

Ecotoxicological characterization of emissions from steel coatings in contact with water

Anna Maria Bell^a, Roland Baier^b, Birgit Kocher^c, Georg Reifferscheid^a,
Sebastian Buchinger^{a,*}, Thomas Ternes^a

^a Federal Institute of Hydrology, Am Mainzer Tor 1, 56068, Koblenz, Germany

^b Federal Waterways Engineering and Research Institute, Section B2 - Steel Structures and Corrosion Protection, Kufßmaulstr. 17, 76187, Karlsruhe, Germany

^c Federal Highway Research Institute, Department V3 - Environmental Protection, Brüderstr. 53, 51427, Bergisch Gladbach, Germany

ARTICLE INFO

Article history:

Received 12 November 2019

Received in revised form

14 January 2020

Accepted 19 January 2020

Available online 23 January 2020

Keywords:

Epoxide

Polyurethane

Leaching

Toxicity

4-*tert*-Butylphenol

Diffusion

ABSTRACT

In order to prevent corrosion damage, steel structures need to be protected. Coating systems achieve this by the isolation of the steel from its environment. Common binding agents are epoxide and polyurethane resins which harden by polyaddition reactions. In contact with water, various organic substances might be leached out and released into the aquatic environment potentially causing adverse effects. So far, no legal requirements are mandatory for the environmental sustainability of coating systems. To characterize emissions from steel coatings, recommendations for the ecotoxicological assessment of construction products were utilized. Seven different coating systems based on epoxide or polyurethane resins were leached in 8 steps (6 h–64 d), followed by the testing of acute toxic effects on bacteria and algae as well as estrogen-like and mutagenic effects. In addition, chemical analysis by GC-MS was performed to identify potentially toxic compounds released from the coating systems. Two systems tested did not show any significant effects in the bioassays. One coating system caused significant algal toxicity, none was found to cause mutagenic effects. The other coating systems mainly showed estrogenic effects and bacterial toxicity. The effects increased with increasing leaching time. 4-*tert*-butylphenol, which is used in epoxy resins as a hardener, was identified as the main contributor to acute and estrogenic effects in two coatings. The release mechanism of 4-*tert*-butylphenol was characterized by two different modelling approaches. It was found that the release from the most toxic coating is not explainable by an elevated content of 4-*tert*-butylphenol but more likely by the release mechanism that – in contrast to the less toxic coating – is controlled not only by diffusion. This finding might indicate a sub-optimal formulation of this coating system resulting in a less stable layer and thus an increased release of toxic compounds.

© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Steel structures such as bridges, wind turbines and lock gates exposed to the environment are subject to corrosion. To improve the durability and thus to ensure a long-term structural stability, respective buildings must be protected. For a passive protection, multi-layered polymer coating systems mainly based on epoxide or polyurethane resins are applied. Primer coatings with functional fillers like zinc dust, zinc phosphate or micaceous iron oxide are

frequently used.

Normative requirements for corrosion protection of steel structures by protective paint systems are specified in ISO 12944 part 1 to 8 (ISO, 2018a). The corrosion protection of the infrastructure of public transport in Germany is additionally regulated by supplementary documents (BAST, 2002; 2012; BAW, 2011; BMVBS, 2009). These guidelines focus on the testing of coating materials with respect to their identity, resistance to corrosion and suitability. However, they do not specifically address the environmental sustainability of coating systems. For instance, the respective ISO standard merely precludes the use of toxic and carcinogenic substances and asks for reduced emissions of volatile organic compounds (VOC), though no specific requirements on testing and evaluation methods are defined.

* Corresponding author. Federal Institute of Hydrology, Department G3 - Biochemistry, Ecotoxicology, Am Mainzer Tor 1, 56068, Koblenz, Germany.
E-mail address: Buchinger@bafg.de (S. Buchinger).

Critical raw materials such as coal tar pitch, asbestos fibers, red lead and chromate have been replaced completely by synthetic PAH-free hydrocarbon resins, glass- or wollastonite fibers and e. g. zinc pigments, respectively. The use of the persistent organic pollutant polychlorinated biphenyl (PCB) as plasticizer in various binding agents was banned already at the end of the seventies (OECD, 1973). Also the application of the biocide and endocrine disruptor tributyltin (TBT) in anti-fouling paints on ships is internationally prohibited since 2008 (IMO, 2001) and poses no longer a risk in currently licensed coating systems. Nevertheless, a large number of further compounds might be released from coatings in contact with water which might cause adverse effects in the aquatic environment. For instance, epoxide resin based coatings are formed by components containing ingredients such as bisphenol A/F, benzyl alcohol, nonylphenols, other phenolic substances or polyamines (Dornbusch et al., 2016; Ellis, 1993; Jin et al., 2015; Paluvai et al., 2014), while highly reactive diisocyanates, polyols as well as plasticizers are used for polyurethane coatings (Akindoyo et al., 2016; Smirnova et al., 2016; Thomas et al., 2017). Both product groups can contain a wide range of volatile organic solvents, metallic pigments, UV stabilizers, biocides and a multitude of other compounds. In addition it has to be pointed out that further unknown potentially toxic compounds might be formed during polymerization (hardening) and the reaction with water.

In a study investigating a variety of 20 biocide-free antifouling coatings, Watermann et al. (2005) reported overall no or just a slight toxicity to luminescent bacteria, whereas in eroding coatings (epoxy- and rosin-based) an elevated toxicity to the cypris larvae of *Balanus amphitrite* was observed. Furthermore, the release of bisphenol A and nonylphenol, especially from epoxy resins, was reported (Watermann et al., 2005). Also significant toxic effects to the algae *Desmodesmus subspicatus* and the earthworm *Enchytraeus albidus* were observed in polyurethane systems for waterproofing (Markl et al., 2017). Moreover, a variety of substances released from a one-component polyurethane resin were identified, which all caused toxicity to *Aliivibrio fischeri* (Luft et al., 2017). The ecotoxicological assessment of anti-corrosion coatings based on epoxy resins was investigated by Vermeirssen et al. (2017). For some coatings, high amounts of bisphenol A with associated estrogenic activities as well as toxic effects to water fleas and luminescent bacteria were reported by this study. Another study addressing three different fire protection coating systems showed quite different toxicity levels in an algal growth inhibition test as well as a luminescent bacteria test (Heisterkamp et al., 2016). However, investigations of anti-corrosion coatings are very limited and no general concept for their investigation is established so far.

A comprehensive ecotoxicological assessment of steel coatings requires an appropriate combination of a reasonable leaching procedure and representative toxicity tests. The German Institute of Structural Engineering (DIBt) has already established a combined testing and evaluation approach for the assessment of effects of construction products approved to be in contact with soil and groundwater (DIBt, 2011). At the international level, the CEN Technical Committee 351 developed a method for a standardized assessment of construction products under the Construction Products Directive (CPD). A study on behalf of the German Federal Environmental Agency (UBA) dealt with the elaboration and validation of an ecotoxicological test battery and contributed to the European harmonization of test methods (Gartiser et al. 2017a, 2017b). Because coating systems for corrosion protection are not mandated under CPD, no legal consequences for the testing of these materials will arise from these guidelines so far.

Against this background the aim of this study was to characterize the ecotoxicological relevance of substance emissions from steel coatings in contact with water by adopting the test

recommendations for the assessment of ecotoxicological properties of eluates of construction products. For this purpose different priming and top coatings containing either epoxide or polyurethane resins as binding agent were systematically examined.

2. Material and methods

In accordance with DIN CEN/TS 16637, the horizontal dynamic surface leaching test (CEN, 2014) was used for the leaching of coated steel plates. The ecotoxicological investigations were targeted on acute toxic effects on bacteria and algae. In addition estrogen-like and mutagenic effects were examined. To identify the potentially toxic compounds released from the coating systems and to elucidate the underlying release mechanism chemical analysis was performed in parallel using GC-MS detection.

2.1. Selection of coating systems and fabrication of test plates

In consultation with the German Federal Waterways Engineering and Research Institute (BAW), five representative anti-corrosion coating products for hydraulic engineering were selected (Table 1). In total, two priming and three top coatings were tested individually and in combination resulting in seven different coating systems that contain either epoxide or polyurethane resins as binding agent. Usually primer and top coatings are tested in combination to capture the overall toxicity of the coating system. Several epoxide-based top coatings, such as top coating (I), are also approved for the use as coating system without primer coating. Therefore, the epoxide-based top coatings were tested additionally in the absence of a primer coating. The coating works were performed or commissioned by the Institute for Corrosion Protection (IKS, Dresden). The respective coatings were applied on the front and back of steel plates with a size of 150 x 160 x 3 mm following the respective instructions provided by the manufacturer of the coatings. The particular layer thickness was defined by the list of approved coating systems of the BAW. The effective dry film thickness of the resulting coating systems was determined according to DIN EN ISO 2808 (ISO, 2007a) and results are listed in SI (see Table SI 1).

2.2. Leaching, sampling and sample preparation

Leaching was conducted in accordance with the dynamic surface leaching test (DSLTL, CEN/TS 16637-2) (CEN, 2014), which is originally intended for the assessment of the release of dangerous substances from construction products. For our purpose the coated plates were attached on nylon strings and dipped into all-glass aquariums (300 x 220 x 240 mm) filled with 3 l deionized water. The containers were covered by a glass plate. According to DSLTL, CEN/TS 16637-2 samples were taken at eight different time points (after 0.25, 1, 2.25, 4, 9, 16, 36 and 64 d) including a complete exchange of the water. Each coating system was investigated as triplicate. Two aquaria without plates served as controls.

On the one hand, individual samples from each sampling date were collected and on the other hand, time-proportional composite samples over a short time (first two sampling steps) and a long time (all sampling steps) were mixed. Water samples were stored in closed, dark glass bottles at 2–8 °C and either processed within 48 h after collection or used for the preparation of the composite sample. Samples that were not examined immediately were kept frozen at ≤ −18 °C until further analysis.

In addition, composite samples were concentrated 1000-fold by solid phase extraction (SPE) using OASIS HLB 6 cc (200 mg) cartridges. The cartridges were conditioned with 2 ml n-heptane (Picograde, Promochem), 2 ml acetone (Picograde, Promochem),

Table 1

Description of investigated coating systems: type of binding agent (EP: epoxide, PUR: polyurethane); NDFT: nominal dry film thickness.

no. of coating system	primer coating				top coating			
	label	type	no. of components	NDFT [μm]	label	type	no. of components	NDFT [μm]
1	A	EP	2	80	(I)	EP	2	200
2	—	—	—	—	(I)	EP	2	200
3	A	EP	2	80	(II)	EP	2	500
4	—	—	—	—	(II)	EP	2	500
5	B	PUR	1	80	—	—	—	—
6	B	PUR	1	80	(III)	PUR	1	2x200
7	—	—	—	—	(III)	PUR	1	2x200

3 × 2 ml methanol (Optigrade, Promochem) and 4 × 2 ml double distilled water and then loaded with 1000 ml of the aqueous samples. After drying, the SPE cartridges were eluted with 4 × 2 ml methanol. Extracts were evaporated, aliquoted and restored either in 800 μl DMSO (ReagentPlus, Sigma-Aldrich) or 200 μl ethanol (Optigrade, Promochem).

2.3. Bioassays

Due to the wide variety of simultaneously tested materials and the time-consuming sampling design of DSLT this study focused primarily on acute toxic effects on the lower trophic levels (bacteria and algae). Since safety data sheets of the tested materials provided indication that compounds with possible specific effects might be present, additionally estrogen-like and mutagenic effects were examined.

2.3.1. Luminescent bacteria

2.3.1.1. Luminescent bacteria assay in cuvettes. The assay utilizes the bioluminescence of the marine bacterium *Aliivibrio fischeri* and quantifies the inhibition of the bacterial light emission after exposure to the test sample as a measure for the acute bacterial toxicity. The standard bioluminescence inhibition assay was carried out according to DIN EN ISO 11348-2 (ISO, 2007c) using the LUMISTox 300 measuring instrument (Hach Lange) and reconstituted liquid-dried bacteria (LCK 482, Hach Lange). Before testing, the aqueous leachates from each sampling date were salinated with sodium chloride ($\geq 99.5\%$, Fluka) to a salinity value of 20. A NaCl solution (2%, m/v) was used as negative control and 3,5-dichlorophenol (97%, Sigma; $c = 4.5 \text{ mg/l}$) served as positive control. The samples were tested as dilution series in geometric sequence with a dilution factor of two and the lowest ineffective dilution (LID) that causes an inhibition of luminescence of less than 20% was used as test result. Thus, the higher the value determined, the higher the toxicity of the sample. The fractional dilution level inducing exact 20% inhibition was calculated by linear interpolation for a better comparability between samples.

2.3.1.2. HPTLC coupled luminescent bacteria assay. For the performance of the HPTLC coupled luminescent bacteria assay, liquid-dried bacteria were reactivated with 4 ml of supplied reactivation solution and cultivated in 200 ml liquid medium for starter and main cultures according to DIN EN ISO 11348-1 (ISO, 2007b) in an Erlenmeyer flask with cap under constant agitation ($350 \pm 50 \text{ rpm}$) for $48 \pm 2 \text{ h}$ at room temperature. On the day of analysis, ethanolic extracts or water samples were sprayed in 5 mm bands using the automatic TLC sampler ATS 4 (Camag) on a HPTLC plate (Silica gel 60 F₂₅₄ glass plates, $20 \times 10 \text{ cm}$, Merck Chemicals, prewashed with Methanol and activated by drying for 30 min at 110°C). Chromatographic development was conducted in the automated developing chamber AMD 2 (Camag) with ethyl acetate (Optigrade, Promochem) and n-hexane (Lichrosolv, Merck) in equal parts after

focusing the samples with methanol. Before the exposition with luminescent bacteria, the solvents on the plates were allowed to evaporate for at least 3 h. Subsequently, the plates were dipped into a suspension of luminescent bacteria with the Chromatogram Immersion Device 3 (Camag) for 1 s at highest speed. The supernatant suspension was removed from the silica surface using a squeegee. The bioluminescence was documented after an exposure time of 11 min by using a cooled 16 bit CCD camera integrated in the Bio-Luminizer (Camag). The quantitative evaluation of black and white images and the calculation of inhibition chromatograms was performed according to Schulz et al. (2017).

2.3.2. Algae

The growth-inhibition test with the green alga *Desmodesmus subspicatus* (SAG 86.81) was carried out according to DIN 38412-33 (DIN, 1991). The growth of the algae in the presence of a test sample is determined by a fluorescence measurement in comparison to a negative control. The incubation was performed in a light thermostat with rotational stand at 23°C and an irradiance of $70\text{--}120 \mu\text{E/m}^2\text{s}$ in test tubes with a testing volume of 4 ml. The density of algae at the beginning of the test was adjusted to $1 \times 10^4 \text{ cells/ml}$. Distilled water was used as negative control and potassium dichromate ($\geq 95\%$, Merck; $c = 0.5 \text{ ml/l}$) served as positive control. To quantify the growth inhibition, the chlorophyll fluorescence was measured with a fluorescence spectrophotometer (Hitachi F-2500) after 72 h. Time-proportional composite samples (short-time and long-time water samples) were tested as dilution series in geometric sequence with a dilution factor of two and the lowest ineffective dilution (LID), that caused a growth-inhibition of less than 20%, was used for the evaluation of test results. Thus, the higher the value determined, the higher the toxicity of the sample.

2.3.3. Ames fluctuation test

The mutagenicity of DMSO extracts was determined with the *Salmonella typhimurium* strains YG 1041 (frameshift tester strain) and YG 1042 (base-pair substitution strain) with the Ames fluctuation test according to ISO 11350:2012 (ISO, 2012). The mutagenic potential is determined by the increase of mutants that reverted to a histidine-independent growth in the presence of the test sample. The test strains were incubated in the presence of ampicillin (sodium salt, Sigma-Aldrich) and kanamycin (solution from *Streptomyces kanamyceticus*, 50 mg/ml in 0.9% NaCl, BioReagent, Sigma-Aldrich). The incubation time of the strain YG 1041 in 384 well plates (Greiner Bio-One) was prolonged from 48 h up to 72 h due to a low number of wells with revertant growth in the positive controls. All samples were tested two times with and without metabolic activation using 2-aminoanthracene (96%, Sigma-Aldrich; $c = 4.14 \times 10^{-8} \text{ mol/l}$) and 2-nitrofluorene (98%, Sigma-Aldrich; $c = 3.78 \times 10^{-8} \text{ mol/l}$) as positive control, respectively. Distilled water served as negative control. The cell density of the overnight cultures was adjusted to 150 FAU (Formazine Attenuation Units) for YG 1041 and 160 FAU for YG 1042 with S9 mix (from rat liver, Harlan

Cytotest Cell Research GmbH). Without S9 mix, 170 FAU for YG 1041 and 80 FAU for YG 1042 were applied. A test sample was regarded as mutagenic if a significant increase in the number of revertant wells was observed compared to the negative control in at least one strain with or without S9 mix.

2.3.4. Yeast estrogen screen

The estrogenicity of ethanolic extracts was investigated according to ISO 19040-1 (ISO, 2018b) using the test strain according to McDonnell et al. (1991b) (1991a) that is based on the strain *Saccharomyces cerevisiae* BJ3505 (protease deficient, MAT α , PEP4:HIS3, prb-1- δ 1.6 R, HIS3- δ 200, lys2-801, trp1- δ 101, ura3-52gal2can1). The cells were exposed at 30 °C for 18 h to the samples, followed by an activation of the human estrogen receptor alpha (ER α) in case of the presence of ER α -agonists in the sample. The activation of the receptor is measured by a reporter gene assay using the *lacZ*-gene - encoding the enzyme β -galactosidase - as the reporting element. Each extract was tested three-fold independently in 96-well microtiter plates (Cellstar, Greiner Bio-One) with four technical replicates each. Ethanol (1%) was used as negative control and a dilution series of 17 β -estradiol (E2) (\geq 98%, Sigma-Aldrich) served as positive control (500–0.66 ng/l) and for calibration. The estrogenic potential of samples was quantified as 17 β -estradiol equivalent concentration (EEQ) and converted to the original aqueous leachates.

2.3.5. Data evaluation and statistics

The statistical analysis of bioassay data was performed using the open source software R (version 3.4.3). The Michaelis-Menten model that is defined by a three-parameter function (Equation (1)) was implemented to fit the time-dependent inhibition of luminescence.

$$f(x) = c + \frac{d - c}{1 + (e/x)} \quad (1)$$

The fits of concentration-response relationships and estimates of EC50 were generated by a five-parameter log-logistic function (Equation (2)).

$$f(x) = c + \frac{d - c}{(1 + \exp(b \times (\log(x) - \log(e))))^f} \quad (2)$$

In either case, the response of respective bioassays was evaluated as a function of concentration x with the parameters c and d as lower and upper response limits, respectively. The parameter e is defined as inflection point, parameter b denotes the relative slope and the parameter f describes the asymmetry of the curve.

The correlations were calculated by means of simple linear regression model (Equation (3)) where b is the intercept and m is the slope.

$$f(x) = mx + b \quad (3)$$

2.4. Chemical analysis

2.4.1. DOC

Dissolved organic carbon (DOC) was determined according to DIN 1484:1997–08 (DIN, 1997) by thermal oxidation coupled with infrared detection (DIMATOC 2000, Dimatec). Prior to measurements, aqueous samples from each sampling date were filtered (0.45 μ m) and acidified with HCl 30% (Suprapur, Merck) to a pH < 2. Potassium hydrogen phthalate (Emsure, Merck) served as control standard.

2.4.2. Zinc

The analysis of zinc was carried out by inductively coupled plasma mass spectrometry (ICP-QQQ-MS, Agilent 8800x). The instrument was equipped with a Peltier cooled Scott spray chamber and a MicroMist atomizer (both Glass Expansion) as well as a quartz torch and nickel cones (both Agilent Technologies). Undiluted water samples (short-time and long-time composite samples) were acidified with 1% (v/v) HNO₃ (65%, Suprapur, subboiled, Merck KGaA) prior to measurement. Helium was used as collision/reaction gas with a flow rate of 5 l/min. SPS-SW1 (Surface Water Level 1, Campro Scientific), SLRS-6 (River Water Certified Reference Material for Trace Metals and other Constituents, NRC) and SRM 1640a (Trace Elements in Natural Water, NIST) were chosen as reference materials for method validation.

2.4.3. GC-MS

To identify compounds released from the coating systems, samples were qualitatively analyzed by gas chromatography coupled with mass spectrometry (GC-MS, Agilent GC7890A with MS 7000 Triple Quad). For this purpose, the ethanolic extracts were diluted with n-heptane (Picograde, Promochem) at least 1:20 before injection of 1 μ l sample. The GC was equipped with a HP-5ms GC-column (Agilent J&W, 30 m \times 0.25 mm \times 0.25 μ m). Helium was used as carrier gas in a constant flow mode (1 ml/min) and the following temperature program was used: initial column temperature 60 °C, held for 2.5 min, increasing by 20 °C/min up to 130 °C and 4 °C/min to 320 °C, held for 1.5 min. Mass spectra were recorded after electron ionization (70 eV) in the range of 50–1000 m/z and were compared to data of a reference library (NIST08). If one or more compounds in a sample matched to certain substances of the library with a high probability (>90%) further identification was employed by matching the samples to the corresponding reference substances in multiple reaction monitoring (MRM) mode. The quantification of all identified targets and further compounds specified in the material safety data sheet was done by an external calibration (Table 2) using standards purchased from Sigma-Aldrich. Additionally, in selected samples, the content of 4-tert-butylphenol (4tBP) was quantified by addition of the internal standard 4-tert-butylphenol-d9 (TRC Canada) prior to extraction. Extracts were kept in n-heptane.

2.5. Identification of release mechanisms

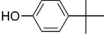

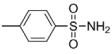
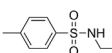

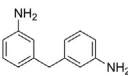
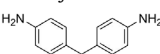
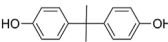
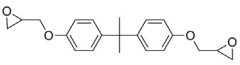
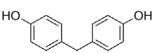
The assessment of the underlying release mechanism for 4tBP according to Annex B of CEN/TS 16637-2 (CEN, 2014) follows a stepwise process along a decision tree and is based on mean concentrations measured in the individual samples. In general, a diffusion-controlled release of a compound is linear with the square root of time (Higuchi, 1961). The elution times defined in the DSLT are selected to result in a specific 3-step release pattern in case of a release by diffusion in which the level of the second and third step is twice the level of the previous step. If the root mean square error (RMSE) between the measured amounts and the diffusion-model described above is less than 0.4, a diffusion-controlled release of compounds is assumed as the main release mechanism.

Furthermore, the release of 4tBP was modelled under the assumption of a one-dimensional diffusion in a plane sheet with an initial uniform concentration. According to Crank (1975), the total amount of diffusing substance M_t released at time t can be expressed as

$$M_t = M_\infty \left(1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left\{ \frac{-D(2n+1)^2 \pi^2 t}{4l^2} \right\} \right) \quad (4)$$

Table 2

Analytical parameters of target compounds. Quantification limits (LOQ) were determined by signal-to-noise method ($S/N \geq 10$). As bisphenol A and 4-*tert*-butylphenol were present in blank samples respective LOQ were calculated from blank values (mean + 10 sd). A comparison of MS spectra obtained from the samples with reference spectra is shown in SI (see Fig. SI 1). The recovery of analytes is also summarized in SI (see Table SI 2).

substance	CAS	retention time [min]	MRM transition (m/z) precursor → product ions	LOQ [mg/l]
4- <i>tert</i> -butylphenol (4tBP)	98-54-4	8.46	149.8 → 134.9, 106.9	0.005
				
3- <i>tert</i> -butylphenol	585-34-2	8.49	149.8 → 134.9, 106.9	0.005
				
4-toluenesulfonamide (4TSA)	70-55-3	15.41	170.8 → 90.8, 106.9 154.9 → 90.8	0.002
				
4-tolueneethylsulfonamide(4TESA)	80-39-7	16.6	183.9, 199.0 → 90.8	0.0005
				
benzo[f]quinolone	85-02-9	19.1	178.9 → 150.9 177.9 → 149.9	0.0005
				
3,3'-methyleneedianiline	19471-12-6	25.7	198.2 → 106.2, 182.2	0.001
				
4,4'-methyleneedianiline	101-77-9	25.87	197.2 → 180.2 198.2 → 182.2, 106.2	0.001
				
bisphenol A	80-05-7	26.9	213.1 → 119.1, 91.1 119.1 → 91.1	0.001
				
bisphenol A diglycidyl ether	1675-54-3	40.7	213.1 → 91.1, 119.1	0.004
				
bisphenol F	620-92-8	24.6	199.9 → 106.9 198.9 → 151.9 182.9 → 114.9	0.002
				

for a non-steady state. Taking the thickness l of coating film into consideration, the diffusion coefficient D and the total amount of diffusing substance M_{∞} after infinite time were determined. Release curves were fitted by minimizing mean squared errors between modelled and measured values.

3. Results

3.1. Time-dependent release of toxic effects

In order to characterize possible ecotoxicological threats to the aquatic environment caused by the use of coatings for corrosion protection, selected materials were evaluated in eight leaching steps over 64 d in total. Acute toxic effects to destruents and primary producers as well as specific effects in the form of estrogenic and mutagenic effects were studied.

The luminescent bacterium *Aliivibrio fischeri* showed an increase of toxicity in all tested coating systems with increasing leaching time, which was most notably within the first leaching steps (Fig. 1 and Fig. SI 2). This finding was confirmed for the most toxic top coating (I) by a luminescent bacteria assay coupled to HPTLC indicating at least one organic compound causing the observed effect

(Fig. SI 3). The peak area of the detected inhibition spots correlated well with the results of the standard bioluminescence inhibition assay ($R^2 = 0.9162$, $p < 0.0002$).

Coating systems with the same top coating showed highly similar effects spanning a wide toxic range from LID ≈ 3 for coating systems 6 and 7 up to LID ≈ 1000 for coating systems 1 and 2. The coating systems 3 and 4 sharing the top coating (II) showed intermediate effects in the range from LID ≈ 20 to 50. The only primer coating tested without a top coating (coating system 5) showed a LID for bacterial toxicity about 10.

The growth of the green alga *Desmodesmus subspicatus* was inhibited only by eluates originating from the primer coating without top coating (Fig. 2). A small reduction of the growth rate was already visible after the short-term elution while undiluted long-term eluates induced cell death so that the apparent growth-inhibition exceeded 100%. The testing of dilution series resulted in a mean EC50 of $59.3 \pm 1.8\%$ of long term eluates from coating system 5.

The Ames fluctuation test provided no evidence of mutagenicity for all tested coating systems (results not shown). In contrast, four of seven enriched eluates from the investigated coating systems showed significant estrogen-like effects (Fig. 3). The samples of

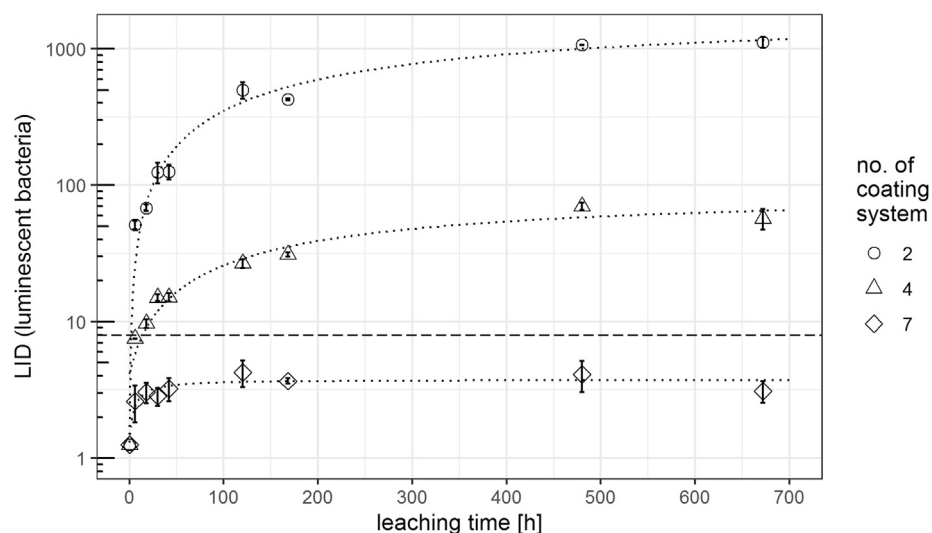


Fig. 1. Time-dependent luminescence inhibition (mean, $n = 3$, error bars indicate SE) in leachates of three selected top coatings for corrosion protection (see SI for further data). Leaching time is displayed as duration of the specific step after the renewal of leachant. Results calculated by linear interpolation are expressed as dilution levels causing 20% luminescence inhibition (lowest ineffective dilution, LID) of *Aliivibrio fischeri*. The dashed line indicates the assessment criterion set by DIBT and dotted lines show fits of data according to the Michaelis-Menten equation (Equation (1)).

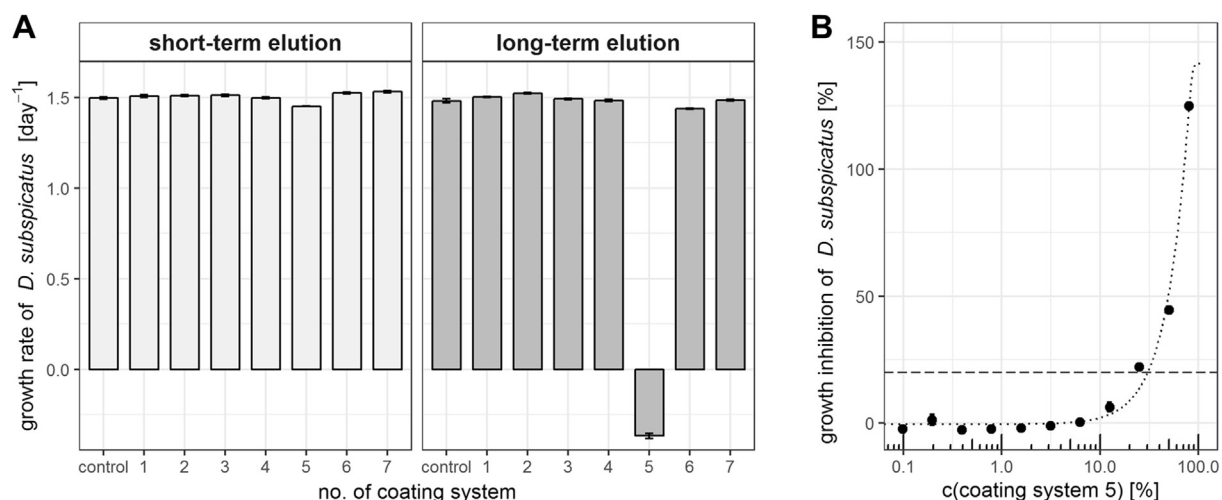


Fig. 2. Effects on growth of the algae *Desmodesmus subspicatus* in leachates of seven anti-corrosion coating systems. A: Average growth rate ($n = 3$, error bars indicate SE) in undiluted leachates. Algae were exposed for 72 h to time-proportional composite samples collected over short (0.25–1 d) and long period (0.25–64 d). B: Concentration-dependent inhibition of algal growth (mean, $n = 3$, error bars indicate SE) of long-term eluates from coating system 5. The dashed line indicates the 20% effect level and dotted line shows the 5-parametric log-logistic fit of data (Equation (2)).

coating systems 1 and 2 showed estrogenic activities after short-term exposure (0.25–1 d) at 4.39 ± 0.42 ng/l and 4.64 ± 0.50 ng/l EEQ, respectively. The effect increased in the corresponding long-term samples (0.25–64 d) about 20–30 times to 144 ± 24 ng/l (coating system 1) and 100 ± 11 ng/l EEQ (coating system 2). Low estrogen-like effects were quantified in the long-term samples of coating system 3 (0.58 ± 0.12 ng/l EEQ) and system 4 (0.87 ± 0.15 ng/l EEQ). No estrogen-like effects were detected in the remaining coating systems.

3.2. Quantified substances and accordance with toxic effects

Time-proportional composite sample extracts were analyzed by GC-MS to identify the potentially toxic compounds released from the coating systems. In total, three of nine suspected compounds

were unequivocally identified and quantified in all three leaching-replica by external calibration (Table 3).

Six substances leached from coating system 5, 6 and 7 remained unidentified (see Table SI 3 for GC-MS scan data). The identified compounds were 4-*tert*-butylphenol (4tBP), 4-toluenesulfonamide (4TSA) and 4-tolueneethylsulfonamide (4TESA). 4tBP was detected only in the leachates of the epoxide-based coating systems 1, 2, 3 and 4, whereas 4TSA and 4TESA leached from the polyurethane resin based coating systems 5, 6 and 7. The highest amounts of 4tBP were quantified in leachates with the highest bacterial toxicity and estrogenicity. Since eluates of coating systems 6 and 7 showed no detectable effects in any of the bioassays applied, further work to determine the contribution of identified compounds to the observed effects was focused on the epoxide resin based coating systems. Extracts of coating systems 1 and 2 showing highest effect

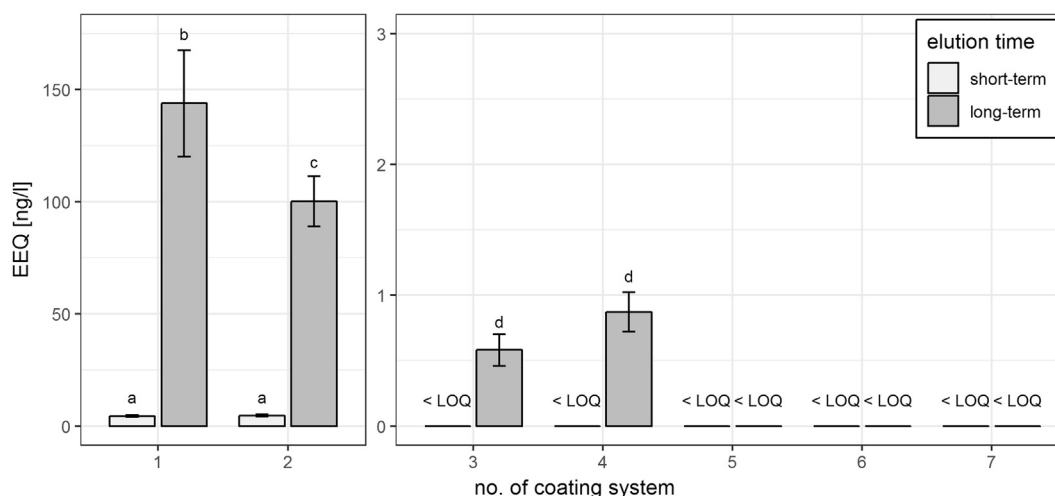


Fig. 3. Mean estrogenic activities ($n = 9$, error bars indicate SE) in leachates of seven anti-corrosion coating systems. Properties of investigated materials are summarized in Table 1. Effects were detected as estradiol-equivalents (EEQ) in time-proportional composite samples over short time (0.25–1 d) and long time (0.25–64 d) with a recombinant yeast estrogen screen. Each coating system was leached three times and each leachate was tested three times. Experiments were performed with enriched samples and the results shown are calculated for the original aqueous leachates (see Fig. SI 4 for full dose-response data). Negative controls showed no effects. Significantly different means are marked with different letters (two-tailed unpaired t -test, $p < 0.05$).

Table 3

Quantified target compounds (mean, $n = 3$) in long-term leachates of seven anti-corrosion coating systems. Contents were measured in enriched samples, results refer to aqueous samples. Peaks that were detected in all replicates but remained unidentified are listed in SI (see Table SI 3).

no. of coating system	4tBP [mg/l]		4TSA [mg/l]		4TESA [mg/l]	
	mean	SE	mean	SE	mean	SE
1	4.3	0.3	< LOQ		< LOQ	
2	2.6	0.2	< LOQ		< LOQ	
3	0.02	0.006	< LOQ		< LOQ	
4	0.03	0.015	< LOQ		< LOQ	
5	< LOQ		0.29	0.03	0.0089	0.0005
6	< LOQ		0.22	0.01	0.0073	0.0003
7	< LOQ		0.004	0.00	< LOQ	

Abbreviations: 4-*tert*-butylphenol (4tBP), 4-toluenesulfonamide (4TSA), 4-tolueneethylsulfonamide (4TESA).

levels were tested for bacterial toxicity and estrogenic activities in comparison to the corresponding concentrations of 4tBP determined in the selected representative leaching-replica. In all cases the samples showed higher effects than the respective standard (Fig. 4). In the bioluminescence inhibition assay, the apparent EC₅₀-values of coating system 1 and 2 expressed in terms of 4tBP were $25.1 \pm 2.7 \mu\text{g/l}$ and $17.13 \pm 0.55 \mu\text{g/l}$, respectively. The EC₅₀-value of the 4tBP tested as single compound was $37.9 \pm 9.9 \mu\text{g/l}$. With respect to estrogenicity, coating system 1 contained $167 \pm 15 \text{ ng/l}$ EEQ whereas the corresponding measured concentration of 4tBP reached an EEQ of $133 \pm 10 \text{ ng/l}$. In coating system 2 in total $94.6 \pm 4.8 \text{ ng/l}$ EEQ were determined from which $58.3 \pm 6.8 \text{ ng/l}$ EEQ could be attributed to 4tBP.

To estimate the total release of 4tBP from the two different top coatings ((I) and (II)) based on epoxide resins, the 4tBP concentrations in the individual samples from each sampling date of coating systems 2 (top coating I) and 4 (top coating II) were analyzed. In both coatings, the 4tBP concentration increased with leaching time (see Table SI 4). Taking into account the leaching volume, the total release of 4tBP was $34.3 \pm 1.2 \text{ mg}$ and $1.97 \pm 0.05 \text{ mg}$ 4tBP from coating system 2 and 4, respectively. The luminescence inhibition of leachates was found to be positively correlated with the 4tBP content in both coating systems (see

Fig. 5).

3.3. Release mechanisms

The concentrations of 4tBP in the individual samples of top coatings (I) and (II) were used for the assessment of the underlying release mechanism according to Annex B of CEN/TS 16637-2 (CEN, 2014). In both cases the release profile showed a 3-step pattern (see Fig. 6) and RMSE was less than 0.40 (0.28 for top coating (I) and 0.10 for top coating (II)). In case of the top coating (I), mean amounts released per plate were 1.1 (step 1), 3.8 (step 2) and 11 mg (step 3). The respective values for top coating (II) were 0.12 (step 1), 0.27 (step 2) and 0.48 (step 3). Compared to top coating (II), the release of 4tBP increases more than 2-fold between the steps in top coating (I).

Assuming diffusion as the only release mechanism, the application of the diffusion equation (Equation (4)) leads to sums of squared errors of 0.015 and 58.5 for the fits of top coating (II) and top coating (I), respectively (see Fig. SI 5 and Table SI 5). D and M_{∞} for coating (II) are $9 \times 10^{-11} \text{ cm}^2/\text{s}$ and 3.9 mg. Due to the lack of fit, meaningful values for these parameters could not be determined for top coating (I).

3.4. Overview and evaluation of ecotoxicological results

The different coating systems investigated were evaluated and compared based on the “principles for the assessment of the impact of construction products on soil and groundwater” suggested by DIBt (2011). According to this concept, the lowest ineffective dilution shall not exceed the dilution stages 8 and 4 in the bioluminescence inhibition assay and algae growth-inhibition test, respectively. Moreover, the eluate must not show any mutagenic potential. Deviating from the proposed range of biological parameters, the toxicity to daphnia and fish eggs as well as the biodegradability of organic constituents were not tested. In addition, estrogen-like effects were examined with a recombinant yeast estrogen screen.

The epoxide resin based coating systems 1, 2, 3 and 4 did neither pass the assessment criteria for bacterial toxicity nor for estrogenicity. The polyurethane resin based primer coating B (system 5)

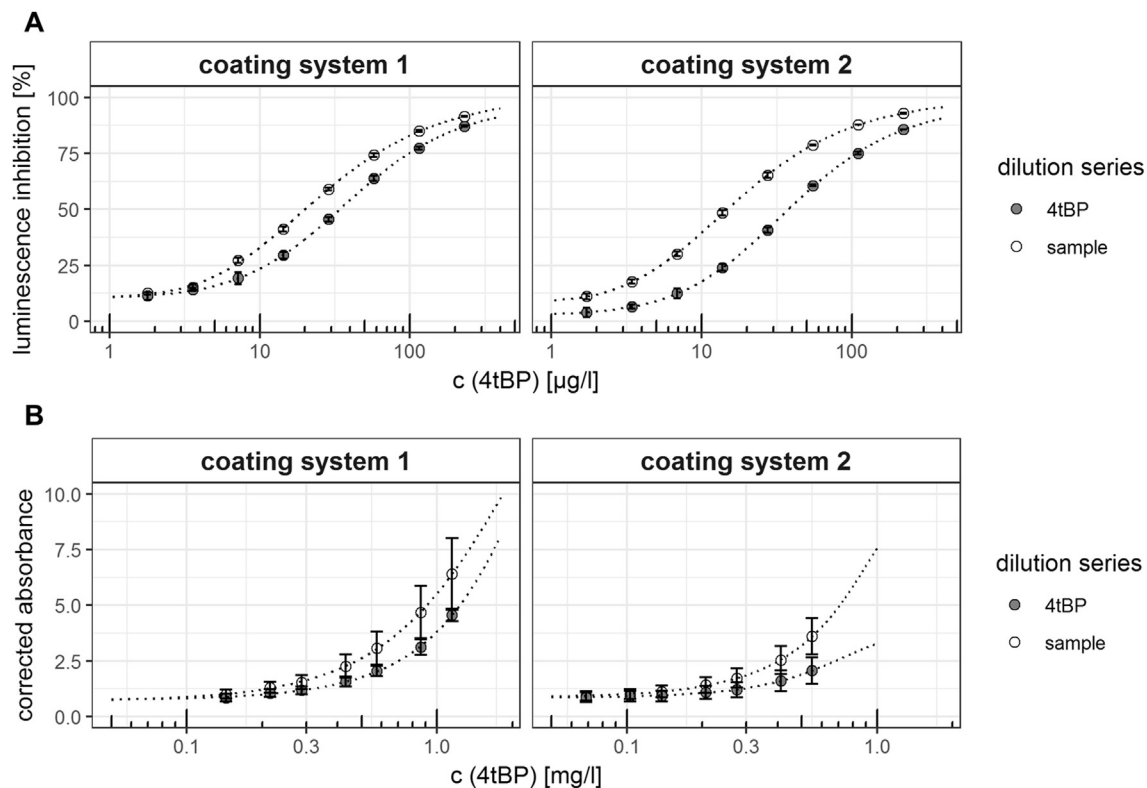


Fig. 4. Concentration-dependent effects of enriched leachates of anti-corrosion coating systems 1 and 2 and 4-*tert*-butylphenol (4tBP). Each representative time-proportional long time sample (0.25–64 d) was tested against its corresponding concentration of 4tBP. Dotted lines show the 5-parametric log-logistic fit of the data (Equation (2)). A: Toxicity effect on luminescent bacteria shown as percentage luminescence inhibition (mean, $n = 3$, error bars indicate SE) B: Estrogenic activities detected as corrected absorbance (mean, $n = 4$, error bars indicate SE) with a recombinant yeast estrogen screen.

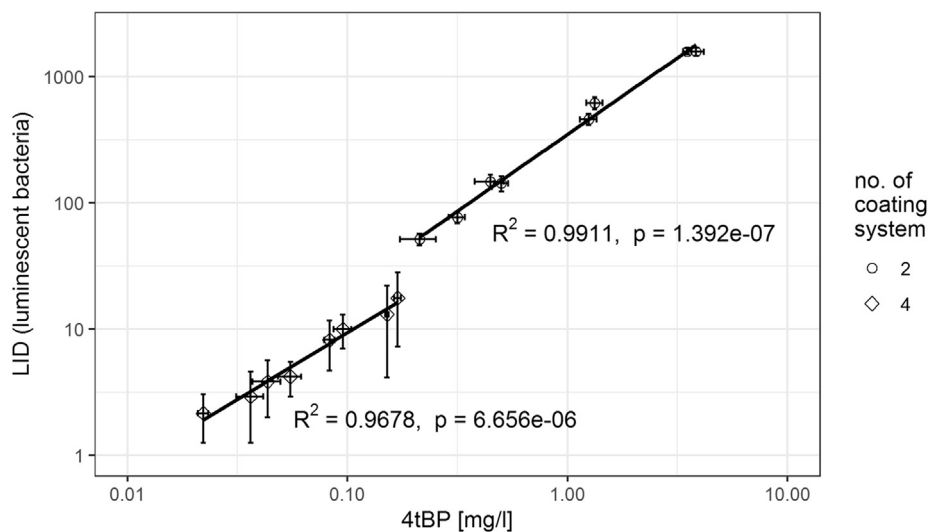


Fig. 5. Correlation of 4tBP content and luminescence inhibition in leachates of two anti-corrosion coating systems ($n = 3 \times 8$). Results of regression tests are shown as adjusted R^2 and associated p-value.

was the only tested coating system which caused toxicity to the green alga *Desmodesmus subspicatus*. With a mean LID of 8, the assessment criterion set by the DIBt was missed by one 1 → 2 dilution step. A reason for this effect may be the elevated zinc content of the respective leachates. The zinc concentration of algal toxic samples ranged between 2.8 and 3.0 mg/l whereas it was below 0.02 mg/l in samples without algal toxicity (see Table SI 6).

Moreover, the observed concentration-response relationship of long-term samples of system 5 agreed well with the toxic properties of zinc. For the same alga a half maximal inhibition of the growth rate was reported at 1.02–1.54 mg/l $ZnCl_2$ (equivalent to 0.49–0.74 mg/l Zn^{2+}) (Eisentraeger et al., 2003). In contrast, the polyurethane resin based systems 6 and 7 did not show any ecotoxicological effects.

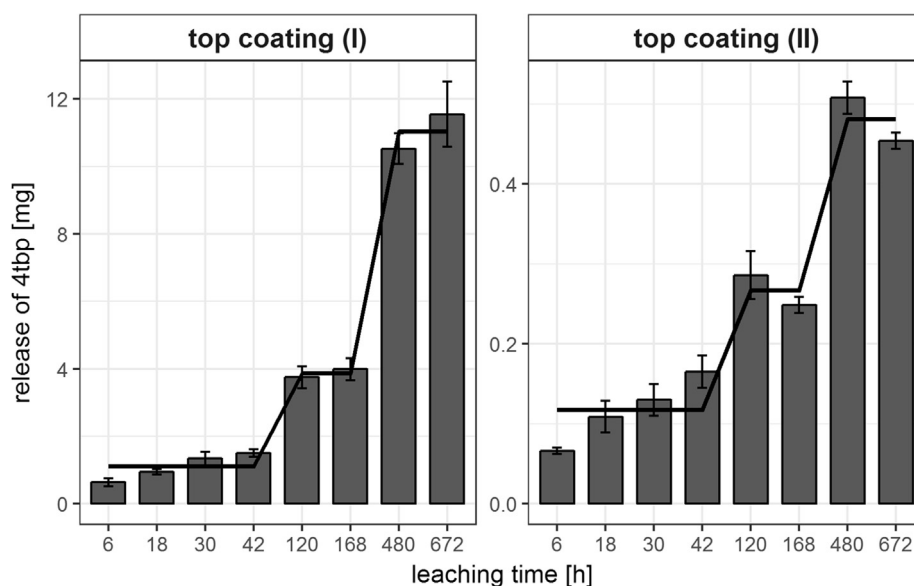


Fig. 6. Time-dependent release of 4tBP (mean, $n = 3$, error bars indicate SE) in leachates of top coating (I) and (II). Leaching time is indicated as the duration of the specific step after renewal of leachant. Solid lines show the arithmetic means of the supposed 3-step release pattern.

4. Discussion

4.1. Release of substances from steel coatings and their environmental relevance

The results of the bioluminescence inhibition assay and chemical analysis show that toxic substances were continuously released from the tested coating systems. As expected, the top coating governed the observed effects and release rates of toxic compounds dropped down over time which becomes apparent by the normalization of determined effect levels of a given time point to the respective leaching time (Fig. SI 6). This trend reflects the decreasing release rate of compounds from the coating. A comparable leaching behavior (high initial release rates decaying with time) was investigated for biocides from façade coatings following a diffusion controlled release (Wangler et al., 2012). Erich and Baukh (2016) proposed a model for biocide release from coatings that was determined by water diffusion, the dissolution of biocides, and their release from the coating by diffusion and advection. However, the decreased release rates are overcompensated by the increasing leaching time resulting in an overall increase in toxic effects over time.

As described, highest concentrations of 4tBP were detected in eluates showing the strongest effects in the bioluminescence inhibition assay and the Yeast Estrogen Screen. By comparing the estrogenic activity of single extracts and respective 4tBP-standards, about 80 and 60% of EEQ could be explained by 4tBP detected in leachates of system 1 and 2, respectively. Analogous to this observation, the inhibition of luminescence in *Aliivibrio fischeri* could be attributed to 4tBP to around 65% in coating system 1 and 45% in coating system 2 (referred to EC50). Thus, 4tBP was identified as a main driver for acute and specific toxicity but it is likely that further substances contribute to the observed effects. However, safety data sheets and chemical analyses gave no indication to the identity of further toxic compounds, further investigations by effect-directed analysis might help to provide additional information about the composition of the leachates. As shown, HPTLC coupled assays offer the advantage to test aqueous samples, thus the loss of individual compounds by prior extraction can be prevented. The extraction

and mass spectrometric analysis of the stationary phase at indicated positions could directly link bioactive zones to toxic compounds (Taha et al., 2015; Weiss et al., 2017). The investigation of bacteriotoxic effects in combination with HPTLC resulted, however, only in one signal near the elution front. This is in agreement with the low polarity of 4tBP. Further compounds with comparable physicochemical properties to 4tBP might co-migrate under the applied conditions. The use of other stationary and/or mobile phases might reveal further bioactive compounds.

Taking into account the composition of the coatings, the theoretical material consumption to prepare a coating of a defined thickness, the actual thickness of the investigated coatings and the concentration of monomeric 4tBP in the hardener component of top coating (II) (coating system 4), a total amount of 2.2 g 4tBP per test plate was calculated, which corresponds to 5.8% (w/w). The total release of 1.97 mg (over eight leaching steps in 64 days) is equivalent to approximately 0.1% of the total amount. Thus, it is likely that despite a general depletion of toxic substances in the coating, a release of compounds might take place over a longer period of time. Although top coating (I) (coating system 2) released in total more than 17-times the amount of 4tBP released from top coating (II), 4tBP was not listed in the available safety data sheet provided together with top coating (I). The 4tBP content of top coating (I) was determined by GC-MS to 2.4% (w/w), this is equivalent to 0.4 g 4tBP per test plate. The total release of 34.3 mg, in turn, corresponds to around 8.6% of the total amount in the coating. Despite a more than five times lower total amount, the top coating (I) lead to a substantially higher release than top coating (II). This is to some degree a counter intuitive finding but might be explained by different release mechanisms and/or mobility of 4tBP in the two polymers. The alkylphenol 4tBP is applied in epoxy resins as a curing agent and for the termination of polyaddition reactions (carbamate formation). Thus it is covalently bound to the polymer structure. A sub-optimal stoichiometry of e.g. monomers and hardener in the recipe of the polymer might result in elevated fractions of 4tBP that is not covalently bound to the polymer structure and thus mobile. An optimization of the recipe might lead to reduced emissions of 4tBP into the environment by an enhanced incorporation in the polymer structure. Besides the benefit for the

environment, the using of smaller quantities could also reduce the cost of the product. Additionally, according to principle 4 of green chemistry (Erythropel et al., 2018) hazardous compounds should be replaced to minimize the toxicity of a product without affecting the desired function. Furthermore, the elucidation of possible release mechanisms revealed distinct differences between the top coatings (I) and (II). As demonstrated for top coating (I), the concentration levels of the second and third step in the release pattern were increased more than two-fold (Fig. 6) and were therefore different than expected for a compound release governed only by diffusion. In contrast, the top coating (II) showed such a behavior indicating a release of 4tBP controlled by diffusion. This finding is supported by the modelling of the 4tBP release using Eq. (4) which resulted in an acceptable accordance between model and measurement only for top coating (II). The determined diffusion coefficient for 4tBP in top coating (II) was $0.9 \times 10^{-10} \text{ cm}^2/\text{s}$ and thus in the same order of magnitude compared to diffusion coefficients of water in epoxy resins ranging between 0.04 and $0.95 \times 10^{-10} \text{ cm}^2/\text{s}$ as reported previously (Li et al., 2006; Liu et al., 2003). The calculated maximal release of 4tBP from the total surface of 498.6 cm^2 was 3.9 mg for this top coating indicating that about 50% of the mobilisable 4tBP was emitted during the release experiment. In contrast to top coating (II) an acceptable fit for the measured 4tBP-release was not possible for top coating (I) indicating a release mechanism that is not only controlled by diffusion. A release of 4tBP by surface wash-off is unlikely. In case of a surface wash-off, a high initial release would be expected in the first and possibly second elution step, followed by substantially lower concentrations in the subsequent eluates (CEN, 2014). This expectation is not reflected in the measurements. Therefore, it might be hypothesized that the release of 4tBP is increased by polymer swelling or even dissolution of the top coating. Independent from the specific release mechanism, the presented results indicate the importance of an optimized formulation to avoid an increased release of toxic compounds to the environment.

The toxicity of 4tBP is well studied for various species together with the evaluation of the environmental hazard by higher-tiered approaches (Wang et al., 2018). With respect to the ecotoxicological assessment of 4tBP, crustaceans were the most sensitive species. The LC50 for *Neocaridina* sp. was reported with 3.61 mg/l and a half maximal inhibition of the reproduction rate to *Daphnia magna* was observed at 4.12 mg/l (Wang et al., 2018). Leachates of top coating (I) reached an average concentration of 4tBP up to 3.85 mg/l , thus emissions from coating system 1 and 2 might pose a threat to crustaceans in the environment. However, the actual surface/volume ratios and dilution effects in rivers have to be taken into account. No risk is to be expected for crustaceans with respect to 4tBP emissions from the other coating systems investigated. In comparison to *Daphnia magna*, algae are less sensitive to 4tBP, the EC50 of seven different algae species ranged between 6.25 and 41.47 mg/l (Wang et al., 2018). Thus, the fact that no toxicity on algae was observed is in agreement with literature.

Although, about 1.5×10^6 fold less potent than 17β -estradiol in the Yeast Estrogen Screen (Routledge and Sumpter, 1997), 4tBP exhibits estrogen-like effects even below a concentration of 1 mg/l (Barse et al., 2006; Jobling and Sumpter, 1993; Meier et al., 2011). In the current study, significant estrogen-like effects could be detected even below 0.02 mg/l 4tBP. An EEQ-based trigger value for the YES assay of about 1 ng/l proposed by Escher et al. (2018) was exceeded by leachates from coating systems 1 and 2. The EEQ levels of the short-term eluates exceeded this trigger value 4 times and the long-term eluates even 93 to 135 times. Based on the investigated estrogenicity, an elevated environmental risk might be present in particular in case of the coating systems 1 and 2. The predicted no effect concentration for freshwater of 0.01 mg/l 4tBP

(ECHA, 2019a) would have been exceeded in all long-term leachates of coating systems 1, 2, 3 and 4. Based on this PNEC and the measured amounts of 4tBP emitted from the two different epoxide resin based top coatings a surface of one square meter of top coating (II) would lead to risk quotient >1 in 3.8 m^3 water. In case of the top coating (I), one square meter would contaminate even 68 cubic meter water. No exceedance of the defined PNEC-value for 4tBP in the environment is reported so far. In the Elbe River, concentrations of 4tBP were reported in levels up to 78 ng/l in water and $75 \text{ }\mu\text{g/kg}$ dry matter in sediment. Water samples of North Sea reached concentrations up to 43 ng/l (Heemken et al., 2001). Further monitoring data for 4tBP is mainly available for Asia ranging from about $0.01 \text{ }\mu\text{g/l}$ in a Japanese river (Inoue et al., 2002) up to $1 \text{ }\mu\text{g/l}$ in the Yangtze River (Liu et al., 2017). 4tBP was detected in fish bile up to $78.2 \text{ }\mu\text{g/l}$, which leads to a bioconcentration factor of 2200 and indicates a possible potential for bioaccumulation (Wu et al., 2016).

The sulfonamides 4TSA and 4TESA leached from coating systems 5, 6 and 7 are supposed to be reaction products of 4-toluenesulfonyl isocyanate (4TSI), which is known as moisture scavenger in polyurethane resin based coatings. As mentioned by Luft et al. (2017) 4TSI can react with ethanol or water to carbamate and form respective sulfonamides after decarboxylation. Both amides are not classified as dangerous. Moreover, ecotoxicological studies are very limited and the ecotoxicological assessments are mainly based on the structure-activity relationship of 2TSA. The EC50 for algae (*Pseudokirchneriella subcapitata*), invertebrates (*Daphnia magna*) and fish (*Salmo gairdneri*) are estimated greater than 100 mg/l 4TSA and the predicted no effect concentration for freshwater has been estimated at 0.15 mg/l (ECHA, 2019b). Leachates of coating system 5 and 6 reached an average concentration up to 0.29 mg/l 4TSA and thereby exceeded the limit value two-fold. 4TSA was also found in leachates of a one-component polyurethane coating in concentrations up to 61 mg/l , while it could not be detected in the rivers Rhine and Mosel (Luft et al., 2017). In samples taken from the Teltow canal in the south of Berlin, the concentration of 4TSA was below the LOQ of $10 \text{ }\mu\text{g/l}$ (Luft et al., 2017). However, 4TSA was found to be present in the aquatic environments of Berlin with up to $1.15 \text{ }\mu\text{g/l}$ and $38 \text{ }\mu\text{g/l}$ in surface and groundwater, respectively (Meffe et al., 2014; Richter et al., 2007).

In contrast to the findings of Vermeirssen et al. (2017), the endocrine disruptor bisphenol A was not detected in eluates of the investigated coatings for corrosion protection. Further ingredients of concern that are known from safety data sheets, such as various isocyanates and amines, were not detected by the GC/MS-screening method applied. The presence of these compounds seems to be less likely, but cannot be excluded since the respective recoveries were not characterized by the use of standards. However, a complete characterization of the chemical composition of the leachates was beyond the scope of this study.

Due to the investigated anti-corrosion coating systems, a local increase of 4tBP and sulfonamides in the aquatic environment might be possible. To clarify, if this input could lead to an exceedance of respective PNECs, further research is required, e. g. by the monitoring of compounds such as 4tBP, before, during and after the application of corrosion protection in the field. In order to finally assess the environmental impact of individual chemicals released from organic steel coatings, the laboratory results need to be transposed to realistic conditions. For this purpose, transfer functions have to be determined, which can predict expected concentrations under natural conditions based on results from leaching experiments performed in trough tests. The simulation model COMLEAM was developed to simulate the discharge of water-mobilisable substances from construction materials into the

environment (Tietje et al., 2018) and could possibly also be applied to organic coating systems. To develop a full picture of aquatic emissions from steel coatings, future investigations should be extended to a broader range of products and also materials (e. g. fluoropolymers, polyester resins). Additionally results on organisms of higher trophic levels such as daphnids and fish would be desirable.

4.2. Recommendations for the assessment strategy of steel coatings

The dynamic surface leaching test in combination with biological test methods allowed the identification of the time-dependent occurrence of ecotoxicological effects and provided reproducible results for the comparison of different anti-corrosion coating systems. Nevertheless, for the characterization of aquatic emissions from steel coatings some amendments should be implemented to increase the efficiency of the leaching procedure as well as the assessment strategy.

The general recommendation for construction products of the technical report CEN/TR 17105:2017 (CEN, 2018) to select only fractions 1 and 2 of dynamic surface leaching test for an ecotoxicological testing cannot be supported against the presented findings. For the investigated coatings, it is evident that a possible ecotoxicological risk would be underestimated if the evaluation would be restricted to the investigation of these short term eluates. The LID values for bacterial toxicity increased about 10-fold from the second to the last sampling in case of top coating (I). In the short time leachates of coating system 3 and 4, no estrogenic effects were detectable, whereas estrogen-like compounds in long time composite samples exceeded the limit of quantification. The same is true for the effect on algae growth that was only significantly detectable in the samples generated by a longer leaching period. To sensitively detect possible adverse effects, it would be advisable to focus on leachates collected over elongated time periods. Samples identified to exhibit toxic effects should be investigated further by the analysis of shorter elution-times to characterize emission rates and to elucidate release mechanisms. The most practicable option would be to choose the fraction with the longest contact time or rather a leachate from a (e. g. four weeks lasting) tank test without water replacement. Thus, the time-consuming sampling design could be avoided in favor of an extended biological test batterie. This would focus the investigations on inorganic and non-volatile organic substances, while unstable and volatile organic compounds are possibly partly lost. But in long term, volatile compounds are potentially less hazardous to water organisms than stable substances with lower vapor pressure due to their dissipation in the water phase. The suggestion presented in the technical report CEN/TR 17105:2017 (CEN, 2018) to select fractions to be investigated by means of organic carbon content is reasonable. The current study demonstrated that e. g. the luminescence inhibition of leachates correlated with DOC concentrations (Fig. SI 7). The assessment criterion of DIBt was considerably exceeded ($LID_{10} > 365$) in all samples with more than 5 mg/l dissolved organic carbon. Fractions with an elevated DOC showed in general an increased aquatic toxicity and therefore should be prioritized for ecotoxicological investigations. However, an assessment based on the DOC alone would be insufficient and too stringent because some samples with elevated DOC-levels showed no toxic effects (Fig. SI 7).

At present, biological test methods are applied to construction products only in particular cases. The ecological safety is mainly verified by the valuation of the composition. If necessary, additionally measured concentrations from leaching tests of single compounds are compared with limit values based on model

calculations (CEN, 2018; DIBt, 2011). The assessment of anti-corrosion coating systems poses a special challenge, as not only starting substances can be hazardous but also compounds formed *in situ* during the polyaddition reactions. Hence a complex mixture of organic substances can possibly be released into the environment. The determination of all mobilisable constituents is difficult to obtain and respective limit values as well as transfer functions are not fully available. For the compulsory assessment of ecotoxicological effects of polymeric products in general and coating materials in particular, it is recommended to assess the cumulative impact of all compounds released into the environment, especially if unknown compounds might be generated by the application. The European Committee for Standardization (CEN, 2018) as well as the DIBt (2011) recommend a minimum test battery for the testing of aquatic ecotoxicity on construction products comprising luminescent bacteria, algae and daphnids. Testing on mutagenicity and toxicity to fish eggs is additionally intended, if formulation indicates respective risks. As in the present study, two of three top coatings showed estrogen-like effects, moreover the investigation of estrogenicity is suggested for the assessment of anti-corrosion coating systems. As long as no standardized approach has been established to evaluate the ecotoxicological test results, the criteria of DIBt using LID limit values are a simple and practicable opportunity also for the comparative assessment of anti-corrosion coating systems.

5. Conclusions

- Anti-corrosion coatings can cause significant ecotoxicological effects. Only two of seven coating systems showed no significant effects in any of the bioassays applied. Based on these results, polyurethane resin based coatings are recommended as top layer.
- 4-*tert*-butylphenol (4tBP) was identified as ingredient of concern and main contributor to acute and toxic effects in epoxide resin based coatings. The predicted no effect concentration for freshwater was exceeded in the leachates of four coating systems. Therefore, it is strongly suggested that 4tBP will be immediately replaced by higher molecular weight phenols.
- The release of a toxic compound is not only determined by its concentration in the coating, but also by the underlying release mechanism. The formulation of coatings should be optimized accordingly.
- The applied test and evaluation concept facilitates the reproducible comparison of steel coating systems without knowing their ingredients and emitting substances. While exhibiting the same anti-corrosion properties, products with less or no impact on the environment can be identified. This can make a contribution to the development of environmentally sustainable formulations. Thus, a combination of ecotoxicological studies and chemical analysis is recommended for an overall comparative assessment.

Author contributions

AMB and SB conceived and designed the study. RB selected the representative coating products for investigations. AMB* coordinated and conducted the experiments. Data analysis was performed by AMB and SB. All authors contributed to the discussion and interpretation of results. The manuscript was drafted by AMB and SB and revised by all authors. All authors read and approved the final manuscript.

* see also acknowledgements

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Ramona Pfänder for technical assistance with the Yeast Estrogen Screen and Ames fluctuation test, Marina Ohlig for the preparation of extracts, Christel Möhlenkamp for GC-MS analyses, Charlotte Molt for DOC measurements and Dennis Ecker for ICP-MS analyses.

The project was funded within the governmental research and development-budget of the German Federal Ministry of Transport and Digital Infrastructure (BMVI).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.watres.2020.115525>.

References

- Akindoyo, J.O., Beg, M.D.H., Ghazali, S., Islam, M.R., Jeyaratnam, N., Yuvaraj, A.R., 2016. Polyurethane types, synthesis and applications - a review. *RSC Adv.* 6 (115), 114453–114482. <https://doi.org/10.1039/c6ra14525f>.
- Barse, A., Chakrabarti, T., Ghosh, T.K., Pal, A.K., Jadhao, S.B., 2006. One-tenth dose of LC50 of 4-tert-butylphenol causes endocrine disruption and metabolic changes in *Cyprinus carpio*. *Pestic. Biochem. Physiol.* 86 (3), 172–179. <https://doi.org/10.1016/j.pestbp.2006.03.006>.
- BAST, 2002. Technische Lieferbedingungen und Technische Prüfvorschriften für Beschichtungsmaterialien für den Korrosionsschutz von Stahlbauten. [Technical terms of delivery and technical test regulations for coating materials for the corrosion protection of steel structures].
- BAST, 2012. Zusätzliche Technische Vertragsbedingungen und Richtlinien für Ingenieurbauten. Teil 4 Stahlbau, Stahlverbundbau. [Additional technical terms of contract and guidelines for civil engineering works. Part 4 steel structures, composite steel structures].
- BAW, 2011. Prüfung von Beschichtungssystemen für den Korrosionsschutz im Stahlwasserbau (RPB). [Guidelines for the testing of coating systems for the corrosion protection of hydraulic steel structures].
- BMVBS, 2009. Zusätzliche Technische Vertragsbedingungen - Wasserbau (ZTV-W) für Korrosionsschutz im Stahlwasserbau (Leistungsbereich 218). [Additional technical terms of contract - hydraulic engineering for hydraulic steel structures (performance category 218)].
- CEN, 2014. Construction Products - Assessment of Release of Dangerous Substances - Part 2: Horizontal Dynamic Surface Leaching Test. <https://doi.org/10.31030/2021527>.
- CEN, 2018. Assessment of Release of Dangerous Substances - Guidance on the Use of Ecotoxicity Tests Applied to Construction Products. <https://doi.org/10.31030/2694085>.
- Crank, J., 1975. *The Mathematics of Diffusion*. Clarendon Press, Oxford, Eng.
- DIBt, 2011. Grundsätze zur Bewertung der Auswirkungen von Bauprodukten auf Boden und Grundwasser. [Principles for assessing the effects of construction products on soil and groundwater].
- DIN, 1991. German Standard Methods for the Examination of Water, Waste Water and Sludge; Bio-Assays (Group I); Determining the Tolerance of Green Algae to the Toxicity of Waste Water (Scenedesmus Chlorophyll Fluorescence Test) by Way of Dilution Series (L 33). <https://doi.org/10.31030/2410166>.
- DIN, 1997. Water Analysis - Guidelines for the Determination of Total Organic Carbon (TOC) and Dissolved Organic Carbon. (DOC). <https://doi.org/10.31030/3042067>.
- Dornbusch, M., Christ, U., Rasing, R., 2016. *Epoxy Resins*. Vincentz Network.
- ECHA, 2019a. Registration Dossier of 4-Tert-Butylphenol. Retrieved from. <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15260/1>. (Accessed 28 June 2019).
- ECHA, 2019b. Registration Dossier of Toluene-4-Sulphonamide. Retrieved from. <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/10745>. (Accessed 1 July 2019).
- 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. [https://doi.org/10.1016/S0147-6513\(02\)00099-4](https://doi.org/10.1016/S0147-6513(02)00099-4).
- Ellis, B., 1993. In: Ellis, B. (Ed.), *Chemistry and Technology of Epoxy Resins*. Springer Netherlands, Dordrecht, pp. 1–36.
- Erich, S.J.F., Baukh, V., 2016. Modelling biocide release based on coating properties. *Prog. Org. Coating* 90, 171–177. <https://doi.org/10.1016/j.porgcoat.2015.10.009>.
- Erythropel, H.C., Zimmerman, J.B., de Winter, T.M., Petitjean, L., Melnikov, F., Lam, C.H., Lounsbury, A.W., Mellor, K.E., Jankovic, N.Z., Tu, Q., Pincus, L.N., Falinski, M.M., Shi, W., Coish, P., Plata, D.L., Anastas, P.T., 2018. The Green ChemisTREE: 20 years after taking root with the 12 principles. *Green Chem.* 20 (9), 1929–1961. <https://doi.org/10.1039/c8gc00482j>.
- Escher, B.I., Ait-Aissa, S., Behnisch, P.A., Brack, W., Brion, F., Brouwer, A., Buchinger, S., Crawford, S.E., Du Pasquier, D., Hamers, T., Hettwer, K., Hilscherova, K., Hollert, H., Kase, R., Kienle, C., Tindall, A.J., Tuerk, J., van der Oost, R., Vermeirssen, E., Neale, P.A., 2018. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. *Sci. Total Environ.* 628–629, 748–765. <https://doi.org/10.1016/j.scitotenv.2018.01.340>.
- Gartiser, S., Heisterkamp, I., Schoknecht, U., Bandow, N., Burkhardt, N.M., Ratte, M., Ilvonen, O., 2017a. Recommendation for a test battery for the ecotoxicological evaluation of the environmental safety of construction products. *Chemosphere* 171, 580–587. <https://doi.org/10.1016/j.chemosphere.2016.12.115>.
- Gartiser, S., Heisterkamp, I., Schoknecht, U., Burkhardt, M., Ratte, M., Ilvonen, O., Brauer, F., Bruckmann, J., Dabrunz, A., Egeler, P., Eisl, A.M., Feiler, U., Fritz, I., König, S., Lebertz, H., Pandard, P., Potschke, G., Scheerbaum, D., Schreiber, F., Soldan, P., Weiss, R., Weltens, R., 2017b. Results from a round robin test for the ecotoxicological evaluation of construction products using two leaching tests and an aquatic test battery. *Chemosphere* 175, 138–146. <https://doi.org/10.1016/j.chemosphere.2017.01.146>.
- Heemken, O.P., Reincke, H., Stachel, B., Theobald, N., 2001. The occurrence of xenoestrogens in the Elbe river and the North Sea. *Chemosphere* 45 (3), 245–259. [https://doi.org/10.1016/S0045-6535\(00\)00570-1](https://doi.org/10.1016/S0045-6535(00)00570-1).
- Heisterkamp, I., Gartiser, S., Kalbe, U., Bandow, N., 2016. Ökotoxikologische Bewertung Reaktiver Brandschutzbeschichtungen [Ecotoxicological assessment of reactive fire protection coatings].
- Higuchi, T., 1961. Rate of release of medicaments from ointment bases containing drugs in suspension. *J. Pharmaceut. Sci.* 50 (10), 874–875. <https://doi.org/10.1002/jps.2600501018>.
- IMO, 2001. International Convention on the Control of Harmful Anti-fouling Systems on Ships.
- Inoue, K., Yoshie, Y., Kondo, S., Yoshimura, Y., Nakazawa, H., 2002. Determination of phenolic xenoestrogens in water by liquid chromatography with coulometric-array detection. *J. Chromatogr. A* 946 (1–2), 291–294. [https://doi.org/10.1016/S0021-9673\(01\)01527-8](https://doi.org/10.1016/S0021-9673(01)01527-8).
- ISO, 2007a. Paints and Varnishes - Determination of Film Thickness.
- ISO, 2007b. 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, 2007c. 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, 2012. Water Quality - Determination of the Genotoxicity of Water and Waste Water - Salmonella/microsome Fluctuation Test (Ames Fluctuation Test).
- ISO, 2018a. Paints and Varnishes - Corrosion Protection of Steel Structures by Protective Paint Systems.
- ISO, 2018b. Water Quality - Determination of the Estrogenic Potential of Water and Waste Water - Part 1: Yeast Estrogen Screen (*Saccharomyces cerevisiae*).
- Jin, F.L., Li, X., Park, S.J., 2015. Synthesis and application of epoxy resins: a review. *J. Ind. Eng. Chem.* 29, 1–11. <https://doi.org/10.1016/j.jiec.2015.03.026>.
- Jobling, S., Sumpter, J., 1993. Detergent components in sewage effluent are weakly oestrogenic to fish: an in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquat. Toxicol.* 27 (3–4), 361–372. [https://doi.org/10.1016/0166-445X\(93\)90064-8](https://doi.org/10.1016/0166-445X(93)90064-8).
- Li, L., Chen, Y., Li, S.J., 2006. Water diffusion behavior in epoxy resins with various fluorine contents. *Appl. Spectrosc.* 60 (4), 392–397. <https://doi.org/10.1366/000370206776593717>.
- Liu, M.J., Wu, P.Y., Ding, Y.F., Li, S.J., 2003. Study on diffusion behavior of water in epoxy resins cured by active ester. *Phys. Chem. Chem. Phys.* 5 (9), 1848–1852. <https://doi.org/10.1039/b208782k>.
- Liu, Y.H., Zhang, S.H., Ji, G.X., Wu, S.M., Guo, R.X., Cheng, J., Yan, Z.Y., Chen, J.Q., 2017. Occurrence, distribution and risk assessment of suspected endocrine-disrupting chemicals in surface water and suspended particulate matter of Yangtze River (Nanjing section). *Ecotoxicol. Environ. Saf.* 135, 90–97. <https://doi.org/10.1016/j.ecoenv.2016.09.035>.
- Luft, A., Broder, K., Kunkel, U., Schulz, M., Dietrich, C., Baier, R., Heininger, P., Ternes, T.A., 2017. Nontarget analysis via LC-QTOF-MS to assess the release of organic substances from polyurethane coating. *Environ. Sci. Technol.* 51 (17), 9979–9988. <https://doi.org/10.1021/acs.est.7b01573>.
- Markl, V., Pflugmacher, S., Reichert, A., Stephan, D.A., 2017. Leaching of polyurethane systems for waterproofing purposes whilst curing. *Water Air Soil Pollut.* 228 (8), 14. <https://doi.org/10.1007/s11270-017-3451-0>.
- McDonnell, D., Nawaz, Z., Densmore, C., Weigel, N., Pham, T., Clark, J., O'Malley, B., 1991a. High level expression of biologically active estrogen receptor in *Saccharomyces cerevisiae*. *J. Steroid Biochem. Mol. Biol.* 39 (3), 291–297. [https://doi.org/10.1016/0960-0760\(91\)90038-7](https://doi.org/10.1016/0960-0760(91)90038-7).
- McDonnell, D.P., Nawaz, Z., O'Malley, B.W., 1991b. In situ distinction between steroid receptor binding and transactivation at a target gene. *Mol. Cell Biol.* 11 (9), 4350–4355. <https://doi.org/10.1128/mcb.11.9.4350>.
- Meffe, R., Kohfahl, C., Hamann, E., Greskowiak, J., Massmann, G., Dünnbier, U.,

- Pekdeger, A.J.E.S., Research, P., 2014. Fate of Para-Toluenesulfonamide (P-TSA) in Groundwater under Anoxic Conditions: Modelling Results from a Field Site in Berlin (Germany), vol 21, pp. 568–583. <https://doi.org/10.1007/s11356-013-1902-8>, 1.
- Meier, S., Morton, H.C., Andersson, E., Geffen, A.J., Taranger, G.L., Larsen, M., Petersen, M., Djurhuus, R., Klungsoyr, J., Svandal, A., 2011. Low-dose exposure to alkylphenols adversely affects the sexual development of Atlantic cod (*Gadus morhua*): acceleration of the onset of puberty and delayed seasonal gonad development in mature female cod. *Aquat. Toxicol.* 105 (1–2), 136–150. <https://doi.org/10.1016/j.aquatox.2011.06.003>.
- OECD, 1973. Decision of the Council on Protection of the Environment by Control of Polychlorinated Biphenyls. OECD/LEGAL/0108.
- Paluvai, N.R., Mohanty, S., Nayak, S.K., 2014. Synthesis and modifications of epoxy resins and their composites: a review. *Polym. Plast. Technol. Eng.* 53 (16), 1723–1758. <https://doi.org/10.1080/03602559.2014.919658>.
- Richter, D., Dunnbier, U., Massmann, G., Pekdeger, A., 2007. Quantitative determination of three sulfonamides in environmental water samples using liquid chromatography coupled to electrospray tandem mass spectrometry. *J. Chromatogr. A* 1157 (1–2), 115–121. <https://doi.org/10.1016/j.chroma.2007.04.042>.
- Routledge, E.J., Sumpter, J.P., 1997. Structural features of alkylphenolic chemicals associated with estrogenic activity. *J. Biol. Chem.* 272 (6), 3280–3288. <https://doi.org/10.1074/jbc.272.6.3280>.
- Schulz, W., Weiss, S.C., Weber, W.H., Winzenbacher, R., 2017. The reciprocal iso-inhibition volume concept: a procedure for the evaluation in effect-directed analysis with thin-layer chromatography - using the thin-layer chromatography-luminescent bacteria assay as an example. *J. Chromatogr. A* 1519, 121–130. <https://doi.org/10.1016/j.chroma.2017.08.076>.
- Smirnova, O., Glazkov, A., Yarosh, A., Sakharov, A., 2016. Fluorinated polyurethanes, synthesis and properties. *Molecules* 21 (7), 10. <https://doi.org/10.3390/molecules21070904>.
- Taha, M.N., Krawinkel, M.B., Morlock, G.E., 2015. High-performance thin-layer chromatography linked with (bio)assays and mass spectrometry - a suited method for discovery and quantification of bioactive components? Exemplarily shown for turmeric and milk thistle extracts. *J. Chromatogr. A* 1394, 137–147. <https://doi.org/10.1016/j.chroma.2015.03.029>.
- Thomas, S., Datta, J., Haponiuk, J., Reghunadhan, A., 2017. Polyurethane Polymers: Composites and Nanocomposites. Elsevier.
- Tietje, O., Burkhardt, M., Rohr, M., Borho, N., Schoknecht, U., 2018. Emissions-und Übertragungsfunktionen für die Modellierung der Auslaugung von Bauprodukten. [Emission and transfer functions for the modelling of leaching from construction products].
- Vermeirssen, E.L.M., Dietschweiler, C., Werner, I., Burkhardt, M., 2017. Corrosion protection products as a source of bisphenol A and toxicity to the aquatic environment. *Water Res.* 123, 586–593. <https://doi.org/10.1016/j.watres.2017.07.006>.
- Wang, L., Liu, J.M., Liu, J.N., Shi, L.L., Wang, Z., 2018. Application of microcosm and species sensitivity distribution approaches in the ecological hazard assessment of 4-tert-butylphenol. *Chem. Ecol.* 34 (2), 108–125. <https://doi.org/10.1080/02757540.2017.1407315>.
- Wangler, T.P., Zuleeg, S., Vonbank, R., Bester, K., Boller, M., Carmeliet, J., Burkhardt, M., 2012. Laboratory scale studies of biocide leaching from facade coatings. *Build. Environ.* 54, 168–173. <https://doi.org/10.1016/j.buildenv.2012.02.021>.
- Watermann, B.T., Daehne, B., Sievers, S., Dannenberg, R., Overbeke, J.C., Klijnsma, J.W., Heemken, O., 2005. Bioassays and selected chemical analysis of biocide-free antifouling coatings. *Chemosphere* 60 (11), 1530–1541. <https://doi.org/10.1016/j.chemosphere.2005.02.066>.
- Weiss, S.C., Egetenmeyer, N., Schulz, W., 2017. In: Reifferscheid, G., Buchinger, S. (Eds.), *Vitro Environmental Toxicology - Concepts, Application and Assessment*. Springer International Publishing Ag, Cham, pp. 187–224.
- Wu, M.H., Pan, C.Y., Yang, M., Xu, B.T., Lei, X.J., Ma, J., Cai, L., Chen, J.S., 2016. Chemical analysis of fish bile extracts for monitoring endocrine disrupting chemical exposure in water: bisphenol A, alkylphenols, and norethindrone. *Environ. Toxicol. Chem.* 35 (1), 182–190. <https://doi.org/10.1002/etc.3176>.