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Occurrence of newly identified plasticizers in handwipes : development and validation of a novel analytical method and assessment of human exposure via dermal absorption

1   # Occurrence of newly identified plasticizers in handwipes; development and validation of

2   a novel analytical method and assessment of human exposure via dermal absorption

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Abstract

A novel analytical method for the monitoring of four newly identified plasticizers, namely di-propylene glycol dibenzoate (DiPGDB), tri-n-butyl trimellitate (TBTM), isooctyl 2-phenoxyethyl terephthalate (IOPhET) and bis 3,5,5-trimethylhexyl phosphate (TMHPh), in handwipes based on pulverization was developed and *in-house* validated. In total, 164 handwipe samples (paired with house dust and human urine) were collected during winter (*n=82*) and summer (*n=82*) 2019 from adults and toddlers living in Flanders, Belgium. Method LOQs ranged from 1 to 200 ng/g. The ranges of $\Sigma_{plasticizers}$ were 70-5400 ng/g for winter and 70-3720 ng/g for summer. The detection frequencies were 39% for DiPGDB, 27% for TBTM and <5% for IOPhET and TMHPh in winter samples and 33% for DiPGDB, 21% for TBTM and <10% for IOPhET and TMHPh in summer ones. The dominant compound in handwipes was DiPGDB, with mean contributions of 74% and 83% for winter and summer, followed by TBTM (24% and 9.2%), TMHPh (1.8% and 8.1%) and IOPhET (<1% and <1%). $\Sigma_{plasticizers}$ concentrations were positively correlated in summer with the use of sanitizer (r=0.375, p<0.05) and negatively correlated in winter with the use of personal care products (r=-0.349, p<0.05). DiPGDB was found positively correlated with the age of the participants (r=0.363, p<0.05) and the time spent indoors (r=0.359, p<0.05), indicating indoor environment as a potential source. Levels of TBTM in handwipes were positively correlated with dust samples collected from the same households (r=0.597, p<0.05), and those detected in toddler handwipes were significantly higher compared to adults (p<0.05). Human daily exposure via dermal absorption was evaluated using the dermal derived no effects level values (DNEL), available in the database of the European Chemicals Agency (ECHA) and estimated using the theoretical bio-accessible fractions per compound. Toddler exposure to TBTM was significantly higher compared to adults (T-test, p<0.05). No risk for adverse human health effects was derived from the comparison with DNELs for all compounds.

1. Introduction

During the last years, several bans, restrictions and replacements of legacy chemicals (e.g. phthalates, bisphenols, etc.) have led to important changes in the composition of goods used indoors. The tendency in replacing phthalates with non-phthalate alternative plasticizers in furnishing and building materials, children toys, and personal care products (PCPs) has steadily increased and brought to light different chemical groups, such as benzoates, dibenzoates, trimellitates, citrates etc. (Hammel et al., 2019). The limited existing information for these new substitutes is leading the interest of the scientific community to perform studies towards the occurrence and the resulting exposure to these compounds (Bui et al., 2016; Kademoglou et al., 2017; Christia et al., 2019).

Recently, four newly identified plasticizers, namely di-propylene glycol dibenzoate (DiPGDB), tri-n-butyl trimellitate (TBTM), isooctyl 2-phenoxyethyl terephthalate (IOPhET), and bis-3,5,5-trimehtylhexyl phosphate (TMhPh), were detected in residential dust from Belgium (Christia et al., 2021b). DiPGDB is a highly solvating plasticizer considered to be the alternative to butyl benzyl phthalate (BBzP) in floorings. It is also used in blends with di-ethylene glycol dibenzoate (DiEGDB) as alternative to di-iso nonyl phthalate (DINP) and likely to di-n-butyl phthalate (DBP) (Plasticstoday, 2021). TBTM is a plasticizer that belongs to the group of trimellitates with main applications in medical devices and parts of car interiors, and it is used also as an additive to building materials, cables, adhesives, nail products and printing inks (ECHA, 2016). The group of trimellitates, including TBTM, is considered a replacement of di-octyl phthalate (DOP), di-iso octyl phthalate (DiOP) and di-ethylhexyl phthalate (DEHP), characterized by good processability which decreases with the increasing length of the alkyl radical (Justia Patents, 2016). Due to their higher resistance to temperature compared to DEHP, trimellitates are preferred in special products where heat resistance is required. IOPhET is used as an additive to adhesives (EPA, 2020). Information regarding this compound remains scarce up to now. TMhPh information is limited similar to IOPhET and it has been referred only as a blowing agent which is applied to enhance the cellular structure during foaming process (PubChem, 2005).

55   The application of alternative plasticizers to products used indoors makes their fate relevant for human exposure

56   (Bui et al., 2016). Inhalation, food ingestion and inadvertent dust ingestion have been reported as main

57   contributors to human exposure, but existing biomonitoring data for these chemicals is limited so far. Few studies

58   have reported that dermal absorption is an important pathway to human exposure, especially for chemicals with

59   higher fractions absorbed by skin and a more lipophilic character (Xu et al., 2016). For example, dermal uptake of

60   polybrominated diphenyl ethers (PBDEs) was found to be the second major exposure pathway after dust ingestion

61   and *in-vitro* studies for organophosphate flame retardants (OPEs) indicated that dermal absorption might be

62   linked to human exposure (Lorber, 2008; Abdallah et al., 2016; Phillips et al., 2018). Skin is the largest organ of the

63   human body, acting as protective barrier to external factors and in some cases allowing substances into the

64   bloodstream, e.g. pharmaceuticals (SPHweb, 2016). Personal habits, like eating food with fingers, smoking, biting

65   nails, may increase the risk of exposure to various pollutants. Especially children are more exposed to pollutants

66   due to hand-to-mouth and object-to-mouth habits (Liu et al., 2017). To estimate the exposure via dermal

67   absorption, handwipes are often used as one of the sampling tools that is representative matrix of dermal

68   exposure, low cost and easy to be collected in a non-invasive way (Stapleton et al., 2012; Xu et al., 2016). It is a

69   relatively recent sampling technique used in studies focused on compounds with structural similarities to the ones

70   reported here, such as flame retardants and other alternative plasticizers (Xu et al., 2016).

71   The present study is linked to our previous work where DiPGDB, TBTM, IOPhET, and TMHPh were quantified in

72   residential dust and the human exposure was assessed via inadvertent dust ingestion (Christia et al., 2021b).

73   During the performed sampling campaigns, paired samples of floor dust, handwipes and urine were collected to

74   evaluate the presence of alternative plasticizers in homes and to which extent the family members were exposed

75   to them. Here, a novel target analytical method for the detection and quantification of four plasticizers in

76   handwipes was developed and *in-house* validated. Possible associations between dust and handwipe

77   concentrations were investigated and the personal habits of the participants were tested as potential exposure

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78   markers. Finally, human exposure via dermal absorption was estimated based on the theoretical bio-accessibility

79   fraction of each plasticizer.

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81   **2. Materials & Methods**

82   Information about chemicals, reagents and equipment applied in every stage of the experimental procedure is

83   included in the supplementary information (SI) (Section S1).

84   2.1 Sample collection

85   2.1.1 Collection of handwipes

86   Twenty five families (n=25) from Flanders, including a total of 82 individuals; $n=49$ adults (males-M: $n=24$ and

87   females-F: $n=25$) and $n=33$ toddlers (aged between 3 and 8 years old) (males-M: $n=19$ and females-F: $n=14$), were

88   recruited for this study. Two sampling campaigns were organized during winter and summer 2019. Sterilized gauze

89   pads (handwipes, dimensions 7.5 x 7.5 cm) were used as the sampling mean. They were cut into two equally sized

90   pieces and cleaned prior to use by submersion in n-Hex:Acetone (3:1 v/v) and ultrasonication (US) for 30 min

91   followed by submersion in isopropanol and US for another 30 min. Afterwards, they were placed in aluminum foil

92   folders and left overnight to dry at room temperature. Each individual handwipe was finally stored in a pre-cleaned

93   glass tube, added with 2.5 mL of isopropanol to keep them humid and facilitate the sampling procedure, and firmly

94   closed with a pre-cleaned polypropylene white cap. The tubes were then stored at room temperature until

95   sampling appointment (Figure S1).

96   All handwipe samples were collected directly by the researcher during the home appointment and the participants

97   were asked not to wash their hands in the hour prior to the visit. Two handwipes were used per participant (one

98   per hand). The entire surface of each hand was wiped, from top to bottom, from wrist to fingertips including the

99   surface between the fingers and the sides of the hand (Stapleton et al., 2008). After sampling, the handwipes of

100   each participant were placed into an aluminum foil folder and inside a plastic zip lock bag. All samples were stored

101   in a portable freezer during the transfer to the laboratory where they were further stored at -20°C pending analysis

102   (Figure S1). All participants were asked to fill in a questionnaire with information related to their age, gender,

103   body mass index (BMI), time spent in homes weekly, frequency of handwashing and use of PCPs. This information

104   was collected and used for further statistical analysis.

105   The collection of paired floor dust was made using a regular vacuum cleaner equipped with nylon socks of 25 μm

106   pore size as reported originally by Harrad et al., 2008 and described in detail by Christia et al., 2021b.

107

108   2.2 Sample preparation of handwipe samples

109   Each sample was removed from the plastic zip-lock bag and transferred to a desiccator where it remained

110   overnight to evaporate the excess of isopropanol. Then, each individual sample was trimmed into small pieces

111   and pulverized for 5 min at the frequency of 35 Hz (Figure S1). After pulverization, the two handwipes per

112   participant were pooled, weighted (average weight of 2 pooled handwipes; 2164±124 mg) and stored into a

113   beaker. The sample preparation protocol was performed according to Christia et al., 2021b after optimization (see

114   SI-Section S2). Briefly, a powder aliquot of approximately 20 mg was weighed in a 5 mL Eppendorf tube and spiked

115   with 100 ng of internal standards (ISs: dibenzyl phthalate-3,4,5,6-d_4 (DBzP-d_4) and di(2-ethylhexyl) phthalate-d_4

116   (DEHP-d_4)). A volume of 2.5 mL of the extraction solvent mixture *n-Hex*:Acetone:MeOH (2:1:1 v/v) and 0.5 mL

117   toluene were added and vortexing (1 min), ultrasonication (5 min) and centrifugation (3500 rpm, 5 min) were

118   performed successively. The supernatants transferred into pre-cleaned glass tubes and the procedure was

119   repeated one more time after the addition of fresh solvents.The supernatants were combined and evaporated in

120   a N_2 evaporator. A volume of 1 mL of solvent mixture *n-Hex*:toluene (1:1 *v/v*) was added and vortexing (1 min)

121   was applied. The clean-up and fractionation steps were performed simultaneously by applying ENVI Florisil®SPE

122   cartridges (500 mg, 3 mL). Three fractions were generated after elution with different solvents. More specifically,

123   the first fraction (F1) was eluted by 10mL *n*-Hex:dichloromethane (4:1 v/v) and discarded as the clean-up fraction.

124   The following fractions F2 and F3 were eluted by 8 mL of EtAc (collection of DiPGDB, TBTM, and IOPhET), and 8

125   mL of MeOH (collection of TMHPh) respectively. Fractions F2 and F3 were collected and evaporated in N_2

126   evaporator. F2 fraction was reconstituted in 50 µL of MeOH and 50 µL of recovery standard (RS) triamyl phosphate

127   (TAP) of concentration 1 ng/µL, whereas the F3 was reconstituted in 50 µL of MeOH and 50 µL of RS $^{13}C_{12}$ Bisphenol

128   S (^{13}C-BPS) of concentration 0.5 ng/µL. After reconstitution, both aliquots were filtered using 0.2 µm pore size

129   centrifugal filters (8000rpm, 5min) and analyzed in ESI+ (F2) and ESI- (F3). A detailed description of the sample

130   preparation protocol is given in Figure S2.

131

132

133   2.3 Instrumental Analysis

134   An Agilent 1290 Infinity Liquid Chromatography (LC) system coupled to an Agilent 6495 Triple Quadrupole Mass

135   Spectrometer (MS/MS) (Agilent Technologies, Santa Clara, CA, USA) was used for the analysis. A Kinetex Biphenyl

136   column (100 × 2.1 mm, 2.6 µm) was used at 40°C for the chromatographic separation. Ultrapure H_2O with 5 mM

137   ammonium formate buffer and (B) MeOH with 5 mM ammonium formate buffer were used as mobile phases for

138   positive electrospray ionization mode (ESI+); wherease (A) ultrapure H_2O with formic acid 0.1% and (B) MeOH

139   with formic acid 0.1% were used for negative electrospray ionization mode (ESI-). The applied flow rate and

140   injection volume were 0.25 mL/min and 5 µL respectively. Information for the chromatographic programs and

141   source parameters are given in Table S1 and Table S2, respectively. The MRM transitions for the targeted

142   compounds, including internal and recovery standards are given in Table S3. The total ion chromatograms (TICs)

143   for quality control (QC) and real sample are given in Figure S3.

144

145   2.4 Quality Assurance & Quality Control

146   Twenty two procedural solvent blanks (BLKs) were analyzed in parallel to the handwipe samples to check for

147   background contamination. Five blanks of pooled pulverized handwipes were analyzed to check for possible

148   contamination coming from the wipe fabric or the apparatus for pulverization. The targeted compound levels in

149   these five samples were below method LOQs (mLOQs). Eight QC samples of pooled handwipes, spiked with 20 ng

150   of the targeted compounds, were analyzed in the sample batches to assure the quality performance of the sample

151   preparation. The mean accuracies and relative standard deviations (RSDs) were 109±10% for DiPGDB, 124±18%

152   for TBTM, 83±16% for IOPhET and 107±9% for TMHPh. The recoveries of ISs were 121±6% for DBzP-d$_4$ and 90±13%

153   for DEHP-d$_4$. All glassware was pre-cleaned by rinsing *n*-Hex and baking at 400 °C for 12 h. Aluminum foil cover

154   was used during the evaporation to eliminate any background contamination. The pair of scissors used for

155   trimming the handwipe samples was rinsed with acetone and MeOH after each sample to avoid cross

156   contamination. Centrifugal filters of 0.2 μm pore size were tested prior use to check any retention or enhancement

157   of the compounds. To control any impurities or additives in the solvents, several solvent injections were

158   performed along the sequences.

159

160   2.5 Data analysis and statistics

161   The built-in Source Optimizer test in the MassHunter Data Acquisition software (version 10.1, Agilent

162   Technologies, Santa Clara, CA, USA) was operated for the optimization of the Jet stream source and funnel

163   parameters during method optimization. The data and statistical analysis were performed by Agilent Mass Hunter

164   software (version B.07.00)and IBM SPSS Statistics software (version 26, SPSS Inc., Chicago, IL, USA) respectively.

165   Independent-sample T-tests and Spearman's rho, were applied for comparisons and correlations of the

166   compounds which were detected in at least 20% of the samples. For the calculation of the descriptive statistics,

167   all values < LOQ were substituted with the detection frequency (df) of the compound in the samples multiplied by

168   the LOQ (df*LOQ) (James et al., 2002). The EPIWEB software (version 4.1) was used to predict the logKow values

169 of the targeted compounds. The method uncertainty, was estimated per compound as reported by Poma at al.,

170 2016 and the full description is given in Section S3.

171

172 2.6 Homogeneity

173 Homogeneity of the pulverized samples was evaluated via Fischer test to allow the analysis of the samples in

174 aliquots of 20 mg. Four aliquots, weighed ~20 mg, were spiked with 400 ng of a mix solution of the targeted

175 compounds, and were analyzed following the protocol described above (*Method 1*). One gauze pad was taken

176 after the step of isopropanol evaporation in the desiccator (Paragraph 2.2) and was trimmed into very small

177 pieces. An aliquot of 100 mg was weighed and transferred into a 50 mL polypropylene tube. The sample was

178 fortified with 2000 ng of standard mix solution of the targeted compounds carefully and equally onto the surface

179 of the fabric. The sample was left to dry and then it was pulverized under the same conditions as applied in the

180 sample preparation (Paragraph 2.2). After pulverization, 4 individual aliquots of ~20 mg were weighed and

181 transferred into 5 mL Eppendorf tubes and analyzed under the same conditions (*Method 2)*.

182 A Fischer test was applied per analyte to test the homogeneity of the methods by using the following equation:

183 $Fcalculated = \frac{\text{Variance 1}}{\text{Variance 2}}$ (3)

184 Where *Variance 1* and *Variance 2* are the squared standard deviations (SDs) in Method 1 and Method 2

185 respectively and Fcalculated > 1. When Fcalculated > Fcritical, the hypothesis of being homogeneous is considered

186 valid.

187

188 2.7 Assessment of human exposure via dermal absorption

189 The estimation of daily intake via dermal absorption was based on the hypothesis where the theoretical bio-

190 accessible fraction of each compound was considered since there is lack of the absorbed by skin fractions for these

9

191   new compunds. The description and calculation of Ba is reported by Dong et al., 2019 and the detailed information

192   is given in Section S4. The equation as reported by USEPA, 2001 was applied after modification:

193   $ADDdermal = Chandwipe * SA * AF * EF * ED/(BW * AT)$ (4)

194   where $C_{handwipe}$ is the concentration of the analyte found in handwipe (converted into ng/cm^2) multiplied by Ba,

195   SA is the exposed hand surface area (cm^2) (109 for male adults, 89 for female adults and 37 for both gender of

196   toddlers) (USEPA, 2011), AF is the fraction of the analyte absorbed by skin (as calculated via theoretical

197   bioaccessibility), EF is the exposure frequency over one year (347 days = 95th percentile of 365 days) (USEPA,

198   2001), ED is the lifetime exposure duration concerning the time stayting active in home (30 years for adults, 2

199   years for toddlers) (USEPA,2001), BW is the average body weight per age group (80±10 kg for male adults, 64±9

200   kg for female adults and 18±4 kg for toddlers) as derived from questionnaires and AT is the average time spent

201   indoors as calculated from the questionnaires.

202

203   **3. Results and Discussion**

204   3.1 Method optimization

205   The method applied in the present study was based on the method previously reported by Christia et al. (2021b)

206   on dust and adapted for the optimal extraction and analysis of handwipe samples. Standards of individual

207   compounds were used to set up the optimal values of dynamic multiple reaction monitoring (dMRM), single ion

208   monitoring (SIM) and quantitative and qualitative transitions with the corresponding collision energies (CEs) due

209   to the introduction of the funnale paramenter in that type of MS. The source and funnel parameters were

210   optimized by the built-in Source Optimizer software of the Mass Hunter data acquisition (see Paragraph 2.5). For

211   the chromatographic separation, the same mobile phases and the analytical column were applied as reported by

212   Christia et al. (2021b). Five sample preparation protocols were tested for achieving the maximum extraction

213   efficiency, combined with the removal of possible interferences. Procedural blanks and handwipe samples were

214   fortified with a mixture of ISs and native compounds. The optimal sample preparation protocol is described above

215   (Paragraph 2.2) and all the applied tests with the calculated recoveries (%) are reported in Section S2 and Figures

216   S4, S5, respectively. The sample preparation technique based on the pulverization process is reported here for

217   the first time and it is a novel approach in the handwipes analysis. The specific technique showed the advantages

218   of (i) reducing the required handwipe mass for the analysis, (ii) requiring considerably lower volumes of solvents

219   during extraction compared to other techniques reported in literature (Stapleton et al., 2008; Xie et al., 2016;

220   Darrow et al., 2017; Liu et al., 2017) and (iii) switching to a less complicated and time consuming method.

221

222   3.2 *In-house* method validation

223   The parameters of linearity, homogeneity, sensitivity, trueness, precision and uncertainty were evaluated for the

224   in-house validation of the method. More specifically, linearity was evaluated by the calibration curves which were

225   were obtained by the analysis of standard solutions of the targeted compounds in MeOH. Six concentration levels

226   were applied within the range 0.003 to 7.1 ng/µL (Table 1). The calibration curve per each analyte was formed by

227   plotting the area ratio of the compound divided by IS against the concentration ratio of the compound to the

228   corresponding IS. A quadratic model was used to demonstrate the correlation between the peak areas and the

229   concentrations for the compounds DiPGDB, TBTM and IOPhET whereas a linear model was used for TMHPh for

230   the same purpose. The correlation coefficient ranged between 0.997 and 0.999, indicating satisfying fits.

231   Homogeneity of the method was proved by applying a Fischer test between two methods as described in

232   paragraph 2.6. Accuracy ranged between 73 to 120% in both methods and the Fcalculated values were lower than

233   the critical ones, as indicated in Table S4.

234   Method sensitivity was evaluated by the limits of detection and quantification for the method (m) and the

235   instrument (i) expressed as iLOD, iLOQ and mLOQ. For that purpose, five procedural blanks (*n=5*) were analyzed

236   and all the compounds were detected within a range 1.7 to 61 ng/g. As mLOQ was defined the value equal to 3

237　times the standard deviation (SD) of each compound detected in the procedural blanks. The instrumental limits

238　of detection (iLODs) and limits of quantification (iLOQs) were calculated per compound from the mean signal to

239　noise ratio (S/N) of the solvent blanks. In detailed, 40 solvent blanks of MeOH:H_2O (1:1 *v/v*) were injected along

240　the sequences. The mean S/N per compound was multiplied by 3 and by 10 for iLOD and iLOQ, respectively and

241　then divided by the slope of the calibration.The iLODs, iLOQs and mLOQs ranges were 0.001–0.19 ng/µL, 0.003-

242　0.58 ng/µL and 1–200 ng/g, respectively (Table 1).

243　The trueness of the method was evaluated by using a pooled handwipe sample. The pooled sample was made

244　from the individual handwipe samples (*n=164*) used during sampling due the non-existence of a certified material

245　for this type of matrix. Five replicates (*n=5*) were fortified at two levels meaningful for the expected concentrations

246　in the samples, namely low level (LL) of 5 ng/g and high level (HL) of 2500 ng/g, and five non-fortified replicates

247　(*n=5*) were analyzed during the same day and between two different days to assess the intra- and inter-day

248　trueness of the method. The trueness was expressed as mean accuracy % per compound and is given in Table 1.

249　The mean values of trueness per analyte were 119±19% for DiPGDB, 119±10% for TBTM, 104±15% for IOPhET and

250　102±10% for TMHPh (Table 1).

251　The precision of the method was estimated for the intra- and inter-day repeatability. Repeatability was equal to

252　the RSD of five replicate analyses for the two levels of fortification, within-a-day and for two different days. The

253　intra-day repeatability was < 20% for all compounds, except for DiPGDB which was 27% for LL fortification level

254　and the inter-day repeatability was < 20% except for DiPGDB and TBTM for which was 25% for LL and 24% for HL,

255　respectively (Table 1).

256　The mean expanded uncertainty (Umean) of the two levels of fortification was calculated and found 21%, 10%,

257　13% and 7% for DiPGDB, TBTM, IOPhET and TMHPh, respectively (Table 1).

258

259　3.3 Concentrations and distribution of targeted plasticizers in handwipes

260   After validation, the method was applied to the analysis of 164 paired samples (*n*=82 winter and *n*=82 summer)

261   and the descriptive statistics and the individual results are given in Table 2 and Table S5, respectively. DiPGDB and

262   TBTM had detection frequencies 39% and 27% in winter samples and 33% and 21% in the summer ones. The rest

263   of the compounds had detection frequencies < 5% in winter and < 10% in summer. The concentration ranges for

264   the $\Sigma_{plasticizers}$ were 70-5400 ng/g for winter samples and 70-3720 ng/g for the summer ones. The concentration

265   ranges (mean) per analyte for the winter sampling were 60-5360 ng/g (320 ng/g) for DiPGDB, 7-2550 ng/g (105

266   ng/g) for TBTM, and 2-255 ng/g (8 ng/g) for TMHPh whereas IOPhET was detected only in 3 samples at 1.7, 1.8

267   and 2.3 ng/g respectively. For the summer sampling the ranges were 60-3715 ng/g (213 ng/g) for DiPGDB, 7-190

268   ng/g (24 ng/g) for TBTM, and 2-1000 ng/g (21 ng/g) for TMHPh, whereas IOPhET was detected only in one sample

269   at 4.2 ng/g.

270   The mean contribution (%) per compound during winter and summer was calculated over the $\Sigma_{plasticizers}$ detected

271   in handwipes. The dominant compound in both seasons was DiPGDB, with mean contributions of 74% and 83%

272   respectively likely due to its wide range of applications and due to the fact that benzoic acid esters in general are

273   among the main replacements of phthalic acid esters. For the rest of the plasticizers, the mean contribution (%)

274   order was TBTM (24%) > TMHPh (1.8%) > IOPhET (0.02%) for winter and TBTM (9.2%) > TMHPh (8.1%) > IOPhET

275   (0.03%) for summer (Table 2, Figure 1). Similar profiles were observed in house dust from the same homes and

276   details on the concentrations and distribution of the compounds are reported in our previous publication (Christia

277   et al., 2021b).

278

279   3.4 Associations between dust and handwipes

280   Spearman correlation coefficients were calculated for the $\Sigma_{plasticizers}$, and the compounds DiPGDB and TBTM with

281   DFs > 20% (excluding the values <LOQ) within the matrices of house dust and handwipes (Table 3). TBTM levels

282   were highly correlated between dust and handwipes in samples collected during winter (r=0.519, p<0.05) and

283   showed a positive trend during summer (r=0.328, p=0.08). $\Sigma_{plasticizers}$ showed a positive trend between dust and

284   handwipe levels measured only summer (r=0.269, p=0.07). These correlations and trends suggest that dust can

285   be an important contributor to human exposure to TBTM in both seasons and to total plasticizers particularly in

286   summer. As previously reported by several studies, there is a significant relationship between indoor chemical

287   concentrations in dust and handwipes (eg. for PBDEs, phathalates). The study of Watkins et al., 2011 showed 2.4

288   times higher concentrations of penta-BDE in the handwipes of participants working in offices with higher

289   concentration of penta-BDE in dust compared to those working in offices with lower concentrations. Positive

290   correlations between dust and handwipes were also found significant for total PBDEs and BDE-153 and for EH-

291   TBB (Stapleton et al., 2012; Stapleton et al., 2014). The phthalates dimethyl phthalate (DMP), diethyl phthalate

292   (DEP), di-iso butyl phthalate (DiBP) and di-n-butyl phthalate (DnBP) were positively correlated between dust and

293   handwipe samples in the study of Giovanoulis et al., 2018 indicating that dermal exposure may be associated with

294   the absorption from dust adhered to the skin.

295   For the $\Sigma_{plasticizers}$ in winter and DiPGDB in both seasons, there were not significant correlations found within dust

296   and handwipe levels.

297

298   3.5 Determinants of plasticizer concentrations in handwipes

299   Several parameters, including seasonal difference, age, gender and personal habits were examined to investigate

300   potential correlations with the levels of plasticizers (Table 4, Figures 2 and 3). During winter, the $\Sigma_{plasticizers}$ was

301   negatively correlated with the use of PCPs indicating other sources of origin for these compounds (r=-0.349,

302   p<0.05). DiPGDB was positively correlated with the age of the participants (r=0.363, p<0.05), thereby adults had

303   higher concentrations of that plasticizer in the handwipes compared to toddlers. DiPGDB levels were positively

304   correlated with the time spent indoors (r=0.359, p<0.05), showed a negative trend with the use of PCPs (r=-0.157,

305   p=0.398) and a positive trend within handwipes and dust (r=0.134, p=0.474). However, due to p>0.05 we cannot

306   suggest that residential dust is the main source of DiPGDB. TBTM concentrations were found significantly higher

307   in toddlers' handwipes compared to adults (T-test, $p<0.05$). In addition, TBTM was found positively correlated

308   with the levels detected in dust ($r=0.519$, $p<0.05$) and negatively correlated with time spent indoors ($r=-0.546$,

309    $p<0.01$). These results might indicate indoor dust as an important source of TBTM, where toddlers are potentially

310   more exposed likely due to crawling habits and playing on the floors. Since the time spent indoors was not

311   statistically linked to the levels of TBTM in handwipes, we could assume that other indoor environments than

312   homes, such as kindergartens, might have contributed to these levels.

313   During the summer season, the $\Sigma_{plasticizers}$ was found positively correlated with the use of sanitizers by participants

314   ($r=0.375$, $p<0.05$) indicating these type of products as a potential source of DiPDGB and TBTM. Since these results

315   refer to samplings which took place one year before the Covid-19 pandemic started, it is possible to hypothesize

316   that the levels of DiPDGB and TBTM might be currently higher, due to the extensive use of hand sanitizers. Two

317   products of seasonal use were added in the summer questionnaires: sunscreen and mosquito repellant. The levels

318   of $\Sigma_{plasticizers}$, DiPGDB and TBTM were found positively correlated to the use of mosquito repellant ($r=0.125$,

319    $r=0.284$, $r=0.008$, $p>0.05$) and negatively correlated with the application of sunscreens ($r=-0.159$, $r=-0.138$,

320    $r=0.038$, $p>0.05$). However these correlations were not statistically significant, and we must remain cautios in

321   formulating further hypotheses.

322

323   3.6 Human exposure via dermal absorption

324   Adults and toddlers were found to be exposed to all plasticizers, except for IOPhET due to its low detection levels

325   in handwipes (<LOQ) and only toddlers were not exposed to TMHPh for the same reason. The ADD_{dermal} values

326   were calculated as mean, median, 5th and 95th percentiles and shown in Table S6 and Figure 4. Based on mean

327   ADD_{dermal} values, the order of compounds was DiPGDB > TBTM > TMHPh for all tested groups of population in

328 winter. During summer, the order was DiPGDB > TBTM > TMHPh for female adults and toddlers whereas for male

329 adults was DiPGDB > TMHPh > TBTM.

330 For DiPGDB, the comparison of mean ADD_{dermal} values among the groups showed that toddlers had 2.2 times

331 higher values compared to male adults (p=0.056) and 3.2 times compared to female adults (p<0.05) during winter.

332 The comparison between the genders of the adult-aged group showed that the mean ADD_{dermal} values of males

333 were 1.5 times higher than those of females (p>0.05). In the summer season, a different pattern was observed for

334 DiPGDB, where the male adults and toddlers had similar levels of mean ADD_{dermal} values (p>0.05), whereas the

335 female adults had 2 times lower values (p>0.05).

336 For TBTM, ADD_{dermal} values in winter season, male and female adults showed similar levels (p>0.05) whereas

337 toddlers had 6 times higher values compared to those of adults (p<0.05). In summer season, mean ADD_{dermal} values

338 of toddlers found 2 and 1.5 times higher compared to female and male adults respectively (p<0.05 and p>0.05).

339 The levels of the latter were found similar (p>0.05). For TMHPh, mean ADD_{dermal} values of females were almost 2

340 times higher compared to males in winter (p>0.05). On the contrary, during summer season males showed 4 times

341 higher values compared to females (p>0.05).

342 Since there is a lack of reference values for the new plasticizers, the dermal derived no effect values (DNEL) were

343 used for DiPGDB and TBTM, whereas for TMHPh the oral DNEL of the structurally similar compound dibutyl

344 hydrogen phosphate was used to assess the risk via dermal absorption. The median, mean and 95th percentile

345 values were found several times lower than the DNELs, indicating no risk for the individual plasticizers via dermal

346 absorption for both adults and toddlers in both seasons (Table S6). However, the combined exposure to the new

347 plasticizers originating from multiple sources in daily life should be further investigated in the future.

348

349 **Conclusions**

16

350 Four plasticizers previously identified in dust were quantified in handwipe samples collected from the same

351 households by applying a newly developed analytical method. The innovative pulverization step significantly

352 reduced the consumption of solvents and facilitated the treatment of this complex matrix. The method was *in-*

353 *house* validated proving that linearity, sensitivity, trueness, and precision for intra- and inter-day were in

354 acceptable ranges. Concentrations of the $\Sigma_{plasticizers}$ were detected up to 5400 ng/g and 3720 ng/g in winter and

355 summer samples, respectively. DiPGDB was the dominant compound found in handwipes, followed by TBTM and

356 TMHPh. Positive correlations between handwipes and dust concentrations indicated that dust is likely a source of

357 origin for these plasticizers in the residential environment. For TBTM, positive correlations between handwipes

358 and dust combined, with the negative correlation between handwipe levels and time spent indoors, might indicate

359 other indoor environments as potential sources for the specific plasticizer. Human exposure via dermal absorption

360 was estimated and toddlers were found more exposed to TBTM and DiPGDB compared to adults. According to

361 the available DNEL values, there was no indication for health risk via dermal absorption of these plasticizers, but

362 combined exposure should be further investigated in the future.

363

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454

455

Table 1. Parameters of the *in-house* validation (as performed for *n=5* replicates).

Compound	Concentration (ng/g)-LL	Trueness (%)	RSD	Concentration (ng/g)-HL	Trueness (%)	RSD
Intra-day						
DiPGDB		124	27		116	12
TBTM	50	116	5	2500	123	14
IOPhET		95	6		114	19
TMHPh		108	11		94	6
Inter-day						
DiPGDB		123	25		113	12
TBTM	50	116	5	2500	121	14
IOPhET		99	11		108	24
TMHPh		113	12		92	10

	Calibration curve range (ng/µL)	Model	R^2	LOQ_{method} (ng/g)	Trueness mean (%)	Umean (%)
DiPGDB		Quadratic	0.997	200	119	21
TBTM	0.003-7.1	Quadratic	0.999	22	119	10
IOPhET		Quadratic	0.999	1	104	13
TMHPh		Linear	0.998	33	102	7

457

458

459   **Table 2.** Descriptive statistics of the targeted plasticizers found in handwipes.

Targeted Analyte	Mean (ng/g)	SD	Median (ng/g)	Min (ng/g)	Max (ng/g)	DF (%)	Contribution (%)	mLOQ (ng/g)
Winter Sampling (n=82)								
DiPGDB	318	775	58	60	5400	39	74	200
TBTM	105	358	7.0	7.0	2550	27	24	22
TMHPh	8.0	33	2.0	2.0	255	3.7	1.8	33
Summer Sampling (n=82)								
DiPGDB	213	507	58	60	3715	21	83	200
TBTM	24	35	7.0	7.0	190	33	9.2	22
TMHPh	21	112	2.0	2.0	1000	8.5	8.1	33

460

461

462 **Table 3.** Spearman correlation coefficients calculated for the concentrations between house dust (Christia et al., 2021b) and
463 handwipes (*p<0.05*)

	House Dust		
Winter	Σplasticizers	DiPGDB	TBTM
Σplasticizers	*0.169*		
DiPGDB		*0.134*	
TBTM			***0.519****
Summer	Σplasticizers	DiPGDB	TBTM
Σplasticizers	*0.269*		
DiPGDB		*0.265*	
TBTM			*0.328*

(Handwipes)

464

Table 4. Spearman correlation coefficients calculated for the concentrations of handwipes and the personal habits of the participants.

Winter	Σplasticizers	DiPGDB	TBTM
Age	*0.178*	***0,363****	*-0.064*
Gender	*-0.036*	*0.152*	*-0.086*
BMI	*-0.169*	*0.008*	*-0.273*
Hours spent indoors/week	*0.045*	***0,359****	***-0,546****
Washing Hands Frequency	*0.254*	*0.307*	*0.125*
Sanitizer	*0.151*	*0.224*	*-0.064*
PCPs	***-0,349****	*-0.157*	*-0.263*
Summer	Σplasticizers	DiPGDB	TBTM
Age	*-0.077*	*-0.396*	*-0.057*
Gender	*-0.039*	*-0.036*	*0.029*
BMI	*-0.168*	*-0.478*	*-0.204*
Hours spent indoors/week	*0.031*	*-0.159*	*-0.025*
Washing Hands Frequency	*-0.049*	*-0.213*	*-0.281*
Sanitizer	***0,375****	*0.162*	*0.168*
PCPs	*0.144*	*-0.222*	*0.257*
Suncream	*-0.159*	*-0.138*	*0.038*
Mosquito Repellent	*0.125*	*0.284*	*0.008*

*p<0.05
**p<0.01

470   **(a)**
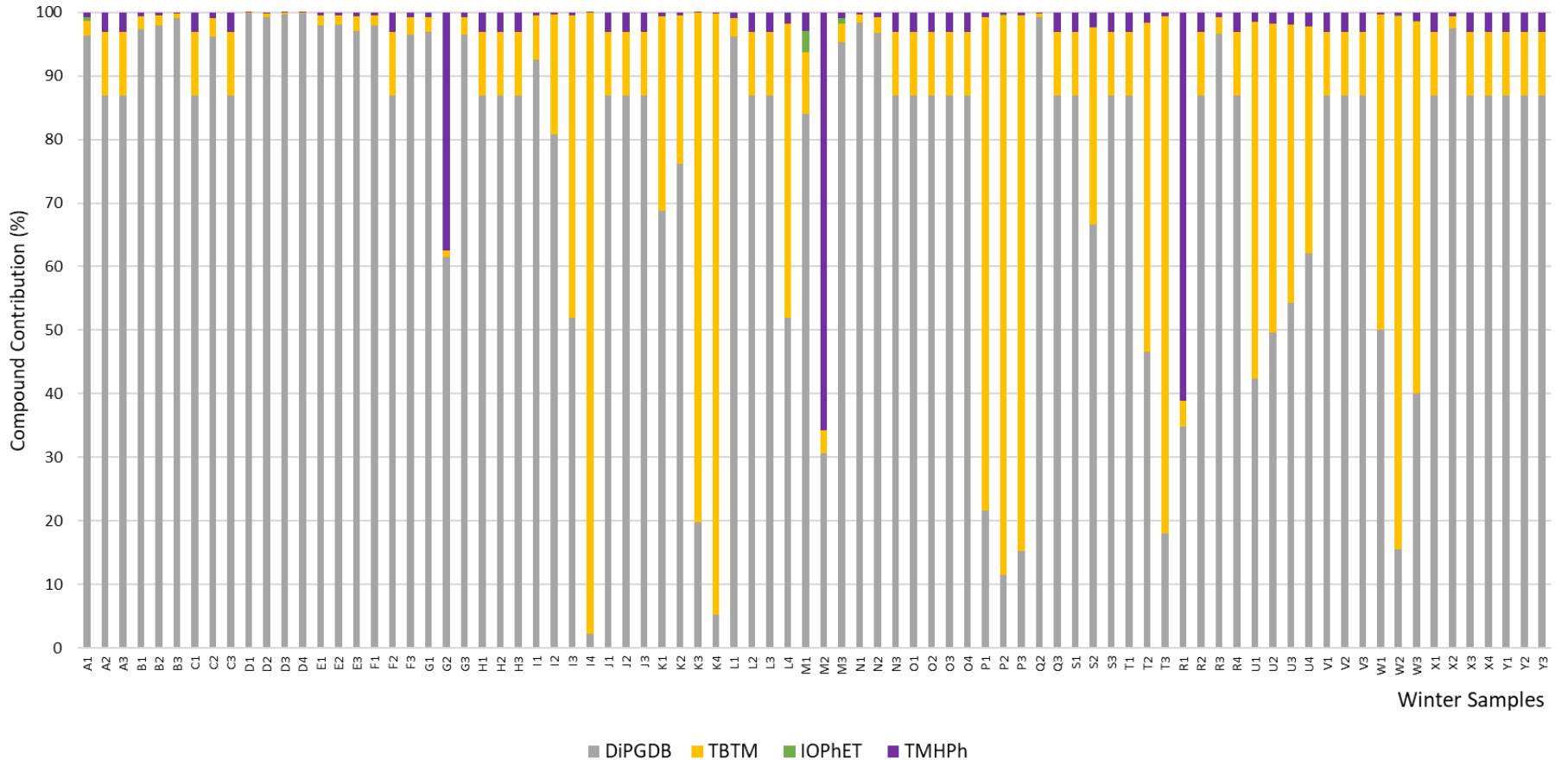
471

472

473   **(b)**
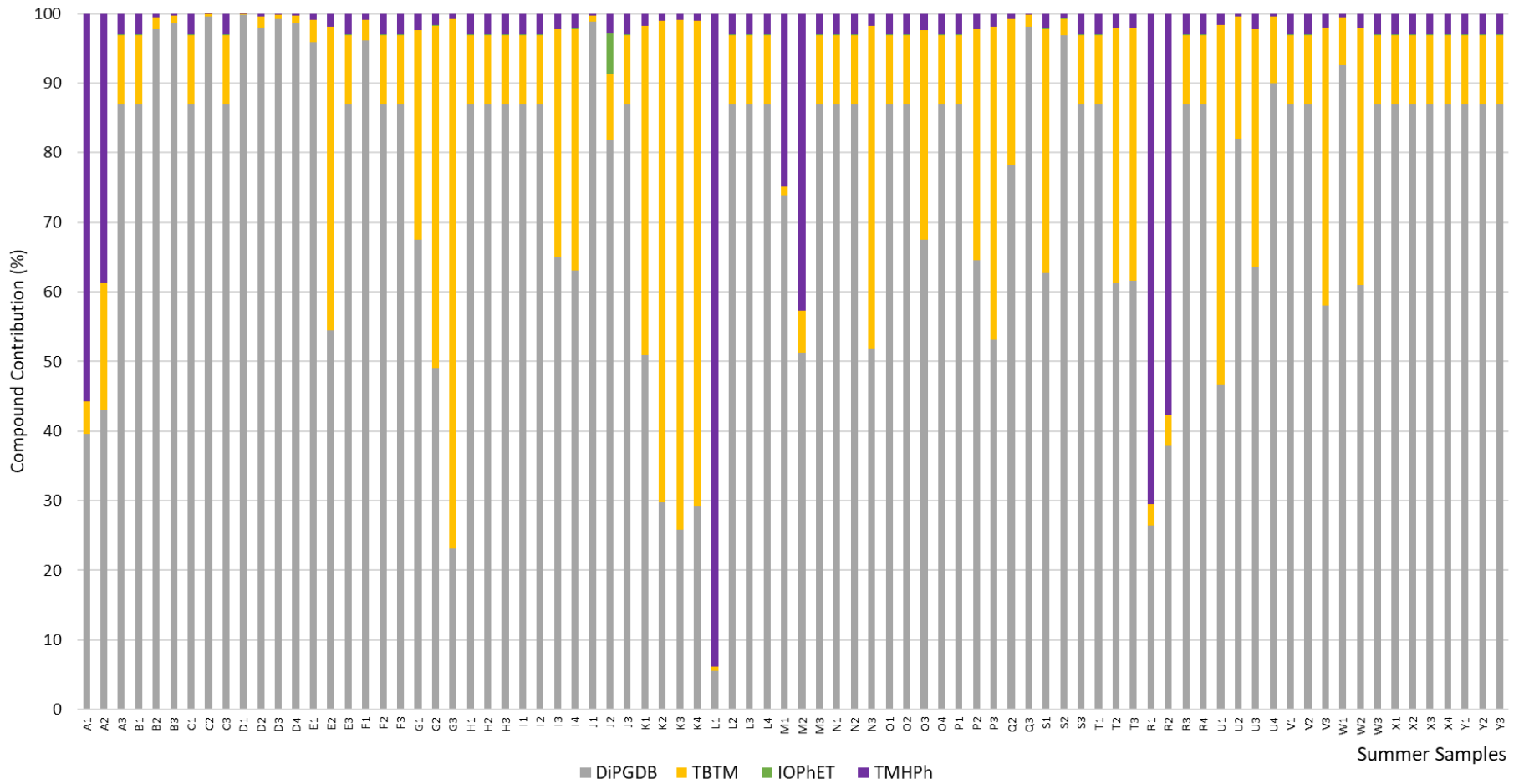
474

475   **Figure 1.** Mean contribution of the targeted plasticizers in (a) winter and (b) summer samples.
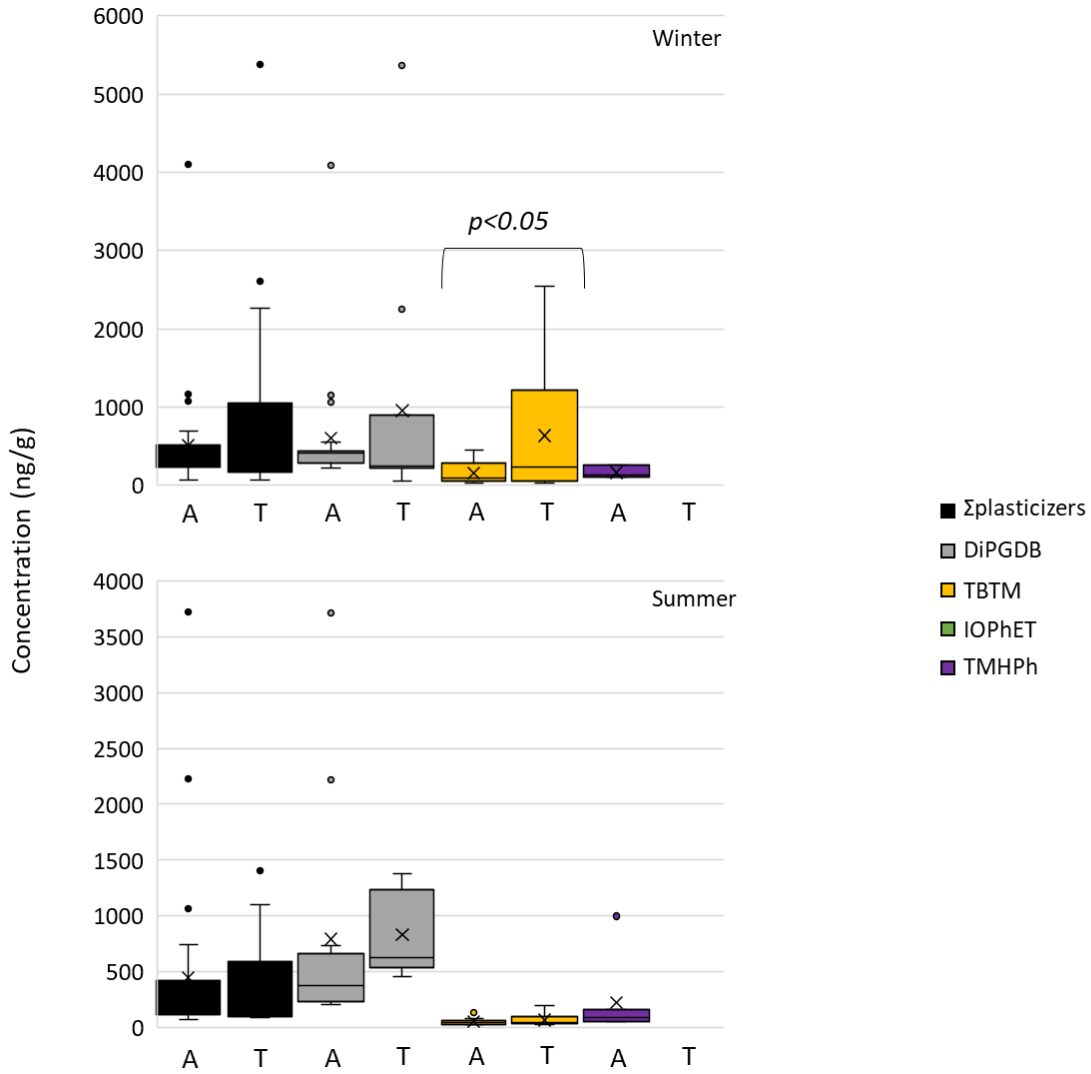
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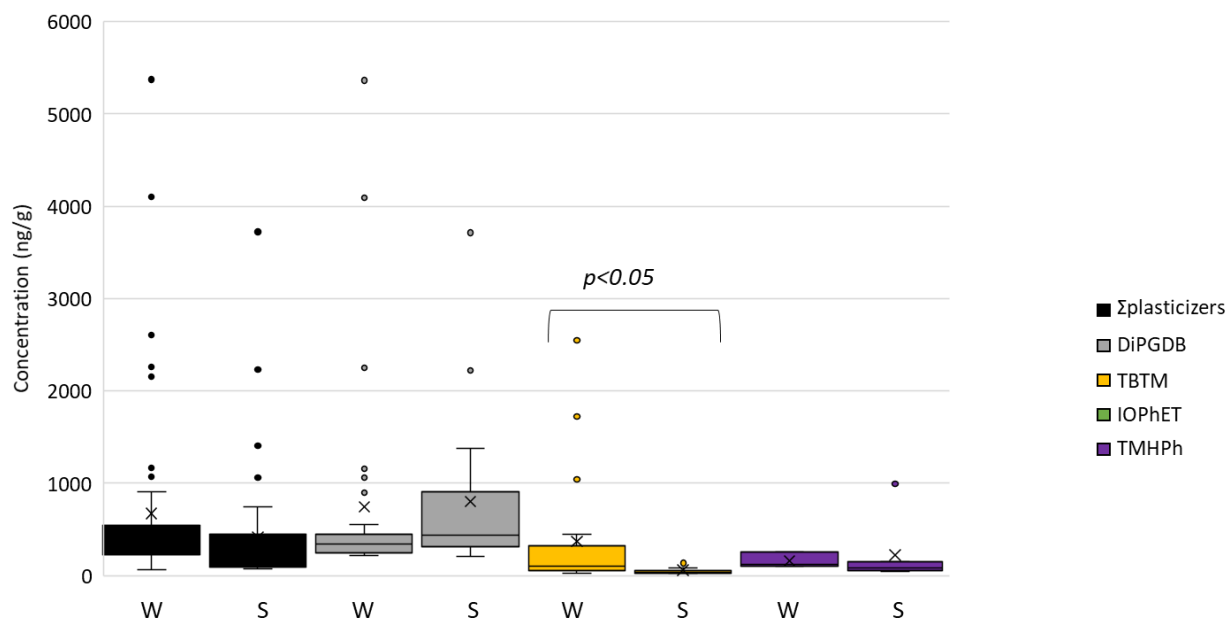
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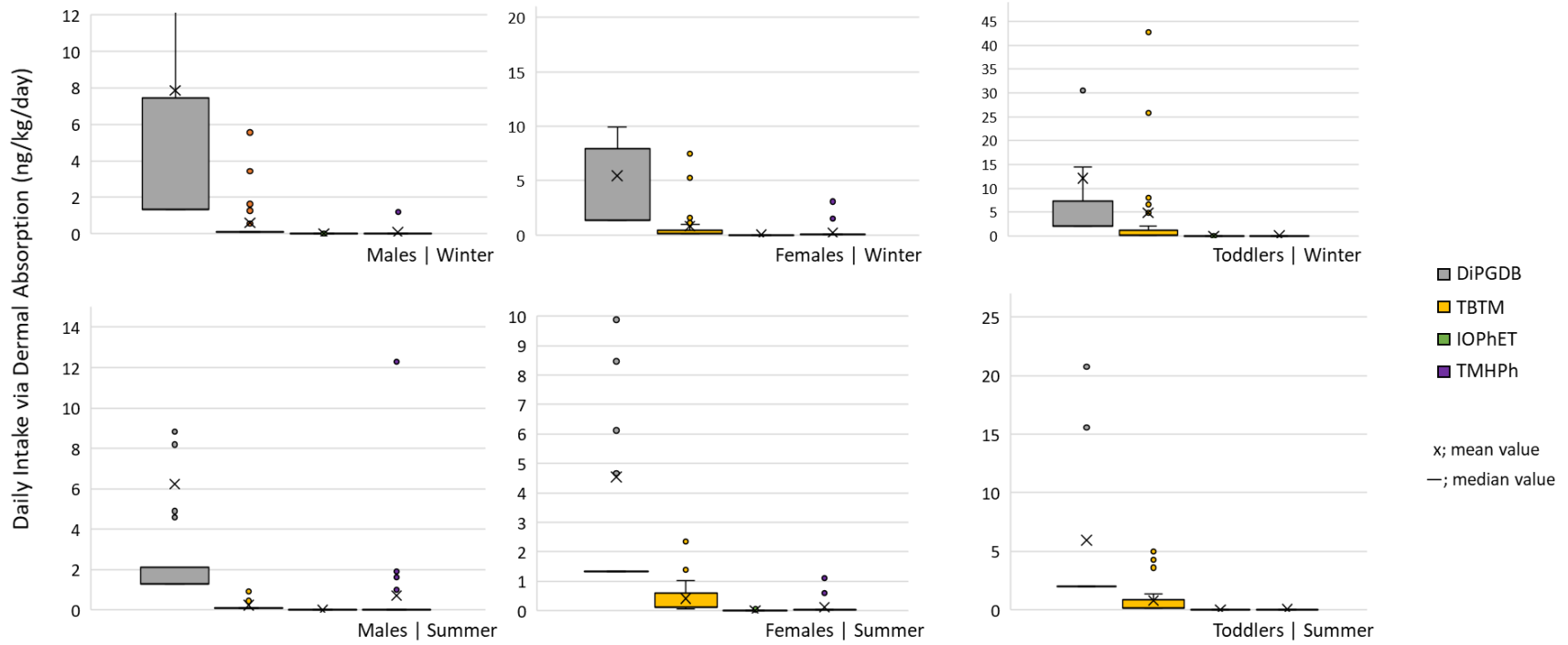478   **Figure 2.** Concentrations of $\Sigma_{plasticizers}$, DiPGDB, TBTM, IOPhET and TMHPh found in winter and summer samples.

479

Figure 3. Concentration of the total and individual plasticizers found in A: adults and T: toddlers.

483

484

Figure 4. ADDdermal (ng/kg/day) calculated for adults and toddlers per season.

1   **SUPPLEMENTARY INFORMATION**

2   Occurrence of newly identified plasticizers in handwipes; development and validation of

3   a novel analytical method and assessment of human exposure via dermal absorption

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10

29 **Section S1.** Chemicals, Reagents & Equipment

30 Isotopically-labelled standards of dibenzyl phthalate-3,4,5,6-d_4 (DBzP-d_4), purity >98%, was purchased from

31 AccuStandard (New Heaven, CT, USA), bis (2-ethylhexyl) phthalate-d_4 (DEHP-d_4), purity >98%, was purchased from

32 Sigma-Adrich (St. Louis, MO, USA) and bisphenol S ($^{13}C_{12}$-BPS), purity >98%, was purchased from LGC Standards

33 (Molsheim, France). The standards of tri-n-butyl trimellitate (TBTM), analytical purity >98%, was purchased from

34 TCI Europe N.V. (Zwijndrecht, Belgium), while bis (3,5,5-trimethylhexyl) phosphate (TMHPh), analytical purity

35 >98%, and isooctyl-2-phenoxy ethyl terephthalate (IOPhET), analytical purity >98%, were custom synthesized (Dr.

36 Vladimir Belov, Max Planck Institute for Biophysical Chemistry (Göttingen, Germany)). The standard of di

37 propylene glycol dibenzoate (DiPGDB), analytical purity 75% (technical grade), was purchased from Sigma Aldrich

38 (St. Louis, MO, USA). Triamyl phosphate (TAP) was purchased from TCI Europe (Zwijndrecht, Belgium).

39 All solvents used for the analyses were of liquid chromatography grade. n-Hexane (*n*-Hex) was purchased from

40 Acros Organics (Geel, Belgium), ethyl acetate (EtAc), dichloromethane (DCM), acetone and toluene were

41 purchased from Merck (Darmstadt, Germany). Methanol (MeOH) was purchased from Fischer Scientific

42 (Loughborough Leics, United Kingdom) and LC-grade ultrapure water (H_2O) was obtained from a PURELAB

43 Flexsystem (18.2 MΩ cm, Milli-Q, Millipore). Eppendorf tubes of 5mL were purchased from Sigma Aldrich (St.

44 Louis, MO, USA). Florisil®ENVI (500 mg, 3 mL) cartridges were purchased from Supelco (Bellefonte, PA, USA).

45 Centrifugal filters (modified nylon membrane) of 0.2 µm pore size were purchased from VWRTM (North America).

46 The pulverizer apparatus Retsch MM500 (serial num:1220161107) equipped with six metal cylinders and six

47 internal metal spheres was obtained from Retsch (Düsseldorf, Germany). The centrifuge apparatus was purchased

48 from Sigma-Aldrich (Saint Louis, Missouri, USA) and the vortex apparatus Reax Top was obtained from VWR

49 (Pennsylvania, USA). The nitrogen evaporator Reacti-Therm III was purchased from Thermo Fisher Scientific

50 (Waltham, Massachusetts, USA).

51

52 **Section S2.** Sample preparation optimization

53 Five different sample preparation tests were applied (Test 01-05). The fortifying levels, vortex time,

54 ultrasonication time, evaporation temperature and pre-clean volumes for the Florisil cartridges were similar

55 among the Tests 01 to 05. The form of the handwipes was different between test01 and the rest of the tests

56 applied. The type of the extraction solvent mixture was different between tests 01, 02 and the rest of tests and

57 the solvent volumes of the elution fractions F1, F2 and F3 were differentiated among tests 03, 04 and 05. The

58 selection of the optimal sample preparation procedure was based on the criteria of recoveries to fall within the

59 range 85-125% and RSDs<20% for ISs and targeted compounds.

60 During Test01, the whole surface of the handwipe sample was transferred into a 50 mL Falcon tube and 8 mL of

61 extraction solvent mix consisting of 5 parts of *n*-Hex:Acetone (3:1 *v/v*) and 1 part of toluene was applied. The

62 elution volume was 12 mL for each fraction.

63 During Test 02, an aliquot ~20 mg of pulverized handwipe was used with 2.5 mL extraction solvent mix of *n*-

64 Hex:Acetone (3:1 *v/v*) and 0.5 mL toluene. The elution volumes of fractions F1, F2 and F3 kept as in Test 01.

65 During Test 03, an aliquot of ~20 mg of pulverized sample was used with 2.5 mL extraction solvent mix of *n*-

66 Hex:Acetone:MeOH (2:1:1 *v/v*) and 0.5 mL toluene. The elution volume of F1 was reduced to 10 mL and the

67 volumes of F2 and F3 remain at 12 mL.

68 Two more tests, Test 04 and Test 05 were applied to finalize the elution volumes of F2 and F3, testing the volumes

69 10 mL and 8 mL, respectively.

70 Procedural blanks and handwipe samples were fortified in Tests 01-03 and only handwipes in Tests 04 and 05 to

71 optimize the elution volumes of F2 and F3.

72 The visualized results are given in Figures S3 and S4 where Test 05 corresponds to the final sample preparation

73 procedure that was selected.

74   **Section S3.** Calculation of the uncertainty

75   The uncertainty of the method (U) for the handwipe analysis was calculated for all the targeted compounds based

76   on Poma et al., 2016[1] and applying the following equations:

77    $Uc = \sqrt{Ur^2 + Ut^2}$ (SI-1)

78   where U_c is the combined standard uncertainty, Ur is the uncertainty of the repeatability, expressed as the

79   standard deviation of the measurements, and Ut is the uncertainty of trueness.

80    $U = Uc * k$ (SI-2)

81   where k is the coverage factor equal to 2 for level of confidence 95%.

82   [1] Poma et.al., 2016. Food Control, 65:168-76. https://doi.org/10.1016/j.foodcont.2016.01.027

83

84   **Section S4.** Calculation of the theoretical bioaccessibility (Ba)

85   The theoretical bioaccessibility (Ba) refers to the fraction of the total amount of a compound that is potentially

86   available for absorption and in the present study was used to estimate the fraction of the contaminant absorbed

87   by skin (AF) value per compound. The Ba is linked to the logKow value of each compound and the estimation per

88   compound was based on the following equation as reported from Dong et al., 2019[2]:

89    $$Ba = a + \frac{(b-a)*(8-logKow)}{8-5} \qquad \text{(SI-3)}$$

90   where Ba is the theoretical bioaccessibility for logKow values between 5 and 8, a and b are the constants assumed

91   as 0.2 and 0.8, respectively. For LogKow < 5, Ba assumed to be 0.8 and for LogKow > 8, Ba assumed to be 0.2.

92   [2]Dong et al., 2019. Environmental Science & Technology, 53:7045-7054. https://doi.org/10.1021/acs.est.9b00280

93 **Table S1.** Chromatographic programs applied in LC analyses.

ESI+	t (min)	A MQ H_2O, 5mM ammonium formate	B MeOH, 5mM ammonium formate
	0	70	30
	1	30	70
	9	0	100
	10	0	100
	10.1	70	30
	12	70	30
ESI-	t (min)	A MQ H_2O, 0.1% formic acid	B MeOH, 0.1% formic acid
	0	70	30
	1	30	70
	9	0	100
	10	0	100
	10.1	70	30
	12	70	30

94

95 **Table S2.** Parameters applied for the source and funnel operation during the analyses.

	ESI+	ESI-
Gas Temperature (°C)	250	250
Gas Flow (mL/min)	17	17
Nebulizer Gas (psi)	45	40
Sheath Gas Temperature (°C)	230	250
Sheath Gas Flow (mL/min)	12	10
Capillary Voltage (V)	3000	3500
Nozzle Voltage (V)	1500	2000
High Pressure RF*	130	150
Low Pressure RF*	160	60

*Funnel parameters

96

97 **Table S3.** Instrumental information on internal standards (ISs) and targeted compounds.

Compound Name	Acronym	Formula	ESI[1]	RT[2] (min)	Precursor Ion (m/z)	Fragmentor Voltage (V)	CAV[3] (V)	Quantitative Product Ion (m/z)	Collision Energy (eV)	Qualitative Product Ion (m/z)	Collision Energy (eV)
ISs											
Bis(2-ethylhexyl) phthalate-d4	DEHP-d4	$C_{24}H_{34}D_4O_4$	+	10.8	395.3	166	5	153.0	10	71.1	10
Dibenzyl phthalate-3,4,5,6-d4	DBzP-d4	$C_{22}H_{14}D_4O_4$	+	7.9	351.1	166	5	91.0	20	181.0	5
$^{13}C_{12}$ Bisphenol S	^{13}C-BPS	$^{13}C_{12}H_{10}O_4S$	-	2.2	261.0	166	5	161.9	20	113.9	25
RS						166	5				
Triamyl phosphate	TAP	$C_{15}H_{33}O_4P$	+	7.4	309.1	166	5	99.0	10	135.0	13
Targeted analytes						166	5				
Dipropylene glycol dibenzoate	DiPGDB	$C_{20}H_{22}O_5$	+	6.6/6.8/7.1	343.0	166	5	163.0	7	105.0	15
Tributyl Trimellitate	TBTM	$C_{21}H_{30}O_6$	+	9.5	379.1	166	5	248.9	15	192.9	30
Iso-octyl 2-phenoxy ethyl terephthalate	IOPhET	$C_{24}H_{30}O_5$	+	11.2	399.1	166	5	261.0	13	149.0	30
Bis (3,5,5-trimethylhexyl) phosphate	TMHPh	$C_{18}H_{39}PO_4$	-	5.1	349.2	166	5	223.1	20	79.0	25

[1]Electrospray Ionization

[2]Retention time

[3]Cell accelerator voltage

98

99   **Table S4.** Fischer test results related to homogeneity of the method.

	SD²			
	DiPGDB	**TBTM**	**IOPhET**	**TMHPh**
Method 1	961	5929	1024	121
Method 2	169	710	197	110
Fcalculated	**5.7**	**8.4**	**5.2**	**1.1**
Fcritical	15.44	15.44	15.44	15.44

100   SD: standard deviation

Table S5. Concentrations (ng/g) of the targeted plasticizers found in winter and summer samples.

	Winter samples				Summer samples			
	DiPGDB	TBTM	IOPhET	TMHPh	DiPGDB	TBTM	IOPhET	TMHPh
A1	277	6.8	1.7	2.0	58	6.8	0.03	82
A2	58	6.8	0.03	2.0	58	25	0.03	52
A3	58	6.8	0.03	2.0	58	6.8	0.03	2.0
B1	327	6.8	0.03	2.0	58	6.8	0.03	2.0
B2	418	6.8	0.03	2.0	372	6.8	0.03	2.0
B3	898	6.8	0.03	2.0	612	6.8	0.03	2.0
C1	58	6.8	0.03	2.0	58	6.8	0.03	2.0
C2	221	6.8	0.03	2.0	2223	6.8	0.03	2.0
C3	58	6.8	0.03	2.0	58	6.8	0.03	2.0
D1	4091	6.8	0.03	2.0	3714	6.8	0.03	2.0
D2	1156	6.8	0.03	2.0	434	6.8	0.03	2.0
D3	2251	6.8	0.03	2.0	1087	6.8	0.03	2.0
D4	5363	6.8	0.03	2.0	622	6.8	0.03	2.0
E1	432	6.8	0.03	2.0	205	6.8	0.03	2.0
E2	435	6.8	0.03	2.0	58	47	0.03	2.0
E3	299	6.8	0.03	2.0	58	6.8	0.03	2.0
F1	430	6.8	0.03	2.0	219	6.8	0.03	2.0
F2	58	6.8	0.03	2.0	58	6.8	0.03	2.0
F3	247	6.8	0.03	2.0	58	6.8	0.03	2.0
G1	281	6.8	0.03	2.0	58	26	0.03	2.0
G2	420	6.8	0.03	255	58	59	0.03	2.0
G3	241	6.8	0.03	2.0	58	192	0.03	2.0
H1	58	6.8	0.03	2.0	58	6.8	0.03	2.0
H2	58	6.8	0.03	2.0	58	6.8	0.03	2.0
H3	58	6.8	0.03	2.0	58	6.8	0.03	2.0
I1	449	34	0.03	2.0	58	6.8	0.03	2.0
I2	424	98	0.03	2.0	58	6.8	0.03	2.0
I3	215	198	0.03	2.0	58	29	0.03	2.0
I4	58	2549	0.03	2.0	58	32	0.03	2.0
J1	58	6.8	0.03	2.0	736	6.8	0.03	2.0
J2	58	6.8	0.03	2.0	58	6.8	4.1	2.0
J3	58	6.8	0.03	2.0	58	6.8	0.03	2.0
K1	224	100	0.03	2.0	58	54	0.03	2.0
K2	313	96	0.03	2.0	58	136	0.03	2.0
K3	426	1727	0.03	2.0	58	165	0.03	2.0
K4	58	1045	0.03	2.0	58	139	0.03	2.0
L1	221	6.8	0.03	2.0	58	6.8	0.03	998
L2	58	6.8	0.03	2.0	58	6.8	0.03	2.0
L3	58	6.8	0.03	2.0	58	6.8	0.03	2.0
L4	58	52	0.03	2.0	58	6.8	0.03	2.0
M1	58	6.8	2.3	2.0	395	6.8	0.03	133

M2	58	6.8	0.03	125	58	6.8	0.03	49
M3	216	6.8	1.8	2.0	58	6.8	0.03	2.0
N1	551	6.8	0.03	2.0	58	6.8	0.03	2.0
N2	262	6.8	0.03	2.0	58	6.8	0.03	2.0
N3	58	6.8	0.03	2.0	58	52	0.03	2.0
O1	58	6.8	0.03	2.0	58	6.8	0.03	2.0
O2	58	6.8	0.03	2.0	58	6.8	0.03	2.0
O3	58	6.8	0.03	2.0	58	26	0.03	2.0
O4	58	6.8	0.03	2.0	58	6.8	0.03	2.0
P1	58	210	0.03	2.0	58	6.8	0.03	2.0
P2	58	450	0.03	2.0	58	30	0.03	2.0
P3	58	323	0.03	2.0	58	49	0.03	2.0
Q2	1063	6.8	0.03	2.0	205	55	0.03	2.0
Q3	58	6.8	0.03	2.0	1380	25	0.03	2.0
S1	58	6.8	0.03	2.0	58	33	0.03	2.0
S2	58	27	0.03	2.0	269	6.8	0.03	2.0
S3	58	6.8	0.03	2.0	58	6.8	0.03	2.0
T1	58	6.8	0.03	2.0	58	6.8	0.03	2.0
T2	58	65	0.03	2.0	58	35	0.03	2.0
T3	58	265	0.03	2.0	58	34	0.03	2.0
R1	58	6.8	0.03	103	58	6.8	0.03	155
R2	58	6.8	0.03	2.0	58	6.8	0.03	89
R3	250	6.8	0.03	2.0	58	6.8	0.03	2.0
R4	58	6.8	0.03	2.0	58	6.8	0.03	2.0
U1	58	77	0.03	2.0	58	65	0.03	2.0
U2	58	57	0.03	2.0	372	80	0.03	2.0
U3	58	47	0.03	2.0	58	31	0.03	2.0
U4	58	34	0.03	2.0	459	49	0.03	2.0
V1	58	6.8	0.03	2.0	58	6.8	0.03	2.0
V2	58	6.8	0.03	2.0	58	6.8	0.03	2.0
V3	58	6.8	0.03	2.0	58	40	0.03	2.0
W1	344	340	0.03	2.0	366	27	0.03	2.0
W2	58	315	0.03	2.0	58	35	0.03	2.0
W3	58	85	0.03	2.0	58	6.8	0.03	2.0
X1	58	6.8	0.03	2.0	58	6.8	0.03	2.0
X2	348	6.8	0.03	2.0	58	6.8	0.03	2.0
X3	58	6.8	0.03	2.0	58	6.8	0.03	2.0
X4	58	6.8	0.03	2.0	58	6.8	0.03	2.0
Y1	58	6.8	0.03	2.0	58	6.8	0.03	2.0
Y2	58	6.8	0.03	2.0	58	6.8	0.03	2.0
Y3	58	6.8	0.03	2.0	58	6.8	0.03	2.0

102

103 **Table S6.** ADDdermal (ng/kg/day) calculated for adults & toddlers per season, based on concentrations in handwipes multiplied by Ba (gender
104 differentiation for adults due to different size of hand surface).

		Winter			Summer			Ba[4]	DNEL[5]
		Adults-M[1]	Adults-F[2]	Toddlers[3]	Adults-M	Adults-F	Toddlers		
DiPGDB	5th perc.	5.01E+00	3.01E+00	7.34E+00	4.67E+00	5.04E+00	1.66E+01	0.8	2.00E+09
	median	7.72E+00	9.57E+00	9.34E+00	8.51E+00	8.47E+00	2.11E+01		
	mean	1.56E+01	1.09E+01	3.54E+01	2.10E+01	1.47E+01	2.82E+01		
	95th perc.	5.22E+01	2.55E+01	1.35E+02	6.64E+01	4.04E+01	4.48E+01		
TBTM	5th perc.	6.94E-01	6.08E-01	4.99E-01	5.94E-01	5.51E-01	6.64E-01	0.61	2.00E+09
	median	1.63E+00	1.61E+00	4.89E+00	9.27E-01	8.86E-01	1.04E+00		
	mean	2.49E+00	2.91E+00	1.42E+01	8.63E-01	1.03E+00	1.72E+00		
	95th perc.	5.13E+00	6.91E+00	5.29E+01	1.09E+00	2.02E+00	4.54E+00		
TMHPh	5th perc.	1.21E+00	1.58E+00	*n.d.*	2.02E-01	6.34E-01	*n.d.*	0.44	2.00E+09[5a]
	median	1.21E+00	2.28E+00	*n.d.*	1.63E+00	8.60E-01	*n.d.*		
	mean	1.21E+00	2.28E+00	*n.d.*	3.37E+00	8.60E-01	*n.d.*		
	95th perc.	1.21E+00	2.98E+00	*n.d.*	1.02E+01	1.09E+00	*n.d.*		

[1]Male adults

[2]Female adults

[3]Male & female toddlers (due to same hand surface size)

[4]Ba values as reported by Christia et.al., 2021 https://doi.org/10.1016/j.chemosphere.2020.127817

[5]Dermal derived no effects level as reported by ECHA (5a; the oral DNEL of the structurally similar dibutyl hydrogen phosphate was used)

n.d.; not detected

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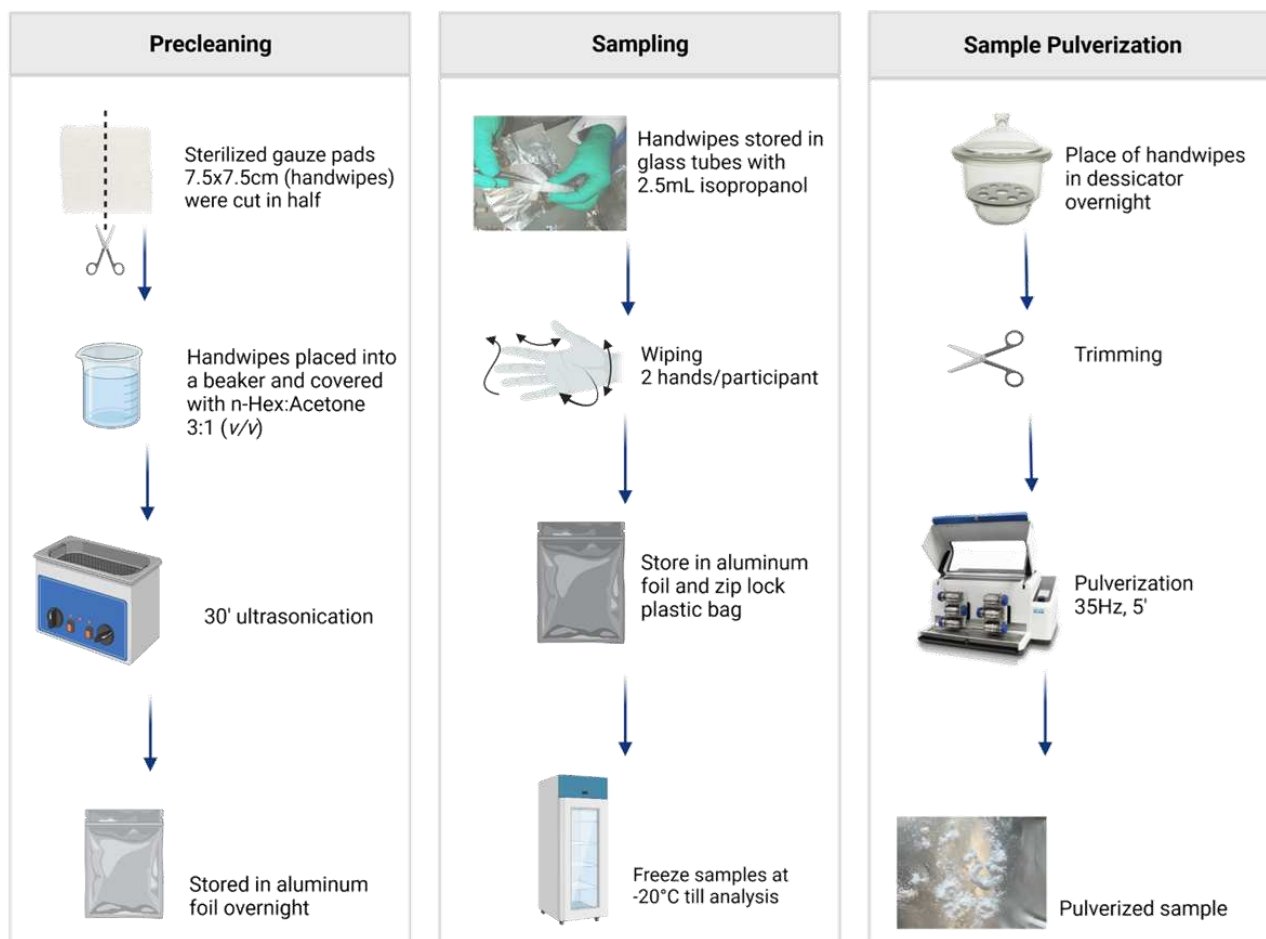
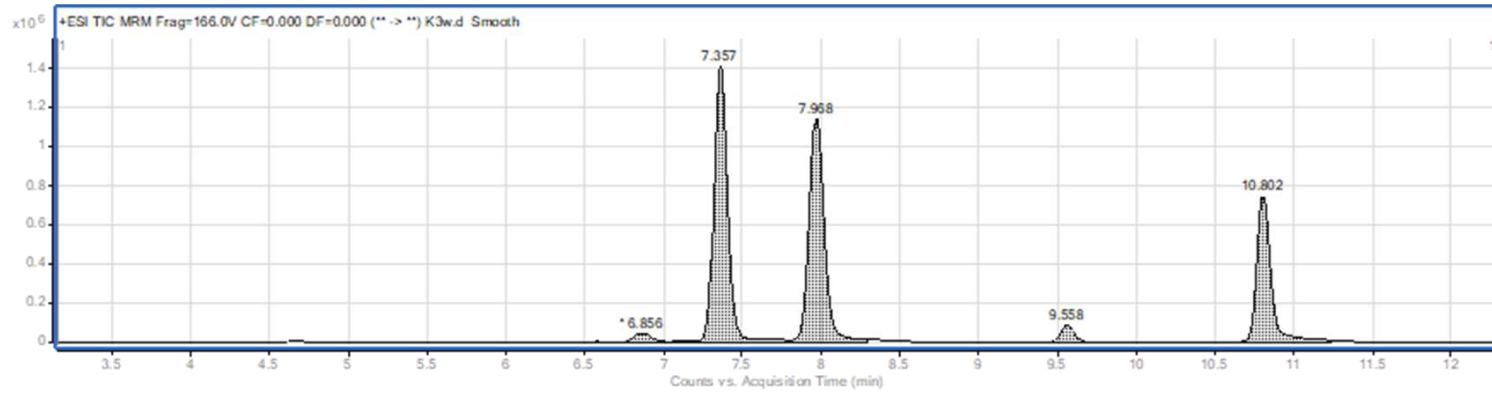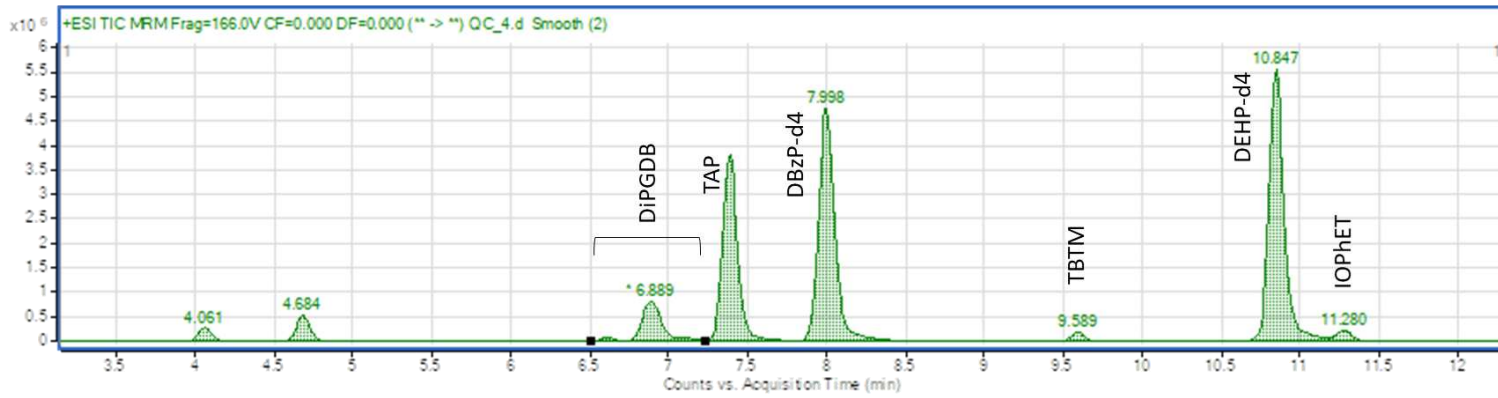**Figure S1.** Precleaning, sampling and pulverization procedures of handwipes.

109

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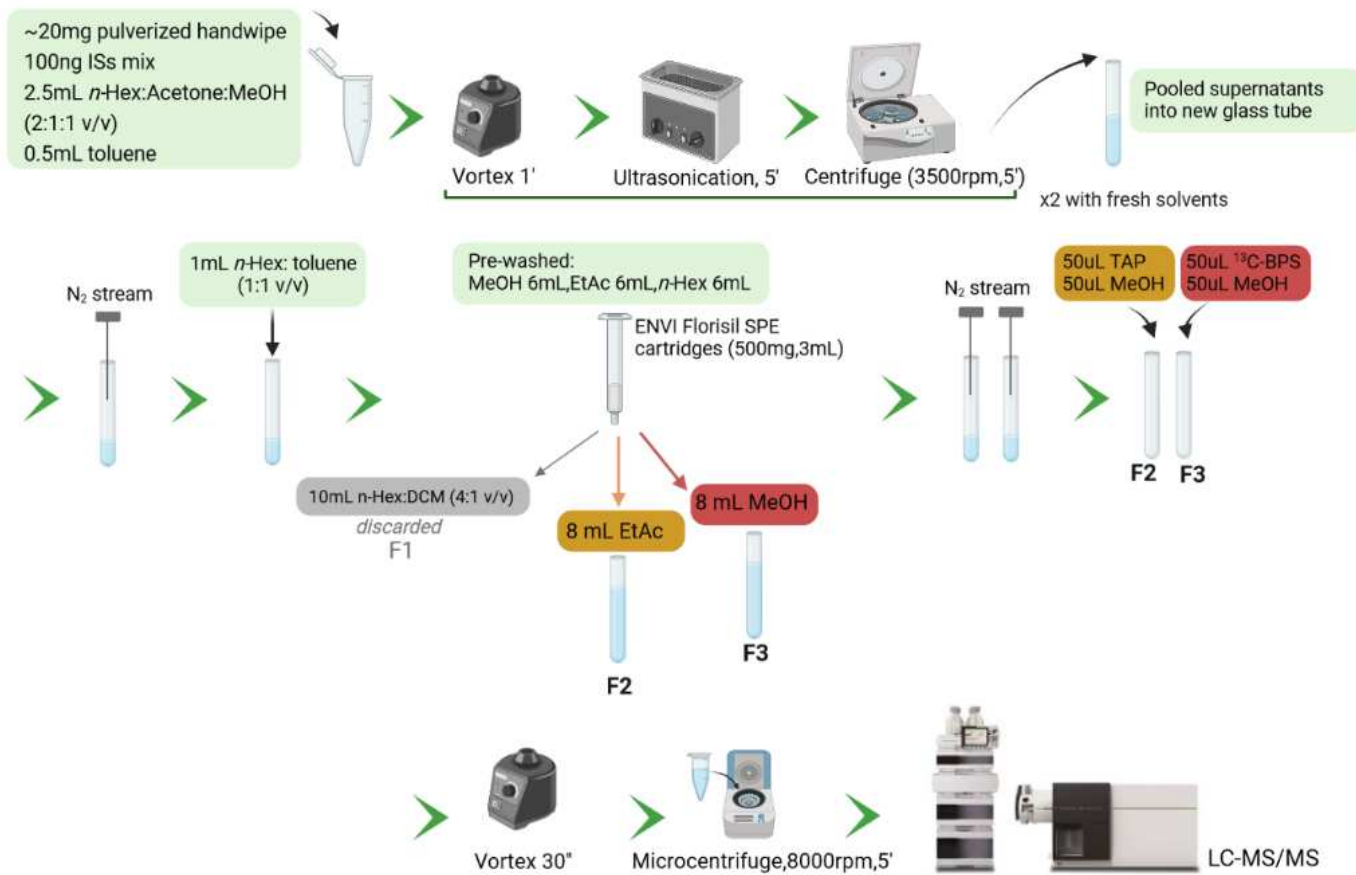
TAP concentration: 1 ng/µL; ^{13}C-BPS concentration; 0.5 ng/µL

112 **Figure S2.** Sample preparation protocol for the analysis of the targeted compounds in handwipes.
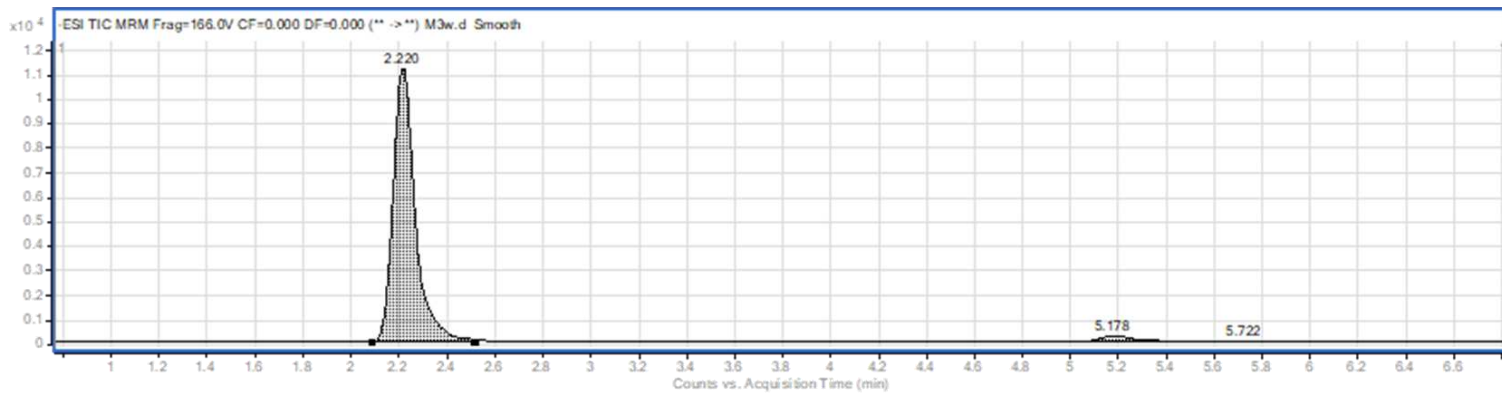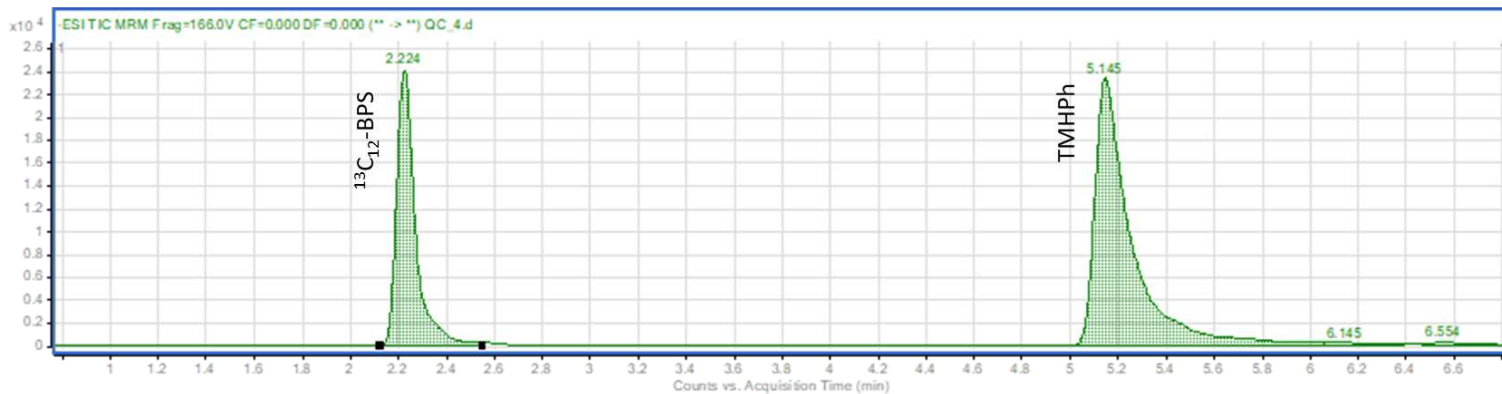
113

114   (a)

QC sample
Real handwipe sample

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116

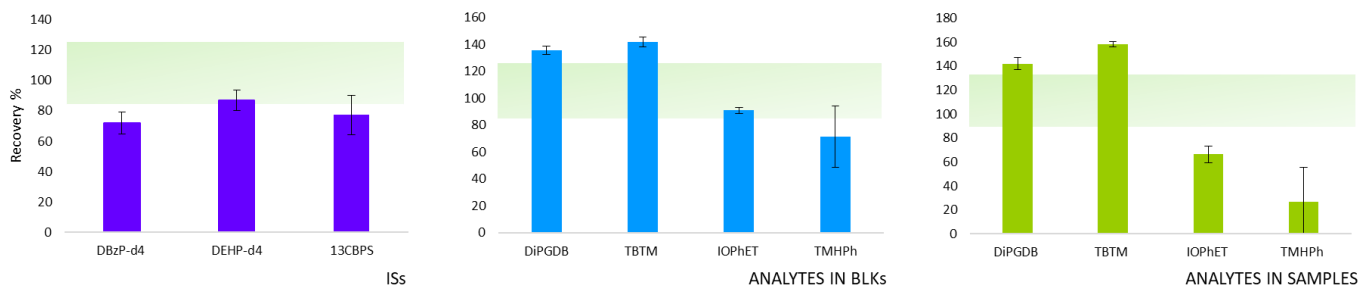117   (b)

QC sample
Real handwipe sample

118

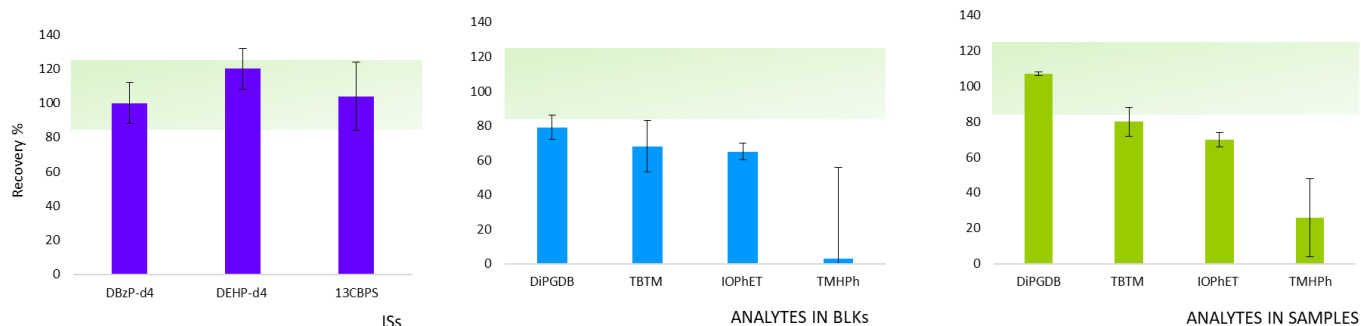119   **Figure S3.** Total ion chromatograms (TIC) of a QC and a real handwipe sample for (a) ESI+ and (b)ESI-. QC spiked mass; 16 ng
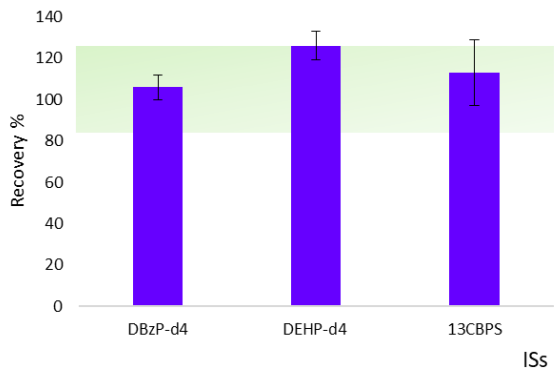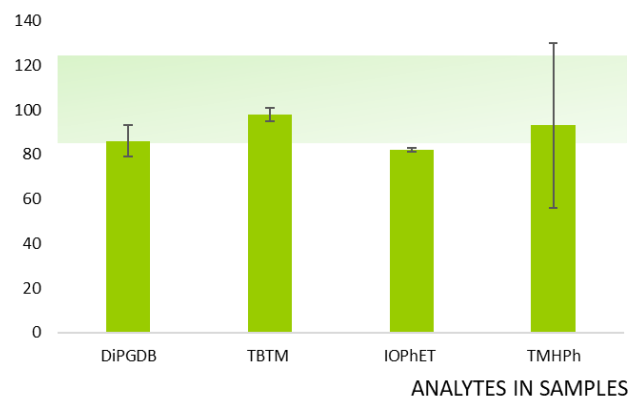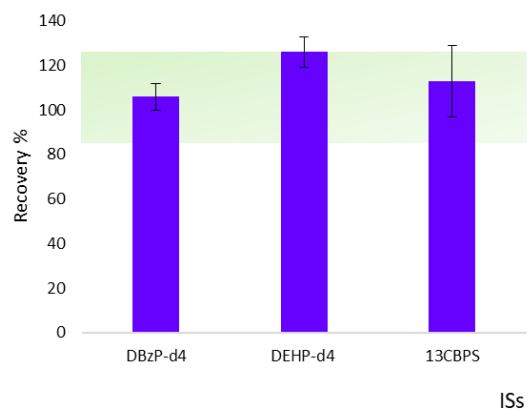
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TEST 01

TEST 02

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TEST 03

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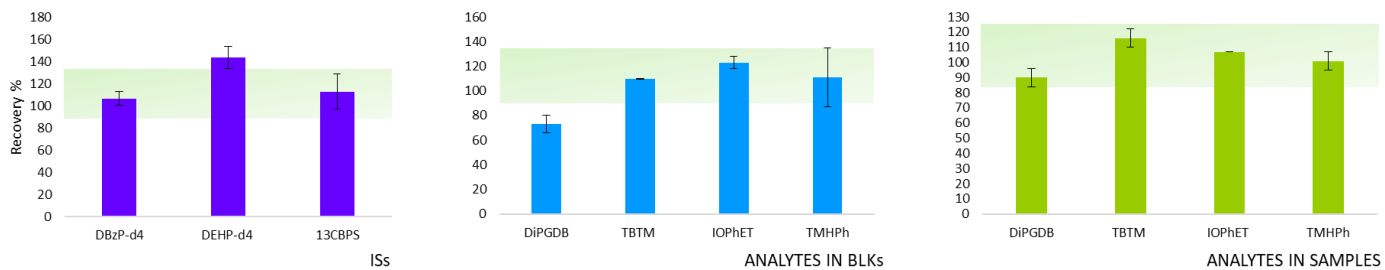
Figure S4. Calculated recoveries (%) of ISs and targeted compounds for Tests01-03 (light green area; accepted recovery range 85-125%).

TEST 04
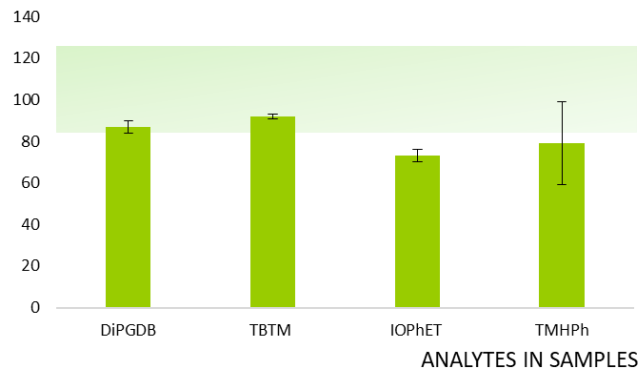
TEST 05

126

127 **Figure S5.** Calculated recoveries (%) of ISs and targeted compounds for Tests04 and 05 (light green area; accepted recovery range 85-125%).