LABPLAS

Land-Based Solutions for Plastics in the Sea

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D6.5 Environmental Risk Assessment (E.R.A.) of plastics in aquatic and terrestrial environments

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Nº	Participant name	Acronym	Country	Type
1	UNIVERSIDADE DE VIGO	UVI	SPAIN	HES
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3	Bundesanstalt fuer Gewaesserkunde	BfG	GERMANY	RTO
4	LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA	INL	PORTUGAL	RTO
5	KATHOLIEKE UNIVERSITEIT LEUVEN	KUL	BELGIUM	HES
6	HELMHOLTZ ZENTRUM FUR OZEANFORSCHUNG KIEL	GEOMAR	GERMANY	RTO
7	NATIONAL OCEANOGRAPHY CENTRE	NOC	UNITED KINGDOM	RTO
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11	ASSOCIACAO PARA O DESENVOLVIMENTO DO ATLANTIC INTERNATIONAL RESEARCH CENTRE	AC	PORTUGAL	RTO
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Executive summary:	According to the standard methodology, environmental risk posed by microplastics was quantitatively characterized using the following expression: Risk Quotient (R) = Exposure Level (EL) / Toxicity Threshold (TT). With that aim, the microplastic concentrations measured in the LABPLAS Project Work Package (WP) 2 in freshwater and marine aquatic environmental compartments were compared with the ecotoxicity thresholds found in the laboratory toxicity tests performed in WP6 using organisms representative of different trophic levels for each environmental compartment. A deterministic approach using an Assessment Factor (AF) and the toxicity threshold of the most sensitive test species, and a probabilistic approach based on the Species Sensitivity Distribution (SSD) curve were both conducted. A very conservative approach maximizing environmental protection was taken. This included using data from hotspots and 95%percentiles, taking a large AF=1000, and toxicity thresholds obtained from most sensitive species, and use of the lower end of the 95% confidence interval. Even under these assumptions, R values were always <1; from 0.004 to 0.7 (deterministic approach) or 0.079 (probabilistic approach). If average values rather than hotspots had been used, R values would further decrease 2 to 3 orders of magnitude. Therefore, levels of **risk in both freshwater and marine habitats** are **not concerning**. Although the

	LABPLAS Project did not sample terrestrial microplastics, a preliminary attempt to quantify risk in soils was made by obtaining exposure levels from published data. In contrast to aquatic results, **for terrestrial agriculture soils R values** obtained with the maximum levels reported in the literature **were >1**. This is partly due to the higher sensitivity of *Eisenia sp.* (earthworm) compared to aquatic model species, but mainly due to the much higher plastic loads reported for some agriculture soils compared to aquatic environments.

Version	Date	Comments
1.0	07.03.2024	First draft
2.0	13.11.2024	Evaluation draft for partners
3.0	28.11.2024	Final version
4.0	14.07.2025	Reviewed with changes to address comments by the PO and reviewer.

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ABBREVIATIONS AND ACRONYMS

Abbreviation / Acronym	Description
AF	Assessment Factor
C	Concentration
CI	Confidence Interval
D	Deliverable
EC	European Commission
EC_{10}	Effect Concentration at which a 10% effect is observed
EL	Exposure Level
EPS	Extracellular Polymeric Substances
ERA	Environmental Risk Assessment
HC_5	Hazardous Concentration for 5% of species
LOEC	Lowest Observed Effect Concentration
MP	Microplastics
NOEC	No Observed Effect Concentration
PS	Poly-Styrene
R	Risk Quotient
SET	Sea urchin Embryo Test
SMP	Small Microplastics (10 to 1000 μm)
SMNP	Small Micro- and Nano- Plastics
spp	Species
std	Standard Deviation
SSD	Species Sensitivity Distribution
TT	Toxicity Threshold
WP	Work Package

1 INTRODUCTION

Despite the numerous benefits plastics provide to society, valid concerns exist regarding the harmful effects posed by their fragmentation into smaller particles. The smaller the size, the higher the risk posed by these particles to organisms and human health. Small micro- and nano-plastics (SMNP) have been found across all environmental compartments, including air, freshwater, marine environments, and soils. For this reason, this document aims to perform a comprehensive ecological risk assessment (ERA) of SMNPs across various environmental compartments.

With this aim, two approaches will be followed. First, according to a mechanistic approach, risk can be assessed using **Risk Quotients** (R), which can be calculated by comparing microplastic (MP) densities in different environmental compartments, as obtained from Work Package (WP) 2 (refer to Deliverables D2.2, D2.3, D2.4, and unpublished data from 2023 cruises) with toxicity thresholds (TT) derived from laboratory tests using organisms representative of different trophic levels in each relevant environmental compartment, as conducted by WP6 (refer to D6.3 & D6.4). Since terrestrial habitats were not sampled for SMNPs in the LABPLAS project, exposure levels will be estimated using data from the literature.

Secondly, a probabilistic approach will be used to compare the distribution of MP abundance detected in the different environmental compartments sampled by WP2 with the Species Sensitivity Distribution (SSD) derived from WP6 laboratory tests for aquatic and terrestrial organisms.

Furthermore, this document refines previous ERA studies by incorporating ecotoxicological data derived from environmental plastics, used to generate secondary microplastics of heterogeneous composition, size and morphology, representative of actual environmental MPs, rather than by resorting to engineered microbeads as most of the earlier studies.

2 METHODOLOGY

The ERA was conducted following the standard methodology established by the United States National Academy of Sciences[1], initially developed for Human Health Risk Assessment to address threats to human health caused by occupational exposure. Risk is quantitatively characterized as a Risk Quotient (R) comparing two estimates: **Exposure Level** (EL) to **Toxicity Thresholds** (TT). Thus, R = EL / TT. The closer the exposure levels are to the toxicity thresholds, the higher the associated risk, with values of R>1 not acceptable.

2.1 Exposure level assessment.

Exposure levels for aquatic habitats were estimated based on the measured SMP concentrations recorded by WP2 in each sampled environmental compartment. In the field, all particles >10 μm were filtered and sent for analysis to the LABPLAS project partner NOC using the methods described in D2.1 and D2.4. For detection with FTIR, our choice of imaging resolution was 25 μm due to analytical time constraints. Mass data were derived from particle counts and bi-dimensional data using two approaches: the estimates provided by the siMPle software (https://simple-plastics.eu/), and those obtained from the actual measurements of mass vs area in plastic particles, down to a maximum dimension of 0.4 mm, conducted by GEOMAR.

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[1] NRC 1983. Risk assessment in the Federal Government: managing the process. National Research Council. National Academy Press, Washington DC.

Since the LABPLAS Project does not target sampling microplastics in terrestrial habitats, EL for soils was obtained from the scientific literature.

2.2 Toxicity threshold assessment

Small, micro and nano plastics (SMNPs) were produced from field plastic samples obtained from three different environmental compartments: marine (shoreline and sea surface), freshwater (river banks and surface water), and terrestrial (soil), according to methods described in D6.1. and D6.3. To identify the hazard and assess the risk posed by these SMNPs, a battery of aquatic and terrestrial ecotoxicological tests (Fig. 1), selected according to internationally accepted standardised methods adapted to plastic toxicity testing, and using organisms representative of different trophic levels and environmental compartments, was conducted.

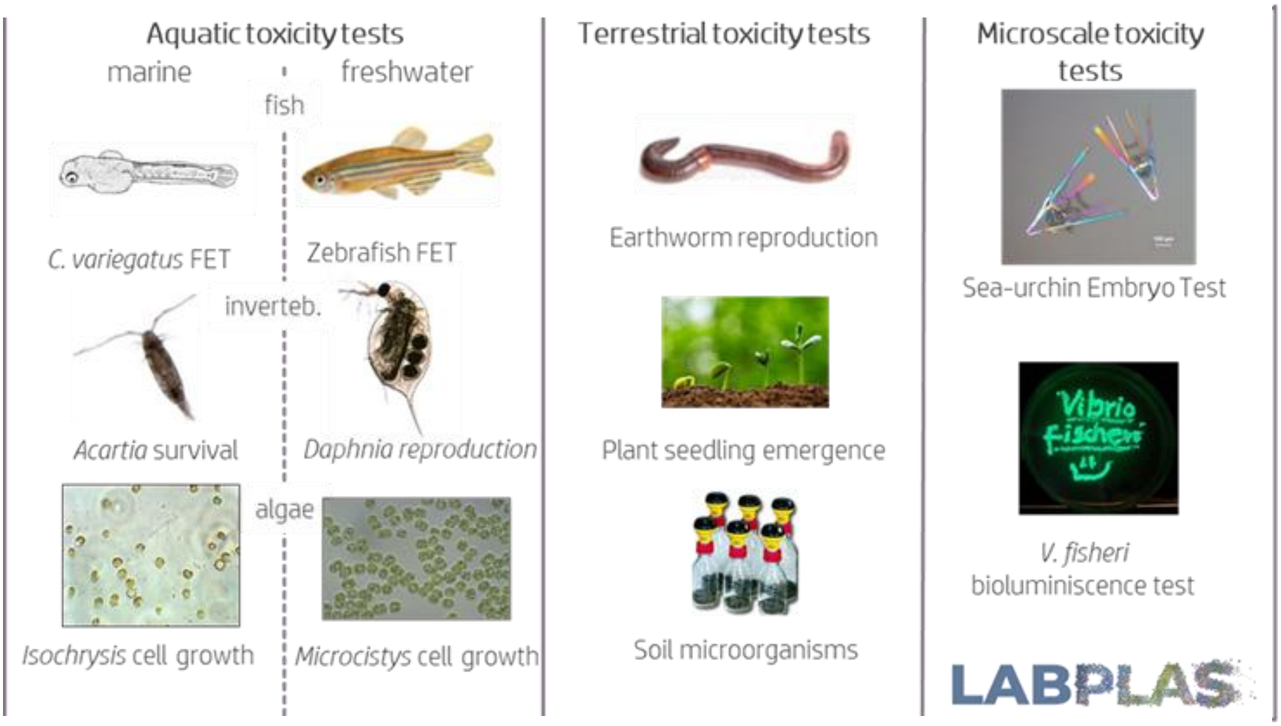

Figure 1. LABPLAS Project plastic toxicity test battery applied to test environmental field samples.

As previously described, the LABPLAS plastic toxicity assessment scheme takes into account two routes of exposure: (1) waterborne chemicals leached from the plastics (Tier I), and (2) plastic particles in contact and/or ingested by the organisms (Tier II) (Figure 3, D6.4). Tier I exposes the organisms to serial dilutions of a leachate obtained by following the standard protocol by Almeda et al (2023), using a load of 10 g of plastic per liter. Leaching time is standardized to 24 h consistently with Dhavamani et al. (2022), who found maximum concentrations of leached plastic additives after 24-48 h, followed by a decrease due to readsorption. Leachate testing allows the detection of effects caused by chemicals leached from the plastics into the water. These chemicals may be part of the original plastic composition or, in the case of environmental plastics, sorbed from the surrounding aquatic medium. Tier II exposes the organisms to plastic particles of a standard size suitable to be ingested by the test species and natural particles of an analogous size range. This size depends on the model organism. For most filter feeders and early stages of aquatic organisms, an upper size limit of 20 µm is suited.

Depending on the completeness of the data sets, TT were estimated according to the following statistical methods. If the data set allowed fitting to a concentration:response non-linear model, the TT was estimated as the EC_{10}, or the concentration of MPs reducing by 10% the biological endpoint recorded. In other cases, treatments were statistically compared to the control and the lowest observed effect concentration (LOEC) was taken as the TT estimation.

For Tier I tests, dilutions were translated into mass concentration units by multiplying the dilution factor by the concentration used to produce the tested leachates, either 1 or 10 g/L.

2.3 Characterization of Risk

Risk was characterized using both a deterministic approach, based on the TT of the most sensitive test species of each habitat (critical value), and a probabilistic approach based on the Species Sensitivity Distribution (SSD) curve.

In the deterministic approach,

$$R = EL \times AF / TT$$

Where AF is an assessment factor (AF) – also called the uncertainty factor – intended to make conservative allowances for the various sources of uncertainty associated with both EL and TT estimation. Typical sources of uncertainty are extrapolation of chronic effects from acute toxicity data, laboratory-to-field extrapolations, or allowances for the representativeness of the chosen test species. Typical values of AF range from 10 to 1000, decreasing as the amount and quality of ecotoxicological information increases. The EC prescribes a value of 10 when long-term effects in three trophic levels are assessed, increasing to a value of 1000 when only TT for acute effects are available. Obviously, high AF values increase the risk of too conservative – and thus, in practice, hardly applicable – estimates of risk.

An alternative probabilistic estimation of risk was also conducted using SSD curves obtained with all the species tested (see Fig. 2). This approach is not limited by the choice of AF and critical values. With that aim, data on TT values for aquatic organisms were fitted to a log-logistic model (Van Straalen & Denneman, 1989). This allowed calculations of the 5th percentile of the TT distribution (HC_5), i.e. the SMNP density affecting less than 5% of the species, and the corresponding 95% CI. If the SMNP levels do not exceed the lower end of the 95% CI of the HC_5, then we can expect with a 95% confidence that less than 5% of the species in the community will be affected, which is considered an acceptable level in the environment (Durán & Beiras, 2017).

3 RESULTS

3.1 Exposure levels

3.1.1 Exposure levels in the river water column

The microplastic abundance of plastic particles between 10 µm and 1 mm in the water of the Thames and Elbe rivers is shown in Fig. 33 of Deliverable D2.3[2]. This dataset was updated with previously unpublished 2023 sampling data. The main statistics of this dataset are compiled in Table 1 below. Total microplastic mass concentrations showed a high variability with no consistent seasonal trends. In 2022, levels were higher in the Thames than in the Elbe, whereas in 2023, when only hotspots were sampled, Elbe showed higher values. To produce a conservative risk assessment, the 95% percentile of the data obtained in each sampling site and the hotspot values were used to calculate the Risk Quotients.

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[2] Statistics were recalculated using the original dataset to correct minor mistakes in the Deliverable and additional 2023 data were added.

Table 1. Abundance of plastic particles between 25 µm and 1 mm in subsurface water of the Thames and Elbe rivers, and the Cecebre reservoir.

25-1000 µm MP	**Thames**		**Elbe**		**Overall**	**Cecebre**	
	winter	**summer**	**winter**	**summer**		**winter**	**summer**
mean (N/m^3)	782	517	506	2,716	1,137	2,287	1,504
mean (µg/m^3)	**922**	**548**	**1,019**	**4,804**	**1,662**	**1,080**	**23,288**
std	*1,876*	*1,014*	*2,087*	*9,238*	*4,520*	*1,275*	*58,291*
geom mean	**151**	**2.0**	**43**	**130**	**44**	**402**	**1,187**
95% perc	**3,173**	**2,142**	**3,674**	**21,244**	**15,973**	**2,788**	**57,062**
hotspot	Ilseworth 2022	Lechlade 2022	Dessau 2023	Cuxhaven 2023	Cuxhaven 2023	Tolva Wint-23	Tolva Sum-23
µg/m^3 in hotspot	**6,118**	**2,807**	**6,366**	**22,041**	**22,041**	**3,013**	**155,409**

3.1.2 Exposure levels in the North Sea surface water

Table 2. Abundance of plastic particles between 25 µm and 1 mm in subsurface water of the North Sea

25-1000 µm MP	**North Sea**		
	AL586 (winter)	**AL596 (summer)**	**Overall**
mean (N/m^3)	446	93	244
std	434	60	317
mean (µg/m^3)	**302**	**1133**	**777**
Std	**450**	**1523**	**1194**
geom mean	**59**	**82**	**71**
95% perc	**653**	**2814**	**2451**
hotspot	Station 1	Station 4	Station 4-summer
µg/m^3 in hotspot	**820**	**3230**	**3230**

3.1.3 Exposure levels in the river sediments

Levels of SMP in the Thames and Elbe river sediments are shown in Table 3 below. Only data in numbers were available for sediment microplastics. Assuming similar morphology of sediment and water surface microplastics, mass was estimated from the median mass/number ratio of water surface microplastics obtained in the Thames and the Elbe rivers, which was 0.343 µg per particle.

Table 3. Abundance of plastic particles between 25 µm and 1 mm in the sediments of the Thames and Elbe rivers.

25-1000 µm MP	**Thames**		**Elbe**	
	T5 London	**T6 estuary**	**E17 Dessau**	**E13 estuary**
mean (N/Kg)	1147	1172	3676	889
std	882	1272	2679	882
maxima (N/Kg)	2117	3362	8713	1258
maxima (µg/Kg)	**726**	**1153**	**2989**	**431**

3.1.4 Estimation of mass using actual weight records

GEOMAR kindly provided unpublished data on the mass:area ratio for a dataset of over 1000 plastic particles (mean size 2.95±3.67 mm) individually measured in terms of maximum dimension, minimum dimension, area and weight. The ratio followed a log-normal distribution with an average value of 0.42 mg/mm^2. The ratio did not show any significant trend to change with particle geometry, with a slope for the ratio vs geometry (assessed as maximum dimension/minimum dimension) close to zero (-0.016). Therefore, we extrapolated this value towards the lower size range of SMP particles analysed in the LABPLAS Project and obtained mass estimations x5.2 times larger than those provided by the siMPle software.

3.1.5 Exposure levels in agricultural soils

The identification and quantification of microplastics in terrestrial environments is beyond the scope of the LABPLAS Project. However, reliable and up-to-date information is available in the scientific literature. Büks & Kaupenjohann (2020) recently reviewed 23 studies of microplastics in land and provided a common upper limit for agricultural sites exposed to sewage sludge and mulching film application of 4.5 mg kg-1 dry soil.

3.2 Toxicity thresholds

Table 4 summarizes the toxicity of the plastic litter samples tested in the LABPLAS Project. These results have been updated from those presented in the previous deliverable (D6.4).

Table 4. Summary of the toxicity tests conducted with micronized environmental plastics sampled in the different areas studied in the LABPLAS Project in Germany (GE), Spain (ES) and the United Kingdom (UK). Test species and endpoints included Vibrio fischeri bioluminescence, sea-urchin embryo test (SET), freshwater and marine cyanobacteria/algae population growth (Microcystis aeruginosa and Skeletonema sp., respectively), freshwater and marine invertebrate reproduction and larval survival (Daphnia magna and Acartia sp, respectively), development of early life stages of freshwater and marine fish (Danio rerio and Cyprinodon variegatus, respectively), soil microbiota respiration, plant seedling emergence (Lepidium sativum) and earthworm reproduction (Eisenia andrei). Results have been arbitrarily classified into categories of increasing toxicity indicated by green, yellow, orange and red. Methods have been thoroughly described in previous deliverables from D6.1 to D6.4. L: leachate test; P: particle test. tested by UVigo.*

Sample			**Microscale test**			**Habitat-specific test**					
Aquatic	Month/ year	**ID**	***Vibrio***	**SET**		**algae**		**invertebrates**		**fish**	
Kiel (GE)	May 22	093	🟩 L	🟨 L		🟩 L		🟩 L		🟩 L	
North Sea (GE)	Apr 23	125	🟩 L	🟨 L	🟩 P	🟩 L		🟩 L	🟩 P	🟩 L	
Cecebre (ES)	Jun 22	095	🟩 L*	🟩 L		🟩 L		🟩 L		🟩 L	
Cecebre (ES)	May 23	134	🟩 L	🟩 L	🟩 P	🟩 L		🟩 L		🟩 L	🟩 P
Elbe (GE)	Feb 23	124	🟨 L*	🟧 L	🟧 P	🟨 L		🟨 L		🟧 L	🟧 P
Elbe (GE)	Jul 23	133	🟩 L	🟩 L	🟩 P	🟩 L		🟩 L		🟩 L	🟩 P
Thames (UK)	Jul 22	127	🟩 L	🟨 L	🟩 P	🟩 L		🟩 L		🟨 L	🟩 P
Thames (UK)	Jul 23	130	🟩 L	🟩 L	🟩 P	🟩 L		🟩 L		🟩 L	🟩 P
Terrestrial	Month/ year	**ID**	***Vibrio***	**SET**		**soil**		**plants**		***Eisenia***	
Abegondo (ES)	Jul 22	110	🟩 L	🟩 L		🟩 L	🟩 P	🟩 L	🟩 P	🟥 L	🟥 P
Abegondo (ES)	Mar 23	129	🟩 L	🟩 L		🟩 L	🟩 P	🟩 L	🟩 P	🟨 L	🟥 P
Abegondo (ES)	Jul 23	131	🟩 L	🟨 L		🟩 L	🟩 P	🟩 L	🟩 P	🟥 L	🟧 P

Aquatic sample ID124 (from the Elbe river banks) and terrestrial sample ID110 (from agricultural soil in Abegondo) were selected as examples of plastic litter showing some toxicity to the aquatic and terrestrial test species used, respectively.

When a concentration:response relationship was found, data were fitted to a toxicity curve, and the EC_{10} (concentration reducing by 10% the endpoint; survival, growth, reproduction) was used as an estimate of the toxicity threshold (TT, mg/L). When data did not allow curve fitting, TT was estimated as the lowest concentration significantly reducing the endpoint (LOEC). When LOEC was not identified, TT was simply delimited as above the highest level tested, and No Observed Effect Concentration (NOEC) was used for computations.

3.2.1 Aquatic species

The TT values obtained for sample ID124 were 154 mg/L for SET, 209 mg/L for copepod larvae, 220 mg/L for *Daphnia*, 431 mg/L for *Microcystis* (EC_{10}), and 490 mg/L for zebrafish (EC_{10}).

Table 5. Toxicity exerted by the most toxic freshwater plastics (ID-124) on the microscale and habitat-specific tests. L: leachate test. All leachates were made up at 10 g/L except for Daphnia (1 g/L). Symbols like those in Table 4.

ID124	**Microscale test**		**Habitat-specific test**			
spp	***Vibrio***	**SET**	**algae**	***Acartia***	***Daphnia***	**zebrafish**
test	L	L	L	L	L	L
EC_{50}	4300 (2.32 TU)	625 (16 TU)	2763 (3.6 TU)	870 (11 TU)	624	700 (14.4 TU)
TT (mg/L)	1433	154	431	209	220	490

3.2.2 Terrestrial species

The TT value obtained for sample ID131 was 2183 mg/L for SET, 14 mg/Kg for *Eisenia* (endpoint: number of juveniles per adult) with particles and 32 mg/Kg for *Eisenia* (endpoint: number of juveniles per adult) with leachates.

Table 6. Toxicity exerted by the most toxic terrestrial plastics (ID-131) on the microscale and terrestrial tests. L: leachate test; P: particle test.

ID131	***Microscale test***		***Habitat-specific test***					
spp	***Vibrio***	***SET***	**Soil microbioma**		**Plant (*Lepidium sativum*)**		***Eisenia andrei***	
test	L	L	L	P	L	P	L	P
EC_{50}	>10,000	9804 (1.02 UT)	>1250 mg/Kg	>2250 mg/Kg	>2250 mg/Kg	>2250 mg/Kg	378 mg/Kg (3.31 TU)	706 mg/Kg
TT	>10,000	2183 (EC_{10})	>1250 mg/Kg	>2250 mg/Kg	>2250 mg/Kg	>2250 mg/Kg	32 mg/Kg	14 mg/Kg

3.3 Deterministic estimation of risk

As explained above, in the deterministic approach, Risk Quotients (R) were calculated as

$$R = EL \times AF / TT$$

Where EL were the maximum concentrations (C) recorded in the LABPLAS Project sites studied (Tables 1 to 3). To follow a conservative approach, in this analysis we will include an AF=1000 (despite chronic effects being recorded), and will use the critical value, corresponding to the most sensitive species from each habitat.

3.3.1 Freshwater sites

TT=220 mg/L (*Daphnia*)

Table 7. Risk Quotient (R) obtained according to the deterministic method for the freshwater sites.

25-1000 µm MP	Thames		Elbe		Overall	Cecebre	
	winter	summer	winter	summer		winter	summer
C (µg/L) 95% perc	**3.173**	**2.142**	**3.674**	**21.244**	**15.973**	**2,788**	**57,062**
R (AF=10)	**0.0001**	**0.0001**	**0.0002**	**0.001**	**0.001**	**0.0001**	**0.0003**
R (AF=1000)	**0.014**	**0.010**	**0.017**	**0.097**	**0.073**	**0.013**	**0.259**
hotspot	**6.118**	**2.807**	**6.366**	**22.041**	**22.041**	**3,013**	**155,409**
R (AF=10)	**0.0003**	**0.0001**	**0.0003**	**0.001**	**0.001**	**0.0001**	**0.007**
R (AF=1000)	**0.028**	**0.013**	**0.029**	**0.100**	**0.100**	**0.014**	**0.706**

Maximum Risk Quotients range from 0.010 to 0.7 for AF=1000, and from 0.0001 to 0.007 for AF=10.

If we estimate mass using the mass:area ratio rather than the siMPle software, R values would range from 0.0005 to 0.5 except for the Cecebre-summer hotspot and AF=1000, when risk would exceed 1 (3.5).

3.3.2 Marine sites

TT=154 mg/L (sea-urchin)

Table 8. Risk Quotient (R) obtained according to the deterministic method for the marine sites.

25-1000 µm MP	AL586 (winter)	AL596 (summer)	Overall
C (µg/m^3) 95% perc	**0.653**	**2.814**	**2.451**
R (AF=10)	**0.00004**	**0.0002**	**0.0002**
R (AF=1000)	**0.004**	**0.018**	**0.016**
hotspot	**0.820** (Station 1)	**3.230** (Station 4)	**3.230** (Station 4-summer)
R (AF=10)	**0.00005**	**0.0002**	**0.0002**
R (AF=1000)	**0.005**	**0.021**	**0.021**

Maximum Risk Quotients (for AF=1000) range from 0.004 to 0.021

If we estimate mass using the mass:area ratio rather than the siMPle software, R values would range from 0.021 to 0.109.

3.3.3 Sediments

Due to the limited amount of micronized environmental plastics, we performed the test with *Lumbriculus variegatus* using leachates from environmentally exposed PS (extracellular polymeric substances (EPS), 1-year exposure in the River Lahn conducted by BfG). Here, a 24-hour leachate induced a significantly reduced growth and reproduction at 1 g/L. Therefore, LOEC was 1 g/L (used as an estimation of TT), and NOEC was 0.5 g/L. Using the maximum SMP levels from Table 3 and AF=1000, Risk Quotients ranged from 0.431 in the Elbe estuary to 2.989 in E17-Dessau. Since these values were obtained based on a single toxicity test, they must be considered very preliminary and demand comprehensive additional research before further discussion.

3.3.4 Soil

Using the lowest TT value obtained for D131 in the *Eisenia* chronic reproduction test with particles (14 mg/Kg) and an AF=10, the Risk Quotient is already >1:

$$R = 4.5 \times 10 / 14 = 3.2 > 1$$

This means that the risk in agricultural soils fertilized with sludge and exposed to mulch film is remarkable for important organisms in soil ecology, such as earthworms. This result deserves further investigation in the future to make sure plastics used in agriculture will not pose unacceptable risks to the structure of soil communities.

3.4 Probabilistic Estimation of Risk

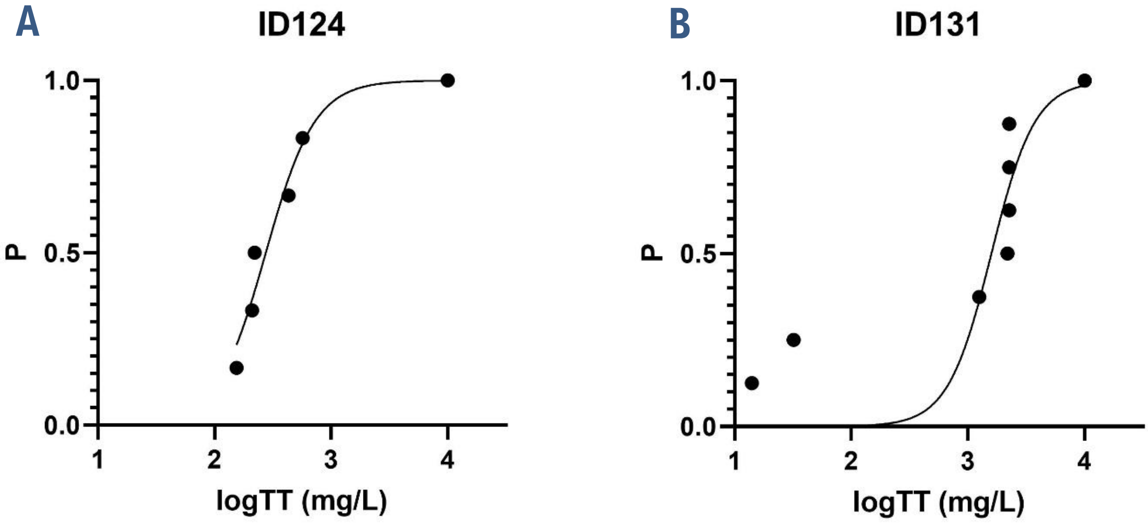

Figure 2. SSD curves for the most toxic plastic samples in aquatic (ID124) (A) and terrestrial (ID131) (B) habitats, respectively. Note that terrestrial data cannot be fit to a log-logistic model.

As shown in Fig. 2A, data on TT values for aquatic organisms fit well into a log-logistic model. This allows calculation of the 5th percentile of the TT distribution (HC5), i.e. the SMNP density affecting less than 5% of the species, with narrow confidence intervals (CI). HC_5= 65.68 mg/L 95% CI: (21.7 – 111.5) mg/L. In contrast, as shown in Fig. 2B, data obtained for terrestrial plastics do not accurately fit this model. HC_5= 446.76 mg/L, but the 95% CI of the HC_5 estimation is unacceptably high (0.0004 - 2125) and does not allow application of this probabilistic approach. Additional ecotoxicological testing of environmental plastics with other terrestrial species beyond those used in the LABPLAS Project is needed to fill this gap.

Assuming a log-normal distribution of the SMP levels in aquatic habitats (see Beiras & Schönemann, 2020), and using the data obtained by NOC in mass units from the Great North Sea sampling (including Thames and Elbe rivers), the probability of encountering SMP levels above 21.7 mg/L (i.e. 21,700,000 µg/m^3) is **R = 0.079**.

Therefore, the probabilistic approach produces a R remarkably similar to the deterministic approach (R=0.073 for the freshwater sites and R=0.016 for the marine sites, using the 95% percentile and AF=1000), highlighting the robustness of the present assessment.

4 DISCUSSION

This deliverable provides, to the best of our knowledge, the best comprehensive effort to assess the risk of plastics in the aquatic and terrestrial environments using a combination of field studies at the European scale to assess the exposure levels, and ecotoxicological bioassays conducted with environmental plastics from the same sites to assess their toxicity thresholds. It is worth highlighting that the environmental levels recorded here include **plastic particles down to 10 µm**. This strongly contributes to shortening the frequently discussed mismatch between the size range of MP recorded in most previous studies (frequently > 0.3 mm) and those reported as posing the most risk to aquatic organisms in laboratory experiments (frequently < 20 µm).

To maximize environmental protection, a number of **conservative assumptions** were adopted in the present analysis, including the following:

- We took the maximum SMP concentrations recorded in the hotspots. In fact, the 2023 sampling was conducted on the hotspots only. Therefore, this affects not only the R values calculated based on the hotspot concentrations but also those calculated based on the 95% percentiles, which for that year do not include SMP values from medium and low levels of pollution.
- In the deterministic approach, we took as TT the critical value, i.e. the value for the most sensitive species and endpoint among all the ecotoxicological bioassays conducted for each habitat.
- In the aquatic assessment, we also took the most toxic environmental plastic sample found (ID-124 from the Elbe). This means we are assuming all plastics detected in WP2 sampling show the toxicity corresponding to the most toxic field sample. However, in practice, most other field plastic samples were several orders of magnitude less toxic.
- A very high AF value was also included (AF=1000) despite information on chronic effects was available.
- In the probabilistic approach, we used the lower end of the 95% confidence interval of the HC_5 rather than the HC_5 itself.

Even under all these extremely conservative assumptions, aquatic plastic always yields R<1, disregarding the approach (deterministic or probabilistic) or method used to estimate mass units from particle size, with the single exception of the Cecebre summer hotspot, estimating mass from the area:mass ratio and using an AF=1000.

These findings are in line with the very low-risk quotients previously obtained for coastal and oceanic ecosystems by Beiras & Schönemann (2020) and by Adam et al. (2019) in freshwaters.

It is remarkable that the large variability in microplastic density was recorded in all sites sampled. Therefore, we should bear in mind that **if average values would have been used**, rather than hotspots, **risk quotient values would further decrease by 2 to 3 orders of magnitude**.

On the other hand, comparison between siMPle counts vs alternative semiautomatic methods conducted in LABPLAS WP3 demonstrates that **siMPle software systematically underestimates the microplastic counts due to limitations of the library used, among other reasons**. This topic is further discussed here: https://labplas.eu/2025/04/21/on-reliability-of-simple-for-quantification-and-characterisation-of-microplastics-in-environmental-samples/

Therefore, using alternative methods would yield higher MP counts and most likely higher risk quotients. Since mass estimations are obtained by assigning a hypothetical third dimension to the areas recorded in the filtered MPs, these mass estimations are submitted to high uncertainty, disregarding the software used, and at this stage, we cannot assess the magnitude of this uncertainty.

In contrast, when **Risk Quotients** are calculated **for agricultural soil habitats** using the very sensitive chronic endpoint of earthworm reproduction and maximum MP levels from the literature, **unacceptably high R values** are obtained, even using a moderate AF value of 10. This deserves further attention in the regulation of plastics used for agriculture, such as mulch films, greenhouses and silos, which were among the terrestrial plastic typologies here analysed.

Eisenia's sensitivity to microplastics was about one order of magnitude higher than the toxicity thresholds for the most MP-sensitive aquatic species. Despite remarkable, this is not the main reason why R values are much higher in soil compared to both freshwater and marine environments. Most of the difference found in R between aquatic and terrestrial habitats is due to the much **higher plastic loads reported**[3] **in some agricultural soils**, compared to the SMP levels recorded by the LABPLAS Project in European aquatic ecosystems.

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[3] Büks & Kaupenjohann (2020) https://doi.org/10.5194/soil-6-649-2020