

E·C·T OEKOTOXIKOLOGIE GMBH





ECT Oekotoxikologie GmbH is a privately owned enterprise that was founded in 1993.

In compliance with Good Laboratory Practice (GLP), we perform standardized ecotoxicological studies in the laboratory as well as at the semi-field and field level. Established test systems cover the aquatic, benthic and terrestrial environment.

In addition to performing standardized tests, we modify existing or develop new methods to improve the characterization of environmental risks caused by chemicals or contaminated matrices such as waste. This activity has in many cases contributed to the establishment of new international guidelines.

The results of our experimental and desk-based studies are used in the environmental risk assessment of industrial chemicals, biocides, pharmaceuticals, plant protection products, wastes and genetically modified organisms.

Through constant involvement in (inter)national research projects and cooperation with universities, ECT keeps up with the state of the art in the field of ecotoxicology and environmental fate of chemicals. Based on broad scientific expertise, we develop strategies to solve problems that may arise during marketing authorization, notification or classification of chemical substances.

Since 1993, ECT has been growing constantly and employs currently about 32 permanent staff. All staff members hold a degree from a university or a technical (high) school. In addition, we host each year several undergraduate and graduate students who often conduct their thesis work with us. Our laboratory, archive and office space covers more than 1500 m² and includes an isotope laboratory.

Our premises are located close to Frankfurt/Main, Germany, within easy reach of the airport of Frankfurt, a central hub in Europe.

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ECT is currently led by two managing directors. Jörg Römbke was among the founders of ECT back in 1993, while Anja Coors joined the management team in 2010. Based on their research interests and scientific experience, they cover different key activities at ECT.

Jörg Römbke is specialized in terrestrial ecotoxicology (particularly method development) as well as in soil ecology and the biodiversity of soil organisms. He has been chairing the ISO working group TC 190 SC4 WG2 since 2012. In addition, he is a member of the SETAC Europe council (2014 – 2017).

Anja Coors has a focus on aquatic ecotoxicology and statistics with extensive experience in mixture toxicity and pharmaceuticals in the environment. She was recently appointed as Honorary Visiting Fellow in the Environment Department of the University of York, U.K.



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Since 1994, ECT has been conducting a broad range of ecotoxicological tests at the laboratory, semi-field and field scale in compliance with Good Laboratory Practice (GLP) and according to international standard guidelines. We are particularly experienced in adapting studies to special requirements as demanded by scientific or regulatory needs.

In addition to the standard test species of the guidelines, we have extensive experience in testing other species to support species-sensitivity distributions. This includes various species of the genus *Lemna* as well as various freshwater crustaceans, cyanobacteria, and algae for the aquatic environment. For the terrestrial compartment, different earthworm, enchytraeid, springtail and plant species are available for testing.

Our testing activities in the field of effect, fate and bioaccumulation include the main environmental compartments water, sediment, sewage sludge and soil. The laboratory space covers about 900 m² and includes an isotope laboratory.

Analytical verification of test substance concentration, which is often required according to standard guidelines, is usually conducted by partners of ECT. Communication lines and GLP monitoring procedures are well established with these long-term partners and ensure a smooth execution of the complete study.

Tests that are not covered by our current portfolio can be subcontracted to our partners and monitored by ECT.

Aquatic Micro-Organisms	Guideline
Activated sludge, respiration inhibition test	OECD 209

Aquatic Primary Producers	Guideline
Freshwater algae and cyanobacteria, growth inhibition test	OECD 201 ISO 8692 DIN 38412-L33
<i>Lemna sp.</i> , growth inhibition test	OECD 221 ISO 20079
Rooted aquatic macrophyte <i>Myriophyllum sp.</i> , growth inhibition test	OECD 239

Aquatic Invertebrates	Guideline
<i>Daphnia magna</i> , acute immobilisation test	OECD 202 ISO 6341 DIN 38412-L30
<i>Daphnia magna</i> , reproduction test	OECD 211 ISO 10706
<i>Chironomus sp.</i> , acute immobilisation test	OECD 235





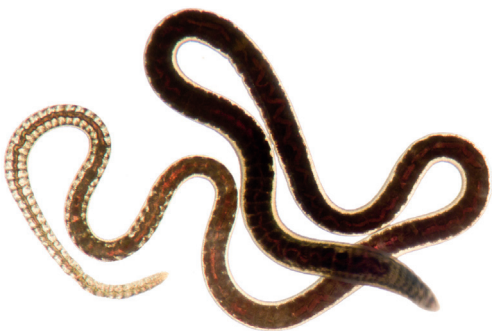
Fish	Guideline
Determination of the acute toxicity of wastewater to zebrafish eggs (<i>Danio rerio</i>)	ISO 15088 DIN 38415-6
Fish embryo acute toxicity (FET) test	OECD 236
Fish, short term toxicity test on embryo and sac-fry stages	OECD 212
Fish, acute toxicity test	OECD 203
Fish, prolonged toxicity test: 14-day study	OECD 204
Fish, juvenile growth test	OECD 215
Fish, early-life stage toxicity test	OECD 210
Fish, complete life-cycle test	Draft Guideline OECD DRP (2008)
Fish, short term reproduction assay	OECD 229
21-day fish assay: A short-term screening for estrogenic and androgenic activity, and aromatase inhibition	OECD 230
Fish, sexual development test	OECD 234



Sediment-dwelling Insects	Guideline
Sediment-water chironomid toxicity test	OECD 218 OECD 219
Sediment-water chironomid life-cycle toxicity test	OECD 233

Sediment-dwelling Oligochaetes	Guideline
Sediment-water <i>Lumbriculus</i> toxicity test using spiked sediment	OECD 225
Sediment toxicity test with <i>Tubifex tubifex</i>	ASTM E1706

Sediment-dwelling Crustaceans	Guideline
<i>Hyalella azteca</i> , sediment toxicity (28 days)	OECD 225 ASTM E1706





Soil Micro-Organisms	Guideline
Determination of soil microbial biomass – Part 1: Substrate-induced respiration method	ISO 14240-1
Determination of soil microbial biomass – Part 2: Fumigation-extraction method	ISO 14240-2
Nitrogen transformation test	OECD 216
Carbon transformation test	OECD 217
<i>Arthrobacter globiformis</i> - Solid contact test	ISO18187

Terrestrial Plants	Guideline
Seedling emergence and seedling growth test	OECD 208
Vegetative vigour test	OECD 227
Measurement of the inhibition of root growth	ISO 11269-1
Chronic toxicity in higher plants	ISO 22030



Terrestrial Invertebrates	Guideline
Earthworm acute toxicity test	OECD 207 ISO 11268-1
Earthworm reproduction test	OECD 222 ISO 11268-2
Enchytraeidae reproduction test	OECD 220 ISO 16387
Collembolan reproduction test	OECD 232 ISO 11267
Predatory mite reproduction test	OECD 226

Terrestrial Field and Semi-field Studies	Guideline
Terrestrial Model Ecosystems (TME)	PERAS (Schaeffer et al. 2009)
Determination of effects on earthworms in field situations	ISO 11268-3 Kula et al. (2006)
Breakdown of organic matter in litter bags in the field	OECD GD 56
Field studies with other soil invertebrates (enchytraeids, mites, collembolans and nematodes)	ISO 11268-3 (adapted) ISO 23611-2 ISO 23611-3 ISO 23611-4 ISO 23611-6
Bait lamina tests to assess feeding activity of soil organisms	ISO 18311





Dung Beetles and Flies	Guideline
Dung beetle laboratory test with <i>Aphodius constans</i> or <i>Onthophagus taurus</i>	OECD GD 122
Dung fly laboratory test with <i>Scathophaga stercoraria</i> or <i>Musca autumnalis</i>	OECD 228
Dung organism field study	Römbke et al. (2010), Jochmann et al. (2011)



Water	Guideline
Bioaccumulation in fish: aqueous and dietary exposure	OECD 305

Sediment
Bioaccumulation in sediment-dwelling benthic oligochaetes
OECD 315

Soil
Bioaccumulation in terrestrial oligochaetes
OECD 317



Test System	Guideline
DOC die-away test	OECD 301 A
CO ₂ -evolution test (Modified Sturm test)	OECD 301 B
Closed bottle test	OECD 301 D
Modified OECD screening test	OECD 301 E
Manometric respirometry test	OECD 301 F
CO ₂ -headspace test	OECD 310
Zahn-Wellens-test	OECD 302 B
Anaerobic biodegradation in digested sludge	OECD 311
Model sewage treatment plant	OECD 303 A
Biodegradation in activated sludge	OECD 314 B
Aerobic mineralization in surface water	OECD 309
Aerobic and anaerobic transformation in aquatic sediment	OECD 308
Aerobic and anaerobic transformation in soil	OECD 307

These tests will
in most cases
be subcontracted
to long-term
partners of ECT.



The ecotoxicological characterisation of waste is part of its assessment as hazardous or non-hazardous according to the European Waste List (EWL, 2000/532/EC and updates). Based on the experience obtained by organizing an international ring test, ECT is perfectly prepared to conduct all tests with solid wastes and their eluates that are useful for the evaluation for the criterion H14P “ecotoxic”.

Since several years, ECT is investigating effluents of wastewater treatment plants in the course of research projects and on behalf of operators. Focus is often placed on directly comparing the effects of various advanced wastewater treatment methods on water quality. We have extensive experience and suitable equipment to investigate wastewater streams by means of chronic tests with aquatic and benthic organisms in temperature-controlled flow-through systems installed directly at the wastewater treatment plant. Test organisms can represent different trophic levels, i.e. aquatic plants such as *Lemna* sp., freshwater crustaceans such as *Daphnia magna*, fish such as *Oncorhynchus mykiss*, and the benthic oligochaete *Lumbriculus variegatus*.



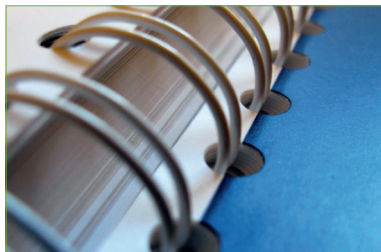
The main emphasis of ECT's consultancy service is on assembling and evaluating data for the environmental risk assessment of industrial chemicals, biocides, pesticides and pharmaceuticals. In addition to compiling existing data, we generate new data by using established (Q)SAR models and by subcontracting and monitoring new studies, where necessary. We also assess the environmental risks caused by mixtures of substances and evaluate the hazardous potential of waste.

In cooperation with partners specialized in the environmental fate of chemicals and human toxicology, we support the preparation and submission of full environmental dossiers.

Based on scientific principles we provide expert opinions for governmental authorities as well as for chemical industry.

Finally, we organize and chair meetings and workshops, where ecotoxicological topics are discussed by stakeholders from various backgrounds.

Examples for such workshops are listed on our webpage.



Our REACH experts at ECT are specialized in the area of ecotoxicology, environmental fate and behavior as well as physico-chemical properties of industrial chemicals. Additional areas are covered through well established and reliable co-operation with ECT's partners.

Prior to the implementation of REACH, ECT has gained experience with industrial chemicals through the compilation and successful submission of more than 30 SIDS Dossiers (Screening Information Data Set) for high production volume (HPV) chemicals in co-operation with companies, consortia and competent authorities. Since 2007, ECT has been involved in the registration of more than 300 substances. Thanks to our engagement in research projects, we are familiar with specific data requirements related to substances of very high concern (SVHC), persistent, bioaccumulative and toxic (PBT) substances, and endocrine disruptors.

ECT supports companies during the entire REACH process by offering the following services:

- » Identification of information requirements and data gaps
- » Data mining and literature search
- » Generating and evaluating e-fate and ecotox data with established (Q)SAR models (e.g. US EPA EPI Suite) and the OECD QSAR Toolbox
- » Data management, evaluation and reporting with IUCLID 6
- » Placing, performing and monitoring of required studies according to GLP
- » Specifying exposure scenarios and conducting the environmental risk assessment
- » Dossier preparation (e.g. technical dossier and chemical safety report)
- » Writing of expert judgements (e.g. within CoRAP)
- » Assessment of substances of very high concern (SVHC): CMR, PBT, vPvB, endocrine disruptors, and equivalent level of concern
- » Classification, Packaging & Labelling (CLP) according to the Globally Harmonized System (GHS)
- » Communication with the competent authorities (e.g. ECHA)



ECT's expert team for services around biocidal products is experienced in preparing and reviewing dossier documents according to the Biocidal Products Directive (BPD, 98/8/EC) such as documents I-IV as well as the IUCLID datasets of various product types (PT). We are familiar with the new Biocidal Product Regulation (BPR, Regulation (EU) 528/2012) and closely follow any updates of the relevant documents and guidance.

We have specialized in the areas of ecotoxicology, chemical fate and behavior, and physico-chemical properties. Our expertise includes exposure and risk assessments by using appropriate Emission Scenario Documents (ESD), the Technical Guidance Document (TGD), the Technical Notes for Guidance (TNsG) and tools such as the European Union System for the Evaluation of Substances (EUSES).

In order to provide our customers with complete dossiers and registration services, ECT closely works together with experienced long-term partners in a formal cooperation called RegisGate.

More information about RegisGate and the offered services can be found at www.regisgate.eu.



Our team of pharmaceutical experts at ECT compiles dossiers for the environmental risk assessment of human as well as veterinary medicines. We are familiar with all parts and tiers of the environmental risk assessment of pharmaceuticals. Based on our experience in national and international research projects, we can provide scientific expert advice particularly in complex cases, higher tier requirements and risk management options.

ECT supports companies during the pharmaceutical registration procedure by providing the following services:

- » Identification of information requirements and data gaps
- » Data mining and literature research
- » Placing, performing and monitoring of required fate and effect studies according to Good Laboratory Practice (GLP)
- » Conducting Phase I and II of the environmental risk assessment for human as well as veterinary pharmaceuticals
- » Dossier preparation (CTD Module 1.6)
- » Providing expert opinion reports on specific questions regarding effects, exposure and risk management
- » Communication with the competent authorities





Our risk assessment team for plant protection products at ECT is experienced in reviewing and summarizing ecotoxicological study reports for the preparation of the respective chapters of the dossier (active substance and formulation) following Regulation (EC) 1107/2009. Our experience covers all sections, i.e. aquatic and terrestrial organisms, as well as higher-tier studies such as field and micro- and mesocosm studies.

We are familiar with the complete set of the FOCUS software and the FOCUS groundwater models that are required for the calculation of Predicted Environmental Concentrations (PECs).

By working on more than 30 pesticide dossiers and reviewing about 190 EU Draft Assessment Reports, we have gathered a broad expertise on possible refinement steps in the environmental risk assessment.

ECT supports companies during the authorization process with particular attention on pesticide fate and behavior in the environment as well as effects on environmental organisms. For a complete handling of Plant Protection Products authorization, we cooperate with our partners specialized in other areas (such as human toxicology).

Our services include:

- » Data gap analysis and development of strategies for additional testing
- » Performing, placing and monitoring of required studies according to Good Laboratory Practice (GLP)
- » Preparation of dossiers for the active substance and formulations
- » Preparation of the fate (Section 5) and ecotoxicity (Section 6) dossier parts
- » Environmental risk assessment and risk refinement
- » Project coordination and communication



MIXTURES

The consideration of mixtures of chemical substances in the regulatory assessment of environmental risks is getting into the focus of competent authorities. Recent developments, particularly in the risk assessment of plant protection products and biocides, require nowadays the evaluation of mixture toxicity in the regulatory process.

Through research projects funded by environmental authorities, we have been involved in the development of concepts that include mixture toxicity considerations in the environmental risk assessment in a pragmatic, manageable and consistent way.

In various research projects, we have conducted experimental studies with mixtures and have applied mixture toxicity concepts to theoretically predict the effects of mixtures based on the effects of their individual components.



Studies on the effects and the fate of chemicals in the context of a formal risk assessment process must generally be conducted according to international guidelines. ECT actively contributes to the development, standardisation and validation of such methods and guidelines. Our activities include the organisation of and participation in international ring tests as well as participation in working groups of international standardisation organisations, in particular OECD and ISO.

ISO (International Organisation for Standardisation)

ISO and its national partners (e.g. the German Institute for Standardisation, DIN) are non-profit associations that are responsible for setting and publishing standards and guidance papers for technical equipment and experimental methods. Members of ECT's staff are active in the Committees ISO/TC 190/SC 4 "Biological methods", especially in the working groups "Effects on soil fauna" (WG 2), "Effects on soil flora" (WG 3), "Effects on soil microorganisms (WG 4), ISO/TC 190/SC 7 (WG 3 "Ecotoxicological characterisation of soils and soil materials" and WG 8 "Bioavailability").

OECD (Organisation for Economic Co-operation and Development)

An important aspect of OECD's Chemicals Safety Program is to coordinate the development, standardisation, validation and publication of Chemicals Testing Guidelines. In this process, expert groups can be established and members of these expert groups are nominated by the National Coordinators of each member country. Staff members of ECT have been nominated as experts in the test guideline sections "Effects on biotic systems" (e.g. testing of endocrine disrupting chemicals with fish and invertebrates) and "Degradation and accumulation".

ECT contributed significantly to the standardisation of the following methods described in international guidelines and guidance papers:

ISO 19204: Soil quality – Procedure for site-specific ecological risk assessment of soil contamination (soil quality TRIAD approach)

ISO 18311: Soil quality – Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms – Bait-lamina test

ISO 18187: Soil quality – Contact test for solid samples using the dehydrogenase activity of *Arthrobacter globiformis*

ISO 23611-6: Soil quality – Sampling of soil invertebrates – Part 6: Guidance for the design of sampling programs with soil invertebrates

OECD 235: *Chironomus* sp., acute immobilisation test

OECD 317: Bioaccumulation in terrestrial oligochaetes

OECD GD 122: Guidance document on the determination of the toxicity of a test chemical to the dung beetle *Aphodius constans*

OECD 233: Sediment-water chironomid life-cycle toxicity test

OECD DRP 95: Detailed review paper on fish-cycle tests

OECD 315: Bioaccumulation in sediment-dwelling benthic oligochaetes

OECD 226: Predatory mite (*Hypoaspis aculeifer*) reproduction test in soil

OECD 228: Dung flies (*Scathophaga stercoraria*, *Musca autumnalis*) laboratory tests

OECD 225: Sediment-water *Lumbriculus* toxicity test using spiked sediment

ISO 17512: Soil quality - Avoidance test with earthworms (*Eisenia fetida/andrei*)

ISO 23611-3: Soil quality - Sampling of soil invertebrates – Part 3: Sampling and soil extraction of enchytraeids

ISO 23611-1: Soil quality - Sampling of soil invertebrates - Part 1: Hand-sorting and formalin extraction of earthworms

OECD GD 56: Breakdown of organic matter in litter bags in the field

OECD 222: Earthworm reproduction test (*Eisenia fetida/andrei*)

OECD 220: Enchytraeidae reproduction test

ISO 16387: Soil quality – Effects of pollutants on Enchytraeidae (*Enchytraeus* sp.)

ASTM 1676: Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with the lumbricid earthworm *Eisenia fetida* and the enchytraeid potworm *Enchytraeus albidus*

ISO 22030: Chronic toxicity in higher plants



*Jörg Römbke and Anja Coors
in the ECT booth at the
SETAC Europe conference
in Glasgow 2013*

We actively participate in SETAC, the *Society of Environmental Toxicology and Chemistry*. SETAC is a not-for-profit organisation with about 6,000 individual members and institutions from all over the globe. SETAC provides a forum where professionals from academia, business and government promote scientific exchange and discuss multidisciplinary approaches to solve environmental problems. ECT has been supporting SETAC as a SETAC Europe partner since 2012. Staff of ECT has actively participated in SETAC advisory groups, workshops and at conferences for many years. Anja Coors served as member of council of SETAC GLB (German Language Branch) for 5 years, including one year (2012) as president. Jörg Römbke is currently member of the SETAC EU council (period 2014–2017).



Our research activities range from rather basic to applied aspects in different areas of ecotoxicology and ecology. We investigate cause-effect relationships of chemicals at various levels of biological organisation, ranging from molecular structures, physiological functions, individuals, and populations to (model) ecosystems. In more applied research projects, we improve existing or develop new testing methods to assess potential impacts of chemicals in the environment.

Another field of our research relates to the improvement of existing environmental risk assessment schemes and the provision of scientific background knowledge to improve regulatory decisions. One example in this area is the assessment of mixtures of chemicals.

In addition, we participate in ecological studies in the context of nature protection and soil biological monitoring activities in Europe and in North and South America (especially Brazil). One example in this research area is the contribution made to the taxonomy of enchytraeids, including their use in soil quality monitoring.

We frequently operate as partner or coordinator of national and international consortia. Our partners in research are universities, national and international research institutions, governmental organisations and companies from the chemical industry.

More information on our completed and currently running research projects and a list of publications in international peer-reviewed scientific journals can be found on our webpage.

INTELLIGENCE-LED ASSESSMENT OF PHARMACEUTICALS IN THE ENVIRONMENT (iPiE)

Active pharmaceutical ingredients (APIs) are biologically active compounds designed to interact with specific pathways and processes in target organisms. Over the past 15 years, a substantial amount of research has been devoted to investigate the occurrence, fate, effects, and resulting risks of APIs in the environment. The research project iPiE (Intelligence-led assessment of pharmaceuticals in the environment, 2015–2018) is continuing this research, funded by the Innovative Medicines Initiative (IMI), a public-private partnership between the European Union and the pharmaceutical industry.

The aim of iPiE is to develop

- » intelligent and efficient testing strategies to identify potential environmental risks of new APIs during the early stages of the development process
- » strategies that enable prioritization of legacy compounds for an environmental risk assessment. These prioritisation approaches should not require extensive experimental testing but apply new or adapted predictive models and exploit existing data obtained e.g. during pre-clinical and clinical development.

Within iPiE, ECT leads the work package “Experimental validation of developed models”, where a number of iPiE partners will verify by experimental testing the frameworks for exposure and effect assessment developed in other work packages. In addition, ECT contributes to other work packages such as the development of guidance for data quality assessment, testing strategies and prioritization. More information is available at i-pie.org.



ECT has expertise in soil biodiversity, especially in the taxonomy of earthworms (Lumbricidae), potworms (Enchytraeidae), and roundworms (Nematoda). In several projects, we have studied ecotoxicological effects on these terrestrial invertebrates in combination with their ecology, distribution, and diversity in the field. In addition, we are active in monitoring studies focusing on the soil quality throughout Germany and Europe. For example, ECT was involved in the preparation of the first map of earthworm distribution in Europe. In addition, we have conducted such studies in North and South America (particularly in Brazil).

Recently, ECT has become involved in the barcoding of invertebrates (e.g. earthworms, enchytraeids, and dung organisms). Barcoding is useful to confirm the species identity in ecotoxicological laboratory tests, which is important for species complexes such as the *Eisenia fetida/andrei* group. In addition, barcoding is a possibility to improve the taxonomic handling of these animals in field tests and monitoring studies. The work is performed in close co-operation with external partners, in particular the company allgenetics (A Coruna, Spain) and the Senckenberg Museum (Frankfurt, Germany).

Finally, ECT is active in the compilation of taxonomic and ecological data for soil organism communities (particularly earthworms and enchytraeids) in publicly available databases, providing robust information regarding their distribution and ecology. The most recent example is our contribution to the German database EDAPHOBASE located at the Senckenberg Museum (Görlitz, Germany).



Eisenia fetida



Eisenia andrei



ECT provides training courses for groups, particularly in terrestrial ecotoxicology. These courses consist of lectures and practical exercises about the fate of chemicals in soil, their effects on non-target arthropods and other soil organisms, statistical data evaluation and quality assurance requirements in ecotoxicological tests at laboratory and field level. Such training has been conducted in the context of (inter-) national training courses in Germany, Finland, the Czech Republic, Portugal, Brazil, Tunisia and Morocco for students and members of environmental agencies and industry.

Together with about 10 other German institutions (mostly universities), ECT is a partner of the postgraduate studies (PGS) initiative in ecotoxicology, jointly organized by the SETAC German Language Branch (SETAC-GLB) and the Society of German Chemists (GDCh).



ECT is experienced in organising workshops and project meetings resulting from our participation in a number of research projects. We offer the following services in our own premises or at other locations:

- » Organization of workshops and conferences
- » Identification and invitation of speakers
- » Invitation of participants
- » Moderation of the workshop or conference
- » Proceedings and summary records

Check out our webpage to see examples of workshops (co)organized by ECT!

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