Environment International 119 (2018) 275–286

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

# (Eco)toxicological maps: A new risk assessment method integrating traditional and *in silico* tools and its application in the Ledra River (Italy)

Giuseppa Raitano[a,*], Daniele Goi[b], Valentina Pieri[c], Alice Passoni[a], Michele Mattiussi[d], Anna Lutman[d], Isabella Romeo[a], Alberto Manganaro[a], Marco Marzo[a], Nicola Porta[a], Diego Baderna[a], Andrea Colombo[a], Eleonora Aneggi[e], Fabrizio Natolino[a], Marco Lodi[a], Renzo Bagnati[a], Emilio Benfenati[a]

[a] *Department of Environmental Health Sciences, IRCCS – Istituto di Ricerche Farmacologiche Mario Negri, Italy*
[b] *Polytechnic Department of Engineering and Architecture, University of Udine, Italy*
[c] *Department of Chemical Sciences, Life Sciences and Environmental Sustainability, University of Parma, Italy*
[d] *ARPA FVG, Italy*
[e] *Department of Chemistry, Physics and Environment, University of Udine, Italy*

A R T I C L E  I N F O

Handling editor: Yong Guan Zhu

*Keywords:*
(Q)SAR
Environmental risk assessment
CECs
Integrating strategy
Wastewater treatment plant
Human risk assessment

A B S T R A C T

Contaminants giving rise to emerging concern like pharmaceuticals, personal care products, pesticides and Endocrine Disrupting Chemicals (EDCs) have been detected in wastewaters, as reported in the literature, but little is known about their (eco)toxicological effects and consequent human health impact. The present study aimed at overcoming this lack of information through the use of *in silico* methods integrated with traditional toxicological risk analysis. This is part of a pilot project involving the management of wastewater treatment plants in the Ledra River basin (Italy). We obtained data to work up a global risk assessment method combining the evaluations of health risks to humans and ecological receptors from chemical contaminants found in this specific area. The (eco)toxicological risk is expressed by a single numerical value, permitting the comparison of different sampling sites and the evaluation of future environmental and technical interventions.

## 1. Introduction

The Directive, 2013/39/EU, which constitutes a framework for EU action on water, confirms the general principles of precaution and prevention set out in the previous Directive issued in 2000 (Directive, 2000). It also establishes the need to expand the list of priority substances to those contaminants that have given rise to new environmental concerns, due to their growing and uncontrolled use. Article 8b (1) of Directive, 2008/105/EC already planned the drafting of a list of those substances or groups of substances for which EU-wide monitoring data were to be gathered for the purpose of supporting future prioritisation exercises. The European Commission made this list available in the implementing decision (EU) 2015/495 of 20 March 2015 (Commission Implementing Decision (EU), 2015).

Wastewaters related to urbanisation, industry and farming alter the composition of the natural resources, introducing contaminants with harmful effects on the ecosystem and also on human health. Wastewater treatment plants (WWTPs) are widely used to convert wastewater into an effluent that can be returned to the water cycle with minimal pollutant impact. In order to avoid environmental pollution by contaminants of emerging concern (CECs), WWTPs need to be constantly monitored and upgraded.

CECs, like pharmaceuticals, personal care products and Endocrine Disrupting Compounds (EDCs) such as estrogens and androgens, have been detected in wastewater (Ribeiro et al., 2015; Cleuvers, 2003; Bendz et al., 2005; Kim et al., 2012; Isidori et al., 2005; Pawlowski et al., 2004; Ferrari et al., 2003; Zhang et al., 2008; Winter et al., 2008; Watts et al., n.d.; Segura et al., 2009; Chang et al., 2011; Bellet et al., 2011) but little is known about their ecotoxicological effects. The current lack of knowledge particularly regards chronic effects, which were rarely investigated (Winter et al., 2008; Fent et al., 2006). Indeed, most conventional urban WWTPs are not specifically designed to remove residual concentrations of organic compounds (Ribeiro et al., 2015) like CECs. Therefore we need much more detailed knowledge about these pollutants and their effects in order to plan targeted interventions and improve the performance of today's tools.

The present work provides a scheme for the overall assessment of the (eco)toxicological and environmental properties of water bodies

* Corresponding author.
*E-mail address:* giuseppa.raitano@marionegri.it (G. Raitano).

https://doi.org/10.1016/j.envint.2018.06.035
Received 20 April 2018; Received in revised form 21 June 2018; Accepted 26 June 2018

aiming to a new combination of water resources management and conservation of the environment and health within consistent integrated water cycles. The project draws on the toxicological characteristics of the Ledra River (Italy) which has modest environmental impact, in order to find a robust method for evaluating residual pollution. The evaluation is based on a single (eco)toxicological index combining the detection of multiple contaminants characterized by different environmental behaviours, (eco)toxicological properties and concentrations. The numerous factors involved mean this is a complex question. However, this approach, once optimized on this river, can then be applied to other water bodies.

We employed the Environmental Risk Index for Chemicals Assessment (ERICA) which is an integrated strategy combining different contributions to the impact of a mixture of contaminants, and based on several risk assessment indices (Boriani et al., 2010; Boriani et al., 2013). ERICA results from the application of these independent indices which are merged. ERICA, in fact, integrates ecotoxicological risk evaluation, human risk assessment and environmental fate and transport into a single value using a dedicated scoring system. Scoring system is based on pollutants characteristics such as the different physico-chemical properties, the Persistent, Bioaccumulative and Toxic (PBT) classification and the toxicological profiles. All these information, coupled with the uncertainty due to missing or uncertain data and risk exceedances, contributes to the definition of the condensed value.

The (eco)toxicological index translates into toxicity the mass flow emitted by a source and is integrated with an environmental risk index to assess the general state of environmental health in an area, reflecting the impact of pollutants on different environmental compartments (soil, water, sediment and air). The ERICA is founded on the risk analysis for ecological and human receptors, as illustrated in international guidelines (European Communities, 2003; Goverment of Canada, n.d.).

In this study we optimized and applied ERICA index focussing on the toxicological and ecological risk indices, assuming a static environmental fate of pollutants only in the water compartment. The optimization involved the development and the use of robust *in silico* methods, to be applied when experimental data were missing. We utilized several freely available models, specific for ecological and human evaluation, and developed some new *ad hoc* models. These tools are intended to fill data gaps through an approach that reduces time and costs. Their use should be fostered in many contexts (European Community, n.d.; https://www.efsa.europa.eu/it/efsajournal/pub/3290; Benigni, n.d.; Mombelli and Ringeissen, 2009; Fjodorova et al., 2008; https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf/e4a2a18f-a2bd-4a04-ac6d-0ea425b2567f; https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9; OECD, n.d.).

The integrated index of toxicological risk related to the water quality (IRW), achieved by this new global risk assessment strategy, describes the general state of health of the Ledra River, depicted by the (eco)toxicological maps.

## 2. Materials and methods

The proposed approach for the integrated toxicological risk of waters from the investigated site includes 7 steps (Fig. 1):

- Water samplings;
- Chemical characterization of pollutants and identification of CECs;
- Hazard evaluation of CECs using literature data and predicted values from existing and new *in silico* models;
- Human and Ecological risk assessment;
- Internal scoring method application;
- Global risk assessment (IRW calculation);
- (Eco)toxicological maps.

### 2.1. Sampling sites

The study area covers the whole Ledra River basin (21 km long), located in the Friuli Venezia Giulia region (Italy). There are numerous WWTPs in this area. Most of them were built during the reconstruction of the Friuli Venezia Giulia region following the 1976 earthquake (Pieri et al., 2012). We selected 15 sampling stations, up- and downstream from the wastewater discharge points (Table 1). Water samples (1 L) were collected in May 2015.

### 2.2. Analytical methods

More than 130 CECs in water samples were monitored by high-performance liquid chromatography-mass speNctrometry (HPLC-MS) analyses. Two validated methods were used for the analysis of pharmaceuticals (Riva et al., 2015; Castiglioni et al., 2005) and illicit drugs (Castiglioni et al., 2006). An API3000 triple quadrupole mass spectrometer (Applied Biosystems – Sciex, Thornhill, Ont., Canada) interfaced to a Series 200 HPLC system (Perkin Elmer) was used for both analyses. Another validated method was used for the quantitation of hormones and phytosanitary compounds (https://ec.europa.eu/jrc/en/news/first-watch-list-emerging-water-pollutants). A method developed by ARPA FVG was used for the analysis of pesticides and additional phytosanitary compounds. A Sciex Q-Trap 6500 mass spectrometer (Ramingham, MA, USA), interfaced with a Shimadzu Nexera2 HPLC system was used for these analyses. For chromatographic separation we used an A C18 CoreShell column (100 × 2.1 mm, 2.7 μm, Supelco Ascentis Express) with two mobile phases: formate/formic acid 0.0033 M (pH 3.7) buffer (MP-A) and acetonitrile (MP-B). The elution gradient was the following: 25% to 50% of MP-B in 2 min; to 95% of MP-B in 3.2 min, hold at 95% for 0.2 min and re-equilibration for 2.8 min at 25% of MP-B. All mass spectrometric analyses were done in multiple reaction monitoring (MRM) mode, measuring the fragmentation products of protonated or deprotonated pseudo-molecular ions of each analyte. Mass spectrometric parameters for the latter method, including source parameters and selected transitions for each analyte are presented in the supplementary material, Table S1.

### 2.3. Experimental property values

For the ecotoxicological characterization of the quantified pollutants we used these free tools: the ECOTOX database (http://cfpub.epa.gov/ecotox/), QSAR Toolbox platform (https://www.qsartoolbox.org/) and Pesticide Properties Data Bases. (PPDB-http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/310.htm). Experimental data were also taken from the literature (Kim et al., 2012; https://www.astrazeneca.com/content/dam/az/our-company/Sustainability/Atenolol.pdf; Czech et al., 2014; Fenet et al., 2012; Kim et al., 2007) and selected on the basis of their compliance with official OECD guidelines (OECD, 2011; OECD, 2004; OECD, 1992; OECD, 2013; OECD, 2012). In the case of multiple data, the lowest value was chosen, according to a conservative approach.

The acceptable daily intake (ADI), chronic oral reference dose (RfD) (US EPA, 1993) for non-cancer health assessment and the slope factor (SF) for cancer evaluation came from several databases, guidelines on Human Risk Assessment (Watts et al., n.d.; Australian Guidelines for Water Recycling, 2008; *Toxicological Relevance of EDCs and Pharmaceuticals in Drinking Water*, 2008) and literature (Snyder et al., 2008; Ho et al., 2013). The main sources were the Integrated Risk Information System (IRIS) database (https://www.epa.gov/iris) from the United States Environmental Protection Agency (EPA), the Risk Assessment Information System (RAIS, https://rais.ornl.gov/cgi-bin/tools/TOX_search?select=chem), and the ISS-INAIL (Istituto Superiore di Sanità and Istituto Nazionale per la Assicurazione contro gli Infortuni sul Lavoro) database available on http://www.iss.it/iasa/index.php?lang=1&tipo=40.