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Eco-toxicity and human estrogenic exposure risks from ·OH-initiated photochemical transformation of four phthalates in water: A computational study

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ABSTRACT

Transformation products (TPs) of emerging organic contaminates (EOCs) in water are still rarely considered in environmental risk assessment, although some have been found to be concern. ·OH is believed as an important reactive species both in indirect phototransformation and advanced oxidation technology. Thus, eco-toxicity and human estrogenic exposure risks of four phthalates and TPs during the ·OH-initiated photochemical process were investigated using computational approach. Four phthalates can be degraded through ·OH-addition and H-transfer pathways. The ·OH-addition TPs were predominant for dimethyl phthalates, while H-transfer TPs were predominant for other three phthalates. Compared with phthalates, ·OH-addition TPs (*o*-OH-phthalates) were one level more toxic to aquatic organisms, and *m*-OH-phthalates exhibit higher estrogenic activity. Although H-transfer TPs were less harmful than ·OH-addition TPs, some of them still have aquatic toxicity and estrogenic activity. Therefore, more attentions should be paid to photochemical TPs and original EOCs, particularly those exhibiting high estrogenic activity to humans.

1. Introduction

Phthalates are widely used as plasticizers in numerous consumer and personal care products, including food packaging, medical devices, cosmetics, and building materials. As such, they have become ubiquitous environmental contaminants (Bertelsen et al., 2013), with several million tons of phthalates produced and used annually worldwide (Feng et al., 2013); large amounts of phthalates are continuously released into the environment during the production, use, and disposal of plastic products. As a result, phthalates are frequently detected in various environmental matrices, including air (Li et al., 2013), soil (Xu et al., 2008) and water (Shi et al., 2012a, 2012b; Xie et al., 2007). When it released to the aquatic environment, phthalates may be transformed into other products. Thus, the aquatic ecosystems and human health are exposed to an unknown cocktail of these chemicals.

Recent studies suggest that most water contaminants may form more toxic byproducts than their parent compounds during the photochemical transformation in surface water, and then increase risks to aquatic ecosystems and human health (An et al., 2014a; Gao et al., 2014b; Potera, 2011). These transformations can also occur in advanced oxidation technology (AOTs) systems (An et al., 2015; Li et al., 2006, 2007) because ·OH is also believed to be an important reactive species in the degradation of organic pollutants in AOTs systems. Recently, it is reported that although some of the transformation products (TPs) are found in aquatic environment at low concentrations, they still can interact with some reactive species in water environments resulting in an adverse impact on the environment (Escher and Fenner, 2011). Accordingly, the environmental presence and the formation of TPs add further complexity to environmental risk assessment. Given this, it is important to study the transformation mechanisms and environmental risks from these ·OH-initiated TPs as well as original compounds during

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the photochemical transformation of phthalates in water.

Endocrine-disrupting and reproductive risks have already been well known for phthalates (Huang et al., 2009; Kaiser, 2005; Li et al., 2010); Moreover, phthalates TPs may significantly contribute to the risk posed by the parent compound, and additional risks to human health may be also associated with phthalate's TPs (Duty et al., 2003). Thus, to assess the exposure risk from single phthalate is insufficient, the risks from its TPs should be also considered. While limited reports were investigated on the fate of phthalates and the health risk imposed by their phototransformation products in water.

Theoretical calculations have been demonstrated to effectively supplement to the experimental results according to our early results (An et al., 2015, 2014b, 2011; Fang et al., 2013). These calculations have successfully predicted the reaction mechanisms and fates, and have provided promising potential methods for the toxicity assessments of EOCs (Gao et al., 2014a, 2014b; Wang et al., 2011). Therefore, in this work, to systematically understand the exposure risk imposed by phthalates and their TPs in water, the $^{\bullet}$OH-initiated transformation of phthalates in water was used as a baseline example to explore the photochemical transformation mechanism by the theoretical calculations, and the environmental fate of phthalates were also considered to assess the eco-toxicity and human estrogenic activity of both original phthalates and their TPs. The study also attempts the structure-dependency toxicity of phthalates as well as their TPs, to help estimate other similar contaminant toxicities. Four phthalates were employed in this study based on their properties as priority control organic pollutants because they are all simplest phthalates, such as dimethyl phthalate (DMP), diethyl phthalate (DEP), dipropyl phthalate (DPP) and dibutyl phthalate (DBP). This study's theoretical data will supply some experimental results to further properly assess the exposure risks of phthalates as well as their TPs to aquatic ecosystems and human health.

2. Computational methods

2.1. Electronic structure calculations

Electronic structure calculations were completed using a Gaussian 03 program (Frisch et al., 2003). The geometry optimization of reactants, products, and transition states (*TS*) were performed using the hybrid density functional B3LYP method with the 6-31G(d,p) basis set (Nicolaescu et al., 2005). This method has been successfully applied to simulate the aqueous reactions of organic pollutants in environmental fields (Nicolaescu et al., 2005; Wang et al., 2012). Harmonic vibrational frequencies were calculated at the same level to identify all stationary points as either minima (zero imaginary frequency) or *TS* (only one imaginary frequency). The minimum energy pathway (MEP) was constructed using intrinsic reaction coordinate (IRC) theory to confirm that each *TS* accurately connected the reactant with the associated product. The mechanism involving single-electron transfer reaction was calculated using Marcus theory (Supplementary information). Single-point energy calculations, including the solvent effect, established the potential energy surface (PES) at B3LYP/6-311++G(d,p) level based on the optimized structures and the conductive polarizable continuum model (CPCM). The reaction kinetics was calculated using transition-state theory (TST), considering solvent cage effects and diffusion-limited effects, and the detail descriptions were given in Supplementary data.

2.2. Eco-toxicity assessment calculations

The acute and chronic toxicities of phthalates as well as TPs were assessed using the "ecological structure-activity relationships" ECOSAR program (ECOSAR, 2014). This allowed for the risk assessments at three trophic levels of aquatic organisms: green algae, daphnia and fish. Acute toxicity is expressed using EC_{50} values (the concentration of tested pollutant leading to 50% growth inhibition of green algae after 96 h) and LC_{50} values (the concentration of tested pollutant leading to 50% dead fish and daphnia after 96 and 48 exposures, respectively). The lowest effect concentration was chosen for the most conservative estimation. The threshold environmental concentrations (TEC) and the exposure risk of target chemicals were assessed using the hazard quotient (HQ) method (Hernando et al., 2006). A detailed description and the eco-toxicity classification are provided in Supplementary information.

2.3. Human estrogenic activity calculations

The human estrogenic activities of phthalates and their TPs were determined using VirtualToxLab software (Vedani et al., 2015, 2012). These simulated and quantified bindings to estrogen receptor β (ERβ), were well known to cause significant changes in target cell estrogen (Grober et al., 2011). The binding affinity is expressed as IC_{50} values (i.e. the concentration at which 50% of ERβ binding was inhibited), and the approach is typically based on human data and can assess the estrogenic risk of the target compounds to human.

3. Results and discussion

3.1. The structure-dependent risk of phthalates

Fig. S1 shows the optimized structures of the four phthalates, including the description of their carbon atom number. There are three typical sites on the benzene ring for four phthalates: meta-(C1), ortho-(C2), and ispo-(C3) positions. The C—H bonds varied slightly across the phthalate side chains. As a result, four phthalates have similar structural parameters and physicochemical properties (Fig. 1). For example, both the octanol-water partition coefficients (LogKow) and molecular polarity slightly increases from lowest to highest in the following order: DMP, DEP, DPP and DBP.

To estimate phthalate exposure risk in aquatic ecosystems, acute and chronic toxicities were investigated first at three trophic levels of aquatic organisms (green algae, daphnia and fish) (Fig. 1). All toxicity values (LC_{50}, EC_{50}, and ChV) for each aquatic organism decreased from DMP to DEP to DPP to DBP. For example, the LC_{50} values for fish were 40.82, 12.47, 3.75 and 1.11 mg L^{-1} for DMP, DEP, DPP and DBP, respectively (Table S1). This suggests that the acute and chronic toxicities at the three trophic levels increased with the order of DMP < DEP < DPP < DBP. The trends were similar for the μ, and LogKow, suggesting the phthalate aquatic toxicity strongly correlated with these two parameters. That is, phthalates with higher μ and large LogKow values are more toxic to aquatic organisms.

As Fig. 1 shows, the acute toxicity values of DMP ranged from 10.0 to 100.0 mg L^{-1} at the three aquatic organisms. According to the European Union criteria (Table S2), DMP is classed as harmful to the three aquatic organisms. The same harmful level was also observed for DEP to fish and daphnia, and a toxic level was obtained to green algae. DPP was classified as toxic level to all three aquatic organisms. A toxic level was also obtained for DBP to fish and daphnia, while DBP with an EC_{50} of 0.49 mg L^{-1} was classified as very toxic to green algae (EC_{50} < 1.0 mg L^{-1}) (Table S1). For chronic toxicity (Fig. 1), although DMP and DEP were not harmful to daphnia, four phthalates still result in adverse effects to other aquatic organisms. Moreover, by comparing the available

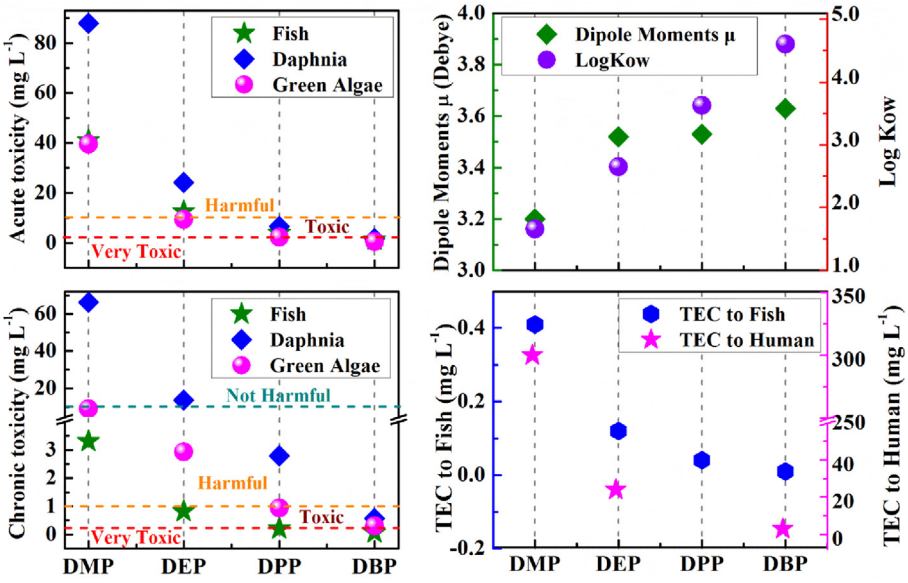

Fig. 1. Properties, aquatic toxicity and the threshold environmental concentration (TEC) of the four phthalates.

experimental data of toxicity values (Table S3), it can be seen that the experimental and calculated data fall into the same toxicity classes, further confirming that this theoretical program is a suitable method to assess the toxicity of phthalates and their TPs.

To further assess phthalate safety in aquatic environments and for human health, the HQ method was also used to calculate the threshold environmental concentrations (TEC) in water. Fish are a human food source, so this trophic level was selected as the model aquatic organism. The TEC for fish were calculated to be 0.14, 0.12, 0.04 and 0.01 mg L^{-1} for DMP, DEP, DPP and DBP, respectively (Fig. 1). When phthalate concentrations exceed these levels, they may impose potential risk to fish and consequently to human health through the dietary exposure. Moreover, they may threaten human health through drinking water exposure because phthalates have been frequently detected in drinking water (Shi et al., 2012a). The TEC to human health is also calculated as only 300, 24 and 3 mg L^{-1} for DMP, DEP and DBP, respectively (Fig. 1), while the TEC of DPP could not be determined because there is no oral reference dose (RfD). Thus, based on these toxicity values, the DPP TEC should be conservatively estimated as similar as DBP. As such, the results suggest that phthalate may impose risks to human health through the drinking water exposure when their concentrations in water exceed the corresponding values.

As for phthalate exposure risk to human health, the estrogenic responses of four phthalates were assessed by examining their binding affinity to estrogen receptor β (ERβ). Data showed that the ERβ-binding affinities of four phthalates were all above 100 mM, indicating that ERβ-mediated endocrine-disrupting effect was negligible. This finding agrees well with previous experimental results, where no phthalate bonding to ERβ was detected (Toda et al., 2004), and further confirms the reliability of the VirtualToxLab calculations used in this work to assess human estrogenic activity of phthalates as well as their TPs. However, the relationship between phthalates and endocrine-disruption remains controversial. The systematically study on the phthalate TPs and their aquatic toxicity as well as estrogenic activity may help resolve the open questions.

3.2. Photochemical transformation patterns of phthalates

3.2.1. Photochemical transformation mechanisms

The ·OH-initiated photochemical transformation of four phthalates were modeled in detail, and grouped as three pathway groups: (i) ·OH-addition leading to the radical adducts formation (*RAF* pathways), (ii) hydrogen atom transfer by ·OH (*HAT* pathways), and (iii) single electron transfer by ·OH (*SET* pathways). Scheme S1 summarizes all phthalate transformation pathways, and Figs. S2–S4 present the optimized geometries of all *TS* and products involved in these pathways. Table 1 lists the computed reaction enthalpies (ΔH) and reaction energies (ΔG) of all pathways. It can be seen that *SET* pathways were non-spontaneous for all phthalate, due to positive ΔG values of 19.32–27.59 kcal mol^{-1}. In addition, these pathways were endothermic processes (ΔH > 22.82 kcal mol^{-1}), while other *RAF* and *HAT* pathways were exothermic processes except carbonyl-·OH-addition pathways (4_{RAF}) of four phthalates with small ΔH of 7.87–9.66 kcal mol^{-1}. As such, from a thermodynamics viewpoint, the *SET* and carbonyl-·OH-addition pathways were less energetically favored than others, and can be ruled out from the initial ·OH reaction steps. This conclusion further theoretically revealed the previous experimental results that the transient absorption spectra of the *SET* intermediates were not observed in the pulse radiolysis of DMP with ·OH (An et al., 2014b). Therefore, there is a very low probability for the electron-transfer reactivity of four phthalates to ·OH.

For the phthalates' *RAF* pathways (Table 2), the energy barriers ($\Delta G^{\neq}$) of 4_{RAF} were calculated as 23.77–28.65 kcal mol^{-1}, and higher by at least 10.33 kcal mol^{-1} compared with benzene-·OH-addition pathways. This confirms that ·OH had difficulty attacking the phthalate's carbonyl groups. However, for the benzene-·OH-addition pathways, $\Delta G^{\neq}$ were obtained with 8.61–9.77, 10.56–10.76, and 13.44–14.20 kcal mol^{-1} for 1_{RAF}, 2_{RAF}, and 3_{RAF}, respectively. $\Delta G^{\neq}$ of the latter pathway was higher than the former two pathways by 4.11–4.83 and 2.68–3.61 kcal mol^{-1}, respectively, thus 1_{RAF} and 2_{RAF} pathways were predicted to be more likely among of four *RAF* pathways. Therefore, for *RAF* pathways, the meta carbon atoms (C1) and ortho carbon atoms (C2) in the benzene ring were most easily attacked position by ·OH, producing the meta ·OH-adducts (·Phthalates-OH_1) and ortho ·OH-adducts (·Phthalates-OH_2) as dominant transformation intermediates, respectively (shown in Figs. S1 and S2-4).

For *HAT* pathways of phthalates, the $\Delta G^{\neq}$ of benzene-H-transfer pathways (1_{HAT} and 2_{HAT}) were calculated as 12.06–12.84 and 16.01–17.73 kcal mol^{-1}, respectively (Table 2). This was higher than

Table 1
Reaction enthalpies ΔH and reaction energies ΔG for ·OH-initiated transformation of the four phthalates (kcal mol^{-1}).

		RAF pathways				HAT pathways						SET pathway
		1_{RAF}	2_{RAF}	3_{RAF}	4_{RAF}	1_{HAT}	2_{HAT}	α_{HAT}	β_{HAT}	γ_{HAT}	δ_{HAT}	
ΔH	DMP[a]	−16.40	−15.21	−12.97	8.52	−4.82	−4.97	−19.41	–	–	–	27.91
	DEP	−16.15	−15.1	−13.2	9.66	−4.84	−4.99	−22.28	−17.12	–	–	26.38
	DPP	−16.29	−14.83	−13.39	8.96	−4.9	−4.93	−22.31	−21.55	−18.14	–	22.82
	DBP	−16.12	−14.58	−13.27	7.87	−4.89	−4.95	−22.36	−21.41	−22.08	−18.48	26.35
ΔG	DMP[a]	−7.42	−5.89	−3.33	18.64	−6.46	−5.13	−20.77				27.59
	DEP	−6.86	−5.85	−3.52	20.76	−6.07	−7.41	−23.61	−18.22			25.96
	DPP	−6.93	−5.63	−4.15	20.37	−6.34	−6.59	−24.74	−24.82	−21.33		19.32
	DBP	−6.75	−5.69	−3.94	19.56	−6.62	−6.61	−24.21	−24.69	−25.51	−21.32	25.32

[a] Data from reference (An et al., 2014b).

Table 2
Free energy barriers $\Delta G^{\neq}$ (kcal mol^{-1}) for the ·OH-initiated transformation of four phthalates.

$\Delta G^{\neq}$	RAF pathways				HAT pathways					
	1_{RAF}	2_{RAF}	3_{RAF}	4_{RAF}	1_{HAT}	2_{HAT}	α_{HAT}	β_{HAT}	γ_{HAT}	δ_{HAT}
DMP[a]	9.57	10.59	14.20	27.38	12.44	15.51	10.60	–	–	–
DEP	9.77	10.56	13.90	28.65	12.84	16.01	10.12	9.7	–	–
DPP	9.56	10.70	13.67	25.02	12.78	17.07	10.22	10.19	8.86	–
DBP	8.61	10.76	13.44	23.77	12.06	17.73	10.07	10.27	7.89	8.77

[a] Data from reference (An et al., 2014b).

alkyl-H-transfer pathways (α ~ δ_{HAT}) by approximately 1.8–6.3 kcal mol^{-1}. Moreover, the former pathways were less exothermic by at least 12 kcal mol^{-1} than the latter pathways (Table 1). This implies that H-transfer from the benzene ring was less favored than from the alkyl group. For the alkyl-H-transfer pathways, DEP Cβ-H-transfer pathway (β_{HAT}) was more likely to occur than the α_{HAT} pathway, because the $\Delta G^{\neq}$ of β_{HAT} was 10.12 kcal mol^{-1}, and was lower by 0.42 kcal mol^{-1} than its α_{HAT}. This means the lowest barrier of *HAT* pathway is that of the H-transfer from the terminal methyl group (−CH_3). As for DPP transformation, the similar trendy was obtained. H-transfer from terminal methyl group (γ_{HAT}) has the highest reactivity, due to the lowest $\Delta G^{\neq}$ of 8.86 kcal mol^{-1}. But DBP showed a different trendy. The highest reactivity was obtained as γ_{HAT} pathway (7.89 kcal mol^{-1}), rather than terminal methyl-H-transfer (δ_{HAT} pathway), with a slightly higher $\Delta G^{\neq}$ (8.77 kcal mol^{-1}).

Based on above mentioned discussion, all of single-electron transfer pathways, two ·OH-addition pathways (3_{RAF} and 4_{RAF}), and two benzene-H-transfer pathways (1_{HAT} and 2_{HAT}) can be excluded from possible phthalate transformation pathways in water. Nevertheless, due to the small difference in the $\Delta G^{\neq}$ of the remaining pathways (1_{RAF}, 2_{RAF} and α ~ δ_{HAT}), their contributions cannot be completely identified based only on the mechanism aspects. As such, the kinetics calculations were also conducted to distinguish each pathway's contribution from each other in the total transformation pathways.

3.2.2. Kinetics calculations

To quantitatively evaluate each major pathway's contribution and explore phthalate transformation fate in water, the reaction kinetics were investigated within a temperature range of 273–313 K. Table S4 presents the calculated second-order rate constants for each pathway as well as the total reactions (k_{total}, the sum of rate constants of each pathway), and the experimental data reported in early references are also summarized for the comparison. Fig. S5 shows that k_{total} as a function of temperature, and the results suggest that k_{total} of phthalate transformation increased as the temperature rose. This suggests that high temperatures could improve ·OH-initiated phthalate transformation in water, and phthalate concentrations could be varied from seasonal water temperature.

Fig. S5 and Table S4 show that, at a fixed temperature, the k_{total} increases as the carbon number of the side chain of four phthalate increases. The k_{total} increases in the order of $k_{DMP} < k_{DEP} < k_{DPP} < k_{DBP}$. The k_{total} for DMP, DEP, DPP and DBP were calculated as 2.66×10^9, 4.40×10^9, 5.80×10^9, and 8.70×10^9 M^{-1} s^{-1} at room temperature, respectively. These calculated rate constants were well matched with the experimental values. Taking DMP transformation as an example, the calculated k_{total} of 2.66×10^9 M^{-1} s^{-1} was the same order of magnitude as the early reported experimental values: $(2.67 \pm 0.26) \times 10^9$ M^{-1} s^{-1} using the competition kinetics method (Wen et al., 2011), and 3.2×10^9 and 3.4×10^9 M^{-1} s^{-1} using pulse radiolysis (An et al., 2014b; Wu et al., 2011), as well as 4.0×10^9 M^{-1} s^{-1} through the fitting the experimental data to the kinetic model (Xu et al., 2009). These consistence results further confirm the reliability of our theoretically calculated data.

Additionally, to estimate rate constants at specific temperatures, the pre-exponential factor, the activation energy and the Arrhenius formulas were established from the rate constant data within the temperature range of 273–313 K (Table S5). The activation energies of four phthalates were calculated as 11.01, 10.95, 10.2, and 9.37 kcal mol^{-1} for DMP, DEP, DPP and DBP transformation, respectively. This means these ·OH-initiated transformation reactions of four phthalates in water can be easily occurred with so small activation energies.

Furthermore, the half-life ($t_{1/2}$) of ·OH-initiated transformation was also calculated within 273–313 K temperature range. Fig. S6 shows that at a specified [·OH] range, the $t_{1/2}$ of four phthalates decreases as the temperature increases, based on [·OH] from 10^{-14}–10^{-18} M in natural waters (Wu and Linden, 2010). For example, at the highest [·OH] of 10^{-14} M in natural waters, the $t_{1/2}$ of DMP increased from 4.8 to 22.7 h as the temperature dropped from 313 to 273 K. This suggests that a decrease of environmental temperature results in an increase $t_{1/2}$ of four phthalates, further confirming the previous experimental observation that longer half-lives are obtained in cold water environments (Staples et al., 1997). Moreover, at a fixed temperature, $t_{1/2}$ of four phthalates increased with the decrease of [·OH]. These results suggested that the $t_{1/2}$ depended more on [·OH] than the temperature, although the increase in the temperature and [·OH] would benefit the phthalates' transformation (Fig. S6). Furthermore, at a fixed temperature and

[·OH], $t_{1/2}$ of four phthalates decrease as the alkyl chain length increases. This implies that as the alkyl chain length increases, the susceptibility of phthalates to ·OH-initiated photochemical transformation increases. For example, at room temperature, with [·OH] up to 10^{-14} M, $t_{1/2}$ values were obtained as 7.2, 3.4, 3.3 and 2.2 h for DMP, DEP, DPP and DBP transformation, respectively. This confirmed that phthalate reaction with ·OH is very important for their transformation reactions in water, particularly at high [·OH] values.

3.2.3. Transformation products

To quantitatively estimate the transformation products of different phthalates in aquatic environments, the temperature dependences of the branching ratios (Γ) were calculated as: $\Gamma = k_{pathway}/k_{total}$, and the results are shown in Fig. 2. For DMP transformation (Fig. 2a), the Γ of 1_{RAT}, 2_{RAT} and α_{HAT} were calculated as 51%, 21% and 28% at room temperature, respectively. Therefore, the ·OH-addition pathway (1_{RAT}) was dominated pathways of DMP transformation to form ·DMP-OH_1. It can also produce stable meta-hydroxylated product on the benzene ring (*m*-OH-DMP) (An et al., 2014b). Ortho-hydroxylated DMP (*o*-OH-DMP) and hydroxymethyl-DMP (α-OH-DMP) were also formed at a lower ratio through the 2_{RAT} and α_{HAT} pathways, respectively. These transformation products were identified by HPLC-MS-MS and doubly confirmed with the authentic standards in our previous experimental research (An et al., 2014b).

The different results were obtained for DEP transformation (Fig. 2b). The stable H-transfer product, β-OH-DEP, was obtained as the main product. Therefore, the hydroxylated product can be easily happened on the side chain than on the benzene ring of DEP, especially on the terminal methyl group (Cβ). This is consistent with the analysis of the energy barrier of these pathways of four phthalates mentioned above. Fig. 2c shows a similar result for DPP transformation. The largest contributor to DPP transformation was obtained for γ_{HAT} pathway, with Γ of 38% at the room temperature, then 1_{RAF}, α_{HAT} and β_{HAT} pathways with Γ of 23%, 15% and 16% respectively. This comparison demonstrates that *HAT* pathways from the terminal methyl group (−CH_3) could be more significant than other pathways for DPP. Meanwhile, *HAT* transformation products decreased in the order of γ-OH-DPP > β-OH-DPP > α-OH-DPP. A different trend was observed for DBP *HAT* pathways (Fig. 2d). That is, the DBP *HAT* transformation products decreased with the order of γ-OH-DBP > δ-OH-DBP > α-OH-DBP > β-OH-DBP. This difference could result from the inductive effect of the electron-withdrawing carbonyl group, and the electron-donating alkyl group may be also responsible for the reactivity trend of DBP.

In summary, for DMP transformation, *RAF* mechanism is obtained as the dominant pathway, while the *HAT* pathways were dominated for other three phthalates. The *RAF* pathway contribution to phthalate transformation was calculated as 72%, 40%, 31% and 26% for DMP, DEP, DPP, and DBP at room temperature, respectively (Fig. S7). With the increase of the alkyl side chain, the role of *RAF* pathway becomes less significant in the ·OH-initiated transformation reactions. For DEP, DPP and DBP, the ·OH attack onto the side alkyl chain are more important than the attack on the benzene ring. The H atom on the end −CH_3 group is the most active position for other three phthalates. Except for DBP, *HAT* reaction from Cγ was the highest position to form the transformation intermediates.

3.3. Exposure toxicity of TPs

3.3.1. Exposure toxicity to aquatic organisms

To systemically understand the exposure risk of these TPs in water, their acute and chronic toxicities to aquatic organisms were both investigated during the photochemical process. For DMP TPs

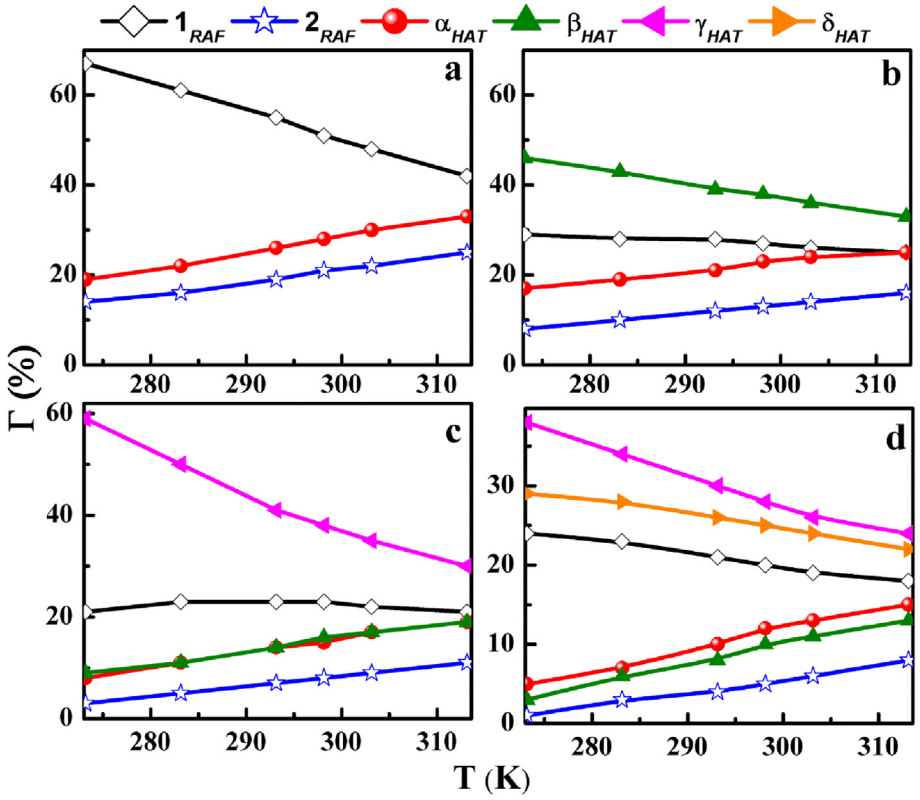

Fig. 2. The contribution ratio of main pathways of four phthalates transformation in the temperature range of 273–313 K: (a). DMP; (b). DEP; (c). DPP; (d). DBP.

(Table S6a), all *RAF* products (*m*-OH-DMP and *o*-OH-DMP) were classified as harmful to three tested aquatic organisms, except *o*-OH-DMP was acutely toxic to daphnia. Moreover, all toxicity values of *RAF* products were 1–56 times lower than that of original DMP, indicating that the eco-toxicity increased through *RAF* pathways. As such, the hydroxylated products on the benzene ring (*RAF* products) were found to be more toxic than original DMP. However, different results were observed for *HAT* TPs. The *HAT* product (α-OH–DMP) was classified as non-harmful to all tested aquatic organisms (Table S6a). The general conclusion is that when DMP is exposed to aquatic environments, *RAF* products are more harmful than *HAT* products, and even more toxic than original DMP. Thus, the adverse effects from *RAF* pathway, hydroxylated products, may raise significant concerns to DMP transformation in water.

For TPs of other three phthalates (DEP, DPP and DBP) (Table S6b–c), a similar conclusion was obtained: the eco-toxicity of TPs were also enhanced by *RAF* pathways compared with *HAT* pathways, as well as compared to the corresponding original phthalates (DEP, DPP and DBP). In particular, several *RAF* products were two levels more toxic than their corresponding original phthalates. As for TPs of DPP (Table S6c), the aquatic toxicity of *RAF* products increased as compared with the two short chain phthalates (DMP and DEP). Moreover, the acute toxicity of *o*-OH-DPP was one level more toxic than *m*-OH-DPP (Table S6c) to three tested aquatic organisms. This implies that hydroxylated products on the ortho-position of benzene ring were more toxic than the TPs in the meta-position. For DBP TPs, both the acute and chronic toxicities of all *RAF* products were classified as very toxic to all three tested aquatic organisms, with the exception of green algae which were located at chronically toxic level (Table S6d). Thus, the aquatic toxicity of *HAT* products cannot be ignored since several TPs could also impose adverse effects on aquatic environments. In particular, as hydroxylated products on the side chain's end $-CH_3$ group, β-OH-DEP, γ-OH-DPP and δ-OH–DBP, were the most harmful species to aquatic organisms among of corresponding *HAT* products.

In summary, for ·OH-initiated transformation of four phthalates, the aquatic toxicity of all TPs increased from DMP to DBP, with similar tendencies as their original compounds. Moreover, all the *RAF* products, especially *o*-OH-phthalates, are more toxic than the parent compounds, but the phenomenon was not seen for the *HAT* products. Several hazardous products may still result from DEP, DPP and DBP transformation although *HAT* products were less harmful than *RAF* products. Among these *HAT* products, the ·OH attack on the end $-CH_3$ group could result in more toxic TPs than on the $-CH_2$ group of other three phthalates. For example, β-OH-DEP, γ-OH-DPP, and δ-OH–DBP are the most harmful *HAT* products to aquatic organisms. Thus, the adverse effect of these phthalate TPs to various aquatic organisms cannot be ignored completely.

3.3.2. Estrogenic activity to human health

TPs of phthalates in water may also alter the endocrine system's normal function. During the photochemical transformation of phthalate, the resulting products referenced above may adversely influence people through the ingestion pathway, and then possibly affect the human endocrine system. To investigate human endocrine-disruption, the estrogenic activities of TPs were also assessed based on the binding affinity to ERβ, which is well known to cause significant changes in target cell estrogen (Grober et al., 2011). Table S7 shows the results of original phthalates, and Fig. 3 and S8 show the TPs interactions with ERβ obtained by the flexible docking. DMP was found to be negligible ERβ-binding affinity; however, its TPs (except *o*-OH-DMP) were found to be potentially hazardous to human health. For example, *m*-OH-DMP and α-OH-DMP with IC_{50} of 743 nM and 59.7 μM (Table S7), were classified as medium and low risk levels, respectively, based on the classification criteria of the binding affinity (VirtualToxLab, 2013). In addition, no binding to ERβ was observed for *o*-OH-DMP, which is consistent with a previous experimental study (Okamoto et al., 2011). These results are all confirmed by the reliability of theoretical method for the estrogenic activity predication.

Thus, it can be concluded that DMP TPs have greater estrogenic activity than the corresponding original DMP, and *RAF* product (*m*-OH-DMP) has more estrogenic activity than *HAT* products (α-OH-DMP). As shown from the binding mode in Fig. 3, DMP and its TPs can form the hydrogen bond with the Arg 346 amino group. Thus two TPs (*m*-OH-DMP and α-OH-DMP) exhibit estrogenic activity due to the formation of another hydrogen bond between hydroxyl group and the carbonyl group of Glu 305, possibly triggering the adverse effects of these TPs' on human health.

Similar results were also obtained for the TPs of other three phthalates (DEP, DPP and DBP) (Table S7). All *o*-OH-phthalates have negligible estrogenic activities, while other TPs have greater estrogenic activity than the original compounds. Furthermore, *RAF* products (*m*-OH-phthalate) have higher estrogenic activity than *HAT* products. For TPs of DEP and DPP, all impose medium risks to humans, except a lower risk of α-OH-DPP. The low binding affinity of α-OH-DPP may result from the hydrogen-bond interaction between the hydroxyl group and the carbonyl group of His 475 (Fig. S8).

Furthermore, the estrogenic activity was enhanced among of DEP and DPP *HAT* TPs, with the activity of α-OH-phthalate lower than β-OH-phthalate, which was both lower than that of γ-OH-phthalates. As for DBP *HAT* TPs, a different trend was obtained. The estrogenic activity was similar for α-OH-DBP and β-OH-DBP, which were both less than δ-OH-DBP and γ-OH-DBP. That is, α-OH-DBP and β-OH-DBP did not show estrogenic activity, but γ-OH-DBP and δ-OH-DBP were found to impose medium and low risks to humans with IC_{50} of 5.89 and 13.8 μM, respectively. Based on the combined results of the branching ratios mentioned above, the estrogenic activities of the *HAT* products have a similar trend as their branching ratios. This means that the increased ·OH attack on the favorable alkyl position will increase the estrogenic activity of *HAT* products.

The above results suggest that during the photochemical transformation of phthalates in water, the TPs have been found to be with endocrine-disrupting potential, and then increase the risks to human health. As for four phthalates studied, *RAF* products (*m*-OH-phthalates) exhibit higher estrogenic activity than *HAT* products, and even possessing higher activity than the original phthalates. Meanwhile, all *HAT* products except α-OH-DBP and β-OH-DBP also possessed estrogenic activity. In particular, the estrogenic activity of γ-OH-DPP exceeded the activity of corresponding original phthalate.

4. Conclusion

A detailed theoretical insight into the ·OH-initiated photochemical transformation of four different phthalates was conducted in water environments. Quantum chemical calculations indicate that ·OH reacted with four phthalates through both ·OH-addition and H-transfer pathways. Kinetics analysis reveal that ·OH-addition TPs are mainly formed for DMP, while H-transfer products are predominant for DEP, DPP and DBP. The eco-toxicity assessments suggest that the aquatic eco-toxicity of phthalates as well as their TPs increased as their alkyl chain length increases. Compared with the original phthalates, hydroxylated products on the ortho-position of phthalates' ring (*o*-OH-phthalates) have greater aquatic toxicity, and hydroxylated products on the meta-position of phthalates' ring (*m*-OH-phthalates) exhibit higher estrogenic activity. Although H-transfer products were less harmful

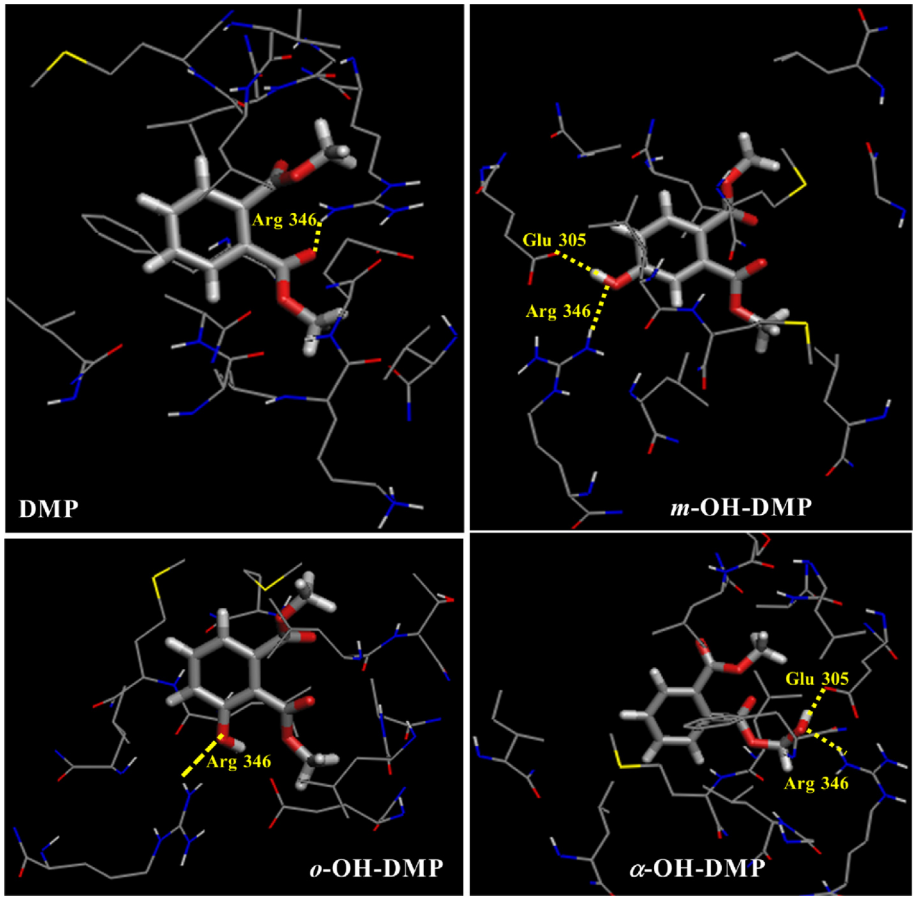

Fig. 3. The interaction pattern of DMP and its transformation products binding to estrogen receptor β (ERβ).

than those ·OH-addition products, they could still be harmful to aquatic organisms and human health. Therefore, these hazardous products should be paid more attention in the future experimental studies and assessment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.08.006.

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