



ECOTOXICOLOGY

Bees and pollinators

► Description of working areas

During the registration process of Plant Protection Products (PPP) it is necessary to provide evidence to enable the safety of the product towards pollinators, i.e. honey bees (*Apis mellifera*), bumble bees (*Bombus terrestris*) and solitary bees (e.g. *Osmia bicornis*). **BioChem agrar** offers a wide range of testing possible side-effects of PPPs, biologicals (including MCPA's), adjuvants and biocides on honey bees, bumble bees and solitary bees based on a long-term experience. Our team is actively involved in several international ring test groups. Thus, we are supporting to develop new testing methods and contribute to generate international guidelines and guidance documents.

Studies on honey bees, bumble bees and solitary bees can be conducted in Germany and/or Spain (in cooperation with **BioChem AGROLOGIA**) throughout the year without being limited to climatic conditions. In-house apiaries at all main locations, run by our own beekeepers provide reliability and independence.

Acute and chronic studies on honey bees, bumble bees and solitary bees as well as larval tests with honey bees are performed in newest, well-equipped laboratories under GLP or non-GLP.

Higher Tier tests are conducted on several crops (i.e. Phacelia, oilseed rape, buckwheat, ...) grown on own fields, prepared with own machinery. As laboratory studies, also higher Tier studies can be conducted in Germany and/or Spain to cover different geographical and climatic regions.

All required analytical verifications of test solution concentrations as well as residue analysis in bee relevant matrices e.g. (flowers, pollen, nectar and honey) can be performed inhouse.

► Regulations/ standards/ guidelines

We are working according to the following regulations:

- OECD No. 213/214: Honey Bee, Acute Contact/Oral Toxicity Test
- OCSPP 885.4380: Honey Bee, Acute Contact/Oral Toxicity Test – MCPA's
- OECD No. 245: Honey Bee, Chronic Oral Toxicity Test (10-Day Feeding)
- OECD No. 237/ GD 239: Honey Bee, Larval Toxicity Test, Single/Repeated Exposure
- EPP0 PP1/170(4) / OECD GD 75 / CEB 230: Honey Bee, Semi-Field/Field Tests
- Oomen et al. (1992): Honey Bee, Brood Feeding Test
- OCSPP 850.3040: Large Colony Feeding Study
- OECD GD 332: Honey Bee, Homing Flight Test
- SANTE/11956/2016 rev. 9: Honey Bee, Maximum Residue Levels in Honey
- OECD No. 246/247: Bumble Bee, Acute Contact/Oral Toxicity Test
- OECD Draft / ICPPR: Bumble Bee, Chronic Oral Toxicity Test (10-Day Feeding)
- Klein et al. (2022)/ ICPPR: Bumble Bee, Semi-Field Test
- OECD Draft / ICPPR: Solitary Bee, Acute Contact/Oral Toxicity Test
- Franke et al. (2020)/ ICPPR: Solitary Bee, Semi-Field Test



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ECOTOXICOLOGY

Non-Target Arthropods (NTA)

► Description of working areas

During the registration process of Plant Protection Products (PPP) it is necessary to provide evidence to enable the safety of the product regarding soil or leaf dwelling non-target arthropods. **BioChem agrar** offers a wide range of testing for possible side-effects of PPPs, biologicals (including MCPA's), adjuvants and biocides on NTA's based on a long-term experience. Our team is actively involved in several international ring test groups. Thus, we are supporting the development of new testing methods and contributing to generate international guidelines and guidance documents

The purpose of these studies is to determine potential side-effects of plant protection products (PPP) on non-target arthropods (NTA).

At Tier I level the parasitic wasp *Aphidius rhopalosiphii* and the predatory mite *Typhlodromus pyri* are tested as most sensitive species. The aim of the studies is to determine effects on mortality and reproduction as main endpoints to calculate the LR50, ER50 and NOER.

In the case effects occur at the Tier I level, it is recommended to conduct higher Level (Tier II) extended laboratory tests and expose NTA's to treated natural substrates, with the aim of determining the LR50, ER50 and NOER with all relevant test species.

To assess the duration of the residual activity of pesticides and the recovery potential of NTA populations, aged-residue tests may be required. NTA's will be exposed to freshly dried or semi-field aged spray residues on treated plants, applied under outdoor conditions. The number of successive bioassays to confirm recovery potential of a NTA population depends on the duration of the residual activity and the extend of effects on survival and reproductive performance. The recovery potential can be concluded if effects on mortality and reproductive performance are below 50% within two successive bioassays.

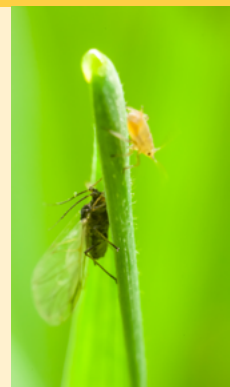


The aging of spray residues could be offered on several model plants (e.g. Citrus, Malus, Phaseolus, Vitis, Hordeum, Zea mays). Treated plants are placed under semi-field (outdoor) conditions with rain protection under a UV-permeable roof from the application until the start of the last bioassay. Our test organisms are bred in-house or purchased from a professional breeder.

Modern equipped labs and many years of expertise are the basics for high quality NTA testing.

Species for Tier I/II/aged residues studies:

- *Aphidius rhopalosiphii*
- *Typhlodromus pyri*
- *Aleochara bilineata*
- *Chrysoperla carnea*
- *Coccinella septempunctata*
- *Orius laevigatus*
- *Poecilus cupreus*
- *Pardosa* spp.
- *Trichogramma cacoeciae*
- *Pieris brassicae*
- *Vanessa cardui*
- *Bombyx mori*



► Regulations/ standards/ guidelines

We are working according to the following regulations:

- Directive 2004/10/EC of 11th February 2004 amending Council Directive 87/18/EEC.
- EFSA 2022: Supporting the development of exposure assessment scenarios for Non-Target Terrestrial Organisms to plant protection products
- Guidance Document on Regulatory Testing and Risk Assessment procedures for Plant Protection Products with Non-Target Arthropods. ESCORT 2 and 3
- Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS

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ECOTOXICOLOGY

Soil organisms

► Description of working areas

During the registration process of Plant Protection Products (PPP) it is necessary to provide evidence to enable the safety of the product towards soil micro and macro-organisms. **BioChem agrar** offers all relevant studies required for the registration of chemical, fertiliser, biostimulant, biological and conventional pesticides.

BioChem agrar is actively involved in several international ring test groups. Thus, we are supporting the development of new testing methods and contributing to generate international guidelines and guidance documents.

Studies on soil organisms can be conducted in Germany (CEZ) and/or Italy (SEZ) in cooperation with CRO partner. In-house breeding of Enchytraeidae, soil mites, collembola and earthworms provide reliability and independence.

Acute, chronic as well as higher Tier field studies can be conducted under GLP or non-GLP.

Higher Tier tests are conducted on bare soil, grassland or relevant crops grown on own field sites and managed with own machinery.

► Regulations/ standards/ guidelines

We are working according to the following regulations:

- **OECD No. 207:** Earthworm, Acute Toxicity Tests
- **ISO 11268-1:2012** Soil quality Effects of pollutants on earthworms Part 1: Determination of acute toxicity to *Eisenia fetida*/*Eisenia andrei*
- **ISO 17512-1:2008** Soil quality
Avoidance test for determining the quality of soils and effects of chemicals on behaviour Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*)
- **OECD No. 222:** Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*)

- **ISO 11268-3:2014** Soil quality
Effects of pollutants on earthworms Part 3: Guidance on the determination of effects in field situations
- **ISO 23611-1:2018** Soil quality
Sampling of soil invertebrates Part 1: Hand-sorting and extraction of earthworms
- **ISO 11268-2:2023** Soil quality
Effects of pollutants on earthworms Part 2: Determination of effects on reproduction of *Eisenia fetida*/*Eisenia andrei* and other earthworm species
- **ISO 23611-2:2006** Soil quality
Sampling of soil invertebrates Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)
- **ISO 23611-5:2011** Soil quality
Sampling of soil invertebrates Part 5: Sampling and extraction of soil macro-invertebrates
- **OECD No. 232:** Collembolan Reproduction Test in Soil
- **ISO 11267:2023** Soil quality Inhibition of reproduction of Collembola (*Folsomia candida*) by soil contaminants
- **OECD No. 226:** Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) reproduction test in soil
- **ISO 21285:2019** Soil quality
Inhibition of reproduction of the soil mite (*Hypoaspis aculeifer*) by soil contaminants
- **OECD No. 216:** Soil Microorganisms: Nitrogen Transformation Test
- **OECD No. 217:** Soil Microorganisms: Carbon Transformation Test
- **ISO/TS 10832:2009** specifies a method to evaluate the effects of pollutants on spore germination of a mycorrhizal fungus, *Glomus mosseae*.
- **ISO 18311:2016** Soil quality
Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms Bait-lamina test

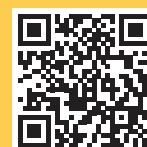


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ECOTOXICOLOGY

Non-Target Terrestrial Plant Testing

One of the requirements for approval of a new pesticide is to ensure that it does not cause unacceptable risks to non-target plants on cropland and adjacent land. Plants of field margins and other adjacent biotopes and ecosystems play an important role in insect, bird and mammal food chains and webs and must be protected.

► Description of working areas

The tests are performed in greenhouse or under semi-field conditions and possibly within a wide range of mono- and dicotyledonous plant species. Our work includes range-finding tests in advance and the corresponding main tests in limit or rate-response design. In our modern greenhouse the air temperature, humidity and illumination are adjusted by an automatic climate control system. A special feature is the cooling system that allows moderate and constant conditions even in hot summers. If necessary, artificial light with a sun-like light spectrum ensures a natural growth of the plants. Each greenhouse chamber has 100 m² and is controlled separately.

► Assessments and Endpoints

- Stage of development (BBCH)
- Emergence/ Survival/ Mortality
- Phytotoxicity/ Visual injury
- Plant height
- Fresh or dry weight determination
- The data are professionally statistically evaluated
- Competent reporting of the results is supplied by our study directors
- Analytical questions can be worked on in-house

► Guidelines

- OECD No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
- OECD No. 227: Terrestrial Plant Test: Vegetative Vigour Test
- OCSPP 850.4100: Seedling Emergence and Seedling Growth
- OCSPP 850.4150: Vegetative Vigour
- EPP0 PP 1/207: Effects on succeeding crops
- Standardized bioassay for the determination of ED10 (NOEL) and ED50 values for herbicides and selected following crops in soil



► Team

Our study directors have special longstanding expertise in the implementation of NTP studies.

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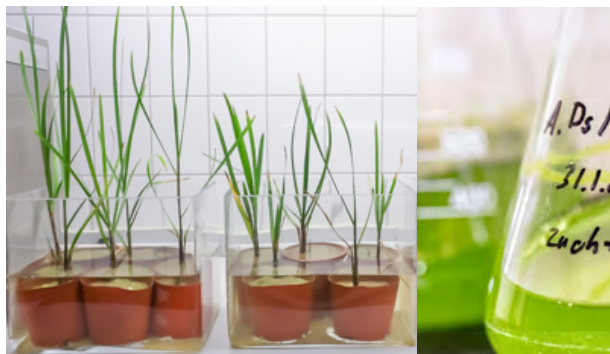
Aquatic tests

► Description of working areas

We are working a broad range of aquatic organisms mainly for the registration of plant protection products, pharmaceuticals, biocides and others, e.g. industrial chemicals (REACH). Besides classical aquatic testing with invertebrates, algae species of various genera and acute fish tests, we conduct macrophyte tests with non-standard species. As alternatives to animal testing, we perform fish embryo and fish cell line tests. All conducted studies are in accordance with international guidelines (OECD/ISO/EPA).

Our team is actively involved in international ring test groups. Thus, we are supporting the development of new testing methods and contributing to generate guidelines and guidance documents.

Benefiting from an in-house analytical department, we offer customized solutions for testing difficult substances and/or applications.



► Regulations/ standards/ guidelines

We are working according to the following regulations:

Acute tests

- OECD 201 algae (various species)
- OECD 202 *Daphnia magna*
- OECD 203 Fish acute (cold and warm water species)
- OECD 235 *Chironomus riparius*

Chronic tests

- OECD 218/219 *Chironomus riparius* (sediment/water spiked)
- OECD 211 *Daphnia magna*
- OECD 233 *Chironomus* Life-Cycle
- OECD 225 *Lumbriculus variegatus*
- OECD 242 *Potamopyrgus antipodarum*
- OECD 243 *Lymnaea stagnalis*

Higher plant tests

- OECD 221 *Lemna sp.*
- OECD 239/238 *Myriophyllum spicatum*
- *Glyceria maxima*

Alternative testing

- OECD 236 Fish embryo test
- OECD 249 Fish cell line test

Biodegradation

- OECD 301 (A - F) ready biodegradability

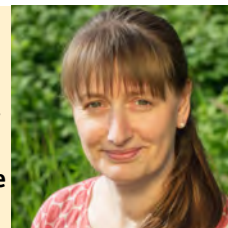
Microorganisms

- OECD 209 Activated Sludge, Respiration Test



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