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Article

Ecotoxicity of phthalic acid ester mixtures on aquatic organisms based on environmental exposure profiles predicted by the Grid-Catchment Integrated Environmental Modeling System

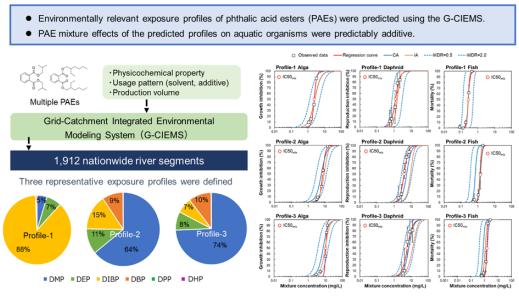
Yusuke ODA[1], Yoshitaka IMAIZUMI[1], Fujiko OZAWA[1], Haruna WATANABE[1], Takahiro YAMAGISHI[1], Kyoshiro HIKI[1,2], Koichi OHNO[1] and Hiroshi YAMAMOTO[1]*

[1] Health and Environmental Risk Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan
[2] Department of Urban Engineering, The University of Tokyo

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ABSTRACT

Phthalic acid esters (PAEs) are a group of compounds composed of structurally similar congeners with diverse physicochemical properties and can therefore form spatiotemporally heterogeneous mixtures in the ambient environment. Developing realistic exposure profiles of PAE mixtures, along with evaluating their combined effects on aquatic organisms, is important for effective ecological risk assessment. In this study, we first predicted the exposure profiles of 12 PAEs in rivers across Japan using the Grid-Catchment Integrated Environmental Modeling System. For shorter-chain PAEs (with alkyl side chains of C1–C6), three representative environmental profiles were selected based on their total concentrations and relative toxic potencies (expressed as dibutyl phthalate [DBP] equivalents), and subchronic ecotoxicity tests using these mixtures were conducted on three aquatic species: algae (*Raphidocelis subcapitata*), daphnids (*Ceriodaphnia dubia*), and fish (*Danio rerio*). In all tested profiles, DBP and diisobutyl phthalate together accounted for over 90% of the toxicity of the PAE mixtures. Regardless of differences in the tested profiles or test species, the observed combined effects of the mixtures generally fell within a factor of two of the predictions made by the concentration addition (CA) model. These results suggest that the combined effects of PAE mixtures can be reasonably predicted using the CA model across a range of mixing ratios. Further efforts to expand environmental monitoring and improve the accuracy of emission estimates for PAEs will be essential for reliably quantifying the ecological risks associated with their combined effects.



Key words: combined effect; phthalic acid esters; short-term chronic toxicity; environmentally relevant exposure profile; Grid-Catchment Integrated Environmental Modeling System

INTRODUCTION

Phthalic acid esters (PAEs) are widely used in a variety of applications, ranging from plastic materials, such as polyvinyl chloride and nitrocellulose, to numerous industrial products, including building materials, paints, adhesives, and food packaging, as well as household items, such as cosmetics, childcare products, and toys (Staples, 2003; Koniecki et al., 2011). PAEs are a group of structurally similar compounds that vary in alkyl chain length and include linear and branched isomers (Staples, 2003). Despite their structural similarities, PAEs exhibit various physicochemical properties, including water solubility, hydrophobicity, sorption behavior, and degradability (Staples, 2003). Given their diverse applications and physicochemical variability, PAEs are expected to occur in the environment as spatially and temporally heterogeneous mixtures, influenced by emission pathways and partitioning behaviors across environmental media.

Regarding the combined effects of multiple PAEs, cumulative risk assessments for human health have been conducted using the hazard index approach, in which exposure levels are divided by toxicity reference values and then summed (Benson, 2009; Kortenkamp and Koch, 2020). For example, Benson (2009) calculated the hazard index for six

* Corresponding Author: yamamoto.hiroshi@nies.go.jp

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PAEs with anti-androgenic activity by deriving adjusted reference doses that accounted for their relative potencies compared to an index compound—di(2-ethylhexyl) phthalate (DEHP)—based on toxicological data from animal studies. This approach is applicable when the combined effects follow the concentration addition (CA) model (e.g., Kortenkamp et al., 2009), assuming that the compounds share the same mode of action. CA-based assessments have advanced in evaluating the endocrine-disrupting effects of PAEs on human health (Benson, 2009; Kortenkamp and Koch, 2020). Several studies have examined the combined effects of PAEs in aquatic organisms, mainly focusing on endocrine-disrupting effects and molecular-level endpoints (e.g., Chen et al., 2019; Hamid et al., 2020). While these studies reported additive, antagonistic, or non-additive effects depending on the endpoints and exposure design, reliable datasets obtained from guideline-compliant standard tests using quantitative apical endpoints relevant to hazard characterization remain limited, thereby constraining their applicability to environmental risk assessment.

In our previous study, we conducted a series of short-term chronic toxicity tests using three aquatic organisms—green alga *Pseudokirchneriella subcapitata*, daphnid *Ceriodaphnia dubia*, and zebrafish *Danio rerio*—to evaluate the ecotoxicity profiles of 12 PAEs with alkyl chain lengths ranging from C1 to C11 (Oda et al., 2025). We found that shorter-chain PAEs (C1–C6) exhibited a monotonic increase in toxicity with the logarithm of the octanol-water partition coefficient (log K_{OW}), whereas no effects were observed for longer-chain PAEs (C8 and above) at concentrations below their aqueous solubility limits (Oda et al., 2025). Furthermore, for six shorter-chain PAEs with available 50% inhibition concentration (IC50) values, mixture toxicity was evaluated under equitoxic conditions based on toxic units (TUs), in which the concentration of each compound was divided by its individual IC50s. The observed combined effects agreed well with those predicted by the CA model (Oda et al., 2025). The similarity in the mode of action among dimethyl phthalate (DMP), diethyl phthalate (DEP), and dibutyl phthalate (DBP) stems from findings that the critical body residue values (expressed in mmol/kg lipid) required to induce acute toxicity in aquatic organisms were comparable to those of polycyclic aromatic hydrocarbons and ketones (Parkerton and Konkel, 2000), supporting the hypothesis that nonspecific narcosis following bioaccumulation may be the primary mode of action. Given the similarity in the mode of action and the observed additive effects, these shorter-chain PAEs can be classified as a single subgroup to which the CA model is applicable. Accordingly, relative potency factors (RPFs) for short-chain PAEs, representing their toxic potency relative to a reference compound (Meek et al., 2011), can be derived based on their linear relationship with log K_{OW}. By adjusting environmental exposure concentrations using these RPFs and summing them, this approach could be applied to the ecological risk assessment of PAE mixtures.

A limitation of our previous work is that the additivity of PAE mixture effects could not be ensured in cases where the individual PAE ratios within the mixtures differed. Spatial variation in concentrations occurs across sites in ambient aquatic environments, and the combined effects can vary depending on the relative proportions or toxic contributions of individual compounds (e.g., Sørensen et al., 2007). Therefore, evaluating the toxicity of PAE mixtures under environmentally relevant exposure profiles is essential; however, available monitoring data for PAEs are still limited. Exposure assessment simulation models can be employed to predict large-scale exposure profiles by estimating environmental emissions based on available information, such as usage patterns and physicochemical parameters.

In this study, we first focused on compiling environmental emissions of 12 PAEs (Table S1) based on physicochemical properties (Table S2), usage patterns, and production volumes. These emission estimates were then input into the Grid-Catchment Integrated Environmental Modeling System (Suzuki et al., 2004; Imaizumi et al., 2018), a high-resolution GIS-based multimedia environmental fate model developed by the National Institute for Environmental Studies (NIES) to predict concentrations in river water across Japan. For the shorter-chain PAEs (C1–C6), their RPFs were derived, and predicted environmental concentrations were adjusted accordingly to estimate site-specific exposure profiles that account for differences in toxicity. Finally, we defined three representative exposure scenarios reflecting distinct environmental profiles and experimentally evaluated the combined effects based on these three exposure scenarios using three aquatic test organisms. The overall schematic workflow of the present study is summarized in Fig. 1.

MATERIALS AND METHODS

PREDICTION OF PAE CONCENTRATION IN RIVER WATER USING G-CIEMS

To predict the environmental concentrations of PAEs, emission estimates were calculated based on use-specific shipment data and corresponding emission factors (Table S1). Shipment volumes were derived from the SPIN (Substances in Preparations in the Nordic countries) database for the years 2013–2017 (Nordic Council of Ministers [http://spin2000.net/], downloaded on 1 December 2021). PAEs were categorized into additive-type and solvent-type uses, and substance-specific emission factors were applied based on the Emission Factor Tables for Risk Assessment under the Chemical Substances Control Law ver. 4 (in Japanese, jointly prepared by the Ministry of Health, Labour and Welfare [MHLW]; Ministry of Economy, Trade and Industry [METI]; Ministry of Environment [MOE], 2018). Publicly available shipment volumes for DBP, DEHP, DINP, and DIDP in FY2020 were obtained from a report by the Japan Plasticizer Industry Association (JPIA, 2022). For substances without shipment data in SPIN (e.g., DPP, DHP), supplementary values were estimated from historical shipment records.

Spatial allocation of emissions was conducted using national population data from G-CIEMS ver.1.2. Physicochemical properties and degradation parameters (Table S2) were compiled from a combination of measured values (*Risk Assessment Report* issued by ECHA [https://echa.europa.eu/information-on-chemicals/information-from-existing-substances-regulation] and *Existing Chemicals Database* issued by OECD [https://hpvchemicals.oecd.org/ui/Default.aspx],

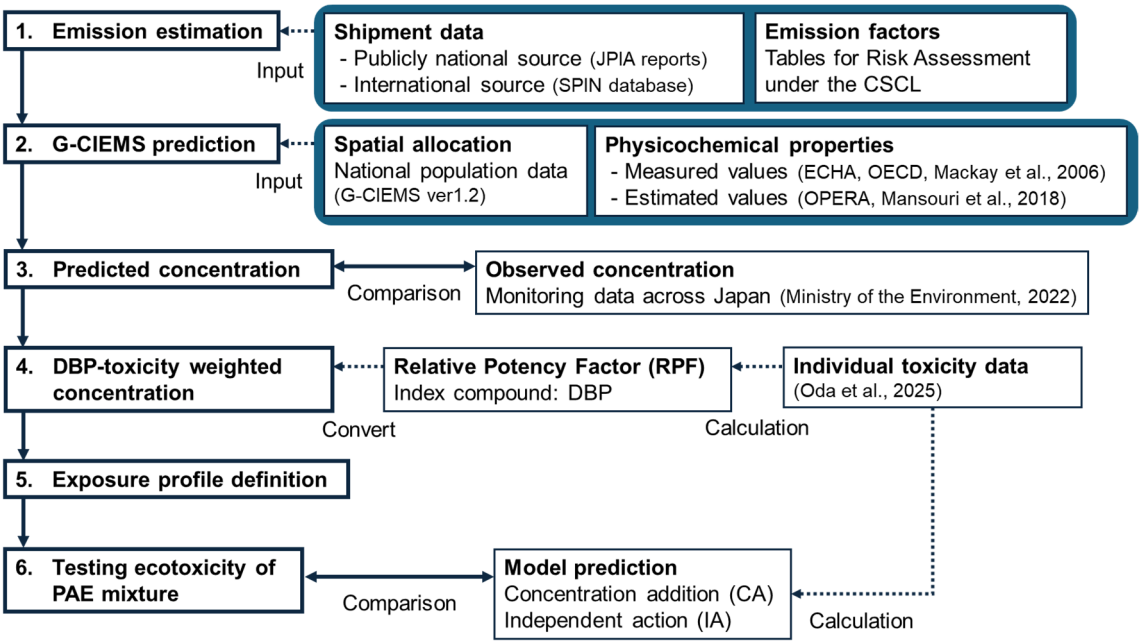


Fig. 1 Schematic workflow of this study

downloaded on 15 February 2023; Mackay et al., 2006) and model estimates by the Open (Quantitative) Structure–Activity/Property Relationship App (OPERA, Mansouri et al., 2018). If environmental fate parameters, such as degradation rates in air, water (rivers and lakes), soil, sediment, and wastewater treatment systems, were not available from the above-mentioned sources, they were assigned based on biodegradability classification and the corresponding degradation rates used in the Uniform System for the Evaluation of Substances (RIVM, VROM & VWS, 2002). Further details on physicochemical and degradation parameter derivation, and emission estimations are provided in the Supporting Materials.

The model predictions were validated by comparing the predicted concentrations of seven PAEs (DMP, DEP, DIBP, DBP, DEHP, DINP, and DIDP) in river water with the observed concentration data from monitoring surveys conducted at 23 sites across Japan in FY2020 (Ministry of the Environment, 2022). Table S3 presents the root mean squared log error, mean bias log error (MBLE), and mean absolute log error (MALE) as statistical metrics describing the model's predictive performance. The uncertainty in the predictions associated with use-specific emission factors was assessed through a scenario comparison with alternative predictions assuming that all PAEs were entirely used as additives (Fig. S1).

CALCULATION OF RPF AND DEFINITION OF EXPOSURE PROFILES

The RPFs were calculated following the WHO/IPCS framework (Meek et al., 2009), using a series of IC50 (50% inhibition concentration) values obtained for six short-chain PAEs (DMP, DEP, DBP, DIBP, DPP, and DHP) from our previous study (Oda et al., 2025). Within the group, DBP was selected as the index compound (RPF = 1.0) because DBP has the highest domestic production and import volume and is supported by a robust set of toxicity data. RPF values of each PAE were calculated for each of the three aquatic organisms by dividing the IC50 of DBP by the IC50 of each compound (Table S4).

The concentrations of the six shorter-chain PAEs predicted by G-CIEMS for all river sites were converted into DBP-toxicity-weighted concentrations by multiplying each by its respective RPF using these RPFs. Subsequently, the DBP-toxicity-weighted concentrations were summed across all PAEs for each organism. To identify representative exposure profiles, we calculated the average DBP-toxicity-weighted concentrations across all rivers (38,268 sites) and focused on the top 5% of sites (1,913 sites) with the highest values. The top 5% of sites were assumed to represent potentially high-risk areas, as they generally included regions with at least one-tenth of the maximum predicted concentration. These areas are inhabited by approximately 30% of Japan's total population, indicating that they are not limited to a few extremely high-concentration sites. Three representative exposure profiles were defined based on the following criteria:

Profile 1: Focusing on DIBP, which showed the highest average contribution to the total DBP-toxicity-weighted concentration among the six PAEs, the relative concentration ratios were calculated as the average of the top 10 sites with the highest DIBP concentrations.

Profile 2: The relative concentration ratios were calculated as the average of the top 5% (1,913 sites) with the highest total DBP-toxicity-weighted concentrations.

Profile 3: Focusing on DMP, which had the highest average predicted concentration among the six PAEs based on G-CIEMS output, the relative concentration ratios were calculated as the average of the top 10 sites with the highest DMP concentrations.

ECOTOXICITY TESTING OF PAE MIXTURES BASED ON EXPOSURE PROFILES

Three aquatic organisms were used: a green alga *Raphidocelis subcapitata*, the daphnid *Ceriodaphnia dubia*, and the zebrafish *Danio rerio*, all of which were maintained under continuous laboratory culture or breeding at NIES. Ecotoxicity testing of each organism was conducted in accordance with the OECD Test Guidelines and the protocols established by Environment Canada: the algal growth inhibition test (OECD, 2006), the *C. dubia* reproduction test (Environment Canada, 2010), and the fish short-term toxicity test on embryo and sac-fry stages (OECD, 1998). Detailed methods, including test strains and culturing conditions, were described in our previous study (Oda et al., 2025).

Concentrations of PAEs in test solutions were quantified using liquid chromatography–tandem mass spectrometry (LC-MS/MS, detailed analysis conditions are described in Oda et al., 2025). For the algal test, PAE concentrations were analyzed at the start and end of the 3-day exposure period, and geometric mean values were calculated. For the daphnid and zebrafish tests, water samples were collected at the start and at each water renewal (every 2 days) twice. Time-weighted average concentrations were then calculated based on these measurements. The measured concentrations of PAEs in the mixture tests are provided in Table S5.

Mixture toxicity tests focused on six PAEs with carbon chain lengths from C1 to C6. Fixed mixture ratios were prepared based on three exposure profiles defined for each test organism. Five concentration levels were tested, corresponding to total toxic unit values of 0.5, 0.7, 1.0, 1.4, and 2.0, with a geometric progression factor of 1.4. The total TU was defined as the sum of individual TUs, where each TU was calculated as the concentration of a compound divided by its respective IC50. Under the assumption of the CA model, a total TU of 1.0 is expected to result in a 50% inhibition effect. For DPP and DHP, when their toxic contributions were less than 0.1% (based on TU), with target concentrations falling below the quantification limits, and their overall contributions were considered negligible (<0.1%), they were excluded from the mixtures.

MODEL PREDICTION AND STATISTICAL ANALYSIS OF THE MIXTURE EFFECT

The observed effects obtained from the mixture toxicity tests were compared with the predicted effects calculated using the CA model, as well as those predicted by the independent action (IA) model, which assumes that each component acts independently, following the procedure described by Backhaus et al. (2000). The predicted effects were calculated according to the following procedure.

For the CA model, the total mixture concentration (ICx_{mix}) that produces an x% inhibitory effect (ranging from 1% to 99%) was calculated using Equation (1):

$$ICx_{mix} = \left(\sum_{i=1}^{n} \frac{P_i}{ICx_i} \right)^{-1} \quad \text{— (1)}$$

where P_i represents the fixed proportion of substance i in the mixture, and ICx_i denotes the concentration of substance i that produces an x% effect when tested individually. Under these conditions, the concentration c_i of substance i in the mixture can be expressed using Equation (2):

$$c_i = P_i ICx_i \quad \text{— (2)}$$

For the predicted effects based on the IA model, when an x% effect was assumed under the CA model, the calculation was performed by substituting c_i into Equation (3):

$$I(c_{\text{mix}}) = 1 - \prod_{i=1}^{n} \left[1 - I(c_i) \right] \quad \text{— (3)}$$

where $I(c_{mix})$ and $I(c_i)$ denote the inhibition (%) at c_{mix} and c_i, respectively. Based on the above calculations, concentration–response curves for both the CA and IA model predictions were generated for x values ranging from 1% to 99%.

The following statistical analyses were conducted using the free software R (version 4.1.3). Inhibitory concentrations (IC_x) of the mixtures and their 95% confidence intervals (CIs) were estimated by fitting the test data to the two-parameter log-logistic model shown in Equation (4) using the drc package (Ritz et al., 2016):

$$f(x) = \frac{1}{1 + \exp\left(b \left[\log(x) - \log(e) \right] \right)} \quad \text{— (4)}$$

where x represents the measured concentration, $f(x)$ is the relative effect at concentration x for each endpoint (expressed as the inhibition rate, with the control set as the upper limit), b is the slope of the concentration–response curve, and e is the inflection point (i.e., the concentration at which $f(x) = 50$).

To assess deviations from additive effects (i.e., CA) observed in the mixture tests, the model deviation ratio (MDR), as defined by Belden et al. (2007), was calculated by dividing $IC50_{mix,CA}$ by the observed IC50. An MDR between 0.5 and 2.0 was interpreted as indicating no marked deviation from the CA prediction.

RESULTS AND DISCUSSION

COMPARISON OF G-CIEMS PREDICTED AND OBSERVED CONCENTRATIONS OF PAES IN RIVER WATER

The predicted concentrations of PAEs in river water by G-CIEMS were compared with observed concentrations at monitoring sites where the survey (Ministry of the Environment, 2022) was conducted (Fig. 2). While relatively small error metrics were obtained for DMP and DEP, larger error values were observed for DBP (Table S3). MBLE showed both positive and negative values across substances, indicating that the predicted concentrations were not consistently biased toward underestimation or overestimation (Table S3). When predictions within a factor of 1/10 to 10 of the measured values are considered reasonable, the proportions of sites falling within this range were 80% (4 of 5 sites) for DMP, 75% (3 of 4 sites) for DEP, 50% (1 of 2 sites) for DIBP, and 50% (3 of 6 sites) for DIDP, indicating that the predictions were generally reasonable. In contrast, all predicted concentrations for DBP were underestimated (i.e., less than one-tenth of the observed concentrations). For DEHP

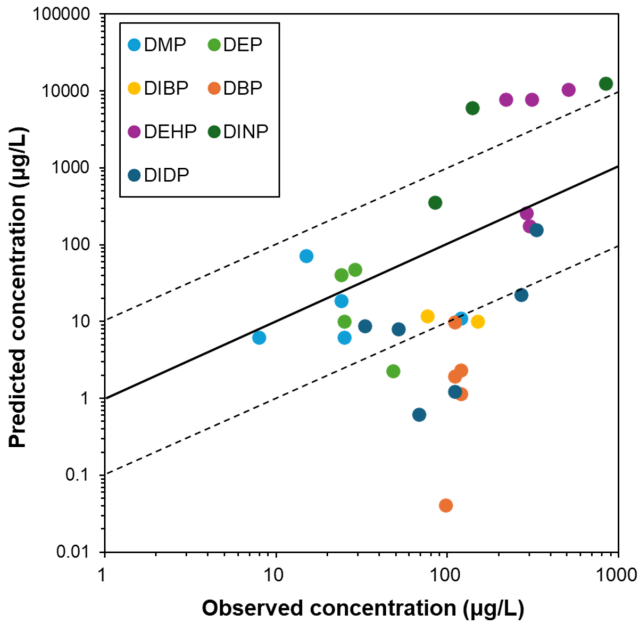


Fig. 2 Comparison of observed and G-CIEMS-predicted concentrations of seven PAEs. The PAEs include dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), di-(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP), and diisodecyl phthalate (DIDP). The solid line represents the 1:1 relationship, whereas the dashed lines indicate a deviation within a factor of 10

and DINP, only 40% (2 out of 5 sites) and 33% (1 out of 3 sites), respectively, fell within the acceptable range, with the remaining sites showing overestimations.

Focusing on longer-chain PAEs (C8 and above), only DIDP exhibited a distinct trend by being consistently underestimated. Although DEHP tends to have a higher vapor pressure and a lower logK_{OW} compared to the other two substances, DINP and DIDP share relatively similar physicochemical properties (Table S2). Therefore, it is unlikely that these properties alone account for the underestimation of DIDP. To explore potential factors affecting the accuracy of emission estimates, we examined the assumed use categories, assigned emission factors, and temporal trends in usage. Regarding use categories, DEHP, DINP, and DIDP were assumed to be used solely as additives (Table S1). For DINP and DIDP, this assumption is supported by NITE-CHRIP use information (in Japanese, https://www.chem-info.nite.go.jp/chem/chrip/chrip_search/systemTop, accessed 9 April 2025), which lists only plasticizer applications. Although DEHP is reported to have additional uses, its shipment volume reported by the Japan Plasticizer Industry Association (JPIA, 2022) is roughly consistent with the production/import quantities reported under the Chemical Substances Control Law (J-CHECK issued by NITE, https://www.nite.go.jp/chem/jcheck/detail.action?request_locale=en&cno=117-81-7&mno=3-1307, accessed 9 April 2025), supporting the validity of the assumption.

As for emission factors, those used for the long-term use stage, as referenced from the CSCL guidelines (in Japanese, MHLW, METI, and MOE, 2018), were calculated using consistent assumptions across substances and were independent of physicochemical properties. However, the emission factors for stages other than long-term use vary with the physicochemical properties. Since these properties likely influence the release rate of plasticizers from products to the environment, the emission factors assigned to longer-chain PAEs may have been overestimated—potentially contributing to the overprediction of DEHP and DINP.

Regarding temporal changes in usage, emissions from long-life products, such as plastics, should ideally be estimated considering product lifespan. However, for simplicity, this study estimated emissions using annual shipment volumes. According to published data from JPIA (2022), shipment volumes from FY2010 to FY2020 changed as follows: DEHP decreased from 142,000 to 88,000 tons, DINP increased from 74,000 to 90,000 tons, and DIDP declined from 4,800 to 1,600 tons. Among them, DIDP showed the most significant decrease in use. This discrepancy between current emissions and historical usage may explain why DIDP was underestimated, unlike DEHP and DINP.

The notably underestimated DBP was assumed to be used primarily in solvent-type applications (86%). However, if the proportion of use as a plasticizer were higher, the emission factor to the aquatic environment would increase severalfold—for instance, assuming 100% use as a plasticizer would result in a 6.7-fold increase (Fig. S1). This adjustment would improve the prediction accuracy, bringing it closer to that of other PAEs, and suggests that the assumed use distribution may have deviated from actual usage. In contrast, for other PAEs, the predicted concentrations were largely insensitive to changes in use-specific emission factors (Fig. S1). Although DEP exhibits a marked difference in emission factors between solvent- and additive-type uses, the predicted concentrations showed only minor changes between the scenarios. This might be explained by atmospheric transport followed by deposition to surface waters being the dominant process for DEP, with direct emissions to the aquatic environment contributing only marginally to the overall riverine concentrations.

The predicted PAE concentrations in river water, which incorporated detailed use information from sources such as SPIN (although based on data from outside Japan), demonstrated a certain degree of validity. However, the results indicate the need for more refined assumptions regarding use categories, emission factors, and product lifespans for some substances. Given the limited availability of monitoring data on PAEs in aquatic environments, future efforts should focus on expanding both the dataset of measured concentrations and the underlying information used for emission estimation.

PREDICTED ENVIRONMENTAL EXPOSURE PROFILES OF SHORTER-CHAIN PAEs

Figure 3 shows the three selected exposure profiles, including the predicted concentrations of shorter-chain PAEs calculated by G-CIEMS and the relative proportions recalculated as DBP-toxicity-weighted concentrations for each test species.

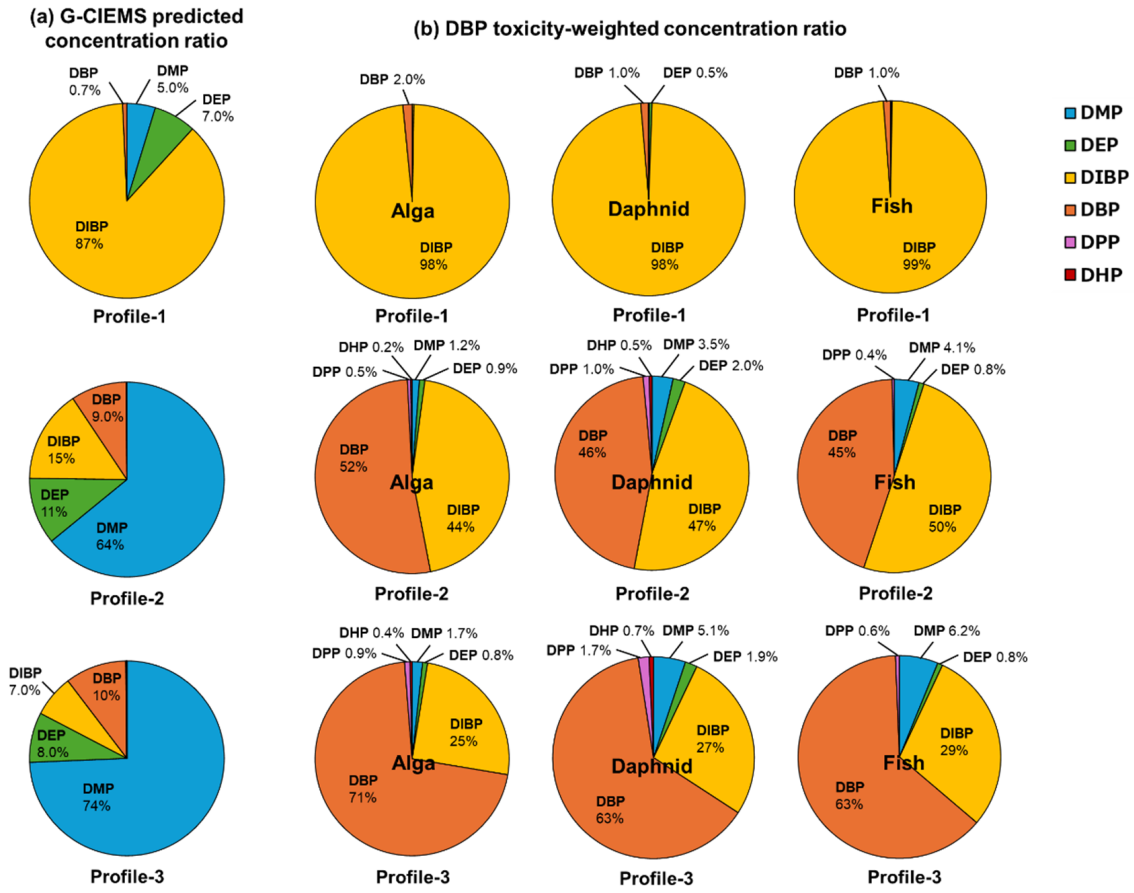


Fig. 3 Relative proportions of shorter-chain PAEs (with alkyl side chains of C1 to C6) in the three selected exposure profiles: (a) concentrations and (b) DBP-equivalent concentrations reflecting toxic potency to alga, daphnid, and fish

Profile 1 was characterized by the predominance of DIBP, as defined on the basis of DBP-toxicity-weighted concentration. In terms of predicted concentration, DIBP accounted for 87% of the total, followed by DMP (7.0%) and DEP (5.0%), whereas DBP represented only 0.7%. When expressed as DBP-toxicity-weighted concentrations, DIBP represented more than 98% across all species, DBP accounted for approximately 1.0%–2.0%, and the contributions of DMP and DEP became < 0.5%.

Profile 2, defined as the average of the top 5% of sites with the highest total DBP-toxicity-weighted concentrations. Regarding predicted concentrations, DMP emerged as the predominant component (64%), followed by DIBP (15%), DEP (11%), and DBP (9.0%). When the predicted concentrations were expressed as DBP-toxicity-weighted concentrations, DBP and DIBP together represented more than 93% across all species, with each contributing approximately 44–52%. DMP ranked next, but their relative contributions were 1.2% in algae, 3.5% in daphnids, and 4.1% in fish.

Profile-3 focused on DMP, which had the highest average predicted concentration across all river sites. In this profile, DMP accounted for 74% of the predicted concentration, followed by DBP (10%), DEP (8.0%), and DIBP (7.0%). In terms of DBP-toxicity-weighted concentrations, DBP and DIBP together comprised more than 90% across all species. Notably, DBP contributed (63%–71%) more than DIBP (25%–29%), in clear contrast to Profile-2. The contribution of DMP was modest, at 1.7% in algae, 5.1% in daphnids, and 6.2% in fish, a pattern similar to that observed in Profile-2.

Overall, while DMP and DEP appeared dominant in terms of predicted concentrations, DBP and DIBP together accounted for over 90% of the total when expressed as DBP-toxicity weighted concentrations, indicating that exposure profiles differed markedly between the two metrics. Although DPP and DHP, which are longer-chain PAEs than DBP and DIBP and relatively more toxic to algae and daphnids, were included in the analysis, their predicted concentrations were 1/1,000 to 1/10 that of other PAEs, and their contributions remained below 1.0% even when adjusted using RPFs.

These findings suggest that DBP and DIBP are the primary contributors to the toxicity of shorter-chain PAE mixtures in the environment. Additionally, the contribution ratios between DBP and DIBP varied notably across exposure profiles. Profile-2 represents locations with relatively higher ecological risk from exposure to the mixture, where DBP and DIBP contributed nearly equally.

MIXTURE EFFECTS OF SHORTER-CHAIN PAES BASED ON EXPOSURE PROFILES

The results of the PAE mixture toxicity tests obtained under each exposure profile or for each test organism were compared with the concentration–response curves predicted by the CA and IA models (Fig. 4). The x-axis in Fig. 4 (mix-

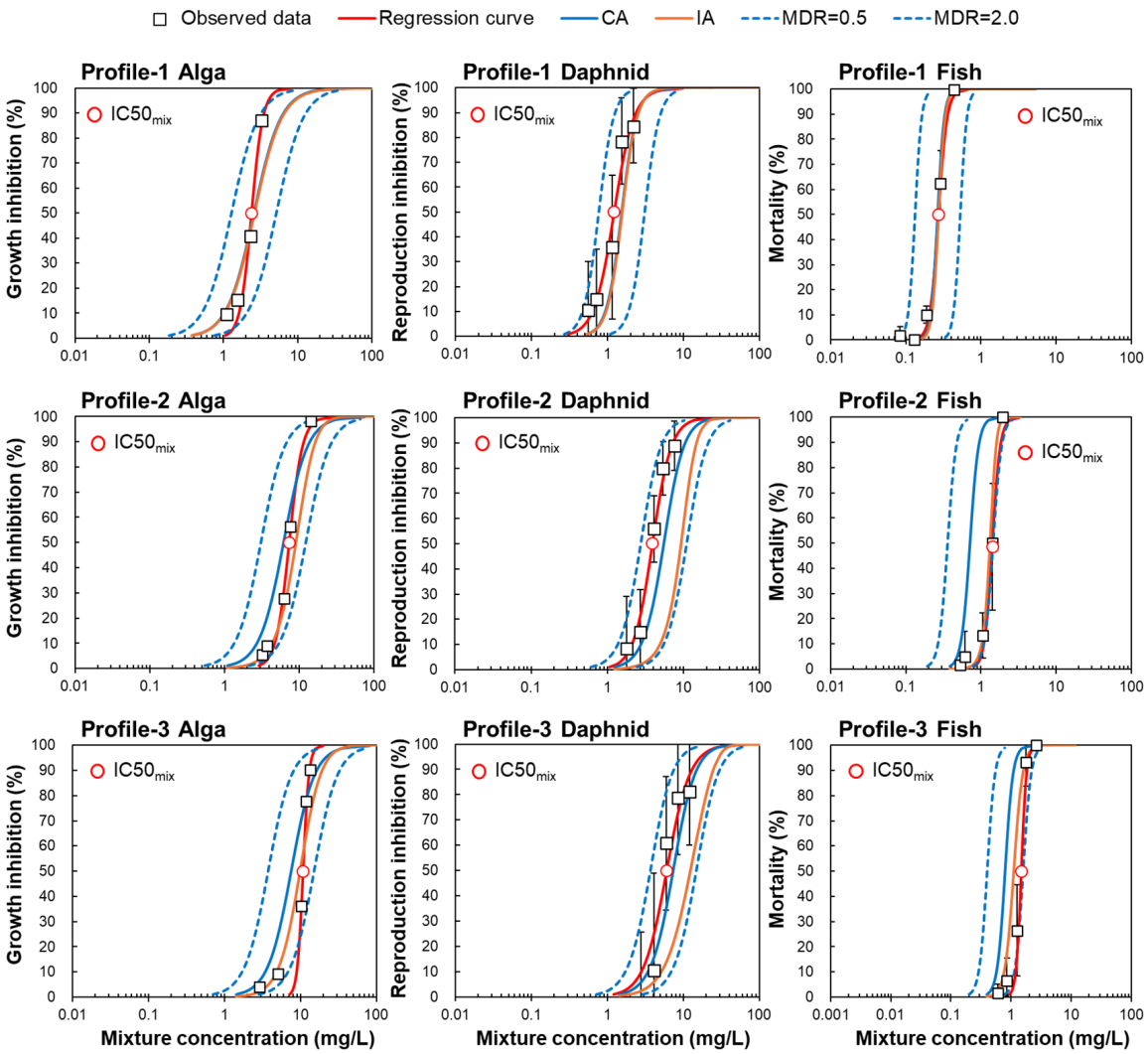


Fig. 4 Concentration–response curves of PAE mixtures for different exposure profiles by test organisms. Regression curves based on observed data (red line with square markers) are compared with predictions from the CA; blue solid line) and IA (orange line) models for each organism. The blue dotted lines indicate where the model deviation ratio is equal to 0.5 or 2.0

ture concentration) represents the total concentration of all component PAEs in the mixture. The experimentally derived IC50 values were compared with the predicted concentrations causing 50% inhibition under the CA model ($IC50_{mix,CA}$) and the IA model ($IC50_{mix,IA}$), and the corresponding MDRs are summarized in Table 1.

In Profile 1, where DIBP accounted for >98% of the predicted mixture toxicity, the predicted concentration–response curves based on both the CA and IA models were nearly identical across all test organisms (Fig. 4). Although the observed effects for algae and daphnids showed slight deviations from the predicted curves, the MDRs were close to 1.0 (Table 1). In Profile 2, where DBP and DIBP each contributed approximately 44%–52% of the overall toxicity, the mixture effects predicted by the CA model were 1.5–2.0 times higher than those from the IA model (Fig. 4). The MDRs for algae and daphnids were 0.837 and 1.42, respectively, whereas the MDR for fish was 0.508, indicating that effects were observed at concentrations nearly twice those predicted by CA (Table 1). The observed concentration–response curve for fish closely matched the IA-predicted curve (Fig. 4), suggesting possible independent or even antagonistic interactions between DBP and DIBP. In Profile 3, where the combined contribution of DBP and DIBP exceeded 90% but the contribution of DBP was more than twice that of DIBP, the CA-predicted effects were approximately 1.3–1.7 times higher than those from IA (Fig. 4). The MDRs were 0.699, 1.22, and 0.531 for algae, daphnids, and 0.531 for fish, indicating a rightward shift (i.e., higher observed concentrations than predicted) for algae and fish (Table 1). In fish, as observed in Profile-2, the mixture effects were approximately half of those predicted by the CA model and slightly lower than those predicted by the IA model.

Our previous study has shown that mixtures of shorter-chain PAEs prepared under equitoxic conditions yield results that closely align with predictions from CA models (Oda et al., 2025). Consistent with these findings, all MDRs in the present study fell within the range of 0.5–2.0, indicating that

Table 1 Comparison of IC50 values among observed data with 95% confidence intervals (CIs), data predicted by additive models (CA and IA), and MDR

Mixture	Organisms	IC50 value [95% confidence intervals] (mg/L)			MDR
		Observed data	CA	IA	
Profile-1	Alga	2.38 [2.22–2.55]	2.57	2.62	1.08
	Daphnid	1.21 [1.13–1.30]	1.54	1.56	1.27
	Fish	0.273 [0.263–0.283]	0.264	0.271	0.967
Profile-2	Alga	7.36 [7.16–7.56]	6.16	9.06	0.837
	Daphnid	3.89 [3.64–4.14]	5.52	9.24	1.42
	Fish	1.42 [1.34–1.50]	0.721	1.33	0.508
Profile-3	Alga	10.8 [10.6–11.0]	7.55	9.85	0.699
	Daphnid	6.05 [5.61–6.48]	7.41	12.5	1.22
	Fish	1.50 [1.43–1.57]	0.796	1.11	0.531

the mixture effects of shorter-chain PAEs under environmental-relevant concentrations did not markedly deviate from CA predictions, regardless of the mixing ratio. Belden et al. (2007) analyzed data from 207 mixture toxicity studies involving CA predictions and found that 88% of MDR values fell within the 0.5–2.0 range, with a central value around 1.0. They proposed using the MDR as an uncertainty factor to account for probabilistic variabilities inherent in mixture effects, such as variability in experimental designs, procedures, and toxicity value estimations. The European Food Safety Authority (EFSA, 2019) also recommends the use of the MDR for the identification and characterization of chemical mixtures. For example, it considers an MDR value exceeding 10 to indicate potential concern for synergy and states that the MDR can be applied as a mixture adjustment factor (EFSA, 2019). However, the MDR threshold remains arbitrarily determined, and a statistical testing approach that considers the distribution of MDR statistics based on probabilistic simulations has also been proposed (Macacu and Guillot, 2020).

We also observed that mixture effects in fish tended to follow IA or even antagonistic patterns. This finding is important from the perspective of toxicological mechanisms. In all exposure profiles tested, DBP and DIBP together accounted for over 90% of the TUs, raising the possibility that these two compounds may exert different modes of action in fish or interact antagonistically. Numerous studies have reported the endocrine-disrupting effects of DBP and DIBP in fish, including suppressed gonadal development (Fairbairn et al., 2012), craniofacial malformations (Jergensen et al., 2019), and decreased gonadosomatic index and plasma estradiol (E2) levels (Chen et al., 2019). For instance, Chen et al. (2019) showed that while individual exposures to DBP (1.13 mg/L) and DIBP (1.04 mg/L) significantly reduced the gonadosomatic index and plasma E2 levels in zebrafish, combined exposure at these concentrations resulted in no observable effects. This indicates an antagonistic interaction rather than simple IA. If these compounds act as estrogens, competitive binding at estrogen receptors could result in such antagonism. Molecular docking simulations have also confirmed DBP's affinity for zebrafish estrogen receptors α and β (Hamid et al., 2020). Although short-term early life stage exposure in TG 212 primarily evaluates integrated apical endpoints such as hatching success and survival, disturbances in estrogen receptor-mediated regulation can also induce developmental toxicity during the early life stages of zebrafish, thereby increasing the risks of malformations and mortality (e.g., Santos et al., 2014). When such receptor-mediated specific effects are considered, it should also be noted that environmentally relevant exposure profiles—and the dominant PAEs contributing to mixture effects—may differ from those represented in the present study. Future studies should investigate the interactions between DBP and DIBP in detail using approaches that consider concentration and mixing ratios, such as isobolome analysis (Sørensen et al., 2007).

A population- and community-level risk assessment of PAE mixtures represents an important next step. Individual test data encompass endpoints such as survival, growth, and reproduction, and their translation into population growth rates would enable predictions of mixture effects at the population level. The species sensitivity distribution approach provides a practical framework for extrapolating mixture effects to broader biological scales (e.g., Posthuma et al., 2019). In community-level risk assessment, however, predictions of mixture effects based solely on individual test datasets still face substantial limitations, as biological interactions among organisms must be considered. Practical validation using experimental ecosystems (e.g., mesocosm approaches), together with evaluations of SSD-based outcomes for multiple chemicals (e.g., Knauer and Hommen, 2013), remains therefore necessary.

CONCLUSION

In this study, we characterized the environmental exposure profiles of PAEs using the GIS-based multimedia environmental fate model G-CIEMS and further derived DBP-equivalent, ecotoxicity-weighted exposure profiles from these predictions. The PAE concentrations predicted by G-CIEMS were generally within one order of magnitude of the observed concentrations; however, DBP tended to be underestimated, highlighting the need to refine assumptions regarding use categories, emission factors, and product lifespans. Among the three defined exposure profiles, DBP

and DIBP accounted for over 90% of the predicted mixture toxicity. Mixture toxicity tests using three aquatic organisms revealed combined effects within a factor of two of the CA predictions. These findings support the applicability of the CA model to multi-component PAE mixtures regardless of the mixing ratio and suggest that cumulative ecological risk assessment of PAE mixtures can be reasonably conducted using the total DBP-toxicity-weighted concentrations.

The workflow for cumulative ecological risk assessment proposed in this study (Fig. 1) is expected to be applicable to emerging contaminants for which environmental detections are still increasing, such as pharmaceuticals and personal care products and per- and polyfluoroalkyl substances (PFAS). Such applications require systematic organization of available information on their uses and physicochemical properties, as well as the accumulation of robust ecotoxicological datasets that ensure the similarity of modes of action.

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DISCLAIMER

The authors employed ChatGPT during manuscript preparation to refine language and readability. The authors carefully checked and edited the text afterward and bear sole responsibility for the published content.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

Text S1, Detailed methods for emission estimation and environmental fate parameters; Figure S1, Ratio of the predicted 99.9th percentile concentrations to the maximum observed concentration under different use scenarios. Scenario 1: predictions based on weighted averages using use-specific shipment volumes from SPIN; Scenario 2: predictions based on emission factors assuming all uses as plasticizers; Table S1, The use proportions of phthalic acid esters (PAEs) by different application categories (solvents and additives), and the weighted average emission factors by different environmental media (air and aquatic environment) calculated based on these proportions; Table S2, Summary of physicochemical properties of phthalic acid esters (PAEs) used in model estimation; Table S3, Statistical metrics for the predictive performance of G-CIEMS; Table S4, Relative potency factors (RPFs) of six PAEs calculated for alga, daphnid, and fish; Table S5, Measured concentrations of the shorter-chain six phthalic acid esters (PAEs) (C1–C6) in different test organisms (alga, daphnid, and fish) and mixtures (Profile 1, 2, 3) at start and end/water renewal and recovery rate.

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ORCID

Hiroshi YAMAMOTO
https://orcid.org/0000-0003-3865-8708
Yusuke ODA
https://orcid.org/0000-0002-6555-1335
Yoshitaka IMAIZUMI
https://orcid.org/0000-0002-2697-5303
Haruna WATANABE
https://orcid.org/0000-0001-5025-1957
Takahiro YAMAGISHI
https://orcid.org/0000-0002-6903-0748
Koichi OHNO
https://orcid.org/0000-0002-4816-5678
Kyoshiro HIKI
https://orcid.org/0000-0001-7898-9486

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