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RESEARCH ARTICLE

Adding knowledge to the design of safer hydrophobically modified poly(acrylic) acids: an ecotoxicological approach

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Abstract

The architecture of hydrophobically modified polymers can be tailored to produce variants with different levels of functionality. This allows industry to apply rational design methods for the development of more environmentally friendly materials. In the present work, the ecotoxicity of six variants of hydrophobically modified poly(acrylic) acids (HMPAA), obtained by changing the crosslinked conformation, insertion position, and length of the hydrophobic groups, was assessed for the (i) bioluminescence production of *Aliivibrio fischeri*; (ii) population growth rate of *Raphidocelis subcapitata* and *Chlorella vulgaris*; (iii) mortality of *Brachionus calyciflorus*; (iv) feeding inhibition, somatic growth rate, reproduction, and mortality of *Daphnia magna*; and (iv) mortality and somatic growth rate of *Pelophylax perezi* tadpoles. The concentrations causing 50% and 20% of effects ($L(E)C_{50\text{ and }20}$, respectively) ranged from 9.64 up to > 2000 $mg{\cdot}L^{-1}$ for all six HMPAA and species. The bacterium *A. fischeri* and tadpoles of *P. perezi* were the most sensitive and most tolerant organisms to the six tested HMPAA, respectively. The computed 5% hazard concentrations (computed on the basis of $L(E)C_{50\ s}$) showed that HMPAA1 (13.0 $mg{\cdot}L^{-1}$) and HMPAA2 (26.1 $mg{\cdot}L^{-1}$) were the most toxic variants, while HMPAA6 (233 $mg{\cdot}L^{-1}$) the least one. These results suggest HMPAA6 (with low crosslink percentage modified by the addition of long and short hydrophobic groups at the surface) to be the most environmentally friendly variant and should be preferentially considered to be used in consumer products, compared to the other five studied variants.

Keywords HMPAA · Ecotoxicity · Freshwater species · Environmentally friendly · Species differential sensitivity · Hazard concentrations

Introduction

Hydrophobically modified polymers (HMP), namely, acrylic acid–derived ones (e.g. HMPAA), were introduced as emulsifiers of personal care and hygiene products (PCPs) as early as in the 1980s (Patil and Ferrito 2013). In the current context of the worldwide SARS-COV-2 pandemic, many of these products have gained an even more leading place in daily routines since thickening properties are highly desirable features intended to increase the time of contact of the product with the skin (e.g. hand sanitizer) and, thus, increasing the probability of eliminating viruses. The escalating utilization on these products demands for an extra attention to the impacts they may provoke to the environment (as this is certainly the last recipient of these compounds), aiming at a sustainable growth and development (e.g. Berardi et al. 2020). Moreover, constituents of PCPs so far fairly lack proper regulation since, due to their high molecular weight, they are considered polymers of low concern (Sanderson et al. 2020). Challenged by the new paradigms such as sustainable development and green chemistry, the industry conceived the concept of "safety-by-design", intending to decrease the risks of harm associated to new industry products that are released to the market (i.e. to produce "greener" products, more environmentally

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and human friendly), without neglecting the efficiency for which they are designed for. Intending an optimized development of these HMP, the "safety-by-design" concept must be addressed at the onset process of the projection/design of the polymers, where several physical and chemical characteristics of the HMP may be fine-tuned (Alves et al. 2018). Notwithstanding, the lack of (eco)toxicological evidence/effects during this process, makes it difficult to characterize these materials as "greener" or not and becomes evident that the concept itself cannot stand alone. Hence, it is then necessary to harmonize the fine-tuning of HMP with an extensive ecotoxicological assessment (e.g. Simões et al. 2021) to reduce potential ecological risks after marketing. Recent evidence identifies acrylic-derived products as products of very low environmental persistence and with residual (or no) risk to the environment (Duis et al. 2021); however, it must be highlighted that they are being constantly used, at very high rates of consumption, and it is likely that this will be the most probable scenario for the upcoming years (e.g. Berardi et al. 2020). Furthermore, despite argued that they tend to precipitate in wastewater treatment plants sludges (Feng et al. 2020), no occurrence of a potential threshold level is mentioned elsewhere (neither on the short nor long term) or even the fate of such sludge and/or the impacts at the site where they are discharged (e.g. Duis et al. 2021). The aforementioned aspects are possibly the reasons for which PCPs have already been designated as emerging contaminants (Gavrilescu et al. 2015; Sanderson et al. 2020), and, recently, their presence was highlighted as being highly amplified in wastewater, sewage and even in surface and underground waters (Yadav et al. 2021). The necessity of implementing this approach (architectural design of polymers at the industry level allied with the ecotoxicological evaluation of these products) stands out as a feasible and reliable approach to tackle some of the knowledge gaps that persist in what concerns HMP, namely, HMPAA, potentially identifying key polymer properties useful in prospective toxicity assessment (rather than the traditional retrospective approach; Sanderson et al. 2020). This is a crucial step to develop accurate "quantitative structure–activity relationship" predictive models and provide information that might be further integrated into regulatory frameworks (Sanderson et al. 2020).

The six variants of HMPAA selected for this study have two types of structure: linear structure (HMPAA2) where each monomer is linked to other two monomers and crosslinked (HMPAA1, 3, 4, 5, and 6) with chemical bonds between polymeric chains. The functional conformations were obtained through the addition of hydrophobic groups, which enhance the amphipathic properties of the polymers providing them unique rheological characteristics that confer a greater stability to solutions when compared with unmodified polymers (Duarte 2011). The hydrophobic groups, when exposed to aqueous medium, tend to associate through intermolecular interactions (decreasing the contact between the water and the hydrophobic groups) leading to an increase in viscosity of the solutions. The increase in viscosity can be described as an osmotic effect and its denominated thickening (Duarte 2011). The thickening of crosslinked polymers and the high viscosity leads to a gel-like conformation and occurs primarily in response to environmental stimuli such as changes in pH, temperature, and ionic strength of surrounding medium (Antunes et al. 2011).

The presented study aimed at evaluating the influence that the conformational alterations in the structure of six HMPAA variants might have in their ecotoxicity and evaluate if any of the HMPAA could be used as a greener alternative to the currently commercialized HMPAA5. To achieve this goal, two tasks were carried out: (i) to perform a battery of standard monospecific bioassays with several key species to derive lethal and sublethal concentrations of each HMPAA polymer and (ii) to integrate the obtained effective concentrations into species sensitivity distribution (SSD) curves, where species were ranked according to their sensitivity allowing the overall determination of which polymer is the eco-friendliest.

Materials and methods

Hydrophobically modified poly(acrylic) acid (HMPAA)

Six variants of hydrophobically modified poly(acrylic) acids (HMPAA) were used. The original HMPAA5 (already commercialized) was supplied by Cognis GmbH as a 30 wt% aqueous solution, while the other five variants (HMPAA1, HMPAA2, HMPAA3, HMPAA4, and HMPAA6) were produced at the Chemistry Department of the University of Coimbra (Portugal) at the same 30 wt% concentration (Fig. 1).

The differences among the six variants were as follows: (i) HMPAA1 is composed of poly(acrylic) acid backbones with intermediate percentage of crosslink and modified by the addition of short and long hydrophobic groups; (ii) HMPAA2 constitutes a linear poly(acrylic) acid derivative composed of poly(acrylic) acid backbone, without crosslink and without long hydrophobic modification; (iii) HMPAA3 is composed of poly(acrylic) acid backbones with low crosslink percentage and with long hydrophobic modification in the surface and with short hydrophobic groups in the crosslinked matrix; (iv) HMPAA4 is composed of poly(acrylic) acid with high percentage of crosslink and modified by the addition of short and long hydrophobic groups inside it; (v) HMPAA5 is composed of poly(acrylic) acid with low percentage of crosslink and modified by the

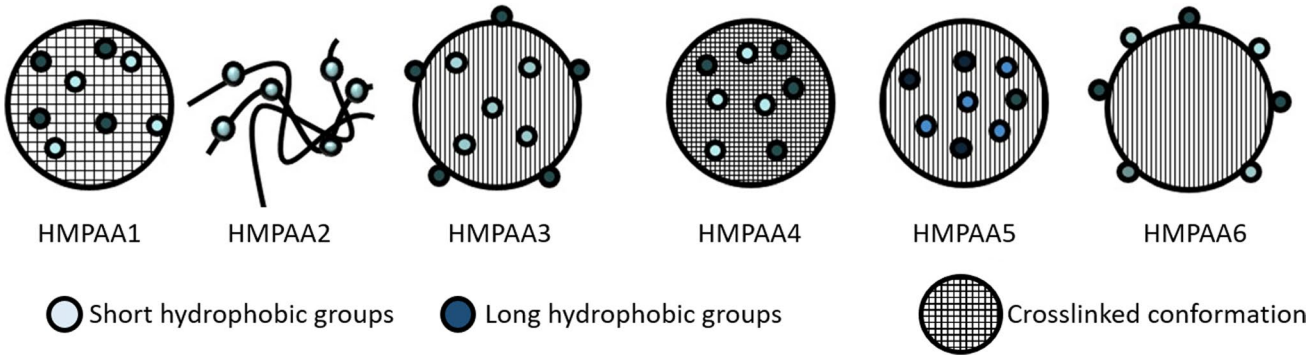

Fig. 1 Schematic representation of the six studied variants of hydrophobically modified poly(acrylic) acids (HMPAA). Light blue circles represent short hydrophobic groups. Dark blue circles represent long hydrophobic groups, and the lines represent the amount of the crosslinked percentage in each variant

addition of short and long hydrophobic groups inside it; and (vi) HMPAA6 is composed of poly(acrylic) acid backbones with low crosslink percentage modified by the addition of long and short hydrophobic groups in the surface of the structure.

For each HMPAA, a stock suspension at a concentration of 2000 mg·L^{-1} was freshly prepared through the dilution of the original 30 wt% aqueous solution with the different test media: MBL (for the algae); distilled water (H_2Od; for the bacterium), ASTM (for the daphnid), ASPM (for the rotifer), and FETAX (for the amphibian) (Nichols, 1973; ASTM 1980; OECD 1998; Dawson and Bantle 1987, respectively). Since the pH of these stock solutions was ~ 4.5, it was adjusted, by adding NaOH (1 M), to match the pH values of the media used and according to the corresponding guidelines and bench protocols: for H_2Od, pH was adjusted to 6.8; for the medium MBL, pH was adjusted to 8.0; for ASTM and ASPM, pH was adjusted to 7.6; and for FETAX, pH was adjusted to 8.0. The concentrations tested in the ecotoxicological assays were then prepared by directly diluting the pH adjusted stock suspension (2000 mg·L^{-1}) with the respective test medium.

The following physical proprieties were measured in the concentrations of 2000 mg·L^{-1} of HMPAA through dynamic (DLS) and electrophoretic light scattering (ELS) in a Malvern Instrument Zetasizer Nano-ZS (Malvern Instruments Ltd, Worcestershire, UK): hydrodynamic diameter, zeta potential, and polydispersity index (PDI). Conductivity and pH were measured with conductivity and pH meters (Wissenschaftlich Technische Werkstätten conductivity 440i and HI 422x-02 pH, PCE Instruments), respectively.

Source of the test organisms

The toxicity of the six variants of HMPAA was assessed for six aquatic species, which included two producers (green microalgae *Raphidocelis subcapitata* and *Chlorella vulgaris*), two primary consumers (*Brachionus calyciflorus* and *Daphnia magna* clone BEAK), a secondary consumer (*Pelophylax perezi*, and a decomposer (*Aliivibrio fischeri*). These species were selected due to their ecological relevance for the aquatic ecosystems and due to the availability of standard and/or well-defined bench protocols. The bacterium *A. fischeri* was selected because it constitutes a time-effective standard assay that is widely used internationally for preliminary and first screening ecotoxicological assessments.

The stock cultures of the freshwater microalgae *R. subcapitata* and *C. vulgaris* were maintained in MBL medium "Woods Hole MBL Medium", at 20 ± 1 °C under continuous and uniform cool-white, fluorescent illumination (100 µE m^{-2} s^{-1}), according to OECD guideline 201 (OECD 2006). The gram-negative, non-pathogenic bacteria *A. fischeri* were obtained as lyophilized from the commercial kit Microtox test®, being supplied by Azur Environmental, and were reconstituted with a reconstitution solution provided by the same company. The neonates of *B. calyciflorus* were obtained after the hatching of commercially available cysts (MicroBioTests, Ghent, Belgium) immediately prior to their use in the assays. Hatching of the cysts was performed for 24 h, at 23 °C, at a constant light intensity of 3000–4000 lx. A culture of *D. magna* was maintained, under asexual reproduction, in laboratorial controlled conditions of temperature (19 to 21 °C) and photoperiod (16:8 h L:D) in ASTM hardwater (American Society for Testing and Materials; ASTM 2002), with the addition of vitamins and the organic additive Marinure 25 (an extract from the algae *Ascophyllum nodosum*; Pan Britannica Industries Ltd., Waltham Abbey, UK) (Baird et al. 1989). Medium was changed every other day, and organisms were fed daily with the green algae *R. subcapitata* (Korshikov) F. Hindák at a concentration of 3.0×10^5 cells mL^{-1} d^{-1}. Neonates from the 3rd to 5th broods were selected to maintain laboratory cultures and carry out the ecotoxicity assays. The egg

masses of *P. perezi* were collected in a small, shallow, freshwater reference pond located at Quinta da Boavista, near the city of Aveiro, Portugal (40°36′16″N, 8°41′48″W) (please see Santos et al. 2013; Venâncio et al. 2019 for detailed information on physical–chemical characteristics of the pond). The egg masses were immediately transported to the laboratory in plastic containers filled with local water. Upon arrival to the laboratory, viable eggs were selected and transferred to the standard medium FETAX (Dawson and Bantle 1987). Organisms were maintained in the laboratory in plastic containers filled with FETAX, with constant aeration, at 23 ± 1 °C and 16:8 h L:D, until larvae hatched and reached Gosner stage 21 (Gosner 1960), which were then used to perform the toxicity assays. Since exposure of the larvae occurred during the period when they present the yolk-sac, thus, without independent feeding, no food was supplied to the organisms.

Ecotoxicological assays

The concentration ranges tested on all the definitive ecotoxicological assays were based on preliminary laboratorial assays and were those that allowed to obtain a dose–response curve, and thus, the estimation of median lethal and/or sublethal concentrations for the later construction of the species sensitivity distribution curves.

Growth inhibition with *R. subcapitata* and *C. vulgaris*

Growth inhibition assays were carried out according to OECD guideline 201 (2006) adjusted to 24-well plates (Moreira-Santos et al. 2004). Both algae species were exposed to a range of eight concentrations of each HMPAA (Table 1), for 72 h under controlled conditions of temperature (23 ± 1 °C) and continuous and uniform cool-white, fluorescent illumination (100 µE/m^2/s), with manual resuspension (by using a micropipette) twice a day. Three replicates per concentration and control (only MBL medium) were performed. The growth of exposed microalgae was determined by measuring the absorbance (ABS) at 440 nm (Jenway, 6505 UV/VIS spectrophotometer, Burlington, USA), as a surrogate for cell density (*CD*; cells mL^{-1}), at the end of the exposure period (Eq. 1; please see Venâncio et al. 2017). For each tested concentration, an additional well containing solely the HMPAA concentration (without the algae) was also assembled aiming at evaluating any potential interference of the polymer on the ABS of the algae (ABS blank), as suggested by the OECD 201 guideline. The average specific growth rate (μ; day^{-1}) (OECD 2006) was then determined, after subtraction of ABS-ABS blank for each test concentration and control, by using Eq. 2:

$$CD\left(cellsmL^{-1}\right) = -17107.5 + (ABS \times 7925.350) \quad (1)$$

$$\mu_{ab} = \frac{\ln D_b - \ln D_a}{t_{b-t_a}}(d^{-1}), \quad (2)$$

where D_b is the cell number at the end of the exposure, D_a is the initial cell number, and $t_b - t_a$ is the time interval in days.

Bioluminescence inhibition with *A. fischeri*

The bioluminescence inhibition assay with the bacteria *A. fischeri* was performed according to the 81.9% basic test protocol, following the detail procedure Microbics Corporation (AZUR Environmental 1998), available in the commercial kit Microtox test® (AZUR Environmental 1998). All exposures and bioluminescence measurements (at 5, 15, and 30 min of exposure) were performed at 4 °C in the Microtox Model 500 Analyser (Microbics Corporation). For each HMPAA variant, a range of nine concentrations (Table 1) plus a control (consisting only of diluent supplied by AZUR) were tested.

Mortality of *B. calyciflorus*

The mortality assay with the freshwater rotifer *B. calyciflorus* was performed according to the standard operation procedure Rotoxkit F (MicroBioTests Inc., Ghent, Belgium) in multiwell plates. Six replicates, with five newly hatched rotifers each, were assigned to all HMPAA concentrations and control (ASPM medium). Incubation of the plates was carried out at a temperature of 25 ± 1 °C, for 24 h, under total darkness. A total of eight concentrations were tested per HMPAA variant (Table 1) plus the control. After the exposure period of 24 h, the number of dead organisms was counted. An organism was considered dead when, after gentle prodding, it remained immobile for 15 s.

Mortality, feeding inhibition, somatic growth rate, and reproduction assays with *D. magna*

The lethal toxicity assays were performed according to OECD guidelines 202 (OECD 2004). Every assay consisted in a set of at least 6 concentrations of each HMPAA plus a control condition (ASTM hardwater medium; Table 1). Assays were carried out for 48 h at 20 ± 1 °C and a 16:8 h L:D photoperiod, with no food addition or medium renewal. Five neonates (6 to 24 h old) were introduced per replicate, consisting in 70-mL glass vessels containing 50 mL of the test solutions; four replicates were carried out per concentration and control. Mortality at 24 and 48 h was assessed, considering an organism dead if immobile during 15 s after gentle prodding. Results obtained with these assays allowed

Table 1 Summarized information of the conditions and range of concentrations used in the bioassays with freshwater species exposed to six variants of hydrophobically modified poly(acrylic) acids (HMPAA). Abbreviations stand for: light (L) or dark (D) cycle, hour (h), day (d), minutes (min), na — not applicable

Test species	Endpoint	Dilution water	Test conditions	Concentrations of HMPAA ($mg{\cdot}L^{-1}$)	Duration of the assay	Geometric factor	Replicates Organisms/replicate	Reference
Chlorella vulgaris	Growth rate	MBL	24:0 h L:D 23 ± 1 °C	160.0; 320.0; 416.0; 540.8; 623; 914; and 1108	72 h	1.2, 1.5 and 2	3 n.a	OECD guideline 201, 2006 Moreira-Santos et al. 2004
Raphidocelis subcapitata	Growth rate	MBL	24:0 h L:D 23 ± 1 °C	160.0; 320.0; 418; 540.8; 623; 914; 1108; and 1330	72 h	1.2, 1.5 and 2	3 n.a	
Vibrio fischeri	Bioluminescence	AZUR diluent	- 4 ± 1 °C	6.96; 13.9; 27.8; 55.7; 114; 223; 446; 891; and 1789	5, 15 and 30 min	2	3 n.a	AZUR Environmental, 1998
Brachionus calyciflorus	Mortality	ASPM	0:24 h L:D 25 ± 1 °C	11.2; 16.8; 24.0; 38.0; 56.8; 85.4; 128; 192; 288; 432; 649; 908; and 973	24 h	1.4, 1.5	6 5 organisms/rep	Rotoxkit F, MicroBioTests Inc
Daphnia magna	Mortality	ASTM	16:8 h L:D 20 ± 1 °C	135; 190; 265; 372; 520; 728; 1020; 1428; 2000	48 h	1.4	4 5 organisms/rep	OECD guidelines 202, 2004
	Feeding rate	ASTM	16:8 h L:D 20 ± 1 °C	78.4; 102; 132; 172; 224; 292; 368; 492; and 640	24 h	1.3	4 5 organisms/rep	Allen et al. 1995
	Somatic growth	ASTM	16:8 h L:D 20 ± 1 °C	102; 132; 172; 224; 291; 368; 492; and 640	72 h	1.3	7 1 organism/rep	Burns 2000
	Reproduction	ASTM	16:8 h L:D 20 ± 1 °C	9.56; 12.4; 16.1; 21.0; 27.4; 35.6; 46.4; 60.2; 78.4; 102; 133; and 172	21 days	1.3	10 1 organism/replicate	OECD 211, 1998
Pelophylax perezi	Growth	FETAX	16:8 h L:D 23 ± 1 °C	368; 492; 640; 824; 1072; 1393; 1811; and 2000	96 h	1.1, 1.3	4 5 organisms/rep	Gosner 1960

establishing sublethal concentrations to perform the sublethal assays (feeding, somatic growth, and reproduction responses).

Feeding inhibition was assessed for 24 h with 4-day-old organisms isolated from 3rd, 4th, or 5th broods (Allen et al. 1995). Groups of 5 neonates were randomly exposed to 30 mL of solution in 50-mL glass vessels. Each test consisted of 7 concentrations of each HMPPAA (Table 1) with four replicates each, a control group (ASTM hardwater), and a blank control (with algae but without daphnids and toxicant to guarantee that initial algal concentrations did not increase significantly over the exposure period). Also, in every concentration, an extra replicate, without daphnids or algae, was performed so that at the end of the assay the absorbance of the HMPAA was subtracted to the absorbance of the algae. Organisms were fed with the green algae *R. subcapitata* (Korshikov) F. Hindák (formerly known as *S. capricornutum*) at a concentration of 3.0×10^5 cells mL^{-1} day^{-1}. Experiments were conducted at 20 ± 1 °C and in total darkness (to avoid the growth of the algae). At the end of the exposure, all organisms were removed, and final cell density was estimated by measuring absorbance at 440 nm, following Eq. 1 (Jenway, 6505 UV/VIS spectrophotometer, Burlington, USA). Then, using cell densities, feeding rates (cells $hour^{-1}$) estimation was made by using Eq. (3) (Allen et al. 1995):

$$Feeding\ rate = \frac{lni - lnf}{t}\ (\text{cellshour}^{-1}) \tag{3}$$

where ln_f is the final cell density, ln_i the initial cell density, and t (hours) the time of exposure.

Somatic growth inhibition assays were carried out with newborns of *D. magna* (from 3rd, 4th, or 5th broods), 6 to 24 h old, which were exposed individually in 50-mL glass vessels filled with 20 mL of test solutions. Seven concentrations were tested, with seven replicates per concentration (Table 1). Medium was supplemented with algae (*R. subcapitata* (Korshikov) F. Hindák at a concentration of 3.0×10^5 cells/mL/day) and the organic extract (*Ascophyllum nodosum*; Pan Britannica Industries Ltd., Waltham Abbey, UK; Baird et al. 1989). Assays were carried out for 72 h at 20 ± 1 °C and a 16:8 h L:D photoperiod. Medium was not changed during the 72 h but was resuspended in order to maintain algal density in the water column. Vessels were checked every day for dead organisms. Organisms were measured at 0 h and 72 h and values converted to daily growth (mm·day^{-1}) considering l_f and l_i the length of organisms at the end and beginning of the assay, respectively (mm), and d (days) the time interval (Burns 2000, Eq. 4).

$$Somatic\ growth = \frac{l_f - l_i}{d}\ (\text{mmday}^{-1}) \tag{4}$$

The 21-day chronic toxicity assays were performed according to the standard protocol OECD 211 (OECD 1998). Ten neonates of *D. magna* (6 to 24 h old), from the 3rd, 4th, or 5th broods, were exposed individually, at 20 ± 1 °C and a 16:8 h L:D photoperiod, to a set of at least 6 concentrations of each HMPAA (Table 1). Seventy-mL glass vessels were used, containing 50 mL of the test solution with the addition Marinure 25 (an extract from the algae *Ascophyllum nodosum*; Pan Britannica Industries Ltd., Waltham Abbey, UK; Baird et al. 1989) and the green algae *R. subcapitata* (Korshikov) F. Hindák (3.0×10^5 cells/mL/day). Organisms were fed every day, and medium was renewed every other day. The intrinsic population growth rate (r, day^{-1}) was also estimated applying the Euler-Lotka equation and standard errors estimation by jackknifing (Meyer et al. 1986; Eq. 5).

$$1 = \sum_{x-0}^{n} e^{-rx} \times l_x \times m_x\ \left(\text{day}^{-1}\right), \tag{5}$$

where x is the age, l_x the probability of survival, and m_x the fecundity of the females until age $_x$.

Growth inhibition of *P. perezi* tadpoles

Five larvae, at developmental stage G21 (Gosner 1960), were exposed per replicate in 6-well plates, filled with 15 mL of solution, for a 96-h period under a temperature of 23 ± 1 °C and photoperiod of 16:8 h L:D. Medium was changed at 48 h, and mortality checked every day, with removal of dead individuals to avoid the growth of microorganisms, which could impair the survival of the remaining alive organisms. During the period of the assay, organisms were not fed as they still rely on egg yolk nutrients. Four replicates were assigned for each concentration and for the control (consisting of FETAX medium). A set of eight concentrations was tested per each HMPAA (Table 1). The initial and final body length of the tadpoles was recorded using a stereomicroscope Leica MS5 with an integrated Microscope Eyepiece Camera (Dinocapture 2.0, Dino-Eye®) to assess somatic growth rate (mm·day^{-1}), using the previously described Eq. 4.

Data analysis

The concentrations (LC_x) causing 10, 20, and 50% of mortality, and respective confidence limits at 95%, were calculated through probit analysis using the software Priprobit Ver. 1.63 (Finney 1971). For the calculation of effect concentrations (EC_x) causing sublethal effects (population growth inhibition, somatic growth inhibition, feeding inhibition, reproduction), and respective 95% confidence limits, a three-parameter log-logistic non-linear model was applied using the Statistica 7.0 software. For bioluminescence inhibition with *A. fischeri*, EC_x values were calculated by using the MicrotoxOmni Azur software (AZUR Environmental, 1998).

The determination of the non-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were performed by one-way analysis of variances (ANOVA) followed by the multicomparison Dunnett's test, comparing all treatments with the respective control, after checking homogeneity of variances (Levene's test) and normality of data (Shapiro–Wilk test). This data is shown in the supplementary material.

Aiming at deriving threshold levels for toxicity ranking of each HMPAA variant, species sensitive distribution (SSD) curves were constructed using the Excel macro file available from United States Environmental Protection Agency (USEPA; available at https://www.epa.gov/ceam/species-sensitivity-distributions). Such approach of ranking species according to their sensitivity allows to establish an association between the concentration of each contaminant and the proportion of species that will be negatively affected by it, as well as identify the most sensitive taxa (European Commission 2011; Posthuma et al. 2019; Liu et al. 2021). Each SSD was created using the herein derived LC_{50} and EC_{50} (e.g. growth, feeding) values for each HMPAA and for the six freshwater species (Liu et al. 2021). The hazard concentration aiming at protecting 95% of species, which corresponds to a hazard concentration that affects 5% of the species in each distribution (HC_5) was then estimated along with the corresponding 95% confidence limits (Posthuma et al. 2019).

Results

Physical characterization of HMPAA

The hydrodynamic diameter of HMPAA varied widely when suspended in the different media (MBL, H_2Od, ASPM, ASTM, and FETAX) (Table 2), and any of the six HMPAA variants presented consistently, across the different media, a lower or higher hydrodynamic diameter. Regarding the zeta potential (ζ-potential, in mV), the majority of HMPAA variants presented values of rapid coagulation or flocculation (< 5 mV) and incipient instability (10 to 30 mV) (Table 2) (ASTM 1985). The zeta potential of the variant HMPAA2 was the only lying within the category of excellent stability, but solely when suspended in ASTM medium (> 60 mV) (ASTM 1985). The majority of PDI values ranged between 0.122 and 0.511 (Table 2), though some reached values above 0.723, which suggests a heterogeneous distribution of the sizes of the HMPAA in suspension. The variant HMPAA2 showed both the lowest (0.07 in H_2Od) and highest (1 in ASTM) values of PDI comparatively to the other HMPAA variants (Table 2). Almost all HMPAA suspensions were negatively charged, with the following exceptions: HMPAA1 when suspended in ASPM, HMPAA2 in MBL, HMPAA4 in H_2Od, HMPAA5 in ASTM and FETAX, and HMPAA6 in FETAX (Table 2). The conductivity values of the HMPAA suspensions varied with the test media, though, the values were similar within test media among the six tested variants (Table 2).

Ecotoxicity tests

The results of the lethal and effective concentrations of HMPAA, for 20 and 50% of effect, computed for the six studied species are shown in Table 3 and at Figure 1S. The NOEC and LOEC values for all species and endpoints are summarized in Table S2. For the two species of producers that were tested, the results showed that for *R. subcapitata*, the HMPAA concentrations causing 50% of growth inhibition ($EC_{50,72\ h}$) ranged between 200 and 1115 mg·L^{-1}.

Table 2 Values of physical parameters measured in the 2000 mg·L^{-1} concentrations for each of the six hydrophobically modified poly(acrylic) acids (HMPAA) in the different tested media (MBL, H_2Od, ASPM, ASTM, and FETAX). Z-average — hydrodynamic diameter (nm). ζ-potential — zeta potential (mV). PDI — polydispersity index. Cond — conductivity (μScm^{-1})

	Z-average (nm)					***ζ-potential* (mv)**				
	MBL	**H_2Od**	**ASPM**	**ASTM**	**FETAX**	**MBL**	**H_2Od**	**ASPM**	**ASTM**	**FETAX**
HMPAA1	685	656	839	737	805	−32.3	−9.1	0.02	−40.8	−0.08
HMPAA2	712	333	390	3353	1272	0.11	−21.3	−24.0	−97.3	−57.5
HMPAA3	797	2081	667	1395	1529	−0.0095	−16.6	−38.4	−0.0251	−53.3
HMPAA4	817	1206	717	1196	997	−0.0563	0.0008	−46.0	−0.006	−45.5
HMPAA5	764	638	678	6801	1083	−29.5	−43.7	−25.6	0.06	0.08
HMPAA6	787	1411	683	1322	1649	−39	−21.3	−0.02	−46.3	0.02
	PDI					**Cond (μScm^{-1})**				
	MBL	**H2Od**	**ASPM**	**ASTM**	**FETAX**	**MBL**	**H2Od**	**ASPM**	**ASTM**	**FETAX**
HMPAA1	0.215	0.243	0.282	0.266	0.254	579	53	348	522	676
HMPAA2	0.133	0.07	0.723	1	0.897	551	49	357	633	307
HMPAA3	0.276	0.419	0.246	0.213	0.511	707	51	362	686	324
HMPAA4	0.211	0.25	0.212	0.257	0.232	539	71	332	338	439
HMPAA5	0.359	0.149	0.485	0.335	0.386	537	54.8	340	662	648
HMPAA6	0.122	0.195	0.175	0.213	0.232	554	53	347	770	250

Table 3 Lethal or effective concentrations causing X% of effect after T time of exposure (minutes, min; hours, h; or days, d) — $LC_{x,T}$ or $EC_{x,T}$ — and respective confidence limits at 95% (in parenthesis), computed for six tested freshwater species exposed to six variants of hydrophobically modified poly(acrylic) acids (HMPAA). n.d., end-point could not be determined since no effect was observed at the highest concentration of 2000 mg·L^{-1}. #highest % of effect observed at the highest concentration of 2000 mg·L^{-1}. *For the growth rate of *Pelophylax perezi*, only EC_{10} and EC_{20} were possible to compute

		HMPAA1	HMPAA2	HMPAA3	HMPAA4	HMPAA5	HMPAA6
Raphidocelis subcapitata	$EC_{50,72\ h,growth}$	**200** (180–240)	**280** (220–360)	**440** (400–480)	**1115** 894–1337	**707** (646–768)	**500** (320–700)
	$EC_{20,72\ h,growth}$	**80.0** (60.0–100)	**248** (188–299)	**360** (320–420)	**291** (231–352)	**360** (347–500)	**320** (120–540)
Chlorella vulgaris	$EC_{50,72\ h,growth}$	**360** (280–440)	**460** (420–500)	**993** (632–1354)	**619** (160–1078)	**842** (432–1253)	**700** (580–820)
	$EC_{20,72\ h,growth}$	**160** (80.0–220)	**360** (300–400)	**620** (480–760)	**140** (60.0–200)	**485** (51.8–918)	**420** (280–540)
Vibrio fischeri	$EC_{50,30\ min,bioluminescence}$	**9.64** (1.72–54.2)	**28.4** (23.8–34.1)	**96.6** (51.4–182)	**101.1** (60.8–168)	**13.8%**#	**1178** (681–1816)
	$EC_{20,30\ min,bioluminescence}$	**0.75** (0.22–61.4)	**9.06** (6.64–30.6)	**20.2** (15.4–22.6)	**31.8** (20.1–43.3)	**13.8%**#	**224** (177.4–286)
Brachionus calyciflorus	$LC_{50,24\ h,mortality}$	**656** (601–715.0)	**56.4** (42.2–91.8)	**361** (320–412)	**906** (622–1412)	**392** (238–1380)	**905** (634–1412)
	$LC_{20,24\ h,\ mortality}$	**566** 484–616	**19.4** 11.2–26.4	**330** -	**214** 0.2–470	**219** 50–350	**640** 280–860
Daphnia magna	$LC_{50,48\ h,mortality}$	**293** (266–323)	**204** (186–224)	**470** (431–513)	**334** (304–367)	**n.d**	**351 (317–378)**
	$LC_{20,48\ h,mortality}$	**240** (206–266)	**174** (149–190)	**406** (352–440)	**282** (238–310)	**n.d**	**262** (211–299)
	$EC_{50,24\ h,feeding}$	**131** (116–145)	**249** (224–274)	**244** (129–359)	**111** (40.2–180)	**38.0** (n.d.)	**281** (189–373)
	$EC_{20,24\ h,feeding}$	**n.d**	**191** (56.0–327)	**177** (146–207)	**n.d**	**n.d**	**252** (199–304)
	$EC_{50,72\ h,somatic\ growth}$	**725** (697–780)	**277** (245–308)	**690** (472–908)	**92.4** (76.4–108)	**138** (26.2–249)	**491** (272–763)
	$EC_{20,72\ h,somatic\ growth}$	**n.d**	**133** (133–162)	**217.4** (108–327)	**n.d**	**91.0** (61.2–121)	**n.d**
	$EC_{20,21d,populational\ growth\ rate}$	**119** (71.2–160)	**123** (114–138)	**44.9** (23.1–69.7)	**9.89** (3.4–18.4)	**48.7** (28.7–72.5)	**n.d**
Pelophylax perezi*	$EC_{20,96\ h,somatic\ growth}$	**n.d**	**1308** (1182–1435)	**651** (552–751)	**943** (846–1038)	**697** (653–741)	**1541** (1441–1642)
	$EC_{10,96\ h,somatic\ growth}$	**1134** (730–1497)	**673** (554–792)	**313** (218–408)	**519** (422–616)	**504** (450–559)	**1149** (10.3–1266)

HMPAA1 was the most toxic variant with an $EC_{50,72\ h}$ of 200 mg·L^{-1}, while HMPAA4, HMPAA5, and HMPAA6 variants were the ones presenting less toxicity to this microalga (Table 3; Fig. 1Sa). For the other tested green microalgae, *C. vulgaris*, the $EC_{50,72\ h}$ values ranged between 360 and 993 mg·L^{-1}, with HMPAA1 exhibiting the highest toxicity ($EC_{50,72\ h}$ of 360 mg·L^{-1}) and HMPAA3 the lowest one ($EC_{50,72\ h}$ of 993 mg·L^{-1}) (Table 3; Fig. 1Sb).

The EC_{50} computed for each HMPAA variant in the bioluminescence inhibition assay with *A. fischeri* ranged from 9.64 up to 1178 mg·L^{-1} (Table 3). The most toxic HMPAA variants to this bacterium were HMPAA1 ($EC_{50,30\ min}$ of 9.64 mg·L^{-1}) and HMPAA2 ($EC_{50,30\ min}$ of 28.4 mg·L^{-1}), while HMPAA6 showed the highest $EC_{50,30\ min}$ (1178 mg·L^{-1}) for this species. Note that it was not possible to calculate the EC_x values for HMPAA5 to *A. fischeri*, though it is expected to be higher than 2000 mg·L^{-1}, and thus meaning that this variant was the least toxic for the bacterium (Table 3; Fig. 1Sc).

Regarding the primary consumers, for the rotifer *B. calyciflorus*, the $LC_{50,24\ h}$ ranged between 56.4 and 906 mg·L^{-1} (Table 3), with HMPAA2 showing the highest lethal toxicity ($LC_{50,24\ h}$ of 56.4 mg·L^{-1}) and HMPAA4 and HMPAA6 the lowest ones ($LC_{50,24\ h}$ of 906 and 905 mg·L^{-1}, respectively; Table 3; Fig. 1Sd). For the other primary consumed species, *D. magna*, the interval of $LC_{50,48\ h}$ computed for the six HMPAA varied between 204 and 470 mg·L^{-1} (Table 3). HMPAA2 was the most toxic ($LC_{50,48\ h}$ of 204 mg·L^{-1}) and

HMPAA5 the least toxic (causing no mortality in daphnids) (Table 3; Fig. 1Se). Regarding the sublethal effects, the ranking of ecotoxicity of the HMPAA varied with the endpoint being monitored. For feeding inhibition of *D. magna* juveniles, the EC_{50}'s ranged between 38.0 and 281 mg·L^{-1}. HMPAA5 was the most toxic variant ($EC_{50,24\,h}$ of 38.0 mg·L^{-1}) and HMPAA6 was the least toxic ($EC_{50,24\,h}$ of 281 mg·L^{-1}) (Table 3; Fig. 1Sf). Relative to *D. magna* somatic growth rate inhibition, the EC_{50}'s showed a wider range, varying between 92.4 and 725 mg·L^{-1}; with HMPAA4 exerting the highest toxicity ($EC_{50,72\,h}$ of 92.4 mg·L^{-1}) followed by HMPAA5 ($EC_{50,72\,h}$ of 138 mg·L^{-1}). HMPAA1 and 3 were the variant that cause lowest effects in somatic growth of *D. magna* ($EC_{50,72\,h}$ of 725 mg·L^{-1} and $EC_{50,72\,h}$ of 690 mg·L^{-1}, respectively) (Table 3; Fig. 1Sg). Relatively to populational growth rate of *D. magna* (only $EC_{20,21d}$ could be determined), HMPAA4 was the most toxic variant ($EC_{20,21d}$ of 9.89 mg·L^{-1}) while HMPAA6 was the least toxic (no effect on reproduction was observed) (Table 3; Fig. 1Sg).

Finally, for *P. perezi*, it was not possible to compute EC_{50} values since all tested concentrations caused an inhibition in somatic growth lower than 50%. Table 3 and Fig. 1Sh present the $EC_{10,96\,h}$ and the $EC_{20,96\,h}$ values for this species corresponding to the no observable effect concentration and lowest observable effect concentration, respectively. The HMPAA concentrations causing 20% of growth inhibition in the tadpoles of *P. perezi* ranged from 651 up to > 2000 mg·L^{-1}. The most toxic variants to *P. perezi* tadpoles were HMPAA3 ($EC_{20,96\,h}$ of 651 mg·L^{-1}) and HMPAA5 ($EC_{20,96\,h}$ of 697 mg·L^{-1}), while HMPAA1 was the least toxic variant (no $EC_{20,96\,h}$ could be estimated even at the highest tested concentration of 2000 mg·L^{-1}) (Table 3; Fig. 1Sh).

Species sensitivity distribution curves and HC_5 values

Overall, the SSD curves showed that the decomposer species *A. fischeri* and the zooplanktonic species *B. calyciflorus* and *D. magna* were consistently at the half bottom of the curves for most HMPAA variants, meaning that they were the most sensitive species (Fig. 2 and Fig. 3). On the other hand, the secondary consumer *P. perezi* was the most tolerant species to all HMPAA variants (no $L(E)C_{50}$ could be computed and presented always on the top of the curves of $L(E)C_{20}$) (Fig. 3).

Table S1 shows the computed HC_5 values as well as curve parameters obtained for each HMPAA variant. When considering the hazard concentrations obtained from the SSD curves constructed with median lethal and sublethal effect

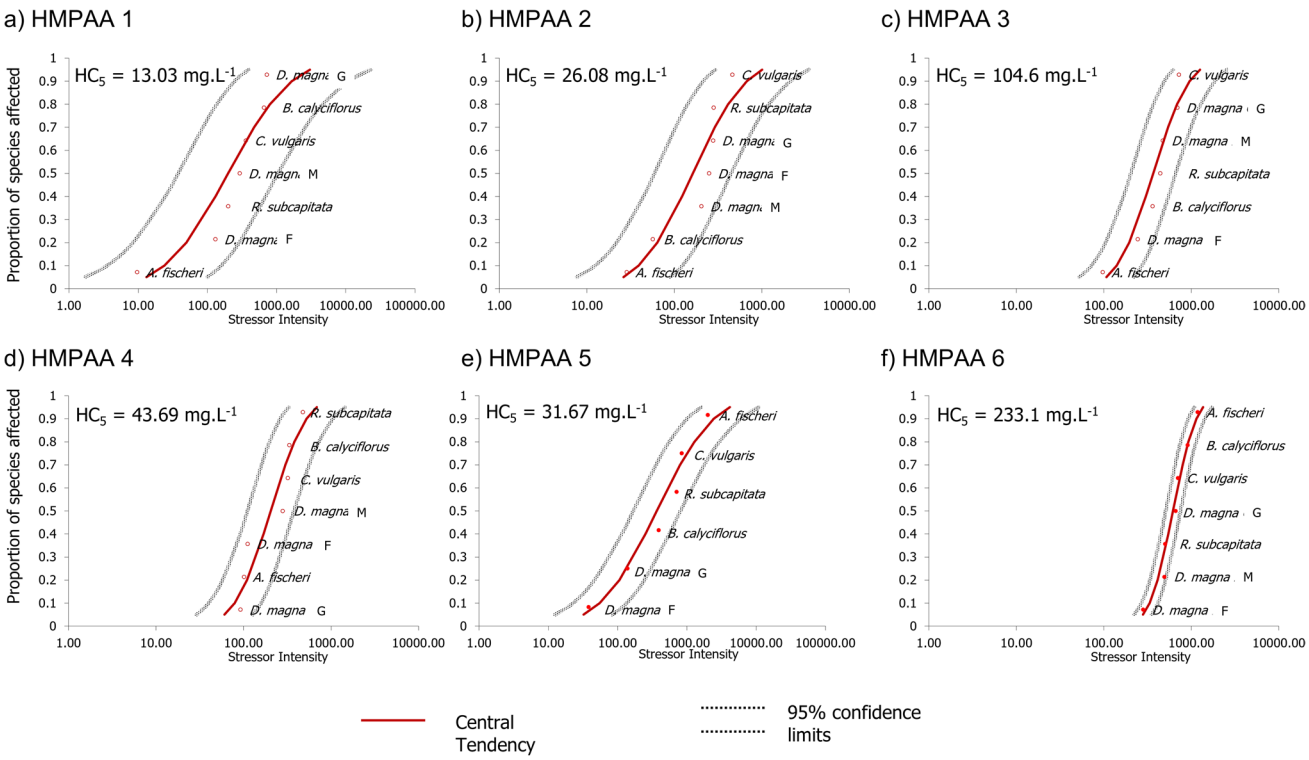

Fig. 2 Species sensitivity distribution curves obtained for each of the six variants of hydrophobically modified poly(acrylic) acids (HMPAA). Curves were constructed based on the median lethal and sublethal effect concentrations (LC_{50} and EC_{50}) computed for six freshwater species. Values for *Pelophylax perezi* are not included because no $L(E)C_{50}$ could be computed for this species. Abbreviations are as follows: F, feeding; G, growth; HC_5, hazard concentration that protect 95% of the species; M, mortality

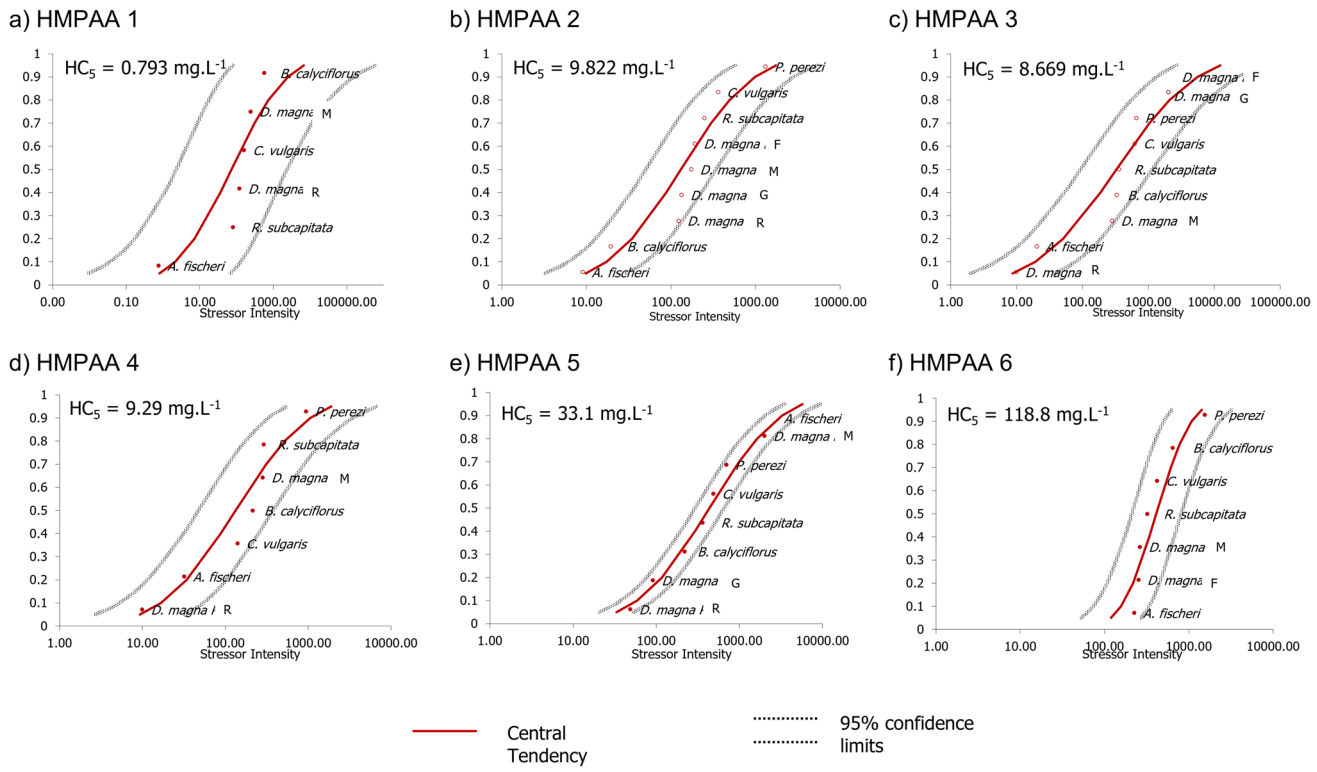

Fig. 3 Species sensitivity distribution curves obtained for each of the six variants of hydrophobically modified poly(acrylic) acids (HMPAA). Curves were constructed based on the lethal and sublethal effect concentrations causing 20% of effect, considered the threshold for effect (LC_{20} and EC_{20}) computed for six freshwater species. Abbreviations are as follows: F, feeding; G, growth; HC_5, hazard concentration that protect 95% of the species; M, mortality

concentrations (LC_{50} and EC_{50}), the HMPAA6 variant was the least toxic variant with an HC_5 value of 233 mg·L^{-1} (with a 95% confidence limit of 186–293 mg·L^{-1}), followed by HMPAA3 with an HC_5 value of 105 mg·L^{-1} (with a 95% confidence limit of 62.4–175 mg·L^{-1}). Despite, it must be noted that the confidence limits of HMPAA3 overlapped with the remaining variants that presented to be the most toxic to the tested freshwater species (Fig. 2 and Fig. 3; Table S1). As for the hazard concentrations computed with the threshold values for effect (LC_{20} and EC_{20}), the HMPAA6 variant stand out, being again the least toxic variant: HC_5 value of 134 mg·L^{-1}, with respective confidence limits of 52.4–269 mg·L^{-1}. All the other five variants presented HC_5 values within the same order of magnitude (< to 20 mg·L^{-1}) (Fig. 2 and Fig. 3; Table S1). The HMPAA1 was consistently the most toxic variant either for HC_5 computed with L(E)C_{50} and L(E)C_{20} (Fig. 2 and Fig. 3; Table S1).

Discussion

Our results indicate that changes in the structural conformation of hydrophobically modified poly(acrylic) acid number 5 (HMPAA5) led to changes in its physical properties (hydrodynamic diameter, zeta potential, PDI, and conductivity) when in aqueous suspensions. Yet, a pattern between structural alterations — changes in shape, increase in crosslink, increase of hydrophobic surface groups, and changes in physical properties — was not found. For instance, the increase of crosslink or an increase in the number of hydrophobic groups outside the HMPAA did not cause a systematic decrease/increase of the variant's hydrodynamic diameter, a result highlighted also by Simões et al. (2021) when studying six variations of quaternized hydroxyethyl cellulose polymers. Thus, it is suggested that additional interactions between the HMPAA and other constituent elements from the mediums such as Na^{2+} or Ca^{2+} ions might have influenced the behaviour of the HMPAA.

A lack of a consistent pattern was also noticeable for the values achieved for physical properties of HMPAA in the different solution mediums. It was expected a greater HMPAA aggregation to occur in media with higher ionic strength such as MBL, since agglomeration of particles is influenced by the sum of repulsive electrostatic forces, interaction of electrical double layer surrounding HMPAA, and the attractive van der Walls forces (Suttiponparnit et al. 2011). A higher ionic strength solution theoretically leads to a smaller electrical double layer thickness and weaker electrostatic repulsive forces promoting aggregation and larger

hydrodynamic size of HMPAA (Suttiponparnit et al. 2011). However, in the presented work, the HMPAAs showed smaller sized when suspended in MBL medium, comparatively to H_2Od, ASTM, and FETAX. This may, in part, be the result of the pH of the medium and its differential interaction with the structures of the six variants, as poly(acrylic) acids might be highly sensitive to small variations in this parameter (Nesrinne and Djamel 2017; Simões et al. 2022). The pH of MBL (and respective stock solution) was around 8 while for H_2Od and ASTM was 6.8 and 7.6, respectively. That higher pH of the MBL medium might stimulate the formation of intra-molecular bonds and, thus, promoting the contraction of the polymer, leading to smaller sized particles as observed elsewhere with hydroxyethyl cellulose polymers (Simões et al. 2022). However, other parameters of the test media (e.g. different chemical composition) are most probably also influencing the size and behaviour of the variants in the aqueous suspensions, since MBL and FETAX exhibit the same pH value, and in the latter medium the HMPAA variants tended to exhibit a higher size.

Moreover, with ionic strength increase it was expected a decrease in zeta potential because of the smaller thickness of the electrical double layer surrounding the HMPs, but the present results did not support this hypothesis. As an example, HMPAA3 and HMPAA4 in MBL medium presented zeta potential values of − 0.0095 and − 0.0563 mV, respectively, and in ASPM (smaller ionic strength) − 38.4 and − 46.0 mV. It is hypothesized that other processes may exercise greater influence over the HMPs behaviour when in aqueous suspensions, namely, the interaction/absorption of ions from the surrounding medium changing not only their aggregation/dispersion properties, but also their electrical charge (Suttiponparnit et al.; 2011). According to this hypothesis, the MBL medium composition rich in ions such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and Cu^{2+} may influence the presented results due to the inherent characteristics of HMPAAs which may have greater or lesser affinity for these ions.

When relating the results obtained from the ecotoxicological assays with the characteristics and conformation of each variant, it was expected that positively charged HMPAAs presented higher toxicity to the tested organisms than negatively charged ones, since biological membranes are negatively charged and thus, there is a higher affinity for opposite charged polymers (e.g. Tripathy et al. 2018; Duis et al. 2021). Still, the results here presented showed that positively charged HMPAAs did not consistently present higher toxicity, either comparing the toxicity of the same HMPAA to different species or comparing different HMPAAs to the same species. As an example, HMPAA6 is only positively charged in FETAX medium, although this variant showed one of the lowest toxicities to *P. perezi*. Moreover, the HMPAA6's EC_x values for *P. perezi* were one of the highest comparatively to other species ($EC_x > 1000$ mg/L). Thus, the surface charge seems not to greatly influence the toxicity of the HMPAA variants. Likewise, also polymers' size could have been one of the discriminating features for polymer toxicity, assuming that smaller polymers could be more easily taken up by organisms and/or cells. However, this was not the case either and, therefore, precluding the establishment of a robust toxicity-structure correlation.

Against this background, pinpointed by previous studies focusing as well on the ecotoxicity of industrially designed polymers (e.g. Martins et al. 2018; Pereira et al. 2018; Simões et al. 2021, 2022), Pereira et al. (2018) strongly suggests that the development of sustainable and ecofriendlier polymers will have to be based above all on the sensitivity of the ecological receptors in detriment of polymers physical–chemical characteristics. Notwithstanding, polymers' conformation must also be appraised since toxicity might be dependent on the way that organisms ingest and incorporate materials and the degradation and metabolization of those compounds. The HMPAA1 differs from HMPAA5, since the first has an intermediate crosslinked conformation and HMPAA5 a low percentage of crosslink. Results showed that an increase in the crosslink conformation led to an increase in toxicity to unicellular organisms (*R. subcapitata*, *C. vulgaris*, and *A. fischeri*). On the other hand, to pluricellular and more complex organisms (*B. calyciflorus*, *D. magna*, *P. perezi*), the increase in crosslink led to a decrease in HMPAA toxicity. This result is most probably related with the way that different organisms internalize the polymers. Despite their high molecular weight and potential inability to cross biological barriers, the interactions of the polymers with the cell surface might still cause structural and functional alterations, for instance, by inducing nanoscale holes in the lipids bilayer and ultimately causing internal metabolic unbalance (e.g. Chen et al. 2009; Fischer et al. 2003). Higher percentage of crosslink can promote intra-molecular interactions promoting the stability of the polymers in solution (Antunes et al. 2011), inducing lower aggregation (smaller sized particles) and therefore higher toxicity of HMPAA1 to unicellular organisms. To pluricellular and more complex organisms, the incorporation of polymer aggregates can still happen easily, for instance, by ingestion and/or through respiratory structures as gills (Bergin and Witzmann 2013) explaining the higher toxicity of HMPAA5 to *B. calyciflorus*, *D. magna*, and *P. perezi* (the first two filter-feeders, and the third with highly developed and sensitive/irrigated respiratory structures) comparatively to others like HMPAA6. Moreover, it must be also considered that pluricellular organisms have internal organs that might metabolize these compounds, possibly leading to the breakdown of the polymer into smaller segments, and thus changing its overall behaviour and toxicity (e.g. Duis et al. 2021).

The HMPAA2 has a different conformation from HMPAA5, while HMPAA5 (and all the other variants) have a spherical conformation; HMPAA2 presents a linear conformation without crosslink. This change in conformation caused an increase in toxicity to all tested species except for *P. perezi*. The linear conformation of HMPAA2 may explain its toxicity. For instance, Wang et al. (2001) thorough analysis on architectural changes on polymers' properties has shown that simpler, linear structures of poly(silyl-ester) suffered a faster initial degradation process, and consequent weight loss, than their structurally more complex analogues (hyperbranched polymers). Such characteristic might make them more available to be incorporated by cells and/or organelles. However, the presence of short aliphatic groups both at the surface and inside HMPAA2 could also be the cause of the change in the polymer toxicity compared to HMPAA5. Yet, HMPAA6 also has short aliphatic groups in the surface of the material and is less toxic to most of the endpoints assessed than HMPAA5. Thereby it is suggested that the presence of short aliphatic groups at the surface of HMPAA2 is not determinant to the increment of the variant toxicity.

Comparing HMPAA5 and HMPAA6 structures, we can notice the change of short and long hydrophobic from the inside to the surface of the HMPAA which may explain in part the overall decrease of the sixth variant toxicity. It is suggested that the presence of hydrophobic groups at the surface of the HMPAAs may limit the number of available sites to bind with receptors in biological membranes. Furthermore, the hydrophobic groups can exhibit lower affinity to bound with cellular membranes reducing the incorporation of these HMPAA into the organisms and consequently reducing their toxicity. The presented results suggest that changes in the conformational structure of HMPAA variants had influence on its ecotoxicity.

Finally, and based on the integration of the data obtained into SSD curves, it is suggested that HMPAA6 is the least toxic variant (HC_5 value of 134 $mg{\cdot}L^{-1}$, with respective confidence limits of 52.4–269 $mg{\cdot}L^{-1}$ for the $L(E)C_{20}$, and HC_5 value of 233 $mg{\cdot}L^{-1}$, with respective confidence limits of 186–293 $mg{\cdot}L^{-1}$ for the $L(E)C_{50}$). Several disadvantages were raised throughout this work, namely, with regard to the availability of environmental concentrations (with which the HC_5 could be compared to), in relation to the argument that these compounds do not present potential toxicity in realistic scenarios or the bias introduced by the lack of standardization of the tests and no strong correlation between the physicochemical characteristics and toxicity. However, and in order to frame the estimated HC_5 values and provide a broader perspective of this work objectives, a rough comparison even with other surfactants is of added value. For instance, the reported environmental concentrations of sodium dodecyl sulfates or cationic surfactants of 1.8 $mg{\cdot}L^{-1}$ and 5.8 $mg{\cdot}L^{-1}$ by Mondal et al. (2021) and Koner et al. (2011), respectively, measured in sewage wastewater in the vicinity of a recreational centre in India, do not seem to pose an immediate threat as they are below the estimated HC_5 values, even considering the already commercially available variant (HMPAA5). Nevertheless, the increased use of these compounds in densely populated areas and/or the proximity to industrial complexes where the production of these detergents occurs may lead to the point release but with very high loads of these compounds into the environment. Kowalska et al. (2005) reported concentrations of anionic surfactants exceeding 1500 $mg{\cdot}L^{-1}$ in effluents from the detergent production industry, while Orlandi et al. (2019) reported values up to 780 $mg{\cdot}L^{-1}$ in wastewater effluents from a spa in India. The latter values, when compared to the HC_5 values derived here (remembering that 20% are already regarded as a threshold for effect), are thus considered of high environmental concern, highlighting here the need to provide more environmentally friendly variants as the aim of the presented work. It is then safe to say that this work provided important data to enrich the ecotoxicological profile of these acrylate-based materials, a key step forward aimed at regulatory decisions.

Conclusion

The results presented show that HMPAA6 is consistently one of the less toxic HMPAA tested for all species. HMPAA6 — whose conformation consists of hydrophobic groups (short and long) on the surface of the HMP — could be an alternative to HMPAA5, which is already commercially available. Therefore, in the context of a more sustainable and greener development, it is suggested that industry should develop HMPAA6-like polymers to reduce the negative impact on biota while maintaining the functionalization of the materials.

As the (eco)toxicity of polymers remains largely underexplored by key regulatory agencies, this work provides important baseline data for deriving critical values to be included in environmental risk assessment agendas for these products in the near future.

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Author contribution JT: Validation, formal analysis, investigation, writing — first draft, review and editing. CV: Validation, formal analysis, investigation, writing — first draft, review and editing. CD: Chemical characterization. FEA: Conceptualization, methodology, writing — review and editing, Funding acquisition. IL: Conceptualization, methodology, formal analysis, writing — first draft and review and editing, visualization, supervision, funding acquisition.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Not required as no human data or samples were used. All experiments with animals (non-vertebrate models and non-independent feeding larval stages of *P. perezi*) were performed in compliance with the 3Rs principle.

Conflict of interest The authors declare no competing interests.

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