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Dioxins and dioxin-like compounds in composts and digestates from European countries as determined by the *in vitro* bioassay and chemical analysis

Martin Beníšek [a], Petr Kukučka [a], Giulio Mariani [b], Gert Suurkuusk [b], Bernd M. Gawlik [b], Giovanni Locoro [b], John P. Giesy [c,d,e,f], Luděk Bláha [a,*]

[a] Masaryk University, Faculty of Science, RECETOX, Brno, Czech Republic
[b] European Commission, DG Joint Research Centre (JRC), Institute for Environment and Sustainability, Unit H.01-Water Resources Unit, Ispra, Italy
[c] Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, SK, Canada
[d] Department of Biology & Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong Special Administrative Region
[e] School of Biological Sciences, University of Hong Kong, Hong Kong Special Administrative Region
[f] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China

HIGHLIGHTS

- Pan-European study of dioxins and dioxin-like compounds in composts and digestates.
- PCDD/Fs, PCBs, OCPs, PAHs and dioxin effects compared in various types of samples.
- Compliance with conservative limits confirmed for most of the samples.
- High added value of the biodetection tools and effect-based monitoring demonstrated.

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ABSTRACT

Aerobic composting and anaerobic digestion plays an important role in reduction of organic waste by transforming the waste into humus, which is an excellent soil conditioner. However, applications of chemical-contaminated composts on soils may have unwanted consequences such as accumulation of persistent compounds and their transfer into food chains. The present study investigated burden of composts and digestates collected in 16 European countries (88 samples) by the compounds causing dioxin-like effects as determined by use of an *in vitro* transactivation assay to quantify total concentrations of aryl hydrocarbon receptor-(AhR) mediated potency. Measured concentrations of 2,3,7,8-Tetrachlorodibeno-*p*-dioxin (2,3,7,8-TCDD) equivalents (TEQ_{bio}) were compared to concentrations of polycyclic aromatic hydrocarbons (PAHs) and selected chlorinated compounds, including polychlorinated dibenzo-*p*-dioxins/furans (PCDD/Fs), co-planar polychlorinated biphenyls (PCBs), indicator PCB congeners and organochlorine pesticides (OCPs). Median concentrations of TEQ_{bio} (dioxin-like compounds) determined by the *in vitro* assay in crude extracts of various types of composts ranged from 0.05 to 1.2 with a maximum 8.22 μg (TEQ_{bio}) kg^{-1} dry mass. Potencies were mostly associated with less persistent compounds such as PAHs because treatment with sulfuric acid removed bioactivity from most samples. The pan-European investigation of contamination by organic contaminants showed generally good quality of the composts, the majority of which were in compliance with conservative limits applied in some countries. Results demonstrate performance and added value of rapid, inexpensive, effect-based monitoring, and points out the need to derive corresponding effect-based trigger values for the risk assessment of complex contaminated matrices such as composts.

* Corresponding author.

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1. Introduction

Composting (aerobic process) and digestion (anaerobic process) can be useful for reduction of various wastes, because these processes can transform organic waste to humus, which is an excellent soil conditioner (Grossi et al., 1998). On the other hand, the presence of or formation of persistent toxicants in composts or digestates is an issue that might limit their widespread use. Use of compost and digestate as amendments to improve fertility increase organic matter, reduce erosion, and improve physical chemical properties of soils such as retention of water (Semple, 2001; Pedra et al., 2007). There are several requirements placed on quality of compost that need to be met to protect the environment from adverse effects of both inorganic and organic contaminants. The most important parameters include minimum content of metals and organic chemicals at toxic concentrations and the absence of pathogens that pose risks to health of humans (Lasaridi et al., 2006). Composts and digestates can be contaminated by various chemicals that can cause adverse effects on humans and wildlife. While heavy metals in composts are relatively well studied and controlled, there is still limited knowledge about the content, fate and effects of organic pollutants. The focus of this study was persistent organic pollutants (POPs), including organochlorine insecticides (OCs), constituents of personal care products, industrial chemicals, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinateddibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (Brändli et al., 2007a,b; Grossi et al., 1998; Hseu, 2004). Some non-persistent organic pollutants, such as lesser molecular mass PAHs, can be degraded during composting (Brändli et al., 2007b), but other contaminants such as PCBs or PCDDs/Fs could accumulate in soil when contaminated compost or digestate is applied repeatedly (Umlauf et al., 2011). In several European countries some limit values for the content of organic pollutants such as PCDDs/Fs, PCBs, PAHs and others have been established (Saveyn and Eder, 2014; WRAP, 2002).

Various toxic effects have been associated with the above mentioned organic pollutants. Probably the most important and most widely studied is activation of the arylhydrocarbon receptor (AhR) which results in dioxin-like toxicity (Sorg, 2013). Toxic potential of dioxin-active compounds is often calculated from the results of chemical analyses using the Toxic Equivalency, TEQ, approach (I-TEQ or WHO-TEQ) (Lee et al., 2013). TEQs, to calculate equivalents of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, (TCDD) (usually expressed in ng of TCDD per kg material) are calculated as the sum of the product of concentrations of individual AhR-active compounds multiplied by their corresponding toxic equivalency factors (TEFs), or relative response factors (Lee et al., 2013; Machala et al., 2001). In addition, TEQ_{bio} can be estimated by use of bioanalytical tools such as ethoxyresorufin *O*-deethylase (EROD) assay (Joung et al., 2007) or transactivation, reporter gene bioassays, such as H4IIE-*luc* cells (Giesy et al., 2002), DR-CALUX (Murk et al., 1996) and others where expression of the reporter gene, luciferase, is up-regulated by exposure to agonists of the AhR.

Both chemical and biological methods used for the analyses of dioxin-active compounds have their specific advantages and disadvantages. Chemical analysis allows assessment of only a limited number of compounds such as the 17 US EPA PCDDs/Fs or 16 US EPA PAHs, while bioanalytical methods measure the integrated potency of all AhR-active compounds present in the sample including also e.g. brominated derivatives (Samara et al., 2009) or various PAH metabolites and analogs (Sovadinova et al., 2006). Chemical analysis can also be costly, especially when considering analyses of PCDD/Fs and co-planar PCBs (Joung et al., 2007). The concept of chemical TEQs is based on additive effects of toxic compounds but these compounds present in complex samples might elicit infra- or supra-additive interactions (Suzuki et al., 2006), which can be detected by the use of biodetection tools. Bioanalytical detection systems can also be more sensitive because they are responding to the complex of chemicals in aggregate instead of each chemical individually. Alternatively, limitations of bioanalytical tools include greater variability which is natural to biological testing systems, a lesser degree of standardization and generally lesser acceptance by regulatory authorities. Nevertheless, the effect-based monitoring is becoming widely applied (Escher et al., 2013; Hecker and Giesy, 2011) and certain biodetection tools have already been standardized and suggested for practical use, including the assessment of endocrine disruptive (estrogenic) (ISO, 2014) or dioxin-like compounds (Hecker and Giesy, 2011).

Concentrations of persistent organic pollutants and PAHs in composts determined by chemical analyses have been reported previously (Grossi et al., 1998; Brändli et al., 2007a,b) and some studies also investigated the dioxin-like activity using the biodetection tools such as H4IIE-*luc* cells (Takigami et al., 2010; Suzuki et al., 2006). However, detailed comparisons of the chemical- and effect-based monitoring of AhR active compounds using the broader set of compost samples are few. In previous studies some PAHs such as benzo[k]fluoranthene, dibenz[a,h]anthracene or benzo[a]pyrene detected in composts (Brändli et al., 2007a) were shown to be potent inducers of AhR *in vitro* (Machala et al., 2001). Also some other PAHs, which may have lesser dioxin-like potentials (IEF – Induction Equivalency Factor) but great abundance in composts (such as fluoranthene and others) could significantly contribute to the dioxin-like effects of the whole sample (Lee et al., 2013). Available data on concentrations of persistent organic pollutants indicate that composts contain congeners with lesser TEFs or IEFs such as octachlorodibenzo-*p*-dioxin or heptachlorodibenzo-*p*-dioxin (OCDD or HpCDD) (Takigami et al., 2010; Muñoz et al., 2013). In addition to PCDDs/Fs and dioxin-like PCBs, other chlorinated compounds could be found in composts including organochlorinated pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), pentachlorobenzene (PeCB) and others (Brändli et al., 2004). Although toxicity of these compounds is not primarily mediated via the AhR, some have been shown to act as weak AhR agonists and could also contribute to the effect of the whole mixture (Mrema et al., 2013). To our knowledge, concentrations of other potential AhR-acting dioxin-like compounds (such as polybrominated diphenyl ethers, PBDEs) have only rarely been investigated in compost or digestates. In summary, the available data indicate that PAHs may be the dominant compounds contributing to the dioxin-like effects of the complex compost samples but the role of different AhR-active compounds has not been studied in detail.

The main objective of the present study was to investigate concentrations of a range of compounds causing dioxin-like toxicity in composts and digestates collected throughout 16 countries in Europe. The present study compared the concentrations of PAHs and diverse chlorinated compounds (indicator PCBs, OCPs, PCDD/Fs and co-planar PCBs) with dioxin-like effects observed *in vitro*.

2. Materials and methods

2.1. Design of the study

The present study investigated various categories of composts such as organic waste from households, green compost from gardens and parks, sewage sludge compost and also some composts and digestates after Mechanical Biological Treatment. Screening