An Irgafos® 168 story: When the ubiquity of an additive prevents studying its leaching from plastics

Hermabessiere Ludovic [1, *], Receveur Justine [2], Himber Charlotte [1], Mazurais David [3], Huvet Arnaud [3], Lagarde Fabienne [4], Lambert Christophe [5], Paul-Pont Ika [5], Dehaut Alexandre [1], Jezequel Ronan [2], Soudant Philippe [5], Duflos Guillaume [1]

[1] ANSES – Laboratoire de Sécurité des Aliments, Boulevard du Bassin Napoléon, 62200 Boulogne-sur-Mer, France
[2] CEDRE, 715 Rue Alain Colas, 29218 Brest Cedex 2, France
[3] Univ Brest, Ifremer, CNRS, IRD, LEMAR, F-29280 Plouzané, France
[4] Le Mans Université, Institut des Molécules et Matériaux du Mans - IMMM-UMR-CNRS 6283, 72085 Le Mans Cedex 9, France
[5] Univ Brest, Ifremer, CNRS, IRD, LEMAR, F-29280 Plouzané, France

* Corresponding author : Ludovic Hermabessiere, email address : ludovic.hermabessiere@utoronto.ca

Abstract :

Plastic pollution is a source of chemical to the environment and wildlife. Despite the ubiquity of plastic pollution and thus plastic additive in the environment, plastic additives have been studied to a limited extend. As a prerequisite to a study aiming to evaluate the leaching of a common additive used as an antioxidant (Irgafos® 168) from polyethylene microparticles, an inventory of the potential background contamination of the laboratory workplace was done. In this study, Irgafos® 168 (tris(2,4-ditert-butylphenyl) phosphite) and its oxidized form (tris (2,4-ditert-butylphenyl) phosphate) were quantified in different laboratory reagents, including the plastic packaging and the powders, using Pyrolysis-GC/MS. At least one form of Irgafos® 168 was detected in all tested laboratory reagents with higher concentrations in caps and bottles as compared to the powders. Additionally, oxidized Irgafos® 168 was also found in the reverse osmosed and deionized water container used in the laboratory. The same profile of contamination, i.e. higher concentration of the oxidized form and higher concentrations in acidic reagents, was observed when comparing the reagent and their respective containers suggesting that the additive is leaching from the container into the powder. Overall, this study demonstrates that the antioxidant additive Irgafos® 168 is ubiquitous in the laboratory workplace. Plastic additives such as Irgafos® 168 can therefore largely interfere and biased ecotoxicological and toxicological studies especially using environmentally relevant concentrations of microplastics. The source, fate and effects of plastic additive from plastic debris should be carefully considered in future studies that require setting up methods to overcome these contaminations.

Graphical abstract
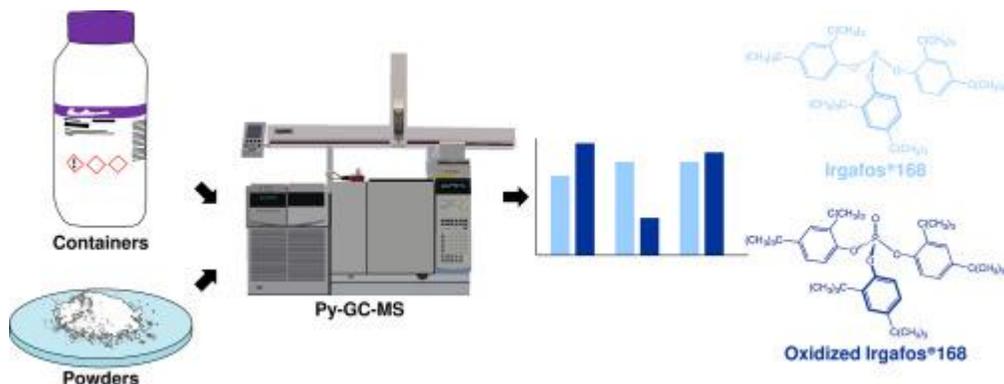
Highlights

► Irgafos 168® quantification was done by Pyrolysis-GC/MS in laboratory reagents. ► Irgafos 168® was quantified in caps, bottle and powders of all laboratory reagents. ► Leaching occurred between the bottle and the powder of the reagents. ► Contamination of the laboratory workplace by this plastic additive was ubiquitous.

Keywords : Microplastic, Additive, Leaching, Pyrolysis, Contamination, Irgafos 168 (R)

1 Introduction

Plastic debris contaminates the environment broadly (Galgani et al., 2015; Horton et al., 2017). With an increasing production of plastic since the 1950's (Geyer et al., 2017; PlasticsEurope, 2019) and a potential surge in production (Geyer et al., 2017), the concentration of plastic debris at the ocean surface will rise (Lebreton et al., 2019, 2018). During its manufacturing, plastic is made with other substances, called plastic additives that are added to confer properties to the polymer (e.g. flexibility, durability, plasticity). As most plastic additives are not chemically bound to the polymeric matrix, they can leach out of the plastic (Hermabessiere et al., 2017) being an additional source of additives in the environment (Al-Odaini et al., 2015). Plastic debris was estimated to be the source of 190 metric tons of 20 plastic additives that entered the oceans in 2015 (De Frond et al., 2019).

The ingestion of plastic particles of various sizes has been demonstrated for a wide range of organisms (Lusher, 2015) and has been linked to higher concentrations of plastic additives in their tissues. For instance, the flame retardant Decabromodiphenyl ether, was transferred to *Puffinus tenuirostris* upon plastic particle ingestion (Tanaka et al., 2015, 2013). Bisphenol A and its analogs were reported to be more concentrated in fish ingesting microplastics than in other fish (Barboza et al., 2020). As some plastic additives are considered endocrine disruptor chemicals (Oehlmann et al., 2009), it is crucial to study the transfer of these chemicals to organisms upon ingestion of plastic particles.

Irgafos® 168 (tris(2,4-di*tert*-butylphenyl) phosphite) (Figure 1) is a plastic additive mainly incorporated into Polypropylene (PP) and Polyethylene (PE) as an antioxidant (Dopico-García et al., 2007). It has been used in food packaging (Cherif Lahimer et al., 2017; Dopico-García et al., 2007), supposedly explaining its detection in frozen vegetable (Tanaka et al., 2003). Irgafos® 168 leached out of food packaging in contact with oil (Garde et al., 1998; Marcato et al., 2003) and its oxidized form, the tris(2,4-di*tert*-butylphenyl) phosphate (Figure 1), leached

65   from PE placed in stirred water (Suhrhoff and Scholz-Böttcher, 2016). Both forms were also

66   detected in plastic pieces used in oyster farming (Gardon et al., 2020) or ingested by seabirds

67   (Tanaka et al., 2019) and detected in indoor dust (Liu and Mabury, 2018; Venier et al., 2018).

68   Irgafos® 168 can be also hydrolysed over time in the aquatic system and one of its degradation

69   products, the 2,4-di*tert*-butylphenol (Figure 1) has been found in plastics collected on Korean

70   beaches (Rani et al., 2017, 2015). A second degradation product (bis(2,4-di*tert*-butylphenyl)

71   hydrogen phosphate) (Figure 1) was reported to impair growth of human cells in culture

72   (Hammond et al., 2013) while Chinese hamster ovary cell lines are also sensitive to this

73   chemical (Kelly et al., 2016; Shah et al., 2016). Oxidized Irgafos® 168 has been detected in

74   one laboratory solvent, likely the result of leaching during chemical storage in the laboratory

75   (Ekpo et al., 2012), it appears necessary to perform an inventory of potential Irgafos® 168

76   contaminations in the laboratory workplace prior to any leaching study, such as those using *in*

77   *vitro* simulated gut conditions as already used to study the desorption of oestrogenic chemicals

78   from plastic items (Coffin et al., 2019). In the present paper, the reduced (tris(2,4-di*tert*-

79   butylphenyl) phosphite) and the oxidized (tris(2,4-di*tert*-butylphenyl) phosphate) forms of

80   Irgafos® 168 were quantified in both the laboratory chemicals used to mimic *in vitro* (*i.e.* Coffin

81   et al., 2019) gut conditions and their containers to account for potential contamination.

82   ## 2   Material and methods

83   ### 2.1   Laboratory reagents

84   Eight chemical reagents used in *in vitro* digestive models were analyzed for trace of Irgafos®

85   168 two forms. These laboratory reagents were exclusively acquired from Sigma-Aldrich (St-

86   Quentin-Fallavier, France) and included: citric acid ($C_6H_7O_8$, reference: 251275), sodium

87   phosphate dibasic (Na_2HPO_4, reference: S7907), sodium cholate hydrate ($C_{24}H_{39}NaO_5$,

88   reference: C1254), calcium chloride dihydrate ($CaCl_2$, reference: C5080), Tris-HCl (reference:

89   T3253), pepsin (reference: 77160), trypsin (reference: T4799) and pancreatin (reference:

90 P1750). The plastic packaging (bottle and cap) were also analyzed. Analyses were only carried

91 out on the container of reverse osmosed and deionized water (reference: 102927G, VWR,

92 Fontenay-sous-Bois, France) used daily in the laboratory (hereafter call: Laboratory water

93 (LW)). Analyses were only carried out on the plastic material of the container of the reverse

94 osmosed and deionized water (reference: 102927G, VWR, Fontenay-sous-Bois, France) used

95 daily in the laboratory (hereafter call: Laboratory water (LW)). The main advantage of using

96 Py-GC/MS for this work was the minimal samples preparation required by this technique (Bart,

97 2001). Unfortunately, this Py-GC/MS technique is not adapted to quantify chemicals in solution

98 as in the water.

99 For each laboratory reagent and each matrix, analyses were done in triplicate.

100 **2.2   Irgafos 168® quantification by Pyrolysis-GC/MS**

101 Methods published by Fries et al., (2013) were applied for the Pyrolysis-GC/MS (Py-GC/MS)

102 analyses. Approximately 500 µg of matrix, *i.e.* plastic packaging or powder, was weighed with

103 0.00001 g precision (XP205, Mettler Toledo, Viroflay, France) then transferred to pyrolysis

104 vials. Py-GC/MS analysis was performed with a 7890N gas chromatograph (Agilent, Little

105 Falls, USA) equipped with a Combipal MPS2 multifunctional injection system (Gerstel, Sursee,

106 Switzerland), used in 'splitless' mode (1 min), and a Gerstel pyrolyser (Sursee, Switzerland).

107 The temperature-controlled cooling injection system was programmed from 50°C (0.3 min) to

108 350°C (2 min) at 12°C/s then the sample was pyrolysed at 700°C for 60 s. During pyrolysis

109 time, the temperature of the interface was maintained at 350°C. The oven temperature program

110 was set from 50°C (2 min) to 320°C (10 min) at 20°C/min. Helium carrier gas used at a constant

111 flow rate of 1.2 mL/min. The capillary column was an HP-5-MS (Agilent, Little Falls, USA):

112 30 m × 0.25 mm × 0.25 µm (thickness). The chromatograph was coupled to a 5975N mass

113 spectrometry detector (Agilent, Little Falls, USA). Quantitative compounds analysis was

114 carried out in single ion monitoring (SIM) mode with the following marker ions for reduced

115 Irgafos® 168 (m/z = 441) and its oxidized form tris(2,4-di*tert*-butylphenyl) phosphate

116 (m/z=316) (minimum of 1.5 cycles/s for dwell time). Compounds were quantified using

117 external calibration curves method ranging from 5 ng to 1000 ng. Irgafos® 168 reduced form,

118 (CAS no: 31570-04-4, Sigma-Aldrich, St-Quentin-Fallavier, France), and oxidized form (CAS

119 no: 95906-11-9, Toronto Research Chemicals, North York, Canada), were used as standards

120 for quantification.

121 **2.3 Statistical analysis**

122 All statistical analyses were performed using R Statistical Software version 3.5.2 (R Core Team,

123 2018) using RStudio version 1.1.463 (RStudio Team, 2016). Effect of matrix (cap, bottle or

124 powder) on reduced and oxidized Irgafos® 168 concentrations was tested using Kruskal-Wallis

125 tests as neither normality (Shapiro-Wilk test), or homoscedasticity (regression residues),

126 requirements were meet. Where significant differences were found with Kruskal-Wallis, the

127 '*agricolae*' package (version 1.2-8) (De Mendiburu, 2017) was used, to perform post-hoc test

128 using the Fisher's least significant difference criterion realized with Bonferroni correction.

129 Concentrations of reduced and oxidized Irgafos® 168 in each matrix were compared using a

130 Student t test after checking the normality of the data and the equality of variances. If variances

131 were not equal, Welch correction was applied. A non-parametric Mann-Whitney test was used

132 for non-normally distributed data. Significant differences were considered when the p-value

133 was below 0.05 and all results are expressed as mean ± 2 Standard Error.

134 **3 Results and discussion**

135 **3.1 Reduced and oxidized Irgafos 168® concentrations: ubiquitous in the**

136 **laboratory workplace**

137 At least one of the forms of Irgafos® 168 was always detected in caps, bottles and powders of

138 the eight-laboratory reagents (Figure 2). For reduced Irgafos® 168, concentrations in caps

139   ranged from under the limit of detection (n.d) in Na_2HPO_4 to 3265 ± 2782 ng/mg in $C_{24}H_{39}NaO_5$

140   (Figure 2A), in bottles between n.d in trypsin and pancreatin and $5{,}493 \pm 24$ ng/mg in pepsin

141   (Figure 2B) and in powder between n.d in pancreatin, $CaCl_2$ and Na_2HPO_4 and $3{,}000 \pm 2{,}507.76$

142   ng/mg in $C_6H_7O_8$ (Figure 2C). For oxidized Irgafos® 168 concentrations in caps were between

143   68.11 ± 23.01 ng/mg in trypsin and 218.13 ± 102.13 ng/mg in Tris-HCl (Figure 2A), in bottles

144   between 16.33 ± 10.48 ng/mg in trypsin and $1{,}023 \pm 634.61$ ng/mg in Na_2HPO_4 (Figure 2B)

145   and in powder between 8.33 ± 8.97 ng/mg in Na_2HPO_4 and 95 ± 157.23 ng/mg in $C_{24}H_{39}NaO_5$

146   (Figure 2C). Irgafos® 168 in its oxidized form was also detected in the container of LW at a

147   concentration of 33.92 ± 34.46 ng/mg (Figure 2B).

148   Overall, both Irgafos® 168 forms were significantly more abundant in plastic pieces, caps and

149   bottles (510.13 ± 257.77 and 710.24 ± 411.08 ng/mg, respectively), than in their respective

150   reagent powders (238.52 ± 216.49 ng/mg) (post-hoc after Kruskal-Wallis, $p<0.05$). When

151   present at concentrations above 100 ng/mg (except for Na_2HPO_4 bottle), reduced form was

152   always found in higher amount than oxidized form in caps, bottles and powder of each reagent

153   (Figure 2). These differences were only statistically significant (t test, $p < 0.05$) for trypsin cap

154   and for pepsin and Tris-HCl bottles (for concentrations above 100 ng/mg) (Figure 2). There was

155   high variability in Irgafos 168® concentrations across matrices (Figure 2). This high variability

156   might be due to the fact that Irgafos® 168 concentration may not be homogeneously distributed

157   in the polymer matrix, especially considering that this type of plastic additive is not chemically

158   bound to the polymer (Hermabessiere et al., 2017). Variability may also occur between batches

159   of the same product as suppliers may use different packaging producers for the same product,

160   who each may potentially use a different mix of additives.

161   Both forms of Irgafos® 168 were ubiquitously detected in the tested laboratory reagents, even

162   in the powders. All laboratory reagents tested here were housed in High-Density PE (HDPE)

163   bottles with PP caps. Additionally, the LW was also stocked into a bag-in-box composed of PE

164   with quantifiable amounts of Irgafos® 168 that might lead to LW contamination over time.

165   Irgafos® 168 is a plastic additive used as an antioxidant in polyolefin with concentration

166   ranging from 0.004 to 3% (wet weight) (European Chemicals Agency, 2019; Hahladakis et al.,

167   2018). Oxidation from the reduced form to the oxidized form is a common phenomenon for this

168   molecule (Yang et al., 2016), thus making the protection of the polymer against oxidation

169   possible (Fouyer et al., 2012). In studies concerning Irgafos® 168 from food packaging, both

170   forms are usually targeted (Dopico-García et al., 2007; El Mansouri et al., 1998; Suhrhoff and

171   Scholz-Böttcher, 2016; Yang et al., 2016). According to Yang et al., (2016) oxidation of

172   Irgafos® 168 can occur either during the extrusion of the polymer, during storage in the dark

173   or during exposure to UV; the latter being the fastest pathway (Yang et al., 2016). Here it is not

174   possible to assess whether the oxidation process of Irgafos® 168 in the laboratory has occurred

175   (i) during storage in the dark as laboratory reagents were stocked in safety cabinets or in the

176   fridge straight from their delivery at the laboratory until their use or (ii) during the production

177   and transport process of both the packaging and the reagent. It was recently suggested that when

178   studying antioxidants, the oxidized form should be primarily targeted as the reduced form will

179   react with oxygen and will not accumulate in the environment (Wu et al., 2019). This is

180   confirmed in the present study as the oxidized Irgafos® 168 was systematically detected in

181   quantifiable amounts whereas its reduced form was under the limit of detection in some

182   laboratory reagents.

183   **3.2   Leaching between plastic bottle to the reagent powder**

184   Concentrations of both Irgafos 168® forms were two times lower in the powder compared to

185   the bottle, suggesting that leaching could probably be a slow, long and most often an incomplete

186   process (Yang et al., 2016) that remain to be further deeply studied. Leaching was mostly

187   dependent on the considered reagent. Indeed, some laboratory reagents demonstrated higher

188   concentrations of reduced Irgafos® 168 (pepsin, $C_6H_7O_8$, Tris-HCl and $C_{24}H_{39}NaO_5$), while

189   others (trypsin, pancreatin, $CaCl_2$ and Na_2HPO_4) showed higher concentrations of oxidized

190   Irgafos® 168. These differences could be linked to factors such as the conservation time and

191   temperature, the age of the products, and their containers. However, it was not possible to

192   retrieve information about reagent production from the manufacturer certificate of origin and

193   analysis. Consequently, retracing the history of the reagents processing prior to their arrival in

194   the lab is important in order to draw conclusions about the leaching process between the

195   packaging and the reagents. Here, it is not possible to rule out whether leaching occurred before

196   lab delivery of the chemicals or during their storage and should only be considered as

197   quantifications in the products before use.

198   Interestingly, the exact same profiles were observed for both Irgafos 168® forms between bottle

199   and powder of each reagent. The form of Irgafos® 168 that was found in a higher concentration

200   in the bottle was also highest in the associated reagent powders suggesting that the content in

201   the powder is principally due to the leaching from the container. This highlights that the

202   leaching process may occur for both forms of Irgafos® 168, as both forms were transferred to

203   the powder in ratios comparable to the container. It is also possible that oxidation occurred after

204   leaching.

205   The ubiquity of Iragfos® 168 in the laboratory workplace could pose challenges when assessing

206   the leaching of plastic additives with environmentally relevant concentrations of microplastics

207   (Lenz et al., 2016). In fact, the results of this study prevented us from performing the

208   aforementioned leaching experiment, that was initially plan to use environmental

209   concentrations of PE fragments loaded with Irgafos® 168. The present study reinforces

210   previous observations (Ekpo et al., 2012) which demonstrates that laboratory reagents could be

211   a significant source of plastic additives contamination in laboratory workplace. For other plastic

212   additives, like phthalates, BPA or nonylphenol, the risk of contamination in the laboratory

213   workplace is well known and already considered in some studies (Net et al., 2015; Soto et al.,

214   1991; Ye et al., 2013). Similar inventory work should be undertaken for other plastic additives

215   commonly used and found in the environment (Hermabessiere et al., 2017). Thereafter,

216   measures can be taken to limit the contamination by plastic additives of the workplace such as

217   using glassware instead of plastic equipment, using water grades, using chemicals supply in

218   glass containers and, if necessary, exchanging data with suppliers to find suitable production

219   and storage procedures.

220   **3.3   Environmental implications**

221   Although it has been demonstrated that wildlife can become contaminated with plastic additives

222   through the ingestion of plastic pieces has been demonstrated (Jang et al., 2016; Tanaka et al.,

223   2015), their contribution to microplastic toxicity is not well understood (Zimmermann et al.,

224   2019). These results emphasize the fact that toxicity of MP with additives remains difficult to

225   assess, due to background noise. Furthermore, pristine MP containing additives may not be

226   environmentally relevant. Indeed, phthalates concentrations from new plastic gear (6.7 - 9.1

227   µg/L) used in oyster pearl farming were far higher in comparison with aged plastic gear (0.4 -

228   0.5 µg/L) (Gardon et al., 2020). When possible, pristine plastic toxicity should be compared

229   with the aged version of the products after long stays in the environment.

230   Nevertheless, the present work highlights that studying plastic additive found in plastic debris

231   is an important topic, especially to understand the fate of additives in the environment. Irgafos

232   168® is not very well studied except for food contact material (Cherif Lahimer et al., 2017).

233   This plastic additive, was recently quantified in indoor dust (Liu and Mabury, 2018; Venier et

234   al., 2018) in various form, and was also found in plastic debris (Figure 1) contaminating marine

235   organisms and seawater (Gardon et al., 2020; Rani et al., 2017, 2015; Tanaka et al., 2019).

236   Testing and understanding the sources, fate and effects of Irgafos 168® in aquatic environments

237 will therefore require new methods to overcome contamination throughout the sampling and

238 analysis in the laboratory.

4 Conclusion

240 In conclusion, the present study demonstrates that a plastic additive used as an antioxidant,

241 Irgafos® 168, was detected in different laboratory materials including plastic packaging and

242 more problematically reagents powders. Reduced and oxidized forms of Irgafos® 168 were

243 ubiquitous in the laboratory workplace at high concentrations, which prevented further study

244 of Irgafos® 168 leaching from plastics in realistic conditions.

Declaration of competing interest

246 The authors declare no competing financial interest.

Acknowledgements

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CRediT authorship contribution statement

426

427 Ludovic HERMABESSIERE: Conceptualization; Investigation; Formal analysis;
428 Visualization; Writing – Original Draft; Writing – Review and Editing
429 Justine RECEVEUR: Investigation
430 Charlotte HIMBER: Resources; Investigation
431 David MAZURAIS: Conceptualization Writing – Review and Editing
432 Arnaud HUVET: Conceptualization; Writing – Review and Editing
433 Fabienne LAGARDE: Conceptualization; Writing – Review and Editing;
434 Christophe LAMBERT: Conceptualization; Writing – Review and Editing
435 Ika PAUL-PONT: Conceptualization; Writing – Review and Editing
436 Alexandre DEHAUT: Conceptualization; Resources; Investigation; Visualization; Writing –
437 Review and Editing
438 Ronan JEZEQUEL: Investigation; Conceptualization; Writing – Review and Editing
439 Philippe SOUDANT: Conceptualization; Writing – Review and Editing; Supervision
440 Guillaume DUFLOS: Conceptualization; Resources, Writing – Review and Editing;
441 Supervision

Figure captions

442

443 Figure 1: Name, CAS number and structure of transformation product of Irgafos® 168 obtained

444 after oxidation and hydrolysis. Schematic drawing adapted from (Liu and Mabury, 2018; Yang

445 et al., 2016)

446 Figure 2: Irgafos® 168 concentration (in ng/mg of matrix) on its reduced (tris(2,4-di-tert-

447 butylphenyl)phosphite) and oxidized (tris(2,4-di-tert-butylphenyl)phosphate) form in caps (A)

448 bottles (B) and powders (C) of used laboratory reagents to create in vitro digestive models.

449 Results are expressed as mean (n=3) + 2 Standard Error. n.d: not detected; LW: Laboratory

450 water. *: $p < 0.05$ and ***: $p < 0.001$ using t test.

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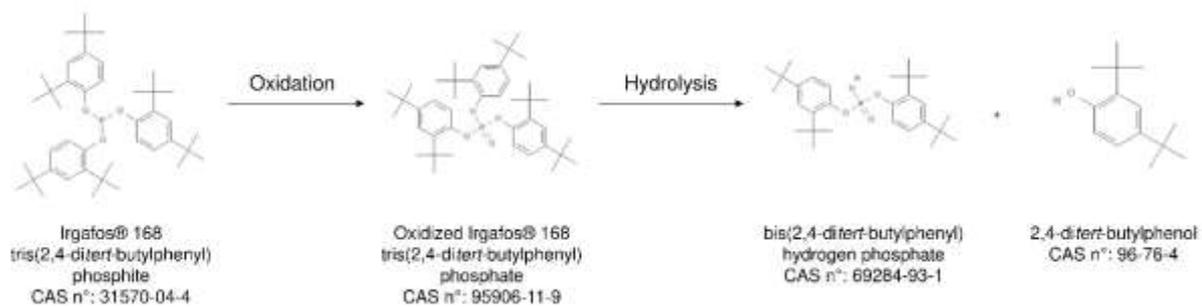
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453 **Figure 1**

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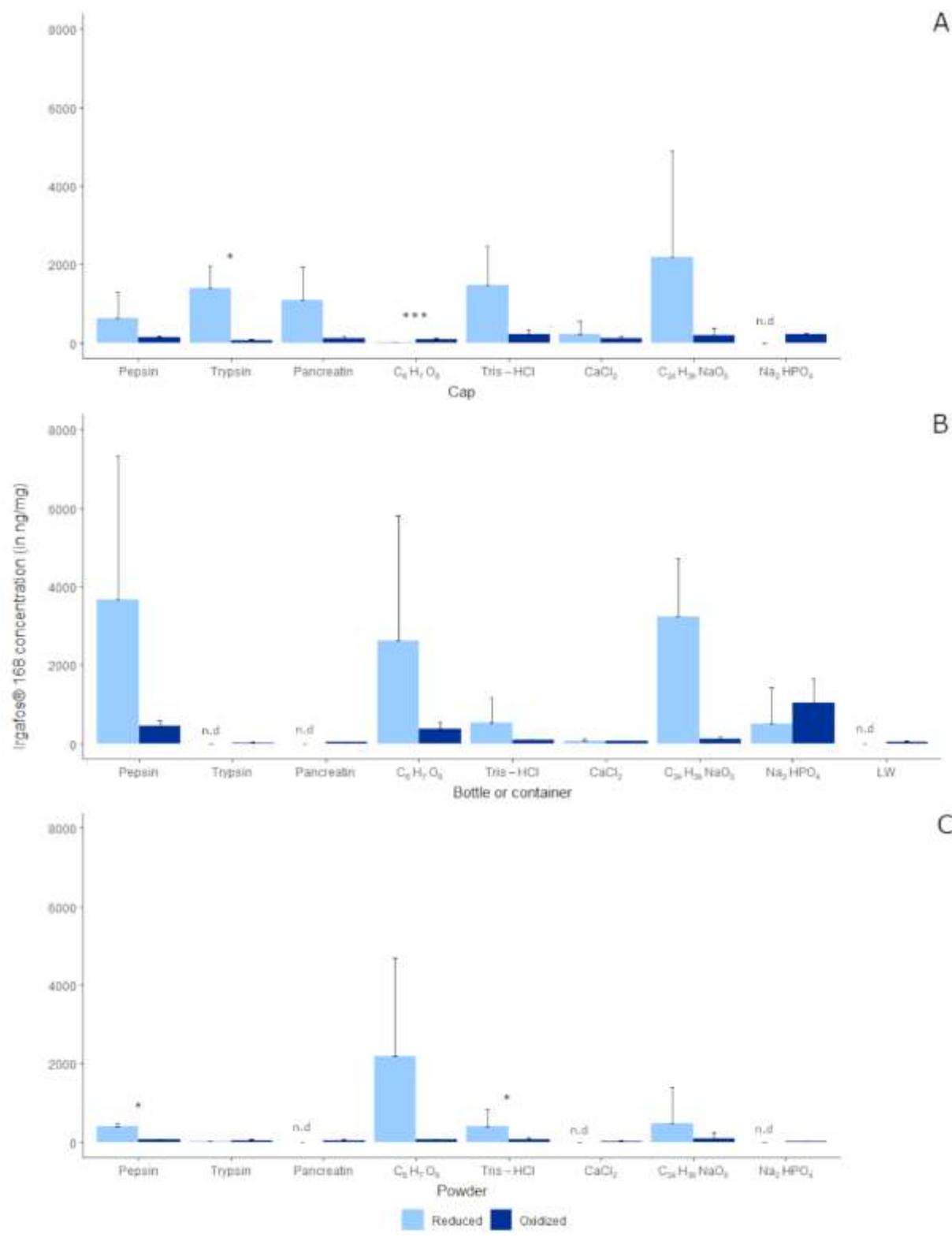
Figure 2

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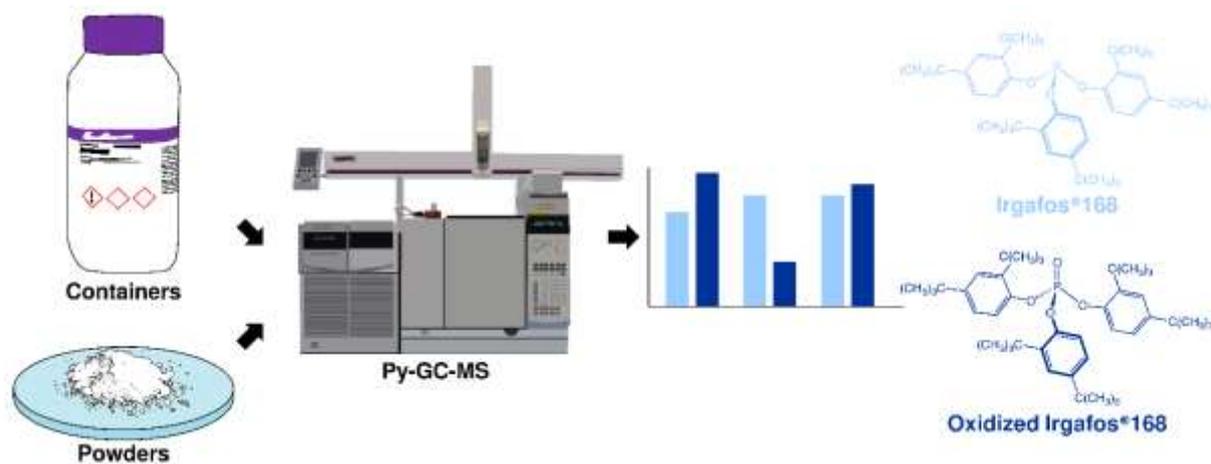
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459   **Graphical abstract**