TDE-C03 – Earthworm Sampling

Territorial bioindicators exclusively for PFAS determination

Version 1.0 | 10 November 2026 | Operational stages: biomass preparation and chemical analysis carried out by suitable facilities.

1. Purpose and scope

Define a simple, repeatable procedure for collecting earthworms from areas proposed for the Lands of Excellence (TDE), preparing their biomass at the sample-preparation facility, and delivering samples suitable exclusively for PFAS determination to the appointed laboratory. The assessment concerns environmental territory and is integrated with other TDE matrices; it is not a wildlife census.

No analyses of metals, pesticides, PCB or other contaminants are performed on earthworms. PFAS thresholds and assessment bands remain solely those already approved in the TDE analytical protocol: this procedure introduces no changes.

2. Strategy and representativeness

• Identify territorial units homogeneous in soil, land use and anthropogenic pressure; do not indiscriminately combine distinct situations.

• For the initial campaign, provisionally plan 3–5 points distributed across the homogeneous unit; this is an initial operational number to be verified in the field, not an ISO requirement.

• Record location, crop/cover type, soil moisture and any soil amendments or ponding. Prefer spring or autumn with moist, unfrozen soil.

• Absence of earthworms does not mean “PFAS absent”: report “biological sample unavailable” and integrate the assessment with other TDE matrices.

3. Essential materials

Clean spade or shovel; measuring tape and 50 × 50 cm guide square; new, clean, non-fluorinated PE/PP sheet; clean gloves verified suitable for PFAS; clean labelled PP/HDPE containers; labels; field record; insulated cooler and cold packs kept separate from the organisms. Avoid PTFE/Teflon and other fluorinated components contacting samples.

4. Field collection – manual method

1. Mark out an approximately 50 × 50 cm square at the selected point.

2. Carefully excavate the upper layer to approximately 20–30 cm, depending on soil structure and cultivation depth.

3. Break up clods on the clean sheet and gently retrieve intact earthworms; do not use electrical current, irritants or extraction solutions.

4. Place live organisms in labelled containers with a small amount of moist soil from the same point; do not waterlog.

5. Record approximate count, any morphological differences and collection difficulties; backfill the excavation.



Figure 1. Manual searching in excavated soil on a clean sheet. Digitally generated illustrative image.

5. Transport to the sample-preparation facility

Transport live organisms as soon as possible in ventilated containers protected from light; keep cool without freezing or placing containers directly on ice. Record collection and receipt dates/times. Do not impose 4 °C as a rigid transport temperature for live organisms.

6. Biological preparation at the sample-preparation facility

6. Keep batches from different origins separate until a documented decision on compositing. Exclude visibly damaged or dying animals and record discards.

7. Gently remove adhering soil with ultrapure water controlled for PFAS blanks, following a consistent procedure.

8. Place organisms without food on a clean, moist substrate verified to be free of significant PFAS contamination. The preliminary gut-purging period is 24–48 hours; record the actual duration and change the substrate when contaminated by excreta.

9. At the end, rinse if needed, remove surface water using a standardized approach and determine purged fresh mass.

10. Prepare a composite sample only from earthworms of the same homogeneous unit and trace their collection points. Freeze and homogenize with suitable equipment; agree in advance with the laboratory whether to deliver frozen or freeze-dried biomass.

11. If freeze-dried, record masses before and after treatment and the fresh/dry ratio, allowing comparison with the mass basis specified by the TDE thresholds.

Analytical note: freeze-drying must not be chosen without laboratory confirmation of the method and results-reporting basis. No chemical preservatives (formalin/ethanol) are

permitted for PFAS samples.



Figure 2. Separation of earthworms from soil clods during sampling. Digitally generated illustrative image.

7. Delivery to the appointed laboratory and essential controls

Element	Operating rule
Requested analysis	Exclusively the PFAS specified in the already approved TDE analytical protocol.
Biomass container	Suitable PP or HDPE verified for the method; cap without fluorinated lining.
Storage	Prepared samples frozen; storage temperature and duration agreed with the laboratory.
Preparation blank	Check preparation water, substrates and equipment, as agreed with the laboratory.
Results	Specify fresh/dry mass and preparation conditions; never apply thresholds to a different mass basis.

8. Short sampling and preparation record

Field	Information to record
TDE code / homogeneous unit	_____
Date, time and operator	_____
Collection points / coordinates	_____

Field	Information to record
Land use, moisture and notes	_____
Number / mass of organisms per point	_____
Receipt at the sample-preparation facility	_____
Washing / substrate / blank	_____
Purging start and end	_____
Purged fresh mass / any dry mass	_____
Sample code / delivery to laboratory	_____

9. Clarifications before protocol implementation

Before the first collection, the preparation manager and the laboratory must agree on (a) minimum biomass for the PFAS panel; (b) suitability of materials and substrates; (c) any freeze-drying; (d) storage and delivery times. An indicative 30–50 g fresh biomass is only a possible preliminary logistical target, not a validated quantity.

The manual method is an operational adaptation for PFAS chemical determination; it is not a full implementation of ISO 23611-1, which also covers other extraction methods for ecological objectives.

10. Technical references

ISO 23611-1:2026, Soil quality — Sampling of soil invertebrates — Hand-sorting and extraction of earthworms: https://www.iso.org/standard/88092.html

Occurrence and Fate of Fluoroalkyl Sulfonamide-Based Copolymers in Earthworms — Bioavailability, Transformation, and Potential Impact of Sludge Application (2024). Study using ultrapure washing, >24 h gut purging, freezing and freeze-drying: https://pmc.ncbi.nlm.nih.gov/articles/PMC11483768/

TDE — Previously approved analytical protocol on PFAS in earthworms (TDE technical document on analytes and thresholds).