



**EARTHWORM
FIELD STUDIES**

STRONGER TOGETHER

Welcome to Eurofins Agrosience Services

We are a leading provider of product development consultancy and technical support to the crop protection industry. Our technical activities involve conducting field and laboratory studies to determine the safety and efficacy of new agrochemicals and crop varieties. With over 25 years of experience, Eurofins Agrosience Services offers outstanding technical knowledge and project management skills. By acquiring a carefully selected range of CRO's, we have created a unique portfolio of expertise that provides analytical, regulatory and field support to plant breeders, agrochemical, biopesticide, biocide and fine chemical manufacturers.

Eurofins Agrosience Services conducts standard and customised earthworm field studies following the international guidelines and current recommendations of the SETAC Working Group. We have experience of specific studies with emphasis on residues and long term trials to monitor long term effects. Earthworm field studies are possible in all crops, grassland and orchards. Due to the seasonal activity of earthworms, field studies can be started in spring and autumn only. The study design allows combining standard studies with residue studies and / or microarthropod studies.

Objective

The objective of this type of field studies is to investigate the occurrence of any significant effect on field earthworm populations following the application of plant protection products (PPPs) in the field. Also, the monitoring of direct effects in the field and earthworm residue studies is sometimes requested.

Importance

Earthworms represent the major group of soil macrofauna in agricultural soils and are important for many soil related processes. Earthworm fauna field testing for ecotoxicological risk assessment is considered for registration procedures of crop protection products in different countries. Within a tiered-test system, field studies will follow the laboratory tests (acute toxicity and laboratory reproduction).

Principle of field studies

The baseline earthworm population density and species distribution will be assessed prior to application. At several times after the application, the earthworm fauna will be assessed for eventual effects and for earthworm population recovery. A standard design includes sampling before application and then at one month, six month and twelve month intervals after application of the test substance. The effect of the test substance will be assessed by comparing the effects on the earthworm population in the treated plots against the population in the control plots. Additionally, earthworm samples, soil samples, litter samples etc. can be taken for residue analysis.

Methodology

Standard earthworm field trials are set up as randomised block design with four replicates per treatment group. For each sampling, four sub-samples are taken per replicate plot resulting in sixteen samples per treatment group and sampling occasion. A toxic reference is included in order to demonstrate the sensitivity of the test system at trial initiation.

Earthworms are sampled using different extraction methods:

- Using the formaldehyde extraction method, a 0.2% formaldehyde solution is uniformly applied at a rate of 10 to 40 L/m² to sample areas within the trial plots. Eventual crop cover on these areas is removed before starting the extraction. After several minutes the first worms appear on the soil surface and can be collected.
- For the extraction of earthworms by hand-sorting, the soil of the sample areas is excavated with a spade and placed in plastic buckets. The soil is sorted by hand and checked carefully for earthworms.
- We recommend using a combined extraction method with hand-sorting of the defined soil volume and subsequent formalin sampling in the excavated hole for all samplings. The advantage of this combined method is that the sampling efficiency is the highest and that species of different life forms and ecological groups are extracted (i.e. anecic, endogeic and epigeic species).

Sample area sizes can be varied to cover different crop scenarios and / or different earthworm densities in the field.

Species identification of earthworms is done in-house down to species level by means of the relevant identification literature.



Earthworm residue sampling, Germany



Earthworm extraction, France



Formalin extraction of earthworms

Available trial sites

Trials can be performed in Northern, Central and Southern Europe. We have experience with earthworm field trials in Sweden, Northern and Southern Germany, Northern and Southern France, Northern and Southern Spain and in Italy.

We have established expert teams in Germany, Northern France (Alsace), Southern France (near Toulouse), Spain (Santiago de Compostela, Canals) and Portugal (Monção).

Special studies

- Earthworm residue studies
- Long-term studies
- Special study designs for persistent compounds

Special skills

- Earthworm determination in our own determination lab by experienced staff
- Residue analytics in our own analytical laboratories
- Long-term co-operating farmers
- Access to appropriate machinery and equipment for specific purposes
- Special application techniques (granule application in furrow or wide spread, application of spray liquid in furrow, fumigation, application of non-soluble substances on carrier substance etc.)
- Potential to develop and adapt test designs to special requests
- Combined design earthworm field study with micro-arthropod study
- Combined design earthworm residue study with arthropod residue study
- Statistics using SAS, Canoco etc.

Participation in working groups, research and publications

- Participation at the SETAC working group for the development of the earthworm field testing guideline "Technical Recommendations for the Update of the ISO Earthworm Field Test Guideline (ISO 11268-3)"
- Earthworm Field Testing: Application Timing a Factor Influencing the Effect of the Toxic reference (SETAC, 2006)
- Earthworm Field Testing: Comparison of different sampling methods (SETAC, 2007)
- Earthworm Field Testing: Comparison of different sampling methods (SETAC, 2008)
- Spacial and temporal distribution of earthworms in an arable field in Southern Germany (SETAC, 2009)
- Spacial and temporal distribution of earthworms in arable fields and its importance for terrestrial risk assessment for earthworms (SETAC, 2010)
- Mustard powder and Allyl-Isothiocyanate (AITC): Possible substitutes for formalin as expellants of deep-burrowing earthworms (SETAC, 2011)



Granule application,
Southern Germany



Residue sampling,
Southern France



Granule application,
Northern Spain

Eurofins Scientific Group

Eurofins Scientific is a life sciences company that serves a wide range of industries including the pharmaceutical, agricultural, food and environmental sectors.

Today the Eurofins Group is a leading provider of analytical services with:

- An international network of 150 laboratories across 30 countries in Europe, the USA, Asia and South America
- About 9,500 staff
- A portfolio of over 40,000 reliable analytical methods
- More than 80 million assays per year to establish the safety, composition, authenticity, origin, traceability, identity and purity of biological substances



Spray application on salad



Earthworm sampling,
Southern France



Earthworm sampling in hops,
Germany