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Currently monitored microplastics pose negligible ecological risk to the global ocean

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Given the rise in plastic production, microplastics (MP) dominate marine debris, and their impact on marine ecosystems will likely increase. However a global quantitative assessment of this risk is still lacking. We conducted an ecological risk assessment of MP in the global ocean by comparing the thresholds of biological effects with the probability of exposure to those concentrations, according to plastic density data adjusted to a log-normal distribution. Levels of MP from 100 to 5000 μm span from <0.0001 to 1.89 mg/L , whereas the most conservative safe concentration is 13.8 mg/L , and probability of exposure is $p = 0.00004$. Therefore large MP pose negligible global risk. However, MP bioavailability, translocation and toxicity increase as size decreases, and particles $<10 \mu\text{m}$ are not identified by current monitoring methods. Future research should target the lowest size fractions of MP and nanoplastics, and use in toxicity testing environmental plastic particles rather than engineered materials.

Inappropriate disposal of solid waste makes the global ocean a sink of plastics, which under environmental conditions fragment into smaller pieces termed microplastics (MP) when they become $<5 \text{ mm}$, the most concerning form of ocean plastic debris^{1,2}. When the issue of MP accumulation in marine compartments emerged in the scientific literature, concern on harmful effects on marine organisms prompted experimental approaches using very high loads of engineered materials such as polystyrene (PS) microspheres, frequently labeled with fluorescent probes^{3–6}. These experiments supported remarkable effects, from reduced ingestion and energy budget to reduced growth of the organisms or their progeny. On that basis, and despite the lack of effects observed in similar reports^{7–10}, and criticism on experimental design of laboratory toxicity testing^{11–13}, MP are repeatedly considered in the scientific literature as a cause of high concern for marine ecosystem health.

In this review we conduct an ecological risk assessment (ERA) of MP in the global ocean according to the standard methodology derived from the National Academy of Sciences paradigm^{14,15} adapted to the marine environment¹⁶. According to the paradigm, risk was characterized by comparing the predicted exposure, derived from the data on MP density in the sea, with the thresholds of toxicity found in laboratory toxicity tests using marine organisms. Unlike previous attempts to assess risk posed by MP^{13,17}, we have considered in our analysis particle size in order to account for the large differences in impact described across the broad range of sizes from 5 mm down to $<1 \mu\text{m}$. This allows refinement of ERA and derivation of environmental quality criteria^{18,19} by introducing in these quantitative estimates plastic particle size as an explanatory variable.

Results

Plastic density in the sea follows log-normal distributions. Tables 1 and S1 compile the density of plastics in surface waters of the global ocean. Reported mean densities of plastics in surface waters ranged from 0.12 to $7250 \times 10^{-3} \text{ Pieces/m}^3$, and from 0.01 to $34.09 \times 10^{-3} \text{ mg/L}$. Similar amounts, in mass units, have been found in subsurface waters (Table 2). Either recorded in numbers or mass units, plastic density frequently shows a log-normal distribution (see Table 1). The smallest dataset²⁰ is normally distributed, and 13 of the remaining 16 datasets fit to log-normal distributions. The exceptions are the three largest datasets^{1,21,22}. In the two large datasets by Law et al.^{1,21}, repetition of the same values in trawls with low abundance of plastics causes non-normality (see Fig. S1a,b). The dataset reported by Goldstein et al.²² has been gathered from different oceanographic cruises, including areas of MP accumulation such as the North Pacific Subtropical Gyre, and areas with lower MP density such as the Eastern Tropical Pacific or Alaska, and the distribution was bimodal, with a Bimodality

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Study	Location	n	10 ⁻³ Particles/m ³				µg/L			
			Mean	Std/CV	Min-max	Log-normal	Mean	Std/CV	Min-max	Log-normal
²³	N Atlantic	11	9.006	9.224/1.02	0.120–30.761	Yes	0.730	1.293/1.77	0.0015–4.509	Yes
²¹	N Atlantic	3803*	42.266	114.801/2.72	0.676–2308.856	No ($p < 0.001$)	0.575	1.561/2.72	0.009–31.400	No ($p < 0.001$)
²⁴	Arctic/N Pacific	157*	94.185	266.034/2.83	2.2–3140.9	Yes	0.131	0.337/2.77	0.0001–2.5191	Yes
²²	Pacific	196	2612.694	4855.95/1.86	1–32,765	No (bimodal)	4.620	8.317/1.80	0.001–52.969	No (bimodal)
²⁵	Mediterranean	36*	1296.67	1983.49/1.53	100–8920	Yes	2.258	3.454/1.53	0.17–22.8	Yes
²⁶	Australia	136*	31.481	46.47/1.48	2.627–287.621	Yes	1.939	2.863/1.48	0.16–17.72	Yes
²⁷	N Pacific	1058*	217.590	1654.47/7.60	1.028–49267.784	No ($p < 0.001$)	2.959	22.501/7.60	0.014–670.041	No ($p < 0.001$)
²⁸	Mediterranean	28*	421.732	848.43/2.01	12.5–3437.5	Yes	6.981	14.046/2.01	0.21–56.91	Yes
²⁰	Mediterranean	10	168.00	103.58/0.62	10–350	Normal	1.374	0.848/0.62	0.08–2.86	Normal
²⁹	Mediterranean	39	1219.27	N.m	N.m	–	2.610	3.786/1.45	0.0025–18.11	Yes
³⁰	Mediterranean	22*	208.182	347.15/1.67	40–1690	Yes	1.704	2.841/1.67	0.33–13.83	Yes
³¹	Mediterranean	33	1305.869	1312.77/1.01	215.98–5783.77	Yes	5.071	5.097/1.01	0.84–22.46	Yes
³²	Mediterranean	74/71*	1002.568	1842.62/1.84	20–11300	Yes	1.751	3.926/2.24	0.011–26.081	Yes
³³	Southern Ocean	5	31	41.9/1.35	99	Yes	N.m	N.m	N.m	–
³⁴	Arctic	21*	534.414	706.945/1.32	5.907–2458.07	Yes	0.652	0.975/1.50	0.004–3.524	Yes
³⁵	Australia	22	195	135.25/0.70	40–470	Yes	0.814	0.569/0.70	0.2–2.0	Yes
³⁶	Arctic	13	128	107/0.84	18–310	Yes	N.m	N.m	N.m	–
³⁷	N Atlantic	28	180	205/1.14	35–870	Yes	0.669	0.556/0.83	0.084/2.525	Yes

Table 1. Plastic density in surface waters; studies for which data from individual trawls were available. When data are reported per sea surface area only sampled volume was calculated from the area of the sampling device trawled inside the water. n, sample size; Std/CV, standard deviation/Coefficient of variation; and n.m., not measured or unavailable data. *Zero values excluded.

Ocean	Cruise/region	Depth (m)	min (µm)	n	Particles/m ³			µg/L	
					Mean	Std	Max	Mean	Max
³⁸ Atlantic	<i>Equatorial 2003–04</i>	0.6	300	88	0.01	n. m	n. m	n. m	n. m
³⁹ N Pacific	<i>NE Pacific 2012</i>	4.5	62.5	34	2080	2190	9180	64.9	286
⁴⁰ N Atlantic	<i>IR & UK 2013</i>	3	250	470	2.46[†]	2.43	22.5	0.71	6.53
⁴¹ Arctic	<i>Barents Sea 2014</i>	6	250	75	2.68[†]	2.95	11.5	0.51	2.17
⁴² N Atlantic	<i>2014</i>	3	10	23	148	131	501	0.066	0.223
⁴³ Arctic	<i>2005 & 2014</i>	0–50	500	38	1.72[†]	0.93	4.52	N. m	N. m
⁴⁴ N Pacific	<i>Off California 2017</i>	5–1000	100	17	4.6	3.1	11	N. m	N. m
⁴⁵ Atlantic	<i>AMT26 JR16001 2016</i>	10–240	25	36	2782	3393	11,693	0.997	2.895
⁴⁶ Arctic	<i>West Greenland 2019</i>	5	10	6	142	85.83	278	0.029	0.051

Table 2. Plastic density in sub-surface waters. When the studies report data for particles < 5 mm (microplastics) separately these values are shown here. Bold indicates measured values. Mass estimates were obtained from particle size data assuming density = 1, cylindrical shape for fibers and spherical shape otherwise. n.m. not measured or unavailable data. [†]> 95% fibers.

Coefficient, BC = 0.621 (Fig. S2, in mass units). The variance in plastic density is large and similar in all datasets, with an average coefficient of variation of 201%.

When all surface plastic density data here reviewed are combined a bell shaped distribution is obtained (Fig. 1a).

Plastics accumulate in the convergence zones of the subtropical gyres. Median values for plastic debris in surface water found in the present review are similar for all oceans (Fig. 1b, in numbers); in mass units 0.16 µg/L in N Pacific, 0.17 µg/L in the Arctic, and 0.18 µg/L in the North Atlantic. Values are higher in the Australian and Mediterranean data-sets, more influenced by coastal sampling sites: 0.92 µg/L for Australia (including both the Indic and Pacific coasts), and 1.02 µg/L for the Mediterranean. On the basis of both original and previously published data gathered from cruises across the global ocean, Cózar et al.⁴⁷ identified zones of accumulation of floating plastic debris in the convergence zones of the five subtropical gyres (N and S Atlantic, N and S Pacific, and Indian oceans), with similar densities ranging from 200 to 500 g/km² across each of the five zones, equivalent to 0.8–2 µg/L. In contrast, mean plastic densities in nonaccumulation zones never exceeded 50 g/km², equivalent to 0.2 µg/L. This means that plastic pollution is a global issue, and position within a particular ocean rather than the ocean itself is more useful to predict plastic density. For example, Law et al.²¹ found a dramatic increase in plastic density in the N Atlantic within a strip at latitudes between 27 and 36°N compared to sites further North or South.

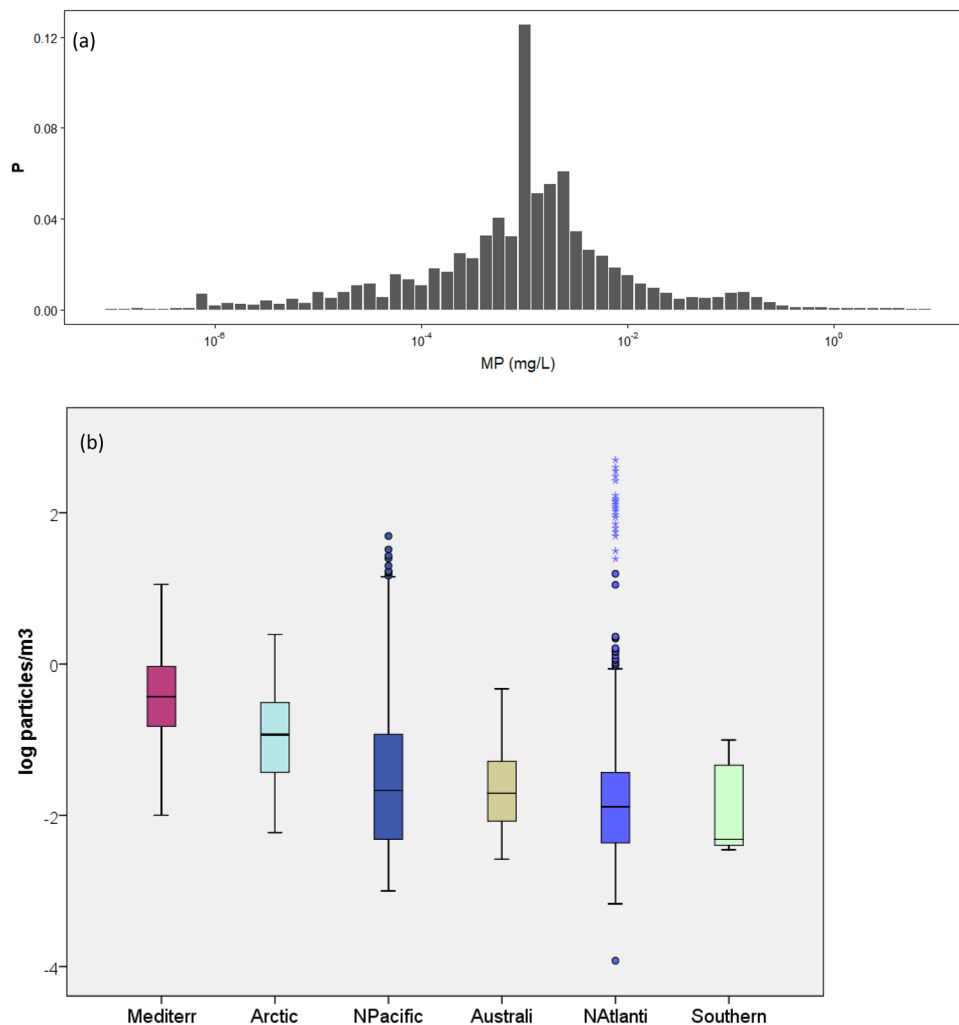


Figure 1. (a) Probability (P) distribution of microplastic density (MP, mg/L in log scale) in seawater from all data reviewed. The 0 mg/L values were excluded. (b) MP density (log particles/m³) in the different oceans sampled ordered from left to right by decreasing median. Horizontal line: median; box boundaries: 25th and 75th percentiles; bars: range of observed values; dots and asterisks: potential outliers.

In coastal regions, plastic densities show very high variability, with largely above average at certain specific areas influenced by riverine discharges such as the Yngtze estuary⁴⁸, or surface currents such as Kuroshio⁴⁹.

Concerning variability within sites, rough wind conditions are reported to decrease floating plastic density^{25,26,32}, as expected in vertically mixed compared to stratified water columns⁵⁰.

Toxicity of plastic particles tends to increase as particle size decreases. The effect of particle size on plastic toxicity was investigated in the dataset obtained from the selected studies compiled in Table S1. Toxicity thresholds (TT, mg/L) increased as particle size (S, μm) increased according to a double-logarithmic regression model ($p < 0.001$, $n = 68$):

$$\log TT = 0.297 + 0.739 \cdot \log S$$

where the slope is significantly > 0 (95% CI: 0.447–1.031). However, the heterogeneity of the biological models and plastic materials used in laboratory toxicity testing were responsible for a low value of $r^2 = 0.28$. When the different plastic particles used in all studies here selected are classified into four size classes, large microplastics (LMP, $> 100 \mu\text{m}$), small microplastics between > 10 and $100 \mu\text{m}$ (SMP-a), small microplastics between 1 and $10 \mu\text{m}$ (SMP-b), and nanoplastics (NP, $< 1 \mu\text{m}$), then a statistically significant ($p < 0.0001$) trend towards increasing toxicity (lower toxicity thresholds) with decreasing particle size can be observed (Fig. 2). Mean TT values are: 7684, 74.1, 58.9, and 4.9 mg/L respectively.

When TT within each size class are fit to species sensitivity distribution (SSD) curves, data fit well to log-logistic functions (Fig. 3, Table 3), allowing calculation of the 5th percentile (HC₅) for the four size classes. HC₅ values are all significantly different among them and, as expected, toxicity increases as particle size decreases.

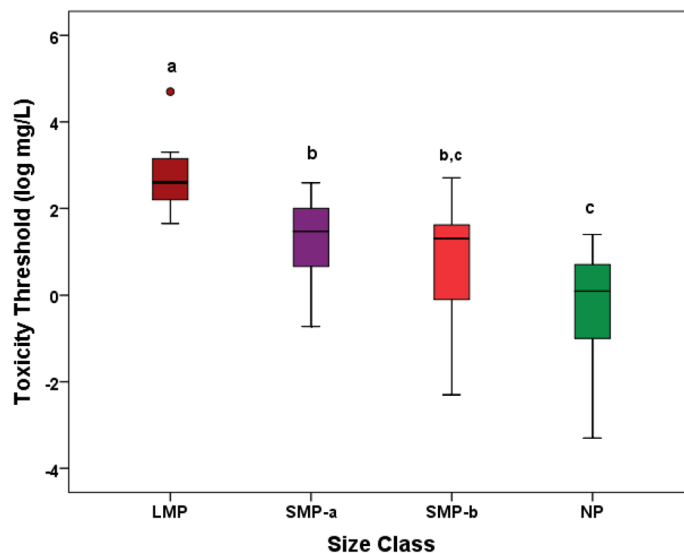


Figure 2. Toxicity thresholds of plastic particles on aquatic organisms. Note the reduction in toxicity threshold, i.e. the increase in toxicity, as particle size decreases. Size classes were defined as: large microplastics (LMP, $> 100 \mu\text{m}$), small microplastics between > 10 and $100 \mu\text{m}$ (SMP-a), small microplastics between 1 and $10 \mu\text{m}$ (SMP-b), and nanoplastics (NP, $< 1 \mu\text{m}$). Horizontal line: median; box boundaries: 25th and 75th percentiles; bars: range of observed values. LMP shows a potential outlier. Different letters indicate significantly different groups (Tukey, $p < 0.05$).

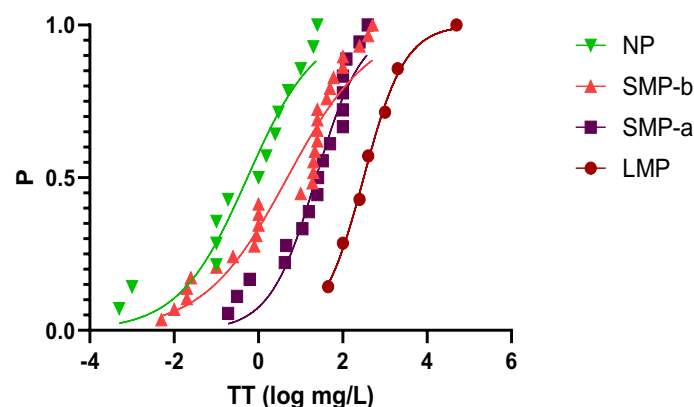


Figure 3. Species sensitivity distribution (SSD) curves fit to the four plastic-size classes, defined as in Fig. 2. Notice lower toxicity thresholds (TT), i.e. higher toxicity, as particle size decreases.

Size class	a (mg/L)	b	n	AIC	HC ₅ (mg/L)	Critical value (mg/L)	Critical value endpoint
LMP	468 (388–548)	1.13 (0.94–1.33)	7	–34.95	16.68 (13.82–19.54)	44.7	<i>Gammarus fossarum</i> growth
SMP-a	28.01 (23.22–32.80)	1.22 (1.03–1.41)	18	–95.69	0.76 (0.63–0.89)	0.19	<i>Sebastes schlegelii</i> growth
SMP-b	6.00 (4.23–7.78)	2.23 (1.92–2.55)	29	–145.72	0.008 (0.006–0.01)	0.01	<i>Crassostrea gigas</i> larval growth of progeny
NP	0.77 (0.54–1.00)	1.84 (1.52–2.16)	14	–72.15	0.003 (0.002–0.004)	0.0005	<i>C. gigas</i> larval ingestion rate

Table 3. Fitting parameters of SSD curves using log-logistic functions (see Material and methods) for different plastic particle size classes: large microplastics (LMP, $> 100 \mu\text{m}$), small microplastics between 10 and $100 \mu\text{m}$ (SMP-a), small microplastics between 1 and $10 \mu\text{m}$ (SMP-b), and nanoplastics (NP, $< 1 \mu\text{m}$).

The HC_5 values are similar in magnitude to the critical values (Table 3), i.e. the lowest concentration for each size class ever found to cause a significant biological effect according to the literature.

Large-size MP currently pose a negligible ecological risk across the global ocean. Risk posed by MP within the range of sizes normally monitored in oceanographic cruises (LMP) can be characterized by obtaining from the SSD curve the maximum levels considered safe in environmental management. This may be estimated from the HC_5 , which would protect 95% of the species in the ecosystem, or more conservatively by the lower end of its 95% CI, which would ensure that protection with a certainty > 95%. The corresponding values are 16.7 and 13.8 mg/L respectively (see Table 3), one order of magnitude above the single highest MP density concentration ever reported in the scientific literature (1.89 mg/L)⁵¹, and ca. 2 orders of magnitude above the highest mean value of all datasets (0.32 mg/L⁵², for S Portuguese coast).

In addition, risk can be more formally characterized by assessing the probability of exposure of marine organisms to MP concentrations above the safe value of 13.8 mg/L. According to the global MP distribution in marine waters (see Fig. 1) this probability is 0.00004. Therefore, on the basis of the current knowledge on environmental levels, and their toxicity quantified in laboratory experiments, the risk posed by LMP is negligible across the whole global ocean, including central oceanic gyres and coastal hot spots.

Risk assessment for small-size MP. Very limited information is available in the scientific literature on the plastic density for particles within the 10–100 μ m range SMP-a, and none for smaller particles. The only study found entirely covering this range of particles gathers data from 23 samples taken by the vessel's underway intake and filtered by 10 μ m mesh⁴². The abundance of plastic particles within this range of SMP found in that study fits well with the comprehensive distribution of abundance vs particle size reported by Isobe et al.⁵¹ (See Fig. S3), and the size distribution is consistent with that reported using a slightly higher threshold of minimum particle size⁴⁵. When data from those studies are pooled together in a single distribution of abundances of particles ranging from 10 to 5000 μ m and abundances are expressed in mass units, then the proportion represented by size class SMP-a is just 0.023%. Pabortsava and Lampitt⁴⁵ though reported a 15-fold higher mass of MP than Enders et al.⁴², resulting in a conservative estimation of 0.35% of SMP-a size MP, in mass. Assuming the distribution across the global ocean (see Fig. 1) is also representative for SMP-a, the resulting risk coefficient, quantified by the probability of exposure to SMP concentrations above the safe value obtained for SMP-a, is even lower than that for LMP ($p = 2.28 \times 10^{-7}$).

Discussion

Currently MP risk assessment is hampered by the mismatch between the size range of MP recorded in the marine environment (frequently > 0.3 mm) and those reported as posing risk to marine organisms in laboratory toxicity tests (frequently < 20 μ m). With these limitations in mind, and on the basis of the current knowledge of MP abundance and effects, the probability for a marine organism to be exposed to concentrations above those considered safe is $p = 0.00004$. Therefore, the risk posed to marine ecosystems by plastic particles within the size range currently monitored by standard methods can be classified as negligible. This is in line with previous assessments^{12,33}.

However, increasing scientific evidence supports that, disregarding biological model, plastic particle size affects their biological effects. The present study found by regression analysis that the logarithm of the toxicity threshold increases with a positive slope ($b = 0.784$; 95% CI: 0.475–1.093) as the logarithm of particle size increases ($p < 0.001$, $n = 62$). Moreover, when plastic particles used in toxicity testing were classified into 4 size classes, the effect of particle size on toxicity was highly significant (ANOVA, $p < 0.0001$), and the dependence of safe concentration (HC_5) on particle size was even more remarkable, with a slope $b = 1.21$ according to the equation:

$$\log HC_5 = 1.21 \log S - 2.10$$

where S is the mean particle size (μ m) for each size class ($r^2 = 0.92$, $n = 4$).

Therefore, the plastic particles with the highest ecological impact are expected to be below the threshold of sizes captured by current standard methodologies of oceanographic sampling. To the best of our knowledge, only four studies attempted sampling of plastic particles below 100 μ m size (see Table 2), and only three of them confirmed the plastic nature of the particles found (by using Raman micro-spectrometry⁴² and linear-array FTIR imaging System). These data allow estimates of SMP-a loads in the water between 0.029 and 2.9 μ g/L. These figures are again well below the safe concentration for 10–100 μ m MP (0.35 mg/L), and even below that for the 1–10 μ m size class (0.005 mg/L) (see Table S2). In conclusion, the scarce data available point also at low ecological risk associated to those small-size MP.

Another remarkable limitation of the ecotoxicological dataset is that only 2 from the 68 TT values here compiled correspond to MP from environmental sources. The other studies work with engineered particles or industrial products that may not be fully representative of secondary MP weathered in the environment. Frequently the tested materials are made of PS, which is not among the three most abundant polymers in the sea⁵⁴, and carry fluorescent chemicals or other dyes intended to facilitate studies of particle ingestion and distribution throughout the organism. Dispersants and preservatives are also common in commercial PS beads. The six most toxic particles, showing TT < 0.1 mg/L in ecotoxicological studies, all corresponded with particles labelled with fluorescent dyes. However, mean TT of dyed versus virgin particles were similar, and no significant differences in toxicity were found between unlabelled and dye-labelled MP ($p = 0.15$; two-way ANOVA with size class and dye as factors). Future research should focus on the toxicity of MP from environmental sources, since their shape and composition is very different from engineered particles, and thus provide estimates of toxicity more

environmentally relevant than artificial materials. In fact, plastic fragments may have a more abrasive surface geometry and cause more physical damage than microspheres. Despite previous identification of the use of PS and other engineered microbeads as a limitation for the environmental relevance of laboratory tests with MP^{11,13}, this microbeads continuous to be used and published.

Various mechanisms contribute to explain the higher impact of smaller particles. Most zooplankton and benthic filter feeders preferably ingest particles between 1 and 20 μm . Jeong et al.⁵⁵ proved that 0.1 μm and 2 μm PS particles were both readily ingested by a marine copepod, but 2 μm particles were more easily egested than 0.1 μm particles, which remained—although at low amounts—in the organism even in the presence of food. Fernández and Albentosa⁵⁶ using marine mussels showed that uptake and residence time in the organism increased as particle size decreased. However, this trend may not hold for smaller size ranges, since Al-Sid-Cheikh et al.⁵⁷ modeling uptake and depuration of 24 nm versus 150 nm particles in scallops, reached the opposite conclusion. This is consistent with a lower threshold for efficient particle capture by filter feeders. There is an upper limit in size for translocation of plastic particles from the digestive system to other tissues, although the value for this limit is an object of debate⁵⁸. Finally, environmental fragmentation of both polyolefins and biopolymers into plastic particles < 1 μm that can diffuse across biological membranes have been reported to account for enhanced deleterious effects on aquatic organisms^{59,60}.

Future field surveys should address plastics < 10 μm , the most toxic and bioavailable fraction for which environmental levels are unknown. Standard methods for monitoring the abundance of this low size ranges are urgently needed in order to conduct a more accurate assessment of the risk posed by plastic pollution in the global ocean.

Methods

Data acquisition. A comprehensive review was conducted on the available scientific data on, first, plastic particle density in surface and subsurface waters of the global ocean, and, secondly, toxicity of plastic particles to aquatic organisms. Concerning surface plastic densities, Song et al.⁶¹ reported that different sampling devices produce differences up to 4 orders of magnitude in floating microplastic abundance. Therefore, in order to allow data intercomparability, surface sampling techniques here reviewed were limited to manta trawl or neuston nets, corresponding to the most common sampling procedures. In those devices mesh size used ranged from 200 to 505 μm , with few exceptions^{62,63} that used nylon mesh of ca. 900 μm . Goldstein et al.²² reported that 98.5% of the particles caught in a 333 μm mesh would also have been caught in a 505 μm mesh, and there were no significant differences in particle size spectra using one size or another ($p=0.22$; Kolmogorov Smirnov test).

Common units chosen for this review were pieces per cubic meter for numerical density, and mg/L for mass density. When weight was not recorded but detailed measurements of particle size were available, plastic mass was estimated from particle size assuming 1 g/cm³ density, cylindrical shape for fibers and spherical shape otherwise. A number of studies report that most microplastics found in marine waters are fibers^{41,64,65}. Due to the lack of data in mass units and difficulties to estimate fiber mass from fiber length data, these studies could not be included in the present risk assessment unless detailed information on particle size distribution in more than one dimension were provided. Unlike fragments, pellets or films, airborne microfibers, for example from laboratory coats, can easily contaminate environmental samples during their analysis, and special precautions advised include working in clean airflow cabinets, normally not available on board of oceanographic vessels, and running filter controls⁴². In fact some studies simply exclude fibers from the records due to the risk of sample contamination^{34,37}.

Marine plastic density data in mass (mg/L) units were compiled in a single database to calculate a probabilistic distribution as described in Adam et al.¹⁷. Briefly, four types of data were found: (I) Studies reporting individual values for every trawl. These data were directly input into the database. (II) Studies reporting mean and standard deviation (s.d.) only. In these cases a log-normal distribution (see below) was modeled using the reported mean and sd as parameters. (III) Studies reporting mean and no s.d. In these cases dispersion was modeled using the mean and the average coefficient of variation of all datasets (201%, see Table 1). The probability distributions described above were pooled in order to generate a single distribution of microplastic abundance in the global ocean.

Concerning toxicity of plastic particles, an ecotoxicological database was built gathering all peer reviewed reports found on the toxicity of artificial polymer particles from 5 mm down to 0.01 μm . This includes the following size classes: LMP (from 5000 μm down to 100 μm), approximately corresponding to the range of sizes reported in oceanographic cruises using conventional plankton sampling devices, small microplastics, from 100 μm down to 1 μm , divided for this study in subclasses SMP-a (100–10 μm) and SMP-b (10–1 μm), and NP (< 1 μm). From the complete ecotoxicological database (114 papers) a subset reporting endpoints with proved ecological relevance affecting population size, i.e. those related with growth, reproduction and survival, was selected. Effects (both stimulant or inhibitory) on enzymatic activities or gene expression were excluded from this selection. Examples of endpoints not directly connected with ecological fitness are induction of antioxidant enzymes or other exposure biomarkers, histological observations, or minor changes in the biochemical composition of tissues. Examples of endpoints with proved ecological relevance are impairment of embryonal development, egg production, growth rate, immobilization or mortality. When the same study reported several ecologically relevant endpoints for the same species, the lowest effective concentration was included in the database. In contrast, when different studies reported the same endpoint in the same organism all values were considered and no attempt was made to judge the more representative testing material, even when values were broadly dissimilar (e.g.^{66,67} for nanoplastics and microalgae, or^{9,68} for small microplastics and bivalves). No attempt was made to judge quality of published data, but studies reporting non-monotonic dose:response

curves (where effects do not increase with dose) or showing no effects at the highest concentration tested when that concentration is comparatively low were discarded.

Statistical methods. The TT reported in the selected studies are compiled in Table S2. Following standard procedures, the cumulative distributions (P) of the TT values, expressed in 260 mg/L, were fit to log-logistic functions according to the equation⁶⁹:

$$P = \frac{1}{1 + 10^{(\log a - \log TT)/b}}$$

where a corresponds to the median of the TT distribution, and $1/b$ is the slope of the curve. From these distributions, termed in the ecotoxicological literature SSD curves, the HC_5 and the 95% confidence intervals were calculated. These parameters are commonly used for derivation of water quality criteria according to standard methods^{18,19,70,71}. Statistical analyses were conducted using IBM SPSS (v. 23) and R (v 3.5.0) statistical software. Effects of size and presence of dye in the particles on log-transformed TT were analyzed by two-way ANOVA. Normal Distribution and homoscedasticity were tested using the Shapiro–Wilk and Levene tests respectively. Bimodal distribution was tested using the Bimodality Coefficient (BC), according to the expression⁷²:

$$BC = \frac{\gamma^2 + 1}{\kappa + \frac{3(n-1)^2}{(n-2)(n-3)}}$$

where γ is the skewness, κ is the excess kurtosis, and n is the sample size. BC values above 0.555 correspond to bimodality.

Log-logistic models were fit using *nls.multstart* package⁷³ and log-normal models using *fitdistrplus* package^{68,74}, both from R⁷⁵.

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Author contributions

R.B. Conception and design of the study, Writing—Reviewing and Editing. A.M.S. Data acquisition and analysis. Software. Original draft preparation.

Competing interests

The authors declare no competing interests.

Additional information

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