



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

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

Ludovic HERMABESSIERE <sup>a, 1, \*</sup>, Justine RECEVEUR <sup>b</sup>, Charlotte HIMBER <sup>a</sup>, David MAZURAS <sup>c</sup>, Arnaud HUVET <sup>c</sup>, Fabienne LAGARDE <sup>d</sup>, Christophe LAMBERT <sup>c</sup>, Ika PAUL-PONT <sup>c</sup>, Alexandre DEHAUT <sup>a</sup>, Ronan JEZEQUEL <sup>b</sup>, Philippe SOUDANT <sup>c</sup> & Guillaume DUFLOS <sup>a</sup>

<sup>a</sup> ANSES – Laboratoire de Sécurité des Aliments, Boulevard du Bassin Napoléon, 62200 Boulogne-sur-Mer, France.

<sup>b</sup> CEDRE, 715 Rue Alain Colas, 29218 Brest Cedex 2, France.

<sup>c</sup> Univ Brest, Ifremer, CNRS, IRD, LEMAR, F-29280, Plouzané, France.

<sup>d</sup> Le Mans Université, Institut des Molécules et Matériaux du Mans - IMMM-UMR-CNRS 6283, 72085 Le Mans Cedex 9, France

\* Corresponding author

[ludovic.hermabessiere@utoronto.ca](mailto:ludovic.hermabessiere@utoronto.ca)

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<sup>1</sup> Present address: Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada

## 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.

## Keywords

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

## 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

from PE placed in stirred water (Suhrhoﬀ and Scholz-Böttcher, 2016). Both forms were also detected in plastic pieces used in oyster farming (Gardon et al., 2020) or ingested by seabirds (Tanaka et al., 2019) and detected in indoor dust (Liu and Mabury, 2018; Venier et al., 2018). Irgafos® 168 can be also hydrolysed over time in the aquatic system and one of its degradation products, the 2,4-ditert-butylphenol (Figure 1) has been found in plastics collected on Korean beaches (Rani et al., 2017, 2015). A second degradation product (bis(2,4-ditert-butylphenyl) hydrogen phosphate) (Figure 1) was reported to impair growth of human cells in culture (Hammond et al., 2013) while Chinese hamster ovary cell lines are also sensitive to this chemical (Kelly et al., 2016; Shah et al., 2016). Oxidized Irgafos® 168 has been detected in one laboratory solvent, likely the result of leaching during chemical storage in the laboratory (Ekpo et al., 2012), it appears necessary to perform an inventory of potential Irgafos® 168 contaminations in the laboratory workplace prior to any leaching study, such as those using *in vitro* simulated gut conditions as already used to study the desorption of oestrogenic chemicals from plastic items (Coffin et al., 2019). In the present paper, the reduced (tris(2,4-ditert-butylphenyl) phosphite) and the oxidized (tris(2,4-ditert-butylphenyl) phosphate) forms of Irgafos® 168 were quantified in both the laboratory chemicals used to mimic *in vitro* (*i.e.* Coffin et al., 2019) gut conditions and their containers to account for potential contamination.

## 2 Material and methods

### 2.1 Laboratory reagents

Eight chemical reagents used in *in vitro* digestive models were analyzed for trace of Irgafos® 168 two forms. These laboratory reagents were exclusively acquired from Sigma-Aldrich (St-Quentin-Fallavier, France) and included: citric acid (C<sub>6</sub>H<sub>7</sub>O<sub>8</sub>, reference: 251275), sodium phosphate dibasic (Na<sub>2</sub>HPO<sub>4</sub>, reference: S7907), sodium cholate hydrate (C<sub>24</sub>H<sub>39</sub>NaO<sub>5</sub>, reference: C1254), calcium chloride dihydrate (CaCl<sub>2</sub>, reference: C5080), Tris-HCl (reference: T3253), pepsin (reference: 77160), trypsin (reference: T4799) and pancreatin (reference:

P1750). The plastic packaging (bottle and cap) were also analyzed. Analyses were only carried out on the container of reverse osmosed and deionized water (reference: 102927G, VWR, Fontenay-sous-Bois, France) used daily in the laboratory (hereafter call: Laboratory water (LW)). Analyses were only carried out on the plastic material of the container of the reverse osmosed and deionized water (reference: 102927G, VWR, Fontenay-sous-Bois, France) used daily in the laboratory (hereafter call: Laboratory water (LW)). The main advantage of using Py-GC/MS for this work was the minimal samples preparation required by this technique (Bart, 2001). Unfortunately, this Py-GC/MS technique is not adapted to quantify chemicals in solution as in the water.

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

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

Methods published by Fries et al., (2013) were applied for the Pyrolysis-GC/MS (Py-GC/MS) analyses. Approximately 500 µg of matrix, *i.e.* plastic packaging or powder, was weighed with 0.00001 g precision (XP205, Mettler Toledo, Viroflay, France) then transferred to pyrolysis vials. Py-GC/MS analysis was performed with a 7890N gas chromatograph (Agilent, Little Falls, USA) equipped with a Combipal MPS2 multifunctional injection system (Gerstel, Sursee, Switzerland), used in 'splitless' mode (1 min), and a Gerstel pyrolyser (Sursee, Switzerland). The temperature-controlled cooling injection system was programmed from 50°C (0.3 min) to 350°C (2 min) at 12°C/s then the sample was pyrolysed at 700°C for 60 s. During pyrolysis time, the temperature of the interface was maintained at 350°C. The oven temperature program was set from 50°C (2 min) to 320°C (10 min) at 20°C/min. Helium carrier gas used at a constant flow rate of 1.2 mL/min. The capillary column was an HP-5-MS (Agilent, Little Falls, USA): 30 m × 0.25 mm × 0.25 µm (thickness). The chromatograph was coupled to a 5975N mass spectrometry detector (Agilent, Little Falls, USA). Quantitative compounds analysis was carried out in single ion monitoring (SIM) mode with the following marker ions for reduced

Irgafos® 168 ( $m/z = 441$ ) and its oxidized form tris(2,4-di*tert*-butylphenyl) phosphate ( $m/z=316$ ) (minimum of 1.5 cycles/s for dwell time). Compounds were quantified using external calibration curves method ranging from 5 ng to 1000 ng. Irgafos® 168 reduced form, (CAS no: 31570-04-4, Sigma-Aldrich, St-Quentin-Fallavier, France), and oxidized form (CAS no: 95906-11-9, Toronto Research Chemicals, North York, Canada), were used as standards for quantification.

### 2.3 Statistical analysis

All statistical analyses were performed using R Statistical Software version 3.5.2 (R Core Team, 2018) using RStudio version 1.1.463 (RStudio Team, 2016). Effect of matrix (cap, bottle or powder) on reduced and oxidized Irgafos® 168 concentrations was tested using Kruskal-Wallis tests as neither normality (Shapiro-Wilk test), or homoscedasticity (regression residues), requirements were met. Where significant differences were found with Kruskal-Wallis, the ‘*agricolae*’ package (version 1.2-8) (De Mendiburu, 2017) was used, to perform post-hoc test using the Fisher’s least significant difference criterion realized with Bonferroni correction. Concentrations of reduced and oxidized Irgafos® 168 in each matrix were compared using a Student t test after checking the normality of the data and the equality of variances. If variances were not equal, Welch correction was applied. A non-parametric Mann-Whitney test was used for non-normally distributed data. Significant differences were considered when the p-value was below 0.05 and all results are expressed as mean  $\pm$  2 Standard Error.

## 3 Results and discussion

### 3.1 Reduced and oxidized Irgafos 168® concentrations: ubiquitous in the laboratory workplace

At least one of the forms of Irgafos® 168 was always detected in caps, bottles and powders of the eight-laboratory reagents (Figure 2). For reduced Irgafos® 168, concentrations in caps

ranged from under the limit of detection (n.d) in  $\text{Na}_2\text{HPO}_4$  to  $3265 \pm 2782$  ng/mg in  $\text{C}_{24}\text{H}_{39}\text{NaO}_5$  (Figure 2A), in bottles between n.d in trypsin and pancreatin and  $5,493 \pm 24$  ng/mg in pepsin (Figure 2B) and in powder between n.d in pancreatin,  $\text{CaCl}_2$  and  $\text{Na}_2\text{HPO}_4$  and  $3,000 \pm 2,507.76$  ng/mg in  $\text{C}_6\text{H}_7\text{O}_8$  (Figure 2C). For oxidized Irgafos® 168 concentrations in caps were between  $68.11 \pm 23.01$  ng/mg in trypsin and  $218.13 \pm 102.13$  ng/mg in Tris-HCl (Figure 2A), in bottles between  $16.33 \pm 10.48$  ng/mg in trypsin and  $1,023 \pm 634.61$  ng/mg in  $\text{Na}_2\text{HPO}_4$  (Figure 2B) and in powder between  $8.33 \pm 8.97$  ng/mg in  $\text{Na}_2\text{HPO}_4$  and  $95 \pm 157.23$  ng/mg in  $\text{C}_{24}\text{H}_{39}\text{NaO}_5$  (Figure 2C). Irgafos® 168 in its oxidized form was also detected in the container of LW at a concentration of  $33.92 \pm 34.46$  ng/mg (Figure 2B).

Overall, both Irgafos® 168 forms were significantly more abundant in plastic pieces, caps and bottles ( $510.13 \pm 257.77$  and  $710.24 \pm 411.08$  ng/mg, respectively), than in their respective reagent powders ( $238.52 \pm 216.49$  ng/mg) (post-hoc after Kruskal-Wallis,  $p < 0.05$ ). When present at concentrations above 100 ng/mg (except for  $\text{Na}_2\text{HPO}_4$  bottle), reduced form was always found in higher amount than oxidized form in caps, bottles and powder of each reagent (Figure 2). These differences were only statistically significant (t test,  $p < 0.05$ ) for trypsin cap and for pepsin and Tris-HCl bottles (for concentrations above 100 ng/mg) (Figure 2). There was high variability in Irgafos 168® concentrations across matrices (Figure 2). This high variability might be due to the fact that Irgafos® 168 concentration may not be homogeneously distributed in the polymer matrix, especially considering that this type of plastic additive is not chemically bound to the polymer (Hermabessiere et al., 2017). Variability may also occur between batches of the same product as suppliers may use different packaging producers for the same product, who each may potentially use a different mix of additives.

Both forms of Irgafos® 168 were ubiquitously detected in the tested laboratory reagents, even in the powders. All laboratory reagents tested here were housed in High-Density PE (HDPE)



bottles with PP caps. Additionally, the LW was also stocked into a bag-in-box composed of PE with quantifiable amounts of Irgafos® 168 that might lead to LW contamination over time. Irgafos® 168 is a plastic additive used as an antioxidant in polyolefin with concentration ranging from 0.004 to 3% (wet weight) (European Chemicals Agency, 2019; Hahladakis et al., 2018). Oxidation from the reduced form to the oxidized form is a common phenomenon for this molecule (Yang et al., 2016), thus making the protection of the polymer against oxidation possible (Fouyer et al., 2012). In studies concerning Irgafos® 168 from food packaging, both forms are usually targeted (Dopico-García et al., 2007; El Mansouri et al., 1998; Suhrhoff and Scholz-Böttcher, 2016; Yang et al., 2016). According to Yang et al., (2016) oxidation of Irgafos® 168 can occur either during the extrusion of the polymer, during storage in the dark or during exposure to UV; the latter being the fastest pathway (Yang et al., 2016). Here it is not possible to assess whether the oxidation process of Irgafos® 168 in the laboratory has occurred (i) during storage in the dark as laboratory reagents were stocked in safety cabinets or in the fridge straight from their delivery at the laboratory until their use or (ii) during the production and transport process of both the packaging and the reagent. It was recently suggested that when studying antioxidants, the oxidized form should be primarily targeted as the reduced form will react with oxygen and will not accumulate in the environment (Wu et al., 2019). This is confirmed in the present study as the oxidized Irgafos® 168 was systematically detected in quantifiable amounts whereas its reduced form was under the limit of detection in some laboratory reagents.

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

Concentrations of both Irgafos 168® forms were two times lower in the powder compared to the bottle, suggesting that leaching could probably be a slow, long and most often an incomplete process (Yang et al., 2016) that remain to be further deeply studied. Leaching was mostly dependent on the considered reagent. Indeed, some laboratory reagents demonstrated higher

concentrations of reduced Irgafos® 168 (pepsin, C<sub>6</sub>H<sub>7</sub>O<sub>8</sub>, Tris-HCl and C<sub>24</sub>H<sub>39</sub>NaO<sub>5</sub>), while others (trypsin, pancreatin, CaCl<sub>2</sub> and Na<sub>2</sub>HPO<sub>4</sub>) showed higher concentrations of oxidized Irgafos® 168. These differences could be linked to factors such as the conservation time and temperature, the age of the products, and their containers. However, it was not possible to retrieve information about reagent production from the manufacturer certificate of origin and analysis. Consequently, retracing the history of the reagents processing prior to their arrival in the lab is important in order to draw conclusions about the leaching process between the packaging and the reagents. Here, it is not possible to rule out whether leaching occurred before lab delivery of the chemicals or during their storage and should only be considered as quantifications in the products before use.

Interestingly, the exact same profiles were observed for both Irgafos 168® forms between bottle and powder of each reagent. The form of Irgafos® 168 that was found in a higher concentration in the bottle was also highest in the associated reagent powders suggesting that the content in the powder is principally due to the leaching from the container. This highlights that the leaching process may occur for both forms of Irgafos® 168, as both forms were transferred to the powder in ratios comparable to the container. It is also possible that oxidation occurred after leaching.

The ubiquity of Iragfos® 168 in the laboratory workplace could pose challenges when assessing the leaching of plastic additives with environmentally relevant concentrations of microplastics (Lenz et al., 2016). In fact, the results of this study prevented us from performing the aforementioned leaching experiment, that was initially plan to use environmental concentrations of PE fragments loaded with Irgafos® 168. The present study reinforces previous observations (Ekpo et al., 2012) which demonstrates that laboratory reagents could be a significant source of plastic additives contamination in laboratory workplace. For other plastic additives, like phthalates, BPA or nonylphenol, the risk of contamination in the laboratory

workplace is well known and already considered in some studies (Net et al., 2015; Soto et al., 1991; Ye et al., 2013). Similar inventory work should be undertaken for other plastic additives commonly used and found in the environment (Hermabessiere et al., 2017). Thereafter, measures can be taken to limit the contamination by plastic additives of the workplace such as using glassware instead of plastic equipment, using water grades, using chemicals supply in glass containers and, if necessary, exchanging data with suppliers to find suitable production and storage procedures.

### 3.3 Environmental implications

Although it has been demonstrated that wildlife can become contaminated with plastic additives through the ingestion of plastic pieces has been demonstrated (Jang et al., 2016; Tanaka et al., 2015), their contribution to microplastic toxicity is not well understood (Zimmermann et al., 2019). These results emphasize the fact that toxicity of MP with additives remains difficult to assess, due to background noise. Furthermore, pristine MP containing additives may not be environmentally relevant. Indeed, phthalates concentrations from new plastic gear (6.7 - 9.1  $\mu\text{g/L}$ ) used in oyster pearl farming were far higher in comparison with aged plastic gear (0.4 - 0.5  $\mu\text{g/L}$ ) (Gardon et al., 2020). When possible, pristine plastic toxicity should be compared with the aged version of the products after long stays in the environment.

Nevertheless, the present work highlights that studying plastic additive found in plastic debris is an important topic, especially to understand the fate of additives in the environment. Irgafos 168® is not very well studied except for food contact material (Cherif Lahimer et al., 2017). This plastic additive, was recently quantified in indoor dust (Liu and Mabury, 2018; Venier et al., 2018) in various form, and was also found in plastic debris (Figure 1) contaminating marine organisms and seawater (Gardon et al., 2020; Rani et al., 2017, 2015; Tanaka et al., 2019). Testing and understanding the sources, fate and effects of Irgafos 168® in aquatic environments

will therefore require new methods to overcome contamination throughout the sampling and analysis in the laboratory.

## **4 Conclusion**

In conclusion, the present study demonstrates that a plastic additive used as an antioxidant, Irgafos® 168, was detected in different laboratory materials including plastic packaging and more problematically reagents powders. Reduced and oxidized forms of Irgafos® 168 were ubiquitous in the laboratory workplace at high concentrations, which prevented further study of Irgafos® 168 leaching from plastics in realistic conditions.

## **Declaration of competing interest**

The authors declare no competing financial interest.

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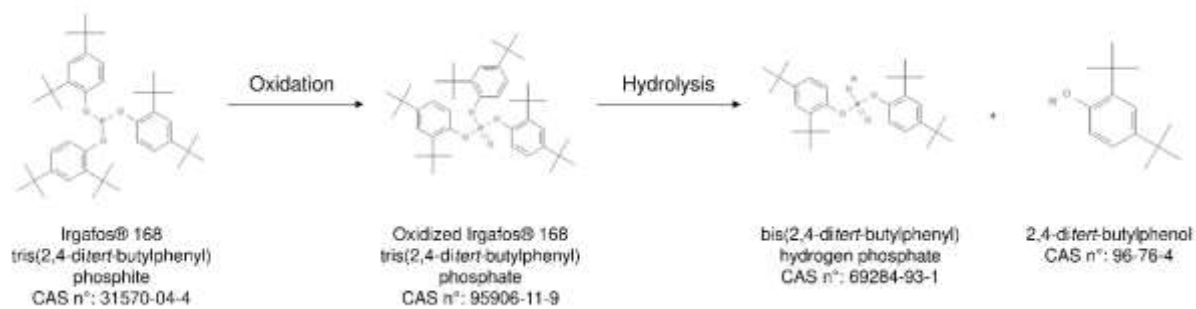
## **CRedit authorship contribution statement**

Ludovic HERMABESSIERE: Conceptualization; Investigation; Formal analysis; Visualization; Writing – Original Draft; Writing – Review and Editing  
 Justine RECEVEUR: Investigation  
 Charlotte HIMBER: Resources; Investigation  
 David MAZURAIS: Conceptualization Writing – Review and Editing  
 Arnaud HUVET: Conceptualization; Writing – Review and Editing  
 Fabienne LAGARDE: Conceptualization; Writing – Review and Editing;  
 Christophe LAMBERT: Conceptualization; Writing – Review and Editing  
 Ika PAUL-PONT: Conceptualization; Writing – Review and Editing  
 Alexandre DEHAUT: Conceptualization; Resources; Investigation; Visualization; Writing – Review and Editing  
 Ronan JEZEQUEL: Investigation; Conceptualization; Writing – Review and Editing  
 Philippe SOUDANT: Conceptualization; Writing – Review and Editing; Supervision  
 Guillaume DUFLOS: Conceptualization; Resources, Writing – Review and Editing; Supervision

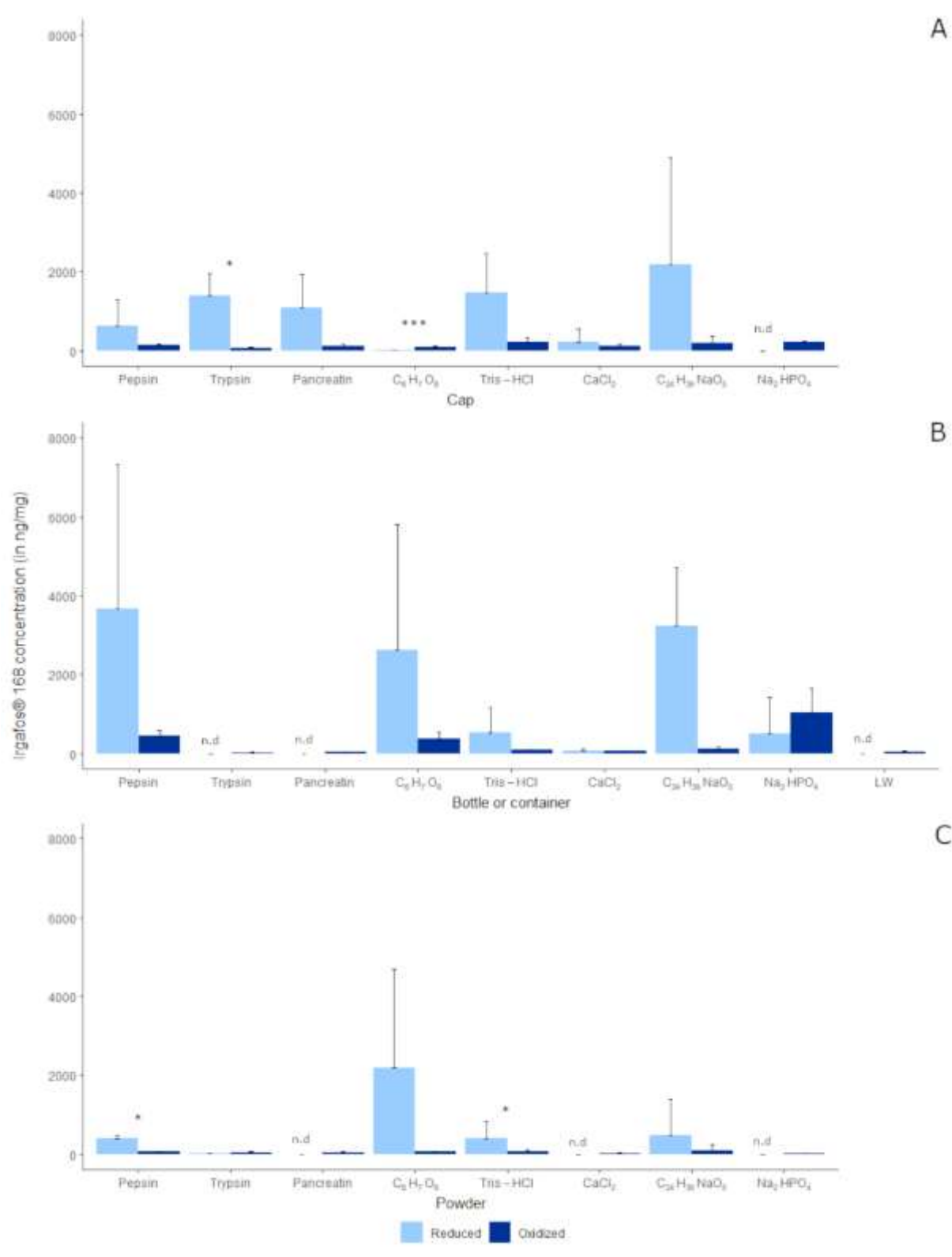
## **Figure captions**

Figure 1: Name, CAS number and structure of transformation product of Irgafos® 168 obtained after oxidation and hydrolysis. Schematic drawing adapted from (Liu and Mabury, 2018; Yang et al., 2016)

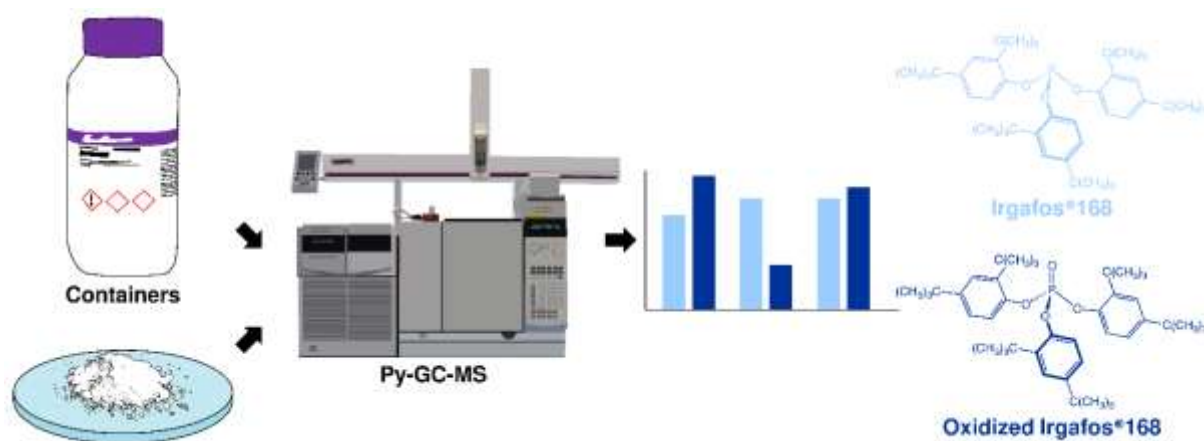
Figure 2: Irgafos® 168 concentration (in ng/mg of matrix) on its reduced (tris(2,4-di-tert-butylphenyl)phosphite) and oxidized (tris(2,4-di-tert-butylphenyl)phosphate) form in caps (A) bottles (B) and powders (C) of used laboratory reagents to create in vitro digestive models. Results are expressed as mean (n=3) + 2 Standard Error. n.d: not detected; LW: Laboratory water. \*:  $p < 0.05$  and \*\*\*:  $p < 0.001$  using t test.



**Figure 1**



**Figure 2**



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459 **Graphical abstract**