Supporting Information

Solving the non-alignment of methods and approaches used in microplastic research in order to consistently characterize risk

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2 figures

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Detailed explanation and example calculation for $EC_{X,Poly}$ and the effect concentration for environmental microplastic $EC_{X,Env}$.

Imagine that a threshold effect concentration of $EC_{X,Mono} = 100 \ \#/L$ is reported, for monodisperse spherical microplastic particles of 0.1 mm. This means that the equivalent volume in a litre is $100 \times 4/3 \times \pi \times 0.05^3 = 0.0524 \text{ mm}^3$. This is the left hand side term in Eq 5. In the next step we calculate the number of particles that would produce the same volume, when these particles have the sizes drawn randomly from a microplastic size distribution limited by the bioavailable (i.e. ingestible) size boundaries set for a certain species, using a Monte Carlo simulation.

For instance, for a species that ingests particles with a width between 0.05 and 2 mm, we keep track of the number of particles ($k_{ingestible}$) that fit within this size window during the (n = 10⁵) iterations during Monte Carlo simulation, and calculate their individual ellipsoid volumes (Eq. 3), by random sampling from L:H and L:W distributions (Figure 1). During the same iterations, masses for each of these imaginary particles are calculated, by multiplying the calculated volumes with individual particle densities sampled from a density distribution function. Total ingestible volume (V_T) is calculated by addition ($V_T = \sum_{i=1}^{i=k} V_{Poly,i}$) and the average volume per particle (V_{Poly}, from the polydisperse distribution) is then calculated using Eq 6. Now, EC_{X,Poly} can be calculated using Eq. 7.

During the Monte Carlo simulation, calculating the fraction of bioavailable particles is needed. This is done by dividing the number of bioavailable particles, by the total number of iterations (e.g. $n = 10^5$). Part of this bioavailability relates to ingestibility, i.e. whether the particle size fits the ingestible range per individual species (Table S1). However, besides correcting for particles to big to be ingested by a species, corrections for unavailability due to density can also be performed. For instance, for pelagic species, it is possible to only take that part of the microplastic continuum into account that has a density equal or smaller than 1 g/cm³. For benthic species that live in or on the sediment layer, the full density distribution can be taken into account if data suggest this is the case. If for instance, due to these two criteria of ingestibility and density, k=250 000 particles defined during the Monte Carlo simulation would be considered bioavailable, then the fraction of bioavailable particles, f_{available}, would be $k/n = 250 \ 000/10^6 = 0.25$. The final effect threshold concentration for 'polydisperse environmental MP' (EC_{X,Env}) then is calculated as EC_{X,Poly}/f_{available} (Eq. 8).

Species	ecies Group Animal size (mm)		Ingestible MP size ^{a)} (μm)	Motivation	References ^{b)}	
Daphnia magna	Zooplankton	5	114.87	Relationship based on maximum		
Daphnia pulex	Zooplankton	3	70.87	size of species: $y = 22x + 4.87$, where y is diameter of largest	Burns, 1986 ¹ ; Gouin, 2020 ²	
Ceriodaphnia dubia	Zooplankton	1.4	35.67	bead (μm) and x is the carapace length (mm).		
Hyalella azteca	Benthic invertebrate	7.8	112	Based on anatomy (size of the mouth opening)	Schmitz et al, 1983 ³	
Hydra attenuata	Benthic invertebrate	10 - 30	400	An MP size class of <400 mm was chosen as the freshly hatched <i>A</i> . <i>salina</i> nauplii that are fed to the <i>H. attenuata</i> are <400 mm in size.	Murphy and Quinn, 2018 ⁴	
Danio rerio	Fish	25	400	General ingestible size given for adult zebrafish, by 3 literature sources.	Avdesh et al., 2012 ⁵ ; Naceur et al., 2008 ⁶ ; Harper and Lawrence, 2008. ⁷	
Artemia franciscana	Zooplankton	0.9	270	MP ingestion demonstrated up to 264 μm.	Jemec et al., 2018 ⁸	
Gammarus fossarum			125	MP in the size range 63–125 μm showed significantly higher ingestion than other particle size treatments. MP of size range 125–250 μm were not ingested.	Straub et al., 2017 ⁹	

Table S1 : Ingestible size ranges.

^{a)} Literature research was performed in order to demonstrate ingestibility and to find plausible ingestible size ranges for the species used in the SSD (Figure 3). Ingestion was demonstrated for all heterotrophic species used in the effect studies (Table S2), except for *Daphnia pulex* and *Ceriodaphnia dubia* where this was based on studies that specifically addressed ingestion as reviewed by Gouin (2020). In all cases it was assumed that there would be no lower size limit with respect to what can be ingested. Therefore the minimum size limit was always set at 1 μ m, being the low boundary for the 1 – 5000 μ m microplastic size continuum. To assess an upper boundary, we considered reported sizes of ingested plastic particles but also of other particles or prey items, and then selected the largest value reported. For autotrophic species used in the SSD that do not feed on particles (e.g., phytoplankton, macrophytes) it was generally assumed that effects relate to the bulk of the material in its totality, e.g. due to reduction of light penetration, nutrient availability and/or general affects due to adherence of the bulk material to the exterior of the organism.

b) Table S1 References

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Reference	Species	ET_reported ¹ (#/L)	Size ² (μm)	V_mp³ (μm^3)	Ingestible ⁴ (μm)	Density⁵ g/cm3	CSF ⁶ (-)	# fraction available ⁷	M fraction available ⁸	V_total ⁹	EC _{X,Poly} ¹⁰	EC _{X,Env} ¹¹
Kokalj, 2018 ¹	A.franciscana	624	(µIII) 183.1	1235512	270	full	0.01 - 1	0.986	7.32E-03	8.03E+09	9.47E+03	9.60E+03
Kokalj, 2018	A.franciscana	5255.5	102.9	219294.7	270	full	0.01 - 1	0.986	7.32E-03	8.03E+09	1.42E+04	1.44E+04
Kokalj, 2018	A.franciscana	18802	63.05	50447.22	270	full	0.01 - 1	0.986	7.32E-03	8.03E+09	1.42L+04 1.17E+04	1.18E+04
Kokalj, 2018	A.franciscana	392.2	264	3703342	270	full	0.01 - 1	0.986	7.32E-03	8.03E+09	1.17L+04 1.78E+04	1.18L+04
Kokalj, 2018	A.franciscana	473.7	247.9	3066278	270	full	0.01 - 1	0.986	7.32E-03	8.03E+09	1.78E+04	1.81E+04
Kokalj, 2018	A.franciscana	1355.5	136.8	515276.4	270	full	0.01 - 1	0.986	7.32E-03	8.03E+09	8.58E+03	8.70E+03
Kokalj, 2018	A.franciscana	253335.2	22.8	14301.96	270	full	0.01 - 1	0.986	7.32E-03	8.03E+09	4.45E+04	4.51E+04
Ziajahromi, 2017 ²	C. dubia	1950	2.5	8.181231	35.67	full	0.01 - 1	0.939	5.44E-05	5.99E+07	2.50E+01	2.66E+01
Ziajahromi, 2017 ²	C. dubia	135	2.5	8.181231	35.67	full	0.01 1	0.939	5.44E-05	5.99E+07	1.73E+00	1.84E+00
Ziajahromi, 2017 ²	C. dubia	275	2.5	197920.3	35.67	full	0.01 - 1	0.939	5.44E-05	5.99E+07	8.53E+04	9.09E+04
Ziajahromi, 2017 ²	C. dubia	120	280	197920.3	35.67	full	0.01 1	0.939	5.44E-05	5.99E+07	3.72E+04	3.96E+04
Jaikumar, 2019 ³	C. dubia	5000	3	14.13717	35.67	full	0.01 - 1	0.939	5.44E-05	5.99E+07	1.11E+02	1.18E+02
Jaikumar, 2019 ³	C. dubia	5000	5.5	33.48652	35.67	full	0.01 - 1	0.939	5.44E-05	5.99E+07	2.63E+02	2.79E+02
Jaikumar, 2018 ⁴	C. dubia	1258925	3	14.13717	35.67	full	0.01 - 1	0.939	5.44E-05	5.99E+07	2.79E+04	2.97E+04
Jaikumar, 2018 ⁴	C. dubia	31622.78	3	14.13717	35.67	full	0.01 - 1	0.939	5.44E-05	5.99E+07	7.01E+02	7.46E+02
Jaikumar, 2018 ⁴	C. dubia	1E+10	5.5	33.48652	35.67	full	0.01 - 1	0.939	5.44E-05	5.99E+07	5.25E+08	5.59E+08
Jaikumar, 2018 ⁴	C. dubia	6309573	5.5	33.48652	35.67	full	0.01 - 1	0.939	5.44E-05	5.99E+07	3.31E+05	3.53E+05
Wu, 2019 ⁵	C. pyrenoidosa	73440.08	157	2026271	5000	full	0.01 - 1	1	1	1.09E+12	1.36E+04	1.36E+04
Wu, 2019 ⁵	C. pyrenoidosa	33997.5	172	2664305	5000	full	0.01 - 1	1	1	1.09E+12	8.27E+03	8.27E+03
Mao, 2018 ⁶	C. pyrenoidosa	9.1E+12	1	0.523599	5000	full	0.01 - 1	1	1	1.09E+12	4.35E+05	4.35E+05
Lei, 2018 ⁷	Danio rerio	2930	70	69036.08	400	full	0.01 - 1	0.990	1.71E-02	1.88E+10	1.06E+03	1.07E+03
Ogonowski, 2016 ⁸	D. magna	28000000	4.1	36.08695	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	9.84E+04	1.01E+05
Ogonowski, 2016 ⁸	D. magna	8600000	2.6	3.537546	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	2.96E+03	3.05E+03
Ogonowski, 2016 ⁸	D. magna	5000000	2.6	3.537546	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	1.72E+04	1.77E+04
Rehse, 2016 ⁹	D. magna	1.14E+09	1	0.523599	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	5.82E+04	5.98E+04
Jaikumar, 2019 ³	D. magna	50000	5.5	33.48652	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	1.63E+02	1.68E+02
Jaikumar, 2019 ³	D. magna	50000	3	14.13717	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	6.89E+01	7.08E+01
Jaikumar, 2018 ⁴	D. magna	1E+11	3	14.13717	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	1.38E+08	1.42E+08
Jaikumar, 2018 ⁴	D. magna	6309573	3	14.13717	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	8.69E+03	8.93E+03
Jaikumar, 2018 ⁴	D. magna	1E+11	5.5	33.48652	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	3.26E+08	3.35E+08
Jaikumar, 2018 ⁴	D. magna	6309573	5.5	33.48652	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	2.06E+04	2.12E+04
Gerdes, 2019 ¹⁰	D. magna	16129352	5	25.15892	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	3.95E+04	4.06E+04
Jemec, 2016 ¹¹	D. magna	3320	300	23561.94	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	7.62E+03	7.83E+03
Aljaibachi, 2018 ¹²	D. magna	159537.5	2	4.18879	114.87	full	0.01 - 1	0.973	9.02E-04	9.99E+08	6.51E+01	6.69E+01
Jaikumar, 2019 ³	D. pulex	50000	5.5	33.48652	70.87	full	0.01 - 1	0.962	2.89E-04	3.21E+08	5.01E+02	5.21E+02

Table S2. Original and rescaled effect threshold concentrations as used in Figure 3A and 3B, respectively.

D. pulex	50000	3	14.13717	70.87	full	0.01 - 1	0.962	2.89E-04	3.21E+08	2.12E+02	2.20E+02
D. pulex	1E+16	3	14.13717	70.87	full	0.01 - 1	0.962	2.89E-04	3.21E+08	4.23E+13	4.40E+13
D. pulex	5011872	3	14.13717	70.87	full	0.01 - 1	0.962	2.89E-04	3.21E+08	2.12E+04	2.20E+04
D. pulex	2E+10	5.5	33.48652	70.87	full	0.01 - 1	0.962	2.89E-04	3.21E+08	2.00E+08	2.08E+08
D. pulex	79432.82	5.5	33.48652	70.87	full	0.01 - 1	0.962	2.89E-04	3.21E+08	7.96E+02	8.28E+02
G. fossarum	16666.5	47.5	21570.63	125	full	0.01 - 1	0.974	1.08E-03	1.20E+09	2.92E+04	3.00E+04
G. fossarum	166665	47.5	21570.63	125	full	0.01 - 1	0.974	1.08E-03	1.20E+09	2.92E+05	3.00E+05
H. azteca	460000	18.5	3315.231	112	full	0.01 - 1	0.972	8.39E-04	9.30E+08	1.60E+05	1.64E+05
H. azteca	500000	18.5	3315.231	112	full	0.01 - 1	0.972	8.39E-04	9.30E+08	1.73E+05	1.78E+05
H. azteca	2500000	18.5	3315.231	112	full	0.01 - 1	0.972	8.39E-04	9.30E+08	8.67E+05	8.92E+05
H. azteca	2500000	18.5	3315.231	112	full	0.01 - 1	0.972	8.39E-04	9.30E+08	8.67E+05	8.92E+05
H. azteca	710	47.5	14922.57	112	full	0.01 - 1	0.972	8.39E-04	9.30E+08	1.11E+03	1.14E+03
H. azteca	2250	47.5	14922.57	112	full	0.01 - 1	0.972	8.39E-04	9.30E+08	3.51E+03	3.61E+03
H. azteca	2250	47.5	14922.57	112	full	0.01 - 1	0.972	8.39E-04	9.30E+08	3.51E+03	3.61E+03
H. attenuata	2642775	200	1610171	400	full	0.01 - 1	0.990	1.71E-02	1.88E+10	2.24E+07	2.26E+07
Lemna minor	4265	71.3	72954.25	5000	full	0.01 - 1	1	1	1.09E+12	2.84E+01	2.84E+01
Lemna minor	3125	96	178072	5000	full	0.01 - 1	1	1	1.09E+12	5.08E+01	5.08E+01
M. flos-aquae	146880.2	157	1013136	5000	full	0.01 - 1	1	1	1.09E+12	1.36E+04	1.36E+04
M. flos-aquae	67995	172	1332153	5000	full	0.01 - 1	1	1	1.09E+12	8.27E+03	8.27E+03
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Column heading footnotes:

¹ Effect threshold concentration as reported, where necessary recalculated into #/L.

² Size selected for the correction. In case of a single size, that size was used. In case of a range while no actual distribution data were provided, the average was used. In case width – length data were provided, the longest dimension was used.

³ Volume per particle.

⁴ Assumption on ingestible size range for the species under consideration, based on the motivation above. For emergent macrophytes and for phytoplankton, no correction for ingestible microplastic was used. Only studies that confirmed ingestion were used. As for effects of a food dilution mechanism (based on ref 17): 78% of the 49 ingestion-related datapoints underlying the SSDs were from studies that explicitly cited food dilution, 4% were from studies that did not report any effect mechanism at all, however used the same species as for which food dilution was cited, bringing the total to 82%. For another 14% also no effect mechanism was reported, however it concerned other yet similar benthic invertebrate species than in the two previous categories. In this case, we just followed de Ruijter et al (2020)¹⁷ who demonstrated based on a

review of 105 studies that food dilution was the dominant explanatory mechanism for effects, which brings the percentage of datapoints with food dilution as plausible effect (while no other mechanism is reported) to 96%. For the remaining 4% of data points (2 datapoints), one study speculated that 'physical effects upon ingestion' caused the observed effect, a mechanism which however also is related to ingestion and ingested volume. The final datapoint (out of 49), reported a biomarker response as effect mechanism. Besides these 49 ingestion-related datapoints underlying the SSD there were 7 datapoints that did not depend on food dilution as they related to algae ad macrophytes.

⁵ In the present implementation, for all species, the full microplastic density continuum was considered bioavailable (indicated with 'full').

⁶ Corey Shape Factor (CSF). All shapes having a CSF between 0.01 and 1 were considered to be of relevance for all species considered.

² Number fraction of the 10⁵ imaginary MP particles randomly drawn using Monte Carlo simulations that fell within the specified ingestible size, shape and density range and or density range ($f_{available}$).

⁸ Mass fraction of the 10⁵ imaginary MP particles randomly drawn using Monte Carlo simulations that fell within the specified ingestible size, shape and density range and or density range ($f_{available}$).

⁹ Total volume of the fraction of the 10⁵ imaginary microplastic particles randomly drawn using Monte Carlo simulations that fell within the specified bioavailable size, shape and density range (V_{Poly} , Equation 6).

¹⁰ Effect threshold concentration for polydisperse microplastic defined by the microplastic probability density functions, such that the total volume of the bioavailable polydisperse microplastic equates to the total volume of microplastic at the original effect threshold concentration ($EC_{X,Poly}$, Eq. 7).

¹¹ Effect threshold concentration for polydisperse environmental microplastic $EC_{X,Env}$, calculated as $EC_{X,Poly}$ divided by $f_{available}$ (Eq. 8).

Table S2 References

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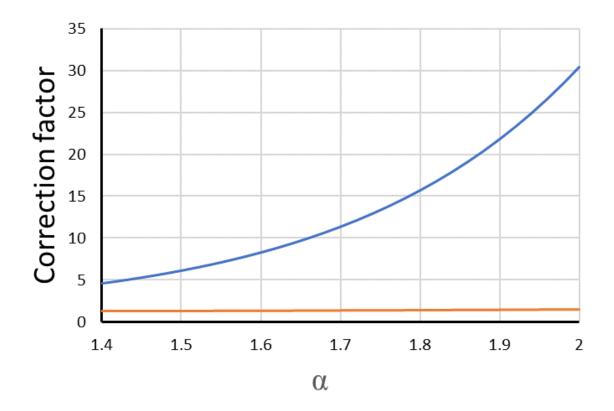


Figure S1: Correction factor required to correct 333-5000 μ m microplastic number concentration data to a default size range of 1-5000 μ m data (blue curve), or 20-5000 μ m (orange curve), as a function of the exponent α in eq. 1.

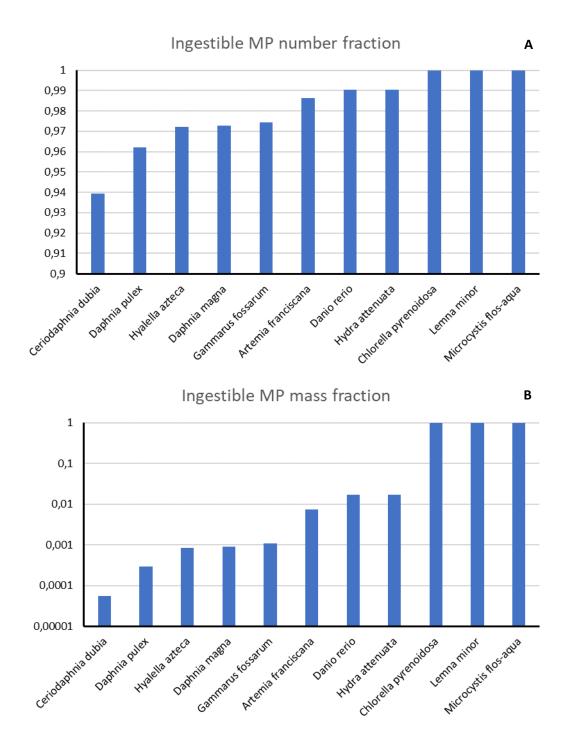


Figure S2: Number concentrations (A) and mass concentrations (B) for the fraction of environmentally relevant polydisperse microplastic (MP) which are ingestible by the 11 species used in the freshwater species sensitivity distributions (SSD).