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## Emerging investigator series: unpacking PET: comparative analysis of leachable and extractable contaminants from virgin and recycled polyethylene terephthalate bottles and textiles

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Plastics, particularly polyethylene terephthalate (PET), are widely used as food contact materials, textiles, and toys. However, their widespread use and potential for human exposure raise environmental and health concerns, particularly regarding the leaching of chemical additives. This study assessed hazardous plastic additives and non-intentionally added substances (NIAS) leached from paired virgin and recycled PET bottles (soda and water) purchased from Michigan and California and from textiles (toys, pillows, and clothing) acquired online or in stores in Michigan and Oregon. Results showed differences in contaminant profiles and concentrations between PET types and products. A total of 12 persistent, mobile, and toxic (PMT) additives, six organophosphate esters (OPEs), and 15 NIAS were detected. Notably, recycled PET (rPET) bottles consistently contained benzene, while virgin PET had higher ethylene glycol and 2-methyl-1,3-dioxolane levels. Additionally, OPEs were detected more frequently in rPET, indicating recycling as a contamination pathway. Geographically distinct contaminant profiles were evident, with Michigan bottles exhibiting elevated benzaldehyde, while California bottles showed higher diethylene glycol levels, suggesting differing manufacturing practices. Textiles exhibited distinct contamination profiles, highlighting a distinct exposure pathway for watersheds through laundry processes. Bioactivity assays with PET product extracts revealed moderate to high hormone receptor antagonism but no clear association with PET type, indicating potential hazardous effects from both virgin and recycled PET products. This study highlights the necessity of continued monitoring of contaminants in PET, including non-intentionally added substances and PMT plastic additives that are not currently regulated.

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### Environmental significance

Our findings demonstrate the pervasive presence of numerous hazardous compounds, including plasticizers and flame retardants, in virgin and recycled PET bottles and textiles. The detection of these substances, particularly their leaching under environmentally relevant conditions and their observed endocrine-disrupting activity, indicates a potential environmental and human exposure risk. This research emphasizes that current practices in plastic additive use led to complex chemical mixtures with potential adverse impacts on water quality, sensitive ecosystems, and vulnerable populations, necessitating a re-evaluation of material safety and recycling processes.

## 1 Introduction

Plastics have become integral to modern life and industry; however, their widespread use has raised increasing concerns regarding the environmental impact of plastic waste on

ecosystems and human health.<sup>1,2</sup> Approximately 33 billion tons of plastic are estimated to be produced by 2050, with most expected to end up in landfills or directly as litter in the environment.<sup>3</sup> To mitigate plastic waste accumulation, substantial reduction in plastic use and increases in recycling rates are urgently required.<sup>1</sup> To date, approximately 10% of the produced plastics are recycled, leading to increasing waste management and environmental pollution challenges.<sup>4,5</sup> The majority of plastic consumption occurs in packaging applications, with polyethylene terephthalate (PET) particularly prevalent due to desirable properties such as transparency, durability, light weight, and affordability.<sup>6-8</sup>

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Encouragingly, PET has some of the highest recycling rates and expected recyclability among plastics.<sup>9</sup> Used frequently in bottles, advancements in bottle return systems and bottle-to-bottle recycling have helped drive these above-average recycling rates for PET.<sup>6</sup> In addition, regulatory measures, such as the Break Free from Plastic Pollution Act (U.S.)<sup>10</sup> have mandated increasing recycled content in PET products.<sup>11</sup> While these are positive developments from a waste and climate impact perspective, concerns remain that recycling could introduce additional potentially hazardous substances including plasticizers, monomers, and degradation by-products, into recycled PET products.<sup>12–14</sup>

To understand potential risks associated with the use of recycled PET products it is essential to understand whether (1) recycled PET contains more or different hazardous contaminants than virgin PET, and (2) whether these contaminants can leach out of the PET into *e.g.* beverages contained in the bottles, or the environment.

Previous investigations into chemical contaminants in PET products have typically either looked at total chemical content (*i.e.* extractable substances) or those that are leachable in the environment, with fewer studies applying both approaches concurrently (Table SA1 and 2, SI A). Moreover, previous studies have mostly focussed on specific groups of contaminants that are detectable with one analytical technique rather than complex contaminant mixtures; let alone the mixture toxicities. Lastly, there is a lack of studies comparing contaminant profiles between virgin and recycled PET.

In this study, we aimed to address these knowledge gaps by evaluating known hazardous plastic additives, non-intentionally added substances (NIAS), and endocrine disruptive properties of the contaminants mixtures leached from paired virgin and recycled PET products including bottles (soda and water) and textiles (plush toys, pillowcases, and children's clothing) purchased in the United States.

## 2 Methods and materials

### 2.1 Sample collection

PET samples included 18 bottles (14 water and 4 soda bottles) purchased from stores in California and Michigan and 8 children's textiles (2 plush toys, 2 pillows, and 4 clothing items) purchased online or from stores in Michigan and Oregon. Half of the samples were recycled PET and half were virgin PET (based on marketing claims), with each recycled PET sample paired to an equivalent virgin PET product. Among recycled PET samples, 69% bore a '100% recycled' label, whereas the remainder offered no indication of recycled-content percentage. Details on the sample preparation are summarised in the SI A (Text SA1).

### 2.2 Extraction and leaching

To understand the mixture of potential contaminants associated with these analysed products, a diverse set of known hazardous plastic additives were analysed, including 124 Persistent, Mobile, and Toxic (PMT) plastic additives, 23 per- and polyfluoroalkyl

substances (PFAS), and 26 organophosphate esters (OPEs) (SI B, Table SB1–3). Fig. 1 illustrate the workflow for leaching and extraction studies on both bottles and textile PET products. Leaching was conducted following a previously published method.<sup>15</sup> Briefly, samples were leached for 34 days on an orbital shaker set to 30 rpm, under a full-spectrum UV light source simulating a day–night cycle (14 hours on, 10 hours off), using filtered lake water collected from Lake Ontario in Toronto, Ontario, Canada (43.6353360, –79.3224297). Samples were collected on days 0, 2, 15, and 34. All samples except children's clothing items ( $N = 22$ ) were leached and analysed for OPEs and PMT plastic additives using high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (HPLC-QToF-MS). Bottles ( $N = 18$ ) were additionally tested for PFAS by HPLC-QToF-MS. A detailed description of the analytical and instrumental methods is presented in the SI-A (Text SA2 and Table SA3).

20 PET-associated NIAS categorised as glycols, aldehydes, aromatic hydrocarbons, and volatile organic compounds (Table SB4) were analysed by an external laboratory<sup>‡</sup> from the plastic samples ( $N = 26$ ). All samples were analysed for NIAS using gas-chromatography coupled with mass spectrometry (GC-MS). Depending on the target analytes, samples were extracted with either tetrahydrofuran or water, while some NIAS were analysed by headspace GC-MS without prior solvent extraction (Text SA3).

Element analysis of antimony was conducted using X-ray fluorescence spectroscopy on both bottles ( $N = 18$ ) and textile products ( $N = 6$ ) (Text SA4).

For the evaluation of endocrine disruptive effects, the bottles and textile products were solvent extracted and screened for nuclear receptor bioactivities using transient transfection luciferase reporter gene assays for disruption of estrogen receptor alpha, androgen receptor, thyroid receptor beta, and peroxisome proliferator activated receptor gamma. Protocols for extraction and toxicity testing are detailed in the SI (Text SA5).

### 2.3 QA/QC

Several measures were taken to avoid contamination of samples with plastic-associated chemicals originating from the laboratory environment and sample preparation. For PMT and OPE samples, acid-washed and baked glassware, and solvent-cleaned glass syringes were used for all sample preparation and collection. For PFAS samples, plastic labware, sample vials, and pipettes were used to avoid the adsorption of these compounds onto glass. Appropriate lake water blanks, method blanks, and sample duplicates were prepared for leachate samples to account for contaminants originating from the matrix or other spurious signals. The limit of detection (LOD) and limit of quantification were calculated as the average blank + 3 times the standard deviation and the average blank + 10 times the standard deviation, respectively. For compounds without detectable blanks, the LOD and LOQ were determined based on a signal-to-noise ratio of 3 and 10, respectively.

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Fig. 1 Workflow for virgin and recycled PET bottles and textiles for leaching and extraction experiments. Bottles were obtained from two states of US: Michigan (MI) and California (CA).  $N$  = the number of samples. Each virgin PET samples paired to an equivalent virgin PET product.

All leaching samples were spiked with appropriate internal standards (Table SA4) to account for variations in sample volume and instrument performance. NIAS analyses were conducted in the external laboratory with accredited methods. All data was blank and recovery corrected.

#### 2.4 Data analysis

Suspect screening for PMT and quantitation for targeted OPEs and PFAS were performed on the full MS scan data using Agilent Technologies MassHunter Quantitative Analysis 10.0, and R (version 4.3.0; R Core Team, 2024) using RStudio (version 2024.04.0 + 735) (details see Text SA6).

The contaminant profiles were evaluated across multiple factors, including PET material source (virgin *versus* recycled), intended product usage (bottles *versus* textile), beverage type, and geographic origin. Statistical tests ( $\alpha = 0.05$ ) were performed to identify any statistically significant difference between those factors above-mentioned (details see Text SA7).

## 3 Results and discussion

For leaching analyses, blanks were detectable in relative peak areas (RPA, defined as the ratio of an analyte's peak area to that of

the internal standard whose retention time is most similar) or concentrations between  $1.12 \times 10^{-2}$  RPA for tris(2-butoxyethyl) phosphate (TBEP) and 7.68 RPA for 1,1'-oxybis[2-methoxyethane] or  $1.82 \text{ ng mL}^{-1}$  (of lake water) for triethyl phosphate (TEP). The suspect screening LODs and LOQs were between  $0.40 \times 10^{-2}$  and  $1.34 \times 10^{-2}$  RPA  $\text{g}^{-1}$  for  $N,N'$ -diphenyl-guanidine and 0.83 and  $2.77 \text{ RPA g}^{-1}$  for 1,1'-oxybis[2-methoxy-ethane]; as well as the quantitative LODs and LOQs were between 0.04 and 0.12  $\text{mg kg}^{-1}$  for TEP and 6.96 and  $0.95 \text{ mg kg}^{-1}$  for 2-isopropylphenyl diphenyl phosphate and 3-isopropylphenyl diphenyl phosphate (2IPDP and 3IPDP), respectively.  $R^2$  values ranged from 0.879 (3IPDP + 4IPDP) to 0.997 (TEP) (median = 0.919) for the 26 targeted OPEs. The linear range for all OPEs was 0 to  $20 \text{ ng mL}^{-1}$ , except for TDCPP, which had a linear range of 2 to  $20 \text{ ng mL}^{-1}$ . Relative standard deviations (RSDs) of the internal standards for OPEs standards and internal standards for PMT plastic additives ranged from 7.0% to 17.5% ( $n = 192$ ). Full method performance details are summarised in Tables SB6 and SB7 in the SI B.

#### 3.1 Identification and characterization of contaminants from PET products

**3.1.1 Detected contaminants.** Across the 26 tested products, twelve PMT plastic additives and five OPEs were detected



in the water leachates, while fifteen NIAS were detected in the solvent extracts of the plastic samples (Table 1). None of the targeted PFAS were detected in the analysed leachates. Detection frequencies ranged from one product (4%) for ethylbenzene and xylene to 25 products (96%) for diethylene glycol (Table 1). OPEs were detected in less than half of the samples with concentrations below the LOQ, indicating that they were not intentionally added to the plastics (Table 1). Exception was TEP that was detectable in concentration at  $0.18 \text{ mg kg}^{-1}$  in a recycled bottle. Amongst NIAS, diethylene glycol and ethylene glycol were most frequently detected (96% and 69%, respectively,  $N = 26$ ), as well as detected in the highest concentrations with up to  $63 \text{ mg kg}^{-1}$  and up to  $15 \text{ mg kg}^{-1}$ , respectively (Table 1). Ethylene glycol is a primary monomer used in PET polymerisation, while diethylene glycol can be either a comonomer introduced to increase flexibility of PET, particular for production of blow-moulded objects such as bottles,<sup>16</sup> or a byproduct of PET production.<sup>17</sup> The high detection of ethylene glycol and diethylene glycol were consistent with previous studies who reported ethylene glycol concentration up to  $6 \text{ mg kg}^{-1}$  in recycled PET bottles from the Netherlands.<sup>18</sup> Similarly, 2-methyl-1,3-dioxolane was found in 62% of the samples with concentrations similar to previously published amounts in recycled PET bottles of between 1 and  $2 \text{ mg kg}^{-1}$  (Table 1).<sup>18</sup>

PMT plastic additives were detected in 91% sample leachates (Table SB5). Melamine was the most frequently detected PMT additive with a detection frequency of 32%. Melamine is commonly used as a non-halogenated flame retardant that can be used as either surface treatment or as fibres in textiles.<sup>19,20</sup> It was recently designated as a substance of very high concern under the EU REACH regulation as a suspected human carcinogen, endocrine disruptor, and potential reproductive toxin.<sup>21</sup> The second most frequently detected plastic additives were benzophenone (diphenyl-methanone) and the PMT OPE trichloropropyl phosphate (TCPP, 3 isomers), both of which were found in 27% of the analysed samples (Table 1). Benzophenone is a UV stabilizer that is used in packaging to prevent photodegradation.<sup>22</sup> It is unclear as to whether recycling may introduce benzophenone contamination, as it has been previously reported in both recycled and virgin PET food packaging.<sup>23</sup> Likewise, the presence of TCPP raised questions about the potential contamination source. Previous studies have shown that flame retardants, like TCPP, can end up in recycled food contact materials due to a lack of oversight on the specific origins of the recycled plastics.<sup>24</sup> However, all detected OPEs were present in trace amounts, indicating that they were not intentionally added to the plastics (Table 1). Further plastic additives found in over 20% of the samples were 1,1'-oxybis[2-methoxy-ethane] and *N,N'*-diphenyl-guanidine which are processing aids/solvents in a variety of plastic production<sup>25</sup> and might therefore be residues from the polymerization or recycling process (Table 1). Generally, the detected PMT plastic additives were processing aids (86%), fillers (64%), intermediates (57%), colorants, lubricants, or plasticizers (43%).

**3.1.2 Influence of product type.** Noticeable differences in contaminant profiles and concentrations were observed between PET bottles and textiles (Fig. 2). A higher variety of plastic additives

and higher concentrations of NIAS were detected in bottles compared to textiles, while the textiles showed a greater diversity of NIAS (Table 1). Ethylene glycol was exclusively detected in PET bottles with a 100% detection frequency, along with 2-methyl-1,3-dioxolane (89%) and benzene (50%) (Table 1). Additionally, all soda bottles contained limonene, a well-known flavour compound frequently detected in PET due to its potential adsorption onto bottle surfaces from the contained beverages.<sup>18,26</sup> In contrast, *m/p*-xylene showed a high detection frequency (87%) in PET textiles, alongside triethylene glycol, styrene, and acetaldehyde, each detected at 50%. Furthermore, formaldehyde (100% detection within textiles) and toluene (87%) had significantly higher detection frequencies in textiles compared to bottles (11% and 6%, respectively) (Fig. SA1). These variations likely originate from differences in polymerisation reagents, additives, and manufacturing conditions specific to each product type. The PMT plastic additive screening results supported this hypothesis (Table 1), identifying ten additives unique to PET bottles. Conversely, PET textiles contained three distinctive additives: dibutyl ester phosphoric acid (75% detection frequency), isoquinoline (50%), and bis(1-methyl-1-phenylethyl) peroxide (25%). Additionally, TCPP exhibited a higher detection frequency in textiles (100%) compared to bottles (11%). In contrast, antimony, a well-known contaminant of concern in PET materials, was detected in significantly higher concentrations in bottles (*t*-test,  $p < 0.05$ , mean =  $244 \pm 49 \text{ mg kg}^{-1}$ ) than in textiles (mean =  $200 \pm 59 \text{ mg kg}^{-1}$ ) (Table SA6).

### 3.2 Virgin vs. recycled PET products

A few differences were found in the contaminant profiles between recycled and virgin PET (Table SA7 and SA8). Ethylene glycol and 2-methyl-1,3-dioxolane showed statistically different concentrations in the analysed bottles (Wilcoxon test and Kruskal-Wallis test,  $p < 0.05$ ) with virgin PET having higher concentrations than recycled PET in both cases. This lower concentration in recycled PET could be a result of loss *via* leaching during bottle usage or intensive cleaning processes in recycling. Benzene, on the other hand, was consistently present in recycled PET bottles, in contrast to only one detection in virgin PET. This discrepancy suggests a potential contamination introduced during recycling. Benzene formation is linked to the thermal step during the recycling process,<sup>18</sup> particularly when combined with chlorine-containing materials like polyvinyl chloride (PVC),<sup>27</sup> which may contaminate PET during recycling due to sorting errors or labelling materials.<sup>28</sup> These results were consistent with previous reports on benzene in PET bottles.<sup>18</sup> The comparatively low concentrations found in our study ( $0.11\text{--}0.24 \text{ mg kg}^{-1}$ ) compared to the literature ( $0.2\text{--}1.8 \text{ mg kg}^{-1}$ ), could be a result of slightly lower PVC contamination in the bottle collection systems.<sup>18</sup> The isolated benzene detection in one virgin PET sample may reflect incidental contamination during manufacture. Although benzene has been reported in virgin PET,<sup>29</sup> given the low detection frequency and small samples size, further investigation is needed.

Similarly, OPEs were found in 54% of the recycled PET products compared to 36% of the virgin PET products (Table SB5). OPEs are used as flame retardants and lubricants in plastic





Table 1 Contaminants detected in recycled (rPET) and virgin PET (vPET) through leaching and extraction

Detection method	Name	Total frequency of detection	Number of detections with concentration range per product group and PET source (mg kg <sup>-1</sup> ) <sup>a</sup>			
			rPET	vPET	rPET	vPET
<b>PMT plastic additives</b>						
Leachate screening via LC-QTOF-MS <sup>c</sup>	Melamine (1,3,5-triazine-2,4,6-triamine)	32%	<b>Bottle (N = 18)</b>		<b>Textile (N = 4)</b>	
	Benzophenone (diphenyl-methanone)	27%	n = 3	n = 4	<LOD	<LOD
	1,1'-Oxybis[2-methoxy-ethane]	23%	n = 2	n = 4	<LOD	<LOD
	N,N'-Diphenyl-guanidine	23%	n = 1	n = 4	<LOD	<LOD
	N-Butyl-benzenesulfonamide	18%	n = 4	n = 1	<LOD	<LOD
	Dibutyl ester phosphoric acid	14%	n = 1	n = 3	<LOD	n = 1
	N,N'-1,2-Ethanediybis[N-(carboxymethyl)-glycine]	14%	<LOD	<LOD	<LOD	<LOD
	1-Methyl-4-nitro-benzene	9%	n = 1	n = 1	<LOD	<LOD
	Isoquinoline	9%	<LOD	<LOD	n = 1	<LOD
	1H-Benzotriazole	5%	<LOD	<LOD	<LOD	<LOD
	2-Methyl-2-[(1-oxo-2-propen-1-yl)amino]-1-propanesulfonic acid	5%	n = 1	<LOD	<LOD	<LOD
	Bis(1-methyl-1-phenylethyl) peroxide	5%	n = 1	<LOD	n = 1	<LOD
	<b>OPEs<sup>b</sup></b>					
Leachate screening via LC-QTOF-MS	Tri-chloropropyl phosphate (TCPP, 3 isomers) <sup>d</sup>	27%	<b>Bottle (N = 18)</b>		<b>Textile (N = 4)</b>	
	Tris(2-butoxyethyl) phosphate (TBEP) <sup>d</sup>	18%	n = 1	n = 1	n = 2	n = 2
	Triethyl phosphate (TEP) (LOQ = 0.122 mg kg <sup>-1</sup> )	14%	n = 3	n = 1	<LOD	<LOD
	Triphenyl phosphate (TPHP) (LOQ = 0.198 mg kg <sup>-1</sup> )	10%	n = 1	n = 1	n = 1	<LOD
	Tri- <i>n</i> -butyl phosphate (TNBP) (LOQ = 0.438 mg kg <sup>-1</sup> )	5%	n = 2	<LOD	<LOD	<LOD
<b>NIAS<sup>d</sup></b>						
Solvent extraction via GC-MS	Diethylene glycol	96%	<b>Bottle (N = 18)</b>		<b>Textile (N = 8)</b>	
	Ethylene glycol	69%	2.6-42.0 (n = 9)	2.3-63.0 (n = 9)	1.3-8.0 (n = 3)	1.3-35 (n = 4)
	2-Methyl-1,3-dioxolane	62%	3.1-7.1 (n = 9)	5.6-15.0 (n = 9)	<LOD	<LOD
	Benzaldehyde	46%	0.10-0.47 (n = 7)	0.25-1.1 (n = 9)	<LOD	<LOD
	<i>n</i> -Nonanal	38%	0.6-4.5 (n = 5)	1.1-4.3 (n = 4)	5.10 (n = 1)	2.0-2.8 (n = 2)
	Formaldehyde	38%	0.12-0.14 (n = 2)	0.1-0.12 (n = 2)	0.22-0.68 (n = 3)	0.12-0.16 (n = 3)
	Toluene	31%	0.1-0.11 (n = 2)	<LOD	0.21-7.3 (n = 4)	0.19-0.58 (n = 4)
	Benzene	31%	0.12 (n = 1)	<LOD	0.12-1.2 (n = 4)	0.23-0.66 (n = 3)
	<i>m/p</i> -Xylene	27%	0.11-0.24 (n = 8)	0.31 (n = 1)	<LOD	<LOD
	Limone	19%	<LOD	<LOD	0.14-0.15 (n = 3)	0.12-0.29 (n = 4)
	Triethylene glycol	15%	6.5-18 (n = 2)	0.99-20 (n = 3)	<LOD	<LOD
	Styrene	15%	<LOD	<LOD	1.6 (n = 1)	1.4-1.7 (n = 3)
	Acetaldehyde	15%	<LOD	<LOD	0.11-0.17 (n = 2)	0.19-0.35 (n = 2)
	Ethylbenzene	4%	<LOD	<LOD	0.17-0.29 (n = 2)	0.15-0.28 (n = 2)
	<i>o</i> -Xylene	4%	<LOD	<LOD	<LOD	0.12 (n = 1)
				<LOD	0.11 (n = 1)	

<sup>a</sup> N = the number of samples tested, n = the number of samples where analytes were detected. <sup>b</sup> Detection > limits of detection (LOD) but < limits of quantitation. The details see Table SB7 in SI B.

<sup>c</sup> Detected via PMT plastic additives screening workflow. <sup>d</sup> LOD details see Table SA5 in SI A; concentration range were from samples where the contaminant was detected, if only detected in one sample, the concentration of the sample was listed instead.

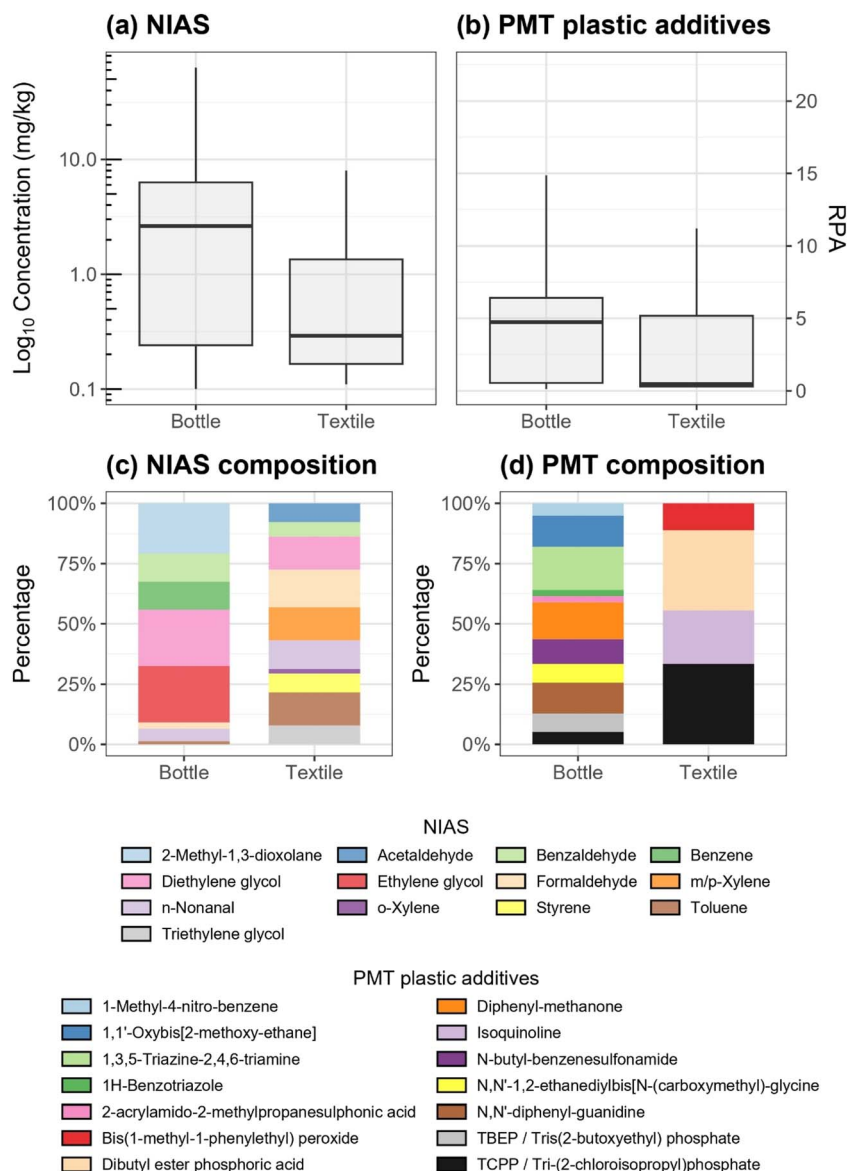


Fig. 2 Comparison of contaminant concentrations and relative composition of NIAS and PMT plastic additives between PET bottles ( $N = 18$ ) and textile products ( $N = 8$ ). Box plots of detected (a) NIAS and (b) PMT plastic additives for bottles and textiles. The y axis of PMT plastic additives is relative peak area (RPA) as the ratio of peak area of analytes to the peak area of the benchmark standard with smallest retention time difference. Stacked-bar charts of percentage composition of (c) NIAS and (d) PMT plastic additives detected in bottles and textile samples.

production and have previously been reported in PET food packaging.<sup>30,31</sup> Current literature lacks direct comparative studies of OPEs in recycled and virgin materials, but our findings suggest a greater likelihood of OPE leaching from recycled PET. All OPEs were found in low concentrations though (nearly all <LOQ), indicating that they likely originated from trace contamination during the recycling process, rather than intentional use/application in the plastic products. Further research should be conducted to investigate the potential sources of OPEs and benzene in the recycling process, particularly regarding the known carcinogenicity of benzene and chlorinated OPEs.<sup>32–34</sup>

Antimony in vPET and rPET were found to be at similar concentration levels ( $258 \pm 59$  and  $231 \pm 34$   $\text{mg kg}^{-1}$ , respectively) with no statistical difference, which aligned with the previous reported content range ( $200\text{--}300$   $\text{mg kg}^{-1}$ ).<sup>35,36</sup>

### 3.3 Geographical influence on contaminants in PET products

PET bottles sampled in this study were purchased from California ( $N = 10$ , including four soda bottles) and Michigan ( $N = 8$ , all water bottles), each with paired recycled and virgin PET sources. Although the exact manufacturing locations are unknown, it is reasonable to assume regionally bottling to minimise transportation cost. Comparative analysis showed distinct geographic differences between the contaminant profiles, which is consistent with the assumption that the two states' bottles have distinctly different origins. Bottles from Michigan showed higher benzaldehyde concentrations with 100% detection frequency and a mean concentration of  $2.9 \pm 1.1$   $\text{mg kg}^{-1}$  ( $n = 8$ ) compared to only one sample from



California with detectable benzaldehyde concentrations of  $0.6 \text{ mg kg}^{-1}$  (Fig. SA2). Benzaldehyde has previously been found in rPET but not vPET,<sup>37</sup> and the reason for elevated benzaldehyde in Michigan bottles, rPET and vPET alike, is unknown. Diethylene glycol, on the other hand, was found in significantly (*t*-test,  $p < 0.05$ ) higher concentrations in California bottles with mean concentrations of  $27.0 \pm 17.0 \text{ mg kg}^{-1}$  ( $n = 10$ ) compared to  $2.0 \pm 0.4 \text{ mg kg}^{-1}$  ( $n = 8$ ) in Michigan bottles. Diethylene glycol has previously been reported as both comonomer<sup>16</sup> and NIAS<sup>38</sup> in PET polymerisation. This suggests that the PET bottles from California, both virgin and recycled, were produced with different polymerisation conditions than the Michigan bottles. In addition, the high variability in California bottles compared to Michigan bottles indicated greater manufacturing variability in California. However, due to the limited sample size, the results should be considered exploratory. A moderate negative correlation ( $R = -0.67$ , Fig. SA3) between shelf life and diethylene glycol concentration was observed in California bottles, suggesting a potential leaching of diethylene glycol from the bottle into the environment or the contained beverage over time. However, this interpretation is complicated by the presence of soda bottles as the soda content may accelerate the leaching process. All soda bottles exhibited lower diethylene glycol concentrations and longer shelf lives than water bottles. The correlation is much less pronounced within each category. Given the small sample size, further investigation is warranted to determine the relative contributions of product contents and shelf life.

Apart from NIAS, six PMT plastic additives were found in bottles from both Michigan and California, accounting for over 65% of the total detected PMTs in each group. However, some variation was also evident. For example, 1,1'-oxybis[2-methoxyethane], a commonly used solvent, was predominantly detected in virgin PET bottles from California. *N*-Butylbenzenesulfonamide, a plasticiser was only detected in bottles, mostly vPET (with 60% detection frequency), from California. Conversely, TCPP and 1-methyl-4-nitro-benzene were solely detected in the bottles from Michigan in both vPET and rPET (Table SB5). This pronounced variation highlights the differing manufacturing practices between the two states and the lack of standardization in PET manufacturing and recycling. This variability is problematic for effective and representative risk assessment of PET materials as well as a potential barrier to the use of recycled materials, because manufacturers have no way of knowing which substances might be present in recycled PET.<sup>39</sup> More standardization in additive uses and design for recyclability could help overcome these challenges, increase recyclability<sup>40</sup> of products, and reduce the presence of hazardous compounds.

### 3.4 Nuclear receptor bioassays

A diverse array of bioactivities was observed across the eight receptor-based assays, with measurable responses in five endpoints: thyroid hormone  $\beta$  receptor (TR) antagonism, estrogen receptor  $\alpha$  (ER) antagonism, ER agonism, androgen receptor (AR) antagonism, and peroxisome proliferator

activated receptor  $\gamma$  (PPAR $\gamma$ ) agonism (Table SA9). Broadly, relatively low ER agonism was observed, with three recycled (17–45%) product extracts and two virgin (13–24%) extracts exhibiting low to moderate agonism. More significant ER antagonism was observed, with six recycled (10–65%) and eight virgin (22–100%) extracts, respectively, exhibiting moderate to high ER antagonism (Table SA9). One recycled material extract exhibited AR agonism and five exhibited antagonism (11–24%), relative to five exhibiting antagonism among the virgin materials (15–35%). Seven extracts both of recycled (11–56%) and virgin (10–57%) materials exhibited low to moderate TR antagonism. Two recycled (13–17%) and two virgin (12–26%) material extracts exhibited a low degree of PPAR $\gamma$  agonism (Fig. SA4). Bottles and textiles extracts exhibited mixed profiles: for example, thyroid hormone receptor  $\beta$  antagonism was higher in textile samples than in bottles, whereas differences in the other receptor responses did not reach statistical significance (*t*-test,  $p > 0.05$ ). Moreover, no clear relationship emerged between PET type (virgin *vs.* recycled) and overall bioactivity. This variability reflects the chemical complexity of both vPET and rPET materials and the potential for PET bottles as well as children's toys and clothes to contain endocrine disruptive chemicals. The detection of effects on multiple receptors highlights the need for follow-up studies employing larger sample sets, other bioactivities, and further characterisation.

## 4 Environmental implication

The results of this study highlight the diversity of hazardous compounds that can be present in virgin and recycled PET bottles and textiles; additionally, the results did not indicate significant contribution of recycling to the overall contamination of PET other than for benzene in bottles. Their presence in general suggests that PET may pose exposure risk to people and the environment *via* either food contact or leaching in the environment. Several NIAS previously reported in PET products and known to migrate<sup>16</sup> were also detected in the solvent extracts in this work. Some were found at lower levels in rPET than vPET, while benzene was detected in every rPET sample but in only one vPET sample, echoing concerns regarding new contaminants introduced by the PET recycling process, in part due to PVC and other foreign materials mixed into PET recycling. The detection of PMTs and OPES including melamine and other flame retardants in water leachate is notable because these were leached under environmentally relevant, aqueous conditions. As such, these substances may pose a more immediate contamination risk to the beverages and environments where these plastics can end up. Child textiles, which can pose contamination risk to watersheds *via* laundry, and exposure risk to children *via* dermal exposure or mouthing, showed a different profile than bottles, with much more abundant styrene, triethylene glycol, acetaldehyde, and formaldehyde. Importantly, recycled and virgin PET and textiles exhibited endocrine disruptive effects in *in vitro* assays, indicating a need to further evaluate the potential environmental and human health risks associated with these materials and their inherent chemical mixtures.



The diversity in detectable additives and NIAS in the analysed items also highlighted the need for standardization of plastic additive uses in plastic products – especially products with high potential for human exposure such as beverage bottles and children's items. Currently, plastic additive use is predominantly driven by producer needs and preferences, ignoring the potential impacts that the resulting chemical cocktails have on product recyclability as well as the health and well-being of our environment and children.

## Author contributions

Yanan Li – formal analysis, investigation, methodology, validation, visualisation, data curation, writing – original draft, writing – review & editing; Eric Fries – formal analysis, investigation, methodology, validation, writing – original draft, writing – review & editing; Gillian Z. Miller – conceptualisation, project administration, resources, data curation; writing – review & editing; Jeff Gearhart – conceptualisation; Dayton McGrail – formal analysis, investigation, visualisation, writing – original draft; Christopher D. Kassotis – formal analysis, investigation, visualisation, writing – original draft; Roxana Sühling – conceptualisation, resources, supervision; writing – original draft, writing – review & editing.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: <https://doi.org/10.1039/d5em00615e>.

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