

DISCUSSION

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EDA-EMERGE: an FP7 initial training network to equip the next generation of young scientists with the skills to address the complexity of environmental contamination with emerging pollutants

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Abstract

The initial training network consortium novel tools in effect-directed analysis to support the identification and monitoring of emerging toxicants on a European scale (EDA-EMERGE) was formed in response to the seventh EU framework program call to train a new generation of young scientists (13 PhD fellows and 1 postdoctoral fellow) in the interdisciplinary techniques required to meet the major challenges in the monitoring, assessment, and management of toxic pollution in European river basins. This 4-year project is of particular relevance considering the multidisciplinary analytical chemistry and biology skills required to investigate the enormous complexity of contamination, effects, and cause-effect relationships. By integrating innovative mode-of-action-based biodiagnostic tools including *in vitro* and *in vivo* tests, transgenic organisms, and 'omics' techniques with powerful fractionation and cutting edge, analytical, and computational structure elucidation tools, powerful new EDA approaches are being developed for the identification of toxicants in European surface and drinking waters. Innovative method development by young researchers at major European universities, research centers, and private companies has been closely interlinked with a joint European demonstration program, higher-tier EDA, and specialized training courses and secondments. Using a simplified protocol based on existing EDA tools, EDA-EMERGE fellows are also being trained to organize and run international and interdisciplinary sampling and monitoring campaigns within selected European river basin sites. Strong networking between academia, the private sector, and leading regulators in the field of river basin management and pollution management ensures the relevance of the research for practice and excellent employment opportunities for the fellows. Additionally, an internationally composed advisory board has been tasked to introduce new perspectives on monitoring, assessment, and management of emerging pollutants within and outside of Europe. The combination of cutting edge science with specialized training in

(Continued on next page)

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(Continued from previous page)

complimentary soft skills is being offered with a strong emphasis on commercial exploitation and media competence which further enhances the employability of the fellows in research, academia, and beyond.

Keywords: Effect directed analysis; Emerging chemical pollutants; EU river basins; Bio-assays; Analytical chemistry; EDA-EMERGE

Background

Water quality in many European rivers improved significantly over the last 30 years, and river waters exhibiting acute lethality to fish or invertebrates are an exception today. However, toxic pressure by pesticides [1], biocides [2], endocrine disruptors [3,4] and many other chemicals [5] still presents a large-scale risk and causes community shifts and the disappearance of sensitive species in many river basins. The European Water Framework Directive (WFD) [6] provides a new holistic approach for the assessment and management of the ecological status of water bodies. However, the WFD chemical monitoring and classification is still centered on the traditional approach of regulating individual chemicals, the priority substances (PS) and the river basin specific pollutants, via compliance with environmental quality standards. This approach, based on chemical analysis of single substances, cannot cover the enormous variety of potentially hazardous chemicals present in the environment, which occur in complex and time variable mixtures. Subsequently, the observation of adverse effects in biota is often not reflected by the occurrence and concentrations of PS but caused by other non-regulated contaminants of emerging concern. In a discussion paper by Christian Daughton, US Environmental Protection Agency (USEPA) stated that 'an ever-expanding universe of unique chemicals, untold numbers of which have yet to be recognized or revealed, continually perfuse our environment and contact living systems' [7]. In 2005, 26 million individual chemical substances were identified and registered in the Chemical Abstract Service (<http://www.cas.org/>), with one-third being commercially available [7]. By June 2013, this number had increased to 72 million. The continuous increase in the diversity of chemicals being used makes identifying those that occur in our environment an increasingly difficult task. The screening of environmental samples, such as extracts of water, sediments, or biota, with liquid or gas chromatography coupled with high-resolution mass spectrometry (LC-HRMS) typically reveals tens of thousands of peaks, the majority of which are unknown.

Nationally and across the member states, there is an increasing awareness that the analysis of PS alone is insufficient to characterize the toxic pressure to aquatic ecosystems and water resources [8,9]. In response, powerful new screening methods are being developed.

These include multi-target analyte approaches together with suspect screening, e.g., the use of transformation prediction models to propose transformation products without available standards and the screening of unknowns including all detected masses or peaks [10]. However, the identification of unknowns, particularly with LC-MS techniques, is very challenging and time-consuming and therefore unlikely to be available as a routine technology for all chemicals in the complex mixtures that are found in the environment. Approaches are required to focus analytical efforts on those chemicals that actually pose a risk or cause an effect. Two of the most prominent methodologies to approach this challenge, effect-directed analysis (Figure 1) and toxicity identification evaluation, apply biological tools to select fractions of a mixture and individual components for identification and structure elucidation efforts according to measurable effects [11-15].

Significant methodological development in EDA has been achieved within the last years as reflected by a recent book on this topic [16]. However, significant bottlenecks remain which are now being addressed by the Marie Curie initial training network (ITN) project, EDA-EMERGE. Research within EDA-EMERGE is directed towards developing powerful new bio-analytical detection tools to significantly improve the analytical power of LC-HRMS-based structure elucidation by interlinking with novel, powerful computer tools, to enhance EDA throughput, and to reduce sample requirements and the risk of artifacts by means of miniaturization and automated online combination of fractionation, biotesting, and chemical analysis. The most important aspect of EDA-EMERGE, however, is its people. This ITN has been designed to produce a new generation of experts that are trained during their PhD studies in biological and chemical tools and the attendant integration in order to unravel and assess complex contamination of the aquatic environment for the sustainable management of European water resources in close collaboration with key players in WFD implementation such as the European Commission Joint Research Centre (JRC), the European Environmental Agency, and the German Federal Environmental Agency.

The EDA-EMERGE consortium includes two universities (Free University of Amsterdam and the RWTH Aachen University), six research centers (Helmholtz

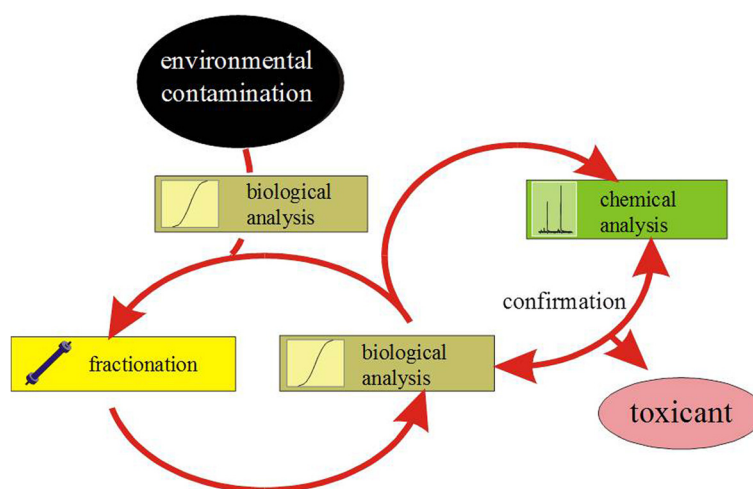


Figure 1 Schematic representation of effect-directed analysis.

Centre for Environmental Research (UFZ), Institut National de l'Environnement Industriel et des Risques (INERIS), Swiss Federal Institute of Aquatic Science and Technology (Eawag), Rudjer Boskovic Institute, Norwegian Institute for Water research, the Italian Institute of Health, the JRC), and five private companies (Environmental Institute, KWR Watercycle Research Institute, WatchFrog, HighChem, and gaiac) (Figure 2). EDA-EMERGE is an outcome of the European Network of Reference Laboratories for Monitoring of Emerging Environmental Pollutants (NORMAN) and strongly benefits from this platform, bringing together 55 partners from science, monitoring, and regulation in 19 countries [17]. Since complex contamination is not exclusively a European problem, EDA-EMERGE is also networking with renowned overseas partners such as advisors from the US-EPA and Environment Canada.

Novel scientific tools

On the basis of the outstanding expertise of their host institutes in EDA, the 13 PhD fellows are developing novel methods for advanced EDA in three work packages, *viz.* bio-analytical tools, chemical tools, and hyphenated tools. A specific focus of bio-analytical tool development will be on vertebrate *in vivo* and *in vitro* models for genotoxicity, mutagenicity [18] and AhR-mediated toxicity, and for endocrine disruption [19]. This will include innovative transgenic models using multi-well-based fluorescent screens for androgenic and corticosteroid disruptors on the basis of extensive experience with tadpole fluorescent screens for thyroid hormone disruptors (Figure 3) and fluorescent zebrafish transgenic embryos for estrogens [20].

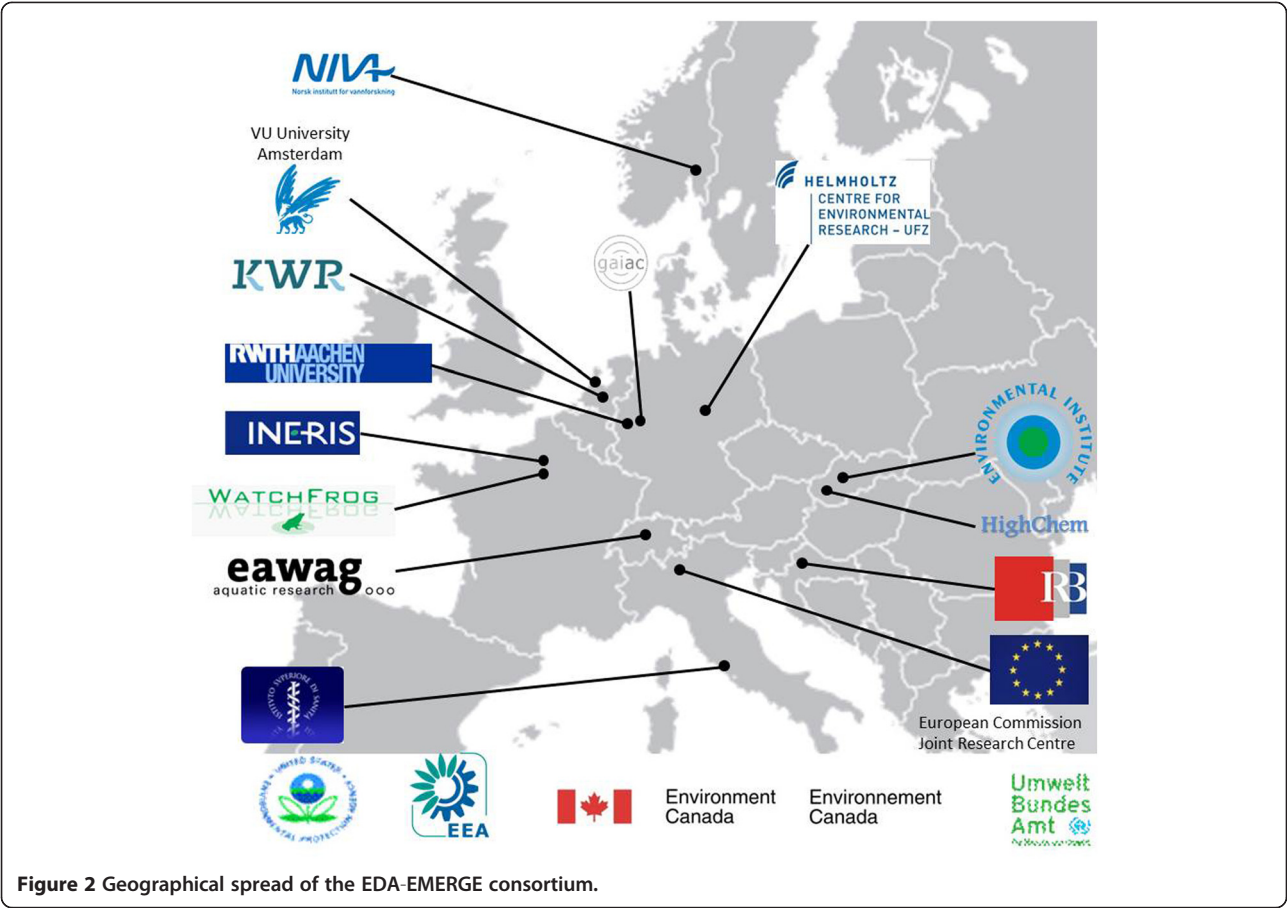
Existing EDA approaches are limited by the lack of knowledge and availability of toxicological endpoints to

direct toxicity isolation and identification. This limitation is addressed by EDA-EMERGE with the investigation of toxicogenomic tools to detect and unravel toxicants with unknown receptors and modes of action. The new approaches include DNA microarray fingerprints in algae [21,22], a proteomic approach in zebrafish embryos previously established in adult fish tissue [23] to direct EDA, and a metabolomics EDA approach using invertebrate and fish cell lines *in vitro*.

Together with the detection of adverse effects, the identification and structure elucidation of components of toxic fractions are a bottleneck in EDA, particularly of polar compounds requiring LC-MS analysis. Tool development focuses particularly on LC-HRMS methods that have successfully been applied by EDA-EMERGE partners for the identification and quantification of glucocorticoid compounds [24], drugs [25], and other polar micropollutants [26]. EDA-EMERGE combines the analytical power of HRMS with computer tools to predict fragmentation, chromatographic retention, and stability [27,28]. Mass spectra of newly identified compounds will feed the community-driven, open-access, accurate mass spectral database MassBank [29] maintained by NORMAN. Significant enhancement of the throughput of EDA towards more routine application will be achieved by automated approaches to directly link fractionation, biotesting and identification [30].

European demonstration program

Applying a simplified EDA protocol featuring a novel on-site large-volume solid phase extraction apparatus with limited fractionation, a selected set of bioassays and chemical screening analysis will be used to characterize about 20 sites within European river basins. This approach combines sites of interest according to sources of

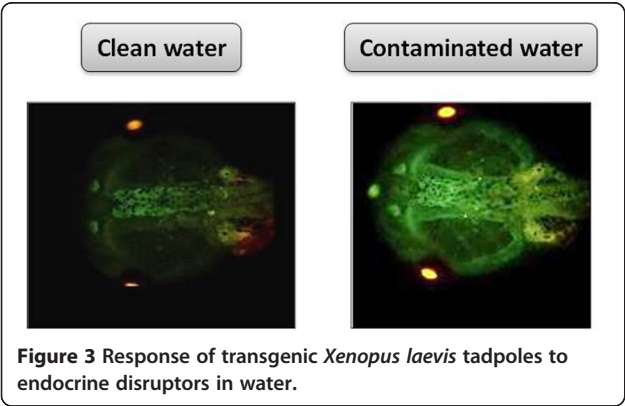


contamination such as municipal wastewater, treated hospital effluents, industrial sites affecting water quality, untreated wastewater entering the river, and reference sites with European sampling stations downstream of impacted headwaters. The European Demonstration Program will allow a comparison of classical monitoring approaches with the new tools and will train the PhD fellows in organizing and running common sampling campaigns on a European scale.

Training program

In line with the major objective of equipping young interdisciplinary researchers with the skills to address the challenges of complex contamination and its effects on aquatic ecosystems and to support WFD implementation, an extensive training program was set up and was enthusiastically followed by the PhD fellows. Although having a primary focus on EDA-EMERGE PhD students, the courses are open to external participants within the attendant maximum capacity, and this opportunity has been intensively utilized by PhD fellows from across Europe.

The formal training program kicked off with a week-long summer school (25 to 29 June 2012). The PhD fellows were lectured by leading scientists within the EDA-EMERGE consortium and beyond on the European regulatory framework, EDA, sampling and sample preparation, separation and fractionation, biodiagnostic tools, and chemical analysis. Additionally, there was also a PC-based workshop on structure elucidation along with virtual case studies. The scientific program was supplemented with a half day excursion to Wechselburg in Saxony where fellows and lecturers learnt about the river Mulde, a previously heavily



contaminated river in a now scenic, tourist-friendly setting, which is slowly recovering (Figure 4). This also provided an informal and relaxed atmosphere for intensive and fruitful networking that significantly promoted EDA-EMERGE progress.

The summer school was followed by several specialized training courses including a course on advanced LC-MS and multivariate statistics at the UFZ, Leipzig, Germany, a course on *in vitro* and *in vivo* assays of endocrine disrupting chemicals using fish models at INERIS, Verneuil-en-Halatte, France, and a course focusing on chemical screening, prioritization of environmental pollutants and data storage in European databases in the Jasna Valley, Slovakia. In total, ten specialized courses complemented by local scientific and soft skill training courses provide a comprehensive supplement to the scientific work in a traditional PhD thesis. An additional component of the training program involves 1- to 3-month secondments (at least one secondment per fellow) hosted by EDA-EMERGE partner institutes to work on aspects of the PhD research where the host institute possesses specialized expertise in analytical techniques or data analysis not typically associated with the home institute. The advantages of the secondment initiative is that, in addition to the specialized skills and expert training acquired, the fellows also broaden their professional network and obtain a different research or working perspective.

Discussion

Within the context of EDA-EMERGE research goals, a number of scientific work packages are being implemented such as developing novel bio-analytical tools, novel chemical tools, hyphenated tools, a higher-tier EDA study, establishing a simplified protocol, and conducting,

monitoring, and sampling within the European Demonstration Programme (EDP). Bio-analytical methods being developed include mechanism-specific screening tools in early life stages of zebrafish, novel fish-based biological tools, and fluorescing vertebrate models for EDA of endocrine disrupting chemicals. DNA microarray fingerprints, a proteomics and a metabolomics approach to direct EDA, are also in progress, linking multiple contaminations with multiple toxicological end points. A particular emphasis is given to toxicogenomic tools including proteomics and metabolomics to achieve unbiased effect detection. These innovative biological tools are supplemented with sophisticated chemical tools for fractionation and structure elucidation. Extensive separation and characterization of contaminants are achieved with novel fractionation tools. Comprehensive multidimensional GC/MS is used for both identification and quantitative screening. Since the identification of polar toxicants is still one of the big bottlenecks of EDA; a specific focus is given to structure elucidation techniques using LC-MS/MS. This will be achieved by developing integrated analytical and computer tools for structure elucidation of polar compounds, including the development of new approaches for retention and fragmentation prediction, diagnostic derivatization techniques, as well as metabolite prediction and identification in treatment processes in the water cycle. Innovative automated high-throughput EDA approaches are in development that directly link fractionation, biotesting, and identification. This includes two-dimensional microfractionation approaches prior to effect detection and chemical analysis.

Beyond these method developments within the individual PhD projects, there are also integrated activities in the scientific work packages focused on a simplified protocol that will be applied in the EDP. This will provide the PhD fellows with experience in planning and implementing large, collaborative, EU-wide sampling campaigns. Standard operational procedures are in development for all steps of the simplified protocol including an integrated large-volume fractionated *in situ* solid phase extraction, a large set of bioassays, and chemical screening analysis for about 300 potentially relevant compounds. The EDP is being performed by PhD pairs (biology and analytical chemistry) from different partner institutions with complementary expertise. Each pair selected a river basin as well as relevant sampling sites representing specific pollution situations and sources and typically including a reference site as well as one integrative sampling site related to monitoring (e.g., the Watch List Study sampling sites).

The fellows have been trained in all relevant techniques such as chemical structure elucidation, chemometrics, sampling/extraction, biotesting, and chemical analysis. The training courses and personalized career development



Figure 4 EDA-EMERGE fellows and lecturers. They were in front of the viaduct in Göhren, Germany bridging the valley of the Zwickauer Mulde River. Completed in 1871 and still in operation, it is one of the most impressive bridges in Germany demonstrating early railway architecture.

plans were designed after a baseline study was performed using an initial training needs analysis survey sent to all EDA-EMERGE fellows at the outset of the project. These specialized technical and scientific training courses together with secondments at partner institutes were supplemented by soft-skill courses such as presentation skills and a film/media course in conjunction with standard local courses such as scientific writing, language skills, and time management offered at most host institutes. According to a basic EDA-EMERGE dissemination plan, ESR fellows have been encouraged from the outset to disseminate their research output via online open-source forums, conferences, and journal publications.

Conclusions

In response to the call to train a new generation of young scientists in the interdisciplinary skills related to EDA and the management of European water sources, EDA-EMERGE provides a highly systematic and comprehensive research and training program within a consortium that brings together key competences from universities, research centers, private companies, and regulatory authorities. A balanced mix of 14 fellows from biology and analytical chemistry backgrounds were linked by research work packages focused on novel analytical tool development. Applications in a simplified protocol and an EDP provided an ideal basis for specialized training and structured public dissemination of the results. Networking is achieved via a range of activities from opening training course participation to external fellows and external experts, a summer school, conference presentations, dissemination and outreach activities as Marie Curie ambassadors, and interaction with other networks to secondments with EDA-EMERGE partners and associated partners.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

WB was responsible for the general design of the project and wrote the first draft of the manuscript. The other authors - SG, TS, MK, MH, MM, JH, KS, JS, AH, JS, ZR, SAA, MS, MC, ML, PL, ST, XO, MS, KT, ACdA, JF, MHW, MA, SK, HH, TBS, CDP, AT, and PS - contributed with specific information concerning their respective methods. All authors helped revise the draft of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The EDA-EMERGE project is supported by the EU Seventh Framework Programme (FP7-PEOPLE-2011-ITN) under the grant agreement number 290100.

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Received: 9 July 2013 Accepted: 10 July 2013

Published: 17 July 2013

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doi:10.1186/2190-4715-25-18

Cite this article as: Brack et al.: EDA-EMERGE: an FP7 initial training network to equip the next generation of young scientists with the skills to address the complexity of environmental contamination with emerging pollutants. *Environmental Sciences Europe* 2013 **25**:18.

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