



Direct toxicity assessment – Methods, evaluation, interpretation



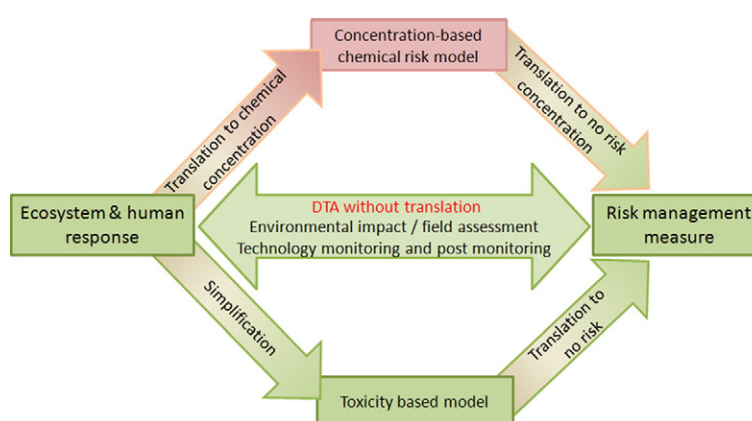
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HIGHLIGHTS

- DTA characterizes environmentally relevant aggregated effects of unknown contaminants
- DTA measures adverse effects without translating the results into concentration
- DTA results may differ from chemically determined hazard information
- The 'no effect sample proportion' is in direct relation with the necessary risk reduction rate
- The equivalency method converts toxicity into reference substance concentration

GRAPHICAL ABSTRACT



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ABSTRACT

Direct toxicity assessment (DTA) results provide the scale of the actual adverse effect of contaminated environmental samples. DTA results are used in environmental risk management of contaminated water, soil and waste, without explicitly translating the results into chemical concentration. The end points are the same as in environmental toxicology in general, i.e. inhibition rate, decrease in the growth rate or in yield and the 'no effect' or the 'lowest effect' measurement points of the sample dilution–response curve. The measurement unit cannot be a concentration, since the contaminants and their content in the sample is unknown. Thus toxicity is expressed as the sample proportion causing a certain scale of inhibition or no inhibition. Another option for characterizing the scale of toxicity of an environmental sample is equivalencing. Toxicity equivalencing represents an interpretation tool which enables toxicity of unknown mixtures of chemicals be converted into the concentration of an equivalently toxic reference substance. Toxicity equivalencing, (i.e. expressing the toxicity of unknown contaminants as the concentration of the reference) makes DTA results better understandable for non-ecotoxicologists and other professionals educated and thinking based on the chemical model.

This paper describes and discusses the role, the principles, the methodology and the interpretation of direct toxicity assessment (DTA) with the aim to contribute to the understanding of the necessity to integrate DTA results into environmental management of contaminated soil and water. The paper also introduces the benefits of the toxicity equivalency method.

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The use of DTA is illustrated through two case studies. The first case study focuses on DTA of treated wastewater with the aim to characterize the treatment efficacy of a biological wastewater treatment plant by frequent bioassaying. The second case study applied DTA to investigate the cover layers of two bauxite residue (red mud) reservoirs. Based on the DTA results the necessary toxicity attenuation rate of the cover layers was estimated.

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1. Introduction

Environmental toxicology is the science of the measurement of the adverse effects of chemicals on individual organisms or complex communities. The probable damage of chemicals to the natural or built environment can be forecasted based on the type and extent of the adverse effects and the chemicals' fate and behavior. The results of environmental toxicology are used to predict environmental hazard and risk posed by certain chemical substances. The hazard of chemical substances derives from their structure, their intrinsic physicochemical, biological and environmental fate properties, but foremost their potential to adversely affect living organisms. The actual impact is based on their interaction with the environment (Gruiz, 2015).

Environmental pollutants are typically complex mixtures of chemically and toxicologically different chemical forms and species, showing variable bioavailability and interactions with the biotic and abiotic compartments of the environment. Direct toxicity assessment (DTA) can characterize the aggregated effects of unknown contaminants in environmental samples.

DTA results directly characterize the health of the habitat of aquatic and terrestrial ecosystems, as well as the efficacy of environmental services, mainly the supporting (nutrient supply and primary production) and regulating services (degradation based processes, such as water purification, element cycling, waste and contaminants decomposition, etc.). DTA detects adverse effects and specifies the necessary scale of their reduction to reach an acceptable environmental quality. Thus the results of DTA are in direct relation to risk, so decision making and risk management can be based on them (Dam and Chapman, 2001; Tinsley et al., 2004).

The main disadvantage of DTA is that the measured toxicity cannot be expressed in concentration, thus it does not fit into the chemical model based risk assessment (RA), and it cannot apply the regulatory screening concentrations.

The basics of soil DTA has been laid down by Torstensson (1993) and Torslov et al. (1997). The first test methods developed for ecotoxicological purposes were based on soil activities, employing biological methods conventionally used in agricultural practice (respiration, enzyme activities, etc.). These biotests can be run as toxicity tests considering living soil as a 'test organism'. The other part of the methodologies includes 'real' environmental toxicity tests i.e. placing the test organism into the medium to be tested. Common test types are bioassays, single species and multispecies aquatic and terrestrial tests, as well as micro- and mesocosms for measuring community level responses (Fernández et al., 2005; Fernandez and Tarazona, 2008; Gruiz et al., 2015b). The newest trends represent/cover the application of contaminant-specific or effect-specific molecular methods (omics based technologies) and the properly selected bioindicators representing species, populations, communities or whole ecosystems (Gruiz et al., 2016, in press).

The acknowledgement and inclusion of DTA into national policies has started in the 90's in several countries with the aim to improve surface water quality by controlling waste water discharge. Such policies entered into force in the US (US WET Policy, 1995; US WET, 2002), in Australia and New Zealand (Dam and Chapman, 2001; ANZECC/ARMCANZ, 2000) or in the UK. UK National Rivers Authority prepared a DTA protocol for discharge control already in 1989 (Hunt et al., 1989). New Zealand laid down standard methods for whole effluent toxicity testing in 1998 (Hall and Golding, 1998), the UK in 2000 (UK DTA, 2000a and , 2000b)

and the Environment Agency (EA) harmonized its DTA protocol with the European IPPC in 2006 (DTA Protocol, 2006). Regarding the test methodologies EA accepted three aquatic test methods for the assessment of whole effluent toxicity to oyster embryo development (Oyster test for DTA, 2007) and to growth inhibition of two algal strains (Algal Test for DTA, 2007 & , 2008). The Monitoring Certification Scheme for Direct Toxicity Assessment was issued by EA in 2010 (MCERTS, 2010) where DTA is recommended for (i) effluent screening and characterization; (ii) monitoring effluent toxicity against a toxicity limit; (iii) assessing the impact of point source discharges on receiving waters; (iv) providing a general quality assessment of receiving waters (for example within monitoring programs). Canada (2012) in its policy for toxicity assessment and control recommends defined toxicity based screening levels.

The policy inclusion of DTA was preceded by intensive research and discussion as well as by several practical applications. The book of the SETAC Workshop on Whole Effluent Toxicity (Grothe et al., 1995) has contributed greatly to the progress. After this time several papers were published on the test procedures (e.g. Luckenbach et al., 2001; Moore et al., 2000a), on application examples (Chapman, 2000; De Vlaming et al., 2000; Tinsley et al., 2004; Wharfe and Tinsley, 2004) and on the variabilities (Moore et al., 2000b; Warren-Hicks et al., 2000; Markle et al., 2000). The ongoing research and application resulted several new ideas and test methods for rapid, in situ DTA of waters and wastewaters. These methodological developments are partly the modifications of conventional laboratory bioassays, such as the rapid Microtox with lyophilized test-organisms (Weltens et al., 2014), rapid bioassays based on algal or other microbiological responses such as photosynthesis, respiration, energy production, enzyme activities with colorimetric or other easy to measure end points (Baran and Tarnawski, 2013).

In addition to these generic toxicity measuring methods more and more contaminant-specific detection methods have appeared based on biomolecules (DNA, RNA, enzymes and immune-molecules) or genetically manipulated organisms carrying substance specific promoters (regulator genes) and reporter genes (built in genes producing the easy to measure signal) (Köhler et al., 2000).

DTA has especially high importance in the risk management of contaminated soils, given that the actual effects and risks in soils can hardly be extrapolated from the toxicity of pure chemicals (Fernández et al., 2005). Several practical trials and environmental applications came to light from the 90's, naming the DTA-based soil tests as contact tests, solid-phase tests, direct contact biotests, interactive bioassays, etc. (Kwan, 1993; Campbell et al., 1997; Chapman, 2000; Gruiz, 2005).

While regulatory assessment of potential environmental contaminants uses hazard information for generic environmental predictions, the management of contaminated land starts with the assessment of an already contaminated actual site, the actual toxicity, i.e. the source of further transport and risk (Toxicity Based Criteria, 1996; Dam and Chapman, 2001). Fig. 1 illustrates the various concepts applied in environmental management and shows the relation of DTA to other risk management models and tools (Gruiz et al., 2015a):

- The chemical model compares the measured concentration to default screening concentrations for each contaminant — one by one.
- The biological model uses tests or studies for measuring the effective concentrations of pure chemicals. Living organisms and known contaminants are placed into artificial or real water or soil — effect assessment or simulation tests.

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