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The use of effect-based methods in determining contaminants of emerging concern in the soil-water system

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The use of effect-based methods in determining contaminants of emerging concern in the soil-water system

Study into the scientific knowledge and practical use of effect-based methods in determining contaminants of emerging concern in the soil-water system

Thesis

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The most alarming of all man's assaults upon the environment is the contamination of air, earth, rivers, and sea with dangerous and even lethal materials

~ Rachel Carson ~

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Abstract

One of the challenges in current and future protection of the soil-water system is dealing with contaminants of emerging concern. Currently, environmental quality standards are missing for contaminants of emerging concern. In addition, the current analytical methods are limited because of the relatively low concentrations, the non-defined standards and the variety of the contaminants.

In this study the possilities of effect-based methods for detecting and evaluating contaminants via their response to the environment are investigated by a literature review and assessed against criteria. The practical interpretation of the application and implementation of effect-based methods in the Dutch soil policy has been reviewed by practitioners in a workshop.

The literature review revealed eight types of effect-based methods that can be applied to the soil-water system. Of these eight types, in vitro bioassays and in vivo bioassays best meet the established criteria.

Both the literature study and the workshop with practitioners showed that effect-based methods must be standardized in order to be widely used in the soil-water system. The translation of measured effects and possible risks is currently unclear. Therefore, standards must be drawn up for effect-based methods with attention to the differences in human and ecological risks.

Keywords: contaminants of emerging concern (CECs) – soil-water system – effect-based method (EBM) – in vitro bioassays – in vivo bioassays – soil policy – practitioners

Samenvatting

Een van de uitdagingen bij de huidige en toekomstige bescherming van bodem en grondwater is het omgaan met opkomende verontreinigingen. Op dit moment ontbreken milieukwaliteitsnormen voor opkomende verontreinigingen. Bovendien zijn de huidige analysemethoden beperkt omdat deze stoffen in relatief lage concentraties voorkomen, gestandaardiseerde analysemethoden ontbreken en opkomende verontreinigingen een grote diversiteit kennen.

In deze studie wordt met een literatuuronderzoek de mogelijkheden van effectmetingen onderzocht voor het detecteren en evalueren van verontreinigingen via hun reactie op het milieu en deze worden beoordeeld aan de hand van criteria. De praktische invulling van de toepassing en implementatie van effectmetingen in het Nederlandse bodembeleid is door professionals beoordeeld in een workshop.

Uit het literatuuronderzoek kwamen acht soorten effectgebaseerde methoden naar voren die kunnen worden toegepast op bodem en grondwater. Van deze acht typen voldoen in vitro bioassays en in vivo bioassays het beste aan de vastgestelde criteria.

Zowel de literatuurstudie als de workshop met praktijkmensen lieten zien dat effectmetingen gestandaardiseerd moeten worden om op brede schaal ingezet te kunnen worden in bodem en grondwater. De vertaling van gemeten effecten en mogelijke risico's is vaak nog onduidelijk. Daarom moeten er normen worden opgesteld voor gemeten effecten met aandacht voor de verschillen in menselijke en ecologische risico's.

1. Introduction

In 2015 the 193 members of the United Nations set up 17 Sustainable Development Goals (SDGs) which express the ambitions for a sustainable society in 2030 (Bebbington & Unerman, 2018). In all of the 17 Goals to achieve environmental sustainability and basic human needs, the soil plays an important role (Keesstra et al., 2016). It is not possible to protect biodiversity, mitigate climate change, preserve the quality of ecosystems and control the quality of water without paying attention to the quality of the soil (Bouma, 2014; Paleari, 2017). Therefore it is important to avoid further chemical and physical land degradation (Keesstra et al., 2018). Chemical degradation includes pollution by inorganic and organic substances. It could affect the biotic and abiotic functioning of the soil, but also the health of humans and animals (Keesstra et al., 2018). The targets in the SDGs include the reduction of the release of hazardous chemicals and materials to air, water and soil, to minimize the adverse effects, to improve the water quality and to reduce the number of deaths and illnesses (Bebbington & Unerman, 2018).

The European Union (EU) is also increasingly aware of the importance of the soil and the protection of it (Paleari, 2017). But currently, soil legislations are fragmented across different governance levels and policy domains and consist of non-strict measures (Paleari, 2017; Virto et al., 2017). The proposal of a Soil Framework Directive in 2006 was rejected after strong opposition from the Dutch government (Glæsner, Helming & de Vries, 2014; Ronchi, Salata, Arcidiacono, Piroli & Montanarella, 2019).

The Netherlands, a member state of the EU, has its regulations to counteract further degradation of the soil (Ronchi et al., 2019). Soil contamination is regulated by different instruments in different situations (SIKB, 2019). Soil investigation has to be done when 1) applying for a building or environmental permit and 2) when there is suspicion the soil is contaminated or could be contaminated by activities (SIKB, 2019). Depending on the application or use of the soil and groundwater there are different standardized packages for contaminants that have to be analysed (SIKB, NEN and Bodem+, 2008). In 2021 a new act will come into force in the Netherlands: the *Omgevingswet*. With the *Omgevingswet* the government wants to simplify and merge spatial development rules. The act stands for a good balance between using and protecting the physical living environment (Rijksoverheid, n.d.). In the new act, an area-oriented approach will be more central instead of a location or case-oriented approach (Rijksoverheid, n.d.). That could have consequences for the handling of contaminants in the soil-water system.

One of the challenges in current and future soil protection is dealing with the large number of known and unknown anthropogenic and naturally chemicals released to the environment, called emerging contaminants or contaminants of emerging concern (CECs) (Gomes et al., 2017; Gravilescu, Demnerová, Aamand, Agathos & Fava, 2015; Noguera-Oviedo & Aga, 2016). The terms emerging contaminants and contaminants of emerging concern are used synonymously in the literature (Gomes et al., 2017; Naidu & Wong, 2013). There is no strict definition of both terms (Sauvé and Desrosiers, 2014). The term emerging contaminants first appeared in Rachel Carson's book 'Silent Spring' (Carson, 1962). She showed the widespread usage of DDT had led to the death and disappearance of many birds (Carson, 1962).
Emerging may refer to the lack of information about the presence and the effects of the chemicals in various environmental compartments. Because of the lack of information about the possible risks to human health or the environment, the contaminants are of concern (Sauvé & Desrosiers, 2014; Naidu, Jit, Kennedy & Arias, 2016).
Sauvé and Desrosiers (2014) defined contaminants of emerging concern "as naturally occurring, manufactured or manmade chemicals or materials which have now been discovered or are suspected present in various environmental compartments and whose toxicity or persistence is likely to significantly alter the metabolism of a living being [...] and which are not yet subjected to regulatory criteria or norms for the protection of human health or the environment".
There are no clear criteria that determine whether a substance falls into the "CEC" category. Sauvé and Desrosiers (2014) make in their review a distinction between 'old' and 'true and really new' emerging contaminants. Gomes et al. (2017) also did and called CECs well-known chemicals with unknown risks. The groups of CECs could be roughly divided in pesticides, pharmaceuticals and personal care products, fragrances, plasticizers, hormones, flame retardants, microplastics, nanoparticles, perfluoroalkyl compounds, chlorinated paraffins, siloxanes, algal toxins and various trace elements (Gomes et al., 2017; Naidu & Wong, 2013; Sauvé & Desrosiers, 2014).

To get a better insight into all chemicals produced and imported into the European Union, the legislation Registration Evaluation and Authorisation of Chemicals (REACH) was set up in 2006 (Penman, Banton, Erler, Moore & Semmler, 2015). The legislation of REACH collects data on chemicals that are produced or imported at greater than one ton per year (Penman et al., 2015). More than 16,500 substances were registered in October 2017 (Schulze et al., 2018). REACH consists of summaries of existing toxicity studies and studies published in the literature (Ingre-Khans, Ågerstrand, Beronius & Rudén, 2019). Woutersen et al. (2018) identified the limited availability of information on low tonnage substances as an important constraint of REACH.

In the Netherlands the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) also makes use of the European regulations for chemical substances REACH (Wintersen, Otte & Traas, 2019). The RIVM has developed a system to identify chemical substances that are relevant for the Dutch policy. At this moment about 1200 substances or substance groups have been identified as 'substances of very high concern'. Dutch companies have to prevent discharges and emissions of 'substances of very high concern' to the environment. If that is not feasible, the emissions must be limited as much as possible (minimization obligation) (RIVM, 2019). The problem of CECs exists because of knowledge gaps and insufficient legislation that impedes decision-making for policy (Naidu, Jit, Kennedy & Arias, 2016; Woutersen et al. 2018). For new discovered CECs there are no standards for environmental quality assessment. For existing CECs standards may need to change due to newly discovered understanding of adverse (biological) effects (Naidu, Jit et al., 2016). Knowledge gaps around CECs are about the presence, occurrence, source, persistence, fate and transport in the environment as well as effects on humans and ecosystems (Sauvé & Desrosiers, 2014; Naidu, Espana, Liu & Jit, 2016). Naidu, Espana et al. (2016) reviewed the area of CECs and existing knowledge gaps in it. According to their review, to manage the problem of CECs the development of new methods to identify CECs is required as well as a more comprehensive environmental risk assessment (Naidu, Espana et al., 2016).

Not only the lack of information about CECs is a problem (Geissen et al., 2015; Naidu, Espana et al., 2016). Geissen et al. (2015) investigated the current state of art and the challenges for monitoring programs of CECs, fate and risk assessment tools for analysing CECs. They conclude detection, identification and quantification of CECs in different environmental compartments is highly challenging (Geissen et al., 2015).

Due to a lack of sensitive analytical methods, it is hard to detect the presence of CECs because of their relatively low levels (usually in levels of µg/L) in samples (Geissen et al., 2015; Naidu, Espana et al., 2016; Noguera-Oviedo & Aga, 2016). Noguera-Oviedo and Aga (2016) have done a review study on key research milestones in the area of CECs. According to Noguera-Oviedo and Aga the lack of analytical methods is due to the slow development of commercial instruments that are capable of detecting CECs. Moreover, the development and application of sensitive analytical methods is expensive and time-consuming (Rasheed, Bilal, Nabeel, Adeel & Iqbal, 2019; Schulze et al., 2018). The production of new chemicals continues and goes beyond the power of current safety monitoring and risk assessment methods (Gravilescu, Demnerová, Aamand, Agathos & Fava, 2015; Noguera-Oviedo & Aga, 2016). The variety of CECs asks for different analytical methods (Vanderford et al., 2014). Moreover, if a contaminant is detected, regulatory criteria or norms are often missing so nothing can be said about possible risks (Naidu et al., 2016). In addition, the existence of CECs in the environment is not static due to changes in production, use and disposal (Geissen et al., 2015).

It seems to be impossible to analyse all CECs and to make regulatory criteria or environmental quality standards for all these contaminants (Escher et al. 2018). Only a list of priority contaminants will say nothing about the adverse effects on the environment (Altenburger et al., 2015; Escher et al., 2018). Individual chemicals could be under traceable levels and a mixture of different chemicals could cause significant effects (Naidu, Espana et al., 2016; Noguera-Oviedo & Aga, 2016).

Gravilescu et al. (2015) also have done a review study on how the problems of CECs can be tackled. They conclude that it is an option to quantify the availability or measure the effects of CECs in soil and sediment for risk assessment and decision making in case of possible contamination of land (Gravilescu et al., 2015). Neale et al. (2017) emphasizes that the use of effect-based methods (EBMs) could be another way of detecting CECs. EBMs could be used as a complementary analytical tool for detecting and quantifying contaminants via their response to the environment (Neale et al., 2017). Brack et al., 2019 defines EBMs as "bioanalytical methods using the response of whole organisms (in vivo) or cellular bioassays (in vitro) to detect and quantify the effects of groups of chemicals on toxicological endpoints of concern".

In studies into CECs in surface waters, bioassays are already successfully applied for monitoring the water quality (Brack et al., 2019). Escher et al. (2018) developed a method that goes beyond existing environmental quality standards for chemicals in surface water. They have derived effect-based trigger values from existing environmental quality standards and existing data about effects caused by single chemicals. In the study of Escher et al. (2018) effect-based trigger values are defined as "thresholds that differentiate between acceptable and poor water quality with respect to the organic micropollutants". With effect-based trigger values acceptable risk could be indicated for complex mixtures of chemicals. The effect-based trigger values are obtained by using in vitro and in vivo bioassays (Escher et al., 2018). Bioassays can prove ecotoxicological risks by identifying effects caused by known or unknown contaminants in samples (De Baat et al., 2019). De Baat et al. tested different bioassays at 45 locations in Dutch surface waters. They selected a bioassay battery that could identify (specific) risks caused by a wide range of chemical contaminants and their transformation products. The bioassay effect was compared with the defined effect-based trigger values from the study of Escher et al. (2018). In 9 out of the 21 applied assays, trigger values were exceeded. For each of the 45 locations a risk assessment could be made by the sum of the effect-based trigger values exceedances. They concluded that EBMs can identify the presence of hazardous contaminants irrespectively being already listed as CECs. According to de Baat et al. (2019) when first using EBMs instead of time-consuming and costly chemical analysis, something can be said about the ecotoxicological risks. After measuring the effects, additional specific analysing tests can be done for targeted identification of groups of contaminants that cause the effects. Knowing which contaminants are causing the effects could be important to take measures for improving the water quality (De Baat et al., 2019).

Neale et al. (2017) also studied a battery of bioassays to detect contaminants in surface water. They found that the exact type of bioassay is not important, but the diversity of bioassays including apical endpoints and specific bioassays relevant for occurring contaminants. Apical endpoints are the observable outcome in a whole organism when it is exposed to a toxicant, like clinical signs or pathologic state (Ecetoc, nd.). The combination of bioassays with apical endpoints and specific bioassays is important to determine the hazard potential of the contaminants.

EBMs could also be an appropriate analytical tool for detecting CECs in the soil-water system. For toxicological use, hundreds of different bioassays are available (Busch et al., 2016). Each of these bioassays measures different aspects at different levels of complexity and specificity. According to the review of Gomes et al. (2017) for evaluating the ecotoxicity of CECs in soil, earthworms (Eisenia andrei) are frequently used and plants like carrots, rice and cucumber.

2. Research design and methodology

2.1. Research objective

The main aim of this research is to identify effective practices for determining the presence and risks of contaminants of emerging concern in the soil-water system without knowing everything about them. The focus will be on the use of effect-based methods. Furthermore, this research intends to provide recommendations on how to draw up a policy to deal with CECs and clarify future research needs.

2.2. Research question

How could effect-based methods be used in detecting contaminants of emerging concern in the soil-water system and how could these methods be integrated into Dutch soil policy?

Sub questions:

1. What EBMs for the soil-water system have been described in the public literature?
2. Which criteria (e.g. policy, technical, practical, financial) determine the suitability of EBMs to be applied in the soil-water system?
3. Which EBMs have the greatest potential to be applied in practice based on the criteria?
4. What type of research questions concerning determination of CECs arise from the Dutch soil policy and what requirements and standards are there for soil research?
5. How should the Dutch soil policy have to change for implementation of EBMs in the soil-water system?
6. How can relevant practical knowledge about the applicability of EBMs in the soil-water system be obtained effectively through a workshop?
7. Which insights and recommendations concerning the use of EBMs in determining CECs arise from practitioners knowledge?

2.3. Research method

Definitions

The study is focusing on the soil-water system in the Netherlands. The focus of this study is on the application of EBMs in the detection of CECs. The problem of CECs in itself falls outside the scope of the study

For the definition of CECs the definition of Sauvé and Desrosiers (2014) is used. CECs are "naturally occurring, manufactured or manmade chemicals or materials which have now been discovered or are suspected present in various environmental compartments and whose toxicity or persistence are likely to significantly alter the metabolism of a living being [..] and which are not yet subjected to regulatory criteria or norms for the protection of human health or the environment". The lack of regulatory criteria or norms means in this study the lack of environmental quality standards.

CECs include, but are not limited to, the following groups of contaminants: pesticides, pharmaceuticals and personal care products, fragrances, plasticizers, hormones, flame retardants, nanoparticles, perfluoroalkyl compounds, chlorinated paraffins, siloxanes, algal toxins, various trace elements (Gomes et al., 2017; Naidu & Wong, 2013; Sauvé & Desrosiers, 2014).

The term effect-based methods is used according to the definition of Brack et al. (2019): "EBMs are bioanalytical methods using the response of whole organisms (in vivo) or cellular bioassays (in vitro) to detect and quantify the effects of groups of chemicals on toxicological endpoints of concern". An important term around EBMs is Mode of Action (MoA). It means the series of key processes when a contaminant interacts with a target site (e.g. receptor) and goes through changes in an organism that causes sublethal or lethal effects (Beyer et al., 2014).

Methodology

This study can be divided into two parts to investigate the usability of effect-based methods in detecting CECs in the soil-water system. The first part consists of a desk study. For the second part a workshop is held with practitioners.

The flowchart (figure 1) shows the research approach step-by-step to answer the research question.

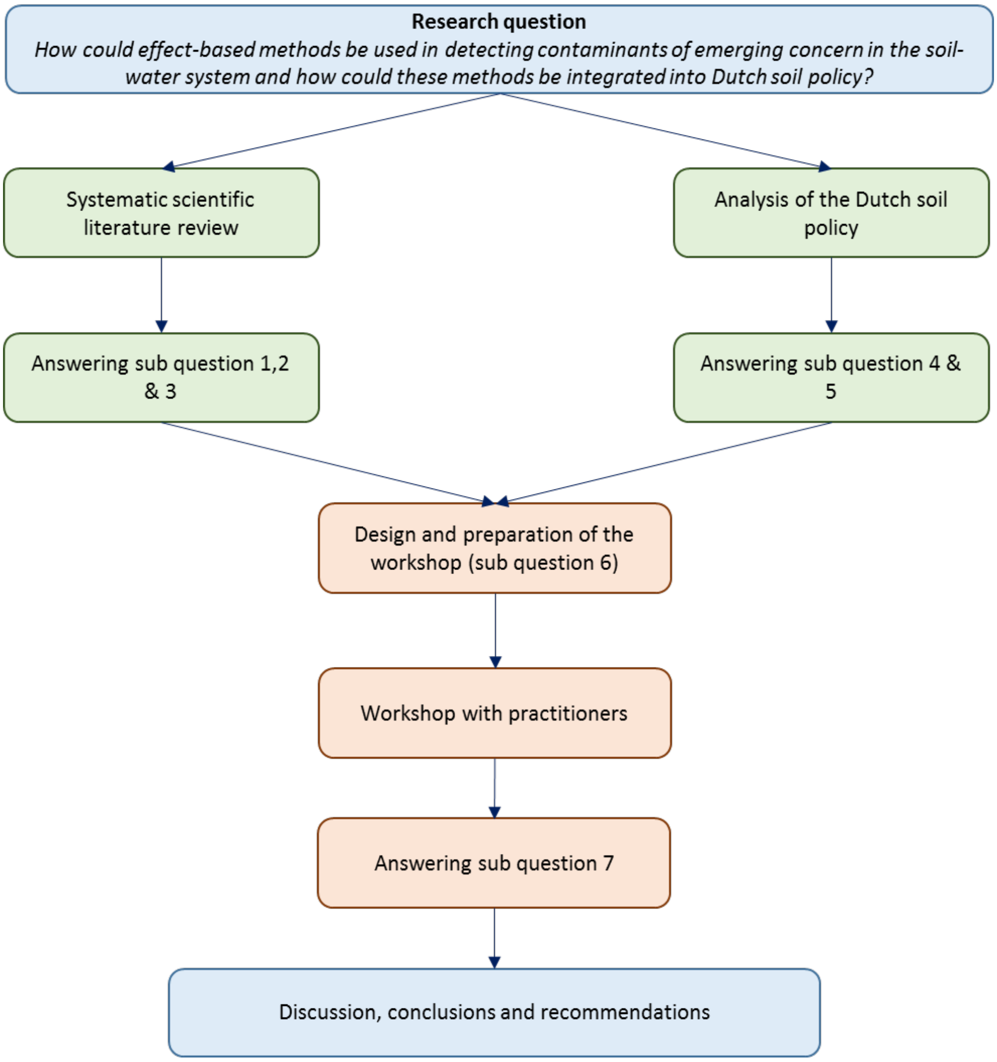

Figure 1. Flowchart

Literature study

The first three sub questions into the possibilities of EBMs are answered by a systematic scientific literature review. Because of the current application of EBMs in surface water also the studies focused on surface water are used. Primarily, the databases Web of Science and ScienceDirect are used. Table 1 gives an overview of the search terms used and appendix 1 gives a complete overview of the literature study. For the literature review 81 articles were found and assessed.

Table 1. Search terms used for the scientific literature review

Database	Keywords
Web of Science	Effect-based methods, effect-based methods AND soil, effect-based methods AND contaminants, effect-based methods AND pollutants, effect-directed analysis, effect-directed analysis AND micropollutants,
ScienceDirect	Effect-based methods AND contaminants, ecological effects AND contaminants of emerging concern, emerging contaminants AND bioassays, emerging contaminants AND effect-based methods, bioassays AND soil, biological early warning system AND soil, earthworms AND emerging contaminants, biosensor AND soil AND emerging contaminants

The conclusions drawn from the scientific literature review are used as basis for the answers to the fourth and fifth sub question. In addition, scientific literature and websites of the government about the Dutch soil policy were used for the answers to the fourth and fifth sub-questions. The answers to the first five sub questions were the input for the preparation of the workshop.

Workshop

The results of the literature review are reviewed by practitioners for the practical feasibility of the use of EBMs in the soil-water system. The knowledge of practitioners is gathered in a workshop. The main goal of the workshop was to make an inventory of how practitioners think about the use of EBMs in the soil-water system. The focus of the workshop was on points of agreement and disagreement, knowledge gaps and what is needed for implementation of EBMs in the soil-water system.

In this study a multi-disciplinary workshop seemed to be an appropriate tool for gathering information from practitioners. In the workshop different kind of practitioners were together so it was easier to get the knowledge in a relatively short time. Other methods, especially interviews, are costly in time and money (McIntosh & Morse, 2015). A workshop is part of a participatory approach (Slocum, 2003). A participatory approach could be useful because in complex problems it is better to have as much knowledge, experience and expertise as possible (Slocum, 2003). The problem of detecting CECs in the soil-water system could be considered as a complex problem (Noguera-Oviedo & Aga, 2016). A workshop also has disadvantages as some participants may be intimidated because of other participants tend to dominate. Therefore, not all participants would give their own opinion.

The workshop is held in an online session on April 23 of 2020, as part of three sessions in the context of a Dutch project around CECs in the soil-water system[1]. The workshop would initially be an in-personmeeting but the outbreak of Covid-19 has turned it into an online session. Microsoft Teams was used as online tool for the workshop.

An online session requires more preparation than aphysical in-person meeting because not everyone can speak at the same time and this must be properly supervised. Sub question 6 is answered through the preparation of the workshop.

Before the workshop has taken place the participants were informed about the topic. All participants received per e-mail a short paper with a description of EBMs in general and how it could be used in the soil-water system so they were all familiar with the topic (see Appendix 2 for the paper).

Besides the short paper the participants were sent three questions by email. They could choose one of three cases and answer the three questions for that particular case. The replies were sent back by e-mail.

The questions were:

1. How could EBMs be useful in this case?
2. What are the advantages and disadvantages of using EBMs in this case?
3. What is needed to implement EBMs in addition to or instead of doing standardized soil investigation in the soil-water system?

The cases are practical examples and could occur in the Netherlands. With practical examples it could be easier for participants to answer the questions and to get useful answers.

The cases were:

a. Moving soil. A quantity of soil must be relocated. It is unclear whether the soil is contaminated and what kind of contaminants the soil could contain.
b. Allotment garden. An allotment garden is laid out at a location where contaminants without norms or regulatory criteria are found.
c. Water-collection area. In a water-collection area surface water from a river infiltrates. There are concerns about the water quality of the river.

[1] The Dutch project about dealing with CECs in the soil-water system is commissioned by UP (*Uitvoeringsprogramma Bodem en Ondergrond*). The consortium consists of several Dutch environmental consulting firms and is supported by several knowledge institutes. The project started in 2018 and will be finished in 2020 (POP UP Opkomende Stoffen, n.d.).

The participants can be divided into three different groups: 12 practitioners, 12 policy officers and 3 researchers. Practitioners are consultants from different fields such as soil, water and waste. Policy officers are all participants that contribute to soil and water policy. The researchers are from a university or research institution.
The duration of the workshop was 90 minutes. The workshop started with a round of personal introductions in which each participant mentioned what his/her background was and which institution or company they were from. Names are not listed because of the European Data Protection Regulation of the European Union which came into force in 2018 (European Commission, 2019). After the introdiction round a presentation of 15 minutes was given about the main outcomes of the literature review. After the presentation, the three cases were discussed with the most striking statements. For each case, one participant was asked to explain one of the answers. These participants were approached prior to the workshop to ask if they would like to explain their answers. The answers that deserved an explanation and the most interesting answers for discussion were chosen. The base was that at least a policy officer and a practitioner would explain an answer. Of the 27 participants, 12 participants answered the questions for one of the cases in advance. Case 1 was answered by 2 practitioners and 2 policy officers, case 2 was answered by 1 practitioner and 2 policy officers and case 3 was answered by 4 practitioners and 1 researcher.
The other participants could respond to the explanation in the chat of the meeting tool. Following the reactions in the chat, the chairwoman of the workshop gave the floor to several participants to explain their reaction. During the workshop no conclusions were drawn as a result of the discussions. Only the different points of view were gathered.
The results of the workshop are used to answer the seventh sub question.

3. Results

3.1. Literature study

The literature review is the basis of the thesis. This chapter first reviews the different EBMs currently available for detecting CECs. Secondly, the different EBMs are subjected to the criteria for application in the soil-water system. At last, a conclusion is made which EBM will have the greatest potential for applying in practice.
In section 3.1.4 and 3.1.5 the focus is on the Dutch soil policy and how it has to change for implementing EBMs in the soil-water system.
The chapter answers the following sub questions:

1. What EBMs for the soil-water system have been described in the public literature?
2. Which criteria (e.g. policy, technical, practical, financial) determine the suitability of EBMs to be applied in the soil-water system?
3. Which EBMs have the greatest potential to be applied in practice based on the criteria?
4. What type of research questions concerning determination of CECs arise from the Dutch soil policy and what requirements and standards are there for soil research?
5. How should the Dutch soil policy have to change for implementation of EBMs in the soil-water system?

3.1.1. Overview of effect-based methods described in literature

EBMs are biological methods that could be done at different levels of biological organization (Connon et al., 2012). Wieczerzak et al. (2016) divided biological methods into two groups: biomonitoring and bioanalytics. The division of Wieczerzak et al. is used for this study. See figure 2 for an overview of the different biological methods.

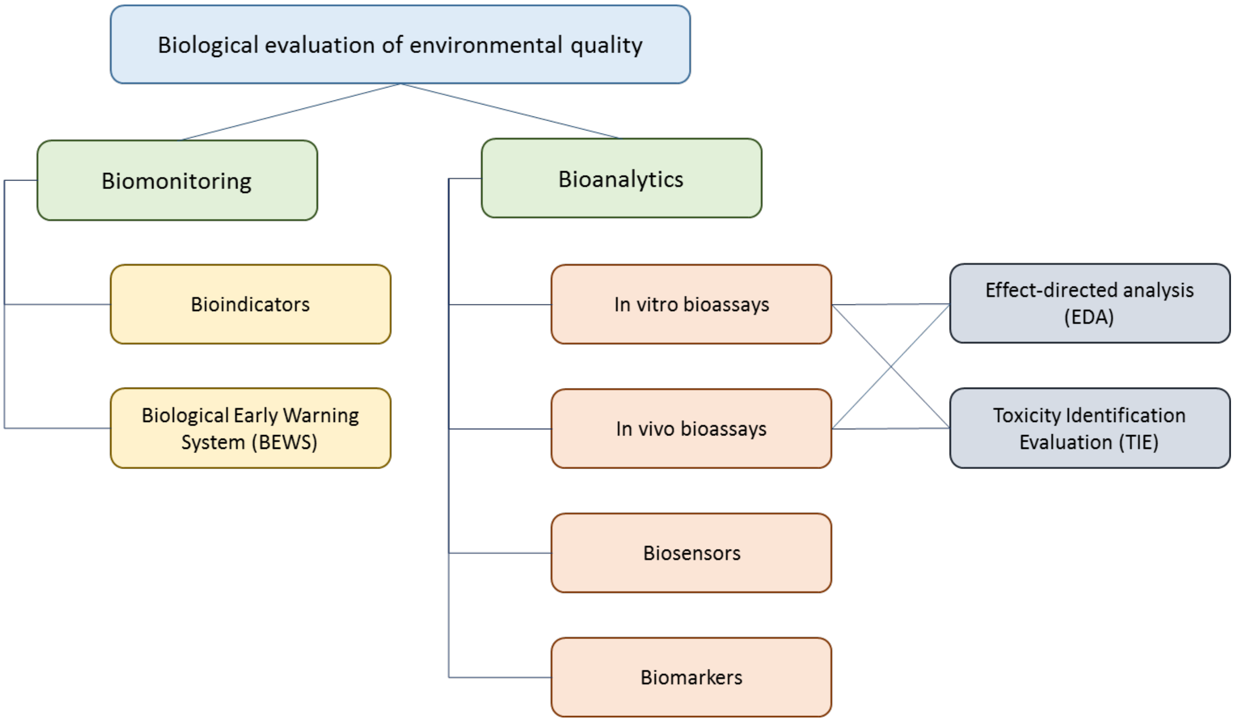

Figure 1. Biological tools for determining CECs in the environment(based on Wieczerzak et al. , 2016)

Biomonitoring is aimed at observing changes or disturbances at different levels in ecosystems by in situ exposure or by using biomarkers of resident organisms (Connon et al., 2012; Wieczerzak et al., 2016). Biomonitoring usually takes place in the field. Examples of biomonitoring are bioindicators and Biological Early Warning Systems (BEWS) (Wieczerzak et al., 2016). For in situ exposure an organism could be deployed as biological early warning system that is based on the reaction of an organism to a contaminant or mixture of contaminants (Allan et al., 2006). With bioanalytics reactions to CECs could be determined by organisms or cell-based parts of organisms and usually takes place in laboratory. Bioassays, biosensors and biomarkers are examples of bioanalytics (Wieczerzak et al., 2016). In the laboratory different kinds of bioassays are exposed to environmental samples or extracts. In vitro bioassays are cell-based and measure specific biochemical effects such as endocrine disruption and genotoxicity (van der Oost et al., 2017a). In vivo bioassays use whole organisms and measure effects such as growth, reproduction, feeding activity, and mortality (Dopp et al., 2019; van der Oost et al., 2017a). Biosensors form a separate group within bioanalytics and could also be a useful tool for detecting CECs (Nguyen, Kwon & Gu, 2017). Biomarkers are reactions of organisms to contaminants and could be based on bioassays (Peakall, 1994)

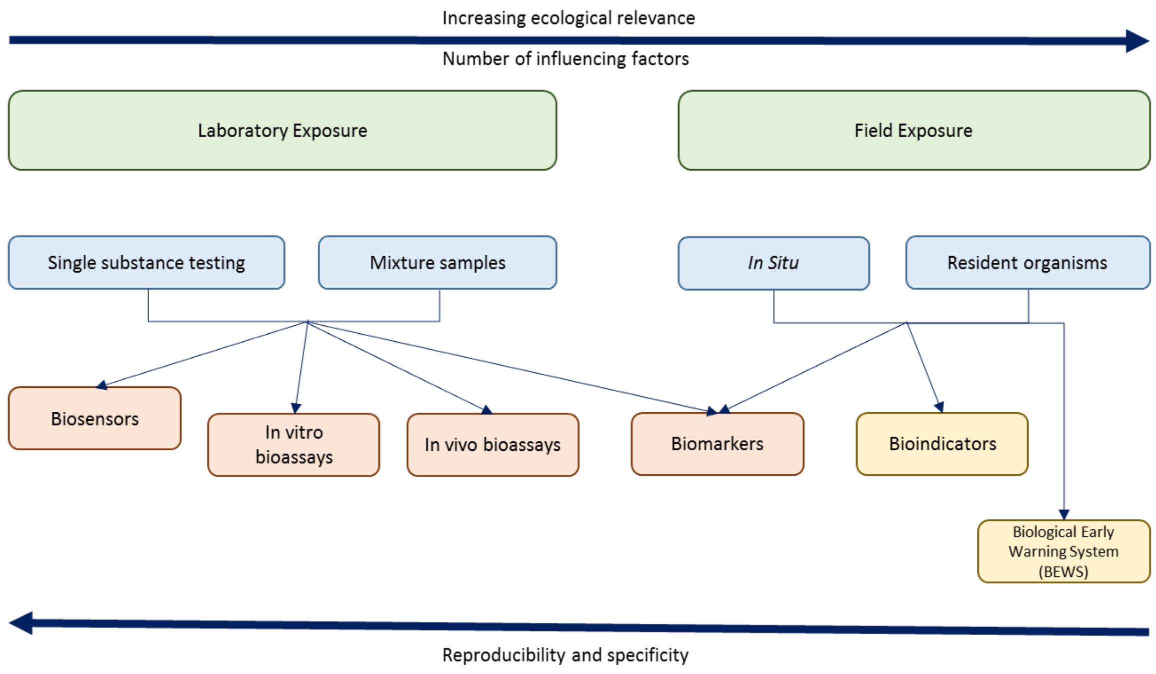

Figure 2. Overview of the different effect-based methods (Connon et al., 2012)

Figure 3 gives an overview of the different EBMs with a subdivision into laboratory exposure and field exposure. The upper arrow indicates how much ecological relevance a method will have and how much it will be influenced by other factors. The lower arrow indicates the reproducibility and specificity of a method. These terms are further explained in section 3.2.

Bioindicators

Research with bioindicators could be performed in situ and consists of observation of species and organisms (Wieczerzak et al., 2016). Values that could be measured are, for example, the total number of free-living organisms or the diversity and structure of community indices (Gutiérrez et al., 2016; Visioli et al., 2013).

In the soil-water system soil nematodes are suitable bioindicators for determining the presence of CECs (Gutiérrez et al., 2016). Gutiérrez et al. (2016) have done a study on soil nematodes in different soil samples with known concentrations of CECs. They have studied different perspectives of the nematodes, such as species composition, biomass, trophic ground and footprint. From the study appeared that the total number of nematodes did not change due to contamination, but the diversity and structure of the nematode community vastly altered. The advantages of bioindicators are that the research can be done in situ and no sample preparation is needed (Wieczerzak et al., 2016). A limitation is that not all organisms are equally sensitive to the same types of disturbances or contamination and could therefore react differently to diverse types of CECs (Martinez-Haro et al., 2015).

Biological Early Warning Systems (BEWS)

A BEWS is, just as bioindicators, a form of in situ biomonitoring and is based on the different responses of an organism to disturbance. These responses are based on behavioural endpoints or behavioural changes (Bae & Park, 2013). That can be at cell level like the changes in neurotransmitters, plasma enzymes, hormones, and energy metabolism (Dell'Omo, 2002). At community or population level changes like biodiversity, energy transfer and population growth can be measured (Chon et al., 2010). For behavioural changes at community or population level, microbial communities are considered to be the first and most swift responders to environmental contaminants (Wahsha et al., 2017). At higher trophic levels in water species likes *Daphnia's*, fish and algae are suitable (Connon et al., 2012). In soil earthworms (*Eisenia fetida*), nematodes (*Caenorhabditis elegans*) can act as early warning organisms (Dominguez-Rodriguez et al., 2020; Fajardo et al., 2019), but also plants (Delerue et al., 2019).

Dominguez-Rodriguez et al. (2020) have done a study on earthworms exposed to contaminated soils. The soil samples were contaminated with 2,4-dichlorophenoxyacetic acid (2,4-D), a commonly used herbicide. The earthworms died in direct contact with the soil samples contaminated with 300 mg/kg herbicide. When earthworms were exposed to extracts of the soil samples on filter paper they survived.

The advantages of using organisms or plants as BEWS are that in the case of studying changes at community or population level no laboratory is needed (Wahsha et al., 2017). The ecological relevance is high. Moreover, BEWS is a cost-effective way of testing the degree of soil contamination (Bae & Park, 2013). Although, the ecological relevance is high, there could be influence from other factors than CECs, such as weather conditions, acidity of the soil (Wahsha et al., 2017). In addition, responses are slow and often imprecise at community and ecosystem level (Bae & Park, 2013).

In vitro bioassays

In vitro bioassays are cell-based and aimed at measuring specific effects. An environmental sample is exposed to a specific bioassay for determining endocrine disruption or genotoxicity (van der Oost et al., 2017a). The measured effect is the result of an interaction between a contaminant and the (part of) cell(s). The type of interaction between a contaminant and its molecular target can be distinguished in nonspecific, specific and reactive toxicity (van der

Oost et al., 2017a). " '*Nonspecific toxicity' refers to the minimum cytotoxicity that a chemical can exhibit not mediated by specific mechanisms (narcosis). 'Specific toxicity' refers to all common mechanisms that involve the selective binding of a chemical to a protein (enzyme or receptor). Mechanisms of action are classified as reactive when covalent bonds are formed between the chemical and its target or when chemical reactions such as oxidative stress are involved*" (van der Oost et al., 2017a). Different kind of in vitro bioassays can be used for determining CECs in environmental samples. Cultured cells, micro-organisms (Devier et al., 2011) and bacterial assays such as *Pseudomonas fluorescens* can be used (Ghosh et al., 2017). Testing cytotoxicity and genotoxicity in soil bioassays with (parts of) *Allium* are suitable (Stapulionyte et al., 2018) and other plant-based bioassays, such as *Vicia faba* (Bhat et al., 2019; Ghosh et al., 2017; Iqbal, 2016).

Altenburger et al. (2018) used a panel of different in vitro bioassays to study the effects of mixtures of contaminants in water samples. Various in vitro bioassays were used for detecting effects like estrogenic response and activation of biotransformation. They have measured both single contaminants and a selection of the same contaminants in a mixture in different laboratories. In 12 of the 14 bioassays combined effects were measured in the mixtures of 12 organic contaminants with different modes of action. From this study appeared that it is possible to determine combined effects from mixture exposure in water monitoring.

In vitro bioassays are suitable to investigate specific mode of actions (Beyer et al., 2014). Using in vitro bioassays for determining effects from CECs is very specific and sensitive. Moreover, it can be used in microplate format so the volume of samples can be reduced (Devier et al., 2011). Compared to in vivo bioassays, in vitro bioassays are simpler, faster and more cost-effective (Ghosh et al., 2017). Especially microbial bioassays are inexpensive, less time consuming and are sensitive to different toxic contaminants (Hassan, van Ginkel, Hussein, Abskharon & Oh, 2016, 2016).

Measuring specific effects has also limitations because the interpretation of the measured effects could be difficult (Brunner et al., 2020). In samples it could only explain a small portion of the overall toxicity (Hong et al., 2016). Compared to in vivo bioassays, in vitro bioassays are lacking complexity (Ghosh et al., 2017). Specific effects are difficult to explain in which contaminants cause the effect (Devier et al., 2011). For effects as cytotoxicity, reactive and adaptive stress less than 1% of the demonstrated effects with bioassays can be explained by known and identified contaminants (van der Oost et al., 2017a). Another limitation of in vitro bioassays is the difficulty of reproducing and repeating the test (Wieczerzak et al., 2016).

In vivo bioassays

In in vivo bioassays whole organisms are used for measuring effects such as growth, reproduction, feeding activity, and mortality (Dopp et al., 2019, Connon et al., 2012). For application in water *Daphnia's*, fish and algae are suitable (Connon et al., 2012). In soil earthworms (*Eisenia fetida*), springtails (*Folsomia candida*), nematodes (*Caenorhabditis elegans, Steinernema carpocapsae*), soil bacterium (*Arthrobacter globiformis*), enchytraeids (*Enchytraeus crypticus*), soil algae can be used (Dominguez-Rodriguez et al., 2020; Fajardo et al., 2019; Garbo et al., 2019; Kim et al., 2018; Richter et al., 2015). Plant bioassays could also be used for in vivo bioassays such as alfalfa (*Medicago sativa*), cress (*Lepidium sativum*), cucumber (*Cucumis sativus*), lettuce, rice, mung bean, oats (*Avena sativa*), oilseed rape (Kim et al., 2018; McGinnes et al., 2019; Richter et al., 2015; Visioli et al., 2013).

In vivo bioassays are more environmentally relevant than in vitro bioassays (Beyer et al., 2014). With in vivo bioassays an estimation can be made of bioavailability of the contaminants present in the environmental sample (Ghosh et al., 2017). Compared to whole organism bioassays plant bioassays are more sensitive to environmental stress. They are easy to handle and store, low-cost and have a good correlation with soil (Visioli et al., 2013). But, according to Delerue et al. (2019) a disadvantage of using plant bioassays is they are also sensitive to other factors. Differences in soil pH and the content of organic matter could influence the results of the tests.

Compared to the use of in vitro bioassays, in vivo bioassays require specialized equipment and operator skills and long acclimatization times. They are labor-intensive, expensive, and time-consuming (Hassan et al., 2016). The effects between different organisms are not uniform so a broad range of bioassays is needed. The effects measured could be soil specific and not comparable with other soil types (Richter et al., 2015). Moreover, the use of higher organisms may also be ethically undesirable (Hassan et al., 2016).

An example of using in vivo bioassays for soil samples is the study of Domínguez-Rodríguez et al. (2020). They used earthworms for testing the toxicity of the herbicide 2, 4-D (2, 4-dichlorophenoxy acetic acid) in soil samples. All earthworms died in direct contact with the polluted soil. No mortality occurred when the earthworms were exposed to the herbicide extracted on filter paper.

Challenges of performing EBMs with both in vitro and in vivo bioassays well are, in first place, the purity of the sample. The test has to be performed immediately after sampling (Xu et al., 2019). Especially for complex environmental samples in each step of sampling, sample preparation and storage of the chemical composition of the sample could change. Thereby its toxicity could also change due to added active compounds via contaminated materials or removed by adsorption to materials. During transport, storage and sample preparation active compounds could be added or removed (Abbas et al., 2019). Another point of attention is the possibility of false-positive or negative results due to naturally occurring compounds, also called matrix effects (Abbas et al., 2019). Effect-directed analysis (EDA) could be a way to separate contaminants from naturally occurring compounds (Abbas et al., 2019). EDA will be explained below. Cytotoxicity could also mask the effect under investigation. Cytotoxicity is an important toxicological endpoint but at high concentration it could mask the specific effect. To achieve a noncytotoxic concentration the sample could be diluted, but this also minimizes the effect of interest (Abbas et al., 2019).

For both in vitro and in vivo bioassays, there is a lack of regulation as to which set of bioassays should be used as standard making it difficult to use them in determining CECs (Brunner et al., 2020).

Biosensors

Biosensors combine a biological component with a physicochemical detector for real-time monitoring. Biosensors are analytical devices, constructed by combining a biological sensing element (e.g., enzymes, antibodies, microorganisms, or DNA) with a transducer to obtain a useable signal output (Hassan et al., 2016).

There are different kind of biosensors; optical, electrochemical, mass-based or colorimetric biosensors. The signal output of a biosensor can be a change in proton concentration, release or uptake of gases, light emission, or absorption. The transducer converts the biological signal into a measurable response such as current, potential, or absorption of light through electrochemical or optical means, which can be further amplified, processed and stored for later analysis (Hassan et al., 2016).

The biological component of a biosensor for use in soil or water can be a whole cell (such as bacteria, microalgae, yeast, or fungi) or based on molecules (Bilal & Iqbal, 2019; Hassan et al., 2016). Rajkumar et al. (2017) used a bacterial biosensor for detecting effects caused by organophosphate pesticides in soil samples.

At this moment, biosensors are not yet applicated at large scale but are further developed for real-world application (Chang et al., 2017). Biosensors could be useful for monitoring chronic toxicity (Nguyen et al., 2017). The technique can provide a rapid, sensitive, real-time, on-site detection and analysis in the field (Wang et al., 2014). That could be advantageous in isolated/remote settings where transportation of test samples is not possible (Bilal & Iqbal, 2019). A biosensor could identify and quantify specific compounds directly in the air, soil or water (Hassan et al., 2016; Kim et al., 2019). The technique can complement classical analytical methods because they can distinguish between bioavailable and unavailable forms of contaminants (Hassan et al., 2016).

A limitation of biosensors is its specificity for only one parameter, such as biological oxygen demand (BOD) (Ghosh et al., 2017). When biosensors will be used in a water body, it will give little information about biological functions or organisms in the water. The environmental relevance is small (Hassan et al., 2016). However, it gives insight into the bioavailability/ bioaccessibility and physical transfer into the test organism when a whole organism is used (Allan et al., 2006).

Biomarkers

Biomarkers are defined as "*a biological response to a chemical or chemicals that give a measure of exposure and sometimes, also, of toxic effect*" (Peakall, 1994). The response could be molecular, cellular, physiological or behaviour changes (Ghosh, Thakur & Kaushik, 2017). The biomarkers have been measured in field-exposed organisms (Wernersson et al., 2015). Biomarkers have the advantage of acting as an early warning system because damage at molecular or cellular level arises earlier than when it is visible on whole organism level (Allan et al., 2006). The World Health Organisation divided biomarkers into three different groups: biomarkers of exposure, biomarkers of susceptibility and biomarkers of effect (Allan et al., 2006). Biomarkers of exposure detect the interaction between a contaminant or its metabolites and target molecules or cells, in a compartment within an organism. Biomarkers of susceptibility detect the ability of an organism to react to the exposure of a specific contaminant, including genetic factors and changes in receptors. Biomarkers of effect measure the biochemical, physiological or other alterations in an organism, which could be measured and linked to possible health alterations due to exposure to a contaminant (Allan et al., 2006). Biomarkers of exposure and biomarkers of effect are suitable for detecting CECs in the environment (Martinez-Haro et al., 2015). The implementation may be based on in vivo bioassays (Ghosh et al., 2017). In water samples hydropsyche larvae are suitable for instance (Previsic et al., 2019). In soil plants (*Lycopersicon esculentum*), earthworms (*Eisenia fetida, Amynthas gracilis*), nematodes, isopods, springtails, gastropods and oligochaetes could be used as a biomarker (Aparicio et al., 2019; Dong et al., 2012; Gong & Perkins, 2016; Lee et al., 2019; Parelho et al., 2018).

Dong et al. (2012) have done a study to the biochemical toxicity of antibiotics. DNA damage and changes in enzyme activities in earthworms (*Eisenia fetida*) were used as biomarkers. They proved DNA damage as biomarker is suitable for determining exposure to low concentrations of antibiotics in terrestrial environment. DNA damage was in this study a more sensitive biomarker than changes in enzyme activities.

Transcriptomics, genomics, metabolomics and proteomics are relatively new discovered and studied biomarkers (Martyniuk et al., 2016; Matich et al., 2019). With these techniques changes in organisms are also considered, but at DNA, RNA, protein and metabolite level (Martyniuk et al., 2016).

Biomarkers can act as an early warning system (Allan et al., 2006; Martinez-Haro et al., 2015). But using biomarkers in detecting CECs requires understanding of the mechanisms in cells when exposed to a mixture of contaminants (Allan et al., 2006). Currently, biomarkers are not understood well enough to make conclusions about the impacts of CECs on organisms (Connon et al., 2012). Moreover, not all organisms are sensitive in the same amount to the same types of CECs, and could therefore react in a different way to various contaminants (Martinez-Haro et al., 2015).

Additional tests

Performing EBMs with bioassays will give insight into the toxicity of an environmental mixture sample. Which contaminants are causing an effect will not be clear with bioassays alone. For identifying the contaminants which cause an effect additional tests could be done such as effect-directed analysis (EDA) and toxicity identification evaluation (TIE) (Allan et al., 2006; Brack et al., 2016; Burgess et al., 2013).

Effect-directed analysis (EDA)

EDA is based on biological and chemical analysis. The toxicity of a mixture of contaminants can be assessed for separated classes of contaminants or matrices deprived of specific classes of contaminants (Beyer et al., 2014). Within this method the emphasis is on organic contaminants. EDA consists of different phases: 1) a biological analysis will be done with in vitro bioassays, 2) the complexity of the mixture will be reduced by fractionation of organic compounds of the sample via column chromatography, 3) a biological analysis with in vitro bioassays will be done with the sub-fractions of the sample, and 4) direct or target chemical analysis for confirmation. The mixture may undergo several rounds of fractionation to reduce the complexity of the mixture (Burgess et al., 2013).

EDA is suitable for municipal and industrial effluents, water, wastewater, pore water, (whole) sediments, technical mixtures, consumer products, biota, soil, crude oil, and suspended solids (Burgess et al., 2013; Hong et al., 2016).

In the projects SOLUTIONS and NORMAN, funded by the European Union, EDA is further developed for application in water (Altenburger et al., 2015; Brack et al., 2012). EDA is increasingly applied in water quality monitoring and will be further improved (Brack et al., 2016). Booij et al. (2014) have done a specific study of using EDA in detecting contaminants that negatively influence the effective photosystem II efficiency in marine microalgae. There are multiple unknown contaminants present in estuarine and coastal water, but the study of Booij et al. (2014) detected several contaminants (atrazine, diuron, irgarol, isoproturon, terbutryn, and terbutylazine) that negatively influence photosynthesis in microalgae.

For determining unknown CECs and the effects these CECs will cause EDA is a valuable method. In addition, transformation products may also be determined (Devier et al., 2011). The method has a very high specificity in toxicity identification (Burgess et al., 2013). The lacking of standard sample preparation methods of various sample matrices is a limitation of EDA (Hong et al., 2016). There could be potential loss of contaminants during the process of extraction and fractionation. Loss of contaminants can also cause because the samples have to be solvent extracts (Li et al., 2017). Although the costs of in vitro bioassays are relatively low, the chemical analysis is relatively expensive (Li et al., 2017).

Last, EDA is less environmentally relevant. The first goal of EDA is targeting the drivers of effects, ecological relevance is a secondary goal (Burgess et al., 2013; Li et al., 2017). The ecological relevance of EDA will increase when in vivo bioassays will be used for biological analysis (Brack et al., 2016).

Toxicity identification evaluation (TIE)

TIE is also based on biological and chemical analysis. The goal of the method is comparable: targeting drivers of effect at which all potential contaminants are considered (Beyers et al., 2014; Burgess et al., 2013). TIE consists of different phases at which the concept lies in removing groups of contaminants until the toxicity of the sample disappears. The suspected contaminants will be identified by analytical chemistry (Beyer et al., 2014). The different phases are 1) biological analysis with whole organism bioassays, 2) characterization of the contaminants that are causing toxicity in the sample in different classes (e.g. non-ionic, organics, metals, ammonia), 3) chemical analysis of the suspected contaminants, 4) identification of the contaminants by the same kind of whole organisms bioassays, 5) confirmation (Burgess et al., 2013).

TIE can be applied to municipal and industrial effluents, pore water and whole sediments (Burgess et al., 2013). In sediment samples the bioassays can consist of amphipods (*Leptocheirus plumolosus*) (Baileys et al., 2016).

Yi et al. (2015) have done a study to the toxicity of sediment with TIE with samples from the river Guangzhou in China. The biological analysis was done with bioassays with midges (*Creontiades dilutes*). From the study appeared the toxicity to midges was mainly caused by metals (Zn, Ni, and Pb) and pesticides (cypermethrin, lambda-cyhalothrin, and fipronils) which were commonly used in these areas. According to Yi et al. it is important to measure the bioavailability of sediment-bound organics for improving the accuracy in TIE.

Just as EDA, TIE is also a valuable method for identifying unknown contaminants that cause effects (Devier et al., 2011). Assessing the nature and magnitude of toxicity mixture is a limitation of both TIE and EDA, "*such as additivity, synergism (i.e. larger effect than expected based on additivity predictions) and antagonism (i.e. smaller effect than expected based on additivity predictions)"* (Beyer et al., 2014). The advantages of TIE relative to EDA are the environmental relevance of TIE, bioavailability is considered, it applies to whole sediment/soil samples, and low costs of chemical analysis (Li et al., 2017). On the other hand, whole organism bioassays are more expensive compared to in vitro bioassays (Li et al., 2017). Although TIE applies to whole sediment/soil samples it is difficult to identify contaminants due to the complexity of the samples (Li et al., 2017). TIE has a high specificity for classes of contaminants and moderate specificity for specific contaminants in the identification phase (Burgess et al., 2013).

3.1.2. Criteria effect-based methods must meet for application in the soil-water system

EBMs are currently not widely used in tests in the soil-water system. In the Netherlands EBMs with in vivo and in vitro bioassays are used in a few cases: 1) part of the Triade test, 2) for an ecological risk assessment, and 3) sometimes as part of an evaluation after remediation (Bodemrichtlijn, 2016). Triade is a test used in ecological risk assessment of contaminated (water)soils and consists of three pillars: 1) chemical analyzes, 2) EBMs (e.g. bioassays) and 3) an ecological assessment (e.g. field surveys) (Brand et al., 2013).

Regulations of how EBMs have to be used are lacking for the use on a bigger scale in the soil-water system. EBMs are used in other fields and therefore standards have been derived. For instance, the regulations from the European Union for EBMs in screening dioxins in feed and food (van der Oost et al., 2017a).
EBMs have been used in surface water for longer than in the soil-water system (van der Oost et al., 2017a). From several studies targeting EBMs in surface water criteria for applications of EBMs could be derived that are also useful for EBMs in the soil-water system (Allan et al., 2006; Brack et al., 2019; Busch et al., 2016; de Baat et al., 2019; van der Oost et al., 2017a). Each of the EBMs described in part 3.1. has been tested against the following criteria:

1. Identification of a broad spectrum of CECs and their metabolites.
2. Good performance of biotests, with quality standards such as accuracy, potential high-throughput capacity, reproducibility, robustness, selectivity, sensitivity and speed.
3. The implementation and applicability of the EBM should be easy and done by routine laboratories. Additional criteria for tests are 1) standardized protocols, 2) a small test volume, 3) tests available in kits and simple to undertake, and 4) no specifically trained personnel or extensive laboratory facilities needed.
4. Relevant and effective sampling of soil and groundwater samples.
5. A good translation of measured effects into actual risks in practice.
6. Cost effectiveness.
7. Decisions can be made about measures when the EBM indicates risks, even without knowing the specific drivers of the risk.

Whether an EBM meets the criteria is determined based on the results of the literature search described in part 3.1.1. Every criterion is scored for every EBM on a scale of 1 (does not meet the relevant criterion at all) to 5 (fully meets the criterion). If it is not clear, the score is determined in relation to the other EBMs or a score of 3 is given.
The results are given in table 2.

1. Identification of a broad spectrum of CECs and their metabolites

In theory every EBM could be suitable for detecting every potential toxic contaminant but it depends on how widely a test is set up. The EBM fully meets the criterion when it is possible to cover the different types of toxicity; at various trophic levels (nonspecific), specific and reactive toxicity (Van der Oost et al., 2017a). For instance, for bioassays it means the design of a good panel of bioassays that covers all kinds of toxicity. Only EDA could not fully meet this criterion because the emphasis of that method is on organic contaminants. Inorganic contaminants could be missed by using EDA.

2. *Good performance of biotests, with quality standards such as accuracy, potential high-throughput capacity, reproducibility, robustness, selectivity, sensitivity and speed*

For each EBM it has been determined to what extent they can meet certain quality standards such as accuracy and potential high-throughput capacity.
When performed well in vitro bioassays and biosensors could fully meet the criterium. In vitro bioassays are very sensitive and specific (Devier et al., 2011). Biosensors are analytical devices and could perform rapid, sensitive and real-time monitoring (Hassan et al., 2016; Wang et al., 2014). Biosensors only measure one parameter and therefore cannot be widely applied. Too many biosensors are needed for a good picture of the toxicity of a test site (Ghosh et al., 2017). Using in vivo bioassays is a bit more difficult because of the use of whole organisms it is less controllable (Hassan et al., 2016). Biomarkers can meet the criterion of good performance partly; there are many variables possible when using biomarkers so reproducibility is difficult. EDA and TIE are both laborious techniques and in the phases of sample preparation and fractionation contaminants can disappear from the sample (Hong et al., 2016). Biosensors and BEWS are performed in situ making it difficult to meet the quality standards.

3. *The implementation and applicability of the EBM should be easy and done by routine laboratories*

Applying EBMs at large scale would mean that the test can be done according to standardized protocols by routine laboratories with no specialized trained personnel needed.
In vitro bioassays could meet this criterion. In vitro bioassays are simpler and faster than in vivo bioassays (Ghosh et al., 2017). In vivo bioassays are more labor-intensive and require specialized equipment and operator skills (Hassan et al., 2016). The use of biosensors can be according to standardized protocols and in small test volume. However, they have to be placed in situ and therefore not every laboratory is suitable for applying biosensors (Bilal & Iqbal, 2019).
For some biomarkers standardized protocols are available but there are many biomarkers to measure (Connon et al., 2012). The use of transcriptomics, genomics, metabolomics and proteomic requires specifically trained personnel and cannot be done by routine laboratories

(Matich et al., 2019). Specialized equipment and operator skills are also needed for the use of EDA and TIE (Li et al., 2017).
Applying bioindicators and BEWS according to standardized protocols is not easy because the test set is in situ. In addition, both are monitoring techniques which means that it takes a long time to perform the test (Wieczerzak et al., 2016).

4. *Relevant and effective sampling of soil and groundwater samples*

Relevant and effective sampling means that soil and groundwater can be sampled in a reliable manner. Van der Oost et al. (2017a) state in their article that concentrations of CECs in water can vary. Depending on the type of soil and the groundwater flow, this also applies to soil and groundwater to a certain extent.
The EBMs that can currently be used for relevant and effective sampling of the soil-water system are in vitro and in vivo bioassays, EDA and TIE (Burgess et al., 2013; Domínguez-Rodríguez et al., 2020). Biomarkers are also suitable for the soil-watersystem but the relevance of the biomarker is not always clear (Connon et al., 2012). Biosensors are suitable for sampling water because it measures a flow. Application of biosensors for soil is still limited and requires more research (Nguyen et al., 2017).
Relevant and effective sampling using bioindicators and BEWS is not suitable for all types of soil and sites. Not every test site features suitable and well-defined bio-indicating plants and animals (Wieczerzak et al., 2016).

5. *A good translation of measured effects into actual risks in practice*

The translation of the testoutcome into practice is a challenge for EBMs. With the use of whole organism bioassays the outcome of the test could be best translated into practice. Although, the test takes place in a laboratory under controlled conditions, other factors are excluded (Wieczerzak et al., 2016).
For the other kind of EBMs it is more difficult to translate the outcome of the test into practice. The test with bioindicators or BEWS will take place in situ and could be influenced by other factors than contaminants. The translation of the effects into practice measured with in vitro bioassays as well as with biomarkers could be difficult. A positive response in an in vitro bioassays could not always be associated with a risk to humans or the environment (Brunner et al., 2019). A very few biomarkers are currently understood well enough to make clear the risks (Connon et al., 2012). The environmental relevance of biosensors is small because only a whole cell or micro-organism is used (Hassan et al., 2016).

6. *Cost effectiveness*

For determining cost-effectiveness the absolute costs have not been used. Nor has any comparison been made with chemical analysis of CECs. Assumptions have been made about the relative costs compared to the other EBMs. In situ tests will be low in costs because no laboratory or expensive equipment is needed just personnel. That will be the case for the monitoring EBMs; bioindicators and BEWS. In vitro bioassays are less expensive than in vivo bioassays because of the use of (parts of) cells compared to whole organisms. Biosensors have yet not been commercialized in the environmental field so they are expensive now (Sadana & Sadana, 2015). The use on a larger scale should help keep the cost of biosensors down. The cost-effectiveness of the use of biomarkers depends on what kind of biomarkers are used. Relative new techniques such as transcriptomics, genomics, metabolomics and proteomic are expensive but it also applies that deployment on a larger scale reduces costs (Alpern et al., 2019). EDA and TIE are relatively expensive methods because of the combination of biological and chemical analysis and sample preparation and fractionation (Burgess et al., 2013).

7. *Decisions can be made about measures when the EBM indicates risks, even without knowing the specific drivers of the risk*

According to Brack et al. (2019) this criterion means that EBMs, for example in surface water upstreams and downstreams of effluents, can be used to indicate the difference between two sites. This can also be applied to the soil-water system in a similar way. It is not necessary the individual contaminants to be known because the results of the EBMs are then compared. EDA and TIE are the best methods for taking measures on the results of the tests because of the drivers of effects are known. The individual contaminants are not known but the classes of contaminants are (Beyer et al., 2014). Because of the use of whole organisms in vivo bioassays are better to make decisions on the results than in vitro bioassays which measure specific effects such as genotoxicity. The risks of measured effects with in vitro bioassays are not clear. That is also the case for biomarkers. Very few biomarkers are currently understood well enough to assess the risks to humans or the environment (Connon et al., 2012). For the monitoring EBMs bioindicators and BEWS decisions could only be made when other external factors are excluded. The environmental relevance of biosensors is low so it is difficult to make decisions when a biosensor measures an effect (Hassan et al., 2016).

Table 2. EBMs tested against criteria

Criteria	1	Broad spectrum of CECs	2	Quality standards	3	Implementation and applicability
EBMs						
Bioindicators	5	All potentially toxic contaminants are involved	1	It is difficult to meet the requirements of reproducibility, accuracy, sensisitivity because the setting is in situ and different for every case	1	In situ
Biological early warning systems (BEWS)	5	All potentially toxic contaminants are involved	1	It is difficult to meet the requirements of reproducibility, accuracy, sensisitivity because the setting is in situ and different for every case	1	In situ
In vitro bioassays	5	All potentially toxic contaminants are involved	5	Very sensitive and specific	5	It is simpler and faster than in vivo bioassays
In vivo bioassays	5	All potentially toxic contaminants are involved	4	Because of the use of whole organisms it is a bit more difficult to meet the quality standards than with in vitro bioassays	3	It require specialized equipment and operator skills, and is labor intensive
Biosensors	5	All potentially toxic contaminants are involved	5	It is a rapid, sensitive, real-time, on-site detection and analysis in the field	3	Biosensors have to be placed in situ but could be perfomed according to standardized protocols, in a small test volume
Biomarkers	5	All potentially toxic contaminants are involved	4	It is not easy to apply on a large scale because there are many variables and possibilities	2	Standardized protocols are not available and difficult to make. Especially transcriptomics, genomics, metabolomics and proteomic requires specifically trained personnel and is not simple to undertake
Effect-directed analysis (EDA)	4	Emphasis on organic contaminants	4	The technique is laborious and therefore not easy to implement	1	It require specialized equipment and operator skills and extensive laboratory facilities
Toxicity identification evaluation (TIE)	5	All potentially toxic contaminants are involved	4	The technique is laborious and therefore not easy to implement	1	It require specialized equipment and operator skills and extensive laboratory facilities

Criteria	4	Relevant and effective sampling	5	Translation testoutcome into practice	6	Cost effectiveness	7	Decisions on measures
EBMs								
Bioindicators	2	It is not suitable for every place and soil type	2	Other factors could influence the results because it is in situ	5	In situ	3	Decisions could be made when other factors are excluded
Biological early warning systems (BEWS)	2	It is not suitable for every place and soil type	2	Other factors could influence the results because it is in situ	5	In situ	3	Decisions could be made when other factors are excluded
In vitro bioassays	5	Suitable for the soil-watersystem	2	Interpretation of effects could be difficult	4	Less expensive than in vivo bioassays	3	It is not clear for all kinds of effects
In vivo bioassays	5	Suitable for the soil-watersystem	5	Environmental relevant	3	Expensive because of the use of whole organisms	4	The effects could better explained than with in vitro bioassys because of the use of whole organisms
Biosensors	1	It measures a flow. At this moment not suitable for soil	2	It measures specific contaminants and environmental relevance is small	3	Low costs	2	Ecological relevance is low and risks are not clear
Biomarkers	4	There are many variables and is therefore not always effective	2	Very few biomarkers are understood well enough and not all organisms are equally sensitive	3	It depends on what kind of biomarkers are used	3	Very few biomarkers are understood well enough
Effect-directed analysis (EDA)	5	Suitable for the soil-watersystem	2	Use of in vitro bioassays. The effects are linked to contaminants	1	The combination of bioassays and chemical analysis	5	The drivers of effects are known
Toxicity identification evaluation (TIE)	5	Suitable for the soil-watersystem	5	Use of in vivo bioassays. The effects are linked to groups of contaminants	1	The combination of bioassays and chemical analysis	5	The drivers of effects are known

Fully meets the criterion	5
Largely meets the criterion	4
Not clear; partly meets the criterion/partly does not meet the criterion	3
Largely does not meet the criterion	2
Does not meet the criterion at all	1

3.1.3. EMBs which have the greatest potential to be applied in the soil-water system

In section 3.2. the different EBMs have been tested against seven criteria. There are several ways to interpret the results.

One of the ways is to add up all scores for each EBM, see table 3. From this it can be deduced that in vitro bioassays and in vivo bioassays have the highest score. These EBMs would have the greatest potential to be applied in the soil-water system according to this way of interpretation.

Table 3. Total scores on the different criteria

EBM	Scores	Total
Bioindicators	5+1+1+2+2+5+3	19
BEWS	5+1+1+2+2+5+3	19
In vitro bioassays	5+5+5+5+2+4+3	29
In vivo bioassays	5+4+3+5+5+3+4	29
Biosensors	5+5+3+1+2+3+2	23
Biomarkers	5+4+2+4+2+3+3	23
EDA	4+4+1+5+2+1+5	23
TIE	5+4+1+5+5+1+5	26

Another way is to assume that not all criteria are equally important. In line with the requirements for current soil investigation in the Netherlands, it can be deduced that the following criteria are most important: 2) quality standards, 3) implementation and applicability, 5) translation risks into practice, and 7) decisions on measures (SIKB, 2016). No EBM fully meets these requirements based on the analyzes of the criteria. When the scores are added up for the above four important criteria, it can be deduced that in vivo bioassays achieve the highest score. In vitro bioassays and TIE also score high in this way of interpretation. See table 4.

If EBMs are applied in a different way than according to current soil investigation, for example according the WFD, other criteria may be more important.

From the interpretation of the scores on the seven criteria, it can be concluded that bioassays, both in vivo and in vitro, have the greatest potential to be applied in the soil-water system. The additional tests EDA and TIE could be valuable here, based on the results of the scores on the criteria.

Table 4. Total scores the main criteria. The highest score is shaded in green, the second-highest score is shaded in light green

EBM	Scores	Total
Bioindicators	1+1+2+3	7
BEWS	1+1+2+3	7
In vitro bioassays	5+5+2+3	15
In vivo bioassays	4+3+5+4	16
Biosensors	5+3+2+2	12
Biomarkers	4+2+2+3	11
EDA	4+1+2+5	12
TIE	4+1+5+5	15

3.1.4. Type of research questions concerning CECs arisen from the Dutch soil policy and the requirements and standards for soil research

In the Netherlands, but also in Europe, attention is mainly paid to CECs in the water sector and much less in other sectors (Lahr et al., 2014). Several major projects on emerging contaminants in water resources management subsidized by the European Union have been carried out in recent years, such as SOLUTIONS and NORMAN (Brack et al., 2012; Brack et al., 2015).

Dutch soil policy focuses on measuring concentrations of contaminants (Brand et al., 2013). EBMs are only used in a few cases. When, based on a site-specific risk assessment, it appears that there are potential risks, the Triade method is applied. When using this method, the contaminants are already known before EBMs are carried out.

No soil policy in the Netherlands focuses on CECs. The *Circulaire bodemsanering*, appendix 6 indicates how to deal with non-standardized contaminants. If the detection value for non-standard contaminants is exceeded, action must be taken by the initiator / competent authority. This can be the municipality, province or a company (Staatscourant, 2013). If the competent authority cannot assess the seriousness or urgency of contamination, the *Rijksinstituut voor Volksgezondheid en Milieu* (RIVM) can determine an intervention value ad hoc. In addition, the RIVM can propose 1) an ad hoc SRC eco (ecotoxicological Serious Risc Concentration); the concentration of a contaminant in the soil, above which the ecotoxicological criterion on which the intervention values are based is exceeded; 2) an ad hoc human SRC (Human Toxicological Serious Risc Concentration); the concentration of a contaminant in the soil,

above which the human toxicological criterion on which the intervention values are based is exceeded; 3) both of the above values. If both values have been derived, the lower of the two values is regarded as an ad hoc intervention value for soil (Staatscourant, 2013).
In the Netherlands, the term "substances of very high concern" (ZZS) is also used. The ZZS list can be used as a tool for companies and permit issuers to ensure that ZZS emissions to the environment are minimized. As of 1 January 2016, a minimization obligation applies to all ZZS. This means that these contaminants should be kept out of the environment as much as possible.
The list of substances of very high concern consists of a combination of the known lists such as the REACH regulation (SVHC substances, substances of very high concern), the OSPAR convention (priority action substances), the priority hazardous substances from the Water Framework Directive (WFD), substances from the CLP regulation (classification, labeling and packaging, substances class CMR 1A and 1B) and the POP regulation (substances in appendices I, II and III). If new substances are added to the lists, they will be included in the RIVM list with ZZS. As indicated above, the ZZS list of the RIVM is therefore larger than just the set of substances of the SVHC list of REACH (RIVM, 2020).
Soil investigation in the Netherlands is an environmental hygiene study into the chemical quality of the soil (and groundwater) at a specific location. Soil investigation has to be done when 1) applying for a building or environmental permit and 2) when there is suspicion the soil is contaminated or could be contaminated by activities (SIKB, 2019). In addition, an environmental hygiene statement is also needed for the soil on which or in which soil or dredging sludge is applied or for the soil or dredging sludge to be used (SIKB, n.d.). Each type of soil investigation has to meet a protocol, drawn up by the Royal Dutch Standardization Institute (NEN, n.d.). In a soil investigation, the soil or dredging sludge is analyzed according to a standardized package for contaminants. The standardized package contains the contaminants for which the chance of exceeding the background values is higher than 5%. The basis for this is a large-scale national study in which all analysis results available at laboratories are compared. There is a standardized package for 1) land soil investigation, 2) the water soil investigation and 3) the testing of soil and dredging sludge and groundwater investigation (SIKB, n.d.). When it is supposed that certain parameters may occur in deviating concentrations but which are not included in the standardized package, the standardized package must be extended with these parameters. If the preliminary investigation shows that there may be a soil load with contaminants that are not part of the standardized package, the investigation into the quality of the soil or dredging sludge or receiving soil must

be expanded to include contaminants that can be present (SIKB, nd). Currently, only chemical analysis to concentrations of contaminants is approved for soil samples in the Netherlands (SIKB, 2016). Only within the Triade method decisions can be made based on EBMs (RIVM, 2007).

3.1.5. Changes needed in Dutch soil policy for implementation of effect-based methods in the soil-water system

The answer to sub-question 5 is based on the conclusions of sub-question 3; in vitro and in vivo bioassays have the greatest potential to be applied in the soil-water system.
The use of EBMs for detecting CECs in the soil-water system requires an adjustment of the current policy in the Netherlands. This requires several steps for which the criteria from section 3.1.2. can be used for (criteria 2, 3, 5 and 7).

A representative study with bioassays requires a selection of bioassays based on the possible effects of relevant contaminants, environmental pressures or expected emissions (Brack et al., 2019; Brunner et al., 2020). Several studies have been conducted in the water sector to compile a set of bioassays on expected effects (Escher et al., 2014; Neale et al., 2017). A set of bioassays should have sufficient sensitivity and distinctiveness and respond to as many groups of contaminants as possible (Wernersson et al., 2015). A set of bioassays could be selected case-by-case. Such a recommendation is also made in the Water Framework Directive (WFD). It states that an optimal set of tools varies on a case-by-case basis (Wernersson et al., 2015). Both a combination of in vitro and in vivo bioassays are used.
As in the current soil policy in the Netherlands, a division is made into soil quality classes. For example, the housing class has different requirements than the industry class (Bodemplus, 2008). The choice for a set of bioassays could be aimed at this.
Standardized protocols must be developed to apply bioassays within the soil policy. The Organization for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO) have already issued standards for the implementation of a large number of bioassays (ISO, 2019). The protocols will have to be formally validated, just as the implementation of the chemical analysis has been validated (SIKB, 2016).
When the application of bioassays is included in the soil policy, an agreement must be reached on what level of bioassays response in soil and soilwater samples is acceptable. So standards will have to be drawn up for every type of bioassay. It should be taken into account that the results of the different bioassays cannot be compared with each other, every bioassay

has different characteristics. Also, not every measured effect will harm ecosystem and human health (Escher et al. 2018). Effect-based trigger values (EBT) are drawn up in the water sector, which distinguishes between acceptable and poor water quality (Escher et al., 2018). In this way, a statistical translation is made between the effects measured with in vitro bioassays and the risks for humans or the environment. The EBTs are derived from existing concentration based environmental quality standards for individual contaminants in combination with measured effects with bioassays (Brack et al., 2019). It could also be drawn up for the soil-water system.

The soil policy can be as follows:

- No exceedances of the EBT measured with the set of bioassays: soil can be used for every use function
- Exceedances above a set target value of the EBT measured with the set of bioassays: soil is not applicable for all types of use
- Exceedances above a set action value of the EBT measured with the set of bioassays: further research into which contaminants are responsible for the measured effect.

Xu, Wei, Wang, Bai & Du (2020) have reviewed several methods to assess the toxicity of water samples based on bioassays. One of these methods is according to the toxicity unit classification system of Persoone et al. (2003). In two steps, the acute toxicity of samples is determined with a set of bioassays that could evaluate the water quality. In the first step, the toxicity is determined on undiluted samples. In the second step, toxicity tests are performed on a dilution series of the samples, using the tests that gave more than 50% effect in the undiluted sample. The results obtained with each bioassay are subject to a value which is described in toxicity units according to the formula TU = 100 / EC50 (TU is toxicity units and EC is the concentration at which there is 50% effect). This value is expressed as the percentage effect of each bioassay to the samples, such as reproduction and inhibition of growth. The results are divided into classes according to Persoone et al. (2003), see table 5. Such a method could also be applied to the soil-water system.

In summary, the implementation of EBMs in the Dutch soil policy means: selection of bioassays, standardized protocols and drawing up standards for the results of the measurements.

Table 5. The toxicity classification based on Toxity Units (TU) values from Persoone et al. (2003)

Class	Toxicity	Description
Class I	No acute toxicity	None of the tests shows a toxic effect
Class II	Slight acute toxicity	The effect percentage observed in at least one toxicity test is significantly higher than that in the control but is below 50% (<1 TU)
Class III	Acute toxicity	The EC50 is reached or exceeded in at least one test, but in the 10-fold dilution of the sample the effect is less than 50% (=1–10 TU)
Class IV	High acute toxicity	The EC50 is reached in the 10-fold dilution for at least one test but not in the 100-fold dilution (=10–100 TU)
Class V	Very high acute toxicity	The EC50 is reached in the 100-fold dilution for at least one test (≥100 TU)

3.2. Workshop

3.2.1. Insights and recommendations from practitioners knowledge

The following tables give an overview of the different answers of participants to the three questions for each of the cases. The first column of the tables indicates to which group the participant belongs. The answer shaded in green is discussed during the workshop. A description of the discussion is provided below the tables.

Table 6. Answers of participants to the three questions for the first case

Case 1. Moving soil	
1. How could EBMs be useful in this case?	
Practitioner	It is depended on the size of the deposit and its future application Using EBMs in this case is very hypothetical because it is always known what the origin of the soil is
Practitioner	EBMs are useful when the soil is applied to a sensitive destination (e.g. a nature reserve or vegetable garden) and if it concerns a large batch of soil. It often concerns different batches with different origins that are used, which may also lead to combination toxicity
Policy officer	Because there are no standards for all CECs (too little knowledge about CECs and its effects on humans and the environment). EBMs could measure immediately the effects of both standardized contaminants and CECs
Policy officer	For assessing the trend or condition of a nature reserve. It has less value for regular application of earth moving or making a site suitable for allotment
2. What are the advantages and disadvantages of using EBMs in this case?	
Practitioner	Advantage: - It gives insight in characteristics of a batch of soil Disadvantage: - Missing standards for EBMs
Practitioner	Advantage: - Knowing whether a possible effect is to be expected Disadvantage: - It often concerns multiple batches of soil from different origins, so the effects can still be different than the EBM has shown
Policy officer	Advantage: - The effects are known immediately because you skip the standardization step Disadvantages:

	- Long-term effects may be missed because those effects are not yet visible. The reliability of the EBMs must also be of sufficient quality - Decisions have to be made if there is a negative effect. If this is not properly arranged, the EBM may not be taken
Policy officer	Advantage: - The effects are known immediately. No model interpretation. - Broad screening instead of measuring at substance level Disadvantage: - Missing standards for EMBs - No regional policy - Knowledge of business (advisory and executive) and the government is limited. Additional costs
Practitioner	Advantage: - It gives also insight in effects of unknown contaminants. - Combination toxicology - EBMs give a more realistic view than chemical analysis of a limited set of contaminants Disadvantage: - The drivers of a measured effect are often unkown
3. What is needed to implement EBMs in addition to or instead of doing standardized soil investigation in the soil-water system?	
Practitioner	Standards based on a biofunction background value / living / industry (biological soil quality map)
Practitioner	Development of a good, quick and inexpensive research method. Development of policy how to deal with negative results from EBMs
Policy officer	Time and resources could be limited for further development and implementation
Policy officer	In the future EBMs will be implemented alongside standard soil testing. To be able to use EBMs in practice, standards must be set for each EBM must be set (depending on the location and use). For example, the degree of hormone disruption in a batch of soil when applied in agricultural areas.

During the workshop the last question was addressed. The question was: 'What is needed to implement EBMs in addition to or instead of doing standardized soil investigation in the soil-water system?'. A practitioner working in soil consultancy gave the following answer: 'Standards based on a biofunction background value / living / industry (biological soil quality map)'. The specific question asked during the workshop was what these standards should be based on. According to the practitioner the most important thing standards have to be based

on is to prevent earth moving from coming to a standstill. Sample matrix (clay, peat, sand, dredging sludge) will already give very different results based on properties with EBMs.
The reactions of other participants were (the group to which the participant belongs is given in brackets after the answer):

- For water quality, so-called effect/signal values have been derived for various objectives. That could also be the case for soil. Mapping the relevant exposure for human is essential (researcher)
- There is a big difference in standards for ecological risks and human risks. For humans, the risks of contaminants in the soil are very different because human do not live in the soil and are not directly exposed to it (researcher)
- Standards based on poisoning are also difficult to trace for the ecosystem (practitioner)
- What about the standards related to the reliability of the measurements? (practitioner)
- In absence of reliability: that is why large uncertainty margins are usually used (standard set much lower) (researcher)

Case 2. An allotment garden

Table 7. Answers of participants to the three questions for the second case

Case 2. An allotment garden	
1. How could EBMs be useful in this case?	
Policy officer	Function-specific testing of the soil quality is possible with EBM. It creates a clear picture of the possibility of performing a certain function (allotment) on / in the relevant soil
Practitioner	Indicate whether measured concentrations are a problem for the function of the soil.
Policy officer	It is (currently) difficult and (needlessly) expensive to investigate the presence of the large number of contaminants that can pose an increased risk.
2. What are the advantages and disadvantages of using EBMs in this case?	
Policy officer	Advantages: - A targeted EMB says much more about the suitability of the soil for one specific function and intended use - Combination toxicology - In borderline cases (a measured content of 0.1 mg below a limit value) an EBM can decide whether or not soil is suitable for a certain function. Disadvantages:

	- An EBM must be performed separately for each location and the function must be defined very well (it have to be known to what question the EBM is performed) - An EBM may raise more questions among users / citizens than it does gives answers, the matter is more complicated than "just testing against standards" and thus more complicated to communicate
Practitioner	Advantage: - The effects of the soil are known Disadvantage: - The cause of the effect does not have to be related to an expected contaminant
Policy officer	Advantage: - It gives a quick indication whether an effect can be expected at a certain level of ecology without first performing a broad standard test Disadvantage: - If an effect is measured, can this be extrapolated to crop cultivation or use of the location? - If no effect is measured, is a possible effect on humans excluded?
3. What is needed to implement EBMs in addition to or instead of doing standardized soil investigation in the soil-water system?	
Policy officer	- EBMs and standard soil investigation must be applied side by side, one does not exclude the other but complements the other - A knowledge platform about the use of EBMs, protocols for performing EBMs - High-throughput and financial feasibility of EBMs - A very clear and well-defined question prior to the EBM - EBMs can be used as a first basis for standards to be developed
Practitioner	Standards for effects have to be defined. The background values of a contaminant must be taken into account
Policy officer	Cooperate with the water sector because they already have more experience with EBMs. To do pilots before entering EBMs

During the workshop the first question was addressed. The question was: ‘How could EBMs be useful in this case?’. A policy officer working as advisor in the soil sector gave the following answer: ‘function-specific testing of the soil quality is possible with EBMs’. The explanation was with EBMs a clear picture is created of the possibility of performing a certain function (allotment) on or in the relevant soil. For what purpose do you want to use an allotment and one should focus the EBM on it, e.g. growing crops for consumption or recreation only.

The reactions of other participants were (the group to which the participant belongs is given in brackets after the answer):

- Focus EBMs on the function you want to perform on a specific piece of land. EBMs can broaden the scope of usage applications. It can be used in addition to standard studies because otherwise you create blind spots (practitioner)
- Comply with existing regulations as much as possible, such as the current classification in quality classes (practitioner)
- One crop can be more sensitive than the other (practitioner)
- It is very important for what purpose an EBM is done and especially from which role (policy officer)
- It is additional information for an overall view (practitioner)

Case 3. Water-collection area

Table 8. Answers of participants to the three questions for the third case

Case 3. Water-collection area	
1. How could EBMs be useful in this case?	
Practitioner	Very useful through sampling surface water
Practitioner	EBMs can only yield something location-specific and over a longer period. Measurement in both source (surface water) and path / object (soil / groundwater) is relevant
Practitioner	EBMs can indicate that something is happening at the location that affects what you perceive
Researcher	EBMs and non-target screening (NTS) can be used as screening methods, in addition to the chemical-analytical methods already used in the drinking water sector. EBMs can demonstrate the presence of unknown contaminants based on the effects that these contaminants may cause in bioassays. The tests can be selected for human health and / or ecological status
Practitioner	EBMs can provide an orienting picture whether there are toxicologically relevant effects on critical endpoints
2. What are the advantages and disadvantages of using EBMs in this case?	
Practitioner	Advantage: - Measurements indicate the harmful contaminants for humans and animals
Practitioner	Disadvantage: - Expensive - How to determine the cause (source) of the measured effect

Practitioner	Advantage: - It becomes clear that something is going on Disadvantage: - It is not known if the measured effect is caused by contamination
Researcher	Advantage: - Effects can also be made visible for (mixtures of) unknown contaminants (provided that the correct tests are applied) Disadvantage: - It is not immediately clear which contaminants are involved (this requires further research, for example through a combination of chemical analyzes, based on knowledge about individual substances and / or effect-directed analysis).
Practitioner	Advantages: - Combination toxicology - A useful tool to get a first impression of possible contamination (provided that the correct bioassays are chosen) - Relatively sensitive Disadvantages: - There is no legal framework for interpretation - Are the correct critical endpoints being considered? - Sample preprocessing and processing is complex - The relationship between bioassay response and risk is obscure
3. What is needed to implement EBMs in addition to or instead of doing standardized soil investigation in the soil-water system?	
Practitioner	Defining standards
Practitioner	Determining the reference value (what is the definition of an undesired effect) and how do you determine whether changed effects cannot be the result of ecological processes
Practitioner	Formulation of hypotheses for expected behavior in the soil of contaminants from certain contaminant groups and which risks can be expected Development of standards for using EBMs
Researcher	Clear guidelines for the selection of endpoints and methods, and the interpretation of the measurement data
Practitioner	EBMs can be used complementarily to, for example, identify hotspots to carry out further (standard) soil investigations. It is important to obtain an additional picture of the significance of a response. A clear perspective for action is therefore a precondition. There must be consensus about the battery of bioassays being applied (and in what situation)

During the workshop the last question was addressed. The question was: ‘What is needed to implement EBMs in addition to or instead of doing standardized soil investigation in the soil-water system?’. A practitioner gave the following answer: ‘EBMs can be used complementarily to, for example, identify hotspots to carry out further (standard) soil investigations. It is important to obtain an additional picture of the significance of a response. A clear perspective for action is therefore a precondition. There must be consensus about the battery of bioassays being applied (and in what situation)’. During the workshop a specific question was where would you take those samples for measuring effects. A researcher answered that it is not about where the measurements are taken but how. For EBMs, it means a good selection of tests and pre-treatments and making agreements about them. EBMs can also be used as a kind of screening and being supplement to other standard tests. Knowledge about background values is indeed useful but close attention have to be paid to the impact of pre-processing.

The reactions of other participants were about the usefulness of EBMs (the group to which the participant belongs is given in brackets after the answer):

- Are EBMs suitable for assessing an individual case? Precisely because standards or comparison is lacking. EBMs can be applied for monitoring because changes over time can be compared and action can be taken where it is needed (policy officer)
- Measuring effects should be done if it is known that a CEC is present (to be able to make a risk assessment). By doing a broad screening, you will look for problems. It will become more complicated. It is interesting from an academic point of view, but in practice (for earthmoving) the results of EBMs can lock it completely (practitioner)
- There is a lack of awareness of what it means when standards are set by the government by measuring smaller quantities and more contaminants (practitioner)
- For many contaminants, no standard has been derived. Applying EBMs is useful then (provided you have derived a standard for this in the future) (policy officer)
- The aim is not to measure more and more contaminants. EBMs can provide a much more realistic picture of unknown contaminants not measured by default but that are potentially harmful (policy officer)
- Still struggling to translate EBMs and risks to reality if it is unknown which contaminants cause effects. Interpretation of effects is very difficult due to the heterogeneity of contaminants in the soil (practitioner)

3.2.2. Summary of the main outcomes of the workshop

Question 1. How could EBMs be useful in this case?

Using EBMs in the case of moving soil is very hypothetical because the origin of soil is always known. EBMs can be useful when applying soil to a sensitive location. In addition, EBMs can be valuable to reflect a trend or condition of a nature reserve. EBMs are useful because it is impossible to analyze all contaminants individually.

Question 2. What are the advantages and disadvantages of using EBMs in this case?

Advantages: It gives a quick indication whether there is an effect or risk. Combination toxicology.

Disadvantages: Standards are missing for EBMs. There is no policy for the application of EBMs. The interpretation of effects could be difficult. The drivers of effects remain unknown.

Question 3. What is needed to implement EBMs in addition to or instead of doing standardized soil investigation in the soil-water system?

The development of a policy on how to deal with the results from EBMs (determining a reference value). Standards must be developed for EBMs.

It should be applied alongside standard soil tests (chemical analysis). It is preferable to cooperate with the water sector because they have already more experience with the use of EBMs.

There are no major differences in outcomes between the different cases. The main concern in the case of moving soil is the results of EBMs will lock it up. In the allotment case, the main focus was on the specific functions of the soil in relation to the tests to be carried out. In the case of water-collection area, EBMs were mainly seen as a screening method.

4. Discussion

This section discusses the study and gives an understanding of the results. The validation of the methods, theoretical and practical implications, and limitations of the study are described.

Validation of the methods

The different EBMs were investigated with a systematic scientific literature review. Three review studies have been used to provide an overview of all EBMs (Allan et al., 2006; Connon et al., 2012; Wieczerzak et al., 2016). They also have investigated the various options for using bioanalytical tools in the detection of contaminants and their ecotoxicological effects to the environment. The focus of Allan et al. (2006) and Connon et al. (2012) was on contaminants in water, EBMs that are specifically applicable to the soil could have been missed. In addition, the studies by Allan et al. (2006) and Connon et al. (2012) are already relatively old. Newer EBMs could therefore also have been missed.

The criteria used to assess the suitability of each EBM have been derived from scientific studies that focus on water and the environment in general (Allan et al., 2006; Brack et al., 2019; Busch et al., 2016; de Baat et al., 2019; van der Oost et al., 2017a). All seven criteria used in this study were used by van der Oost et al. (2017a). They have derived the criteria from the European Union's requirements for bioanalytical methods in screening of feed and food for dioxin-like chemicals. There is no general list of criteria used to test suitability of research methods in soil or in the environment. Because the EBMs in the different sectors may be performed in different ways and are therefore interpreted in different ways, a comparison with other studies is difficult.

A workshop was used as a method to obtain the knowledge of practitioners. A workshop is not often used in scientific studies, which makes comparisons with other studies difficult. In this study, a workshop turned out to be a well-designed method for gathering knowledge from many different people in a relatively short time. The workshop was attended by 27 participants, 24 of which were policy officers and practitioners. Although the composition of the group was varied, it is difficult to estimate whether the group of practitioners is representative of all practitioners in the Netherlands. In addition, it is not clear whether these participants had the right expertise because EBMs are currently not widely used in Dutch soil policy. In addition, only 16 of the 27 participants have given their opinion during the workshop. That could be the people who are most familiar with the subject and possibly very much in favor or against using EBMs. Not everyone dares to express their opinion, which could be even stronger in an online session than in a physical meeting. To prevent that not every participant of the workshop would give his opinion or that certain participants would dominate, information was already collected prior to the workshop. This made it possible to form a picture of the insights of practitioners in advance. Another way to collect practitioners knowledge is through a survey. More people can be reached with a survey. A disadvantage of a survey is that participants do not hear the views of other participants. On the other hand, the number of participants in a workshop is lower than in a survey. By better mapping the knowledge and points of view of participants, a workshop could be given several times with different participants. Also, more practitioners from the water sector could be involved in a workshop because they may have more expertise from EBMs.

Theoretical and practical implications and limitations

The classification of EBMs in biomonitoring and bioanalytics by Wieczerzak et al. (2016) was used as a guideline in this study. The literature review revealed that the collective name EBMs is used in different ways and that multiple classifications are possible. According to Brack et al. (2019), EBMs are bioanalytical methods using in vitro and in vivo bioassays, while the review studies by Allan et al. (2006), Connon et al. (2012) and Wieczerzak et al. (2016) use the term EBMs for all methods that measure effects including bioassays. Van der Oost et al. (2017a) makes a division into biomarkers of organisms exposed in the field and bioassays with laboratory organisms or cell lines exposed to samples. For example, biomarkers can also be performed with bioassays (Ghosh et al., 2017). Bioindicators and biomarkers are sometimes used interchangeably, while Wieczerzak et al. (2016) makes a clear distinction between the two methods. Biomarkers can be used as an early warning system, suggesting overlap with BEWS (Allan et al., 2006; Martinez-Haro et al., 2015). These differences are not important in assessing individual EBMs. However, because the distinction is not always clearly given in scientific research, confusion can arise as to which type of EBM is meant. This can have consequences for the assessment against the various criteria, because the examples mentioned score differently. For example, biomarkers score higher on the criteria than BEWS. Biomarkers are changes at the level of organisms, while BEWS can also mean changes at the population level. If BEWS is always carried out on the basis of biomarkers, BEWS is more applicable in the soil-water system than how they now meet the criteria.

In vitro and in vivo bioassays seem to be most applicable in the soil-water system. Much research has been done into the application of bioassays, whether or not in combination with EDA or TIE. Half of the articles found concerned studies into the application of bioassays, whether or not in combination with EDA or TIE. The other EBMs have been studied less because they seem less applicable at first glance. It is unclear whether they are actually less applicable. Perhaps too little research has been done on this.

Although a lot of research has been done on the application of bioassays (Brack et al., 2019; van der Oost et al., 2017a), Allan et al. (2006) suggest that there is not one method that is suitable for all cases in detecting CECs. According to Allan et al. (2006), the choice of a method depends on the information required. This approach seems difficult to implement in the current soil policy since the current soil research is standardized (SIKB, n.d.). Standardization of research and reproducibility seems to be one of the most important requirements for soil research in the current soil policy (NEN, n.d .; SIKB, n.d.). The EBMs for which standardization and reproducibility are easy to implement better meet the criteria. These are mainly the EBMs that can be applied in the laboratory. While the ecological relevance of the EBMs performed in the laboratory is lower than the EBMs performed in situ such as bioindicators and BEWs (Connon et al., 2012). The higher ecological value of an EBM can be important in assessing whether CECs have negative effects on the environment. Although, when using bioindicators and BEWS, consideration must be given to the influence of external factors which in turn makes interpretation of the results more difficult (Wahshsa et al., 2017).

The literature study shows that the criterion "translation measured effects into practice" is a difficult point when applying EBMs. It was also found in vivo bioassays can best translate the testoutcome into practice (Wieczerzak et al., 2016). But not every measured effect will harm ecosystem and human health (Escher et al., 2018). In the water sector effect-based trigger values (EBT) are drawn up, which distinguish between acceptable and poor water quality. At the moment no research has been done for a comparable translation of effects measured in the soil-water system. The translation of effects into practice also appears to be an important limiting factor in the application of EBMs for practitioners. The lack of standards for the measured effects appears to be a reason for practitioners not to apply EBMs in the future. This could be overcome by the application of EDA and TIE. In line with the current soil policy, these methods seem to be suitable because the drivers of certain effects are identified with EDA and TIE (Burgess et al., 2013). The question is to what extent the high costs, duration and laboriousness of these methods are a limiting factor, because targeted measures can be taken in response to the results of EDA and TIE. In order to avoid the problem of standards for contaminants and for measured effects, a choice can be made to strive for a certain ecological quality of the soil-water system without a standard being derived for each individual contaminant or measured effect. This requires a greater adjustment of the current soil policy and it will take more time and effort to achieve this.

5. Conclusions

This study has investigated the possibilities of using EBMs in detecting CECs in the soil-water system. The study provides an overview from scientific literature of the different EBMs which could be used. The different EBMs are assessed by criteria. The insight and recommendations from practitioners are obtained by a workshop.

The research question was:
How could effect-based methods be used in detecting contaminants of emerging concern in the soil-water system and how could these methods be integrated into Dutch soil policy?

This study shows different possibilities for using EBMs in detecting CECs in the soil-water system which could be divided in monitoring and analytical methods. Analytical methods were found to be most suitable in detecting CECs. Of these, in vitro and in vivo bioassays best meet the criteria set for soil research. To gain more insight into which contaminants cause the effects, the studies can be extended with EDA and TIE to determine the drivers of the effects. EBMs can supplement the current standard soil investigations to gain more insight into the occurrence and risks of CECs. Based on the literature review, the current soil policy and the practitioners knowledge it can be concluded that to implement EBMs in the Dutch soil policy, EBMs should be standardized. Standards should be drawn up for the measured effects. Additional research will be required to understand the measured effects in relation to possible risks to human and environment. Policy will have to be drawn up as to which (measured) effects are still acceptable and when measures must be taken or when follow-up research is necessary.

6. Recommendations

Based on the results of the literature study, the Dutch soil policy and the insights and recommendations of practitioners a number of recommendations can be derived.

1. At this moment the relationship between measured effects with EBMs and the potential risks to people and the environment is unclear. More research is needed into this relationship, especially focused on the soil-water system. The studies that have been conducted into the relationship between measured effects and potential risks mainly focus on water. The risks of CECs in the soil can be very different from the risks for, for example, surface water from which drinking water is obtained. In addition, the risks for humans and risks for the environment will also have to be considered. At a certain concentration of CECs in the soil, the effect may apply directly to earthworms and indirectly or not to humans.
2. The implementation of bioassays have to be standardized before a broad application can take place in the soil-water system.
3. Much more research has been done in the water sector and these results can serve as input for the soil-water system. Bioassays are already being used in the water sector to measure the biological quality of water (van der Oost et al., 2017a). The measured effects are therefore already translated into practice. The application of bioassays in water is now mainly focused on the toxicity of (a mixture) of substances on aquatic organisms. This approach could also be useful for the soil-water system and soil practitioners could learn from the water sector.
4. This research has shown that in vitro and in vivo bioassays are most suitable for application in the soil-water system. Other EBMs score less highly on the criteria but have also been studied less. It can be valuable to study also the other EBMs, because other EBMs, for example, have a higher ecological relevance. There are now also several conventional soil studies, all of which are applied. Various techniques are also possible with EBMs and perhaps it is better to assess which EBM is most suitable for each situation. For this, the other EBMs should be better investigated.
5. This study only looked at Dutch soil policy. In follow-up research it can be valuable to look more internationally. How do other countries deal with CECs in the soil-water system? Are EBMs used in other countries? Which EBMs? Soil practitioners in the Netherlands could learn from that. It is also possible to opt to pursue a more

international soil policy, such as in the WFD of the European Union (Wernersson et al., 2015).

7. References

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Appendix 1. Overview of systematic scientific literature review

Database	Keywords	Number of articles	Articles sort by:	Publication years	Number of abstracts assessed	Articles included	NB.	Articles
Performed on 19-11-2019								
WoS	Effect-based methods	1359	Relevance	2010-2019	100	8		Connon et al., 2012; Escher et al., 2015; Escher et al., 2018, Brack et al., 2018; Brack et al., 2019; Altenburger et al., 2019; Doyle et al., 2015; Brand et al., 2013
WoS	Effect-based methods soil	38	Relevance	2010-2019	38	1		Brand et al., 2013
WoS	Effect-based methods contaminants	25	Relevance	2010-2019	25	4 (1)	1 article from referencelist of another article	Altenburger et al., 2018; van der Oost et al., 2017; Li et al., 2018; Shao et al., 2019; Hollender et al., 2019
WoS	Effect-based methods pollutants	30	Relevance	2010-2019	30	3		Altenburger et al., 2018; Li et al., 2018; Altenburger et al., 2019;
WoS	Effect-directed analysis	401	Relevance	2010-2019	100	11		Weiss et al., 2011; Weller, 2012; Brack et al., 2016; Burgess et al., 2013; Altenburger et al., 2015; Tousova et al., 2017; Simon et al., 2015; Devier et al., 2011; Brack et al., 2018; Brack et al., 2015; Hong et al., 2016
WoS	Effect-directed analysis micropollutants	22	Relevance	2010-2019	22	3		Tousova et al., 2017; Brack et al., 2015; Dopp et al., 2019
Sciencedirect	Effect-based methods contaminants	10879	Relevance	2015-2019	100	7		Oberg & Leopold, 2019; Aminot et al., 2019; Rasheed et al., 2019; Fischer et al., 2017; Martin-Pozo et

	of emerging concern							al., 2019; Gogoi et al., 2018; Matich et al., 2019;
Sciencedirect	Ecological effects contaminants of emerging concern	3696	Relevance	2015-2019	50	5		Matich et al., 2019; He et al., 2018; Rasheed et al., 2019; Maltby et al., 2018; Gogoi et al., 2018
Performed on 06-12-2019								
Sciencedirect	Emerging contaminants bioassays	1508	Relevance	2015-2019	150	27		Di Paolo et al., 2016; Xu et al., 2019; Altenburger et al., 2018; Tousova et al., 2019; Taheran et al., 2018; Vethaak et al., 2016;, Ghosh et al., Osorio et al., 2018; Gogoi et al., 2018; He et al., 2016; Pedrazzani et al., 2018; Abbas et al., 2019; Shao et al., 2019; Marshall et al., 2017; König et al., 2016; Iqbal, 2016; Wieczerzak et al., 2016; Li et al., 2016; Garbo et al., 2019; Pochiraju et al., 2019; Bilal & Iqbal., 2019; Dopp et al., 2019; Richter et al., 2015; Yang, 2017; Brunner et al., 2019; Martinez-Haro et al., 2015; Neale et al., 2017
Performed on 20-12-2019								
Sciencedirect	Emerging contaminants effect-based methods	21597	Relevance	2015-2019	250	15		Matich et al., 2019; Privisic et al., 2019; Lamastra et al., 2016; Aminot et al., 2019; Oberg & Leopold, 2019; Martin-Pozo et al., 2019; Naidu, Espana et al., 2016; McGinnis et al., 2019; Gogoi et al., 2018; Naidu, Jit et al., 2016; Richardson & Kimura, 2017; Rasheed et al., 2019; Fischer et al., 2017; Noguera-Oviedo & Aga, 2016; Osorio et al., 2018
Performed on 17-01-2020								
Sciencedirect	Bioassays soil	4134	Relevance	2016-2020	125	12		Domínguez-Rodríguez et al., 2020; Stapulionyte et al., 2019; Fajardo et al., 2019; Zhang et al., 2018; Guo et

								al., 2017; Delerue et al., 2019; Kim et al., 2018; Kim et al., 2020; Bhat et al., 2019; Aparicio et al., 2019; Iqbal, 2016; Wieczerzak et al., 2016;
Performed on 31-01-2020								
Sciencedirect	Biological early warning system soil	2326	Relevance	2015-2020	100	4		Wahsha et al., 2017; Parelho et al., 2018; Musculo et al., 2015; Lee et al., 2019; Edge et al., 2020
Sciencedirect	Earthworms emerging contaminants	766	Relevance	2010-2020	100	5		Djerdj et al., 2020; Gong & Perkins, 2016; Bilal et al., 2019; Naidu et al., 2016; Dong et al., 2012
Sciencedirect	Biosensor soil emerging contaminant	761	Relevance	2010-2020	50	4		Hoon Kim., 2019; Bilal & Iqbal, 2019; Naidu et al., 2016; Farré et al., 2012

Appendix 2. Short paper for participants of the workshop

De grote hoeveelheid (nieuwe) stoffen die in het milieu en daarmee ook in de bodem terecht komen vraagt om een andere aanpak dan de standaard stoffenmeting. Voor veel opkomende stoffen zijn nog geen normen afgeleid. Daarnaast ontbreekt er van veel opkomende stoffen kennis over de hoeveelheden, persistentie en verspreiding in het milieu. Bovendien zijn de effecten op mens en milieu niet altijd bekend.

Het lijkt onmogelijk om alle opkomende stoffen te analyseren en regelgevende criteria of milieukwaliteitsnormen voor al deze stoffen te maken. Daarbij zegt een norm niets over welke effecten een stof kan hebben op mens of milieu. Bovendien kan een stof onder de norm aanwezig zijn, maar door een mengsel met andere stoffen wel aanzienlijke effecten veroorzaken.

Effectmetingen zijn een andere, veelbelovende manier om (risico's van) opkomende stoffen te detecteren. Effectmetingen worden al in water toegepast als aanvulling op de chemische analyses, om de kwaliteit van oppervlaktewater en drinkwater te monitoren. In de bodem zijn effectmetingen met bioassays onderdeel van het bodemonderzoek Triade.

Met effectmetingen kunnen de aanwezigheid en risico's van opkomende stoffen aangetoond worden zonder dat exact bekend is welke stoffen (in welke concentraties) er in het monster aanwezig zijn. Effectmetingen met zowel in vitro als in vivo bioassays lijken voor de bodem het meest geschikt. In vitro bioassays bestaan uit cellen die specifieke effecten meten zoals hormoonverstoring en genotoxiciteit. In vivo bioassays bestaan uit volledige organismen en meten effecten zoals groei, voortplanting en sterfte. Er zijn aanvullende technieken beschikbaar om te detecteren welke stof voor een bepaald effect verantwoordelijk is.

Hoewel effectmetingen, evenals chemische analyses, ook nadelen hebben, kan deze andere manier van onderzoek een waardevolle aanvulling zijn op de huidige standaard bodemonderzoeken.

Na een inleidende presentatie gaan we in deze workshop in groepjes de toepasbaarheid en bruikbaarheid van effectmetingen onderzoeken aan de hand van een casus. Op basis van de geïdentificeerde kansen en belemmeringen voor het gebruik van effectmetingen in de praktijk, stellen wij binnen het POP-UP-project een advies op voor eventuele beleidsaanpassingen en techniekontwikkeling. Deelnemers van de workshop kunnen de bestaande kansen alvast mee naar huis nemen en verzilveren in hun dagelijkse praktijk.