−39.9807	−44.1985	−25.3253	−23.7186	−40.1406
CBG	−30.7416	−33.2241	−22.0712	−19.3301	−18.2183
SHBG	−37.1562	−40.2857	−25.6923	−24.2684	−21.2266
TBG	−34.1400	−35.8789	−22.3594	−20.5038	−40.5303
TTR	−35.2280	−37.1439	−20.6632	−23.8367	−30.1745

Overall, TAP might disrupt these three hormone receptors: GR, TRα and TRβ. TAPO might interfere with the normal binding of these

Table 9
Interaction sites, main types and distances between residues at the active sites of thyroid hormones biosynthesis-related enzyme IYD and TAP, TAP's major metabolites.

Receptor	Ligands	Interaction sites	Main types	Distances (Å)
IYD	TAP	Arg 100, Arg 104	Conventional hydrogen bond, Pi-Anion	Lig – Arg 100 (2.58) Lig – Arg 104 (3.24)
	TAPO	Arg 100, Arg 104	Conventional hydrogen bond	Lig – Arg 100 (1.79) Lig – Arg 104 (2.59)
	PHT	Arg 100, Ser 102, Arg 104, Thr 237	Conventional hydrogen bond, Pi-Anion	Lig – Arg 100 (2.84) Lig – Ser 102 (2.65) Lig – Arg 104 (3.59) Lig – Thr 237 (1.88)
	DETP	Arg 104, Lys 182, Tyr 184	Conventional hydrogen bond	Lig – Arg 104 (1.98) Lig – Lys 182 (2.43) Lig – Tyr 184 (2.90)
	DEP	Arg 100, Arg 101, Ser 102, Arg 279	Conventional hydrogen bond, Attractive charge, Salt bridge	Lig – Arg 100 (1.88) Lig – Arg 101 (4.46) Lig – Ser 102 (2.07) Lig – Arg 279 (2.60)

Table 11
Interaction sites, main types and distances between residues at the active sites of hormone transport proteins and TAP, TAP's major metabolites.

Receptors	Ligands	Interaction sites	Main types	Distances (Å)
HSA	TAP	Lys 199, Arg 222	Conventional hydrogen bond	Lig – Lys 199 (2.66) Lig – Arg 222 (3.00)
	TAPO	Lys 199	Conventional hydrogen bond	Lig – Lys 199 (2.05)
	PHT	Arg 257, Ser 287, Ile 290	Attractive charge, Conventional hydrogen bond, Amide-Pi Stacked	Lig – Arg 257 (4.58) Lig – Ser 287 (1.87) Lig – Ile 290 (4.54)
	DETP	Arg 257	Conventional hydrogen bond	Lig – Arg 257 (2.12)
	DEP	Lys 199, Arg 218, Arg 222	Conventional hydrogen bond, Attractive charge	Lig – Lys 199 (1.85) Lig – Arg 218 (2.82) Lig – Arg 222 (2.61)
SHBG	TAP	Phe 67, Asn 82, Met 139	Pi-Pi Stacked, Conventional hydrogen bond, Pi-Sulfur	Lig – Phe 67 (4.89) Lig – Asn 82 (2.59) Lig – Met 139 (4.16)
	TAPO	Phe 67, Asn 82, Met 139	Pi-Pi Stacked, Conventional hydrogen bond, Pi-Sulfur	Lig – Phe 67 (4.84) Lig – Asn 82 (2.17) Lig – Met 139 (4.29)
	PHT	Asp 65, Phe 67, Asn 82	Pi-Pi Stacked, Conventional hydrogen bond	Lig – Asp 65 (2.15) Lig – Phe 67 (4.08) Lig – Asn 82 (2.78)
	DETP	–	–	–
	DEP	Phe 67	Pi-Anion	Lig – Phe 67 (4.78)
TBG	TAP	Tyr 20, Lys 270, Asn 273, Arg 381	Pi-Cation, Conventional hydrogen bond	Lig – Tyr 20 (4.67) Lig – Lys 270 (3.17) Lig – Asn 273 (3.04) Lig – Arg 381 (3.44)
	TAPO	Arg 381	Pi-Sigma	Lig – Arg 381 (2.75)
	PHT	Gln 238	Conventional hydrogen bond	Lig – Gln 238 (1.98)
	DETP	Asn 273	Conventional hydrogen bond	Lig – Asn 273 (1.99)
	DEP	Ser 266, Lys 270	Conventional hydrogen bond, Attractive charge	Lig – Ser 266 (2.55) Lig – Lys 270 (2.75)

Table 12
The lowest interaction energy (kcal/mol) between TAP, TAP's major metabolites and hormone receptors.

Receptors	TAP	TAPO	PHT	DETP	DEP
GR	−36.8425	−38.0438	−21.8705	−20.8930	−21.3411
MR	−35.5446	−38.6282	−22.7501	−21.5424	−25.8509
AR	−37.9152	−40.0815	−23.0466	−22.0322	−24.1372
CAR	−36.3142	−39.2319	−20.4643	−20.8157	−20.8015
ERα	−34.9721	−38.6160	−24.3489	−21.2301	−22.1135
ERβ	−33.3428	−34.8708	−26.2235	−23.3019	−26.1649
ERRα	−31.3311	−34.1177	−25.3990	−23.7727	−22.8410
ERRγ	−25.5320	−27.9839	−27.5525	−24.5199	−26.6702
PR	−36.6287	−40.2356	−24.5775	−21.9644	−26.5485
TRα	−40.2179	−40.5868	−30.3521	−22.5194	−37.7211
TRβ	−41.7089	−42.7165	−24.8326	−22.2293	−30.8696
β1AR	−35.2497	−41.6788	−26.0393	−23.9079	−22.3257
β2AR	- 35.5131	−36.7502	−24.5873	−22.1916	−24.1935
D2DR	−35.6015	−37.3540	−21.2402	−22.1884	−21.1554
D3DR	−33.9438	−37.0953	−25.2388	−23.5515	−24.8556
D4DR	−37.7654	−36.5579	−21.6463	−21.5246	−18.5749

receptors GR, MR, AR, CAR, ERα, PR, TRα, TRβ, and β1AR to their respective native ligands. Although the binding of PHT and PR had higher binding interaction energy, there were many strong chemical bonds between them. Thus, PHT might have adverse effects on the normal physiological function of PR. DEP might interact with TRα and should be able to be considered as a potential thyroid hormone disruptor.

We also verified the accuracy of the molecular docking results to ensure the accuracy of the parameters and method selection during the molecular docking process. The evaluation index was the value of RMSD. The smaller the RMSD was, the closer the docking results of TAP and its major metabolites with the target proteins were to the structure of the original ligands and the target proteins. See Table S1 for the specific results. As shown in Table S1, the CDOCKER molecular docking results of Discovery Studio 2019were credible and acceptable.

4. Discussion

4.1. Overview of TAP and its major degradation metabolites

Organophosphate pesticides, which were widely used in agriculture, were highly toxic and commonly ingested with suicidal intent in developing countries (Henretig et al., 2019). Similarly, TAP has been widely used globally for more than 40 years (Holden et al., 2001; JMPR, 2002). China's pesticide risk monitoring results also indicated that TAP had high risks or risk uncertainty in terms of toxicity, residue, and environmental safety. The Ministry of Agriculture of China has banned the use of TAP in vegetables and started re-evaluation study of TAP. However, TAP was still registered in China for pest control in rice, cotton, and grassland. And TAP was often detected in agricultural products, foods, and environmental soil and water samples (Bhandari et al., 2019; Hong et al., 2019; Kumari and John, 2019). The biological half-life of TAP in the body was short (JMPR, 2002), and TAP was rapidly metabolized to the specific metabolites TAPO and PHT, and the non-specific DAP metabolites DETP and DEP under the action of a series of metabolic detoxification enzymes (Bock and Their, 1976; Schwalbe-Fehl and Schmidt, 1986; Wang et al., 2015).

Approximately 75% of OPs registered in the market were metabolized to the DAP metabolites (Shomar et al., 2014; Ueyama et al., 2015). At the same time, OPs were also degraded into DAPs such as DETP and DEP due to the metabolism of the plants and the environmental factors and were present in fruits and vegetables (Zhang et al., 2008). A certain concentration of chlorpyrifos pesticide was sprayed on the green string bean grown in the field, and the residues of DEP could still be detected in the samples collected on the 21st day after spraying (Pan et al., 2012). Interestingly, some organophosphorus flame retardants and plasticizers could also be degraded and metabolized to DEP (Chu and Letcher, 2018; Li et al., 2017; Reemtsma et al., 2011; Sun et al., 2018). The correlation between OPs with diseases established by epidemiological studies mainly based on the contents of their metabolites including DEP in the urine (Katsikantami et al., 2019). Epidemiologic

Table 13
Interaction sites, main types and distances between residues at the active sites of hormone receptors and TAP, TAP's major metabolites.

Receptors	Ligands	Interaction sites	Main types	Distances (Å)
GR	TAP	Asn 564, Phe 623, Tyr 735, Cys 736	Conventional hydrogen bond, Pi-Pi T-shaped, Pi-Sulfur	Lig – Asn 564 (2.68) Lig – Phe 623 (5.01) Lig – Tyr 735 (5.33) Lig – Cys 736 (2.08)
	TAPO	Leu 563, Tyr 735, Cys 736	Pi-Pi T-shaped, Amide-Pi stacked, Pi-Sulfur	Lig – Leu 563 (4.73) Lig – Tyr 735 (5.93) Lig – Cys 736 (3.62)
	PHT	Met 560, Asn 564, Phe 623, Met 646	Conventional hydrogen bond, Pi-Pi T-shaped, Pi-Sulfur	Lig – Met 560 (2.25) Lig – Asn 564 (2.07) Lig – Phe 623 (5.23) Lig – Met 646 (5.19)
	DETP	Asn 564	Conventional hydrogen bond	Lig – Asn 564 (1.93)
	DEP	–	–	–
MR	TAP	Phe 829	Pi-Pi T-shaped bond	Lig – Phe 829 (5.01)
	TAPO	Met 807, Phe 829	Pi-Sulfur, Pi-Pi T-shaped	Lig – Met 807 (4.00) Lig – Phe 829 (5.04)
	PHT	Asn 770, Cys 942	Conventional hydrogen bond, Pi-Lone pair, Pi-Sulfur	Lig – Asn 770 (2.26) Lig – Cys 942 (4.57)
	DETP	Asn 770	Conventional hydrogen bond	Lig – Asn 770 (1.91)
	DEP	Arg 817	Attractive charge	Lig – Arg 817 (4.75)
AR	TAP	Leu 704, Phe 764, Met 780	Pi-Sigma, Pi-Sulfur	Lig – Leu 704 (2.70) Lig – Phe 764 (4.59) Lig – Met 780 (4.94)
	TAPO	–	–	–
	PHT	Asn 705, Phe 764	Conventional hydrogen bond, Pi-Pi T-shaped	Lig – Asn 705 (1.87) Lig – Phe 764 (5.98)
	DETP	Asn 705	Conventional hydrogen bond	Lig – Asn 705 (2.13)
	DEP	–	–	–
CAR	TAP	Phe 161, His 203, Tyr 326	Pi-Pi T-shaped, Pi-Sulfur, Pi-Pi Stacked	Lig – Phe 161 (5.03) Lig – His 203 (4.92) Lig – Tyr 326 (4.98)
	TAPO	Phe 161, His 203, Tyr 326	Pi-Pi T-shaped, Pi-Pi Stacked	Lig – Phe 161 (5.10) Lig – His 203 (4.70) Lig – Tyr 326 (4.98)
	PHT	Phe 161, Thr 225, Phe 243	Pi-Pi T-shaped, Conventional hydrogen bond, Pi-Pi T-shaped	Lig – Phe 161 (4.88) Lig – Thr 225 (2.04) Lig – Phe 243 (5.69)
	DETP	Tyr 224, Thr 225	Pi-Sulfur, Conventional hydrogen bond	Lig – Tyr 224 (5.86) Lig – Thr 225 (1.90)
	DEP	Phe 234	Pi-Anion	Lig – Phe 234 (3.87)
ERα	TAP	Met 343, Thr 347, Met 528	Conventional hydrogen bond, Pi-Sulfur, Pi-Sulfur	Lig – Met 343 (5.42) Lig – Thr 347 (2.72) Lig – Met 528 (4.88)
	TAPO	Met 528	Pi-Sulfur	Lig – Met 528 (5.38)
	PHT	Arg 394, Phe 404	Conventional hydrogen bond, Attractive charge, Pi-Pi T-shaped	Lig – Arg 394 (2.22) Lig – Phe 404 (5.09)
	DETP	Thr 347, Cys 530	Conventional hydrogen bond	Lig – Thr 347 (2.62) Lig – Cys 530 (2.45)
	DEP	–	–	–
PR	TAP	Met 756	Sulfur-X	Lig – Met 756 (3.26)
	TAPO	Met 759, Phe 778, Met 909	Pi-Sigma, Pi-Pi T-shaped, Pi-Sulfur	Lig – Met 759 (2.80) Lig – Phe 778 (5.36) Lig – Met 909 (5.44)
	PHT	Gln 725, Met 756, Met 759, Arg 766, Phe 778, Met 801	Conventional hydrogen bond, Pi-Sulfur, Attractive charge, Pi-Pi T-shaped	Lig – Gln 725 (2.17) Lig – Met 756 (4.49) Lig – Met 759 (4.37) Lig – Arg 766 (2.36) Lig – Phe 778 (5.38) Lig – Met 801 (5.40)
	DETP	Leu 718, Trp 755	Conventional hydrogen bond, Pi-Sulfur	Lig – Leu 718 (1.97) Lig – Trp 755 (5.61)
	DEP	Gln 725	Conventional hydrogen bond	Lig – Gln 725 (2.44)
TRα	TAP	Met 256, Met 388	Pi-Sulfur, Sulfur-X	Lig – Met 256 (3.03) Lig – Met 388 (5.72)
	TAPO	Phe 218	Unfavorable acceptor-acceptor	Lig – Phe 218 (2.80)
	PHT	Arg 228, Ser 277	Conventional hydrogen bond, Attractive charge	Lig – Arg 228 (1.93) Lig – Ser 277 (2.33)
	DETP	–	–	–
	DEP	Arg 228, Ser 277	Conventional hydrogen bond, Attractive charge	Lig – Arg 228 (2.48) Lig – Ser 277 (2.16)

(*continued on next page*)

Table 13 (*continued*)

Receptors	Ligands	Interaction sites	Main types	Distances (Å)
TRβ	TAP	Met 313, Met 442	Pi-Sulfur	Lig – Met 313 (5.82) Lig – Met 442 (4.83)
	TAPO	Met 313, Met 442	Pi-Sulfur	Lig – Met 313 (5.73) Lig – Met 442 (5.21)
	PHT	Met 313, Asn 331	Conventional hydrogen bond	Lig – Met 313 (1.85) Lig – Asn 331 (2.35)
	DETP	Met 310	Sulfur-X	Lig – Met 310 (3.50)
	DEP	Arg 282, Asn 331	Attractive charge, Conventional hydrogen bond	Lig – Arg 282 (5.14) Lig – Asn 331 (2.00)
β1AR	TAP	Trp 117, Phe 201, Phe 306	Pi-Sulfur, Conventional hydrogen bond, Pi-Pi T-shaped	Lig – Trp 117 (4.74) Lig – Phe 201 (2.64) Lig – Phe 306 (5.05)
	TAPO	Phe 201, Ser 212, Phe 306	Pi-Pi T-shaped bond, Conventional hydrogen bond	Lig – Phe 201 (4.93) Lig – Ser 212 (2.30) Lig – Phe 306 (5.10)
	PHT	Asp 121, Phe 307, Asn 329	Conventional hydrogen bond, Pi-Pi T-shaped bond	Lig – Asp 121 (1.94) Lig – Phe 307 (5.59) Lig – Asn 329 (2.30)
	DETP	Asp 121	Conventional hydrogen bond	Lig – Asp 121 (2.05)
	DEP	Asn 310	Conventional hydrogen bond	Lig – Asn 310 (2.97)

studies established the association between OPs and disturbed hormone levels based on urinary DEP measurements, especially the disorder of serum sex hormone levels and thyroid hormone levels in OPs exposed population (Bernieri et al., 2019; Omoike et al., 2015; Panuwet et al., 2018; Shrestha et al., 2018; Wang et al., 2017a,b).

4.2. Endocrine disrupting effects of TAP in vivo and in vitro

TAP exposure can affect the thyroid hormone levels and the expression of *TRα*, *TRβ*, *Dio1*, *Dio2*, *tsh*, *ERα*, *ERβ1*, *ERβ2* in zebrafish embryos (Wu et al., 2018). *In vitro* cell experiments showed that TAP had the potentials to disrupt the estrogen receptor (ER), aromatic hydrocarbon receptor, constitutive androstane receptor (CAR), pregnane X receptor, and vitamin D receptor signaling pathways (https://pubchem.ncbi.nlm.nih.gov/compound/32184) (Table S2).

The effects of TAP on reproductive activity were mainly explored in female rats (Sharma et al., 2015a) and the effects on the offsprings were also studied under pre-conceptional exposure to TAP (Sharma et al., 2015b; Bhanot and Sangha, 2018). TAP exposure altered serum content of estrogen and progesterone, elevated the peroxidation and apoptosis in ovary at the dose of 1/10th, 1/20th, and 1/40th of LD_{50} (Sharma et al., 2015a). *In utero* and lactational exposure to acceptable daily intake level of TAP influenced testis development and functions in the male offsprings, which were characterized by a significant fall in sperm count, sperm motility, plasma testosterone levels, and histopathological alterations in testis (Bhanot and Sangha, 2018). However, non-significant changes in reproductive indices such as gestational length and pup viability were observed in the offsprings in female rats under preconceptional exposure of 30 days to 1/10th and 1/20th of LD_{50} of TAP (Sharma et al., 2015b).

4.3. Potential disrupting effects of TAP and its major metabolites on endocrine hormones biosynthesis and transport

EDCs, also known as endocrine disruptors, were chemicals that could alter endocrine function by mimicking, blocking, or interfering with the production, metabolism, or action of hormones in the body (The Lancet Diabetes & Endocrinology, 2019). Molecular docking can reveal the potential of EDCs (phthalate esters) to inhibit the enzymes of the glucocorticoid biosynthesis pathway (Ahmad et al., 2017), and unraveling the molecular targets of bisphenol A and S in the thyroid gland (Berto-Júnior et al., 2018). EDCs also disturbed hormone-binding globulins and transport proteins in the blood, such as the interfering effects of EDC on HSA (Hill et al., 2018; Peng et al., 2016), CBG (Sheikh and Beg, 2017), SHBG (Hazarika et al., 2019; Liu et al., 2016; Sheikh and Beg, 2019; Sheikh et al., 2016b, 2016c), TBG (Ren et al., 2016), and TTR (Grimm et al., 2013; Hill et al., 2018; Kar et al., 2017; Ren et al., 2016; Xin et al., 2018; Zhang et al., 2015, 2016a; Zhang et al., 2018a,b,c) *in silico*, affecting hormone binding and transport, and the levels of bioavailable free hormones.

Our results also suggested that DEP, the non-specific metabolite of organophosphorus pesticides, can strongly interact with the enzymes CYP11A1, CYP11B2 and CYP19A1 related to steroid hormones synthesis, which might affect steroid hormones (adrenocortical steroid hormones and/or sex hormones) production. TAPO can strongly interact with the enzyme CYP21A2 related to adrenocortical steroid hormones synthesis, which might disturb the production of glucocorticoids and/or mineralocorticoids. Previous studies have also found that CYP11A1, CYP11B2, CYP19, and CYP21 were targets of EDCs-induced adrenocortical toxicity and endocrine disruption in steroidogenic pathway (Harvey et al., 2007; Harvey, 2016). DEP can interact strongly with IYD, indicating that DEP might be a potential thyroid hormone endocrine disruptor. In addition, our study indicated that TAP, TAPO and DEP might disrupt the functions and activities of the enzymes AAAD and PNMT, which were related to catecholamines biosynthesis in adrenal medulla (Kvetnansky et al., 2009, 2013), thereby disturbing the production of adrenaline in the adrenal medulla. The neonicotinoid insecticide imidacloprid can facilitate TH transcription and PNMT mRNA expression to enhance catecholamine synthesis in PC12D cells (Kawahata and Yamakuni, 2018). High dietary phosphoric acid increased plasma phosphate concentration, blood pressure, and pulse count. This mechanism might involve changes in sympathetic adrenaline activity (Mohammad et al., 2018). Phosphoric acid and DEP were similar in chemical structure. Therefore, TAP and its major metabolites might exert endocrine disrupting effects by disturbing the biosynthesis processes of hormones in the endocrine glands.

Based on these results of our research, we speculate that TAP, TAPO, and DEP can affect the binding transport of HSA with adrenocortical hormones, sex hormones, and thyroid hormones through intermolecular interaction with HSA. TAPO can also disturb the circulation of sex hormones by binding with SHBG, and DEP might affect the transport of thyroid hormones in the blood by interacting with TBG. Predictably, TAP and its major metabolites might also interact with hormone-binding globulins and transport proteins in the blood, thereby affecting the levels of free hormones available in the peripheral circulation. Similarly, it can interfere with the negative feedback regulation of endocrine hormones.

4.4. Potential disrupting effects of TAP and its major metabolites on the binding of endocrine hormone receptor proteins

In addition to being regulated by their natural hormone ligands binding, nuclear hormone receptors were also affected and regulated by exogenous environmental contaminants (Wang et al., 2017a,b), EDCs (Balaguer et al., 2017; Le Maire et al., 2010; Ruiz et al., 2017; Sharma et al., 2018; Usman and Ahmad, 2019), foods and their components (Cozzini and Spyrakis, 2017; Goto, 2019; Zhang et al., 2018c), drugs (Cozzini and Spyrakis, 2017), and toxics (Choudhuri et al., 2017), among others, and can cause transcription and expression of the downstream target genes, leading to a series of physiological and pathological phenotypes. Steroid hormone receptors (GR, MR, AR, CAR, ERα, ERβ, and PR) and thyroid hormone receptors (TRα and TRβ) also played a series of important roles in maintaining normal growth and development, reproduction, energy metabolism, inflammation, and immunity in humans and animals (Conroy-Ben et al., 2018; Lu et al., 2018; Pande et al., 2019; Weikum et al., 2017; Zhang et al., 2018b). EDCs, including pesticides, could interfere with hormone receptors *in vivo*, *in vitro* and *in silico* (Chen et al., 2018), such as GR (Khan et al., 2017; Prasanth et al., 2010; Sarath Josh et al., 2016; Zhang et al., 2016c), MR (Khan et al., 2017), AR (Chen et al., 2019; Conroy-Ben et al., 2018; Khan et al., 2017; Sarath Josh et al., 2016), CAR (Verma et al., 2017), ERα (Conroy-Ben et al., 2018; Zhang et al., 2018b), PR (Khan et al., 2017; Sarath Josh et al., 2016; Sheikh et al., 2016a), and TR (Li et al., 2010; Lu et al., 2018; Ren et al., 2015; Xin et al., 2018; Zhang et al., 2016b), among others, thus affecting their biological effects. Dysfunction of the monoaminergic neurotransmission was implicated in major depressive disorder and other neuropsychiatric conditions (Liu et al., 2018). Therefore, the study of the interaction between EDCs and hormone receptors was an important technique for screening EDCs. The molecular docking simulation method, as a computational toxicology research approach based on the interaction between EDCs and receptors, has been increasingly widely applied (Cavaliere and Cozzini, 2018; Chen et al., 2018; Vuorinen et al., 2015).

By investigating the binding abilities of TAP and its major metabolites to hormone receptors, we found that TAP might disrupt these three hormone receptors: GR, TRα and TRβ. TAPO might interfere with the normal binding of these receptors GR, MR, AR, CAR, ERα, PR, TRα, TRβ, and β1AR to their respective native ligands. Although the binding of PHT and PR had higher binding interaction energy, there were many strong chemical bonds between them, so PHT might have adverse effects on the normal physiological function of PR. DEP might interact with TRα and act as a potential thyroid hormone disruptor. Obviously, TAP and its major metabolites might also affect the transcription and expression of downstream genes by destroying the binding abilities of hormone receptors to their native hormone ligands, leading to a series of adverse biological effects. Further, we could also give an example of validation here. When docking RU486, an antagonist of GR (Chen et al., 2018; Weikum et al., 2017), with GR (Fig. S21), it was found that RU486 could form the Pi-Pi T-shaped, Pi-Sulfur with Phe623 and Cys736 of GR, respectively. RU486 also formed Alkyl, Pi-Alkyl bonds with GR at the sites including Leu563 and Tyr735 in GR. These sites were in common with TAP, indicating that TAP might inhibit the binding of glucocorticoids with GR.

5. Conclusions

The endocrine disrupting effects of TAP led to only a few research advances *in vivo* and *in vitro*. The correlation between DEP levels in human urine and endocrine hormone disorders had also been investigated in several epidemiological studies, but there was no direct causal supporting evidence. The purpose of this study was to understand the mechanism of molecular interactions of TAP and its four major metabolites (TAPO, PHT, DETP, and DEP) with endocrine hormone-related proteins and enzymes using molecular docking simulation *in silico*. All three compounds (TAP, TAPO, and DEP) showed high binding affinity with more proteins and enzymes than PHT and DETP. TAP might interfere with the endocrine function of the adrenal glands, including the biosynthesis of corticosteroids and catecholamines. TAP might also bind strongly with GR and TR, affecting the normal physiological function of these hormone receptors. TAPO might disrupt the normal binding of AR, ER, PR, and β1AR to their natural hormone ligands. The binding of PHT and endocrine hormone-related proteins and enzymes had higher binding interaction energies, PHT and PR could form many strong chemical bonds. Analysis of the molecular docking results of DETP showed that there was no strong molecular interaction between DETP and all endocrine hormone-related proteins and enzymes involved in this study, suggesting that DETP was unlikely to be an EDC. DEP might affect biosynthesis of steroid hormones (adrenocortical steroid hormones and/or sex hormones) and thyroid hormones. At the same time, DEP might disrupt the binding and transport of thyroid hormones in the blood and the normal binding of thyroid hormones to their receptors. Together, these suggested that TAP and DEP might have endocrine disrupting activities and were potential EDCs.

TAP remains a commonly used organophosphorus pesticide in many developing countries, with high residual and high exposure risks. DEP was a non-specific metabolite of diethyl thiophosphate and diethyl phosphate organophosphorus pesticides, and even some organophosphorus flame retardants and plasticizers could also be metabolized to DEP. Moreover, DEP not only can be produced by metabolism in the human body, but can be present in agricultural products, foods, and the environment. The human body could be exposed to DEP through various ways such as diet and the environment. Therefore, based on our findings, it was necessary to be more cautious and rigorous in the usage and residue limit standards of organophosphorus chemicals.

Conflicts of interest

The authors declare that no conflicts of interest in this article.

Declaration of interests

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

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Appendix A. Supplementary data

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