



Use of a nanoplastic carrier for assessing the aquatic toxicity of an organo-phosphite polymer additive[☆]

Marica E. Schiano^a, Carlos Edo^b, Enrique Blázquez-Blázquez^c, María L. Cerrada^c, Francisca Fernández-Piñas^{d,e}, Roberto Rosal^{b,*}

^a Dipartimento di Farmacia, Università Degli Studi di Napoli Federico II, Via D. Montesano, 49, I-80131, Naples, Italy

^b Department of Chemical Engineering, Universidad de Alcalá, E-28871, Alcalá de Henares, Madrid, Spain

^c Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC), Juan de la Cierva 3, 28006, Madrid, Spain

^d Department of Biology, Faculty of Science, Universidad Autónoma de Madrid, E-28049, Madrid, Spain

^e Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM), Universidad Autónoma de Madrid, C Darwin 2, 28049, Madrid, Spain

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ABSTRACT

This work reports the production of nanoplastics (NPs) from polypropylene (PP) free of the antioxidant Irgafos® 168 (IRG) and alkane oligomers (ALK). PP pellets were milled into a powder with particle sizes in the 100–500 µm range. Additives and oligomers were removed using dichloromethane, and the powder exposed to UV irradiation, followed by filtration through 1 µm filters. PP suspensions, free of antioxidant and oligomers, were reloaded with IRG and ALK to their original commercial concentrations. This approach allowed testing the aquatic toxicity of IRG at concentrations compromised by water solubility limits. Toxicity assays using the cladoceran *Daphnia magna* with 24–48 h immobilization of neonates as endpoint showed toxicity for NPs containing IRG, with EC₂₀ (48 h) in the 1.8–3.5 mg/L range, that corresponded to IRG exposure <1.2 µg/L. Suspensions of PP containing ALK, but not IRG, exhibited low toxicity (EC₂₀ > 20 mg/L). The results allowed estimating the toxicity of IRG with a EC₅₀ value of 3.3 ± 1.1 µg/L. Assays with different proportions of IRG and its oxidized form showed no differences. This work demonstrated the aquatic toxicity of IRG, for which there were no previous data, and developed a method for testing the toxicity of non-polar additives without being limited by their solubility.

1. Introduction

Plastics have become essential for modern life, playing pivotal roles in many industries and everyday products. With yearly production figures over 400 Mt, the lifecycle of plastic is still far from circular in spite of the efforts made to increase the share of recycled plastics (PlasticsEurope, 2024). Therefore, large quantities of unmanaged waste are produced, significantly contributing to widespread plastic pollution (Geyer et al., 2017; Jambeck et al., 2015). The environmental challenges associated with plastic misuse and waste have been extensively documented, particularly concerning microplastics (MPs). These tiny particles, measuring less than 5 mm in their largest dimension, are ubiquitous in our environment and have been detected in all ecosystems, including the most remote areas (GESAMP and Kershaw, 2015; González-Pleiter et al., 2020; Rosso et al., 2024). Most MPs result from the breakdown of

larger plastic items, such as the packaging materials, tyre abrasion, and the wearing of synthetic textiles when exposed to mechanical stress and photochemical degradation (Zhang et al., 2021). Plastic fragmentation results in progressively smaller sizes and when these particles reach sizes below 1 µm, they are typically referred to as nanoplastics (NPs) provided they have been generated from larger particles and exhibit colloidal behaviour (Gigault et al., 2018).

Commercial plastic materials incorporate different chemical additives to improve their performance during processing or use. The list of additives is extensive, including over 10,000 additives that serve as antioxidants, plasticizers, light stabilizers, antistatic agents, flame retardants, and pigments among other roles (Hahladakis et al., 2018). A recent study showed that a significant number of such additives can be of potential concern due to their hazardous properties, which include ecotoxicity, bioaccumulation potential and environmental persistence

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* Corresponding author.

E-mail address: roberto.rosal@uah.es (R. Rosal).

(UNEP, 2023). Surprisingly some are in use, even for food contact applications (Wiesinger et al., 2021). Furthermore, numerous substances have not been thoroughly characterized regarding their potential ecological impact (Maddela et al., 2023). Additives are essential for the performance and durability of plastics during their lifecycle, but as plastics degrade in the environment, they are eventually released creating a secondary source of chemical pollution (Luo et al., 2022).

Plastic products also incorporate a series of non-listed substances (NLS) or non-intended added substances (NIAS), which include catalyst remnants, solvents, and products of incomplete polymerization (Ariosti, 2022). Like additives, NLS and NIAS are typically not chemically bound, so they can migrate and potentially leak into the environment. The photochemical processes associated with the weathering and fragmentation of plastics accelerate the release of NLS and NIAS, eventually posing toxic risks to the surrounding biota (Gunaalan et al., 2020). This topic has been extensively addressed in the context of food contact substances (Geueke et al., 2023). Specifically, ultraviolet (UV) irradiation has been applied as a nonthermal method to improve the safety and shelf life of numerous food products without the quality losses often associated with thermal processing methods. However, UV irradiation may trigger the formation of degradation products from packaging additives and facilitate the release of NLS and NIAS potentially causing undesirable flavours or even toxicity in food products (Nerin et al., 2013).

Many plastic additives have low water solubility, which is generally desirable as it reduces the potential leaching of highly non-polar substances into aquatic environments. Additives are often designed for high compatibility with the polymer, ensuring minimal solubility in the materials they contact (Hahladakis et al., 2018). However, the low water solubility of many additives makes it difficult to perform risk assessments. The use of co-solvents has drawbacks, and solvent-free methods are challenging and not always feasible (Green and Wheeler, 2013). Additionally, hydrophobic additives do not all dissolve in the same chemical solvent, which limits meaningful comparisons and constrains research to saturation thresholds (Weyman et al., 2012). In this study, we aimed to address these challenges by incorporating the additive into a polymeric nanocarrier derived from commercial PP, the same polymer used in commercial formulations. This approach using nanoplastic carriers can be readily adapted for toxicity testing of other non-polar, insoluble substances on aquatic organisms.

Irgafos® 168, tris(2,4-di-*tert*-butylphenyl)phosphite, IRG, is a phosphite additive commonly used as antioxidant and stabilizer in the production of plastics. Specifically, it prevents the degradation of polymers caused by heat and oxygen exposure during processing and use upon conversion into its oxidized form, tris(2,4-di-*tert*-butylphenyl) phosphate, IRGox (Hermabessiere et al., 2020). IRG reacts with hydroperoxides formed by oxidation of polymers and other additives preventing process induced degradation. There are potential concerns regarding the use of IRG and related chemicals due to the inherent neurotoxicity associated with organophosphates (Tsai and Lein, 2021). This toxicity stems from their ability to bind to acetylcholinesterase, thereby phosphorylating the enzyme's active site (English and Webster, 2012). Based on the data available from the studies performed so far, it was estimated that daily intakes would be considerably below the acceptable rate intake meaning the absence of safety concerns (Markley et al., 2023). However, the solubility of IRG in water is very low (<5 µg/L at 20 °C), a fact that severely limits the information on its aquatic toxicity.

Here, we used polypropylene (PP) nanoplastics free of IRG and alkane oligomers (ALK) contained in a commercial PP as carriers to perform aquatic ecotoxicity assays. The nanocarrier was reloaded with IRG and ALK, jointly and separately. With this strategy, we aimed to test the toxicity of IRG and ALK contained in commercial PP to the cladoceran *Daphnia magna*. The data obtained allowed us deriving effect concentration corresponding to early responses (EC₁₀, EC₂₀) as surrogates for no observed effect concentration (NOEC) as well as an estimation of the EC₅₀ value for IRG.

2. Methods

2.1. Materials

Commercially available metallocene-catalysed isotactic polypropylene, (C₃H₆)_n, PP, melt flow index of 15 g/10 min (ISO 1133-1:2022), 4 ± 1 mm pellets in clear colour, with average molecular weight ~250,000 g/mol, was obtained from LyondellBasell (Rotterdam, Netherlands). PP was subject to Soxhlet extraction with dichloromethane (CH₂Cl₂, Sigma-Aldrich, >99.5 %) for 8 h. This process led to a material free of additives and oligomers, denoted as PPd hereinafter, and an extract (EXT) that includes substances soluble in CH₂Cl₂, from which the solvent was removed in a rotary evaporator. The extract was completely dried in a chromatographic vial under nitrogen flow and accurately weighed. An aliquot was re-dissolved in a specific volume of chloroform containing 4'-Pentyl-4-biphenylcarbonitrile (CAS 40817-08-1), as internal standard for further quantification using gas-chromatography as indicated below. Alkanes (ALK) were isolated from the extract by elution with hexane using a silica column activated for 24 h at 150 °C. The resulting fractions were analysed by GC-MS to verify the absence of IRG and IRGox. Those fractions that exclusively contained alkanes were pooled to carry out the exposure experiment, as indicated below. Irgafos® 168, IRG, tris(2,4-di-*tert*-butylphenyl)phosphite, [(C₄H₉)₂C₆H₃O]₃P, 98 % was acquired from Sigma-Aldrich. The content of alkane oligomers in PPd was below 2 % compared to the same material before extraction.

2.2. Generation of nanoplastics

The generation of nanoplastics (PP-NPs and PPd-NPs, <1 µm) was performed by combining mechanical breakdown followed by UV irradiation. The combination of both treatments is required to reach the desired concentration of NPs after filtering through 1 µm filters. First, plastic pellets were made brittle using liquid nitrogen and mechanically milled using a blender to obtain particle sizes in the 100–500 µm range. Then, the particles were subjected to UV irradiation in MilliQ water (20 g in 500 mL) using a medium pressure mercury vapour lamp, Heraeus TQ 150 (150 W), in a magnetically stirred photoreactor. Radiation dose and spectrum were measured using an Ocean Insight SR radiometer. The lamp spectrum is provided in Fig. S1 (Supplementary Material, SM). The average UV irradiance in the vessel was 93 mW cm⁻², and the irradiation time was set at 72 h, resulting in a fluence (UV dose) of 3 mJ cm⁻². After irradiation, the suspension was filtered with 1 µm glass fibre filters (Millipore) and stored in glass bottles that had been previously washed with ultrapure water at least three times and heated in a muffle furnace at 400 °C for 4 h to ensure the absence of particle contamination. The suspensions of PP and PPd were prepared from stock suspensions (NPs, filtered through 1 µm glass fibre filters) with concentrations greater than 100 mg/L. The concentration was calculated from the total carbon measured as TOC and converted into the mass concentration of the polymer using the mass of the structural unit of PP (85.7% carbon).

PPd-NPs were reloaded with the extract obtained after treatment with CH₂Cl₂, EXT, and separately, with the alkanes (ALK) obtained as indicated before and Irgafos® 168 (IRG) in order to mimic the composition of commercial PP. Accordingly, the following materials were obtained: PPd + EXT, PPd + ALK, PPd + IRG and PPd + ALK + IRG. Aliquots of the stock suspensions were reloaded with EXT, ALK, IRG, and ALK + IRG, maintaining the same concentration found in commercial PP (1.57 mg ALK/g PP and 350 mg IRG/kg PP), and then diluted for exposure experiments. To ensure homogeneity, the stocks were dispersed using a Sonics VibraCell ultrasound disperser (BioBlock Scientific). The concentration of the test suspensions was rechecked before use using the same procedure.

All tested materials are suspensions of NPs, <1 µm, a detail omitted in what follows for the sake of clarity. The re-additivation process was intended to separately test the toxicity of ALK, IRG and their

combination using the same polymeric PP matrix that would originate their exposure to the biota. Both ALK and IRG are highly water insoluble. However, they show high affinity towards PPD (evidenced by high log octanol-water partition coefficients as indicated below), which acts as a carrier in its nanoplastic suspension, allowing the exposure of the biota to otherwise impossible to test hydrophobic materials. Virgin PP (100–500 μm), which includes IRG and ALK in its formulation, was also processed with UV irradiation in the same way indicated before for PPD to check the possible toxic effects derived from the photochemical breakdown of ALK, IRG or the polymer backbone.

2.3. Analyses

The suspensions of nanoplastic plastics were analysed using Dynamic Light Scattering (DLS) and ζ -potential at 25 °C in a Malvern Zetasizer Nano ZS apparatus. The contents of carbon and nitrogen were assessed using a Multi N/C 3100 Series TOC-/TNb analyser (Analytic Jena). TOC was measured for all stocks and checked for the diluted suspensions used in exposure assays to ensure accurate exposure concentrations.

The analytical determination of IRG and its oxidation product, IRGox was carried out using a HP 6890 GC System equipped with an Agilent HP 5973 Mass Spectrometer. The separation was performed using a DB5-HT capillary column (15 m \times 250 μm , 0.1 μm) with helium as carrier gas at a flow rate of 1 mL/min. Ionization was performed by electron impact at 70 eV. The procedure was used to determine IRG concentration in PP, PPD, and in the reconstituted suspensions containing IRG. The content of IRG in PPD was below the quantification limit of the technique.

The chemical changes in the polymer and the characterization of the extraction products were performed by Fourier transform infrared spectroscopy (FTIR) using a total attenuated reflectance device (ATR-FTIR). Spectra were recorded in a PerkinElmer Spectrum Two Spectrophotometer and a Nicolet iS20 Spectrometer with a resolution of 4 cm^{-1} .

Calorimetric analyses were performed in a TA Instruments Q100 calorimeter connected to a cooling system with sample weights ranging from 6 to 8 mg range and in a temperature interval from 20 to 200 °C at a scanning rate of 20 °C/min.

2.4. Toxicity tests

The acute immobilization of the cladoceran crustacean *Daphnia magna* (MicroBioTests, Belgium) was tracked following the OECD Test Guideline 202 and the ISO Standard 6341 (ISO, 2012; OECD, 2004b). Hatching of ephippia was performed in Standard Freshwater Medium (FW) at 23 \pm 1 °C and continuous illumination under 6000 lux of irradiance for 72 h. Then, the organisms were exposed for 48 h to different concentrations of test suspensions prepared immediately before every toxicity assay. The dispersed stock suspensions were diluted in FW, also used as control, to obtain the appropriate testing concentrations. Both positive (viability) and negative controls for CH_2Cl_2 were performed in FW medium. All concentrations and blanks were tested in at least three independent assays. After 24 h and 48 h exposure, the number of immobilized daphnids was recorded as toxicological endpoint. Neonates unable to swim after gentle agitation of the exposure media for 15 s were considered immobile, even if they could still move their antennae. The relative toxicity of the samples was expressed as the percentage of immobilized organisms compared with the controls.

In this work, we used EC_x (the concentration corresponding to x-percent response) to determine the effect concentrations. EC_x , with a certain low x-value (EC_5 , EC_{10} or EC_{20}) is generally used as a surrogate for no observed effect concentration (NOEC), particularly in situations in which data cannot be obtained with enough accuracy (Tanaka et al., 2018). The selection of the value for x that is a more suitable analogue for NOEC is somewhat controversial (Beasley et al., 2015). In this work, we calculated EC_{10} and EC_{20} fitting data to the following logistic model:

$$E(c) = \frac{A}{1 + \left(\frac{x}{100 - x} \frac{c}{\text{EC}_x} \right)^b} \quad [1]$$

where $E(c)$ is the survival after exposure to a toxic concentration “c” for a given period (24 or 48 h), “b” is a coefficient denoting the steepness of the dose-response curve, and “A” is the expected fraction of viable organisms in controls (in this work $A = 1$). Dose-response curve fitting was performed with the statistical software R “drc” analysis package, version 3.0-1 (Ritz et al., 2016).

The joint toxic effect of several compounds in combined exposure can be related to their individual effects in separate exposures. If the compounds (A and B) are sufficiently different, their overall effect can be determined by the probability of the individual effects occurring independently (Backhaus and Faust, 2012):

$$E_{A+B} = 1 - (1 - E_A)(1 - E_B) \quad [2]$$

This relationship will be used below to obtain an estimation for the ecotoxicity of IRG.

3. Results and discussion

The content of extractable products in raw PP was 1.92 mg/g (1.57 \pm 0.02 mg ALK/g of PP and 350 \pm 10 mg IRG/kg of PP). Therefore, most of the extractable materials consisted of short chains of PP as revealed by the FTIR spectra shown in Fig. S2 (SM). In it, the stretching vibrations of $-\text{CH}_2$ and $-\text{CH}_3$ are clearly visible at 2850, 2920, and 2950 cm^{-1} , together with the symmetrical and asymmetrical bending of $-\text{CH}_3$ bonds at 1376 and 1460 cm^{-1} , all of them typical features of PP infrared spectrum. Besides, the absorptions at 840, 1000, and 1165 cm^{-1} are characteristic vibrations of terminal unsaturated CH_2 groups from isotactic PP. No apparent moieties associated to IRG such as the C-O-P band at 1212 cm^{-1} , were detected, which can be attributed to the low concentration of IRG relative to ALK (Hernández-Fernández et al., 2023).

3.1. Nanoplastic carrier

Fig. 1 presents the characterization results of PPD-NPs. DLS measurements (Fig. 1A) show the presence of nanoplastics in the few hundred of nanometre size when dispersed in MilliQ water (median size 375 nm). We performed additional filtration experiments on NP suspensions using 100 nm PTFE filters (Puradisc, Whatman), which determined that over 50 % of the particle mass (measured as TOC) could be attributed to particles smaller than 100 nm. Therefore, the observed hydrodynamic size partly consists of aggregates of smaller particles. This result is consistent with the high log octanol-water partition coefficient of alkane oligomers. Similarly, the log octanol-water partition coefficient of Irgafos 168 (15.5) is very high, which explains why neither IRG nor IRGox could be detected above their quantification limit (0.3 mg/L) in the liquid phase (Lynch et al., 2022). The zeta potential was -3.6 ± 1.5 mV (pH 7.0), slightly negative. This is a result commonly observed in water-hydrophobic interfaces due to the preferential affinity of hydroxide anions for the surface layer compared to hydronium (Roger and Cabane, 2012). The results corresponded to a suspension with tendency to form aggregates. A similar hydrodynamic size was observed for PPD suspensions in the FW medium used for *D. magna* exposure together with a background of the colloidal suspension provided by the FW itself, in the 1–4 μm range (Fig. S3, SM). The size of plastic particles in suspension was considerably smaller than the algae usually employed as food in *D. magna* immobilization assays (Rist et al., 2017). Therefore, the daphnids have been exposed to particles that can be internalized.

Fig. 1B shows the FTIR spectrum of the PPD carrier. For this analysis, a suspension was concentrated to form aggregates suitable for ATR-FTIR analyses. Along with the bands attributed to PP, the infrared spectrum

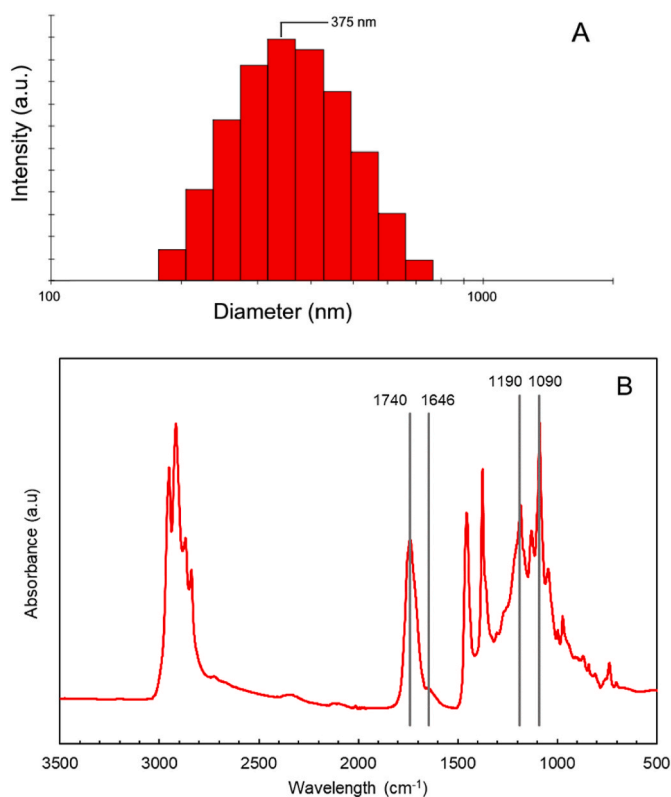


Fig. 1. Characterization of PPD (NPs, <1 μm) particles used as carrier: (A) DLS particle size; (B) FTIR spectrum.

shows several moieties associated with photooxidation. Under UV irradiation in the presence of oxygen, polyolefins undergo photooxidation reactions that start with the formation of O₂-polymer charge transfer complexes ultimately leading to a variety of oxygenated compounds including carboxylic acids, esters, lactones, alcohols and many more (Grause et al., 2020). The bands from oxygenated moieties are clear, especially the absorption in the 1740 cm⁻¹ range attributed to the stretching vibration of C=O bonds, most likely from esters. The band at 1646 cm⁻¹ corresponds to C=C bonds, and the bands at 1090 cm⁻¹ and 1190 cm⁻¹ can be attributed to C-O stretching and OC-O stretching respectively (Rjeb et al., 2000).

Figs. 2 and 3 present results from the characterization of PP and PPD particles with sizes ranging from 100 to 500 μm after irradiation treatment (+) and PP before irradiation (-). FTIR spectra (Fig. 2) showed no

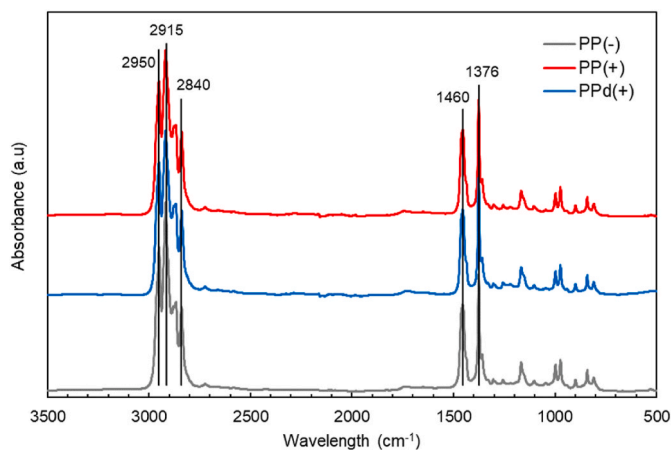


Fig. 2. FTIR spectra of PP and PPD particles after irradiation treatment (+) and PP before irradiation: PP(-).

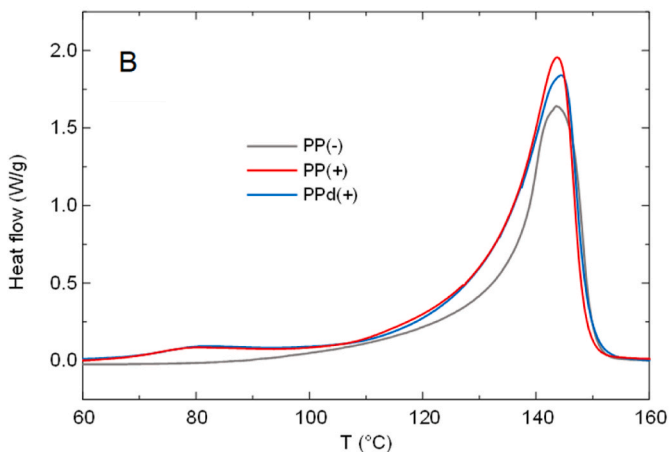
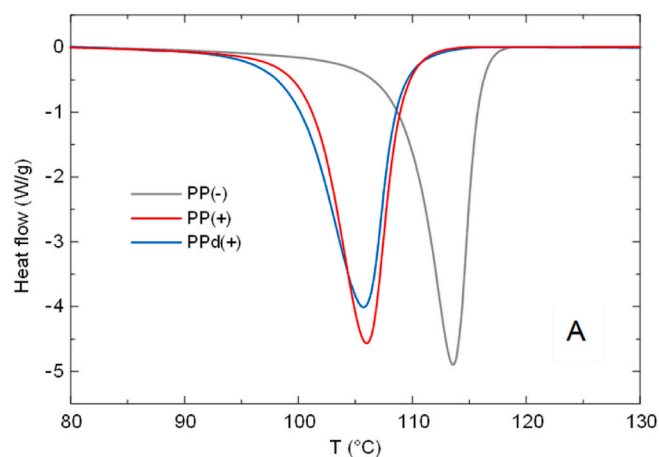


Fig. 3. DSC of PP and PPD particles after irradiation treatment (+) and PP before irradiation: PP(-).

difference among irradiated and non-irradiated particles. This is likely because altered and oxidized domains tend to detach from the surface of PP and PPD particles, leaving a material with same chemical structure as the parent raw polymer. Particle disintegration via surface cracking and delamination is a phenomenon observed both in laboratory and environmentally exposed plastics (Menzel et al., 2022; Nakatani et al., 2023). Conversely, DSC shows a clear shift in the crystallization temperature to lower values in irradiated materials compared to the pristine PP(-), as seen in Fig. 3A. This outcome can be explained by the fact that metallocene isotactic propylene simultaneously crystallizes in two crystalline polymorphs. When it degrades, chain scission occurs due to the tacticity errors of PP, and its ability to form orthorhombic crystals is reduced (Blázquez-Blázquez et al., 2019). In addition, this chain scission with subsequent formation of shorter macrochains can also be deduced from the appearance of a small endothermic peak in PP(+) and PPD(+) samples at around 80 °C during the heating run, indicating that a portion of their chains melt, because of their shorter length, at temperatures lower than the main peak, whose location is similar for both specimens (Fig. 3B).

3.2. Toxicity assays

PPd was used to recreate PP particles (NPs) with the same content of alkane oligomers (ALK) and/or antioxidant additive (IRG) found in the later. Therefore, we prepared suspensions of PPD-NPs (<1 μm) with 1.57 mg/g of ALK (PPd + ALK), 350 μg/g of IRG (PPd + IRG), 1.92 mg/g of the extract obtained from PP (PPd + EXT), and a combination of ALK and IRG in the same proportion found in raw PP (PPd + ALK + IRG). All

these materials were tested for toxicity using *D. magna* 48 h immobilization assays performed as explained before. Fig. 4 shows the toxicity responses for increasing concentrations of PP, PPd and reloaded PPd suspensions of NPs. The concentrations (in mg/L) refer to the whole mass, but essentially correspond to the polymer (PP or PPd).

The results showed a limited effect of PPd NPs, but a considerable toxicity exerted by raw PP NPs (suspension of particles <1 μm in both cases). In fact, PP NPs reached EC₅₀ for *D. magna* 48 h immobilization at 9.1 ± 0.7 mg/L, whereas the EC₅₀ for PPd NPs (free of ALK and IRG) was >50 mg/L, the highest assayed concentration (Fig. 4A). Low toxicity was also observed for PPd + ALK (the blend reconstructed without IRG, Fig. 4B). However, in suspensions containing IRG, the toxic response was clear and consistent with that observed for PP NPs, which were irradiated but not subjected to the prior removal of ALK and IRG.

Table 1 lists EC₂₀ values along with their respective confidence intervals (calculated as the standard error of the parameter estimate). Both EC₂₀ and EC₁₀ can serve as analogues for NOEC. However, while they are generally preferred for laboratory single-species toxicity data, the choice of EC₁₀ or EC₂₀ remains controversial (Beasley et al., 2015; EFSA, 2009). Therefore, we also calculated EC₁₀, which are included in Table S1 (SM) along with the values for the fitting parameter “b” (Eq. (1)).

As shown in Fig. 4 and Table 1 and S1, NPs containing IRG exerted considerable toxicity towards *D. magna* at carrier concentrations in the 1.8–3.5 mg/L range (which corresponded to a concentration of IRG <1.2 μg/L). The EC₅₀ (48 h) of PP NPs was 9.1 ± 0.7 mg/L for which, the concentration of IRG was 3.2 ± 0.3 μg/L. The calculation using the EC₅₀ values for PP, PPd + IRG, PPd + EXT and PPd + ALK + IRG, all of them with the same concentration of IRG yielded a median effect value for

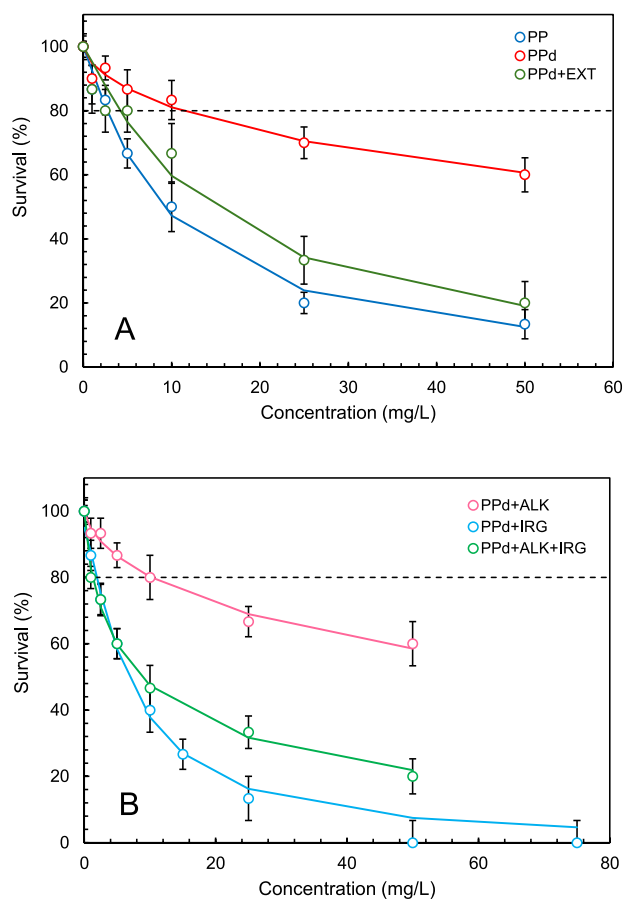


Fig. 4. Dose-effect relationship for the tested suspensions of NPs after 48 h exposure. Error bars represent standard deviation. The dashed line represents EC₂₀.

Table 1

EC₂₀ in mg/L ± standard deviation (*D. magna*) for the materials (NPs, <1 μm) tested in this work.

	mg/L ± standard deviation	
	EC ₂₀ , 24 h	EC ₂₀ , 48 h
PP	32.6 ± 9.0	2.3 ± 0.2
PPd	>50	24.6 ± 9.4
PPd + ALK	41.4 ± 4.7	21.2 ± 2.6
PPd + IRG	4.5 ± 0.2	1.8 ± 0.2
PPd + EXT	7.5 ± 0.9	3.5 ± 0.5
PPd + ALK + IRG	15.3 ± 3.3	2.2 ± 0.2

carriers containing IRG of 3.5 ± 1.5 μg/L, close to its reported solubility limit. The comparison between PP and PPd + IRG, PPd + EXT, and PPd + ALK + IRG showed no differences in toxicity that could be attributed to degradation products from the scission of alkane oligomers and additives upon irradiation. If such differences had existed, the toxicity levels of PP would have been significantly different from those of PPd + IRG, PPd + EXT, and PPd + ALK + IRG, but this was not observed. The key difference among the samples is that in PP, the oligomers and additives were exposed to irradiation, whereas in PPd + IRG, PPd + EXT, and PPd + ALK + IRG, they were not, with concentrations remaining the same.

However, the concentration of IRG in PPd-loaded NPs does not represent the individual expected effect of IRG if *D. magna* were exposed in the absence of PPd NPs. The individual effect of IRG can be estimated assuming that the toxic effect of PPd NPs or PP oligomers is independent to that of IRG (Eq. (2)), which is reasonable considering the structural differences between plastic particles and the antioxidant molecule. In this case the probabilistic model allows determining the effect of IRG (E_{IRG}) from that of PPd NPs (E_{PPd}) and IRG-loaded PPd NPs ($E_{PPd+IRG}$):

$$E_{IRG} = 1 - \frac{(1 - E_{PPd+IRG})}{(1 - E_{PPd})} \quad [3]$$

PP, PP + EXT or PP + ALK + IRG could have been used instead of PPd + IRG, although in that case, the effect of oligomers (ALK) should have been taken into account. Eq. (3) can be easily computed using the output of the logistic model given in Eq. (1) with the values for parameter b shown in Table S1. This procedure allows to estimate the EC_x for IRG. EC₂₀ yielded a value of 1.2 ± 0.3 μg/L. The model described by Eqs. (2) and (3) is generally suitable for explaining the co-occurrence of compounds with completely different molecular structures and modes of action, such as NPs and additives. Additionally, the model allows for estimating the EC₅₀ for IRG even if one of the compounds (PPd) did not reach 50 % inhibition within the experimental range. The estimated EC₅₀ was 3.3 ± 1.1 μg/L, which is consistent with the IRG concentration in carriers containing IRG at the EC₅₀ level (3.5 ± 1.5 μg/L). The same calculation for ALK, comparing PPd + ALK and PPd, allows an estimation for the toxic effect of ALK, which was EC₅ > 78.5 μg/L (EC₁₀ already falls outside the range of concentrations studied).

This work also shows that freshwater organisms can be exposed to levels above the solubility limit of toxic compounds through interaction with non-polar carriers, either NPs or other types of non-polar particles existing in co-exposure. Besides, to exert toxicity towards *D. magna*, the IRG included in PP-NPs must leach out the nanoparticles, at least in the gastrointestinal tract of the cladoceran. The same can be said for the IRG added to the PPd nanocarrier. In this case, the interaction between IRG and PPd NPs can be attributed to hydrophobic partitioning interaction and van der Waals forces in view of the neutral character of IRG and the hydrophobicity of the carrier (Yu et al., 2019). The fact that the observed toxicity was similar for PP, PPd + IRG, PPd + EXT and PPd + ALK + IRG suggests that leaching out kinetics is not playing a significant role, probably because of the small size of NP particles. Otherwise, the effect of the ‘naturally’ loaded PP NPs should be lower than those of the IRG-loaded PPd NPs, for which IRG should be more accessible.

Finally, the ratio between the non-oxidized and oxidized forms of the additive, IRG and IRGox, has been assessed after exposure by GC-MS. In PPd + IRG, the ratio was approximately 0.5 (0.38–0.45 range), while in PPd + ALK + IRG and PPd + EXT, the ratio was close to the unity (1.0–1.2 range). This result suggests that IRG was oxidized more efficiently in the absence of PP oligomers, which might exert a protective role against IRG oxidation. The same effect could explain that the toxicity of PPd + IRG was higher at high concentrations (lower survival ratio) than that of PPd + EXT and PPd + ALK + IRG (Fig. 4 and Tables 1 and S1, SM). Although no specific results have been obtained on the toxicity of IRGox, these results suggest a similar effect of both compounds on the cladoceran *D. magna*.

It is important to note that, up to the present, no data were available on the freshwater toxicity of IRG. Existing reports did not indicate any short-term aquatic toxicity. This includes a 96 h test with the fish *Brachydanio rerio* and a 24 h *D. magna* immobilization assays at nominal concentrations >100 mg/L as well as a 72 h exposure assay of the green alga *Scenedesmus subspicatus* at a nominal concentration of 75.2 mg/L (OECD, 2004a). However, in all cases these concentrations greatly exceeded the water solubility limit of IRG (<5 µg/L at 20 °C) and, therefore, they are not representative of true exposure concentrations. It is believed that the oxidized form of IRG might generate reactive oxygen species leading to oxidative stress. However, a recent study found that IRGox decreased the overall oxidative levels in C18-4 mouse spermatogonial cells (Wang et al., 2022). IRG and its degradation products have been associated with endocrine-disrupting properties that could potentially affect reproductive health and development (Shi et al., 2020). The potential bioaccumulation and persistence of IRG is clear from its high octanol/water partition coefficient and although experimental evidence is still scarce, IRG has been identified as candidate for Stockholm convention (Herzke et al., 2023).

The toxicity of NPs remains controversial. Some studies report various effects from NP exposure, such as oxidative stress, changes in enzymatic activities, impaired growth, and histopathological damage. However, only a limited number of these studies have used realistic NPs derived from the breakdown of larger particles, and in these cases, only limited lethal effects have been observed. Concerning polyolefins, a recent study exposed *D. magna* to different types of polyethylene NPs at low concentrations over their lifespan and found effects on survival only after 30–40 days of initial exposure (Ekvall et al., 2022). To our knowledge, there are no other studies on PP NPs. For larger particles, no effects have been observed when exposing *D. magna* to PP fibers (4–24 µm) at up to 100 mg/L or PP fragments (10–100 µm) at 50 mg/L (Jemec-Kokalj et al., 2022; Renzi et al., 2019).

The exposure to non-polar compounds in water near their solubility limit is a complex task, as chemicals exhibiting hydrophobic characteristics have a higher likelihood of adhering to non-polar surfaces in experimental devices. In this work, we exploited this property to perform exposures at concentrations near or beyond their solubility limit. Certainly, the ingestion of plastic particles loaded with the pollutant increases the probability of *D. magna* to suffer adverse outcomes. Therefore, it is important that the size of plastics (and other particles present in the medium in which the compound could partition) falls within the range of ingestible matter for test individuals.

In summary, this work showed that IRG can induce significant toxicity at concentrations near its solubility limit (NOEC, EC₁₀, EC₂₀). Moreover, freshwater organisms can be exposed to levels above the solubility limit through interaction with non-polar carriers, not only the plastic particles that primarily contain them but also other types of non-polar particles existing in co-exposure. We have also demonstrated that PP-derived NPs can be used as a carrier to test the toxicity of non-polar compounds, specifically, but not necessarily limited to, additives commonly used in their manufacture. This procedure paves the way to obtain toxicological data for plastic additives to freshwater organisms, many of which have not yet been toxicologically assessed (Takada and Karapanagioti, 2019; UNEP, 2023; Wiesinger et al., 2021).

Understanding the behaviour of these additives and their interactions with polymer fragments is essential for developing effective strategies to minimize plastic pollution and safeguard ecosystems and risks to human health.

4. Conclusions

In this work, we demonstrated that a colloidal solution of NPs (<1 µm), produced through UV irradiation of PP that had been freed of additives and oligomers, can be used as carrier to assess the toxicity of non-polar additives with low solubility, which are not currently tested for aquatic toxicity. The so-produced NPs, PPd, were reloaded with the phosphite antioxidant Irgafos®, a common additive contained in raw PP, as well as the alkane oligomers extracted from commercial PP pellets. Our results showed that PP (<1 µm) and PPd NP suspensions containing Irgafos® 168 exerted considerable toxicity towards *D. magna* (24–48 h immobilization) at carrier concentrations in the 1.8–3.5 mg/L range which contained up to 1.2 µg/L of Irgafos® 168. In contrast, suspensions of PPd NPs without the additive displayed considerably lower toxicity. Our findings allowed us to estimate the toxicity of Irgafos® 168 with EC₂₀ and EC₅₀ values of 1.2 ± 0.3 µg/L and EC₅₀ 3.3 ± 1.1 µg/L respectively (*D. magna* immobilization, 48 h). This is the first time the aquatic toxicity of this widely used organo-phosphite polymer additive has been determined.

CRedit authorship contribution statement

Marica E. Schiano: Writing – original draft, Methodology, Investigation. **Carlos Edo:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Enrique Blázquez-Blázquez:** Writing – review & editing, Methodology, Investigation. **María L. Cerrada:** Writing – review & editing, Supervision, Conceptualization. **Francisca Fernández-Piñas:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Roberto Rosal:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2024.124837>.

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