Project no. SSPI-CT-2003-511135

ERAPharm

Environmental Risk Assessment of Pharmaceuticals

Specific Targeted Research Project

Thematic Priority: 1.1.6.3 ‘Global Change and Ecosystems’

Publishable Final Activity Report

Period covered: 01 Oct. 2004 − 30 Sept. 2007

Date of preparation: 21 December 2007,
Date of revision: 23 June 2008, due to changes in legal names of partners

Start date of project: 01 October 2004 Duration: 3 years

Project coordination team: Thomas Knacker, Karen Duis, Anja Coors
Project coordinator organisation: ECT Oekotoxikologie GmbH

Project logo:	
Project no.:	**SSPI-CT-2003-511135**
Title:	**Environmental Risk Assessment of Pharmaceuticals**
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PROJECT EXECUTION

Project objectives

Research on the fate and effects of pharmaceuticals in the environment has become an important issue in recent years and progressed significantly. Yet, a number of uncertainties still need to be elucidated before risks can be fully evaluated. Therefore, the overall objective of ERAPharm was to further advance existing knowledge and procedures for use in the environmental risk assessment (ERA) of human and veterinary pharmaceuticals. The addressed specific objectives were

- to investigate previously unstudied major exposure routes of pharmaceuticals into the terrestrial and aquatic (freshwater and marine) environment
- to investigate the factors and processes affecting the fate of pharmaceuticals in soils and surface water and to develop testing and modelling approaches for the evaluation of pharmaceuticals in the future
- to develop a scenario-based exposure assessment system for pharmaceuticals considering a range of region-specific usage characteristics and climatic conditions as well as exposure of soils, surface water, sediment and groundwater
- to explore the use of bioanalytical assays for an initial hazard screening and mode-of-action classification of pharmaceuticals
- to evaluate whether and how information on pharmaco- and toxicodynamics in mammalian species can be used to predict effects on organisms in the environment
- to modify and refine test methods in order to detect the effects of long-term, low-level exposure to pharmaceuticals on aquatic and terrestrial organisms (bacteria, invertebrates and fish) at the individual, population and community level
- to investigate whether and to what extent environmentally relevant concentrations of selected pharmaceuticals cause effects in environmental organisms
- to explore, as far as possible, fate and effects of selected transformation products and develop pragmatic approaches for assessing these
- to develop, based on the obtained results, guidance on how to improve the environmental risk assessment of pharmaceuticals including a web-based tool to support the ERA process

Contractors involved:

1) ECT Oekotoxikologie GmbH (ECT), *Germany*
2) AstraZeneca UK Ltd. (AstraZeneca), *United Kingdom*
3) Brunel University (UBRUN), *United Kingdom*

4) Bundesanstalt für Gewässerkunde (BfG), *Germany*
5) Centre National du Machinisme Agricole du Génie Rural des Eaux et des Forêts (Cemagref), *France*
6) University of York (UoY), *United Kingdom*
7) The Danish University of Pharmaceutical Sciences (DFU), since 1 January 2007 Kobenhavns Universitet (KU), *Denmark*
8) Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz (Eawag), *Switzerland*
9) Geotechnisches Institut AG (GI AG), *Switzerland*
10) Utrecht University (UU), *The Netherlands*
11) Instituto Nacional de Investigación y Tecnologia Agraria y Alimentaria (INIA), *Spain*
12) National Environmental Research Institute (NERI), since 1 January 2007 Aarhus Universitet (AU), *Denmark*
13) Umweltbundesamt (UBA), *Germany*
14) Canadian Water Network (CWN), *Canada*

Summary of the work performed

The work addressing the specific objectives of ERAPharm was organised in nine work packages and four working groups. The working groups addressed specific aspects of the environmental risk assessment (ERA) of pharmaceuticals and proposed improvements, namely (i) how to target the ERA by using information from mammalian studies and higher-tier test approaches, (ii) how to better assess, model and consider partition and persistence of pharmaceuticals in the environment, (iii) how to identify pharmaceuticals likely to pose a high risk below current action limits and how to use alternative endpoints in the ERA, and (iv) how to identify transformation products and assess their effects and exposure. A considerable amount of the experimental work focussed on three case study compounds: two human pharmaceuticals, the ß-blocker atenolol and the anti-depressant fluoxetine, and the veterinary parasiticide ivermectin. Yet, further human and veterinary pharmaceuticals were investigated in the individual work packages.

Fate assessment

Fate and exposure of human and veterinary pharmaceuticals were investigated by using methods of analytical chemistry for both radio-labelled and non-labelled compounds, and modelling approaches. For selected pharmaceuticals, analytical methods were developed and adapted for various environmental matrices, i.e. water, soil, sediment, dung, manure, invertebrate tissue, and fish plasma, and were validated by inter-laboratory comparison studies for surface water, sediment, wastewater and soil. The established methods were used to study sorption, partitioning, transformation and biodegradation at the laboratory scale with water, marine and freshwater sediments, soil and sludge. Based on these results, recommendations were proposed how to improve current standard guidelines for the fate assessment of pharmaceuticals. For example, when investigating the partitioning behaviour of a pharmaceutical, the choice of the test matrix (e.g. poultry litter, cattle manure, sediment, or soil) should be related to the respective environmental compartment, the predominant use of the pharmaceutical (e.g. in poultry or cattle) and the storage conditions of the waste (aerobic or anaerobic). The applicability of the octanol-water partition coefficient, K_{ow}, for fate predictions such as bioaccumulation was questioned for the mostly ionisable pharmaceuticals, whereas the alternative of using liposome-water distribution coefficients appeared promising. Biodegradation of human pharmaceuticals might be underestimated by the ready biodegradability test currently requested in the European regulatory guideline. Sewage treatment simulation tests may better predict removal of pharmaceuticals in waste water treatment plants and therefore might help to avoid triggering of unnecessary effects

testing. Finally, the extensive information gained in the water-sediment partitioning tests performed according to OECD 308 should be better exploited for the ERA compared to the limited usage the guideline currently makes of the results of this requested test.

In addition to laboratory studies, the fate of ivermectin was studied at the semi-field scale by means of three different types of terrestrial microcosms in which also effects on soil organisms and potential leaching were investigated. In two large field studies, the fate of ivermectin was further assessed in a Northern and a Mediterranean European region, thereby taking into account the geographic and climatic diversity of Europe. These studies explored a previously unstudied but realistic exposure route by following the excretion and field degradation of ivermectin in dung of subcutaneously ivermectin-treated cattle. Results indicated that leaching of ivermectin from dung to soil is of little relevance and confirmed high persistence of ivermectin in dung under field conditions. In addition to the planned work program, field experiments were undertaken to evaluate the fate of human pharmaceuticals in municipal biosolids following application by different recommended practices to agricultural fields which represent additional, previously unstudied exposure routes. Even after long time periods, pharmaceuticals were detected at low levels in the run-off from the fields after broadcast application. Yet, other field application practices proved to avoid pharmaceutical-contaminated run-off.

Several transformation products of the human pharmaceutical atenolol, the veterinary pharmaceutical ivermectin and tetracycline antibiotics were isolated during the fate studies, subsequently identified and the pathways for their formation further explored.

The data obtained within ERAPharm provide an extensive overview on the behaviour of pharmaceuticals in various environmental matrices such as water, freshwater and marine sediments, soil, dung and sludge under different environmental conditions. By using these experimentally determined fate data and information compiled from the literature, existing models originally developed to predict physico-chemical properties as well as runoff, transport, and leaching of chemicals or pesticides were evaluated and adapted for use with pharmaceuticals. Comparison of the measured concentrations of β-blockers in the Glatt river watershed with respective predictions derived by the GREAT-ER model revealed that the default dilution factor of 10 to extrapolate from effluent to surface water concentrations, as given in the current guideline on the ERA of human pharmaceuticals, might not be protective enough and should be updated by using georeferenced data on discharge, precipitation and population densities across Europe. As a result of evaluating existing region-specific European exposure scenarios for pesticides in the FOCUS model, three new scenarios were identified as being not sufficiently covered in the existing framework although being relevant for veterinary pharmaceuticals: hilly areas with cool and wet climate, foothills of mid-altitude mountain ranges, and plains with a continental climate and heavy soils. In order to better simulate transport of pharmaceuticals applied in liquid manure or sewage sludge, it was recommended to modify the modelling tool MACRO in FOCUS by enabling defining the applied volumes and loads, allowing for changes in hydraulic conductivities in the surface layer and accounting for particle facilitated transport.

Effects assessment

To explore methods for an initial hazard screening of pharmaceuticals, various ß-blockers and parasiticides were tested in a mode-of-action based battery of bioassays which included estimation of baseline toxicity, uncoupling of energy transduction, specific effects on photosynthesis, estrogenic and androgenic activity, reactive toxicity, and genotoxicity. A model was developed as a pragmatic approach to estimate the toxicity of transformation products through quantitative structure activity relationship (QSAR) and mixture toxicity predictions and applied for a large range of pharmaceuticals. Although the increase in hydrophilicity due to human metabolism resulted generally in lower toxicity, this approach

illustrated that metabolites add to the overall toxic potential of the mixture.

To explore effects on bacterial communities and potential development of resistance due to low-level long-term exposure, methods to perform both terrestrial and aquatic microbial microcosm studies were established and applied to test antibiotics from different structural classes. In these studies, effects on the microbial community were detected by means of functional parameters such as arginine ammonification and enzymatic assays, whereas effects on community structure were identified by means of community-level physiological profiling and molecular methods. Molecular methods were also used to estimate the occurrence of resistance genes. Structural changes detected at the molecular level were observed at antibiotic concentrations where only slight functional changes were apparent, whereas the potential of antibiotics to increase the level of antibiotic resistance in the test systems appeared to be low, especially at environmentally realistic concentrations.

An extensive dataset on the toxicity of ivermectin and abamectin on dung and soil organisms was obtained by a range of single-species laboratory tests. Existing laboratory tests with soil invertebrates and plants were adapted and two new dung fauna testing protocols were developed. In three different semi-field tests and two field studies with dung of ivermectin-treated cattle, the effects of ivermectin on dung fauna and soil arthropods were assessed under different environmental conditions. Based on the gained experience, it was concluded that the assessment of effects of pharmaceuticals on terrestrial ecosystems is in general not restricted by a lack of suitable test methods. Yet, guidance on when and how to use higher-tier studies for the terrestrial compartment is needed and was developed within the project. The importance of developing and using tests on dung fauna was confirmed by the observation that dung flies revealed to be the most sensitive organisms in both field studies, whereas soil arthropods, with collembolans being the most sensitive, were only affected at much higher ivermectin exposure concentrations under laboratory conditions. Climatic conditions appeared to interact with the effects on organism-mediated dung decomposition, which supports the importance of a regional assessment of parasiticides and the relevance of dung decomposition as endpoint. Changes in community structure of dung and soil organisms (e.g. dominance spectrum or species number) were identified as additional promising endpoints. In general, field studies appeared to be a reasonable tool to directly assess the impact of parasiticides. However, there is a need for more methodological standardisation of such studies to ensure repeatability and comparability. In particular, study site size and/or replication should be large enough to account for the low number of individuals and high fluctuation in dung fauna.

Effects of the three selected case study compounds on aquatic species were assessed in laboratory tests covering a broad range of species (nematodes, algae, snails, crustaceans, insects and fish). In addition to tests conducted according to standard protocols as required in the guideline for the ERA of pharmaceuticals, effects were also assessed in non-standard tests by using non-standard species (e.g. snails), multi-species systems (e.g. microcosms), non-standard endpoints (e.g. trans-generational effects, behaviour). Stronger effects of fluoxetine and atenolol in the second generation of exposed *Daphnia magna* than in the first indicated that non-standard endpoints can be more sensitive than standard endpoints. High toxicity of ivermectin towards aquatic invertebrates was detected in standard toxicity tests and confirmed using a set-up in which aquatic organisms were exposed to ivermectin-spiked cattle dung in a water-sediment system, representing another previously unstudied, yet relevant exposure route. In general, non-standard test species or multi-species systems did not demonstrate a higher sensitivity for the case study compounds than the standard tests required according to the guidelines. Ivermectin proved to be an exception, since effects on collembolan abundance in a long-term semi-field test turned out to be the most sensitive endpoint in the terrestrial compartment and a multi-species test with exposure via spiked dung showed effects below the detection level of the chemical analysis. However, the inclusion of additional taxonomic groups such as molluscs for the aquatic effects assessment

still appears advisable due to the physiological and reproductive features of these organisms, which are not represented by the current test organisms.

The mammalian-fish leverage model aims at estimating the risk of pharmaceuticals for fish based on human therapeutic doses and environmental exposure in order to guide further effects assessment. Using information from pharmacological dossiers and additional results from long-term fish studies, this model was evaluated for the ß-blocker atenolol and revealed to be applicable. One key assumption of this model, the presence of similar receptors in mammalian and fish species, was verified through identification of adrenergic receptors in fish by molecular methods.

Environmental risk assessment

A scientific opinion paper was prepared which summarizes in a concise way the recommendations regarding the ERA of pharmaceuticals. A targeted environmental risk assessment of pharmaceuticals was proposed by pursuing the same principles as for established ERAs (e.g. risk characterisation by comparing compartment specific exposure and effects, tiered approach) but by taking advantage of the available non-environmental data and the specific properties of pharmaceuticals, i.e. biologically active substances. The targeted ERA should minimize the generation of new data, reduce the uncertainties of risk characterisation and allow the selection of cost effective tests. A potential scheme for assessing the risks of transformation products in a timely and cost-effective manner was developed. The proposed approach uses information on identity and excretion obtained in the developmental phases of pharmaceuticals to guide further steps, e.g. fate and effects assessment. According to the proposed approach, explicit risk assessment of a transformation product is advocated if it is identified as being more persistent, more mobile or more toxic than the parent compound.

The extensive dataset generated on aquatic and terrestrial long-term toxicity of the case study compounds as well as on fate-relevant parameters were compiled to be used in the ERA in addition to data available in the literature. The aim of the case studies was to perform exemplary environmental risk assessments according to and reaching beyond current European guidelines. This approach facilitated addressing specific gaps in the current guidelines and to provide scientific guidance on how to deal with these issues. For the parasiticide, the ERA conducted strictly according to guideline would indicate a risk for surface water. However, based on additional tests beyond those requested by the guideline, a risk was also indicated for the terrestrial compartment. For atenolol, no risk was indicated, even when more sensitive non-standard endpoints were used for deriving hazard quotients. For fluoxetine, the preliminary calculation resulted in a very broad range of refined predicted environmental concentrations due to considerable uncertainty in input parameters, which presents a challenging issue for the final decision on whether fluoxetine poses a potential risk to the environment.

Finally, a web-based tool, named PharmaEcoBase (http://pharmaecobase.lyon.cemagref.fr/), was developed and implemented in order to aid in the ERA of pharmaceuticals using most up-to-date scientific data.

Impact of the project

The exemplary environmental risk assessments (ERAs) for the case study compounds and the results of the four working groups integrating the conclusions of the performed work and recommendations on advancing the ERA of pharmaceuticals will be published as a special edition of an international peer-reviewed journal. In addition, the prepared scientific opinion paper will be made available to the interested public, the scientific community, representatives from the pharmaceutical industry and from competent authorities responsible

for the regulation of pharmaceuticals. The international conference "*Pharmaceuticals in the Environment*" organised by ERAPharm in September 2007 in York, U.K., served as an excellent opportunity to share the results obtained by the project and critically discuss the conclusions and recommendations with the scientific community as well as stakeholders.

During the project, ERAPharm members have been involved in international committees such as advisory and professional interest groups of the *Society of Environmental Toxicology and Chemistry* (SETAC) and Pellston workshops. Advanced test protocols for assessing fate and effects of pharmaceuticals were developed and disseminated, e.g. draft guidelines for effects testing with dung fauna to be adapted by the *Organisation for Economic Co-operation and Development* (OECD). Specific input was also provided by submitting proposals on improvements of the ERA of pharmaceuticals to working groups of the *European Food Safety Authority* (EFSA) and the *European Medicines Agency* (EMEA).

The partnership policy of ERAPharm has been to include representatives from competent authorities, the pharmaceutical industry, national research institutes, universities and SMEs. In addition to the partners of the consortium, stakeholders – representatives from competent authorities and pharmaceutical industry – participated in ERAPharm meetings and actively contributed to the working groups. These opportunities to meet and discuss developments in the ERA of pharmaceuticals in an early phase were highly esteemed by all partners and fostered fruitful discussions on a high scientific level. Representatives of the Canadian competent authority regarded for example their participation as very helpful in the ongoing development of Canadian environmental regulations for personal care products and pharmaceuticals.

In summary, ERAPharm significantly advanced the scientific knowledge on the fate and effects of pharmaceuticals and provided expert guidance on improvements of the ERA as it is expressed in the high number of peer-reviewed publications, conference contributions and input into international committees dealing with various aspects of the environmental risk assessment of pharmaceuticals.

Co-ordinator contact details

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DISSEMINATION AND USE

Peer-reviewed publications

- Jacobsen, A.M. & Halling-Sørensen, B. (2006). Multi-component analysis of tetracyclines, sulfonamides and tylosin in swine manure by liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* 384, 1164-1174.
- Escher, B.I., Bramaz, N., Richter, M. & Lienert, J. (2006). Comparative ecotoxicological hazard assessment of beta-blockers and their human metabolites using a mode-of-action-based test battery and a QSAR approach. *Environ. Sci. Technol.* 40, 7402-7408.
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sewage treatment plants. *Water Res*. 41, 1614-1622.

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- Garric, J., Vollat, B., Duis, K., Péry, A., Junker, T., Ramil, M., Fink, G. & Ternes, T.A. (2007): Effects of the parasiticide ivermectin on the cladoceran *Daphnia magna* and the green alga *Pseudokirchneriella subcapitata*. *Chemosphere* 69, 903-910.
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Other publications

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- Press release ('Neue Ansätze für eine verbesserte Beurteilung möglicher Umweltrisiken von Arzneimitteln'), available at *www.erapharm.org/downloads/*
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- Knacker, T. (2007): Umweltrisikobewertung von Humanpharmaka – Inhalte und Ziele des von der EU geförderten Projektes ERAPharm. *Pharm. Ind*. 69 (4): 404-406.

- Knacker, T.: Presentation of ERAPharm and final project results in the *newsletter* of *Network of reference laboratories for monitoring of emerging environmental pollutants* (NORMAN).
- Schmitt, H. (2007): Antibiotika als Umweltkontaminanten – Effekte auf Mikroorganismen, *Münchener Beiträge zur Abwasser-. Fischerei- und Flussbiologie*, Band 58, Bayerisches Landesamt für Umwelt (Ed.), p. 177-186.

Conferences and workshops organised by ERAPharm partners

- Workshop on '*Sulfonamide antibiotics in the environment – fate and effects*', organised by H. Schmitt. Utrecht University, Utrecht (The Netherlands), 09 February 2006.
- Session on 'Environmental risk assessment of pharmaceuticals: The state-of-the-art' chaired by T. Knacker and A. Boxall. *SETAC Europe 16th Annual Meeting*, The Hague (The Netherlands), 07-11 May 2006.
- Workshop on 'Exposure modelling and monitoring – use of data and models' chaired by M. Ramil. *Joint DIA/HESI/SAPS Conference on Environmental Assessment of Human Medicines*, Stockholm (Sweden), 22-23 May 2006.
- Workshop on 'Human pharmaceuticals: risk for freshwater ecosystem' organised by J. Garric in the framework of a national program aiming to prioritise human pharmaceuticals in a freshwater monitoring program. Lyon (France), 30 June 2006.
- FORUM-Conference *'Umweltbewertung von Humanpharmaka'*, organised by T. Knacker, J. Koschorreck and T. Ternes, Düsseldorf (Germany), 18-19 September 2006.
- Workshop on '*Ecological risk assessment of pharmaceuticals*' organised by C. Metcalfe, Viamede Resort, Stoney Lake, Ontario (Canada), 07 October 2006.
- Workshop on '*Usage and environmental fate of veterinary pharmaceuticals*' organised by M. Schneider, Dübendorf (Switzerland), 17 January 2007.
- Workshop on'*Emerging contaminants in the environments. Exposure, fate, effects, risk assessment and mitigation measures*' organised by T. Ternes and C. Metcalfe, Koblenz (Germany), 30 March 2007.
- '*International Conference on Analysis of Emerging Contaminants in the Environment*' organised by A. Boxall, York (U.K.), 7-9 March 2007.
- International conference on '*Pharmaceuticals in the Environment*' organised by ERAPharm, York (U.K.), 19-21 September 2007.

Presentations at national and international conferences and meetings

- Boxall, A.: Fate of pharmaceuticals in the environment. Platform presentation, Society of Chemical Industry (SCI) meeting '*Pharmaceuticals in the environment: fate, effects and regulation*', London (U.K.), 01 March 2005.
- Fenner, K.: Challenges in exposure modelling for pharmaceuticals. Platform presentation, Society of Chemical Industry (SCI) meeting '*Pharmaceuticals in the environment: fate, effects and regulation*', London (U.K.), 01 March 2005.
- Knacker, T., Liebig, M., Duis, K. & Klaschka, U.: European efforts concerning risk assessment, research and source control for PPCPs. Platform presentation, Environment Canada Workshop on '*PPCPs in the environment – towards the development of a national research strategy*', Canada Centre for Inland Waters, Burlington, Ontario (Canada), 10-11 March 2005.
- Boxall, A.B.A.: Fate of veterinary medicines in the environment. Platform presentation, *TETSOIL meeting*, Vienna (Austria), 19 May 2005.
- Alder, A.C., Richle, P., McArdell, C.S. & Giger, W.: Betablockers in municipal wastewaters in Switzerland. Poster, *SETAC Europe 15th Annual Meeting*, Lille (France), 22-26 May 2005.
- Duis, K., Ternes, T.A., Fenner, K., Escher, B., Schmitt, H., Römbke, J., Garric, J., Hutchinson, T., Boxall, A. & Knacker, T.: ERAPharm − a project for improving future environmental risk assessment of pharmaceuticals. Poster, *SETAC Europe 15th Annual Meeting*, Lille (France), 22-26 May 2005.
- Krogh, K.A., Löffler, D., Ternes, T, Halling-Sørensen, B. Determination of parasiticides in waters, soil and sediment using LC-MS/MS. Platform presentation, *SETAC Europe 15th Annual Meeting*, Lille (France), 22-26 May 2005.
- Sánchez, P., Ortiz, J. & Tarazona, J.V.: Hazard assessment of pharmaceuticals on terrestrial ecosystems. Platform presentation, *SETAC Europe 15th Annual Meeting*, Lille (France), 22-26 May 2005.
- Schmitt, H., van Beelen, P., Hamscher, G., Stoob, K. & Seinen, W.: Antibiotic usage in veterinary farming and antibiotic resistance. Platform presentation, *SETAC Europe 15th Annual Meeting*, Lille (France), 22-26 May 2005.

- Tarazona, J.V., Alonso, E., Gónzalez-Doncel, M. San-Andres, M.I., Carbonell, G.: Use of toxicokinetic/toxicodynamics information for predicting bioaccumulation and critical body burdens of pharmaceuticals in aquatic organisms. Platform presentation, *SETAC Europe 15th Annual Meeting*, Lille (France), 22-26 May 2005.
- Escher, B.: Mode-of-action based ecotoxicological hazard assessment. Platform presentation, *The National Research Institute for Environmental Toxicology* (Entox), University of Queensland, Brisbane (Australia), 10 June 2005.
- Jensen, J.: Risk management or further investigations of VMPs? – A scientist's look on various options. Platform presentation, *AnimalPharm Conference*, Amsterdam (The Netherlands), 03 October 2005.
- Fenner, K.: Current state of the art and challenges in exposure assessment for pharmaceuticals (ERAPharm). Platform presentation, EMEA Conference on '*Environmental risk assessment for human and veterinary medicinal Products*', London (U.K.), 27-28 October 2005.
- Knacker, T., Duis, K., Escher, B., Hutchinson, T., Tarazona, J., Ternes, T., Apel, P., Koschorreck, J. & Boxall, A.: Environmental risk assessment for pharmaceuticals − challenges from a scientific point of view. EMEA Platform presentation, Conference on '*Environmental risk assessment for human and veterinary medicinal products*', London (U.K.), 27-28 October 2005.
- Escher, B.I., Bramaz, N., Lienert, J. & Richter, M.: Comparative ecotoxicological hazard assessment of beta-blockers and their human metabolites. Platform presentation, *SETAC North America 26th Annual Meeting*, Baltimore, U.S.A., 13-17 November 2005.
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- Ramil, M., Fink, G., Löffler, D. & Ternes, T.: Fate of pharmaceuticals in sediments. Platform presentation, *SETAC North America 26th Annual Meeting*, Baltimore, U.S.A., 13-17 November 2005.
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