### Data alignment

#### Text for main manuscript

Monodisperse laboratory-based effect concentrations (e.g. 40-micron polyethylene fragments) were re-scaled to a default size range (e.g. 1 - 5,000 microns) according to Kooi et al (2021) and described in detail in the **supplemental information**. Re-scaling effect concentrations (*EC*) to a default size range allows direct comparison to exposure concentrations (*C*) for a default size range (which may also be re-scaled) (equation 1) (Kooi et al 2021).

**Equation 1**

Where *ECenv andCenv* are the environmentally realistic effect concentration and occurrence concentrations, respectively; *CFbio (or meas)* is a dimensionless correction factor the bioavailable effect concentration (*bio*) or the environmentally-monitored concentration (*meas*); and *ECpoly andCmeas* are the polydisperse effect concentration and (unaligned) environmental concentration, respectively. Environmental concentrations are aligned to an upper (*UL,D;* µm*)* and lower default size range (*LL,D;* µm) based on the power law slope of the particle occurrence in the environment based on size (*a*, unitless), with the upper limit (*UL, meas;* µm) and lower limit (*LL, meas;* µm) defined by the size limits of quantification of the monitoring method employed (equation 2).

**Equation 2**

Equation 2 is used to align both measured environmental concentrations and polydisperse effect concentrations to a default size range (1 – 5,000 µm). Conversion of a monodisperse effect concentration (*ECmono*) to a polydisperse effect concentration (*ECpoly*) requires consideration of the ecologically relevant metric (ERM) *x* believed to be responsible for the observed toxicological effects (where *x* = volume for food dilution or *x* = surface area for translocation) (equation 3).

**Equation 3**

Where *µx, poly* is the mean value for ERM *x* for polydisperse particles within a given bioavailable size distribution, and *µx, mono* is the mean value for ERM *x* for the monodisperse effect concentration from the literature test data. When the suspected effect mechanism depends on ingestion of the particle, particles that are too large to be ingested by the organism of interest (i.e. wider than the mouth opening) are considered biologically unavailable (i.e. food dilution mechanism of action) and are excluded from alignment. Further, in the case of toxicity depending on tissue translocation, particles too large to be translocated through tissue (>83 microns) are considered biologically unavailable and excluded from alignment. In the case of organisms that have mouth openings smaller than 83 microns, their mouth opening size is used as the upper limit for alignment.

#### **Text for Supplementary**

##### Alignment of Monodisperse Effect Concentrations to Polydisperse Environmental Concentrations

To illustrate the data alignment of converting monodisperse effect concentrations to polydisperse, environmentally realistic concentrations, a real example from the literature is used. Take for example, a NOEC of 562 particles/L for a chronic toxicity study for *Daphnia magna* conducted using 40 µm-long polyethylene fragments (Jaikumar et al 2018).

Given an upper limit (UL) and lower limit (LL) of the measured and default size range, a dimensionless correction factor () for measured environmental concentrations or for bioavailable effect concentrations may be calculated, which rescales the measured (M) or bioavailable (B) number concentrations for a certain size range to the number concentration for the microplastics default (D) size range (e.g. 1 to 5,000 um) according to the power law distribution for length (L) with slope in Table S4 of Kooi et al (2021).

In order to convert the *monodisperse* effect concentration to a *polydisperse* mixture of microplastic particles, a correction must occur which takes into consideration the ecologically relevant metric (ERM) (Koelmans et al 2017). For a given ERM, the threshold may be related to both mono- or polydisperse particles interchangeably so long as the total magnitude of ERM remains the same using equation S1 (Koelmans et al, 2020).

**Equation S1.**

In this example of a monodisperse effect concentration for *Daphnia magna*, is in particle count per volume (i.e. 562 particles/L), and is the mean value of the ERM of interest in this study, which in the case of particle count, is 1. Particles follow a power law regime in the marine environment, so may be calculated using equation S2 (Kooi et al 2021).

**Equation S2.**

Where relates to the power law distribution of microplastic particles in the marine environment (i.e. particle length; 2.07; Kooi et al 2021), and UL and LL are the upper and lower limits of bioavailability for this species/life stage/effect metric, respectively. In this example, the organism of interest is *Daphnia magna*, which has an average body length of 0.5 cm, and an estimated maximum ingestible particle width of 115 µm particles based on average mouth size opening (Jâms et al 2020). Therefore, would be equivalent to 115 µm, and would be the lower default size range (1 µm). Note that if bioavailability for an ERM of interest is based on a different parameter (for example, tissue translocation potential), would be equivalent to that size (i.e. 83 µm). for particles for the 1-115 µm fraction in the marine environment is calculated as 4.35.

Now that , , and are known for this example, the *bioavailable* *polydisperse* effect concentration may then be calculated using equation S3:

**Equation S3.**

for this example is 129.4 particles/L. Now that the *bioavailable*, *polydisperse* effect concentration is known for this example, in order to relate this threshold effect concentration to an *environmentally relevant* (e.g. 1 - 5,000 um) range of particles (; particles/L) a further correction must occur using equation S4 (Kooi et al 2021).

**Equation S4.**

Where is a dimensionless correction factor for the bioavailable effect concentration. Equation S4 rescales the bioavailable effect concentration (particles/L) to an environmentally relevant concentration for the microplastics default (D) size range (e.g. 1 to 5,000 um) according to the power law distribution for length (L) with slope in the marine environment (i.e. 2.07) (Kooi et al, 2021). is calculated using equation S5. (Kooi et al 2021):

**Equation S5.**

In this example, the organism of interest (*Dapnhia magna*) has an estimated maximum ingestible size range () of ~115 µm-wide particles, and would be the default lower size range 1 µm. Further, the upper limit (UL,D) and lower limit (LL,D) of the default size range are 5,000 and 1 µm, respectively. equates to 1.006. Finally, the is calculated for particles/L as an ERM using equation S4 ( = 130 particles/L)."

###### Volume

In the case of an ERM of interest being volume, is equivalent to the average volume of a the monodisperse particle (i.e. ), calculated using equation S6.

**Equation S6.**

Where is the volume for a given particle *i*, and *a*, *b*, and *c* are radii along the principal axes, corresponding to one-half times the length, width, and height of an ellipsoid. Upper and lower limits of bioavailability for volume correspond to the maximum ingestible size of particles and the lower limit of the size range to be aligned to (1 µm).

Equation S6 can be applied to fragments, thin films, microbeads, spheres, or fibers given a known length to width ratios for such shapes, with the height assumed to be equivalent to 0.67 x width (Kooi et al 2021). Width to length ratios differ for microplastics differ by compartment, with averages ranging from 0.67 to 0.77 (Kooi et al 2021). Averaged values may be used to estimate the volume of polydisperse environmental mixtures of microplastics. Substituting for the length to width ratio of a particles, the formula simplifies to:

**Equation S7.**

Where is the length of the particle (µm) and is the width:length ratio of the particle (unitless). For the example of a 40 µm PE fragment, the length was reported by the authors () (Jaikumar et al 2018), however the width was not directly reported, so a default value of 0.77 is used, which is the average for marine surface water (). Accordingly, is calculated using equation S6 ( 208 um^3).

Using equation S2, is calculated using the volume of particles that are bioaccessible (based on mouth opening size and particle length, i.e. 115 µm) and the volume of particles for the lower limit of the size range of interest (1 – 5,000 µm). The upper and lower bioavailability limits in volume are VUL = 316,000 µm3 and VLL = 0.208 µm3, respectively. may now be calculated given these limits, using an alpha value of 1.48 for volume in the marine surface water environment (Kooi et al 2021), which equated to = 314.9 µm 3.

Now that , , and are known for this example for volume, the *bioavailable* *polydisperse* effect concentration for the volume ERM may then be calculated using equation S3 = 371.42 particles/L. Again, to relate this *bioavailable*, *polydisperse* effect threshold () to an environmentally relevant *polydisperse* mixture of particles for the volume ERM, an additional correction must be applied (equation S4) to rescale the effect threshold to the environmental size range of interest (e.g. 1-5,000 µm) using , identical as for particles = 373.7 particles/L.

### Surface Area

For surface area as an ERM, is equivalent to the average surface area () of the *monodisperse* particle for the effect threshold, calculated using equation S8.

**Equation S8.**

With a, b, c being equal to 0.5x length, 0.5x width, and 0.5x height, respectively. For the example of a 40 µm PE fragment, the length was reported (), however the width was not reported by the authors, so a default value of 0.77 is used (), which is the average for marine surface water, and height is assumed to be 0.67 times the width () (Kooi et al 2021). Surface area is calculated for this example and equates to = 2,880 µm2.

Since the probability distribution of ERM (surface area) follows a power law regime, the mean ERM value for the polydisperse particles, , can be calculated. For surface area of environmentally disperse particles, UL and LL are calculated using the equation for the surface area of an ellipsoid (equation S8). SAUL = 23,838 µm2 and SALL = 1.8 µm2.  may now be calculated given these limits, using an alpha value of 1.98 for surface area in the marine surface water environment (Kooi et al 2021), which equates to: = 207.29 µm2.

Now that , , and are known for this example for surface area, the *bioavailable* *polydisperse* effect concentration for the surface area ERM may then be calculated, and equates to: 7,824 particles/L.

Again, to relate this *bioavailable*, *polydisperse* effect threshold () to an environmentally relevant *polydisperse* mixture of particles for the surface area ERM, an additional correction must be applied to rescale the threshold to the size range of interest (e.g. 1-5,000 µm) using , identical as for particles. Therefore, = 7,872 Particles/L.

### Mass

In the case of an ERM of interest being total mass, is equivalent to the average mass of a the monodisperse particle (i.e. ), calculated as follows (equation S9):

**Equation S9.**

Where *m* is the mass (µg), *p* is density (g/cm3), *V* is volume (um3) - which is calculated by the cube of the radius of each particle (i.e. 1/2 \* length, or 40 µm for this example), and additional conversion factors for g to ug (1 *x* 106) and cm3 to um3 (1 *x* 10-12). For the example of a 40 µm polyethylene fragment, the volume was calculated above (~ ), and the density of polyethylene was is estimated to be 0.935 g/cm3. is calculated to be 0.0124 µg.

Since the probability distribution of ERM (mass) follows a power law regime, the mean ERM value for the polydisperse particles, , can be calculated by first calculating the lower and upper ingestible masses of particles based on the length of the ingestible particle. The *UL* and *LL* are respectively defined as the upper and lower limit in ERM (mass) for which the mean is calculated, and is the power law exponent of mass. In the case of marine surface water, an of 1.32 is utilized (Kooi et al 2021).

For mass, *UL* and *LL* are mass-based upper and lower limits of bioaccessibility based on the width of particles, respectively. To estimate mass-based limits based on size, the volume of bioaccessible particles is first calculated using the equation for the volume of an ellipsoid, then multiplied by the average density of particles in the 1-5,000 µm distribution in the environmental compartment of interest (e.g. surface marine water: 1.10 g/cm^3) (Kooi et al 2021). Therefore, MUL = 0.35 µg and MLL = 2.3 *x* 10-3 µg. may now be calculated given these limits, using an alpha value of of 1.32 for the marine surface water environment (Kooi et al 2021), equating to: = 0.0017 µg.

Now that , , and are known for this example for mass, the *bioavailable* *polydisperse* effect concentration for the mass ERM may then be calculated, which is equivalent to: 4,023.2 particles/L. Again, to relate this *bioavailable*, *polydisperse* effect threshold () to an environmentally relevant *polydisperse* mixture of particles for the mass ERM, an additional correction must be applied to rescale the threshold to the size range of interest (e.g. 1-5,000 um) using , identical as for particles. Accordingly, = 4,048 Particles/L.

### Specific Surface Area

In the case of an ERM of interest being specific surface area, is equivalent to the surface area of a 40 µm PE fragment (i.e. ) divided by the mass (i.e. ), calculated as follows:

**Equation S10**

Where *SA* is the surface area (um^2) of the particle, and *m* is the mass (µg). Accordingly, = 2.32 *x* 10-5 um2/µg.

Since the probability distribution of ERM (specific surface area) follows a power law regime, the mean ERM value for the polydisperse particles, , can be calculated, where UL and LL are respectively defined as the upper and lower limit in ERM (ssa) for which the mean is calculated, and is the power law exponent of specific surface area. For example, marine surface water has an of 1.98 (Kooi et al 2021). For specific surface area, *UL* and *LL* are area/mass-based upper and lower limits of bioaccessibility based on the width of particles, respectively. To estimate area/mass-based limits based on size, the volume of bioaccessible particles is first calculated using the equation for the surface area of an ellipsoid, then divided by the lower and upper bioavailable mass of particles in the 1-5,000 µm distribution in surface marine water, as calculated above. Accordingly, SSAUL = 68,500 um2/µg ; SSALL = 7,880,000 um2/µg.

may now be calculated given these limits, using an alpha value of of 1.98 for the marine surface water environment (Kooi et al 2021), which equates to 337,000 um2/µg.Now that , , and are known for this example for specific surface area, the *bioavailable* *polydisperse* effect concentration for the specific surface area ERM may then be calculated, equivalent to: 386.21 particles/L.

Again, to relate this *bioavailable*, *polydisperse* effect threshold () to an environmentally relevant *polydisperse* mixture of particles for the specific surface area ERM, an additional correction must be applied to rescale the threshold to the size range of interest (e.g. 1-5,000 um) using , identical as for particles. Accordingly, = 388.6 Particles/L.

## Aligning Occurrence Data for Risk Characterization

Given an upper limit (*UL*) and lower limit (*LL*) of the measured (*M*) and default size range (*D*), a dimensionless correction factor () for measured environmental concentrations may be calculated, which rescales the measured (*M*) number concentrations for a certain size range to the number concentration for the microplastics default (*D*) size range (e.g. 1 to 5,000 µm) according to the power law distribution for length (*L*) with slope (Kooi et al, 2021).

The following equation (equation S11) for is identical to the rescaling equation for effect concentrations () as discussed above (equation S4) except the bioavailable fraction of particles is denoted as *UL,B* and *LL,B* on the denominator (Koelmans et al., 2020; Kooi et al., 2021).

**Equation S11.**

As an example, a measured marine surface water concentration of 10 particles/L for particle size ranges 300 - 5,000 µm is used. To compare this unaligned concentration to the environmentally relevant (1 - 5,000 um) effect threshold (EC\_env) in particles/L (calculated above), is first calculated using equation S11, which equates to 470.3. The resulting correction factor (; unitless) is then multiplied by the measured concentration () to obtain a rescaled exposure number concentration using equation S4, which equates to 4,703 particles/L.

This measured rescaled environmental concentration may then be directly compared to the rescaled effect thresholds for various ERM to determine if risk is present using the traditional risk quotient formula (effect concentration divided by environmental concentration), whereby a ratio greater than one is indicative of a likelihood risk, and a ratio less than one is indicative of a low likelihood of risk.

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| --- | --- | --- |
| *ERM* | *ECenv* | *ECenv / Cenv* |
| Particles | 130 | 36 |
| Volume | 374 | 13 |
| Surface Area | 7,872 | 0.60 |
| Mass | 4,048 | 1.2 |
| Specific Surface Area | 389 | 12 |

In this example, the rescaled environmental concentration is higher than the rescaled effect thresholds for all ERMs except for surface area, therefore if risk occurs for this species, it would likely be through a a food-dilution based mechanism (i.e. ERM = volume) or other ERM predicted by mass or specific surface area, as opposed to a tissue translocation mechanism of action (i.e. ERM = surface area).