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► **To cite this version:**

Ludovic Hermabessiere, Justine Receveur, Charlotte Himber, David Mazurais, Arnaud Huvet, et al.. An Irgafos® 168 story: when the ubiquity of an additive prevents studying its leaching from plastics. Science of the Total Environment, 2020, 749, pp.141651. 10.1016/j.scitotenv.2020.141651 . anses-02915461

HAL Id: anses-02915461

<https://anses.hal.science/anses-02915461v1>

Submitted on 14 Aug 2020

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1 **An Irgafos® 168 story: when the ubiquity of an additive prevents studying**
2 **its leaching from plastics**

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15 Published in *Science of The Total Environment* and available online at
16 <https://doi.org/10.1016/j.scitotenv.2020.141651>

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17 **Abstract**

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

38 **Keywords**

39 Microplastic; Additive; Leaching; Pyrolysis; Contamination; Irgafos 168®

40 **1 Introduction**

41 Plastic debris contaminates the environment broadly (Galgani et al., 2015; Horton et al., 2017).
42 With an increasing production of plastic since the 1950's (Geyer et al., 2017; PlasticsEurope,
43 2019) and a potential surge in production (Geyer et al., 2017), the concentration of plastic debris
44 at the ocean surface will rise (Lebreton et al., 2019, 2018). During its manufacturing, plastic is
45 made with other substances, called plastic additives that are added to confer properties to the
46 polymer (e.g. flexibility, durability, plasticity). As most plastic additives are not chemically
47 bound to the polymeric matrix, they can leach out of the plastic (Hermabessiere et al., 2017)
48 being an additional source of additives in the environment (Al-Odaini et al., 2015). Plastic
49 debris was estimated to be the source of 190 metric tons of 20 plastic additives that entered the
50 oceans in 2015 (De Frond et al., 2019).

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

59 Irgafos® 168 (tris(2,4-*di*tert-butylphenyl) phosphite) (Figure 1) is a plastic additive mainly
60 incorporated into Polypropylene (PP) and Polyethylene (PE) as an antioxidant (Dopico-García
61 et al., 2007). It has been used in food packaging (Cherif Lahimer et al., 2017; Dopico-García et
62 al., 2007), supposedly explaining its detection in frozen vegetable (Tanaka et al., 2003).
63 Irgafos® 168 leached out of food packaging in contact with oil (Garde et al., 1998; Marcato et
64 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-ditert-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-ditert-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-ditert-
79 butylphenyl) phosphite) and the oxidized (tris(2,4-ditert-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 (C₆H₇O₈, reference: 251275), sodium
87 phosphate dibasic (Na₂HPO₄, reference: S7907), sodium cholate hydrate (C₂₄H₃₉NaO₅,
88 reference: C1254), calcium chloride dihydrate (CaCl₂, 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-ditert-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 met. 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 \pm 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 $\text{C}_{24}\text{H}_{39}\text{NaO}_5$
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 $\text{C}_6\text{H}_7\text{O}_8$ (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 $\text{C}_{24}\text{H}_{39}\text{NaO}_5$
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, C₆H₇O₈, Tris-HCl and C₂₄H₃₉NaO₅), while
189 others (trypsin, pancreatin, CaCl₂ and Na₂HPO₄) 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 $\mu\text{g/L}$) used in oyster pearl farming were far higher in comparison with aged plastic gear (0.4 -
228 0.5 $\mu\text{g/L}$) (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.

239 **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.

245 **Declaration of competing interest**

246 The authors declare no competing financial interest.

247 **Acknowledgements**

248 Ludovic Hermabessiere is thankful to ANSES (French Agency for Food, Environmental and
249 Occupational Health & Safety) and the Hauts-de-France Region for the financial support of his
250 PhD. The present work was funded by the ANR Nanoplastics project (ANR-15-CE34-0006)
251 and has been financially supported by the European Union (ERDF), the French State, the French
252 Region Hauts-de-France and Ifremer, in the framework of the project CPER MARCO 2015-
253 2020. The authors are also thankful to Kennedy Bucci, Hannah De Frond, Rachel K. Giles and
254 Clara Thaysen for their helpful revision of English and their comments on the manuscript.

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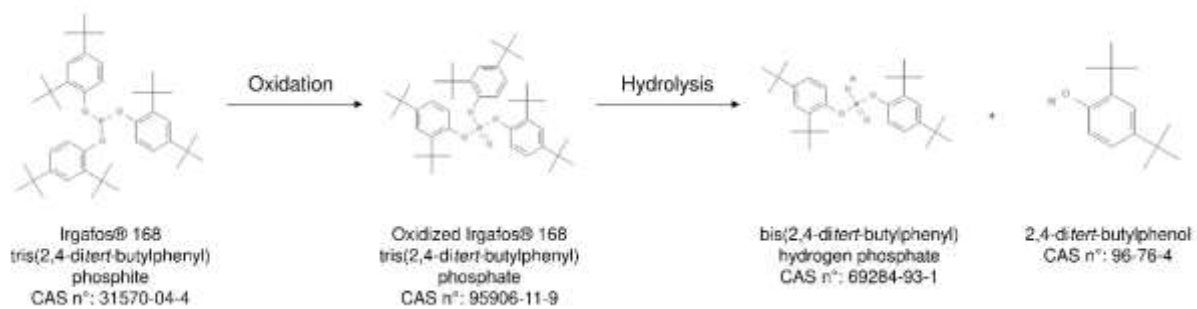
440 Guillaume DUFLOS: Conceptualization; Resources, Writing – Review and Editing;
441 Supervision

442 **Figure captions**

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.

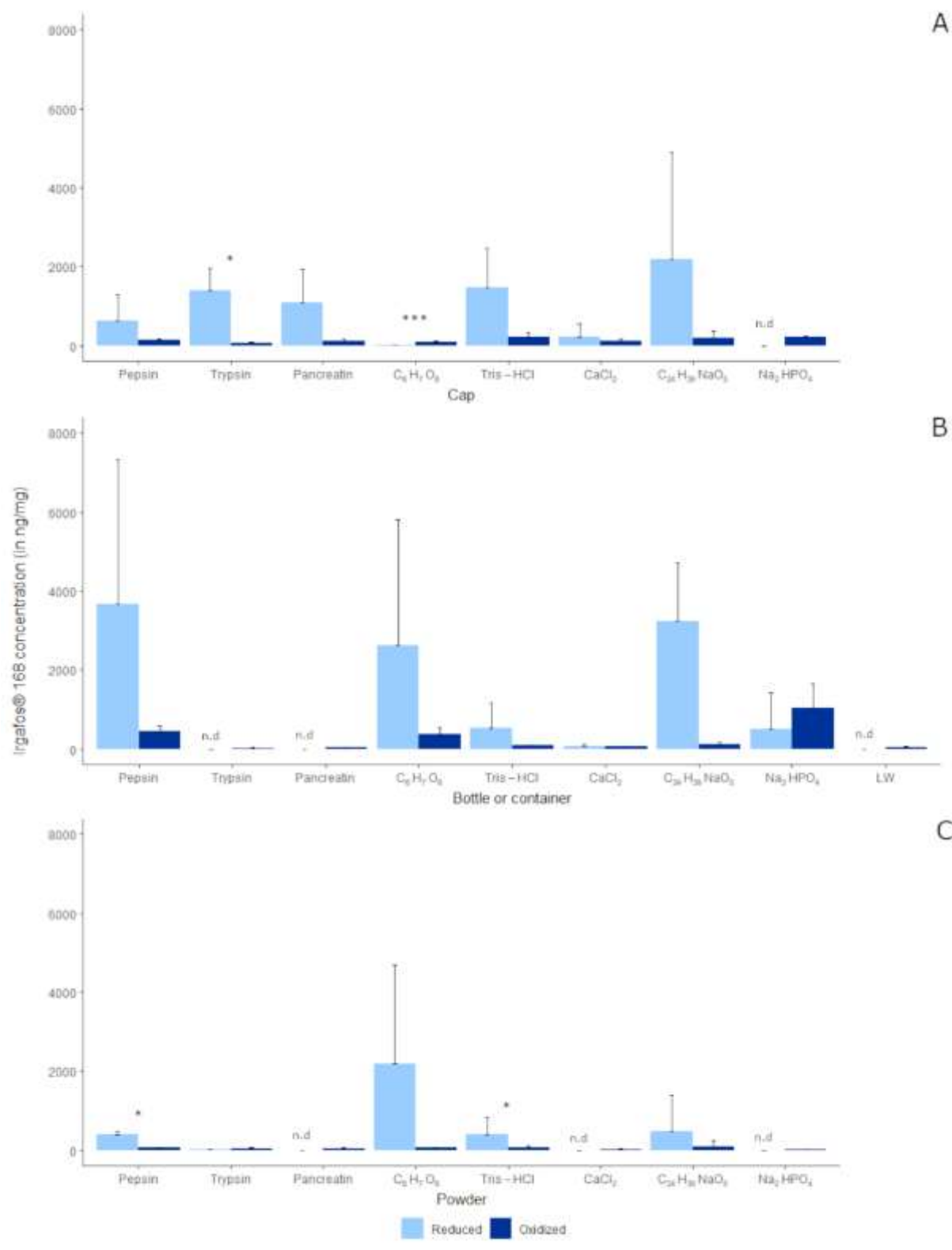
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453 **Figure 1**

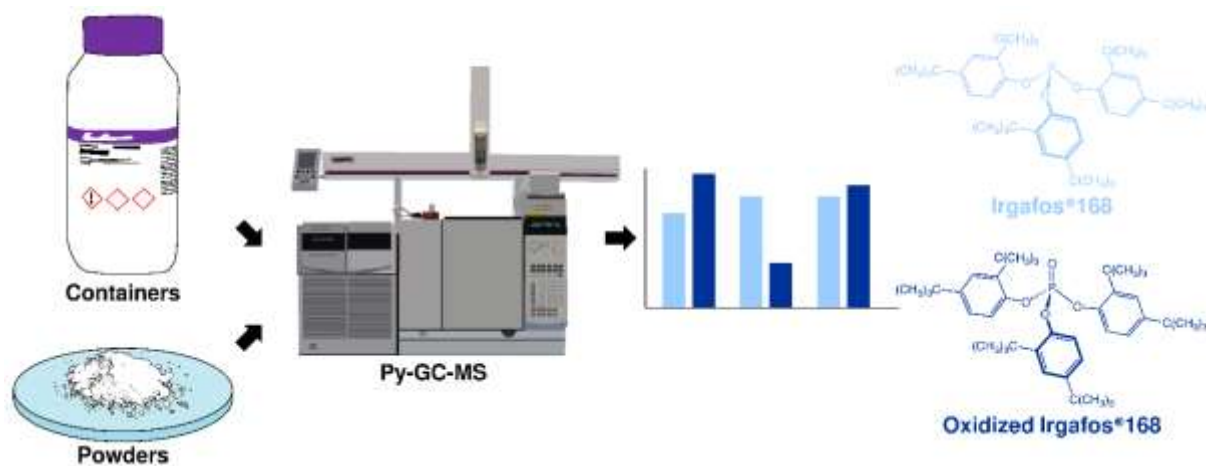
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456 **Figure 2**

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458

459 **Graphical abstract**