A Probabilistic Risk Framework for Microplastics Integrating Uncertainty Across Toxicological and Environmental Variability: Development and Application to Marine and Freshwater Ecosystems

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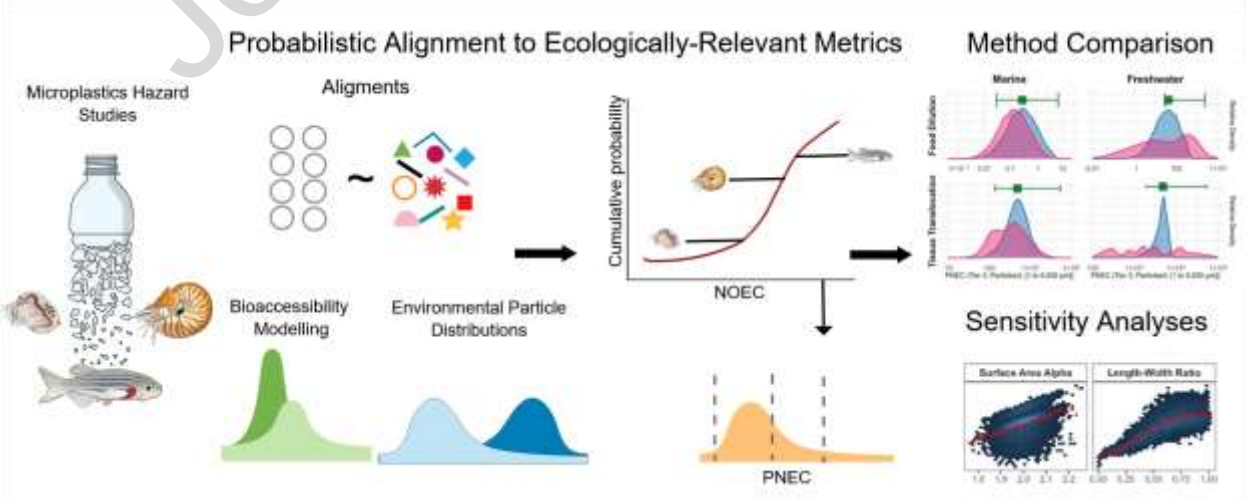

Abstract

Quantitative risk assessment for microplastics (MPs) is complicated by misalignments between environmentally relevant particles and those used in toxicity studies. Previous approaches addressed this using ecologically relevant metrics (ERMs) and species sensitivity distributions (SSDs), but did not propagate uncertainty from particle-trait alignments or intraspecies variability. Here, we present a novel probabilistic framework that propagates uncertainty through ERM alignments using Monte Carlo (MC) simulation, paired with a modified probabilistic SSD model (PSSD++). Using high-quality data from the updated Toxicity of Microplastics Explorer (ToMEx 2.0), we compared hazard thresholds derived by three approaches: traditional SSD, MC+SSD, and PSSD++. PSSD++ consistently produced the most health-protective median thresholds and lowest 5^{th}-percentile values, which generally exhibited the widest relative confidence intervals. MC+SSDs produced the narrowest uncertainty ranges. Uncertainty was greater for food dilution than for tissue translocation, and greater for freshwater environments than marine. Sensitivity analysis identified ERM-alignment parameters as the dominant drivers of threshold variability, contributing up to two orders of magnitude difference. This framework emphasizes the importance of propagating alignments uncertainty in MP risk assessments and highlights key research needs, including improved models for tissue translocation and more representative environmental particle characterizations.

Keywords

Microplastics; Probabilistic Risk Assessment; Hazard Characterization; Meta-Analysis; Ecotoxicology

Highlights

- Novel probabilistic ERA framework (PSSD++) for microplastics thresholds
- Integrated Monte Carlo uncertainty propagation into ERM alignments
- Applied framework to ToMEx 2.0, the largest MP toxicity database
- PSSD++ yields more precautionary but more uncertain thresholds
- ERM choice dominates threshold uncertainty across environment

Glossary

Acronyms

- **ABS** – Acrylonitrile Butadiene Styrene
- **AICc** – Corrected Akaike Information Criterion: model selection metric adjusted for small samples
- **DOM** – Dissolved Organic Matter: natural organic compounds in water
- **ECx** – Effect Concentration at x%: concentration causing x% effect (e.g., EC50)

- **ERA** – Ecological Risk Assessment: framework for evaluating potential adverse effects of contaminants
- **ERM** – Ecologically Relevant Metric: particle- and species-specific measure of MP exposure effects
- **HCx** – Hazard Concentration for x% of species: e.g., HC5 = protective for 95% of species
- **HONEC** – Highest Observed No Effect Concentration
- **ICx** – Inhibitory Concentration at x%: concentration at which x% inhibition occurs
- **LCx** – Lethal Concentration at x%: concentration causing x% mortality
- **LHS** – Latin Hypercube Sampling: efficient parameter space sampling method
- **MC** – Monte Carlo: repeated random sampling method for probabilistic simulation
- **MLE** – Maximum Likelihood Estimation: statistical method for parameter estimation
- **MP** – Microplastic: plastic particles <5 mm in size
- **NIAS** – Non-Intentionally Added Substances: impurities/byproducts in plastics
- **NOEC** – No Observed Effect Concentration: highest concentration at which no adverse effects are observed
- **PA** – Polyamide
- **PE** – Polyethylene
- **PET** – Polyethylene Terephthalate
- **PNEC** – Predicted No Effect Concentration: threshold below which no adverse ecological effects expected
- **PP** – Polypropylene
- **PS** – Polystyrene
- **PSSD** – Probabilistic Species Sensitivity Distribution: variant of SSD preserving species-specific variability
- **PSSD+** – Probabilistic Species Sensitivity Distribution Plus: adds uncertainty factors and chronic NOECs
- **PSSD++** – Modified PSSD+ used in this study, incorporating uncertainties from ERM alignments via MC simulations
- **PTFE** – Polytetrafluoroethylene
- **PUR** – Polyurethane
- **PVC** – Polyvinyl Chloride
- **RCI** – Relative Confidence Interval: (95th percentile – 5th percentile) / median
- **SSDs** – Species Sensitivity Distributions: represent variation in species sensitivity to a stressor
- **ToMEx 2.0** – Toxicity of Microplastics Explorer v2.0: expanded MP toxicity database

Field-Specific Terms and Definitions

- **Allometric Model** – Relates organism traits (e.g., body size) to physiological metrics
- **Bioaccessibility** – Fraction of MPs that can be absorbed based on size, shape, species
- **Bootstrapping** – Resampling method for estimating distribution uncertainty
- **Eco-corona** – Biomolecule layer on MP surfaces affecting behavior and toxicity
- **Empirical SSD** – SSD built from raw, untransformed toxicity data
- **Entanglement** – Physical hazard mechanism where fibers trap/interfere with organisms
- **Filter Feeders / Deposit Feeders** – Feeding strategies affecting MP exposure pathways
- **Fluorescent/Raman Spectroscopy** – Techniques to detect MPs via light interactions

- **Granuloma** – Localized inflammation from persistent particles like MPs
- **Power Law Exponent** – Describes frequency-size relationship of particles
- **Smoothed Distribution** – Continuous approximation of probability density
- **Step-Function Distribution** – Piecewise approximation for empirical distributions
- **Translocation** – Movement of particles from gut to tissues/circulatory system

Introduction

Microplastics (MPs), generally defined as plastic particles smaller than 5 mm [1, 2], represent a diverse and complex class of contaminants [3]. Their increasing prevalence, persistence, and bioavailability are raising significant concerns amongst governmental bodies [4, 5, 6]. This has led to increased pressure to develop a reliable, transparent ecological risk assessment (ERA) framework to inform effective risk management for decision-making. Despite recent advancements, significant challenges persist in MP ERA frameworks, particularly in accurately representing the diverse characteristics and behaviors of these contaminants.

A key challenge in MP ERA frameworks is the mismatch between exposures in hazard studies and actual environmental exposures. Discrepancies exist in particle size, shape, density, polymer types, chemical composition, and eco-coronas [7, 8, 9]. Methods have been developed to align MP exposure and effect data based on particle traits, enabling ERAs despite differing particle distributions [10, 11]. This alignment-based approach, combined with species sensitivity distributions (SSDs), informed MP management thresholds in aquatic ecosystems using toxicity data published <2021 [12]. However, confidence in these thresholds is low-medium due to limitations in underlying hazard studies, including limited environmental relevance of particles used (mostly monodisperse spheres or fragments), few studies meeting quality criteria, and insufficient toxicity mechanistic understandings [12]. Additionally, quantifiable uncertainties from alignments were not fully characterized, as models relied on single, deterministic values rather than complete probability distributions. These uncertainties limited their applicability in environmental risk management decisions, such as the risk characterization and regulatory decision for San Francisco Bay waterbodies adopted by the California State Water Resources Control Board [13, 14].

Thanks to the recent update of the MP toxicity database used in the ERA approach employed by [12] some uncertainties in MP ERAs may now be reduced. The updated Toxicity of Microplastics Explorer version 2.0 (ToMEx 2.0) database addresses some of the previous limitations, containing over twice the data of its predecessor. It features greater diversity in particle traits (*e.g.*, approximately 370 additional fiber data points and 8 new polymers), along with 26 new freshwater and 32 new marine species [15]. Furthermore, adding a significant number of freshwater species enablesenables thederivation of thresholds for marine and freshwater environments separately.

Another limitation of the ERA method employed by [12] and others lies in the dependence of SSDs on modelling the variation of species sensitivities to MP exposure. While the SSD approach has been used to derive environmental quality standards since the 1980's in the United States and Europe [16, 17], they have been criticized for their reliance on theoretical distributional assumptions that ignore a prior distribution of raw biological responses - and likely misrepresent the variability in biological responses [18], in addition to collapsing species-specific data into single deterministic values - obscuring uncertainties and intraspecies/interlaboratory variability [19, 18]. Analysis of large ecotoxicity databases demonstrate that this 'intertest variability' alone contributes roughly a threefold spread in effect concentrations, yet standard SSD practices average out this variability, confounding test-level noise with true interspecies differences [20]. To address these limitations, the probabilistic species sensitivity distribution (PSSD) method was developed. PSSD involves generating a probabilistic toxicity distribution for each species, which are then combined into an SSD for a specific ecosystem, preserving raw biological variability and distributions [21]. The PSSD has been further refined to incorporate probabilistic elements related to uncertainty factors, harmonizing toxicity endpoints into chronic, no-effect concentrations (NOECs) [22]. This 'PSSD+' method has been applied to MPs [23], but it has not yet incorporated non-alignment uncertainties into its model framework.

Here, we present an enhanced mechanistic ERA framework for MPs to both improve the reliability of threshold derivation and populate the assessment with the best available toxicological and environmental data. This framework probabilistically incorporates uncertainties in bioaccessibility, environmental particle distributions, inter-laboratory variability, and uncertainty factors through Monte-Carlo (MC) simulations, which we refer to as a PSSD++. We demonstrate the application of this PSSD++ approach using quality-screened toxicity studies from the ToMEx 2.0 database for freshwater and marine environments for both food dilution and tissue-translocation-mediated effect mechanisms (environments and ERMs treated separately), and provide sensitivity analyses to identify influential parameters and better understand the multi-dimensional nature of the assessment. Last, we assess the representativeness of the MP toxicity data through comparison to environmental occurrence data we extracted from the literature. The application of this framework here is provided as a detailed proof-of-concept, and it is recommended that further applications be applied using site-specific environmental particle distribution data, amongst other considerations (e.g., inclusion/exclusion of species; biological organization of endpoints included; etc.).

Materials and Methods

Hazard Data

This study utilized aquatic ecotoxicity data from the ToMEx 2.0 database, a publicly available dataset containing approximately 13,000 data points extracted from nearly 300 studies [15]. This dataset includes annotations for quality criteria, initially developed by [10] and refined in [12]. We applied the same filtering criteria as in [12] based on quality, biological organization (population, individual, tissue, cellular, subcellular), and organismal groups (*e.g.*, plants, algae, fish), according to the relevant threshold tier (i.e., tiers 1 – 4), which progress from highly protective assumptions based on all biological endpoints to more predictive, higher-confidence thresholds based on organismal- and population-level endpoints) and ecologically relevant metric (ERM) (supplemental information).

Unlike conventional chemicals, ERMs for plastic debris are not single concentrations (*e.g.*, mg/L). Instead, they represent particle- and species-specific effect mechanisms, encompassing physical characteristics (*e.g.*, size, shape, density) and chemical composition relevant to the environment [7]. Consistent with [12], we focused on 'food dilution' and 'tissue-translocation-mediated effects' as ERMs. Only data with defined effect metrics (*i.e.*, NOECs, lowest observed effect concentrations [LOECs], effect concentrations [ECx], inhibitory effect concentration [ICx], and lethal effect concentrations [LCx]) were used. Intermediaryl exposure concentrations thatthat did not specify a dose-response point (e.g., concentrations reported between LOECs and ECx/LCx values) and highest observed no effect concentrations (HONECs) were excluded [12]. Aligning toxicity data to ERMs requires particle-level environmental monitoring data, for which we used the freshwater and marine surface water data published by [24]. This dataset is restricted to particles ≥ 1 µm, so we only aligned hazard data that exposed organisms to particles ≥ 1 µm. Following [12], only particle-based studies were used – with studies involving the addition of sorbed and/or dissolved chemical toxicants excluded. Further details on ToMEx 2.0 and the threshold population methods for freshwater and marine environments can be found in [12] and [15], as well as in the supplemental information.

Occurrence Data

To evaluate how representative the MPs in the ToMEx 2.0 toxicity dataset are of environmental conditions, we compared their particle traits and relative abundances to those found in marine and freshwater environments. We conducted targeted literature searches for environmental datasets (i.e., Zenodo, Mendeley Data; search term: "microplastics," searched March–May 2024) and published summaries (i.e., Google Scholar, searched July 2024) reporting MP characteristics. For each sample or site, we extracted the proportions of different polymer types (grouped into polyethylene/polyethylene terephthalate/polyester [PE/PET/Polyester], polypropylene [PP], polystyrene [PS], polyamide [PA], polyurethane [PUR], polyvinyl chloride [PVC], polytetrafluoroethylene [PTFE], and others), shapes (spheres, fragments, fibers), and average particle lengths and widths. These traits were selected because they are most commonly reported and used in ERM alignments. PE, PET, and polyester were grouped, as they were already pooled in most source studies. Other characteristics known to influence toxicity—such as biofouling [25], surface charge [26, 27], chemical composition including additives and non-intentionally added substances (NIAS) [28, 29], and surface functional groups [30, 31]—were not included, as they were rarely reported. Where available, raw data were used; otherwise, values were extracted from figures using the R package *metaDigitise* [32].

In total, we compiled MP trait data from 87 environmental samples (53 freshwater, 36 marine) across 12 published sources [33, 34, 35, 24, 36, 37, 38, 39, 40, 41, 42, 43] (see Excel table in supplementary information). We compared these environmental MP characteristics to those in ToMEx 2.0 using non-metric multidimensional scaling (NMDS), a technique for visualizing complex multivariate data in reduced dimensions [44]. As we did not apply quality screening (e.g., [45]) to the environmental data, these comparisons may be influenced by unassessed study biases.

Tissue Translocation Bioaccessibility Modelling

We identified studies in ToMEx 2.0 that determined whether or not tissue translocation of MPs occurred in exposed organisms, and extracted information associated with MP physical characteristics for each species. Tissue translocation observations were often reported as binary (*i.e.*, translocated or not), and in the case of the three studies that reported percentages of administered particles translocated, any amount greater than zero was considered as translocated for the purposes of this assessment. Data from these studies were used to model the probability of tissue translocation for MPs based on particle length using binomial logistic regression, with the logit link function: $log(p / [1-p]) = \beta_0 + \beta_1 X_1$, where p is the probability of the event occurring, β_0 is the intercept, β_1 is the regression coefficient, and X_1 is particleparticle length. To estimate the uncertainty in the threshold particle length associated with a 50% probability of translocation, we used bootstrap resampling (n = 10,000 simulations).

Ecotoxicological threshold modelling

Two novel approaches were developed and applied here to derive MP ecotoxicological thresholds in this study, and were compared to the traditional SSD-based approach developed previously in [12] (with the correction described in [46]). All three approaches used the ERM-based alignment approach described in [12]((with the minor modification for polydisperse mixture bioaccessibility modelling described in the supplementary information and in [15]) and apply identical tier filters, ERM definitions, and size ranges (1 to 5,000 µm).

The four-tier threshold framework applied here follows [12], where each tier applies a different set of biological filters and species-level data-collapsing rules, producing a protective-to-predictive

gradient of thresholds. Tiers 1 and 2 ("protective" tiers) include all biological levels of organization and collapse multiple toxicity values for each species to the 1st quartile. Tiers 3 and 4 ("predictive" tiers) restrict inputs to organismal- and population-level endpoints and collapse species data to the median. The lower three tiers use the HC5, but Tier 1 differs in that the threshold is taken as the lower 5th-percentile confidence bound of the SSD rather than the median HC5 estimate. Tier 4 uses the HC10 to reflect increased confidence in higher-level biological responses.

The two novel approaches handled these ERM uncertainties probabilistically (Table 1). MethodMethod 1 (MC+SSD) applies the full ERM/SSD-based workflow from Thornton-Hampton et al. [15] butbut introduces MC simulations to propagate uncertainty in bioaccessibility limits and environmental particle-trait distributions.Method 2 (PSSD++) uses the same MC-aligned dataset as Method 1 but replaces SSD fitting with a modified PSSD+ framework that also propagates intraspecies variability and assessment-factor uncertainty (Table 1).

All modelling components are linked through a unified sequential workflow: bioaccessibility models provide ingestion and tissue-translocation limits; these limits and particle-trait distributions are used to align each toxicity value to its ERM; and the aligned dataset (either single-value or MC-propagated) is then passed to the selected threshold-modelling method (traditional SSD, MC+SSD, or PSSD++). Only the MC methods propagate uncertainty in bioaccessibility and ERM alignment; the traditional SSD method retains deterministic alignments. For each environment, tier, and ERM, we calculated relative confidence interval widths (RCI = (95th–5th percentile)/median) as a standardized measure of uncertainty [47].

Method 1 (MC+SSD):

Method 1 combines MC simulations for uncertainty propagation on alignments with SSD threshold derivation, as in [12]. SSDs for unique ERM, threshold tier, and environment combinations were derived for each MC simulation, forming distributions of threshold values that represent alignment uncertainty. This differs from [48] by probabilistically treating all parameters (instead of just power law values).

Latin Hypercube Sampling (LHS) was used to construct probability distributions for MC ERM alignments, ensuring efficient and uniform sampling across multidimensional parameter space. LHS, which is advantageous for complex models and recommended for microplastics [, 49, 50], utilized the *sobol_matrices()* function from the *sensobol* R package [51] to generate a Sobol sequence. This sequence yielded 589 samples across 16 parameters, totaling 9,423 simulations, with each parameter transformed into its respective probability distribution.

Environmental MP probability distribution data for ERM alignments, as in [12], came from [24]. Alpha power law values were sampled from normal distributions based on [24] mean and standard deviation, while length-width ratios were sampled from a truncated normal distribution [24] with bounds of 0.0001 to 1. Bioaccessibility limits were based on a pre-existing ingestible size model and a derived tissue translocation logistic regression model. Ingestion bioaccessibility used the [52] allometric model, with Beta and Intercept values sampled from normal distributions (means/standard errors: 0.9341 ± 0.1376; 1.1200 ± 0.3222, respectively, from their codebase). Species- and life stage-specific body lengths from [15] were used. Tissue translocation bioaccessibility limits were estimated by deriving the particle length associated with a 50 % probability of translocation from the fitted logistic regression

model described above by solving for the midpoint (-β0/β1).) To capture uncertainty, MP particle lengths were sampled from the fitted regression model by generating normal distributions forfor the logistic regression's intercept (β1) and slope (β0) parameters using their associated standard errors. Values outside 1-500µm were removed for biological plausibility and consistency with the 1µm default lower limit, resulting in a heavily right-skewed truncated ratio distribution (Figure 1).

All probability distribution parameters are in Table S4 and visualized in Figure 1. To reduce extreme outlier influence, z-scores were calculated for thresholds per environment/tier/ERM. Values with |z-score| > 3 were removed (0.01% to 2.2% occurrence; Table S6).

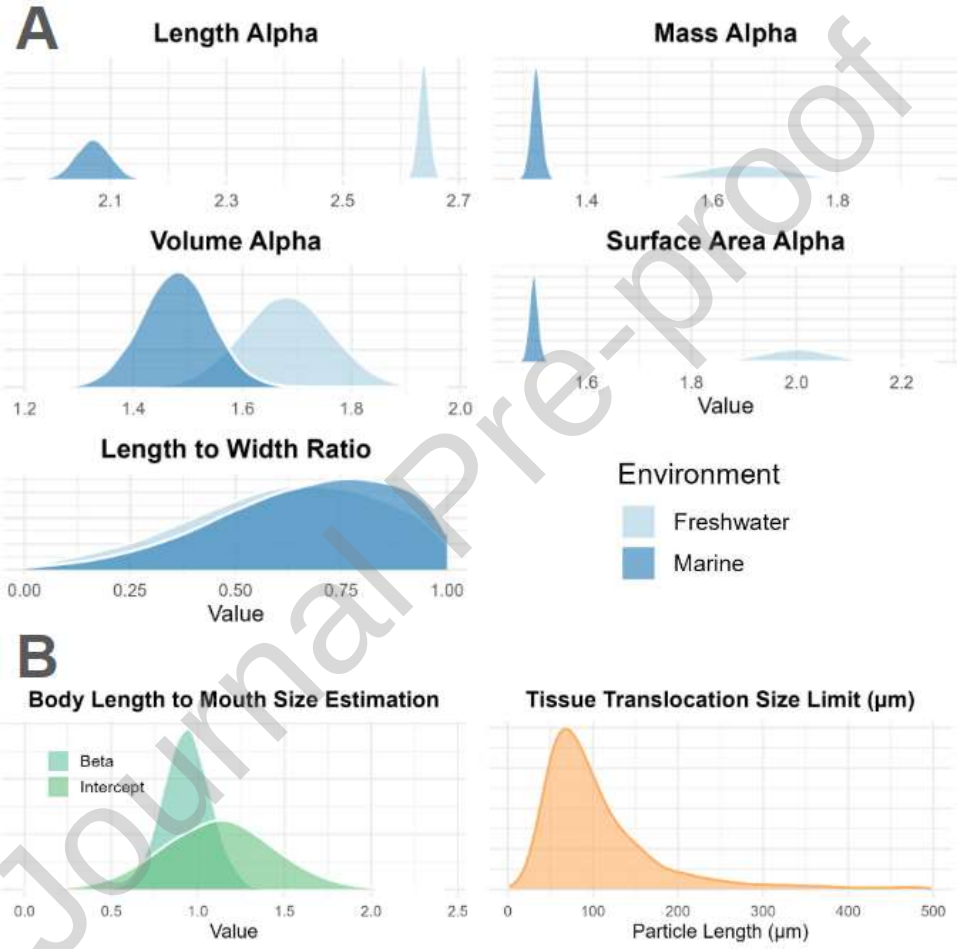

Figure 1. Distributions of ERM alignment and re-scaling parameters used in MC simulations. **(A)** Power law (alpha, unitless) values for particles (*i.e.*, length, mass, volume, and surface area), and particle length to width ratios (unitless scale from 0 to 1) are environment-specific (dark blue = marine, light blue = freshwater), are from [24]. **(B)** Values used to estimate mouth size openings of organisms based on their body lengths using the allometric equation derived in [52]. The tissue translocation size values represent the sizes of microplastics (µm) with a predicted 50% probability of translocating tissues based on the generalized linear model with the lowest AICc (*i.e.*, logistic regression; binary translocation ~ particle length; see supplementary information)**.** All species, life stages, organism groups, environments, polymers, and shapes were included in the generalized linear model used to estimate translocatable microplastic particle sizes. The distribution of values was generated from 10,000 simulations.

Method 2: Probabilistic Species Sensitivity Distribution (PSSD)++

Method 2 (PSSD++) combines MC-aligned toxicity values (from Method 1) with a modified PSSD+ approach. Unlike standard SSDs, PSSD(++) uses MC simulations to estimate uncertainty for each species' mean, considering data distribution, inter-laboratory variation, and assessment factors (exposure time, dose descriptors). While SSDs assume a community response distribution(*e.g.*, log-logistic, log-normal, etc.) [53], PSSD(++) uses an empirical approach, avoiding the issue of communities not following single distributions [21, 53 54].

We further modified PSSD+ to incorporate variability from ERM alignments derived probabilistically from the MC simulations for each species/environment, rather than a blanket intra-laboratory coefficient of variation (i.e., 0.3 as applied in [22]). Our approach resulted in intraspecies coefficient of variation values ranging between 0.08 to 2.7, with wider variability when aligning to food dilution than to tissue translocation ERMs (see supplemental information for details). We also addressed two technical issues enabling derivation of tiered thresholds and incorporation of large MC datasets (details in Supplementary Information). We only applied this approach to tiers 3 and 4 of the [12] framework, as tiers 1 and 2 collapse species-level data to a single point estimate (1st quartile), which precludes the probabilistic species-level distributions required by the PSSD++ method.

MC simulations on an Intel Core i7 with 64 GB RAM (12 cores) took approximately 5 hours to generate SSD-based thresholds, utilizing LHS for efficient sampling and robust uncertainty propagation. PSSD++ generations, using single-threading on the same system, took about 40 minutes per environment/ERM, totaling 2.7 hours. Due to RAM limitations, 300 PSSD++ simulations were run per analysis, rather than the typical 10,000 as employed in [21] and [55], which was deemed sufficiently stable after iterative analyses.

Table 1. Comparison of the traditional Species Sensitivity Distribution (SSD) method (as applied in [12]) with the two novel probabilistic approaches developed in this study (i.e., Monte Carlo [MC] + SSD and probabilistic SSD [PSSD++]). ERM = ecologically relevant metric; AF = assessment factor; HCx = hazard concentration for x% of species.

Feature ↓ Method →	**Traditional SSD [12; 15]**	**MC+SSD**	**PSSD++**
Uncertainty treatment	Deterministic; no propagation of ERM-alignment uncertainty	Probabilistic via MC sampling of alignment parameters	Probabilistic; incorporates uncertainty in ERM alignments, intraspecies variability, and species-level aggregation
Distributional assumption(s)	Parametric SSD (e.g., log-logistic/log-normal, etc.)	Parametric SSD (e.g., log-logistic/log-normal, etc.)	MC-generated SSD (nonparametric)
Alignment inputs (i.e., ERM, bioaccessibility limits, particle traits)	Single-value alignment	MC-propagated alignment distributions	MC-propagated alignment distributions

Species-level representation	1 point per species (tier-dependent)	1 point per species per MC simulation (also tier-dependent)	Full distribution per species (propagated variability + alignment + AF uncertainty)
Assessment factor (AF) treatment	Deterministic; fixed AFs applied directly	Deterministic AFs applied (after MC)	Probabilistic; AF uncertainty incorporated into species-level MC distributions
Output	HCx with 95% CI reflecting parametric SSD uncertainty	Distribution of HCx reflecting combined alignment and parametric SSD uncertainties	Distribution of HCx reflecting alignment, species-level, and AF uncertainties

Sensitivity Analyses

We conducted four sensitivity analyses on thresholds to understand alignment multidimensionality, disentangle species and environmental sensitivities, determine individual study influence, and evaluate study quality filters. We performed a one-at-a-time sensitivity analysis using MC simulation to explore the influence of ERM-alignment parameters, visualizing relationships with hexagonal density scatterplots for representative Tier 2 freshwater and marine thresholds. We also assessed whether marine or freshwater species are inherently more or less sensitive by generating thresholds using freshwater species while holding other parameters constant (e.g., using marine environmental microplastics distribution data). A leave-one-out sensitivity analysis determined individual study influence, systematically removing studies and recalculating thresholds, as a single data point can disproportionately affect HC values. Finally, we assessed the influence of quality filters (technical and risk assessment criteria from [12] using a leave-one-out approach on representative Tier 2 thresholds for both food dilution and tissue translocation ERMs in marine and freshwater environments. All leave-one-out sensitivity analyses were conducted relative to the default study/species filters for each tier, as described earlier.

Results

Database coverage

Organismal data covered in the ToMEx 2.0 dataset

ToMEx 2.0 contains approximately an even split between toxicity data for freshwater and marine species, with 144 studies for freshwater species (*n* = 63 species; 6,555 data points), and 149 toxicity studies for marine species *(n* = 101 species; 6,243 data points). However, approximately 90% of all toxicity data in ToMEx 2.0 was not used in threshold modelling due to the data points failing one or more of the following conditions: the study did not meet minimum quality criteria from [12]; an inapplicability of species (*e.g.*, plants and bacteria were not included); the exposure particles being outside of the default (*i.e.*, 1 to 5,000 µm) or bioaccessible (species/ERM-dependant) size range; or the effect metric being an intermediary exposure concentration or a HONEC. Specifically, just 16 studies for freshwater species (*n* = 10 species; 140 data points), and 16 studies for marine species (17 species; 235 data points) were used for any of the four-tiered thresholds, with the majority of data points requiring some adjusting using uncertainty factors (*i.e.*, to translate from acute to chronic, or from various effect metrics to NOECs), with 71% of marine data points and 84% of freshwater data points requiring a composite uncertainty factor between 2 and 100 for tiers 1 and 2 (Table S1). The proportions of chronic vs. acute studies was consistent for tiers 1&2 (36% chronic) and 3&4 (28% chronic) for freshwater species, while for marine species, proportions varied dramatically between these tiers (76% chronic for tiers 1&2; 27% chronic for tiers 3&4) (Table S1).

While the overall amounts of data were comparable between environments, the representation of biological diversity was significantly greater for the marine thresholds, with 8 distinct organismal groups included in the marine thresholds, compared with just 3 for freshwater thresholds. Of the marine data used in any thresholds, there were 21 genera represented: *Oryzias*, *Crustacea*, *Echinoderm*, *Mytilus*, *Diaphanosoma*, *Pseudechinus*, *Cnidaria*, *Hydra*, *Tigriopus*, *Ciliophora*, *Strombidium*, *Karenia*, *Rotifera*, *Brachionus*, *Pinctada*, *Paracentrotus*, *Crepidula*, *Tripneustes*, *Parvocalanus*, *Centropristis*, *Skeletonema*. Of the freshwater data that we used in any threshold, there were only 7 genera represented: *Daphnia*, *Ceriodaphnia*, *Moina*, *Hyalella*, *Oryzias*, *Oncorhynchus*, *Raphidocelis*, *Chlorella*, and *Danio*. For biological levels of organization, the majority of the data for both freshwater and marine species were for individual-level and sub-cellular responses (Figure 2).

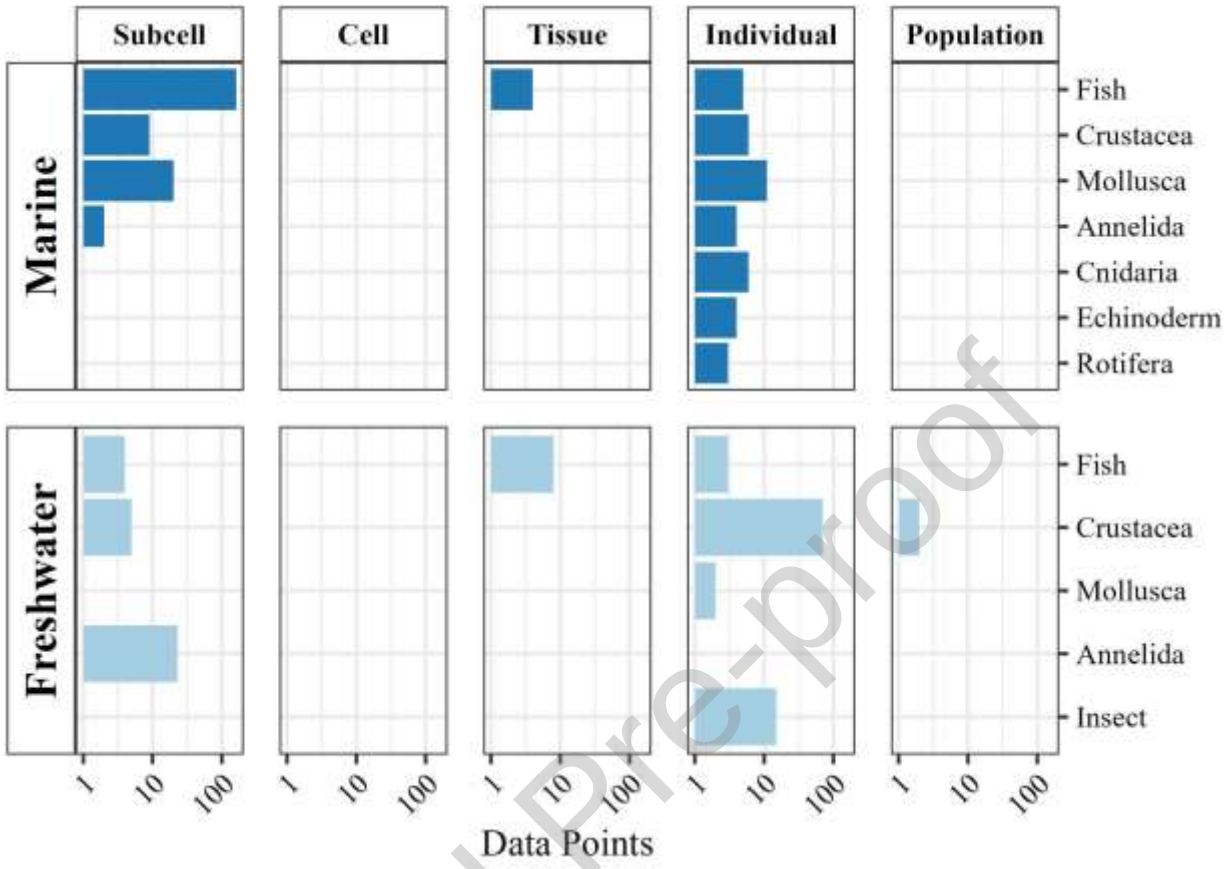

Figure 2. Barplot showing microplastic hazard data used for threshold derivation in this study, faceted by environment and biological levels of organization. Organismal groups are separated in rows. Note that the x-axis is in log10 scale.

MP Particle Trait Hazard Data Representativeness

A comparison was made between the characteristics of MPs used in toxicity tests (ToMEx 2.0) and those more commonly found in environmental samples (Figure 3). The NMDS analysis revealed differences between the MPs used in toxicity tests and those present in freshwater and marine environments (Figure 3). It is important to note that, although the NMDS ordination plot is a useful visualization of these multivariate differences, the apparent proximity of some environmental samples to the ToMEx 2.0 centroid primarily reflects the compression of high-dimensional particle-trait variation into two axes rather than true similarity to the spherical, polydisperse laboratory particles represented in the database.

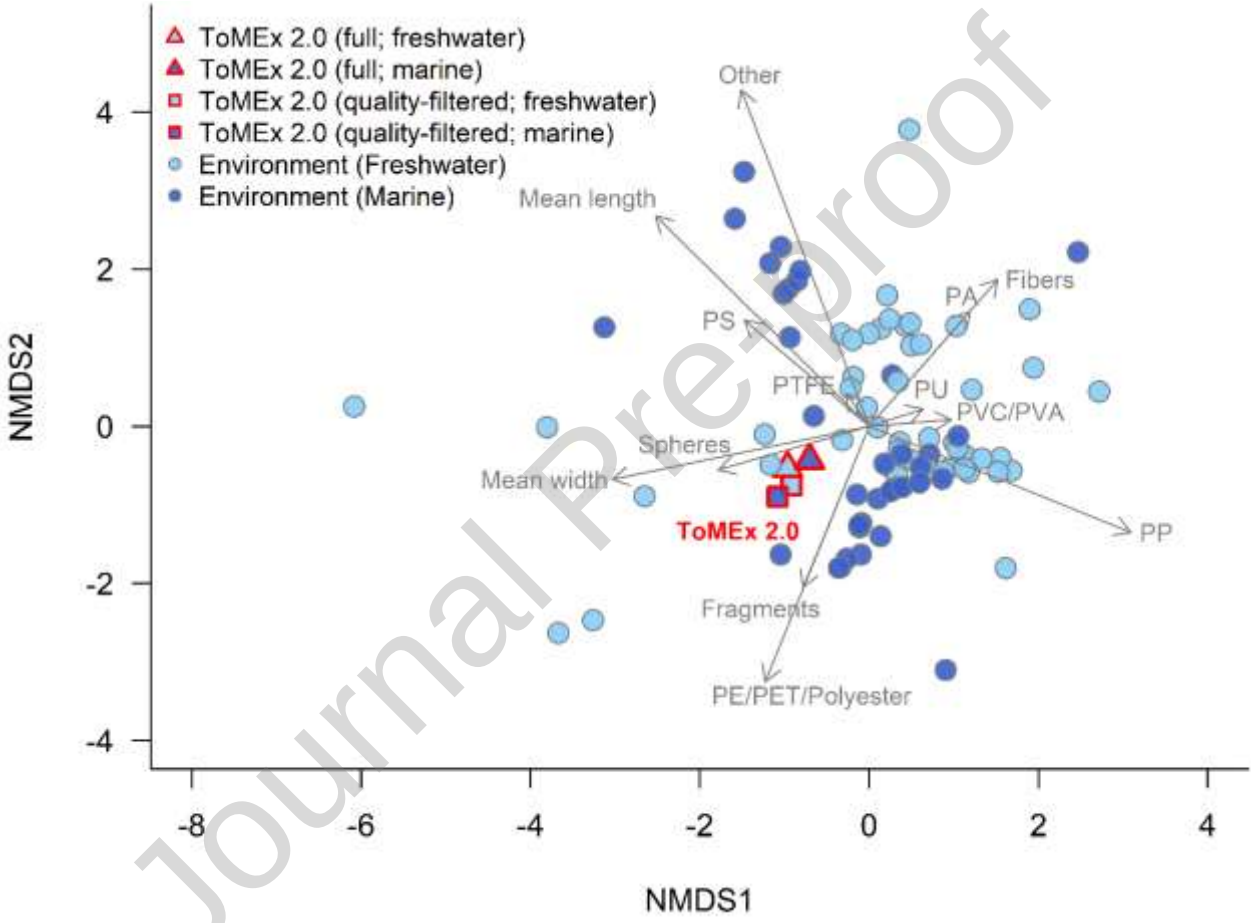

Figure 3. NMDS scatterplot demonstrating the representativeness of MP particle traits in ToMEx 2.0 compared to MPs from environmental samples. MP particle trait data analyzed here included relative proportions of different polymer types, shapes (fragments, spheres, fibers), and size measures (mean length, median length, surface area). Triangles: full ToMEx 2.0 data, squares: ToMEx 2.0 data (quality score filtered), circles: environmental samples, light blue: freshwater, dark blue: marine. Arrows show increasing values for the respective particle characteristics. Arrow lengths reflect the strength of the association with the NMDS axes.

Toxicity tests (full ToMEx 2.0 data) involving both marine and freshwater organisms were strongly associated with the use of spheres (34% of experiments) and fragments (39% of experiments), and PS (24% of experiments) and PE/PET/Polyester (43% of experiments) polymers. The relative abundances of

spheres and fragments, and PS and PE/PET/Polyester MPs in the environment were comparatively lower overall ($median_{(spheres)}$: 0%, $range_{(spheres)}$: 0-77%; $median_{(fragments)}$: 22 %, $range_{(fragments)}$: 0-94 %; $median_{(PS)}$: 4%, $range_{(PS)}$: 0-69%; $median_{(PE/PET/Polyester)}$: 36%, $range_{(PE/PET/Polyester)}$: 0-100%). Conversely, PP (median proportion: 14%) was primarily associated with MPs from environmental samples but was largely absent from toxicity tests (6% of particles). Concerning particle size, the mean length and width of particles used in the toxicity tests was smaller ($mean_{(length)}$ ± SD: 250 ± 834 µm, $mean_{(width)}$ ± SD: 135 ± 481µm) than in environmental MPs ($mean_{(length)}$ ± SD: 596 ± 748 µm, $mean_{(width)}$ ± SD: 124 ± 233 µm, however these differences are likely due to monitoring artifacts - i.e., the size distributions of measured particles are highly influenced by the mesh sizes of filters used, and size limitations of the analytical method(s) used. A notable underrepresentation of fibers was observed in ToMEx 2.0, making up 10% of particles in marine studies and 7% of particles in freshwater studies (8% overall), in contrast to its predominance in environmental samples, with median proportions of 49% and 85% in marine and freshwater studies (overall median: 77%), respectively.

Tissue Translocation Data Extraction and Modelling

We identified 27 studies within the database reporting tissue translocation measurements in exposed organisms, comprising 54 unique observations (*i.e.*, the unique combination of MP characteristics and species/tissues), with 36 confirmed observations of translocation, and 18 reported non-translocations. The vast majority of studies used spheres (18), with a limited number using fragments (5) or fibers (4). Particle lengths in these studies ranged from 0.024 µm to 1,500 µm, with a median length of 5.5 µm (mean length = 63 µm; 1st quartile: 0.25 µm, 3rd quartile: 60.8 µm). Polystyrene was the most commonly-used polymer in these studies (15), followed by polyethylene (4), unreported polymer types (4), polyamide (2), a proprietary composition from Cospheric (4), polyvinyl chloride (1), and polyester (1), with the remaining studies investigating a mix of polymers including acrylonitrile butadiene styrene, the natural polymer cotton, polyester, polyethylene terephthalate, polypropylene, polyethylene co-vinyl acetate, and polyacrylonitrile. Fish were the most commonly investigated species in these studies (19), followed by mussel (3), with the following species having just one study each: crab, crustacean, mollusk, oyster, and shrimp. Liver was the most commonly investigated tissue (5 studies), with the following tissues also reported (studies often reported translocation in multiple tissues): whole body, fillet, embryos, head, yolk sac, brain, eyes, ovaries, gonads, spleen, kidney, pancreas, circulatory system, cytoskeleton, muscles, and skin.

The binomial logistic regression analysis of tissue translocation MP data revealed a significant inverse relationship between MP particle length and the probability of tissue translocation (Figure 4). Distinctions according to particle characteristics (*e.g.*, polymers, shapes) or organismal traits (*e.g.*, species, tissues, etc.) were not considered in these analyses due to the relatively small size of the dataset. Therefore, the association between particle size and translocation probability that we derived here was generalized across all particle types and organisms in our bioaccessibility modelling. MP particle length was a significant predictor of tissue translocation (logistic regression: z = ; p = 0.03; Figure 4). The model yielded an intercept (β_o) of 1.34 (SE = 0.40) and a slope coefficient (β_1) of -0.015 (SE = 0.0067) for particle length (µm), indicating that as particle length increases, the log-odds of translocation decrease. The root mean square error of this model was 0.53 log-odds units. The truncated ratio distribution of particle lengths at which there is a 50% probability of translocation across tissues derived using these parameters

was lognormal, with a median value of 88 µm, a mean value of 128 µm, an interquartile range of 62 to 131 µm and a 95% confidence interval of 36 to 302 µm (Figure 4).

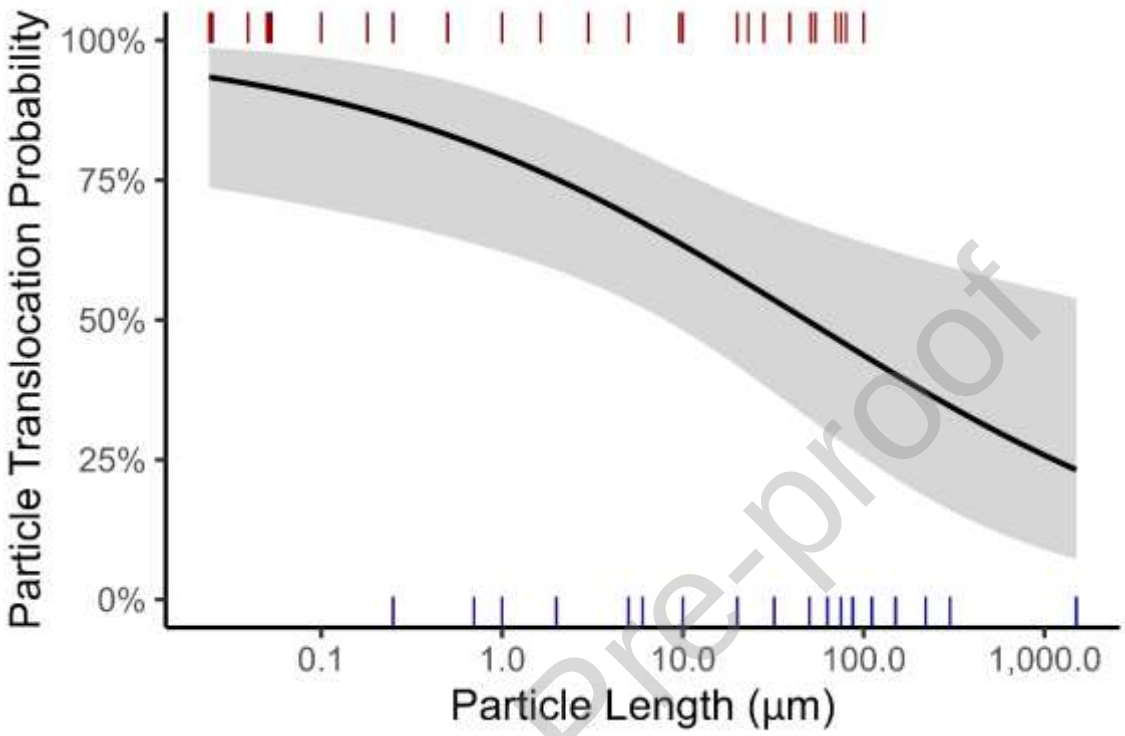

Figure 4. The binomial logistic regression model was fit to MP tissue translocation data. The y-axis represents the probability of translocation, and the x-axis is the MP particle length (µm) (note the log10 scale). The ribbon surrounding the regression line represents the 95% confidence interval. Individual data points for tissue translocation study findings (binary) are represented with red bars on top (in the case of translocation being demonstrated) and blue bars on the bottom (in the case of translocation not being demonstrated).

Probabilistically-Derived Thresholds

Thresholds derived using the three approaches—SSD, MC+SSD, and PSSD++—ranged from 1×10^{-5} to 137 particles/L for food dilution, and from 0.81 to 9,510 particles/L for tissue translocation-mediated effects across all tiers and environments (Table 2). While median threshold values were broadly in the same order across methods, SSD and MC+SSD results were within 20% of one another, whereas PSSD++ thresholds were consistently lower—by up to 2-fold—with the largest differences observed in marine environments (Table 2).

Despite the similarity in medians, the methods diverged in how they captured uncertainty. SSD-derived thresholds exhibited the widest relative confidence intervals (RCIs), except for Tier 3 freshwater food dilution, where PSSD++ had the highest RCI (Table S10; Figure 6). For all tiers and ERMs, the 5th percentile values were lowest for PSSD++, while its 95th percentile values were often intermediate—lower than SSD but higher than MC+SSD. Although this pattern might initially suggest that PSSD++ captures a broader range of uncertainty, the wider RCIs observed for SSD thresholds largely reflect uncertainty

introduced through parametric model fitting, especially in cases with limited species counts or wide intra-species variability. By contrast, the PSSD++ approach relies on empirical species-level distributions rather than parametric SSD fits, which can reduce upper-tail variability and result in narrower RCIs despite propagating more process-level uncertainty. Additionally, the use of 300 PSSD++ simulations (due to RAM constraints)- fewer than the iteration counts typically employed in PSSD analyses- may limit sampling of the extreme tails and thus underestimate the full uncertainty range. Consequently, while PSSD++ thresholds for some cases (e.g., Tier 3 freshwater food dilution) varied substantially- up to 24-fold across the confidence interval (Table S8)- direct comparisons of RCI widths across methods should be interpreted cautiously.

Threshold distributions from the PSSD++ method varied by ERM and environment but showed relatively minor differences across tiers (Figure 5; Figure 6; Figure S13A). Intra-species data availability and integrated variability varied significantly by species and environment, while remaining consistent across ERMs (Figure 5). Freshwater tissue translocation thresholds were highly positively skewed, with long left tails, while marine thresholds exhibited minimal skew. Kurtosis was highest for tissue translocation thresholds overall, especially in marine systems. Freshwater thresholds had higher RCIs (8 to 59) than marine thresholds (3 to 9) across ERMs and tiers. Among ERMs, food dilution thresholds had higher uncertainty (RCIs: 8 to 59) than tissue translocation thresholds (RCIs: 3 to 18) (Table S8). Within each ERM and environment, Tier 4 thresholds consistently showed lower RCIs than Tier 3, likely reflecting greater robustness of higher percentile (HC) values. Marine thresholds also showed a higher percentage of statistical outliers (±1.5 × IQR) than freshwater thresholds for both ERMs (Figure S5).

In summary, while all methods yielded similar median thresholds, PSSD++ consistently produced the lowest medians and 5th-percentile threshold values, reflecting its more precautionary treatment of uncertainty. However, PSSD++ did not consistently show the widest uncertainty bounds; SSD-based thresholds generally had the largest RCIs, with PSSD++ exceeding them only in specific cases (e.g., Tier 3 freshwater food dilution). This reflects differences in how uncertainty is estimated, as well as the limited number of PSSD++ simulations, which may constrain tail sampling. Despite consistent trends in thresholds between methods, results are not statistically different due to the relatively high variability in the underlying data.

Table 2. Proposed four-tiered SSD-based ecotoxicological microplastics thresholds for freshwater and marine environments based on food dilution and tissue translocation-mediated effects ERMs derived using the traditional SSD-based approach [12; 15]; the MC+SSD approach, and the PSSD++ approach. Values represent median threshold values from the probabilistic assessment, with the values in parentheses corresponding to the 95% confidence intervals. Values are reported in particles/L (aligned to particle lengths of 1 to 5,000 µm).

	Environment→	**Marine**		**Freshwater**	
Tier ↓	**ERM → Method ↓**	**Food Dilution**	**Tissue Translocation**	**Food Dilution**	**Tissue Translocation**

Tier1 (95% CI)	**SSD[a]**	**$4.8x10^{-5}$** (NA)	**0.82** (NA)	**0.4** (NA)	**93** (NA)
	MC+SSD[b]	**$1.0x10^{-5}$** ($1.0x10^{-5}$ to $1.0x10^{-4}$)	**0.81** (0.21 to 1.8)	**0.4** (0.03 to 4)	**100** (66 to 180)
	PSSD++[c]	**NA[c]**	**NA[c]**	**NA[c]**	**NA[c]**
Tier2 (95% CI)	**SSD[a]**	**$2.6x10^{-3}$** ($4.8x10^{-5}$ to 1.1)	**13** (0.82 to 380)	**4.9** (0.4 to 150)	**720** (93 to 23,000)
	MC+SSD[b]	**$2.6x10^{-3}$** $2.3x10^{-4}$ to $2.2\ x10^{-2}$)	**13** (6.4 to 30)	**4.1** (0.34 to 38)	**710** (460 to 1,100)
	PSSD++[c]	**NA[c]**	**NA[c]**	**NA[c]**	**NA[c]**
Tier3 (95% CI)	**SSD[a]**	**0.30** (0.03 to 7.3)	**490** (140 to 5,300)	**42** (28 to 2,400)	**4,800** (1,900 to 54,000)
	MC+SSD[b]	**0.28** (0.029 to 2.2)	**480** (160 to 1,200)	**36** (3.4 to 250)	**4,600** (2,000 to 6,700)
	PSSD++[c]	**0.140** (0.01 to 1.2)	**320** (74 to 1,400)	**31** (0.14 to 1,500)	**2,150** (167 to 38,000)
Tier4 (95% CI)	**SSD[a]**	**1.5** (0.22 to 44)	**1,600** (430 to 16,000)	**140** (63 to 6,700)	**9,500** (3,200 to 120,000)
	MC+SSD[b]	**1.3** (0.15 to 9.5)	**1,550** (605 to 3,820)	**120** (14 to 770)	**9,500** (4,100 to 14,000)
	PSSD++[c]	**0.64** (0.081 to 5.0)	**710** (190 to 2,500)	**140** (6.3 to 1,800)	**6,400** (420 to 56,000)

[a]95% confidence intervals for SSD-based thresholds are the bootstrap uncertainties of distributions fit to the species data at the HC5 for Tiers 2 and 3, and HC10 for Tier 4. Because Tier 1 is the lower 95% confidence interval of the SSD at the HC5, no further uncertainty estimates can be provided for that tier and this approach.
[b]95% confidence intervals for MC+SSD-based thresholds represent the distribution values at those percentiles for the HC5/HC10 SSD values for the corresponding tier.
[c]Median values from PSSD++ values are presented, alongside the 5th and 95th percentiles at the HC5/HC10 in the PSSDs for Tiers 3 and 4, respectively. Since data collapsing is not applied in the PSSD++

approach, values are not presented for Tiers 1 and 2 (which use the 1st quartile of each species' data to populate their SSDs).

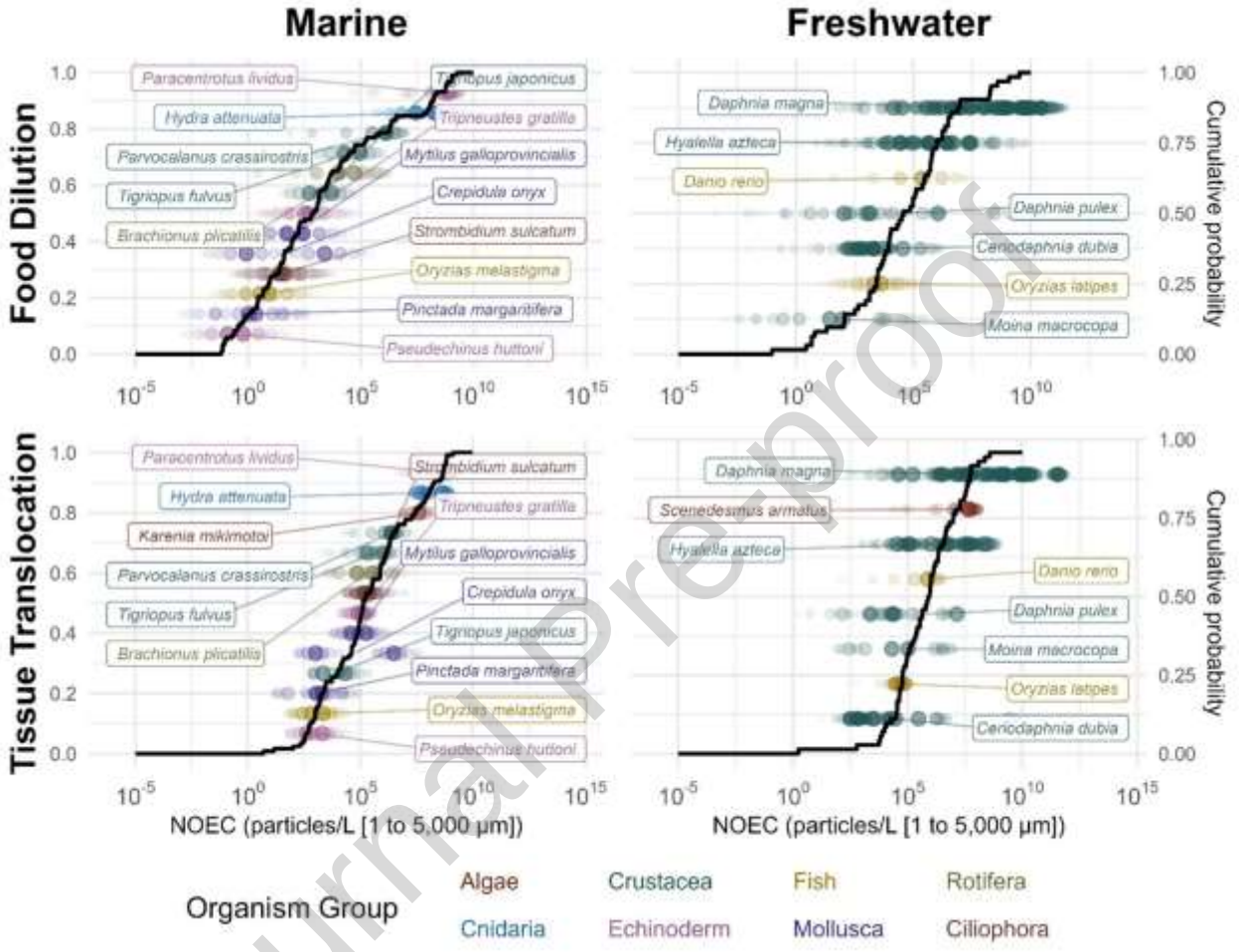

Figure 5. Modified Probabilistic Species Sensitivity Distribution (PSSD++) of MPs for tissue translocation-mediated effects (top row) and food dilution (bottom row) ERMs, for marine (left column) and freshwater (right column) ecosystems. Labels and colored points denote individual species with colors corresponding to their organismal group. Each NOEC for each species is plotted such that the median aligned value is darker and larger, with the distribution of aligned values from the MC simulation appearing smaller and with lighter shading. Values up to the 99.99th and 0.0001th percentile values from the MC-aligned datasets are visible. For data-rich species in which numerous studies were used, overlapping points demonstrate the low variability between the aligned values (*e.g.*, *Daphnia magna*). The probabilistic distribution for each PSSD++ is shown with a black line.

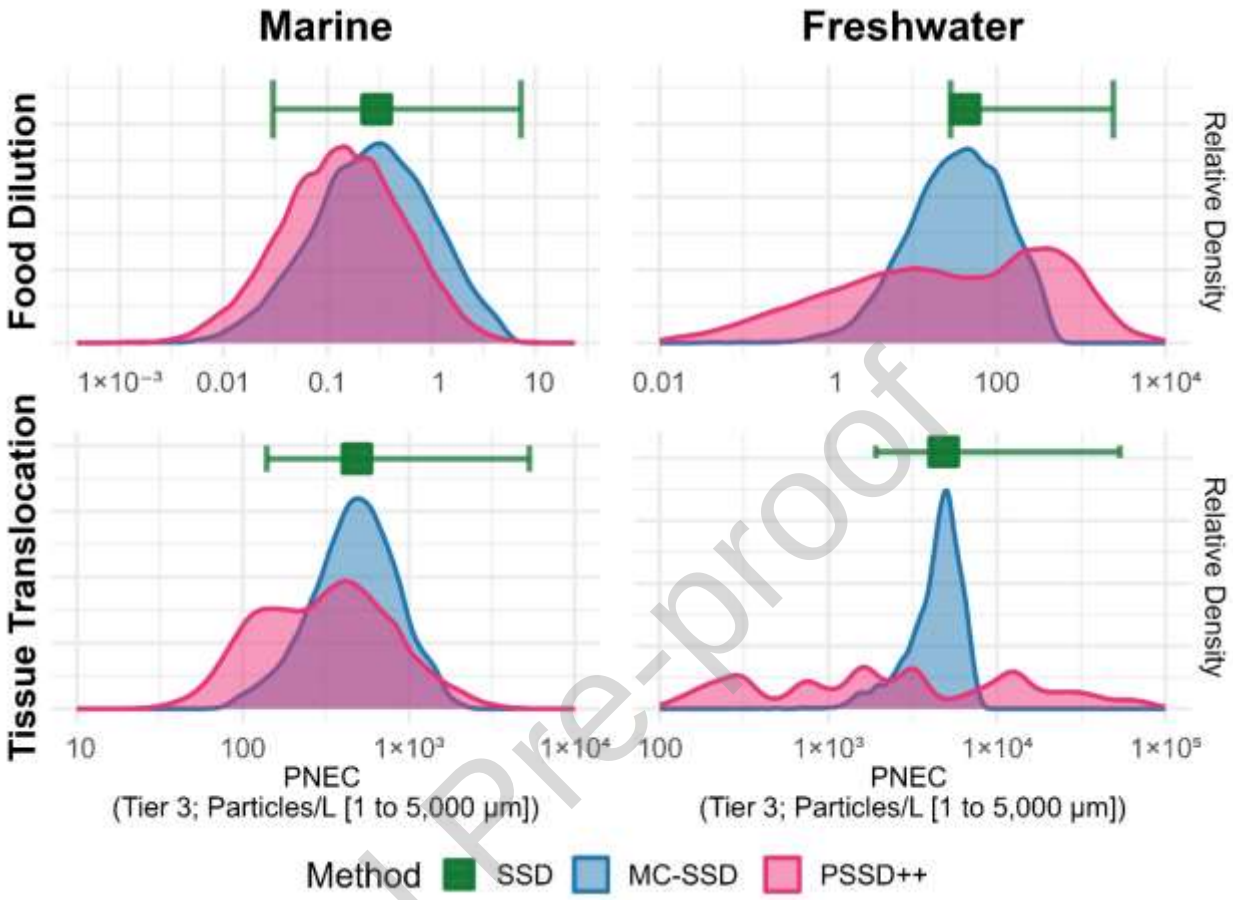

Figure 6. MP PNEC values for Tier 3 are compared between different modelling methods for marine and freshwater ecosystems, and food dilution and tissue translocation ERM. PNECs derived using the two probabilistic modelling methods are shown with smoothed distributions, with blue denoting MC+SSD (*i.e.*, "method 1") and pink denoting PSSD++ (*i.e.*, "method 2"). The traditional SSD-based approach is shown with a green point and error bars representing the 5th and 95th percentile values of the bootstrapped uncertainty for the SSD.

Sensitivity Analyses

Alignments

The alignment sensitivity analysis revealed a strong influence of several MP particle characteristics used in the alignments, as well as bioavailability modelling parameters on the resulting thresholds, which varied according to ERM and environment. For food dilution, the power law for particle volume had the strongest influence on the thresholds for both marine and freshwater environments and exhibited a first-order exponential-like positive relationship (Figure 7). The MP particle length-to-width ratio exhibited a weak, positive association with the resulting threshold (Figure 7). No apparent relationships were observed between other parameters that aligned toxicity values to the food dilution ERM and the resulting thresholds, including the ingestion bioavailability model parameters (Figure 7).

In the case of tissue translocation, the following parameters strongly influenced the resulting thresholds: tissue translocation bioavailability model size limit, surface area power law alpha value, and particle length-to-width ratio (with a stronger influence on freshwater thresholds than on marine) (Figure 7). A strong inverse exponential relationship between the tissue translocation limit and threshold was present for the marine environment, with a noticeably weaker, somewhat linear relationship for the freshwater environment (Figure 7). For the surface area power law alpha value, there was a positive linear relationship with the resulting threshold. In the case of the length-to-width ratio of particles, no trend was observed with marine thresholds, however for freshwater there was a strong, positive, non-linear relationship (~3rd-degree polynomial) (Figure 7). The parameters involved in the allometric body length to plastic ingestion model had weak negative relationships to the threshold (Figure 7).

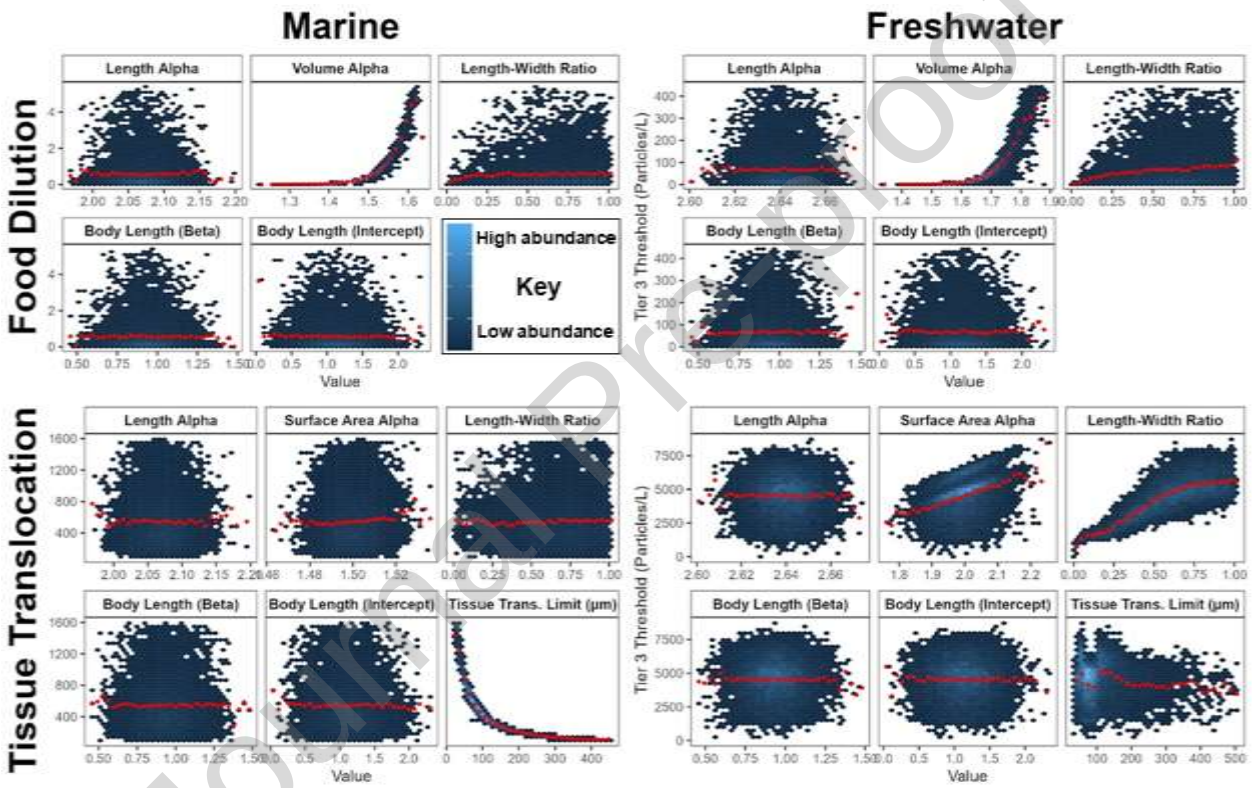

Figure 7. Hexagonal density scatterplots of ERM alignment parameters and their influence on a representative tier of the ecotoxicological thresholds (*i.e.*, Tier 3) for two ERMs, *i.e.*, Food Dilution (left column) and Tissue Translocation-Mediated Effects (right column), derived from Marine (top row) and Freshwater (bottom row) environments. Each relevant parameter involved in the alignment/rescaling of the threshold is presented as a hexagonal density scatterplot. Values for each parameter are on the x-axis (alpha values and length-to-width ratios are unitless; tissue translocation size limit is measured in µms, and the body length to mouth size opening estimation parameters are in mm). The y-axis represents the threshold value measured in particles/L. Lighter colors indicate a higher density of obtained values (*i.e.*, x and y) from the MC simulations, with the red dots indicating the median for a given x-y pair to illustrate trends.

Environment

Marine toxicity thresholds were consistently lower than freshwater thresholds across all ERMs/tiers, with greater differences at lower tiers. For example, the marine food dilution threshold for tier 1 was ~40,000 times lower than its freshwater counterpart, compared to ~100 times lower for tier 4. Tissue translocation showed smaller differences between environments compared to food dilution, with the marine threshold being ~100 times lower for tier 1, and just ~6 times lower for tier 4.

Sensitivity analysis revealed that alignment parameters primarily drove these differences, accounting for 1 to 2 orders of magnitude. Freshwater-aligned values were always higher. For instance, marine Tier 2 thresholds for food dilution (2.6x10-3 particles/L) and tissue translocation (13 particles/L) increased ~10 to 100 times when freshwater parameters were applied (0.25 and 110 particles/L, respectively). Even after controlling for alignment parameters, freshwater thresholds remained 1.3- to 300-fold higher than marine thresholds, with differences inversely related to the tier. For example, when marine distribution parameters were used, freshwater species showed 300x higher food dilution and 19x higher tissue translocation thresholds for Tier 1, but only 4.3x and 1.3x higher for Tier 4, respectively.

Studies and Quality

The leave-one-out sensitivity analysis conducted on individual studies demonstrated varying sensitivities of thresholds by tier, ERM, and environment (Figure 8). Overall, thresholds for food dilution were more strongly influenced by individual studies than the thresholds for tissue translocation, and freshwater environments were relatively less volatile than marine thresholds. Leave-one-out sensitivity distributions were all strongly positively skewed, with the vast majority of studies (70%) having minimal influence by themselves on the thresholds (fold-change between -0.5 and +2). Across all threshold tiers, ERMs, and environments, there were a small number of studies that had strong influences. Specifically, 15% of studies would increase the threshold by more than double when they were removed, with 2.4% of studies causing more than a +10-fold change (Figure 8). On the opposing end of the spectrum, there were 7.8% of studies that would have caused the thresholds to decrease by more than one-half when removed (Figure 8).

Freshwater tissue translocation thresholds were the least sensitive to the removal of a single study, with the most influential study on the low end being a ~30% lower Tier 4 (removal of Jaikumar et al. [56]'s *Ceriodaphnia* and *Daphnia sp.* study), and on the high end, a +3.7-fold change for Tier 1 (removal of Mizukami-Murata et al. [57]'s *Raphidocelis subcapitata* study) (Figure 8). Marine thresholds were far more sensitive to the removal of single studies, and had a maximum fold-change of +83 and +18 for food dilution (Tiers 1 and 2, respectively) and +15 for tissue translocation (Tier 1) when Capolupo et al. [58]'s study on *Mytilus galloprovincialis* is removed (Figure 8). Capolupo et al. [58]'s influence is substantially greater than all other studies for all thresholds, environments, and ERMs, with the next most influential studies overall being Kim & Rhee et al. [59]'s study on *Moina macrocopa* (+34-fold change for freshwater food dilution tier 1) and Richardson et al. [60]'s study on *Pseudechinus huttoni* (+12-fold change for marine food dilution tier 1). The marine food dilution threshold was also the most sensitive on the low end of the distribution, with the removal of Beiras et al. [61]'s study on *Brachionus plicatilis, Tigriopus fulvus,* and *Paracentrotus lividus* resulting in a -0.4-fold change for Tier 2.

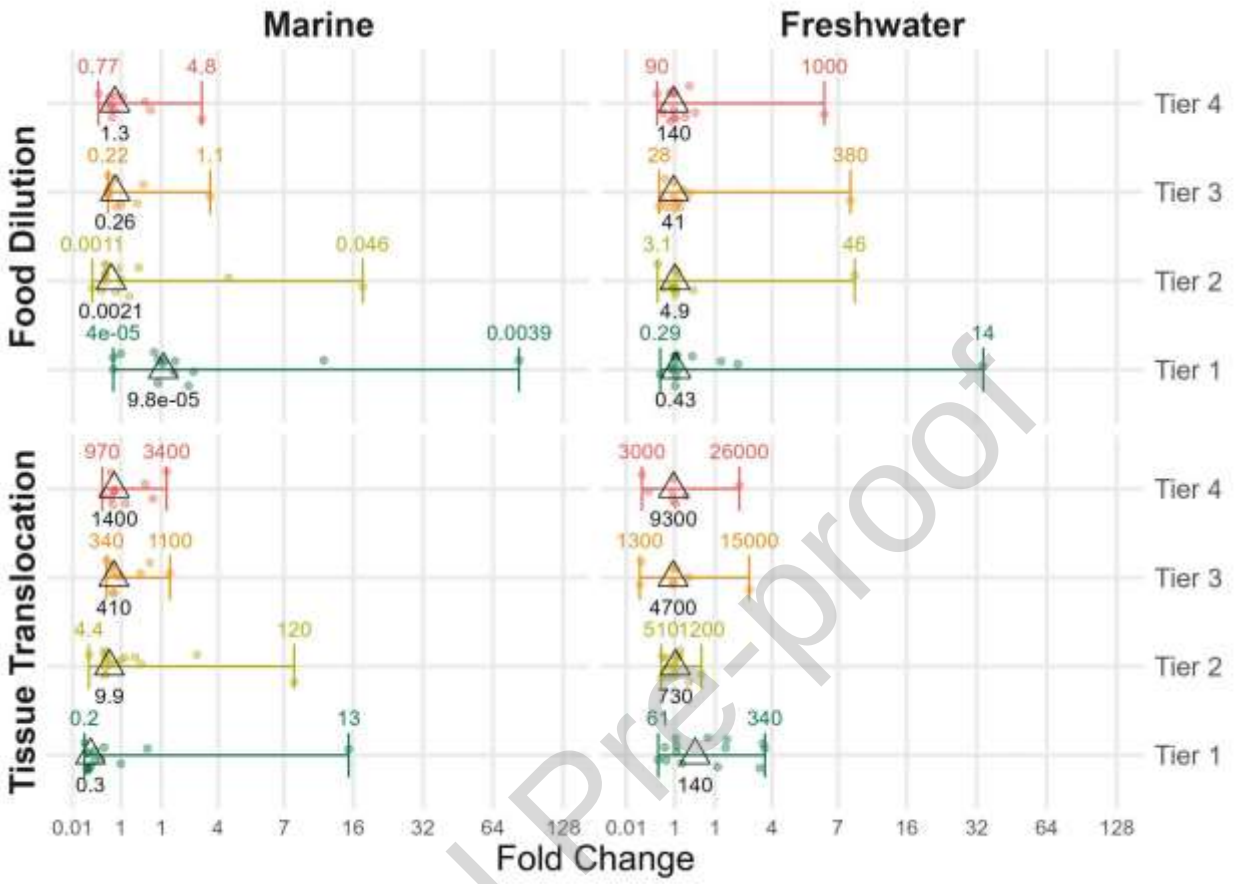

Figure 8. Sensitivity analysis of ecotoxicological thresholds based on leaving one study out from the derivation at a time. Each point represents the fold-change of the threshold when a single study is removed (*i.e.*,[threshold without study/threshold with all studies] - 1). Points are jittered vertically to show the distribution of points. Minimum and maximum threshold values based on leaving a single study out are annotated with text. In all cases, the majority of studies have minimal influence on thresholds (*i.e.*, fold change < 2); however, several tiers/environments/ERMs are highly sensitive to at least one study.

Discussion

This is the first study to have propagated the uncertainties involved in the particle-associated ERM alignment-based approach to deriving thresholds for MPs. While the refined PSSD++ approach developed in this study represents a substantial improvement over traditional SSD-based methods for assessing hazards of MPs, particularly in its ability to propagate uncertainty of particle-associated ERM alignments, bioaccessibilities, intra-species variabilities, and assessment factors, several key limitations remain, which may be separated by unquantifiable and quantifiable uncertainties. The unquantifiable uncertainties stem from constraints in the underlying data rather than the analytical methodology itself. First, the ToMEx 2.0 toxicological dataset, while the most comprehensive available, still underrepresents the diversity of MP characteristics present in natural environments. Second, the bioaccessibility model for tissue translocation could be more accurate by reducing simplifications that may not reflect biological complexity and addressing issues with the underlying studies. As a result, while the analytical framework itself is robust and flexible, its predictive power remains tightly coupled to the quality and representativeness of available input data. In contrast, quantifiable uncertainties and variabilities – driven by ERM alignments and propagated through our novel modelling process - are well characterized by the PSSD++ method, and comparisons with alternative modelling approaches highlight the advantages of probabilistic methods in capturing these sources of variability.

Data representativeness

Although the ERM alignment framework is designed to reduce dependence on raw particle characteristics used in toxicity tests- thereby mitigating some issues of representativeness- this approach only captures ingestion-related food dilution and tissue translocation effects [11, 12]. Many other mechanisms through which MPs influence toxicity, such as chemical leaching (e.g., [62, 63, 28]), particle charge and surface chemistry [64, 65, 66], eco-corona formation [7, 8, 67], weathering [68, 25, 69, 70, 71], and shape-dependent physical interactions (e.g., entanglement [72]), fall outside the two ERMs used here. Because these mechanisms can influence toxicity independently of the assumed ERMs, unrepresentative particle characteristics in toxicity studies reduce the reliability of derived thresholds even after alignment. Thus, understanding the extent and nature of these data gaps remains critical for evaluating the robustness and generalizability of our approach.

The ToMEx 2.0 dataset reveals critical gaps and persistent inconsistencies between the characteristics of MPs used in laboratory toxicity tests and those found in aquatic environments [73]. Notably, differences in toxicities between polymers and shapes -if any- are poorly represented. For example, fibers may be more toxic than non-fibers [74, 75], and some polymers may be more toxic due to intentionally-added chemicals (e.g., bisphenol A, phthalates, 6PPD) or NIAS such as residual oligomers, monomers, industrial chemicals, pharmaceuticals, and pesticides [62, 63, 28]. While comprehensive testing of all polymers and formulations is neither feasible nor necessary [76], a more representative dataset of MPs to which organisms are exposed would improve risk assessment reliability.

Toxicity tests predominantly used PS particles, followed by PE, while other environmentally relevant polymers (PET, polyester, PP, PVC, PTFE) were underrepresented despite their prevalence and high production volume [77, 78, 79]. MPs from bio-based plastics were also underrepresented, despite their growing use [77, 80] and limited environmental data [81]. Tire wear particles containing synthetic rubbers and plasticizers were rarely tested (with just one study's toxicity data used in this assessment [82]), despite their widespread presence [83].

Shapes of MPs in toxicity tests also diverged from environmental observations. Spheres were predominantly used, while fibers - highly abundant in natural environments - were rarely considered. This is concerning given fibers' increased risk of entanglement and ingestion. Studies have reported the high prevalence of MP fibers ingested by organisms such as crustaceans [84], mussels [85], fish [86], and other taxa from both freshwater and marine environments. Fragments, more common in environmental samples than in toxicity tests, may elicit different biological responses due to their greater physical obstruction potential, enhanced gut disruption, their greater likelihood of internal laceration, and higher cellular toxicity from roughness [87, 88, 89].

Particle size inconsistencies were also identified, which may influence uptake, tissue translocation, digestive transit, excretion, and toxicological outcomes [90, 91], underscoring the need for realistic size distributions that reflect environmental conditions to improve ecological relevance. While the alignments accounted for the bioaccessibility aspect to some extent, and the different size ranges used in experiments incorporated some size-specific toxicity differences, some differences may still remain.

Beyond polymer type, shape, and size, the lack of weathering and other environmental modifiers limits ecological relevance. Features like dissolved organic matter (DOM) or biofilms—ubiquitous in nature—were rarely reported, especially in freshwater tests. These factors can alter MP behavior, bioavailability, and toxicity by influencing aggregation, surface charge, or interactions with contaminants [68, 25, 69, 70, 71]. Similarly, surface charges and functional chemical groups were considered in few studies, despite their influence on particle reactivity and toxicity [64, 65, 66].

This updated framework did not address plastic-associated chemicals, including additives, NIAS, and sorbed chemicals, which can leach and cause toxicity [92]. Despite efforts to characterize these chemicals [28, 63] and model their transfer via MPs in food webs [93], a lack of chemical monitoring data hinders aquatic risk characterization [94]. The framework also did not consider plastics as vectors for pathogens, which can harbor and transport microorganisms and antibiotic-resistant bacteria [95, 96]. Future ERA frameworks should integrate risks from both particle-induced hazards and those from chemicals and pathogens.

The availability and cost of commercial MPs, and challenges in manufacturing and characterization, likely contribute to discrepancies between MPs used in toxicity testing and those in the environment. Analytical limitations and costs also restrict surface charge and functional group characterization. This divergence underscores the need for future research to incorporate a broader, more environmentally realistic range of MP characteristics into experimental designs. Including diverse polymer types, shapes (especially fibers), realistic size distributions, and environmentally relevant conditions (e.g., DOM, biofilms) will enhance the ecological validity of toxicity assessments.

Tissue Translocation Modelling

Translocation—the movement of particles from the digestive tract into tissues and potentially systemic circulation—is governed by particle traits (size, shape) and organism-specific factors like age and internalization mode [97, 98]. This study used a data-driven model to estimate size-dependent translocation probabilities, finding smaller particles to have higher uptake, and incorporated model uncertainty via MC simulations—an advance over previous single-value approaches. Below, we discuss the biological plausibility of this model and discuss its room for improvement to more accurately account for bioaccessibility via translocation. Principally, a more nuanced model implemented in our probabilistic framework could improve accuracy; however, the development of such a model is constrained by the limited studies reporting continuous translocation data, limited representation of the diversity of MP particle traits and biological organisms, and the lack of fit-for-purpose quality criteria to evaluate studies.

The model's biological plausibility is supported by mechanistic evidence: studies report MP translocation from 24 nm to 100 µm, with a median 50% probability at 88 µm – consistent with the 83 µm value derived in [12], which used the same modelling approach, but a smaller dataset for derivation. Submicron to micron-sized MPs can cross epithelial barriers via endocytosis, paracellular transport, or transcytosis through microfold cells [99, 100], though the maximum translocatable size is debated. The size limit for endocytosis varies by mechanism: clathrin- and caveolin-mediated endocytosis typically transport particles up to 200 nm and 50–100 nm, respectively [101, 102]; macropinocytosis can accommodate up to ~5 µm, and phagocytosis (by immune cells) can handle >20 µm [103]. Some studies report even larger particles crossing barriers [104, 105, 99, 106, 100], and field studies have observed translocation of particles up to 567 µm [107, 108, 109]. Granuloma formation may allow larger MPs to enter muscle tissue [106], but more research is needed. Since the tissue translocation limit is inversely related to threshold values, including larger MPs in the model may underestimate thresholds.

The logistic regression model applies a binary size cutoff for translocation, which simplifies the continuous nature of biological uptake. However, using logistic regression with MC simulations to propagate uncertainty is a key improvement over prior methods (e.g., [12]), as it better captures biological variability. A binary model was used due to limited data: only 3 of 25 studies reported uptake percentages [106, 110, 87]. Future models could benefit from integrating organism-specific traits (e.g., gill presence, feeding mode such as filter, deposit, or predation), which influence exposure and uptake [111, 112], and consider multiple compartments such as the human physiologically based kinetic model developed in [50].

Currently, the model uses only size to predict translocation, not accounting for shape, surface charge, or modifications, due to the predominance of pristine spheres in the database (18 studies vs. 5 on fragments and 6 on fibers/mixes). Fibers may have distinct uptake/retention due to flexibility and slower gut passage [89]. Surface charge and modifications, often induced by weathering or bio/eco-coronas, can significantly affect translocation via endocytosis, phagocytosis, and paracellular transport [113, 114, 65, 67]. As most studies used pristine particles, the model may underestimate environmental MP translocation.

Methodological challenges also complicate translocation assessment. Self-contamination is a major uncertainty, as MPs are pervasive in labs and can be introduced via air, clothing, or equipment [115], leading to false positives and overestimated translocation rates [116]. While lab exposures allow better contamination control, they remain susceptible to biases, especially with fluorescently labeled particles [117]. Detection methods (fluorescent/Raman microspectroscopy) must account for background fluorescence, spectral overlap, and detection limits [118]. Sample preparation (e.g., aggressive digestion, filtration) may also affect MP detection or distribution in tissues, introducing artifacts [119]. Developing quality criteria for translocation studies was beyond this study's scope, but future work should address this.

These findings highlight the complex interplay of MP traits, organismal factors, and environment in translocation. Incorporating model uncertainty yields more realistic probability distributions and robust threshold estimates, but further improvements are needed. Future research should standardize detection protocols, improve contamination controls, and expand datasets to refine models and enhance ecological risk assessments. Longitudinal studies on long-term accumulation and toxicokinetics of translocated MPs are also crucial for understanding broader ecological and human health impacts.

Quantified Drivers of Threshold Magnitude and UncertaintyAcross all tiers, environments, and modelling approaches, three factors consistently shaped the magnitude and uncertainty of derived MP thresholds: (1) the ERM to which toxicity data were aligned, (2) the modelling framework used to propagate alignment and biological uncertainties, and (3) the environmental particle trait distributions used in the alignments. While analytically distinct, these factors are tightly interrelated, and considering them together provides clearer insight into the primary sources of variability in the resulting thresholds.

ERMs

Across all combinations of tiers, environments, and modelling approaches, the effect mechanism (*i.e.*, ERM) had the most consistent influence on both the magnitude and relative uncertainty of the derived thresholds. As described above, food dilution thresholds were consistently lower than tissue translocation thresholds across all model types; however, this increased sensitivity was accompanied by greater normalized uncertainty, with food dilution thresholds exhibiting higher RCIs than their tissue translocation counterparts. This contrast is likely driven by differences in how bioaccessibility is modeled: food dilution thresholds depend on species-specific ingestion bioaccessibility estimates (i.e., using body length measurements from various sources, and the model from [52]), which introduce variability across both species and particle traits and includes a wide range of particle sizes (8 µm to 70,273 µm) whereas tissue translocation is based on a species-agnostic logistic model that uses a narrower and more stable size range (i.e., 88 µm or the mouth size opening - whichever is smaller), reducing the variability introduced by different rescaling values.

Modelling Techniques

While there were minimal differences in magnitude between thresholds derived using different modelling techniques, there were marked differences in their uncertainties. Probabilistic approaches

(MC+SSD and PSSD++) revealed clearer distinctions between ERMs, often showing non-overlapping 95% CIs where the traditional SSD-based method did not (a pattern that aligns with an earlier assessment in [12]). This suggests that these newer methods may be better suited for capturing the variability inherent in species response and bioaccessibility modelling by accounting for uncertainty due to alignments. In contrast, the environmental compartment (marine vs. freshwater) had a relatively smaller influence on normalized uncertainty, although marine thresholds were generally lower and slightly more variable in absolute terms. These results reinforce that ERM selection is the dominant factor shaping both the central estimates and the uncertainty distributions of MP hazard thresholds.

Environments

The environmental context (marine vs. freshwater) significantly influenced the magnitude and variability of derived MP thresholds. Unlike [12], which combined data due to limited sample size, the expanded ToMEx 2.0 database allowed for independent SSD development for each environment (with sufficient species, $n \geq 5$; [120]). This enabled more tailored threshold derivation and investigation into species/parameter differences. While some studies hypothesize that marine thresholds can be extrapolated from freshwater data (e.g., [121]), our findings suggest that such extrapolation may be unreliable. Specifically, marine thresholds were consistently lower than freshwater thresholds by approximately 0.5 to 2.5 orders of magnitude across all tiers and ERMs. Our alignment parameter control sensitivity analysis indicates that these differences are primarily driven by environmental MP particle trait distributions (*e.g.*, size, shape, density) used in alignments, though some residual differences could be attributed to inherent species sensitivity. The apparent greater sensitivity for marine species could potentially be explained by the following: (1) marine species are more sensitive to microplastics than freshwater species; (2) the MP characteristics to which we aligned the toxicity measurements had toxic traits that were not accounted for with the alignments (e.g., presence of NIAS, etc.), with more toxic particles used in marine environment studies; or (3) the number of species populating the SSDs were too few to reliably detect differences (an effect documented in [121]). Unfortunately, insufficient information is available at this time to determine which of the three explanations (or additional explanations) are correct.

The strong influence of environmental MP particle traits on the thresholds underscores the importance of using site-specific, high-resolution environmental data when applying this ERA framework. The generic particle distributions used here (i.e., [24]) were selected for demonstration purposes, and it is recommended that distribution data that most closely resembles the environment of interest (ideally, site-specific monitoring data) be used to derive thresholds intended for risk characterization, when possible [122]. MP sources and fate vary between systems, resulting in heterogeneous particle distributions [123, 124]. For example, the power law exponents for particle lengths from [24] (2.07 in marine surface waters for the Netherlands) differ substantially from values reported by [125] (2.67 in marine environment), while the freshwater surface water values reported in [24] (2.6 in freshwater surface waters for the Netherlands) are comparable to freshwater surface water values reported in [126] (2.4-2.7 in the size range of 5 to 300 µm for the St Louis Estuary and Western Lake Superior, USA). Methodological differences could also partially explain these differences, as the sampling and analysis

methods used in these studies were not identical, in addition to the data analysis technique used to derive the values. Specifically, [24] applied maximum likelihood estimation (MLE) followed by bootstrapping - avoiding size binning - while [126] and [125] used binned data. MLE-based approaches, by incorporating all observations, are considered more robust for estimating underlying size distributions [127].

Influence of Individual Studies and Quality Criteria

Sensitivity analyses showed minimal impact from quality screening, as highly influential studies remained. A few studies disproportionately influenced thresholds, with some changing by over an order of magnitude (e.g., 83-fold for marine food dilution Tier 1). This is higher than Mehinto et al. [12]'s 4-fold maximum. This volatility isn't due to more species in Tiers 1 and 2, but the framework itself: Tiers 1 and 2 use the 25th percentile toxicity value for a given species, while Tiers 3 and 4 use the median. Additionally, Tier 1 is based on the 5th percentile confidence interval of the modelled SSD, resulting in expected high volatility.

Lower tier thresholds are more impacted by individual studies partly due to the inclusion of sub-organismal endpoints, unlike upper tiers (organismal and population-level only). This explains the high influence of [58] on marine thresholds, which reported lysosomal, neurological, and immunological effects in mussels (*Mytilus galloprovincialis*) exposed to 1.5 ng/L (100 particles/L) 3 µm PS spheres. However, increased sensitivity at lower biological levels doesn't explain the 7 to 14-fold difference in freshwater food dilution thresholds, where [59] reported intergenerational effects on water flea (*Moina macrocopa*) survival at 100 ng/L (1 µm PS spheres).

The freshwater tissue translocation ERM, though generally more robust (2.7-3.7-fold change), was heavily influenced by [74]. This study included fibers, which were more toxic than beads in the same experiment and caused developmental deformities in *Ceriodaphnia dubia*, potentially due to entanglement [74]. This raises uncertainties in applying the ERM-based framework, as effects from other means (e.g., entanglement) might be misrepresented, given that food dilution and tissue translocation are assumed for all theoretically bioaccessible particles. Such mixed modes of action underscore the value of complementary approaches that explicitly integrate particle characteristics into SSD estimation. For example, [128] applied a Bayesian hierarchical SSD framework to microplastics, demonstrating that particle size and exposure medium (i.e., freshwater vs. marine) can be incorporated directly into hazard modelling. Their findings highlight how trait-explicit SSDs may complement the ERM-based approach used here, particularly when multiple mechanisms of toxicity may be operating simultaneously.

Conclusion

This study advances MP ERA by fully propagating uncertainty across all components of threshold derivation. By integrating MC-based ERM alignments with a modified PSSD approach, we provide a transparent and defensible framework for deriving MP hazard thresholds. Applied to the largest MP toxicity database available to date (ToMEx 2.0), this framework enabled the generation of tiered thresholds across marine and freshwater ecosystems, alongside detailed sensitivity analyses to assess the influence of alignment parameters, data quality, and individual studies.

ERM selection was the primary driver of threshold magnitude and uncertainty, with food dilution ERMs producing more protective but less stable thresholds than tissue translocation ERMs. Differences between marine and freshwater thresholds were driven largely by MP particle distributions rather than species-specific sensitivities, underscoring the importance of incorporating site-specific environmental data when applying this ERA framework.

PSSD++ thresholds - which incorporate alignment uncertainty, intraspecies variability, and assessment-factor uncertainty - produced more realistic and precautionary estimates than deterministic SSDs. However, additional research could further enhance the representativeness, precision, and ecological realism of MP ERAs, including: 1) a toxicity database with broader representation of MP types present in the environment (especially fibers, PP, PET, and tire wear particles); 2) high-quality translocation studies reporting continuous uptake data across a wider range of MP traits (particularly fragments, fibers, and surface-modified particles resembling biofouling conditions); 3) an updated ERA framework that considers non-ingestion-based mechanisms of toxicity (*e.g.*, entanglement from fibers), the impacts of plastic-associated chemicals, and plastics as vectors for pathogens; and 4) generation of additional high quality toxicity studies - ideally including replication studies of the highly influential studies observed in this assessment.

Overall, this probabilistic ERA framework offers a scalable and transparent approach to MP threshold derivation. Continued improvements in toxicity and monitoring data, as well as bioavailability modelling, will further increase the trust and adoption of MP risk thresholds for use in environmental risk assessment and management.

Data statement

All data and code used and produced in this paper are publicly available at this GitHub repository https://github.com/ScottCoffin/ToMEx2.0_EcoToxRisk, with large files (>100 mB) produced by the Monte-Carlo and PSSD++ simulations available at Zenodo (https://doi.org/10.5281/zenodo.16740504).

Declarations

Scott Coffin reports a relationship with The Moore Institute for Plastic Pollution Research that includes: board membership. **The following authors have nothing to declare:** Luan de Souza Leite, Win Cowger, Lidwina Bertrand, Kazi Towsif Ahmed, Andrew Yeh, Mariella Siña, Stephanie Kennedy, Bethanie Carney Almroth, Ezra Miller, Anna Kukkola, Andrew Barrick

Author Contributions: CRediT

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Luan de Souza Leite: Writing- Original Draft; Writing - Review & Editing; Visualization
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Stephanie Kennedy: Project Administration; Writing - Review & Editing
Kazi Towsif Ahmed: Writing- Original Draft; Writing - Review & Editing; Visualization
Andrew Yeh: Writing - Review & Editing
Mariella Siña: Investigation; Writing - Review & Editing
Magdalena Mair: Data acquisition/data mining (environmental samples), literature search, coding, formal analysis, visualization, writing (results), review
Anna Kukkola: Data curation; Writing - Original Draft; Writing - Review & Editing
Bethanie Carney Almroth: Writing - Original Draft; Writing - Review & Editing
Ezra Miller: Conceptualization; Writing - Original Draft; Writing - Review & Editing

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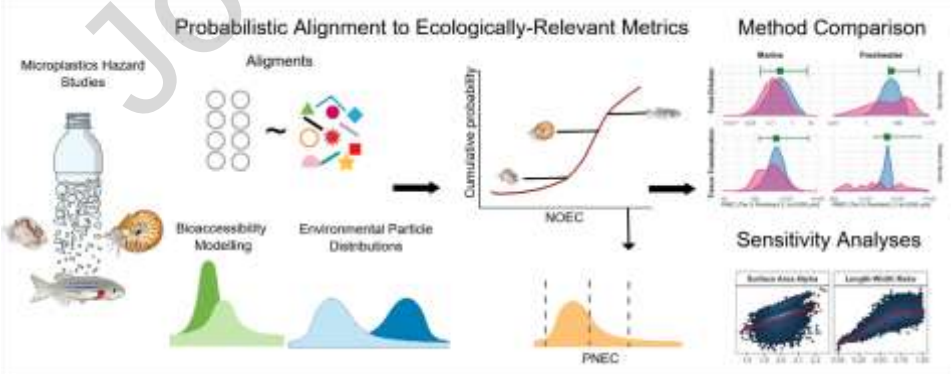
Declaration of Competing Interest

Scott Coffin reports a relationship with The Moore Institute for Plastic Pollution Research that includes: board membership. The following authors have nothing to declare: Luan de Souza Leite, Win Cowger, Lidwina Bertrand, Kazi Towsif Ahmed, Andrew Yeh, Mariella Siña,Stephanie Kennedy, Bethanie Carney Almroth, Ezra Miller, Anna Kukkola, Andrew Barrick, Magdalena Mair

Environmental Implication

This study introduces a novel probabilistic framework that propagates uncertainty across particle- and species-specific alignments, toxicity mechanisms, and environmental compartments to derive ecologically relevant hazard thresholds for microplastics. By integrating mechanistic endpoints and Monte Carlo simulations at every stage of data harmonization, the approach provides a more transparent and robust foundation for ecological risk assessment, with resulting thresholds being more protective and realistic. The accompanying sensitivity analysis reveals which parameters contribute most to uncertainty, highlighting key knowledge gaps and guiding future research priorities for more targeted data generation and risk refinement.

Graphical abstract



Highlights

- Novel probabilistic ERA framework (PSSD++) for microplastics thresholds
- Integrated Monte Carlo uncertainty propagation into ERM alignments
- Applied framework to ToMEx 2.0, the largest MP toxicity database
- PSSD++ yields more precautionary but more uncertain thresholds
- ERM choice dominates threshold uncertainty across environment