

Corrosion protection products as a source of bisphenol A and toxicity to the aquatic environment



Etiënne L.M. Vermeirssen ^{a, *}, Conrad Dietschweiler ^b, Inge Werner ^a, Michael Burkhardt ^b

^a Swiss Centre for Applied Ecotoxicology Eawag-EPFL, 8600 Dübendorf, Switzerland

^b HSR University of Applied Sciences, Institute of Environmental and Process Engineering (UMTEC), 8640 Rapperswil, Switzerland

ARTICLE INFO

Article history:

Received 10 April 2017

Received in revised form

27 June 2017

Accepted 2 July 2017

Available online 3 July 2017

Keywords:

Construction materials

Epoxy resins

Leaching

Bisphenol A

Toxicity

CALUX

ABSTRACT

Steel components are typically treated with anti-corrosion coatings like epoxy or polyurethane resins to protect the integrity and functioning of steel. Such resins may contain substances, such as bisphenol A (BPA), that have caused concern in a human and environmental toxicological context. We investigated the release of toxicity from four anti-corrosion coatings used in hydraulic and civil engineering. Resins were applied onto glass plates and leachate samples produced by horizontally shaking the plates in water for 7 days. Two experiments were conducted, one with a 1 day and one with a 7 day curing period. Using a suite of bioassays, we tested samples for: agonistic and antagonistic effects on various mammalian nuclear receptors; inhibition of photosynthesis and growth in algae; inhibition of bacterial bioluminescence; and inhibition of water flea reproduction. Concentrations of BPA, bisphenol F and various BPA transformation products were determined by chemical analysis (LC-MS/MS). Bioassay results were evaluated using a scheme developed by DIBt (Centre of Competence for Construction, Berlin, Germany). Three products induced responses in one or more of the measured endpoints and toxicity profiles varied markedly in intensity across products. One product released high amounts of BPA which was associated with effects on nuclear receptor transactivation, requiring a more than 700-fold dilution for effect induction to fall below 20%. The same product was also the most toxic to water flea reproduction, requiring ca. 70-fold dilution for effects to fall below 20%. Another product was highly toxic in terms of bacterial bioluminescence, particularly after a shorter curing time, requiring a ca. 1/300-fold dilution for effects to fall below 20%. The third product required a 22-fold dilution for inhibition of water flea reproduction to drop below 20%. Results show that anti-corrosion coatings based on epoxy resins can be a source of toxicity to the aquatic environment. The fact that some products are more toxic than others highlights opportunities for the development of low risk formulations and products with better environmental performance. Finally, the DIBt scheme provides a useful starting point to develop further ecotoxicity guidelines for testing and data evaluation of leachates from construction materials.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Steel is an essential component in many types of construction and it is used in large amounts. Examples of steel construction elements are beams or plating of bridges, pillars and sheet piling. When steel is exposed to damp conditions or is in contact with water – especially water with elevated chlorine content – there is a high risk of corrosion. Consequently, corrosion protection is applied to maintain the structural integrity of steel; it ensures stability and safety of constructions over time and is an economic way to

preserve functioning, durability and safety of steel construction materials.

Corrosion of steel can be prevented in various ways (Hansson, 2011; Schweitzer, 2006; Poursaee, 2016). A frequently used method is the application of coatings, such as paints and resins. In Germany, anti-corrosion coatings are applied to ca. 80% of corrosion protected surfaces (Bayer et al., 2010). Amounts used in Switzerland are in the range of 315–490 tons (Burkhardt et al., 2015). A wide array of such coatings are available on the market and include: epoxy resins, two component-polyurethane, one component-polyurethane, poly(vinyl)ester resins and fluoropolymers coatings (Schweitzer, 2006; Qian et al., 2015).

Although paints and resins are a cost-effective way of protecting

* Corresponding author.

steel, some compounds used in formulations of corrosion protection products are associated with (eco)toxicological concerns. Bisphenol A (BPA), which has been shown to affect reproduction in mammals and other vertebrates (including fish; [Chow et al., 2012](#); [Vandenberg et al., 2012](#); [Wright-Walters et al., 2011](#)), is an example of a suspect ingredient used in corrosion protection product formulations. Some products can contain more than 50% of BPA or related compounds (e.g. BPF; [Schweitzer, 2006](#)). Furthermore, BPA can be supplemented as an additive to the hardener to improve product properties like processing ability or surface properties. This points to the possibility that anti-corrosion coatings may be a source of BPA related toxicity to the aquatic environment.

Recently, several national and international organisations started to address the issue of ecotoxicological effects caused by compounds that leach from construction materials. This includes reports on ecotoxicological testing of construction materials by CEN (European Organisation for Standardisation; [CEN, 2017](#)) and the German Federal Environment Agency ([Gartiser et al., 2016](#)) as well as a guideline developed in Germany by DIBt (Centre of Competence for Construction, Berlin, Germany; [DIBt, 2011](#)) to assess the impact of construction products on soil and ground water. Furthermore, international standards have been adopted to produce leachate samples from construction materials (e.g. [CEN, 2011](#)) which facilitates the possibilities for testing eluates with bioassays in a reproducible fashion. Currently, however, leachate samples are mainly used for chemical analysis and appraisal of chemical constituents as well as to determine basic parameters such as pH, TOC (total organic carbon) and conductivity. Although the functioning and effectiveness of polymer coatings on steel are well established, at the moment, little is known about the leaching of compounds from such coatings (or other materials used in construction, see e.g. [Vé Wiewel and Lamoree, 2016](#)). Moreover, little information on possible effects of eluates from polymeric steel coatings on e.g. bacteria, algae and water fleas exists at BAW (Federal Waterways Engineering and Research Institute, Karlsruhe, Germany; R. Baier, pers. comm.).

The main aim of our study was to explore the possible ecotoxicological effects of epoxy resin-based anti-corrosion coatings. To select relevant products, we first performed a market survey of product types used in Switzerland ([Burkhardt et al., 2015](#)). Out of a range of products, four were selected on the basis of market share and possible presence of compounds of concern (i.e. BPA). In the DIBt scheme for the environmental assessment of certain construction materials, a combination of bacteria, water flea and algae tests are specified as a suitable test battery for evaluating leachates from construction materials ([DIBt, 2011](#)). We used this test battery as a basis for our current investigation and extended the suite of tests with nuclear receptor transactivation assays ([van der Linden et al., 2008](#)). The main reason to include a CALUX (Chemical Activated Luciferase gene eXpression) panel was to cover possible effects caused by compounds such as BPA, known to be estrogenic, and to detect the potential presence of other endocrine disruptors. Eluates were analysed by means of LC-MS/MS to quantify BPA as well as bisphenol F (BPF) and BADGE (BPA diglycidyl ether); all of these are typical epoxy resin components; [Schweitzer, 2006](#)) and we determined EC50 (50% effect concentration) values for BPA and BPF for various tests. With this information, we could link possible effects in bioassays with BPA concentrations in the leachate samples by calculating toxic units ([van der Ohe and de Zwart, 2013](#)). Furthermore, as environmental quality standards (EQS) have been developed for BPA in surface waters, we used the measured BPA concentrations in eluates to assess the risk the tested products may pose to the aquatic environment.

Results from algae, water flea and bacterial tests were evaluated along the scheme suggested by DIBt. This scheme suggests a 20%

effect level as a threshold for significant effects and proposes maximum allowable dilutions required to reduce effects in algae, bacteria and water fleas below this 20% threshold. Although CALUX assays are not covered by the scheme, we also evaluated CALUX data at the 20% effect level.

2. Materials and methods

2.1. Selection of four relevant corrosion protection products

Market research was conducted for steel construction and steel water construction for Switzerland to establish corrosion protection product diversity, application data and information on sale volumes and prospects ([Burkhardt et al., 2015](#)). Out of the various available products, four epoxy resin-based products were selected after a prioritisation that focused on the potential for toxicity as well as market share. We evaluated chemical relevance by studying the technical and safety data sheets and took into account discussions with producers and BAW (responsible for product registration in Germany). Products were supplied by the producers and subsequently anonymised as Products 1 to 4.

2.2. Eluates of anti-corrosion coatings

Glass plates (8 by 12.5 cm; a surface area of 100 cm²) were used as inert substrate for epoxy resins. Epoxy resin applications typically involve the following layering: a steel base, a zinc-based primer and an epoxy resin coating. In this study we opted for an inert carrier (i.e. glass) as we aimed to address the leaching of toxicity from epoxy resins only. By selecting glass as a carrier, we reduced complexity and avoided possible confounding factors that could result from leaching when using more complex layering.

Products were prepared according to technical data sheets provided by the producers and applied using a brush (see [Fig. S1](#)). The required thickness of the product layer (300–800 µm) was achieved by taking into account the density of the components, the mixture ratio of the components and the (wet) weight of applied product. Resins were left to cure for 1 day (double the time to achieve touch dry according to product specifications). In a second trial, the curing time was 7 days (maximum drying time according to product specifications). Following curing, plates were weighed again to determine the dry weight of the applied product.

Leaching was performed by placing the coated glass plates in closed glass containers filled with 100 mL of nanopure water (conductivity < 5 µS/cm). The glass containers were placed in the dark and shaken at 30 rpm for 7 days at room temperature. To obtain sufficient amount of leachate for all bioassays as well as chemical analysis, 11 replicates were produced per product and replicate eluates were combined.

2.3. Chemical analysis of leachate samples

Basic chemical parameters that were measured included pH, electrical conductivity and TOC. In addition, chemical analysis was conducted by Bachema (Schlieren, Switzerland) to measure BPA, BPF as well as BADGE and two of its hydrolysis products BADGE·H₂O and BADGE·2H₂O. Briefly, samples were injected and separated using liquid chromatography. Identification and quantification of compounds was accomplished using electrospray ionisation in positive and negative modes and tandem mass spectrometry (LC-MS/MS, one mass for qualifier and another mass for quantifier). Quantification was further verified using standard addition to samples. The calibration of BPF was set with the isomer 4,4'-Bisphenol F. The limit of quantification (LOQ) of BPA and BPF was 1 µg/L each.

2.4. Bioassay suite

2.4.1. Nuclear receptor transactivation assays – CALUX panel

CALUX assays are based on the human osteosarcoma U2OS cell line to which exogenous receptors were transferred (e.g. human estrogen receptor alpha for ER α -CALUX; for further details see: van der Linden et al., 2008, Gijsbers et al., 2011 and van der Linden et al., 2014). Samples generated after a 1 day curing period were tested using six different CALUX assays: 1) ER α -CALUX (estrogenic effects); 2) anti-AR-CALUX (anti-androgenic effect); 3) anti-PR-CALUX (anti-progestagenic effects); 4) Nrf2-CALUX (oxidative stress); 5) P53-CALUX (genotoxicity); 6) PPAR γ 2-CALUX (insulin sensitivity). Samples after 7 days of curing were only tested with: ER α -CALUX, anti-AR-CALUX and anti-PR-CALUX (as no effects were observed in the other three CALUX assays).

CALUX assays were performed by BDS (Biodetection Systems; Amsterdam, The Netherlands) according to a recent ISO standard (ISO, 2014) which involves direct testing of aqueous samples (i.e. as opposed to testing of extracts or samples dissolved in DMSO). Samples were serially diluted in triplicate on 96-well assay plates. Furthermore, each CALUX plate was run with a dilution series of a receptor specific agonistic or antagonistic reference compound (e.g. 17 β -estradiol for ER α -CALUX; see SI for further details on reference compounds).

2.4.2. Inhibition of bioluminescence in bacteria – Microtox

The assay with *Aliivibrio fischeri* was performed as described in Escher et al. (2008). Nine parts of leachate sample were mixed with one part ten-fold concentrated assay medium. Subsequently a two-fold sample dilution series was made and 100 μ l of each dilution series was transferred in triplicate to a 96-well plate and mixed with 100 μ l of a bacteria solution. This allowed for a maximum sample concentration in the assay of 45%. A dilution series of 3,5-dichlorophenol served as the positive control on each plate and each plate contained eight negative controls (nanopure water).

2.4.3. Inhibition of photosynthesis activity and algal growth rates – combined algae test

The assay with *Pseudokirchneriella subcapitata* was performed as described in Escher et al. (2008). Leachate sample was mixed with concentrated assay medium and a two-fold sample dilution series was made (150 μ l) in triplicate on a 96-well plate and 150 μ l of algae culture in assay medium was added. This allowed for a maximum sample concentration in the assay of 44%. Each plate contained a duplicate dilution series of diuron (the reference compound in the test) and eight negative controls (nanopure water).

2.4.4. Inhibition of reproduction in water fleas – *Ceriodaphnia dubia* assay

Tests using *Ceriodaphnia dubia* were conducted according to ISO/CD 20665 (ISO, 2005) and AFNOR T90-376 (AFNOR, 2000) and performed by Soluval Santiago (Couvet, Switzerland). The dilution medium and control water was prepared by mixing 25% of Evian mineral water, 25% of Elendt M4 medium (Elendt and Bias, 1990) and 50% of deionised water, supplemented with selenium and vitamin B12. Food consisted of a mixture of yeast, digested fish flake suspension (TetraMin[®]) and green algae (*P. subcapitata* and *Chlorocella* sp.).

Water fleas from an in-house culture were exposed to dilution

series of samples and mortality (immobilisation) and inhibition of reproduction (Equation (1)) were assessed over 7–8 days. Each dilution was tested with 12 replicate containers containing 10 ml of test solution and one water flea each (20 replicates were used for controls). The maximum tested sample concentration was 90%. Dose-response curves were fitted with Prism (GraphPad Prism[®], GraphPad Software, La Jolla, USA) to determine the 20% effect level of inhibition of reproduction or the LC20 (lethal concentration for 20% of the population at 7/8 days).

$$\text{inhibition (\%)} = \left(1 - \frac{\text{average n offspring in sample}}{\text{average n offspring in control}} \right) \times 100 \quad (1)$$

2.5. Establishing 50% effect concentrations for BPA and BPF

For CALUX assays, aqueous stock solutions of BPA and BPF were used (10^{-4} M with ca. 1% ethanol content) and for algae and bacteria, ethanolic stock solutions were used (2×10^{-3} M). Dilution series were tested with three technical replicates and dose-response data from BPA and BPF were used to determine EC50 values for both compounds. To derive the EC50 for BPA and water flea reproduction we extracted data from the literature (Tatarazako et al., 2002) and fitted a dose-response curve (see Fig. S2).

2.6. Data evaluation of in vitro assays

We used Prism to fit dose-response data of reference compounds (positive controls) to a log logistic function (Equation (2)). Subsequently, all data (i.e. data from reference and samples) were normalised using the control data as the anchor point for the bottom (0% effect) and the maximum fit for the top (100% effect; Equation (3)). Next, all data were refitted with Equation (2) to derive dose-response curves of the normalised data. Control data, typically $n = 8$, were also used to establish LOQs as 10-fold the standard deviation of the controls.

$$\text{induction} = \text{bottom} + \frac{(\text{top} - \text{bottom})}{1 + 10^{(\log(\text{EC}50) - \log(\text{concentration})) \cdot \text{slope}}} \quad (2)$$

$$\text{normalised effect (\%)} = \frac{(\text{induction} - \text{bottom})}{(\text{top} - \text{bottom})} \times 100 \quad (3)$$

From the dose-response curves, we interpolated the required sample dose to reach a 20% effect level (the effect level suggested in the DIBt scheme for in vivo assays; DIBt, 2011). CALUX data were interpolated at the 20% effect level of the reference compound (or positive control, PC) and not the EC20 of the sample because sometimes super-induction (effects >100%) was observed (Sotoca et al., 2010; see Fig. S3).

ER α -CALUX assay data of samples were also interpolated at the 10% level of the reference compound (positive control, PC10; EPA, 2009; see also Kunz et al., 2017). PC10-values were then used to determine 17 β -estradiol equivalent (EEQ) concentrations in the samples (Fig. S3).

We calculated toxic units (TUs, Equation (4)) to determine the extent to which BPA contributed to the observed toxicity of several endpoints (i).

$$\text{toxic unit} \left(\text{TU}_{\text{BPA-effect } i} \right) = \frac{\text{BPA concentration in the sample dilution that causes 50\% effect } i}{\text{EC50 of BPA for effect } i} \quad (4)$$

3. Results and discussion

3.1. Elevated BPA concentrations in some leachate samples

BPA concentrations in leachate samples were relatively low for three products (<100 µg/L), but reached 10 mg/L for Product 3 tested after 7 days of curing and almost 7 mg/L after 1 day of curing (Table 1). The emissions calculated are about 1 mg/m² and 0.7 mg/m² BPA after 7 days and 1 day of curing, respectively. BPA concentrations of 7 and 10 mg/L are ca. 30'000 to 45'000-fold higher than the AA-EQS proposed for BPA in surface waters (0.24 µg/L; Swiss Centre for Applied Ecotoxicology Eawag-EPFL, 2016). To produce drinking water using only natural treatment methods, the IAWR (International Association of Water Works in the Rhine Basin; IAWR, 2008) recommends a lower target of 0.1 µg/L for endocrine active compounds such as BPA. This IAWR target value is exceeded up to 100'000 times by BPA in eluates from Product 3. It has to be noted, however, that we measured high BPA concentrations (7 and 10 mg/L) under test conditions which are worst-case due to the relatively low volume to surface ratio (i.e. 10 L/m²). Thus, under field application conditions (e.g. coated steel piling in a water body), dilution will typically be orders of magnitude higher. Notwithstanding the typically much larger dilution in the environment, results clearly show that Product 3 released considerable amounts of BPA. This raises concern with respect to risks to the environment when dilution is not sufficient, for example, when BPA release occurs in small ponds or other relatively stagnant groundwater bodies.

Overall, chemical analysis shows that emission profiles from the four products are diverse. Three of four tested products hardly released BPA (or BPF). This points to significant differences between the products and a potential to improve formulations with respect to risks associated with BPA release. BPA emissions could, for example, be reduced by reducing BPA content in formulations. The safety data sheet of Product 3 gives indications of large amounts of BPA used. Alternatively, a more robust reaction process of epoxy resins components may reduce the available free BPA in the product and thus reduce BPA emissions (this may have contributed to low emissions seen for Products 1 and 2). Although, following product aging or adverse product treatments, part of the BPA may eventually always be released from BPA containing products (see Bruchet et al., 2014; e.g. for polycarbonate, see Koehler et al., 2003).

It is interesting to note that no BPF was released above the LOQ even though it is a typical ingredient in epoxy resins (Schweitzer, 2006). However, BPF content may be very low (or not present) in

the four tested products. BADGE is another typical epoxy resin component. Interestingly, BADGE release by Product 3 did not exceed the LOQ (of 0.1 mg/L) and thus BADGE release was at least a factor 70–100 lower than measured BPA release (i.e. 7–10 mg/L; see Table 1). Conversely, Product 4 released BADGE in the absence of detectable BPA concentrations. Also this observation underlines the fact that the emission profiles across the four tested products are diverse.

In this study, we tested each curing condition (i.e. 1 or 7 days) only once. It thus remains to be seen, how reproducible the concentrations that we obtained will prove to be. For instance, BPA release from Product 3 was slightly higher after a longer than after a shorter curing period (10 versus 7 mg/L). Also TOC release and conductivity from Product 3 were higher after a longer curing period. These observations are unexpected. One would assume a more complete curing after a longer drying time and consequently less release of BPA or TOC (as well as a lower conductivity; Table 1). This assumption is supported by TOC data from the other three products, where TOC was always lower after a longer curing period. The TOC and BPA-release results from Product 3 may point to the fact that slight differences during preparation of the resin mixture can affect release of compounds from the final product. As we only made the products twice (once for each curing period), we do not have information on the variability of BPA release from repeated independently prepared resin mixtures. Under ideal preparation and application conditions, release from corrosion protection products may be low. However, emissions may be higher when preparation is less robust and application occurs under adverse field conditions. This may be the case, for example, when the applied coatings rapidly get into in contact with groundwater, when they are applied at low temperatures in the winter season or when they are applied under tidal conditions at offshore installations.

3.2. Results from CALUX assays agree well with measured BPA concentrations

Samples from Product 3 were the most active samples in CALUX assays. Less than 1% of sample from Product 3 (7 days of curing) was sufficient to induce a 20% effect level in the ER α - and anti-AR-CALUX assays (Table 2; Fig. 1 shows ER α -CALUX dose-response curves; see SI for all dose-response data). The activity observed in ER α -, anti-AR- and anti-PR-CALUX for Product 3 can be explained with the measured BPA concentrations. For example, the EC50 for BPA in ER α -CALUX was determined as 0.055 mg/L (Table S1). The

Table 1

Chemical analysis data from eluates, generated over 7 days, from four corrosion protection products following either 1 or 7 days of product curing.

| | Conductivity (µS/cm) | TOC (mg/L) | BPA (µg/L) | BPF (µg/L) | BADGE (µg/L) | BADGE·H ₂ O (µg/L) | BADGE·2H ₂ O (µg/L) |
|-------------------------------|----------------------|------------|------------|------------|--------------|-------------------------------|--------------------------------|
| After 1 day of curing | | | | | | | |
| Control | 6.8 | 2.1 | <1 | <1 | <1 | <1 | <1 |
| Product 1 | 6.3 | 9.3 | 2 | <1 | 3 | 8 | 10 |
| Product 2 | 42.2 | 12.5 | 2 | <1 | 18 | 37 | 26 |
| Product 3 | 25.0 | 19.7 | 6'890 | <100 | <100 | <100 | <100 |
| Product 4 ^a | 5.3 | 3.7 | <100 | <100 | 268 | 565 | 246 |
| After 7 days of curing | | | | | | | |
| Control | 3.8 | 0.9 | <1 | <1 | <1 | <1 | <1 |
| Product 1 | 4.2 | 3.3 | <1 | <1 | 13 | 13 | 7 |
| Product 2 | 73.0 | 11.2 | 2 | <1 | 5 | 10 | 9 |
| Product 3 | 46.7 | 37.1 | 10'400 | <100 | <100 | <100 | <100 |
| Product 4 | 6.6 | 0.8 | <10 | <10 | 38 | 128 | 46 |

The pH of all samples was between 6 and 7. Abbreviations: Total organic carbon (TOC), bisphenol A (BPA), bisphenol F (BPF), and bisphenol A diglycidyl ether (BADGE), bisphenol A (2,3-dihydroxypropyl)-glycidyl-ether (BADGE·H₂O) and bisphenol-A-bis (2,3-dihydroxypropyl) ether (BADGE·2H₂O). The label "<" indicates values below the limit of quantification (LOQ). LOQs vary across samples (1, 10 or 100 µg/L) and depend on the amount of sample that was injected to allow for the quantification of dominant compounds.

^a This sample was generated together with the samples after 7 days of curing.

Table 2

Percentage of sample required to induce a 20% effect level – compared to the positive control (PC20)^a – in six CALUX assays. Four products were tested and eluates were generated over 7 days following either 1 or 7 days of product curing.

| | ER α | Anti-AR | Anti-PR | Nrf2 | P53 | PPAR γ 2 |
|-------------------------------|-------------|---------|------------------|------|-----|-----------------|
| After 1 day of curing | | | | | | |
| Control | – | – | – | – | – | – |
| Product 1 | 9.4 | 12 | 14 | – | – | – |
| Product 2 | – | 66 | 44 | – | – | – |
| Product 3 | 0.4 | 1.7 | 9.5 | – | – | – |
| Product 4 ^b | – | – | – | – | – | – |
| After 7 days of curing | | | | | | |
| Control | – | – | – | – | – | – |
| Product 1 | 33 | 50 | 44 | X | X | X |
| Product 2 | – | 65 | 67 | X | X | X |
| Product 3 | 0.2 | 0.1 | 8.4 ^c | X | X | X |
| Product 4 | – | – | – | X | X | X |

^a For ER α -CALUX the reference is 17 β -estradiol; for anti-AR and anti-PR-CALUX, with reference compounds flutamide and RU486, the reciprocal PC20 is used i.e. the sample percentage at which the maximum effect of the reference is inhibited by 20%.

^b This sample was generated together with the samples after 7 days of curing.

^c Cytotoxicity was observed above sample concentrations of 20%. X: the sample was not tested, because after 1 day of curing, no effects were observed (no measurable effect, PC20 > 100%, is indicated by “–”).

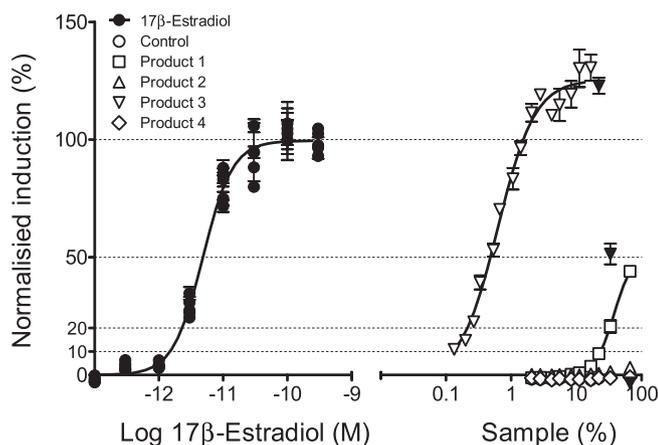


Fig. 1. ER α -CALUX dose-response curves of eluates from four corrosion protection products tested after 7 days of curing. Each product as well as the control was tested on one plate against a 17 β -estradiol (E2) reference. Solid lines show fits (Equation (1)) to pooled E2 data and data from the products (average and standard deviation of triplicates). Product 3 was cytotoxic upwards of 20% sample tested, filled triangles were not used for fitting. Dotted lines indicate 10, 20, 50 and 100% effect levels of the E2 reference.

BPA concentration at the PC50 (percentage of sample required to induce a 50% effect level compared to the positive control; see also Fig. S3) effect level of the Product 3 sample following 7 days of curing was 0.050 mg/L (i.e. 10.4 mg/L times 0.48% sample = 0.050 mg/L; see Table 1 for concentration data and Table S2 for PC50 values). This translates into a $TU_{BPA-ER\alpha}$ of 0.9 (Equation (4)). TU -values of BPA in anti-AR- and anti-PR-CALUX were between 1.3 and 2.3 ($TU_{BPA-anti-AR} = 1.3$; $TU_{BPA-anti-PR} = 2.3$). TU_{BPA} -values around 1 indicate that BPA caused most of the observed effects in CALUX assays. There may be other compounds in the Product 3 samples that influenced CALUX results, however, they would have been masked by BPA and obviously minor mixture components compared to BPA.

The CALUX dose-response curves of Product 3 show clear super induction (Fig. 1 and Fig. S3). This is another indication that supports the fact that BPA caused effects. The BPA standard we tested also showed super induction (Fig. S5) and the fact that BPA causes

super induction (possibly by stabilisation of luciferase) has been described in the literature (Sotoca et al., 2010).

Samples from Product 1 and 2 were the next most active samples in CALUX assays. Samples from Product 4 did not induce any effects in any of the six CALUX assays. Although Product 1 and 2 also released BPA, concentrations were not high enough to explain effects in the CALUX assays ($TUs \ll 0.1$).

EEQs were determined at the 10% effect level and could be derived from dose-response data from samples from Products 1 and 3. After 1 or 7 days of curing, EEQs in samples from Product 1 were 4.8 and 2.0 ng/L, respectively. These levels are typical for treated sewage effluent (Jarošová et al., 2014). Samples from Product 3 and after 1 or 7 days of curing reached EEQs of 160 and 280 ng/L, respectively. These levels are very high and a cause for concern. ER α -CALUX EEQ-based trigger values have been suggested for surface water and range between 0.2 and 0.4 ng/L (Jarošová et al., 2014). The highest EEQ measured for a sample from Product 3 (280 ng/L) exceeds the lowest trigger value (0.2 ng/L) by a factor of 1'400. This observation is yet another indication that Product 3 is problematic in terms of its adverse ecotoxicological potential.

3.3. Inhibition of bacterial bioluminescence

Three tests used in this study are similar (though not identical) to tests used to evaluate construction materials in a scheme developed by DIBt (DIBt, 2011; Fig. S4). As a first test of eluates from construction materials, the scheme foresees the use of a cuvette based Microtox method with *A. vibrio* (ISO, 2009a; ISO, 2009b; ISO, 2009c). We applied the same type of assay, however, we used a modified (96-well) version of the Microtox (Escher et al., 2008). Within the DIBt scheme, effects on inhibition of bacterial bioluminescence have to be reduced to below 20% with a sample dilution factor that needs to be lower than eight-fold. Product 1 clearly failed this criterion. After 7 days of curing (the recommended curing time by the producer) the criterion was exceeded more than 160-fold. This means, an almost 1'300-fold sample dilution was required for effects to drop below 20% (see Table 3 and Fig. 2 for dose-response curves). Product 3 is a borderline case: after 7 days of curing the criterion was marginally exceeded, whereas after 1 day of curing the criterion was met (Table 3). Even maximal samples doses (i.e. 45%) of Products 2 and 4 did not inhibit bacterial bioluminescence above 20% (Fig. 2).

Product 3 released large amounts of BPA and thus we calculated TUs for the bacterial (bact.) bioluminescence endpoint and the eluates from Product 3. After 1 day of curing the $TU_{BPA-bact.}$ was 0.43 and after 7 days of curing the $TU_{BPA-bact.}$ was 0.21. This indicates that BPA explains less than half of the observed toxicity and that there may be other active compounds released from Product 3. The origin of the significant toxicity in eluates from Product 1 remains unknown. The toxicity is not associated with BPA, as Product 1 did not release measurable amounts of the compound. For example, it is known that anti-corrosion coatings typically contain polyamines and other phenolic compounds besides BPA and BPF (Schweitzer, 2006). Effect-directed analysis (Brack, 2003) could be used in future studies to identify the toxic substance(s) in leachates from Product 1.

3.4. Inhibition of reproduction in water fleas

As a second test, the DIBt scheme requires a 48 h *Daphnia magna* immobilisation assay (ISO, 2011), whereas we used a 7 day reproduction test using a different species of water flea, namely *C. dubia*. A four-fold dilution is permitted to reduce effects on water fleas to below 20%. Eluates from three products exceeded this limit after 7

Table 3

Required dilutions for anti-corrosion coating leachate samples to reduce effects below 20% in three CALUX assays and three further bioassays. Four products were tested and eluates generated over 7 days following either 1 or 7 days of product curing.

| | ER α -CALUX | anti-AR-CALUX | anti-PR-CALUX | Inhibition of algal growth | Inhibition of bacterial bioluminescence | Inhibition of water flea reproduction |
|-------------------------------|--------------------|---------------|---------------|----------------------------|---|---------------------------------------|
| After 1 day of curing | | | | | | |
| Control | – | – | – | – | – | – |
| Product 1 | 11 | 8 | 7 | – | 1'282 | 5 |
| Product 2 | – | 2 | 2 | – | <2 | 2 |
| Product 3 | 259 | 57 | 11 | 4 | 4 | 10 |
| Product 4* | – | – | – | – | – | 3 |
| After 7 days of curing | | | | | | |
| Control | – | – | – | – | – | 2 |
| Product 1 | 3 | 2 | – | – | 171 | 8 |
| Product 2 | – | 2 | – | – | 2 | 22 |
| Product 3 | 468 | <u>766</u> | 12 | 10 | 8 | 67 |
| Product 4 | – | – | – | – | – | 2 |

An underlined value indicates that extrapolation was used (see Fig. S9 for full dose-response data).

days of curing and two products exceeded the limit after 1 day of curing (Table 3 and Fig. 2). Particularly Product 3 caused a significant exceedance, with an almost 70-fold dilution requirement to reduce effects on reproduction below 20%.

To determine the role of BPA in the toxicity of Product 3 we calculated the TU_{BPA-C.dubia} which was only 0.2 after 1 day of curing and 0.1 after 7 days of curing. The relatively large difference from a TU of 1 may be caused by other compounds contributing to water flea toxicity in the Product 3 sample (i.e. high TOC levels were observed for Product 3, Table 1). However, in the case of water flea reproduction, there is uncertainty associated with the TUs. Both the experimental data (i.e. standard deviation bars in Fig. 2) and the data extracted from Tatarazako et al. (2002), vary (the coefficient of variation for the EC25 of BPA is ca. 10%; Tatarazako et al., 2002). Thus, there are two significant sources of variability involved with TU calculations. More importantly, although both Tatarazako et al. (2002) and this study used the same species (*C. dubia*), there may be differences in sensitivity between test strains. A 1.5-fold difference in sensitivity has been reported for three *D. magna* strains towards malathion (Toumi et al., 2015), for example. A two-fold sensitivity difference between daphnia strains used for chemical testing and sample testing will directly translate in a two-fold difference in the TU.

3.5. Inhibition of algal growth rates

As a third test, the DIBt scheme involves a 72 h algae growth test in flasks (DIN, 1991) with *Desmodesmus subspicatus*, whereas we used a 24 h growth rate test on a 96-well plate with a different species, *P. subcapitata* (Escher et al., 2008). Only Product 3 induced slight toxicity toward algae growth, however, the algae growth criterion was not exceeded after 7 days of curing nor after 1 day of curing (Table 3; Fig. 2). No effects on algae growth rate were observed for the other products. The TU_{BPA-algae}-values for Product 3 after 1 and 7 days of curing were 0.25 and 0.18 respectively; meaning that BPA likely contributed to the observed effects.

3.6. "Miniaturised" bioassays to facilitate ecotox studies on construction products

As highlighted above, we used different tests than those outlined in the DIBt scheme (DIBt, 2011; Fig. S4). The main reasons are: 1) cost savings, 2) having additional endpoints, and 3) enhanced sensitivity. Tests on 96-well plates and with *C. dubia* require less sample compared with tests run in cuvettes, flasks or with *D. magna*. It is cheaper and easier to generate such small sample

volumes. At the same time, smaller test volumes allow more samples or more dilutions to be tested simultaneously (i.e. multiple sample dose-response curves fit on a single 96-well plate). In terms of additional endpoints, the combined algae test also covers effects on photosystem II inhibition (samples did not cause effects, data not shown) and *C. dubia* tests provide information on acute effects (immobilisation) as well as on the chronic reproduction endpoint. Particularly for water fleas, the chronic endpoint is generally more sensitive than the acute endpoint (see Toumi et al., 2015 for data on *D. magna*). In fact, the LC50 values in our study were always larger than EC50 values on reproduction (see SI).

Escher et al. (2008) discuss the comparability between large volume and 96-well plate tests in terms of algae and bacterial bioluminescence. It was concluded that changing from flasks to 96-well plates (algae) and from cuvettes to 96-well plates (bacteria) did not affect the performance of the assays (for further data on algae see also Eisentraeger et al., 2003). This is encouraging, however, as we used different algae and water flea species than the DIBt scheme (as well as a different application area), a comparison of our results with the DIBt scheme has to be done with caution. It will only be possible to extend the DIBt scheme towards other application areas and with other as yet not standardised assays (including various CALUX assays) after an in-depth evaluation of the various assays types and their respective endpoints.

3.7. Extrapolating results from leachates to risks in the environment

Although the samples of all but one product (i.e. Product 4) produced significant effects in one or more bioassays, it is not straightforward to transpose toxic effects observed in the lab to risks in the environment. This is highlighted in the DIBt scheme (DIBt, 2011), as a transfer function is needed to link toxicity results from leachate experiments to real world exposure. A transfer function does not exist for anti-corrosion coatings. Therefore, we do not attempt to extrapolate our lab leachate results to risks in the environment. Nonetheless, our results clearly demonstrate that different products have different toxicity signatures. For example, Product 3 was problematic in terms of release of BPA, which was associated with effects in various bioassays (e.g. CALUX). It is also clear that Product 1 releases significant toxicity to bacteria and the remaining two products appear to pose much lower risk. Finally, Product 4 was completely unproblematic in all tests, regardless if a 1 day or 7 days curing procedure was applied.

Our experiments serve as an indicator for the relevance of epoxy resin anti-corrosion coatings as a source of toxicity to the aquatic environment. More studies are required to confirm and broaden

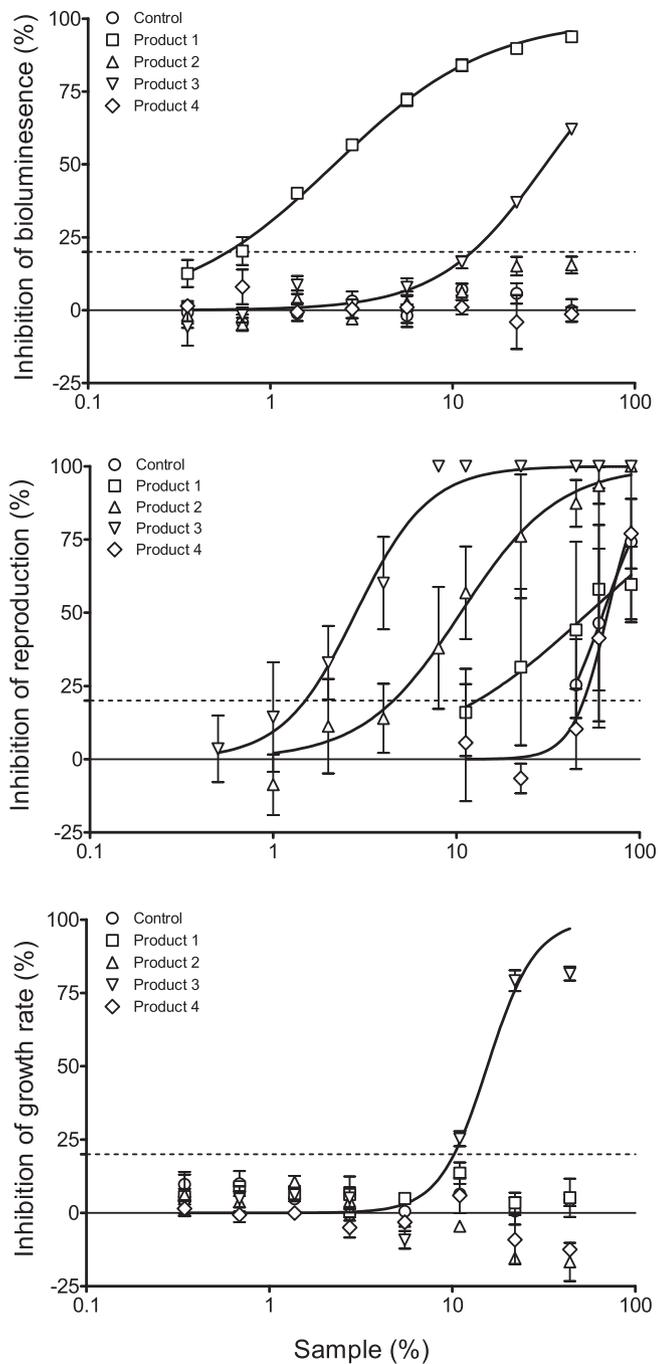


Fig. 2. Dose-response curves of eluates from four anti-corrosion coatings tested after 7 days of curing in three bioassays. Top: inhibition of bacterial bioluminescence in *A. vibrio* (average and standard deviation of triplicates); middle: inhibition of water flea reproduction in *C. dubia* (average and standard deviation of 12 replicates); bottom: inhibition of algal growth rate in *P. subcapitata* (average and standard deviation of triplicates). Solid lines show log-logistic fits to the data (Equation (1)) and the dashed lines indicate 20% effect levels.

the database. Furthermore, it would be advantageous to define protection goals as well as assay specific criteria to better evaluate results. In this light, the DIBt scheme can serve as a good starting point. For example, transfer functions (to extrapolate from lab to real-world exposure scenarios) could be developed further and the scheme could be extended to cover additional and relevant mechanisms of actions such as those that address endocrine disruptive compounds.

4. Conclusions

- Epoxy resin based anti-corrosion coatings can leach significant amounts of toxicity
- Some coatings are more toxic than others, this points to opportunities for the development of low risk formulations and products with better ecotoxicological properties
- One of four tested products released large amounts of bisphenol A (BPA)
- Under worst-case leaching conditions, proposed BPA water quality criteria were greatly exceeded

Author contributions

Study design: CD, ELMV, IW, MB. Experimental work: CD*. Market research: CD, MB. Data analysis: ELMV. Discussion and interpretation of results: all. Drafting of manuscript: ELMV. Revising manuscript: all.

*see also acknowledgements and method sections.

Acknowledgements

Algae tests and bacterial bioluminescence assays were performed by Barbara Ganser, Andrea Schifferli and Sereina Gut (Swiss Centre for Applied Ecotoxicology Eawag-EPFL). The project was funded by the Swiss Federal Office for the Environment (FOEN).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2017.07.006>.

References

- AFNOR, 2000. Water Quality - Determination of Chronic Toxicity to Ceriodaphnia Dubia in 7 Days - Population Growth Inhibition Test (T90-376).
- Bayer, F., Capell, A.W.H., Gormanns, G., Nicolai, O., Pflugfelder, J., 2010. Korrosionsschutz von Stahlbauten durch Beschichtungssysteme. Deutsches Lackinstitut GmbH, Frankfurt am Main.
- Brack, W., 2003. Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures? *Anal. Bioanal. Chem.* 377 (3), 397–407.
- Bruchet, A., Elyasmino, N., Decottignies, V., Noyon, N., 2014. Leaching of bisphenol A and F from new and old epoxy coatings: laboratory and field studies. *Water Sci. Technol. Water Supply* 14 (3), 383–389.
- Burkhardt, M., Dietschweiler, C., Vermeirssen, E., Ganser, B., Schifferli, A., Werner, I., 2015. Organische Beschichtungen im Schweizer Stahlwasserbau und deren Ökotoxizität (Report for the Swiss Federal Office for the Environment FOEN, Bern).
- CEN, 2011. EN 16105 Paints and Varnishes — Laboratory Method for Determination of Release of Substances from Coatings in Intermittent Contact with Water.
- CEN, 2017. Guidance on the Use of Ecotoxicity Tests Applied to Construction Products. CEN.
- Chow, W.S., Chan, W.K.-L., Chan, K.M., 2012. Toxicity assessment and vitellogenin expression in zebra fish (*Danio rerio*) embryos and larvae acutely exposed to bisphenol A, endosulfan, heptachlor, methoxychlor and tetrabromobisphenol A. *J. Appl. Toxicol.* 33, 670–678.
- DIBt, 2011. Grundsätze zur Bewertung der Auswirkungen von Bauprodukten auf Boden und Grundwasser. Deutsches Institut für Bautechnik.
- DIN, 1991. Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung; Testverfahren mit Wasserorganismen (Gruppe L); Bestimmung der nicht giftigen Wirkung von Abwasser gegenüber Grünalgen (Scenedesmus-Chlorophyll-Fluoreszenztest) über Verdünnungsstufen (L 33).
- Eisentraeger, A., Dott, W., Klein, J., Hahn, S., 2003. Comparative studies on algal toxicity testing using fluorometric microplate and Erlenmeyer flask growth-inhibition assays. *Ecotoxicol. Environ. Saf.* 54 (3), 346–354.
- EPA, 2009. EPA 740-C-09-006 Endocrine Disruptor Screening Program Test Guidelines OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)).
- Elenndt, B.-P., Bias, W.R., 1990. Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing Effects of the optimization of culture conditions on life history parameters of *D. magna*. *Water Res.* 24, 1157–1167.
- Escher, B.I., Bramaz, N., Mueller, J.F., Quayle, P., Rutishauser, S., Vermeirssen, E.L.M., 2008. Toxic equivalent concentrations (TEQs) for baseline toxicity and specific

- modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples. *J. Environ. Monit.* 10, 612–621.
- Gartiser, S., Heisterkamp, I., Schoknecht, U., Burkhardt, M., Ratte, M., 2016. Empfehlungen für eine Testbatterie zur ökotoxikologischen Bewertung der Umweltverträglichkeit von Bauprodukten. Umweltbundesamt, Dessau-Roßlau, Germany, p. 163.
- Gijsbers, L., Man, H.-Y., Kloet, S.K., de Haan, L.H.J., Keijer, J., Rietjens, I.M.C.M., van der Burg, B., Aarts, J.M.M.J.G., 2011. Stable reporter cell lines for peroxisome proliferator-activated receptor γ (PPAR γ)-mediated modulation of gene expression. *Anal. Biochem.* 414 (1), 77–83.
- Hansson, C.M., 2011. The impact of corrosion on society. *Metall. Mater. Trans. A* 42 (10), 2952–2962.
- IAWR, 2008. Danube, Meuse and Rhine Memorandum 2008, Internationale Arbeitsgemeinschaft der Wasserwerke im Rheineinzugsgebiet.
- ISO, 2005. Water Quality - Determination of Chronic Toxicity to Ceriodaphnia Dubia (ISO/CD 20665).
- ISO, 2009a. Water Quality - Determination of the Inhibitory Effect of Water Samples on the Light Emission of *Vibrio Fischeri* (Luminescent Bacteria Test) - Part 1: Method Using Freshly Prepared Bacteria (ISO 11348-1:2007).
- ISO, 2009b. Water Quality - Determination of the Inhibitory Effect of Water Samples on the Light Emission of *Vibrio Fischeri* (Luminescent Bacteria Test) - Part 2: Method Using Liquid-dried Bacteria (ISO 11348-2:2007).
- ISO, 2009c. Water Quality - Determination of the Inhibitory Effect of Water Samples on the Light Emission of *Vibrio Fischeri* (Luminescent Bacteria Test) - Part 3: Method Using Freeze-dried Bacteria (ISO 11348-3:2007).
- ISO, 2011. Water Quality — Determination of the Inhibition of the Mobility of *Daphnia Magna* Straus (Cladocera, Crustacea) — Acute Toxicity Test.
- ISO, 2014. Water Quality - Determination of the Estrogenic Potential of Water and Waste Water - Part 3: in Vitro Human Cell-based Reporter Gene Assay (ISO/CD 19040-3).
- Jarošová, B., Bláha, L., Giesy, J.P., Hilscherová, K., 2014. What level of estrogenic activity determined by in vitro assays in municipal waste waters can be considered as safe? *Environ. Int.* 64 (0), 98–109.
- Koehler, K.E., Voigt, R.C., Thomas, S., Lamb, B., Urban, C., Hassold, T., Hunt, P.A., 2003. When disaster strikes: rethinking caging materials. *Lab. Anim.* 32 (4), 24–27.
- Kunz, P., Simon, E., Creusot, N., Jayasinghe, B.S., Kienle, C., Maletz, S., Schifferli, A., Schönlaue, C., Ait-Aïssa, S., Denslow, N.D., Hollert, H., Werner, I., Vermeirssen, E.L.M., 2017. Effect-based tools for monitoring estrogenic mixtures: evaluation of five in vitro bioassays. *Water Res.* 110, 378–388.
- Poursaee, A., 2016. Corrosion of Steel in Concrete Structures. Elsevier, Amsterdam.
- Qian, Y., Li, Y., Jungwirth, S., Seely, N., Fang, Y., Shi, X., 2015. The application of anti-corrosion coating for preserving the value of equipment asset in chloride-laden environments: a review. *Int. J. Electrochem. Sci.* 10 (12), 10756–10780.
- Schweitzer, P.A., 2006. Paint and Coatings Applications and Corrosion Resistance. Taylor & Francis Group, Boca Raton, Florida.
- Swiss Centre for Applied Ecotoxicology Eawag-EPFL, 2016. <http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards/> (Last Accessed 10 April 2017).
- Sotoca, A.M., Bovee, T.F.H., Brand, W.N.V., Boeren, S., Murk, A.J., Vervoort, J., Rietjens, I.M.C.M., 2010. Superinduction of estrogen receptor mediated gene expression in luciferase based reporter gene assays is mediated by a post-transcriptional mechanism. *J. Steroid Biochem. Mol. Biol.* 122.
- Tatarazako, N., Takao, Y., Kishi, K., Onikura, N., Arizono, K., Iguchi, T., 2002. Styrene dimers and trimers affect reproduction of daphnid (*Ceriodaphnia dubia*). *Chemosphere* 48 (6), 597–601.
- Toumi, H., Boumaiza, M., Millet, M., Radetski, C.M., Camara, B.I., Felten, V., Ferard, J.-F., 2015. Investigation of differences in sensitivity between 3 strains of *Daphnia magna* (crustacean Cladocera) exposed to malathion (organophosphorous pesticide). *J. Environ. Sci. Health, Part B Pest., Food Contam. Agric. Wastes* 50, 34–44.
- van der Linden, S.C., Heringa, M.B., Man, H.-Y., Sonneveld, E., Puijker, L.M., Brouwer, A., van der Burg, B., 2008. Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays. *Environ. Sci. Technol.* 42 (15), 5814–5820.
- van der Linden, S.C., von Bergh, A.R.M., van Vught-Lussenburg, B.M.A., Jonker, L.R.A., Teunis, M., Krul, C.A.M., van der Burg, B., 2014. Development of a panel of high-throughput reporter-gene assays to detect genotoxicity and oxidative stress. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 760, 23–32.
- van der Ohe, P.C., de Zwart, D., 2013. In: Féard, J.-F., Blaise, C. (Eds.), *Encyclopedia of Aquatic Ecotoxicology*. Springer Science+Business Media Dordrecht, The Netherlands.
- Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.-H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr. Rev.* 33 (3), 378–455.
- Vé Wiewel, B., Lamoree, M.H., 2016. Geotextile composition, application and ecotoxicology - a review. *J. Hazard. Mater.* 317, 640–655.
- Wright-Walters, M., Volz, C., Talbott, E., Davis, D., 2011. An updated weight of evidence approach to the aquatic hazard assessment of Bisphenol A and the derivation a new predicted no effect concentration (Pnec) using a non-parametric methodology. *Sci. Total Environ.* 409 (4), 676–685.