ECOTOXICOLOGICAL ASSESSMENT OF SELECTED CONSTRUCTION MATERIALS: GALVANIC ANODES AND ORGANIC COATINGS FOR CORROSION PROTECTION

Ökotoxikologische Bewertung ausgewählter Baumaterialien: galvanische Anoden und organische Beschichtungen zum Korrosionsschutz

von

Anna Maria Bell
aus Koblenz

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Fachbereich 3: Mathematik/Naturwissenschaften
Universität Koblenz-Landau

Gutachter:
Prof. Dr. Thomas Ternes
Prof. Dr. Werner Manz

Prüfungskommission:
PD Dr. Carola Winkelmann
Prof. Dr. Thomas Ternes
Prof. Dr. Joachim Scholz

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SUMMARY

Buildings and infrastructures characterize the appearance of our cultural landscapes and provide essential services for the human society. However, they inevitably impact the natural environment e.g. by the structural change of habitats. Additionally, they potentially cause further negative environmental impacts due to the release of chemical substances from construction materials. Galvanic anodes and organic coatings regularly used for corrosion protection of steel structures are building materials of particular importance for the transport infrastructure. In direct contact with a water body or indirectly via the runoff after rainfall, numerous chemicals can be released into the environment and pose a risk to aquatic organisms. Up to now, there is no uniform investigation and evaluation approach for the assessment of the environmental compatibility of building products. Furthermore, galvanic anodes and organic coatings pose particular challenges for their ecotoxicological characterization due to their composition. Therefore, the objective of the presented thesis was the ecotoxicological assessment of emissions from galvanic anodes and protective coatings as well as the development of standardized assessment procedures for these materials.

The possible environmental hazard posed by the use of anodes on offshore installations was investigated on three trophic levels. To ensure a realistic and reliable evaluation, the experiments were carried out in natural seawater and under natural pH conditions. Moreover, the anode material and its main components zinc and aluminum were exposed while simulating a worst-case scenario. The anode material examined caused a weak inhibition of algae growth; no acute toxicity was observed on the luminescent bacteria and amphipods. However, an increase of aluminum and indium levels in the crustacean species was found. On the basis of these results, no direct threat has been identified for marine organisms from the use of galvanic aluminum anodes. However, an accumulation of metals in crustaceans and a resulting entry into the marine food web cannot be excluded.

The environmental compatibility of organic coating systems was exemplarily evaluated using a selection of relevant products based on epoxy resins (EP) and polyurethanes. For this purpose, coated test plates were dynamically leached over 64 days. The eluates obtained were systematically analyzed for their ecotoxicological effects (acute toxicity to algae and luminescent bacteria, mutagenic and estrogenic effects) and their chemical composition. In particular, the EP-based coatings caused significant bacterial toxicity and estrogen-like effects. The continuously released 4-*tert*-butylphenol was identified as a main contributor to these effects and was quantified in concentrations exceeding the predicted no effect concentration for freshwater in all samples. Interestingly, the overall toxicity was not governed by the content of 4-*tert*-butylphenol in the products but rather by the release mechanism of this compound from the investigated polymers. This finding indicates that an optimization of the composition can result in the reduction of emissions and thus of environmental impacts - possibly due to a better polymerization of the compounds.

Coatings for corrosion protection are exposed to rain, changes in temperature and sun light leading to a weathering of the polymer. To determine the influence of light-induced aging on the ecotoxicity of top coatings, the emissions and associated adverse effects of UV-irradiated and untreated EP-based products were compared. To that end, the investigation of static leachates was focused on estrogenicity and bacterial toxicity, which were detected in the classic microtiter plate format and in combination with thin-layer plates. Both materials examined showed a significant decrease of the ecotoxicological effects after irradiation with a simultaneous reduction of the 4-*tert*-butylphenol emission. However, bisphenol A and various structural analogues were detected as photolytic degradation products of the polymers, which also contributed to the observed effects. In this context, the identification of bioactive compounds was supported by the successful combination of *in-vitro* bioassays with chemical analysis by means of an effect-directed analysis. The presented

findings provide important information to assess the general suitability of top coatings based on epoxy resins.

Within the scope of the present study, an investigation concept was developed and successfully applied to a selection of relevant construction materials. The adaptation of single standard methods allowed an individual evaluation of these products. At the same time, the suitability of the ecotoxicological methods used for the investigation of materials of unknown and complex composition was confirmed and the basis for a systematic assessment of the environmental compatibility of corrosion protection products was created. Against the background of the European Construction Products Regulation, the chosen approach can facilitate the selection of environmentally friendly products and contributes to the optimization of individual formulations by the simple comparison of different building materials e.g. within a product group.

ZUSAMMENFASSUNG

Gebäude und Infrastrukturen prägen das Bild unserer Kulturlandschaften und erbringen essentielle Dienstleistungen für die menschliche Gesellschaft. Sie wirken sich jedoch auch unweigerlich auf die natürliche Umwelt aus, z.B. durch die strukturelle Veränderung von Lebensräumen. Überdies gelten sie aufgrund der Freisetzung chemischer Inhaltsstoffe aus den eingesetzten Baumaterialien als potentielle Verursacher negativer Umweltauswirkungen. Galvanische Anoden und organische Beschichtungen, die an Stahlbauwerken regelmäßig zum Schutz vor Korrosion zum Einsatz kommen, sind als Baumaterialien für die Verkehrsinfrastruktur von besonderer Bedeutung. In direktem Kontakt mit einem Wasserkörper oder indirekt über den Abfluss nach einem Niederschlagsereignis können zahlreiche Chemikalien in aquatische Lebensräume emittiert werden und eine Gefahr für Wasserorganismen darstellen. Zur Beurteilung der Umweltverträglichkeit von Bauprodukten existiert bislang kein einheitlicher Untersuchungs- und Bewertungsansatz. Zudem stellen galvanische Anoden und organische Beschichtungen aufgrund ihrer Zusammensetzung besondere Herausforderungen an deren ökotoxikologische Charakterisierung. Ziel der vorliegenden Arbeit war es daher, die Gefährdung der aquatischen Umwelt durch galvanische Anoden und Korrosionsschutzbeschichtungen mithilfe ökotoxikologischer Untersuchungen zu beurteilen und standardisierte Bewertungsverfahren für diese Materialien zu entwickeln.

Die Untersuchung des möglichen Umwelteinflusses durch die Anwendung von Anoden an Offshore-Anlagen erfolgte auf drei trophischen Ebenen. Um eine möglichst realistischste und zuverlässige Abschätzung zu gewährleisten, wurden die Experimente in natürlichem Meerwasser und unter natürlichen pH-Bedingungen durchgeführt. Zudem erfolgte die Exposition gegenüber dem Anodenmaterial und deren Hauptbestandteilen Zink und Aluminum unter Simulation eines Worst-Case-Szenarios. Das untersuchte Anodenmaterial verursachte eine schwache Hemmung des Algenwachstums; auf die getesteten Leuchtbakterien und Flohkrebse zeigte es keine akute Toxizität. Allerdings wurde eine Erhöhung der Aluminium- und Indiumgehalte in den Krebsen festgestellt. Auf Grundlage dieser Ergebnisse wurde keine direkte Gefahr für marine Organismen durch den Einsatz galvanischer Aluminium-Anoden identifiziert. Eine Anreicherung von Metallen in Krebstieren und ein daraus resultierender Eintrag ins marine Nahrungsnetz kann jedoch nicht ausgeschlossen werden.

Die Umweltverträglichkeit organischer Beschichtungssysteme wurde exemplarisch für eine Auswahl relevanter Produkte auf Basis von Epoxidharzen (EP) und Polyurethanen bewertet. Dazu wurden beschichtete Probeplatten schrittweise über 64 Tage ausgelaugt. Die gewonnenen Eluate wurden systematisch auf ihre ökotoxikologischen Effekte (akute Toxizität gegenüber Algen und Leuchtbakterien, mutagene und estrogenartige Wirkungen) und chemische Zusammensetzung analysiert. Dabei zeigten sich insbesondere die EP-basierten Beschichtungen durch die Verursachung erheblicher bakterieller Toxizität und estrogenartiger Wirkung auffällig. Als primärer Urheber dieser Effekte wurde das kontinuierlich freigesetzte 4-*tert*-Butylphenol identifiziert, dessen Konzentration in allen Proben die *predicted no effect concentration* für Süßwasser überschritt. Gleichzeitig hat sich gezeigt, dass die Gesamttoxizität nicht durch den Gehalt an 4-*tert*-Butylphenol in den Produkten bestimmt wird, sondern vom Freisetzungsmechanismus dieser Verbindung aus den untersuchten Polymeren abhängig ist. Diese Ergebnisse deuten darauf hin, dass eine Optimierung der Zusammensetzung, beispielsweise aufgrund einer besseren Polymerisation der Inhaltsstoffe, zu einer Reduzierung von Emissionen und damit zu einer verminderten Belastung der Umwelt führen kann.

Regen, Temperaturwechsel und Sonneneinstrahlung können zur Verwitterung polymerer Korrosionsschutzbeschichtungen führen. Um den Einfluss lichtbedingter Alterung auf die Ökotoxizität von Deckbeschichtungen zu erfassen, wurde die Emissionen und damit verbundene negative Auswirkungen von UV-bestrahlten und unbehandelten EP-basierten Produkten miteinander verglichen. Nach statischer Auslaugung stand dabei die Untersuchung estrogenartiger und bakterien-

toxischer Wirkungen im Fokus, die sowohl im klassischen Mikrotiterplattenformat als auch in Kopplung mit Dünnschichtplatten detektiert wurden. Beide untersuchten Materialien zeigten nach Bestrahlung eine signifikante Abnahme der ökotoxikologischen Effekte bei gleichzeitiger Verringerung der Freisetzung von 4-*tert*-Butylphenol. Jedoch wurden auch Bisphenol A und verschiedene Strukturanaloga als photolytische Abbauprodukte der Polymere nachgewiesen, die ebenfalls zur beobachteten Wirkung beitrugen. Die Identifizierung bioaktiver Inhaltsstoffe konnte dabei durch die erfolgreiche Kombination der *in-vitro*-Bioassays mit chemischen Analysen im Sinne einer effektgeleiteten Analytik unterstützt werden. Die vorliegenden Ergebnisse liefern wichtige Hinweise für die Beurteilung der generellen Eignung von Deckbeschichtungen auf Basis von Epoxidharzen.

Das im Rahmen der vorliegenden Studie entwickelte Untersuchungskonzept konnte erfolgreich auf eine Auswahl relevanter Baumaterialien angewendet werden. Die gezielte Anpassung einzelner Standardmethoden erlaubte dabei eine individuelle Produktbewertung. Gleichzeitig wurde sowohl die Zweckmäßigkeit der angewendeten ökotoxikologischen Methoden für die Untersuchung von Materialien unbekannter und komplexer Zusammensetzung bestätigt als auch die Basis für eine systematische Bewertung der Umweltverträglichkeit von Korrosionsschutzprodukten geschaffen. Vor dem Hintergrund der Europäischen Bauprodukteverordnung kann der gewählte Ansatz dem einfachen Vergleich verschiedener Baumaterialien z.B. innerhalb einer Produktgruppe dienen und damit die Auswahl umweltverträglicher Produkte vereinfachen und zur Optimierung einzelner Rezepturen beitragen.

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Chapter 1

GENERAL INTRODUCTION

1.1 BUILDINGS AND INFRASTRUCTURE AS POSSIBLE SOURCES OF ENVIRONMENTAL POLLUTION

The global mass of objects made by humans was estimated to exceed the mass of all taxa living on the Earth in 2020 (Elhacham et al. 2020). This impressively demonstrates the human dominance on the entire planet and implies its significant impact on the Earth's environment. With a total of 1,100 Gt, buildings and infrastructure are the prevalent categories in this global analysis. In Germany, the area used for human settlements and transport infrastructure reached around 51,500 km^2 in 2019; this equals to 14.3% of the total area of Germany (Destatis 2020). This results in a built-up area of approximately 22,000 km^2 considering an average sealing degree of 45%. Road traffic accounts for more than half of the space required in the transport sector whereas air, rail and ship traffic together take up less than a tenth (see Figure 1.1). Alone at the federal level, around 40,000 bridges and tunnels at 51,000 km roads, 26,000 bridges and tunnels at 33,000 km rail lines as well as 2,300 bridges and hydraulic engineering structures at 7,300 km waterways are maintained in Germany (BMVI, personal communication, January 18, 2021). Accordingly, (infra)structures can be considered as highly relevant in terms of their mass, their consumed land area as well as their number. Besides the expected negative ecological effects of accompanying traffic, energy consumption and surface sealing, the infrastructure and buildings itself can pose an environmental risk. A wide variety of structures and materials are deployed that potentially can release corresponding organic and inorganic ingredients or its degradation products into the environment and cause ecotoxicological effects.

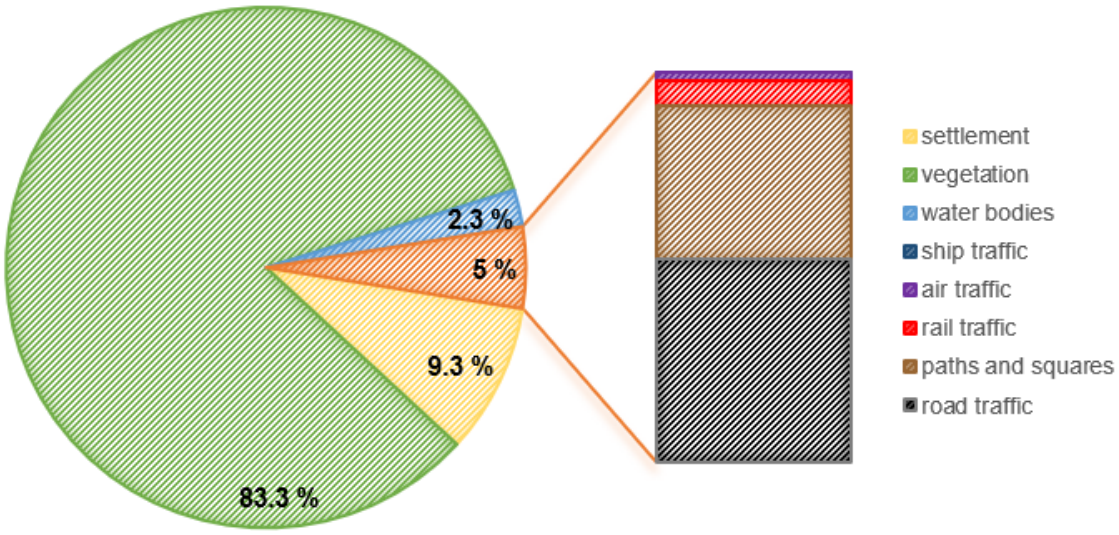

Figure 1.1: Relative area requirement of the different types of land use in Germany, 2019 (Destatis 2020).

It is commonly known that building materials can have multiple impacts on the human health. Asbestos is one of the most important building pollutants and can cause asbestosis and lung cancer (LaDou 2004). Similarly, man-made mineral fibers widely used e.g. in rock or glass wool for thermal and acoustical insulation were suspected to pose a carcinogenic risk to humans as a consequence of lung deposition (IARC 2002). Furthermore, indoor pollution can be caused by volatile organic compounds emitted from wood-based materials, sealants, adhesives, paints, etc. resulting in allergic reactions and effects on the respiratory tract or immune system (Mølhave 1991). A prominent example for a substance class released from building products and presenting an environmental hazard are biocides. In order to protect against growth of algae and fungi, they are frequently applied in façades but can pollute soil and water (Vermeirssen et al. 2018). As well, lead-based paints applied

for the protection of wooden and metallic surfaces until the late twentieth century are classified as environmentally hazardous and are considered as very toxic to aquatic organisms (ATSDR 2020).

1.2 Corrosion protection of transport infrastructure

In the field of public transport, steel structures are of particular importance. Examples for major steel constructions as part of the transport infrastructure are steel bridges in railway and road construction, gravel troughs in railway construction, offshore structures such as wind turbines as well as locks and weirs in hydraulic engineering. Not at least of its high strength with low construction weight, the high durability and good recyclability, steel is a widely used building material (Duggal 2009). However, metallic structures are susceptible to corrosion - an alteration process of metals caused by the chemical reaction with their environment (Greene 2020). The annual costs of corrosion in industrialized countries amount up to 4% of the gross domestic product (Koch 2017). Besides the economic damage, the destruction of valuable infrastructure by corrosion results in safety impairments and aesthetic failures. Therefore, the prevention of corrosion is of great importance which can be achieved by active and passive protection methods. Galvanic anodes are counted among the active protection methods that lead to a negative polarization of the steel by electron transfer and thus protect against oxidation. Due to the independence of any external power source, the easy installation and operation as well as no or little requirements for maintenance, galvanic anodes are preferably applied to structures immersed in water like support structures of offshore installations (Reuben 1994). In contrast, passive protection methods as organic coatings act as a physical barrier between the metal and the corrosive environment. Corrosion control by organic coatings is a widely used and cost-effective method that has a broad range of technical applications and simultaneously allows an aesthetic design of metallic structures (Schweitzer 2009). Depending on prevailing conditions, even formulations with special characteristics as resistance to extreme pH conditions (Møller et al. 2017) or self-healing properties (Zhang et al. 2018) are applied. However, besides the desired effect of protecting metallic (infra)structures against corrosion, active and passive methods have in common to potentially release harmful substances into the environment. The comprehensive assessment of the possible environmental impact caused by corrosion protection measures requires the investigation of ecotoxicological effects of according emissions. However, a consistent investigation and evaluation concept is still missing. As detailed in section 1.5 the aim of the present thesis was to develop methods for the ecotoxicological assessment of active and passive corrosion protection measures and their application to selected products. Therefore, the fundamental operating principles and chemical composition of both approaches are explained in the sections 1.2.1 and 1.2.2. The section 1.3 summarizes the existing regulative framework for construction products in general and section 1.4 provides a general overview about the use of ecotoxicological methods for the assessment of chemical compounds and environmental samples.

1.2.1 Galvanic anodes

In the presence of an electrolyte, the conductive connection of two different metals leads to the formation of a galvanic cell. Driven by the difference in potential, the less noble metal acts as an anode and is oxidized under the continuous transmission of electrons to the more noble metal that acts as cathode and is consequently protected against oxidation. This principle can be utilized for corrosion protection by connecting a steel structure to be protected by a less noble metal (HTG 2009). While the corrosion is relocated from the steel structure to the galvanic anode, the anode material is steadily consumed and releases respective metallic ions into the environment (see Figure 1.2).

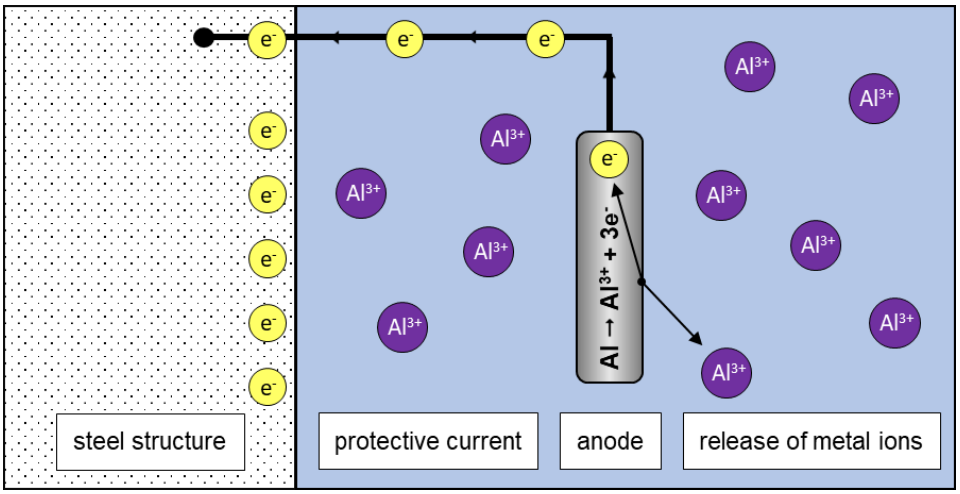

Figure 1.2: Operation principle of a galvanic aluminum anode for corrosion protection and the concurrent release of metal ions into the environment, adapted from HTG (2009).

Depending on the surrounding medium, the anode material differs in composition. In general, alloys based on magnesium, aluminum or zinc are suitable reactants for iron and low-alloy steels. Due to the comparatively low weight and high electrochemical capacity, the corrosion protection of offshore structures as wind turbines is mainly realized by aluminum anodes (Bardal 2005). To avoid the passivation of anode material and to preserve its effectiveness over a long term, aluminum is alloyed with activating elements such as zinc or indium (Oluwole 2012). Besides intentionally added metals, galvanic anodes often contain further elements originating from impurities in raw materials. Reese et al. (2020) quantified 26 elements in different aluminum-based anode materials in total of which only six are regulated as component in galvanic anodes by international standards. The detailed composition of a galvanic anode determines its current capacity and operating voltage (Singh 2014). The potential difference between the anode material and the structural steel drives the continuous protective current. In case of aluminum the potential difference amounts to 0.30 to 0.37 mV and the average current capacity of according anodes equals about 2,700 Ah/kg (HTG 2009).

The mass of the anode required for the effective corrosion protection primarily depends on the surface area of the structure to be protected and the pursued protection period. Wind turbines are usually designed for a lifetime of 20 to 25 years (Bouty et al. 2017). The majority of offshore wind farms in Europe is founded on monopiles (WindEurope 2019). This hollow, cylindric steel structures on average have a diameter of 4.8 m, a length of 51.4 m and a weight of 421.4 t (Negro et al. 2017). At a rough estimate, the necessary mass of aluminum anode material is 13 t per monopile resulting in an annual release of 45 t aluminum and 2 t zinc in a windfarm with 80 wind turbines and one substation (Kirchgeorg et al. 2018). Thus, a large amount and a wide variety of elements are emitted to the marine environment that potentially constitute a hazard to marine organisms.

1.2.2 COATINGS

Organic coatings are disperse systems of varying composition that are mostly applied in liquid form, harden by cross-linking reactions and thus form a thin and continuous polymeric layer on its substrate (Sander 2010). The corrosion protection of steel structures by protective paint systems is standardized at international and European level by DIN EN ISO 12944 (part 1 to 9) (ISO 2017), in which appropriate coating systems are proposed depending on the corrosive action of environmental

conditions. Overall, 45,000 t of anti-corrosive coatings in the amount of 179 million Euros were marketed in Germany in 2019 (VdL 2020). A total of 218 corrosion-protective coating products are currently approved for the corrosion protection of steel structures in the responsibility of the Federal Ministry of Transport and Digital Infrastructure [Bundesministerium für Verkehr und digitale Infrastruktur, BMVI].

The protective function is usually ensured by multilayered systems (Knudsen and Forsgren 2017). The primer coating provides the adhesion of the system to the metal surface and the corrosion resistance by directly preventing the contact of the metal to corrosive agents like water, ions and oxygen. An optional intermediate coating increases the thickness of the system and serves as an additional diffusion barrier. The top coating offers both, mechanical and chemical resistance and is responsible for the desired visual appearance. In principle, every layer of a coating system is composed of a binder, pigments, fillers, additives, and solvents (Popov 2015). In many cases, all constituents are combined only by mixing of different components in a specific proportion.

The binder forms the polymeric backbone of the coating, binds the other components and adheres to the substrate, i.e. the metal surface (Wicks 2007). The binder type largely determines the properties of the final coating system and is therefore used to classify the different coating materials. Predominantly, synthetically produced resins are used to manufacture anti-corrosive coatings, most commonly epoxies and polyurethanes (Sander 2010). Further binding agents are alkyd resins, polyvinyl chloride, epoxy esters, acrylic resins, polyaspartics, alkyl silicate, polysiloxanes and polyester resins. Binder originating from a condensation reaction between epichlorohydrin and molecules containing hydroxy-groups are summarized as epoxy resins (Dornbusch 2016). The majority of epoxides arises from the reaction with bisphenol A (BPA) and thus is based on bisphenol A diglycidyl ether (BADGE) or corresponding oligomers of various molecular weight. The final polymerization and curing of the coatings take place in presence of appropriate hardeners. Typically, aliphatic polyamines are used to link epoxy resins by polyaddition (see Figure 1.3). In contrast, the cross-linking of polyurethanes can be induced either with or without the addition of a hardener (Meier-Westhues 2019). Both variants are based on highly reactive isocyanates (aliphatic or aromatic) and cure under formation of the eponymous urethane or urea groups (see Figure 1.4). One-component coatings contain prepolymers with two or more isocyanate groups and the curing reaction is triggered by atmospheric moisture. The polymerization of two-component polyurethane coatings is caused by reactions between isocyanates and compounds with reactive hydrogen-containing groups. Typically, these are addition reactions between diisocyanates and macropolyols (e.g. polyether, polyester, polyacrylic).

Pigments are insoluble coloring components of a coating material and can be of organic or inorganic nature (Bayer 2020). Besides their primary purpose, some pigments have additional functions, for example certain metallic pigments have anticorrosive properties. In the past, toxic lead- and chromate-based pigments were frequently used, modern coatings often contain zinc as dust, zinc phosphate or zinc oxide instead. Coating fillers can influence the mechanical properties and are used to increase the volume of a layer. For this purpose, the natural occurring micaceous iron oxide is commonly used. Together with the binder and pigments, the filler forms the solid part of an anticorrosive coating. Moreover, a wide range of additives is used to improve the properties of the coating and to fulfill various functions (Sander 2010). For example, catalysts for cross-linking reactions, flow modifier, biocides, flame retardants, UV stabilizers or plasticizers are added to the formulation. To dissolve the film forming ingredients and enable the processing of a coating, solvents are used. These volatile organic compounds determine the viscosity of the varnish and control the drying of the coating film. Depending on the characteristics of applied binder type, different solvents such as aliphatic or aromatic hydrocarbons, alcohols, ketones, esters or glycol ethers are used.

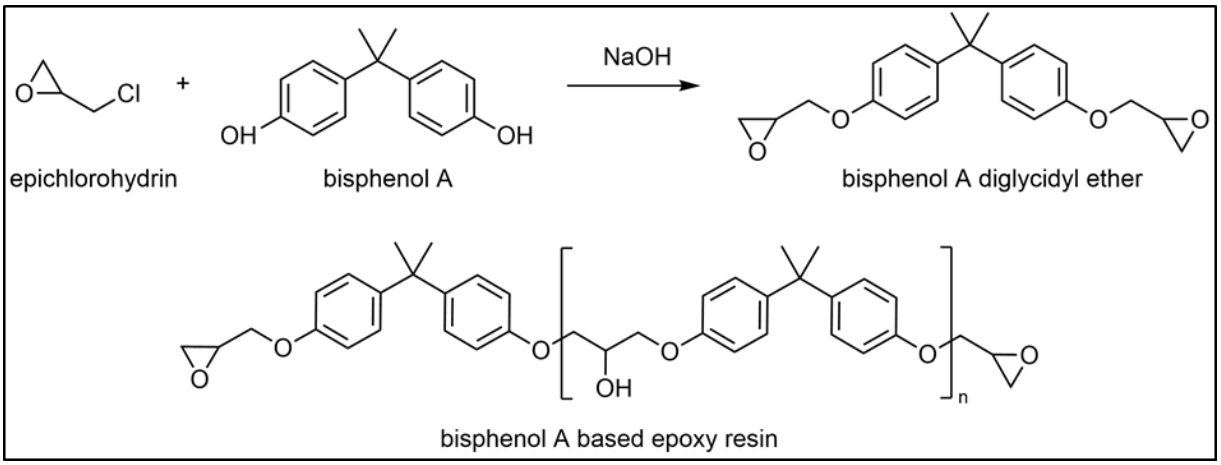

Figure 1.3: Reaction between epichlorohydrin and bisphenol A yielding in bisphenol A diglycidyl ether and basic structure of bisphenol A based epoxy resins.

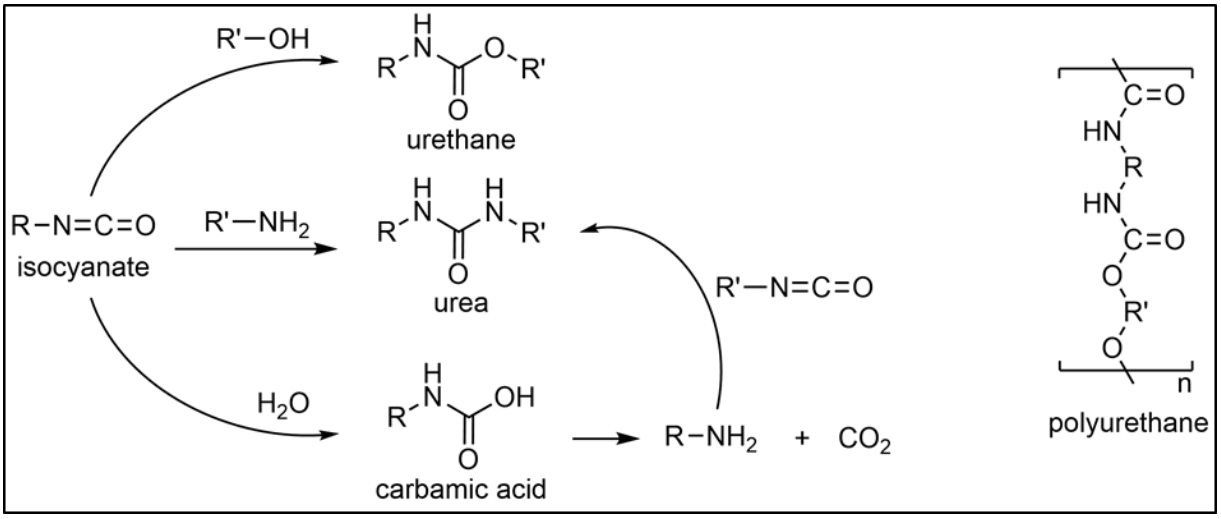

Figure 1.4: Isocyanate cross-linking reactions and generalized structure of a polyurethane basing on a diol and a diisocyanate.

As described above, organic coatings are very complex mixtures and due to the polymerization process have an unspecific final composition. In addition to the numerous constituents, further undefined compounds can be formed as during the weathering of coatings. The degradation of polymeric structures by UV radiation deserves particularly mention since the negative impact on durability is frequently documented (Knudsen and Forsgren 2017). The chemical breakdown caused by sunlight can lead to e.g. an aesthetic change of the coating system, the cracking and delamination of layers or a reduced coating thickness. Thus, the coated steel structures can potentially emit a multitude of known and unknown compounds that pose a possible risk to the environment.

1.3 Environmental compatibility of construction products in the legal context

The environmental compatibility of construction products and structures is requested by the amended European Construction Products Regulation No. 305/2011 (CPR). Buildings must not be a threat to the environmental quality during construction, use and demolition in particular by the

release of dangerous substances into air, water and soil (EU 2011). To ensure that construction products meet this basic requirement, horizontal harmonized test and assessment methods are under development. For example, technical specifications regarding the leaching of dangerous substances from construction products (TS 16637, part 1 to 3) and the chemical analysis of relevant eluates (e.g. TS 17195 and TS 17332) were published by the working groups of the technical committee CEN/TC 351 (Construction Products: Assessment of release of dangerous substances). Furthermore, a guidance on ecotoxicological analysis of construction products and their eluates (TR 17105) was proposed. To achieve the status of an European standard, the new test methods have to be successfully validated. For this purpose, round robin tests, for example with concrete and mortar (Nebel and Spanka 2013) as well as rubber granulate and sheets (Gartiser et al. 2017), were initiated. Currently, methods and criteria evaluating the ecotoxicity of (construction) products are elaborated as part of a research project commissioned by the Federal Environment Agency (FKZ 3719 37 3020). However, so far, no method was published as a standard and legally binding limit values for the assessment of ecotoxicological test results are not available yet. Moreover, some construction products are not mandated by the CPR and therefore excluded from the scope of the developed test and assessment methods. For example, metals and organic coatings on metals are not covered by the mentioned regulations.

On national level, performance data on ecotoxicity of construction products is requested only in Germany. The German institute of structural engineering [Deutsches Institut für Bautechnik, DIBt] published an investigation and evaluation concept for assessing the impact of construction works on soil and groundwater (DIBt 2011) following the regulatory practice in the field of waste management. In a first stage, all constituents of a construction product are determined either by the declaration of the manufacturer or chemical analyses. If concerns about the environmental compatibility of relevant constituents are arising on basis of defined exclusion criteria, additional tests are requested in a second stage. To this end, the respective construction product is leached under laboratory conditions and the eluate is assessed regarding general parameters (e.g. pH and conductivity) and substance parameters including the content of individual mobilizable substances and sum parameters. Only if general characteristics of the eluate are impaired and mobilized substances exceed defined safety thresholds or lead to an elevated TOC content, biological parameters, i.e. biodegradability and ecotoxicological effects are determined. For the evaluation of the impact of construction products on the aquatic environment, assessment criteria are defined for the acute toxicity to luminescent bacteria, algae, water fleas and fish/fish eggs, the mutagenic potential as well as the biodegradability of organic constituents.

Basically, legal demands on the environmental compatibility of construction products can also be derived from legislation regarding water and soil conservation. In simple terms, an environmental risk by chemical pollution is assessed on the basis of limit values which are defined for single substances and must not to be exceeded. Examples for legally binding limit values are the environmental quality standards for surface waters according to directive 2008/105/EC, the no effect levels for ground water derived by the LAWA [Bund-/Länderarbeitsgemeinschaft Wasser] or the precautionary levels of Federal Soil Protection Act [BBodSchG]. Moreover, the use of certain substances and mixtures is prohibited or restricted e. g. by chemical legislation like the REACH-regulation (No. 1907/2006), the POPs-regulation (No. 2019/1021) or biocide-regulation (No. 528/2012). As only a limited number of chemicals is regulated so far, the evaluation of environmental compatibility by limit values can only capture a small part of possibly harmful substances released from construction products and therefore does not allow for a comprehensive assessment.

In addition to European and national regulations, for instance the Federal Highway Research Institute (BASt) and the Federal Waterways Engineering and Research Institute (BAW) published

numerous guidelines and test specifications regulating the use of building materials in the context of public transport. Relevant rules for corrosion protection materials include the additional technical terms of contract for hydraulic engineering ZTV-W (BMVBS 2009) and for civil engineering works ZTV-ING (BASt 2012), the technical terms of delivery and testing for coating material for the corrosion protection of steel constructions TL/TP-KOR-steel structures (BASt 2002), the code of practice for cathodic corrosion protection in hydraulic steel engineering MKKS (BAW 2015), the VGB/BAW-standard for corrosion protection for offshore wind structures (VGB 2018) and the guidelines for the testing of coating systems for the corrosion protection of hydraulic steel structures RPB (BAW 2011). All these regulations aim to ensure the identity, the resistance to corrosion and the suitability of products. However, neither of the named regulations provide uniform testing methods and criteria for the qualification of certain construction products nor pose any requirements for their environmental compatibility.

As the detailed composition of building materials is mostly unknown – usually only limited information is publicly available as safety data sheets – and the identification of all mobilizable substances by chemical analyses is challenging, the assessment of the environmental compatibility based on compound-specific approaches generally reaches its limitations. At the same time, materials like galvanic anodes and organic coatings used for corrosion protection pose a particular challenge for the leaching methods and/or the chemical analysis. The solubility of metallic materials such as galvanic anodes is particularly affected by the pH value of the surrounding medium and not controlled by diffusion. Therefore, the release of potentially toxic substances from metallic products cannot be determined using established leaching tests. Organic coatings like anti-corrosion coatings mainly consist of two reacting components and can release many unknown substances in addition to their actual ingredients. This leads to chemically complex samples that can be characterized analytically only with great effort. The mentioned challenges created the need for alternative or adapted investigation and assessment approaches. Against this background, the effect-based assessment using ecotoxicological methods is an important complementary element to a compound-based assessment of construction materials.

1.4 Ecotoxicological assessment of chemical compounds and mixtures

Ecotoxicological methods aim to measure the response of living organisms to the exposure to chemical substances. Adverse effects can refer to all biological levels such as molecules, cells, organisms, populations or ecosystems and are specific for the investigated substance and species. Conventional *in-vivo* approaches include standardized laboratory tests with single aquatic and terrestrial species (Bidwell 2020). To consider the natural diversity of organisms and habitats, usually several representatives of different trophic levels (decomposer, producer, consumer) are used. In this context, a distinction can be made between acute (short-term) and chronic (long-term) effects. Acute tests address the survival and/or growth of the organism whereas chronic tests address e.g. development, sexual differentiation and/or reproduction in general. In contrast, cellular and subcellular *in-vitro* techniques are used for the detection of specific effects such as hormone-like activity, mutagenicity or photoinhibitory effects. Thus, appropriate bioassays can also provide mechanistic information about the observed effects and reduce the need for animal testing (Rehberger et al. 2018).

The results of the toxicity testing are the basis for the derivation of limit values for the hazard assessment of chemicals. Assuming that an ecosystem is always as sensitive as the most sensitive species, for example, the predicted no effect concentration (PNEC) is extrapolated by applying a safety factor to the lowest effective concentration measured in any of the tests applied. To estimate the environmental risk for aquatic or terrestrial ecosystems, the respective PNEC is compared with

the measured (or predicted) environmental concentrations. The described concept is applied, for instance, in the assessment of the chemical safety of new notified substances under the REACH-regulation (ECHA 2016).

In general, ecotoxicological test systems capture the cumulative toxicity of a sample – including additive, synergistic and antagonistic effects – and consequently enable the assessment of individual compounds as well as of complex chemical mixtures (Fent 2013). The toxicity of a chemical compound is primarily determined by its bioavailability, i.e. the property to be uptaken by an organism and to impact a biological target structure such as cell membranes, receptors, enzymes and DNA. Beside biotic factors like age, nutritional status and developmental stage of a species, the bioavailability of a toxicant can be affected by several abiotic environmental conditions. For example, metals can show a particularly complex environmental behavior and their solubility and mobility is determined by the predominant metal species (Reeder et al. 2006). The chemical speciation, in turn, is influenced by several parameters like pH, dissolved organic carbon, water hardness, and temperature (Gensemer and Playle 1999). The complex aspect of bioavailability is inherently addressed by ecotoxicological methods – a low bioavailability of a potentially harmful compound would lead to a lower toxic potential. This is an advantage especially in case of complex environmental mixtures that can contain hundreds to thousands of chemicals. If an environmental sample causes no toxic effects, it can be concluded that no harmful and bioavailable compounds are present in the sample.

A series of bioassays have been developed for the characterization of wastewater and are implemented in the German Waste Water Ordinance [Abwasserverordnung]. To assess the quality of discharges originating from diverse industrial and commercial sectors, the response of aquatic organisms upon the exposure to respective effluents is determined. For example, requirements on the toxicity to fish eggs, daphnids, algae and luminescent bacteria as well as on the genotoxic potential are defined. Appropriate standardized methods enable the estimation of possible adverse effects on the environment not only in wastewater but in most aqueous samples even if their composition is unknown. Furthermore, ecotoxicological methods offer the possibility for a simple comparison of different samples. Thus, investigations focused on toxicity-based instead of chemical-specific methods are suggested as a practicable approach for the assessment of the environmental compatibility of galvanic anodes and organic coatings.

1.5 Objectives and thesis outline

In 2016, the BMVI formed the “Network of Experts” as a new format of departmental research that works on challenges and future questions with respect to transportation and infrastructure aiming to foster resilient and sustainable transport modes. In this context, the Federal Highway Research Institute (BASt), the Federal Institute of Hydrology (BfG), the Federal Waterways Engineering and Research Institute (BAW), the Federal Maritime and Hydrographic Agency (BSH) and the Federal Railway Authority (EBA) combined their competencies to investigate construction- and structure-related emissions into water and soil. One overarching aim is the development of simplified and standardized investigation- and assessment procedures for building materials associated with the federal transport infrastructure. The research project is targeting both, the resistance and the environmental compatibility of buildings and infrastructure. Results will be collected in a database in order to inform stakeholder about the possible environmental risks posed by construction products and to provide a decision basis for the comparison and selection of products from an environmental point of view. In the first project phase, the emphasis was put on the investigation of galvanic anodes and organic coatings for corrosion protection and will be gradually extended to other important product groups in the further course.

The objectives of the present thesis were aligned to respective research questions of the BMVI-Network of experts and focused on the assessment of ecotoxicological impacts on the aquatic environment. In total, three experimental studies have been conducted to answer the central research issues divided into the following main topics:

Environmental impact of emissions from aluminum anodes
The continuous development of the renewable energy leads to the increased expansion of offshore wind farms. This is accompanied by the application of large quantities of aluminum anodes for corrosion protection and the inevitable release of contained metals to the marine environment. Little is known about the possible impact of this galvanic anodes and its main components aluminum and zinc on marine and especially sediment-dwelling species. Thus, the assessment of a possible environmental impact was the overarching aim of the first study. The experiments included the investigation of the acute toxicity of the anode material as well as its main components aluminum and zinc to marine organisms of three trophic levels. To achieve effect data under most realistic conditions and mimic the worst-case exposition near offshore installations, standard test procedures were adjusted. For this purpose, test species were exposed in natural seawater at natural pH level to maximal soluble metal concentrations or dissolved plus precipitated fractions. Detailed information on the conducted study is given in *Chapter 2*.

Environmental impact of emissions from anti-corrosion coatings
Steel coatings are applied to large areas and across all modes of transport to protect respective infrastructure against corrosion. As organic coatings consist of a great diversity of ingredients and reaction products formed during hardening, a large number of organic substances might be released in contact with water. So far, only a few studies were conducted to assess the environmental impact of emissions from steel coatings. To close this knowledge gap, selected coating systems based on epoxide or polyurethane resins were leached stepwise over 64 days according to TS 16637-2 developed in the context of CPR. Resulting emissions were characterized regarding to their ecotoxicological effects and chemical composition. Drivers of the observed ecotoxicological effects were identified and quantified. Furthermore, release mechanisms of harmful components were investigated and modelled to allow a risk assessment of organic coatings for corrosion protection. *Chapter 3* describes this evaluation of the environmental compatibility of selected anti-corrosion coating systems and the development of a general assessment strategy in detail.

Environmental impact of emissions from UV aged epoxy coatings
The top coating of an anti-corrosion coating systems is permanently exposed to environmental influences during its service life of up to over 25 years. Despite their known susceptibility to UV degradation, coatings basing on epoxy resins are frequently used as upper layers. It is already known that the chemical breakdown caused by sunlight can lead to structural and performance changes. Whether the ecotoxicity of UV aged coatings is changed as well, was part of the research questions in this present study. For this purpose, two epoxy coatings were examined with and without artificial UV aging for their bacterial toxicity and estrogenicity. In addition to the tests in 96-well format, HPTLC coupled bioassays were applied to detect the toxicity profiles of leachates. In combination with chemical analysis using LC-MS, individual bioactive fractions were examined in terms of an effect-directed analysis. The investigations on the impact of emissions from UV aged epoxy coatings are comprehensively described in *Chapter 4*.

Chapter 5 summarizes the main results of the studies, draws final conclusions and gives an overview for future research needs.

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Chapter 2

DOES GALVANIC CATHODIC PROTECTION BY ALUMINUM ANODES IMPACT MARINE ORGANISMS?

Anna Maria Bell, Marcus von der Au, Julia Regnery, Matthias Schmid, Björn Meermann, Georg Reifferscheid, Thomas Ternes, Sebastian Buchinger

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ABSTRACT

Background: Cathodic protection by sacrificial anodes composed of aluminum-zinc-indium alloys is often applied to protect offshore support structures of wind turbines from corrosion. Given the considerable growth of renewable energies and thus offshore wind farms in Germany over the last decade, increasing levels of aluminum, indium and zinc are released to the marine environment. Although these metals are ecotoxicologically well studied, data regarding their impact on marine organisms, especially sediment-dwelling species, as well as possible ecotoxicological effects of galvanic anodes are scarce. To investigate possible ecotoxicological effects to the marine environment, the diatom *Phaedactylum tricornutum,* the bacterium *Aliivibrio fischeri* and the amphipod *Corophium volutator* were exposed to dissolved galvanic anodes and solutions of aluminum and zinc, respectively, in standardized laboratory tests using natural seawater. In addition to acute toxicological effects, the uptake of these elements by *C. volutator* was investigated.

Results: The investigated anode material caused no acute toxicity to the tested bacteria and only weak but significant effects on algal growth. In case of the amphipods, the single elements Al and Zn showed significant effects only at the highest tested concentrations. Moreover, an accumulation of Al and In was observed in the crustacea species.

Conclusions: Overall, the findings of this study indicated no direct environmental impact on the tested marine organisms by the use of galvanic anodes for cathodic protection. However, the accumulation of metals in e.g. crustaceans might enhance their trophic transfer within the marine food web.

Keywords: galvanic anodes, metal toxicity, metal uptake, *Corophium volutator*, seawater

2.1 Background

Offshore installations, such as wind turbines, are founded on support structures that mainly consist of steel. In order to ensure sufficient durability of submerged substructures, appropriate corrosion protection strategies are required. Because of its comparatively low weight and high electrochemical capacity, aluminum-zinc-indium alloys are commonly applied as sacrificial (i.e., galvanic) anodes for cathodic corrosion protection of steel in the marine environment [1]. The galvanic anode and the steel are combined to an electrochemical local element resulting in a continuous oxidation of the galvanic anode and thus transfer of electrons to the steel that exhibits a higher standard electrode potential [2,3]. As numbers of offshore wind farms increase to meet the goals of the European strategy for a “prosperous, modern, competitive and climate neutral economy” [4], an increasing amount of galvanic anodes is expected to be used in the marine environment. More than 5,000 offshore wind turbines have been installed across 12 European countries until 2019, which corresponds to a total capacity of around 22 GW. The majority (77%) of this capacity is located in the North Sea [5]. The necessary amount of a certain anode material mainly depends on its desired life time and the surface area (i.e. foundation type) that requires protection against corrosion [6]. A conservative estimate based on calculations from Kirchgeorg et al. [7] reveals a current annual release of around 1900 t aluminum and 90 t zinc solely to the North Sea. This evaluation assumes the exclusive installation of aluminum anodes (Al-Zn-In alloy) with a life time of 25 years on uncoated monopile foundations without considering offshore substations or converter platforms. By the end of 2030 the wind capacity in Europe is expected to grow to 76 GW [5], which more than triples the need of corrosion protection as well.

The release of metals into the marine environment due to the use of galvanic anodes raises questions regarding potential environmental effects on marine organisms either directly or along the food chain across trophic levels [8]. In laboratory experiments, Deborde et al. demonstrated a significant increase of suspended and dissolved metal fractions in the water phase. The total concentrations reached up to 7280 µg/l Al and 360 µg/l Zn during the activation period of the galvanic anode [9]. A field study in a French harbor reported elevated Al concentrations up to 32.5 g/kg in sediments sampled close to galvanic anodes. Furthermore, up to 380 mg/kg acid-soluble Al was detected [10]. This increased proportion of a labile Al fraction might indicate a higher bioavailability of Al released by galvanic anodes. Leleyter et al. observed a total Al concentration of 29 g/kg in natural sediments contaminated by aluminum anodes [11]. Moreover, galvanic anodes caused local elevations of Zn concentrations, both in sediment and dissolved in seawater. Up to 23 µg/l dissolved and 300 µg/g sediment associated Zn was measured in enclosed marinas on the south coast of England [12]. During a monitoring campaign at the Port of Calais, Caplat et al. found elevated levels of Zn, Cu, Al and In due to the use of galvanic anodes. At undisturbed - e.g. no dredging activities - sampling points near the anodes up to 371 mg/kg Zn, 56 mg/kg Cu, 49,501 mg/kg Al and 12.6 mg/kg In were detected in the sediment [8].

Despite the ubiquitous application of galvanic anodes and the subsequent release of metals, little is known about the consequences for benthic organisms in marine environments. Adverse effects of aluminum anodes were first observed in sea urchin *Paracentrotus lividus* causing changed fertilization rates and the induction of mitotic abnormalities at concentrations of 3 µM and 0.1 µM anode-derived Al^{3+}, respectively [13]. In the digestive glands of the mussel *Mytilus edulis,* an accumulation of up to 1706 mg/kg Al released from galvanic anodes was observed that was reversible after the transfer of organisms to uncontaminated water [14]. A number of ecotoxicological studies on Al characterize the impact of acidification on the solubility and release of Al from e.g. insoluble hydroxides, which might lead to a deterioration of soil and freshwater quality. In this context, a variety of plants and freshwater organisms were examined and showed a broad range of sensitivities depending on

pH and Al speciation [15,16]. In this context, compared to invertebrates, fish have been proven to be particularly sensitive to Al exposition, in neutral to basic water a no effect concentration of 100 µg/l Al was reported for trouts. In contrast, marine studies on the ecotoxicological impact of galvanic anodes are scarce [17]. So far, very little attention has been paid to studying the toxicity and accumulation of Al in benthic organisms. Insoluble and particle-reactive hydroxide ($Al(OH)_3$) and aluminate ($[Al(OH)_4]^-$) species dominate in natural seawater at its common pH around 8 [18] and could particularly cause a threat to marine sediments and sediment-dwelling organisms. The partitioning of Al in sediments is dominated by mineral-bound species such as aluminosilicates in feldspars, mica or clays. Leleyter et al. reported a mineral associated content of Al of 91 to 96% in various natural sediments and suggested that the labile fraction in turn mainly consists of Al oxides (95%) [11,19].

Unlike Al, Zn – as the second main component of galvanic anodes – serves as an essential micronutrient, which could become toxic at higher concentrations. Several studies observed toxic effects of Zn to a variety of organisms such as plants, fish and microorganisms [20-22]. Most recent research pays attention to the toxicity of nanoscale zinc oxide. In this context Wong et al. showed that marine crustaceans were more affected than diatoms and the majority of effects was related to dissolved Zn^{2+} ions. For the amphipod *Elasmopus rapax* a half maximal effect concentration of 0.80 mg/l was determined, whereas the growth of the marine diatom *Thalassiosia pseudonana* was inhibited to 50% at 3.48 mg/l dissolved Zn [23]. At pH values around 8, the free ion (Zn^{2+}) and the mononuclear hydroxide ($[ZnOH]^+$) are prevalent in the aqueous phase [24]. If the maximum soluble concentration is exceeded, Zn can also precipitate as $Zn(OH)_2$ [25]. However, adverse effects of Zn released from aluminum anodes in the marine environment are mainly expected due to its dissolved species, as Zn is a minor component in these anodes and local saturation is most likely not reached.

More research is needed to adequately assess the environmental impact of galvanic anodes on the marine environment, in particular with regard to sediment-dwelling marine organisms. Therefore, the main objective of this study was to investigate the acute toxicity of metals released from galvanic aluminum anodes on various trophic levels in the marine environment using the following marine standard test organisms: the sediment-dwelling mud shrimp *Corophium volutator*, the marine diatom *Phaeodactylum tricornutum* and the luminescent bacterium *Aliivibrio fischeri*. Besides the acute toxicity testing, a basic uptake experiment using dissolved material from a galvanic anode was carried out at laboratory scale to mimic worst-case exposure conditions of sediment-dwelling organisms near offshore installations. Due to the pH-dependent precipitation of aluminum hydroxide after the release of Al from anodes in seawater, this batch experiment focused on sediment toxicity and a potential uptake of Al, Zn and In by *C. volutator*.

2.2 METHODS

2.2.1 RATIONALE OF EXPOSURE SCENARIOS

Different exposure scenarios for bacteria, algae and amphipods were used because of the following rationale. Bacteria and algae belong to the marine plankton and are most likely exposed to released metal ions that are present in dissolved form under natural conditions. Therefore, only the soluble fraction of the dissolved anode material was used for the exposure of bacteria and algae. Similarly, only the soluble fractions of the ionic Al and Zn standards were used for these tests. In case of the dissolved anode material and Al, the assays were performed as limit tests, i.e. exposure at saturation concentration, due to the low solubility of Al at the pH of natural sea water. In case of *C. volutator,* the testing was done with the total amount of metal ions, because the sediment dwelling *C. volutator*

is likely to be exposed to both, the dissolved and the precipitated fraction of the metal ions that sediment on the seabed.

2.2.2 PREPARATION OF TEST SOLUTIONS

The natural seawater used in all experiments originated from the amphipod collecting site mentioned in section 2.2.2.3. For the testing a stock solution (10 g/l) of a commercial galvanic aluminum anode (Al-Zn-In alloy, casted in terms to VG 81257:2009 by Raguse + Voss Metallgießerei GmbH, Germany; $95 \pm 1 \cdot 10^4$ mg/kg Al; $5 \pm 1 \cdot 10^4$ mg/kg Zn, 203 ± 1 mg/kg In) was prepared by dissolving filings of the anode in 30% NaOH (w/v) (Merck Suprapur). Al^{3+} and Zn^{2+} were used as single-element standards (Alfa Aesar Plasma Standard Specpure, 10 g/l Al in HCl; SCP Science PlasmaCAL Custom Standard, 1 g/l Zn in 5% HCl) in the various bioassays for the testing of single elements. Prior to the start of each bioassay, aliquots of the respective metal stock solutions were adjusted to pH 8 with either 30% NaOH (w/v) (Merck Suprapur) or 30% HCl (w/v) (Merck Suprapur, subboiled), resulting in the formation of precipitates. For experiments with the sediment-dwelling amphipods, the saturated solutions with the precipitate were dosed to filtered (Pall VacuCap 90 Filter Unit, 0.45 µm) natural seawater at the desired total bioassay concentration. In contrast, toxicity testing of Al and the anode material to algae and luminescent bacteria was designed as a limit test using the maximal seawater soluble metal concentrations at natural pH. For this purpose, the pH adjusted stock solutions were added to natural seawater in the ratio of 1 to 1000 and stirred for 24 h until an equilibrium of Al in the medium was reached (Fig SI 2.1 and Table SI 2.1). Subsequently, the filtered solution (Nalgene Rapid-Flow Bottle Top Filter with SFCA Membrane, 0.45 µm) was used for the respective limit test. $ZnCl_2$ (Merck Emsure) with a maximal concentration of 100 mg/l Zn^{2+} in natural seawater was used to characterize the dose-response relationship of Zn for algae toxicity. For the testing of bacteria and algae, Al and Zn concentrations were measured before the addition of test organisms as well as at the beginning and the end of toxicity testing by ICP-MS.

2.2.3 BIOASSAYS

2.2.3.1 Luminescent bacteria

The bioluminescence inhibition assay was performed according to DIN EN ISO 11348-2 [26] with liquid-dried bacteria (LCK 482, Hach Lange). This standardized test was conducted in glass cuvettes after the reconstitution of the bacteria and measured using a LUMIStox 300 instrument (Hach Lange, Germany). 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 addition of bacteria to the filtered test solutions reduced the maximal soluble metal concentration in the assay to 80% of the previously dissolved concentration due to dilution. The initial Al and Zn concentrations were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) as described in section 2.2.4. Each limit test was performed with four replicates for the dissolved aluminum anode as well as the aluminum standard. Every experiment was repeated three times in total. Natural seawater was used as negative control and 3,5-dichlorophenol (4.5 mg/l) served as the positive control.

2.2.3.2 Algae

The growth inhibition test using the marine algae *P. tricornutum* (SAG1090-1a) was based on DIN EN ISO 10253 [27]. The incubation was performed in an incubation shaker (Infors Multitron Pro) at 20 °C and 120 rpm in polycarbonate Erlenmeyer flasks with a testing volume of 50 ml. Test vessels were continuously illuminated with 60 – 120 µmol photons/(m^2*s). To simulate the natural speciation and bioavailability of the investigated metals, tests were conducted in natural seawater at pH 8.1 without the addition of chelating agents or trace metals. Merely, 15 mg NO_3^-/l and 1.5 mg PO_4^{3-}/l were added to the growth medium to prevent nutrient limitation of algae growth. An exponentially growing pre-culture (up to 10^4 cells/ml) was measured with a fluorescence spectrophotometer (Hitachi F-2500) and adjusted to $5x10^3$ cells/ml. The inoculation of the test solutions diluted the metal concentration in the assay to 80% of the previously dissolved concentration. Al and Zn concentrations were measured at the beginning and the end of the exposure by ICP-MS. Single concentrations of the dissolved aluminum anode and aluminum standard or dilution series of Zn (test concentration 10 to 1 mg/l) were investigated as triplicates. Every experiment was repeated three times in total. Natural seawater was used as negative control and 3,5-dichlorophenol (2.5 mg/l) served as the positive control. The chlorophyll fluorescence of each sample was determined daily (excitation wavelength 435 nm; emission wavelength 685 nm, plate reader Tecan Infinite M200 Pro). The evaluation of the growth-inhibition was performed after 72 h.

2.2.3.3 Amphipoda

The DIN EN ISO 16712 standard [28] for the determination of acute toxicity of sediment to amphipods was adapted to study the selected metals. In addition to the classic exposure for 10 days in the presence of sediment, this test was performed according to procedures used for positive controls, i.e. water-only exposures for 3 days, as a worst case scenario reflecting a direct contact of the organisms to the precipitated Al. This exposure was performed under identical conditions and with the same procedural steps except that no sediment was added to the test vessels so that the organisms cannot avoid the exposure to the precipitated metals by digging into the sediment. The test species *Corophium volutator* had been collected from uncontaminated reference sites at Sylt and Norderney, Germany, respectively. Corresponding sediment and seawater for exposure and control experiments originated from the same collection site (physicochemical characterization ensured consistent sediment and water quality, Table SI 2.2). The background concentrations in the sediment fractions < 20 µm ranged from 48,900 to 60,500 mg/kg Al and from 150 to 221 mg/kg Zn. The dissolved amount of Al and Zn in seawater was always below the limit of quantification (0.02 mg/l Al and 0.11 mg/l Zn). The sediment was sieved through a 1 mm screen prior usage to remove native macroorganisms. Accordingly, the seawater was filtered to 0.45 µm (Pall VacuCap 90 Filter Unit). After acclimatization to laboratory conditions, 20 individuals were added to 1 l beakers filled with 300 g sediment (approx. 2 cm depth) and 700 g test solution or 900 g test solution without sediment. The incubation was performed under constant aeration and lighting at 15 °C. At the end of respective testing periods, the content of test vessels was sieved and living organisms were counted. Al and the dissolved aluminum anode were investigated at nominal concentrations of 1, 10 and 100 mg/kg seawater. The toxicity of Zn was characterized at nominal concentrations of 0.1, 1 and 10 mg/kg seawater. Nominally dosed concentrations in this bioassay were not accompanied by chemical analyses of seawater. Two to six independent replicates were tested for each concentration level and exposure type (with and without sediment). Each experiment accompanied by negative controls with only seawater (with and without sediment). To assess the sensitivity of the test populations, ammonium chloride (30–150 mg/l NH_4^+) served as reference toxicant (without sediment).

2.2.3.4 Uptake of metals by *C. volutator*

The possible uptake of metals from galvanic anodes was investigated using *C. volutator*. As toxicity tests with *C. volutator* according to DIN EN ISO 16712 [28] provided not enough sample material for chemical analysis, the experimental set up was scaled up from 1 l beakers to 5 l aquariums with about 200 individuals per tested exposure concentration. All other test parameters, such as concentration levels and exposure time, remained the same. More specifically, the investigations were carried out in presence and absence of sediment and at nominal concentrations of 1, 10 and 100 mg dissolved aluminum anode per kg seawater. Due to the high amount of mud shrimp required for analysis in this basic uptake experiment, exposure was not carried out in replicate. At the end of the testing period, test organisms were kept for at least 1 h in fresh seawater for gut purging and were subsequently anaesthetized by aeration with CO_2. The gut passage time of sediment for *C. volutator* was previously verified on multiple individuals by the observation under a stereomicroscope. Preliminary tests showed that precipitates adhere to plumose setae (i.e., the hair-like structures of the pereiopods and the pleopods of the test organisms). Thus, attached particles had to be removed prior to the determination of metal accumulation by ICP-MS in order to prevent an overestimation of metal contents. After wash conditions had been optimized (Fig SI 2.2 and Table SI 2.3), exposed organisms were washed with 0.5% HNO_3 under agitation at 250 rpm for 30 min to ensure sufficient removal of attached material. Subsequently, organisms were rinsed with ultra-pure water, shock frozen in liquid nitrogen, freeze-dried and homogenized for 1 min at 20 Hz with a mixer mill (Retsch M400).

2.2.3.5 Statistical analysis

The statistical analysis of bioassay data was performed with the open source software R using the core distribution, version 3.4.3 and R-Studio, version 1.1.383.

After checking for normal distribution with Shapiro-Wilk's test and homoscedasticity with F-test, an unpaired, two-sided t-test (normally distributed, homoscedastic data), Welch's t-test (normally distributed, not homoscedastic data) or Mann-Whitney U test (data not normally distributed) was used to determine statistical differences in ecotoxicological effects. In case of statistically significant differences in t-test, the strength was calculated. All tests were performed at the significance level $\alpha = 0.05$.

The fit of concentration-response relationships and the estimate of the half maximal effective concentration (EC50) were calculated by a five-parameter log-logistic function (equation 2.1) by means of the extension package *drc* (version 3.0.1) [29].

$$\boldsymbol{f(x) = c + \frac{d-c}{\left(1+\exp\left(b\times(\log(x)-\log(e))\right)\right)^{f}}} \qquad \textbf{(2.1)}$$

The response is evaluated as a function of the 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.

2.2.4 CHEMICAL ANALYSES

Al, Zn and In concentrations in seawater samples were analyzed by ICP-MS (7700 ICP-MS, Agilent Technologies, Inc., USA) according to Düster et al. [30]. Samples were diluted at least 1:10 to reduce salt concentrations and thus matrix load. If analyte concentrations exceeded the linear range of the calibrated system (i.e., > 200 µg/l), samples were diluted. All samples were analyzed as triplicates and mean values were provided for statistical analysis. The following reference materials were used for method validation: SPS-SW1, SPS-SW2 (Spectrapure Standards, Norway) and SRM 1640a (NIST, USA).

Prior to the analysis of Al, Zn and In in the bulk of *C. volutator* by ICP-MS, amphipods were dissolved using microwave pressure digestion (turboWave, MLS GmbH, Germany) as summarized in Table SI 2.4. For the digestion 50 mg of freeze dried, ground and homogenized amphipods, 0.6 ml HNO_3 (65%, Suprapur, subboiled, Merck KGaA, Germany) and 0.4 ml HCl (30%, Suprapur, subboiled, Merck KGaA, Germany) were used. All samples were digested in triplicate and analyzed as described above. In addition, NCS ZC73034 (China National Analysis Center for Iron & Steel, China), a prawn standard, and SRM 2976 (NIST, USA), a mussel tissue standard, were digested and measured as reference materials. All procedures were optimized to obtain a recovery of 100 ± 10% for the analytes in the reference material.

2.3 RESULTS

2.3.1 ACUTE TOXICITY

In case of testing with *A. fischeri*, no significant effects were detected for any of the tested materials (Table SI 2.5). In case of *P. tricornutum*, the dissolved anode and Al at saturation concentration at pH = 8.1 caused a comparable and statistically significant decrease of the growth rate (Fig 2.1 and Table SI 2.6) with an average growth inhibition of 28.3 ± 6.3% and 26.0 ± 2.6%, respectively. The mean exposure concentration of Al from the dissolved anode material was 818 ± 19 µg/l (824 µg/l at the beginning and 817 µg/l at the end of exposure), while the determined concentrations of zinc and indium were below their respective detection limits of 32 µg/l and 29 µg/l in seawater. When tested as a single element, the mean exposure concentration of Al was 880 ± 24 µg/l (899 µg/l at the beginning and 874 µg/l at the end of exposure). Additionally, the effect of Zn on algal growth was characterized as a concentration-response curve at dissolved concentrations from 0.9 up to 8 mg/l (Fig 2.2 and Table SI 2.8). Applied metal concentrations quantified by ICP-MS are summarized in Tables SI 2.7 and SI 2.9. Higher concentrations could not be tested without decreasing the pH value of the medium. Under pH conditions of the investigated natural seawater, the EC50 of Zn^{2+} for *P. tricornutum* was determined as 5.70 ± 0.33 mg/l (with a fixed maximum growth inhibition of 100%).

In case of *C. volutator*, the investigated anode material caused no acute toxicity. However, the single elements Al and Zn showed significant effects at the highest tested concentrations (100 mg/kg aluminum, 10 mg/kg zinc) as illustrated in Fig 2.3 and Table SI 2.10. In case of Al, experiments in the absence of sediments exhibited higher effects on *C. volutator* mortality (max. mortality of 17.5 ± 4.2%) compared to exposure in the presence of sediment. On the contrary, Zn caused the highest mortality of *C. volutator* (52.5 ± 7.5%) in the presence of sediment. In the absence of sediment, the mortality was 30 ± 5.4%. Statistically significant differences between exposures with and without sediments were only detected in case of the exposition with 100 mg/kg aluminum (two-tailed, unpaired Mann-Whitney U test, p = 0.017).

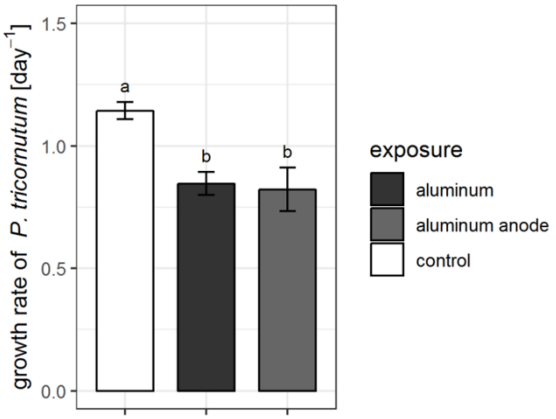
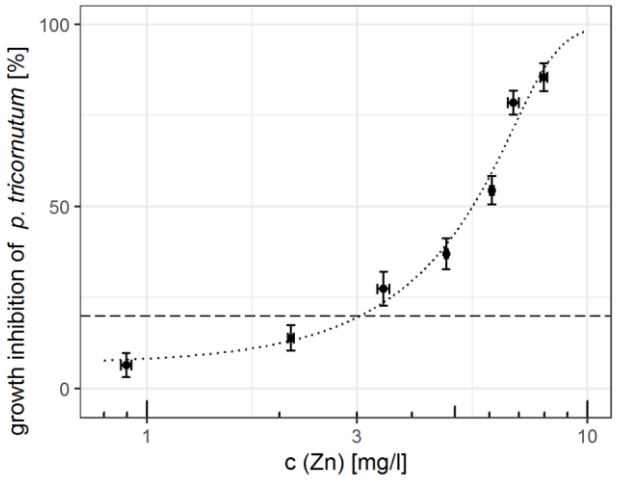


Fig 2.1: Average growth rate of *P. tricornutum* during limit testing of aluminum and the aluminum anode. Diatoms were exposed for 72 h to maximum soluble metal concentrations (n = 3, error bars indicate SE) under natural seawater pH conditions. Significantly different means are marked with different letters (two-tailed unpaired t-test, p < 0.05).

Fig 2.2: Concentration-dependent growth inhibition of *P. tricornutum* during zinc exposure for 72 h (mean ± SE, n = 3). The dashed line indicates 20% effect level and dotted line shows 5-parametric log-logistic fit of data (*b* = -10.3, *c* = 6.8, *d* = 100, *e* = 7.99, *f* = 0.20; equation 2.1).



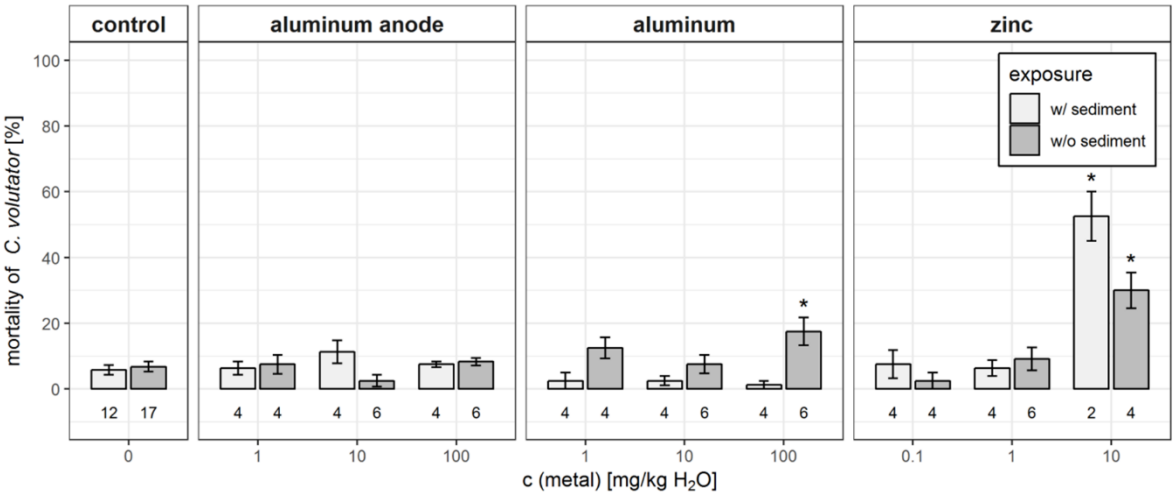


Fig 2.3: Average toxicity (± SE) caused by the dissolved galvanic anode and its main components aluminum and zinc to C. volutator. Test organisms were exposed to the different metal concentrations for 10 days with (w/) sediment and 3 days without (w/o) sediment. The numbers below the bars represent the number of replicates of each experiment. Experiments with statistically significant differences to the corresponding negative control are marked with an asterisk (two-tailed unpaired t-test, p < 0.01). See Table SI 2.11 for complete data of statistical evaluation.

2.3.2 UPTAKE OF METALS

To study the potential uptake of the three components Al, Zn and In of galvanic aluminum anodes, concentrations of these metals were determined in *C. volutator* after exposure to dissolved galvanic anode material in the presence and absence of sediment, respectively. The results are shown in Fig 2.4, Fig 2.5, Table SI 2.12 and Table SI 2.13. No increase of residual metal content compared to the control group was observed in *C. volutator* in the experiments with sediment. In contrast, all investigated elements revealed increased concentrations in *C. volutator* after exposure without sediment. The extent of accumulation indicated differences among the studied metals. As expected, given its prevalence in the dissolved galvanic anode solution, Al showed the highest concentrations in the mud shrimp after exposure. Concentration increased up to 2,200 mg/kg dry weight, which resulted in Al levels five times above those in the unexposed control group.

Although In represents a minor constituent of the tested galvanic anode, it showed the highest relative enrichment in mud shrimp. At the highest exposure level, the measured In concentration in *C. volutator* increased 136-fold compared to the negative control. The lowest enrichment was found for Zn. The Zn concentration in the test organisms was elevated by approximately 28% at the highest exposure level compared to the negative control. In summary, dissolved galvanic anode concentrations in seawater showed a positive correlation with residual metal concentration in biota. However, enrichment expressed no linear relationship in terms of applied test concentration of the dissolved galvanic anode. Nevertheless, the highest enrichment was observed at the highest exposure concentration (Fig 2.5).

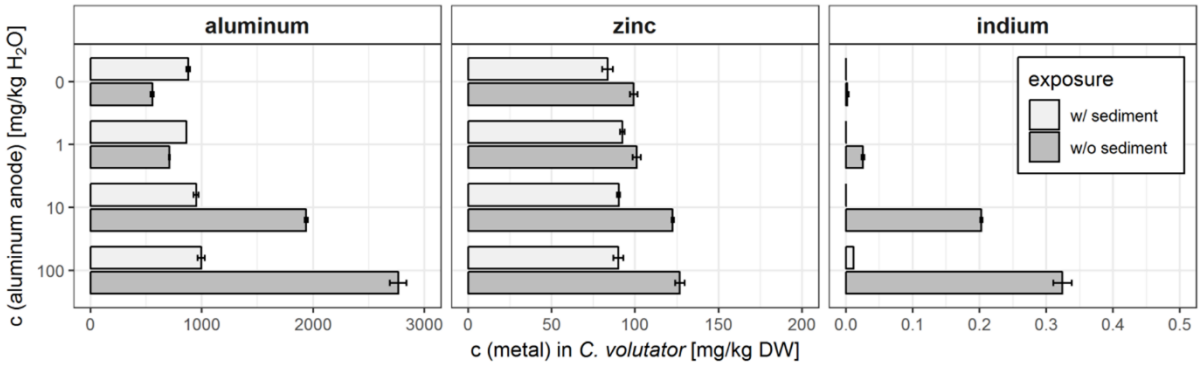


Fig 2.4: Average Al, Zn and In concentrations (± SE of technical triplicates) in *C. volutator*. The exposure with the dissolved aluminum anode proceeded for 10 days with sediment and 3 days without sediment. At the end of the testing period, the organisms were washed with 0.5% HNO_3 for 30 min to remove adherent metal precipitates from exoskeleton, freeze-dried, homogenized, digested and subsequently analyzed by ICP-MS.

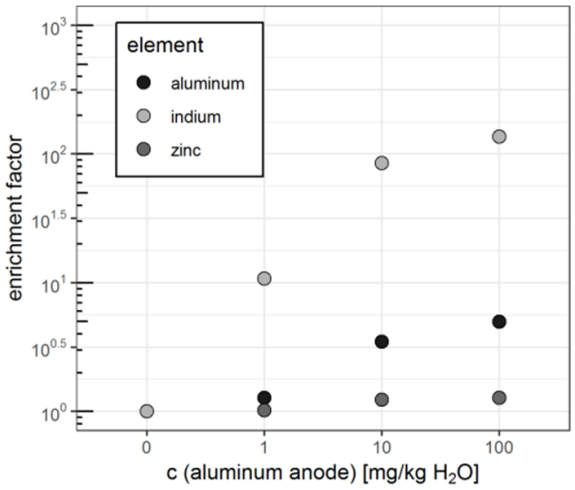


Fig 2.5: Enrichment factor in *C. volutator* after exposure to dissolved aluminum anode material. Results are shown for the exposure with three different concentrations for 3 days in the absence of sediment. Analyte residue concentrations are based on dry weight of organisms. The enrichment factor represents the ratio of analyte residues detected in exposed compared to non-exposed organisms.

2.4 Discussion

In order to assess potential ecotoxicological risks, a worst-case exposure of three marine organisms from different trophic levels, i.e. the marine algae *P. tricornutum*, the bacterium *A. fischeri* and the amphipod *C. volutator* was performed for aluminum, zinc and the dissolved galvanic anode. For *P. tricornutum*, an EC50 value for zinc of about 42 mg/l for exponentially growing algae in f/2 medium was reported [31]. The respective EC50 value of 5.34 mg/l determined in the present study is eight-fold lower. In contrast to the EC50 previously published, no further compounds such as vitamins and other trace metals that are present in the f/2 medium were added to the current experiments to achieve effect data under most realistic conditions. These differences might explain the higher Zn sensitivity for *P. tricornutum* reported here in comparison to previous studies. Deborde et al. reported a maximum increase of dissolved Zn content to around 200 µg/l during the anode activation period in a tank experiment [9]. Based on the 20% effect concentration (EC20, around 3 mg/l) as an estimate for the boundary between the lowest and no observed effect concentration (LOEC and NOEC), the resulting risk quotient is about 0.07, which indicates that there is no acute risk for *P. tricornutum* from the use of galvanic anodes in marine environments. In comparison to the freshwater green algae *Pseudokirchneriella subcapitata*, the marine algae *P. tricornutum* seems to be more tolerant for Zn^{2+}. The reported EC50 for growth inhibition of the freshwater algae at pH = 7.5 is 16.3 µg/l [32].

The observed growth inhibition of *P. tricornutum* at Al concentrations around 850 µg/l is in agreement with Gillmore et al., who reported an EC10 of 920 µg/l for growth inhibition of the same algae under similar conditions [33]. During the anodic dissolution in a tank experiment Deborde et al. measured dissolved aluminum concentrations of 0.2 to 0.5 µg/l, which was in the range of the natural background concentration [9]. Thus, effects on growth of marine algae caused by Al seem unlikely. The same conclusion was drawn by Zhou et al., who reviewed the aluminum toxicity to marine phytoplankton [17]. However, Gillmore et al. showed that diatoms with higher silicon content in cell walls are more sensitive [33]. For *Ceratoneis closterium* and *Minutocellus polymorphus* a 10% inhibition of the growth rate was observed already at Al concentrations of 69 and 440 µg/l, respectively. Based on these numbers, a growth inhibition of *C. closterium* might occur in close proximity to the galvanic anodes when the released aluminum is not yet further diluted by the surrounding water. The presented results indicate furthermore that the inhibition of algae growth by the dissolved galvanic anode reported in the current study is mainly caused by aluminum, as the observed effects do not differ significantly between the exposure to the dissolved galvanic anode and aluminum alone. Thus, possible mixture effects of metals released from a galvanic anode on the growth of *P. tricornutum* are unlikely.

No acute toxicity for any of the tested materials was observed for *A. fischeri*, indicating a lower toxicity of metals released from galvanic anodes for marine bacteria compared to algae. However, due to the scarcity of literature about Al toxicity on bacteria, it is challenging to extrapolate this finding to the field. A varying sensitivity for other marine bacteria is to be expected. In this respect it is interesting that recent studies have shown that aluminum can even have a stimulative effect on the growth of different cyanobacteria [34,35]. Nevertheless, it can be concluded that bacterial toxicity is unlikely to be the main driver for an environmental risk assessment of galvanic anodes because species from other trophic levels showed higher sensitivities.

Corophium volutator showed only a significantly increased mortality at nominal Al concentrations of 100 mg/l in the exposure without sediment. Although not directly indicated by a comparison of the mortality in the controls with and without sediment, the toxicity observed in the absence of sediment might be caused by a higher general stress level of the organisms when exposed solely in water. A further explanation might be the possibility for the organisms to avoid the exposure to the precipitated

$Al(OH)_3$ after digging into the sediment or absorption of the element to the sediment. This observation suggests a protective function of the sediment for benthic organisms. Furthermore, toxic Al^{3+} ions might be sequestered by the sediment lowering the bioavailability of Al for the exposed organisms. On the other hand a saturated Al concentration might be expected because of the precipitated $Al(OH)_3$ that can replenish the Al aquo complexes in solution. The formation of colloidal $Al(OH)_3$ and various polymeric Al(III) cations in dependence of the surrounding pH is challenging for the assessment of the bioavailability of Al species. The chronic toxicity of aluminum to freshwater organisms was successfully predicted by multiple linear regression [36] and a biotic ligand model [37]. However, a valid model is currently not available for marine ecosystems.

In case of Zn, a reduction of toxicity due to the presence of sediment was reported by Bat et al., the EC50 values for Zn-induced mortality of *C. volutator* was 14.12 mg/l in the presence and 9.79 mg/l in the absence of sediment [38]. Although exposure times differed between the published (four days for both setups) and the current experiments, the values for the observed mortality are in good agreement with the previous study (about 50% mortality at 10 mg/l zinc). The comparatively higher toxicity for exposure in the presence of sediment in the current work might be explained by the longer exposure times for this setup compared to the earlier report. Concentrations of total Zn during the anode activation period in a tank experiment ranged from 28.3 to 360.0 µg/l [9]. Under consideration of this maximum release and a roughly estimated NOEC of 1 mg/l, the risk quotient for zinc ranges from 0.03 to 0.36. Conradi & Depledge reported values for chronic Zn exposure in sediment over up to 107 days [39]. They found a significantly decreased survival at Zn concentrations of 800 µg/l, which is more than two times above the highest Zn concentration determined during the tank experiments of Deborde et al. [9].

To assess the potential uptake of the metals by *C. volutator,* the metal composition with respect to aluminum, zinc and indium of this organism was analyzed before and after exposure to the three different concentrations of the dissolved galvanic anode. Interestingly, the maximum enrichment factor of the three metals in biota reflects the ratio between the metal content in the galvanic anode and the natural biota-level of the metals in the mud shrimp. If normalized to Zn = 1, the ratio between metal concentrations in the galvanic anode and the non-exposed shrimp was 3.4 for aluminum and 170 for indium. This corresponds well to the normalized maximum enrichment factor of 3.8 for aluminum and about 105 for indium. In other words, the metal composition in biota after the exposure reflects the metal composition of the galvanic anode indicating that there is no specific mechanism in *C. volutator* that would favor the uptake of one of the investigated elements over the other. The uptake of potentially toxic metals by aquatic organisms can take place in general across the integument, through the respiratory surfaces (e.g. gills), or the gut after ingestion of contaminated food [40]. The mud shrimp *C. volutator*, due to its filter and deposit feeding activity, is exposed to the particular risk of particle-associated contaminants and therefore the uptake of metals from precipitates in the current experiments. Analysis of the exposed organisms provided valuable additional information but raised the question, where the enriched elements are located, i.e., in or at the organism. As Rainbow & Luoma described, metal toxicity is not necessarily related to the total accumulated amount in the organism, because the metals are frequently stored in detoxified forms [41]. The removal of metals from cytoplasm by complexation and accumulation in spherical precipitates in membrane bound vesicles or vacuoles is a well-known detoxification strategy of crustaceans [40]. The same applies to *C. volutator*, where e.g. zinc-rich granules could be observed in hepatopancreas [42]. Alternatively, the elements released by the galvanic anode might be absorbed at the shell of the crustacea. In fact, the biosorption of various metal ions to chitin is used as an approach to purify water from e.g. mining sites [43,44]. It has been demonstrated that chitin can remove metal ions from aqueous solutions by adsorption to functional groups such as hydroxyl- and amino-residues

[45,46]. Based on modeling results, Tarpani et al. proposed chemisorption of Al^{3+} to chitin [44]. A strong binding of metal ions to chitin might explain why the elements were detected in elevated levels despite the acidic washing step. However, the bulk analysis of exposed organisms alone provides no further information. To elucidate the localization of the accumulated metals, methods allowing a special analysis such as laser ablation ICP-MS [47-49] should be applied. The reported enrichment of metal ions by *C. volutator* – either due to uptake or adsorption – might promote a trophic transfer in the marine environment. Benthic invertebrates usually serve as food for larger invertebrates, fish and also birds. As previously reported, aluminum in freshwater can be transferred from a primary to a secondary consumer [50]. In particular with respect to the potential use of areas surrounding offshore wind farms for aquaculture [51,52], further studies are required to investigate the direct and indirect uptake of metals released from galvanic anodes by marine organisms for a risk assessment on environment and human health.

2.5 Conclusion

Based on the assessment of the acute toxicity to three organisms of different trophic levels, a direct environmental threat by the use of galvanic anodes for cathodic protection of wind turbine support structures in the marine environment was not indicated. Toxicity thresholds for *C. volutator, A. fischeri* and *P. tricornutum* determined in worst case exposure scenarios were in most cases at least one order of magnitude higher compared to the concentrations of aluminum and zinc that are expected to be released during cathodic protection. However, a growth inhibition of marine algae in close proximity to an operating galvanic anode might occur if the dilution of the released metal ions occurs by diffusion only. The observed accumulation of aluminum and indium in crustacea might facilitate their enhanced entry into the food chain. To develop a broader picture of the environmental impact of galvanic anodes in marine environments, future investigations should be extended to higher trophic levels, the potential trophic transfer of respective metals and possibly more sensitive chronic endpoints. If sublethal or developmental assays resulted in more significant effects, also the investigation of mixture toxicity could be useful extension of the presented work.

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Chapter 3

ECOTOXICOLOGICAL CHARACTERIZATION OF EMISSIONS FROM STEEL COATINGS IN CONTACT WITH WATER

Anna Maria Bell, Roland Baier, Birgit Kocher, Georg Reifferscheid, Sebastian Buchinger, Thomas Ternes

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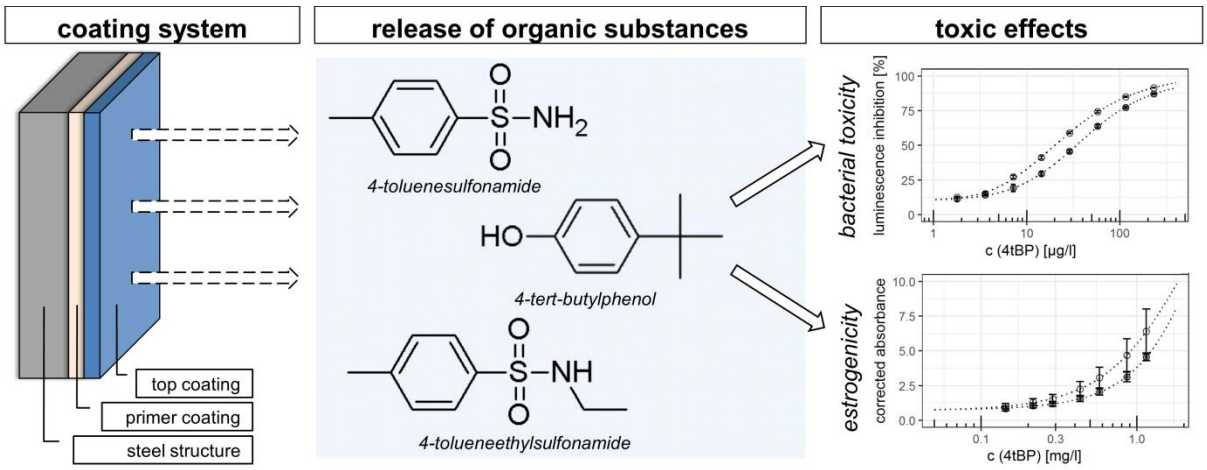


ABSTRACT

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.

Keywords: epoxide, polyurethane, leaching, toxicity, 4-*tert*-butylphenol, diffusion

3.1 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.

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 antifouling 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 amphritrite* 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.

3.2 Materials & 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.

3.2.1 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 3.1). 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 × 160 × 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 3.1).

Table 3.1: 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

3.2.2 LEACHING, SAMPLING AND SAMPLE PREPARATION

Leaching was conducted in accordance with the dynamic surface leaching test (DSLT, CEN/TS 16637-2) (CEN, 2014), which is original 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 × 220 × 240 mm) filled with 3 l deionized water. The containers were covered by a glass plate. According to DSLT, 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 6cc (200 mg) cartridges. The cartridges were conditioned with 2 ml n-heptane (Picograde, Promochem), 2 ml acetone (Picograde, Promochem), 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).

3.2.3 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.

3.2.3.1 Luminescent bacteria

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 (≥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 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.

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 ± 50 rpm) for 48 ± 2 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 × 10 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 BioLuminizer (Camag). The quantitative evaluation of black and white images and the calculation of inhibition chromatograms was performed according to Schulz et al. (2017).

3.2.3.2 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 70–120 µE/m^{2}s in test tubes with a testing volume of 4 ml. The density of algae at the beginning of the test was adjusted to 1×10^4 cells/ml. Distilled water was used as negative control and potassium dichromate (≥ 95%, Merck; c = 0.5 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.

3.2.3.3 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 = 4.14×10^{-8} mol/l) and 2-nitrofluorene (98%, Sigma-Aldrich; c = 3.78×10^{-8} 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.

3.2.3.4 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, MATα, PEP4:HIS3, prb-1-δ1.6 R, HIS3-δ200, lys2-801, trp1-δ101, 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 (ERα) in case of the presence of ERα-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) (≥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.

3.2.3.5 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 3.1) was implemented to fit the time-dependent inhibition of luminescence.

$$\boldsymbol{f(x) = c + \frac{d-c}{1+(e/x)}} \qquad \textbf{(3.1)}$$

The fits of concentration-response relationships and estimates of EC50 were generated by a five-parameter log-logistic function (equation 3.2).

$$\boldsymbol{f(x) = c + \frac{d-c}{\left(1+\exp\left(b\times(\log(x)-\log(e))\right)\right)^{f}}} \qquad \textbf{(3.2)}$$

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 3.3) where *b* is the intercept and *m* is the slope.

$$f(x) = mx + b \quad (3.3)$$

3.2.4 CHEMICAL ANALYSIS

3.2.4.1 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 µm) and acidified with HCl 30% (Suprapur, Merck) to a pH < 2. Potassium hydrogen phthalate (Emsure, Merck) served as control standard.

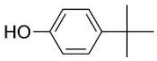
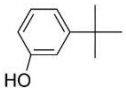
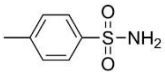
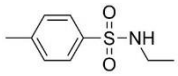
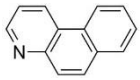
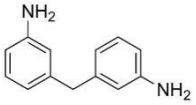
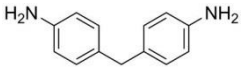
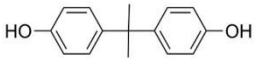
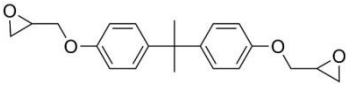
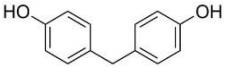
3.2.4.2 Zinc

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) HNO_3 (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.

3.2.4.3 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 µl sample. The GC was equipped with a HP-5ms GC-column (Agilent J&W, 30 m × 0.25 mm × 0.25 µm). 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 3.2) using standards purchased from Sigma-Aldrich. Additionally, in selected samples, the content of 4-*tert*-butylphenol (4*t*BP) was quantified by addition of the internal standard 4-*tert*-butylphenol-d9 (TRC Canada) prior to extraction. Extracts were kept in n-heptane.

Table 3.2: Analytical parameters of target compounds. Quantification limits (LOQ) were determined by signal-to-noise method (S/N ≥ 10). As bisphenol A and 4-*tert*-butyphenol 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 Figure SI 3.1). The recovery of analytes is also summarized in SI (see Table SI 3.2).

substance	CAS	retention time [min]	MRM transition (m/z) precursor → product ions	LOQ [mg/l]
4-*tert*-butylphenol (4*t*BP) 	98-54-4	8.46	149.8 → 134.9, 106.9	0.005
3-*tert*-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'-methylenedianiline 	19471-12-6	25.7	198.2 → 106.2, 182.2	0.001
4,4'-methylenedianiline 	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

3.2.5 *Identification of release mechanisms*

The assessment of the underlying release mechanism for 4*t*BP 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 4*t*BP 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

$$\boldsymbol{M_t = M_\infty \left(1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} exp\left\{\frac{-D(2n+1)^2\pi^2 t}{4l^2}\right\}\right)} \qquad \textbf{(3.4)}$$

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.

3.3 Results

3.3.1 *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 (Figure 3.1 and Figure SI 3.2). 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 (Figure SI 3.3). 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 (Figure 3.2). 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 ± 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 (Figure 3.3). The samples of 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 to 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.

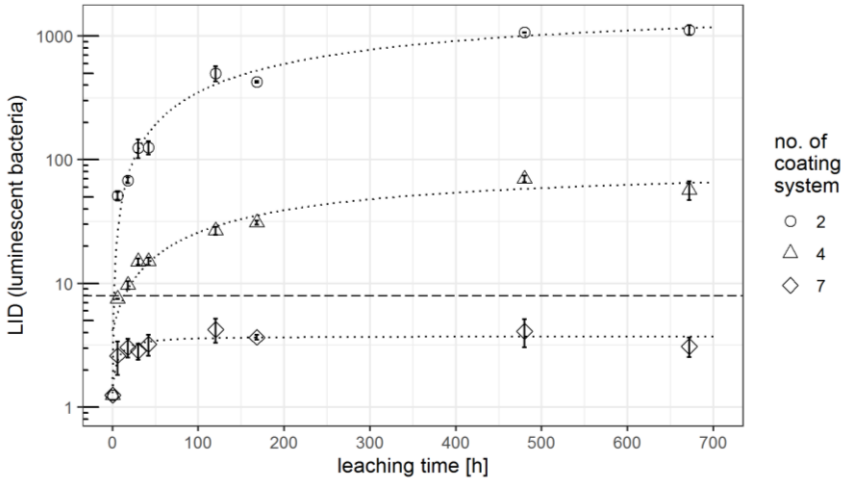


Figure 3.1: Time-dependent luminescence inhibition (mean, n = 3, error bars indicate SE) in leachates of three selected top coatings for corrosion protection (see SI for further data). Leaching time is displayed as duration of the specific step after the renewal of leachant. Results calculated by linear 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 3.1).

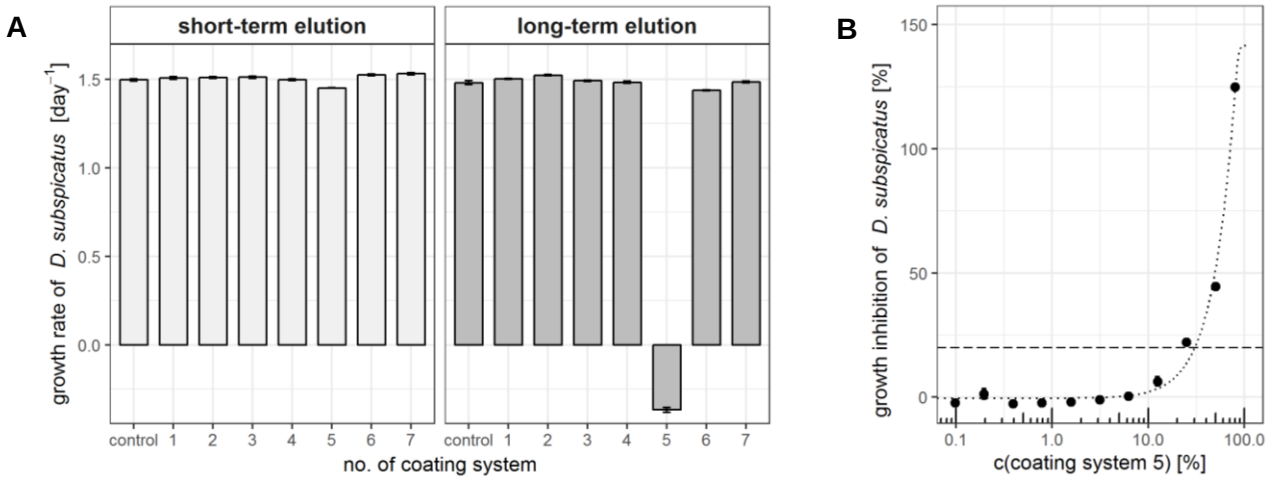


Figure 3.2: Effects on growth of the algae *Desmodesmus subspicatus* in leachates of seven anti-corrosion coating systems. *A*: Average growth rate (n = 3, error bars indicate SE) in undiluted leachates. Algae were exposed for 72 hours to time-proportional composite samples collected over short (0.25–1 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 3.2).

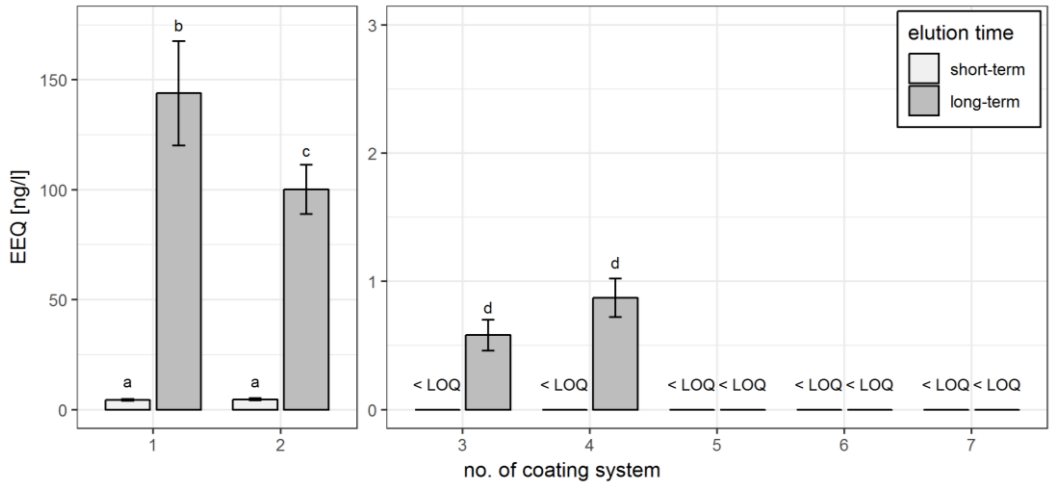


Figure 3.3: Mean estrogenic activities (n = 9, error bars indicate SE) in leachates of seven anti-corrosion coating systems. Properties of investigated materials are summarized in table 3.1. Effects were detected as estradiol-equivalents (EEQ) in time-proportional composite samples over short time (0.25–1 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 Figure SI 3.4 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).

3.3.2 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 3.3). Six substances leached from coating system 5, 6 and 7 remained unidentified (see Table SI 3.3 for GC-MS scan data). The identified compounds were 4-*tert*-butylphenol (4*t*BP), 4-toluenesulfonamide (4TSA) and 4-tolueneethylsulfonamide (4TESA). 4*t*BP 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.

Table 3.3: 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 3.3).

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

no. of coating system	4*t*BP [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	

The highest amounts of 4*t*BP 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 levels were tested for bacterial toxicity and estrogenic activities in comparison to the corresponding concentrations of 4*t*BP determined in the selected representative leaching-replica. In all cases, the samples showed higher effects than the respective standard (Figure 3.4). In the bioluminescence inhibition assay, the apparent EC50-values of coating system 1 and 2 expressed in terms of 4*t*BP were 25.1 ± 2.7 µg/l and 17.13 ± 0.55 µg/l, respectively. The EC50-value of the 4*t*BP tested as single compound was 37.9 ± 9.9 µg/l. With respect to estrogenicity, coating system 1 contained 167 ± 15 ng/l EEQ whereas the corresponding measured concentration of 4*t*BP reached an EEQ of 133 ± 10 ng/l. In coating system 2 in total 94.6 ± 4.8 ng/l EEQ were determined from which 58.3 ± 6.8 ng/l EEQ could be attributed to 4*t*BP.

To estimate the total release of 4*t*BP from the two different top coatings ((I) and (II)) based on epoxide resins, the 4*t*BP 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 4*t*BP concentration increased with leaching time (see Table SI 3.4). Taking into account the leaching volume, the total release of 4*t*BP was 34.3 ± 1.2 mg and 1.97 ± 0.05 mg 4*t*BP from coating system 2 and 4, respectively. The luminescence inhibition of leachates was found to be positively correlated with the 4*t*BP content in both coating systems (see Figure 3.5).

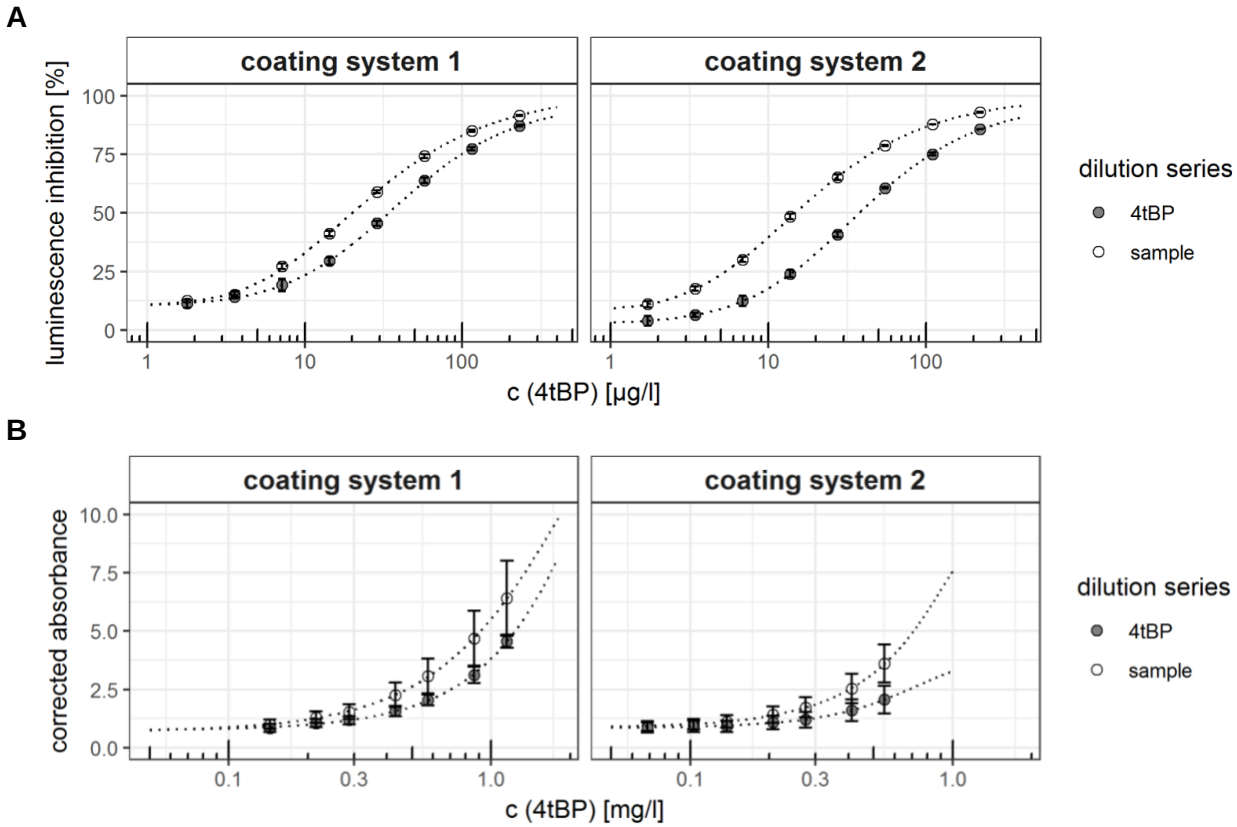


Figure 3.4: Concentration-dependent effects of enriched leachates of anti-corrosion coating systems 1 and 2 and 4-*tert*-butylphenol (4*t*BP). Each representative time-proportional long time sample (0.25–64 d) was tested against its corresponding concentration of 4*t*BP. Dotted lines show the 5-parametric log-logistic fit of the data (equation 3.2). *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.

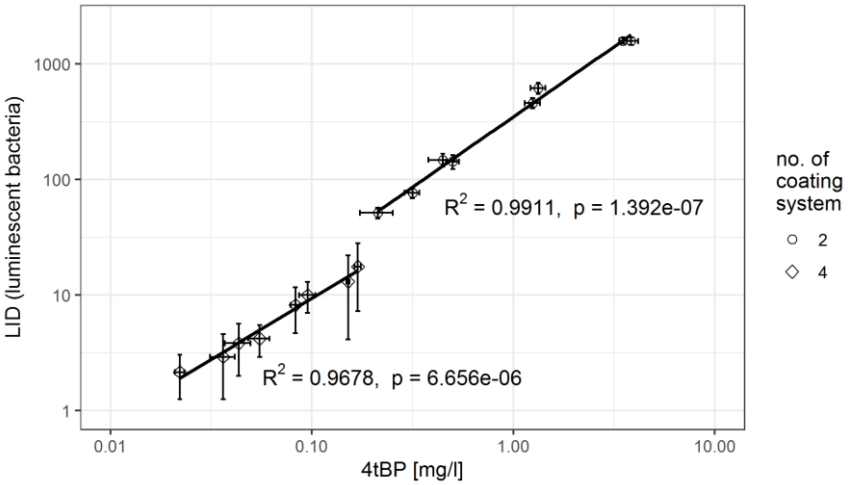


Figure 3.5: Correlation of 4*t*BP content and luminescence inhibition in leachates of two anti-corrosion coating systems (n = 3 × 8). Results of regression tests are shown as adjusted R^2 and associated p-value.

3.3.3 RELEASE MECHANISMS

The concentrations of 4*t*BP 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 Figure 3.6) 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 4*t*BP 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 3.4) 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 Figure SI 3.5 and Table SI 3.5). D and M_∞ for coating (II) are 9×10^{-11} cm^2/s and 3.9 mg. Due to the lack of fit, meaningful values for these parameters could not be determined for top coating (I).

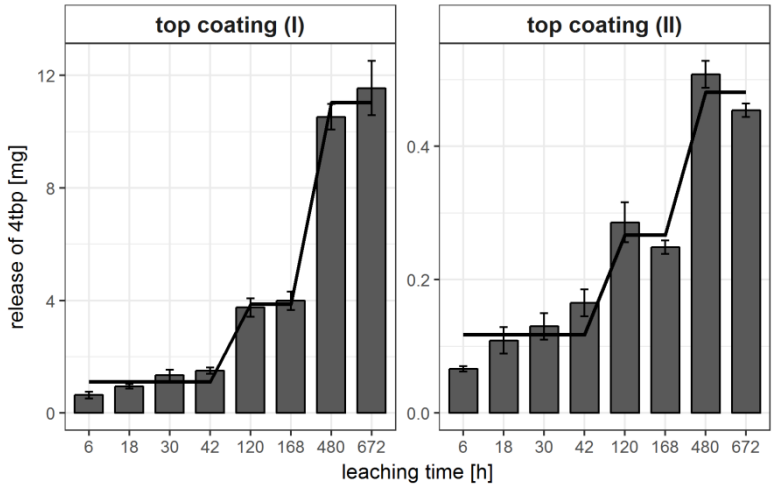


Figure 3.6: Time-dependent release of 4*t*BP (mean, n = 3, error bars indicate SE) in leachates of top coating (I) and (II). Leaching time is indicated as the duration of the specific step after renewal of leachant. Solid lines show the arithmetic means of the supposed 3-step release pattern.

3.3.4 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) 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 3.6). 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.

3.4 Discussion

3.4.1 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 (Figure SI 3.6). 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 4*t*BP 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 4*t*BP-standards, about 80 and 60% of EEQ could be explained by 4*t*BP detected in leachates of system 1 and 2, respectively. Analogous to this observation, the inhibition of luminescence in *Aliivibrio fischeri* could be attributed to 4*t*BP to around 65% in coating system 1 and 45% in coating system 2 (referred to EC50). Thus, 4*t*BP 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 4*t*BP. Further compounds with comparable physicochemical properties to 4*t*BP 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 4*t*BP in the hardener component of top coating (II) (coating system 4), a total amount of 2.2 g 4*t*BP 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 4*t*BP released from top coating (II), 4*t*BP was not listed in the available safety data sheet provided together with top coating (I). The 4*t*BP content of top coating (I) was determined by GC-MS to 2.4% (w/w), this is equivalent to 0.4 g 4*t*BP 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 4*t*BP in the two polymers. The alkylphenol 4*t*BP 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 suboptimal stoichiometry of e.g. monomers and hardener in the recipe of the polymer might result in elevated fractions of 4*t*BP that is not covalently bound to the polymer structure and thus mobile. An optimization of the recipe might lead to reduced emissions of 4*t*BP 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 (Figure 3.6) 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 4*t*BP controlled by diffusion. This finding is supported by the modelling of the 4*t*BP release using equation 3.4 which resulted in an acceptable accordance between model and measurement only for top coating (II). The determined diffusion coefficient for 4*t*BP in top coating (II) was 0.9×10^{-10} cm^2/s and thus in the same order of magnitude compared to diffusion coefficients of water in epoxy resins ranging between 0.04 and 0.95×10^{-10} cm^2/s as reported previously (Li et al. 2006, Liu et al. 2003). The calculated maximal release of 4*t*BP from the total surface of 498.6 cm^2 was 3.9 mg for this top coating indicating that about 50% of the mobilizable 4*t*BP was emitted during the release experiment. In contrast to top coating (II) an acceptable fit for the measured 4*t*BP-release was not possible for top coating (I) indicating a release mechanism that is not only controlled by diffusion. A release of 4*t*BP 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 4*t*BP 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 4*t*BP 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 4*t*BP, 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 4*t*BP 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 4*t*BP emissions from the other coating systems investigated. In comparison to *Daphnia magna*, algae are less sensitive to 4*t*BP, 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 17*β*-estradiol in the Yeast Estrogen Screen (Routledge and Sumpter 1997), 4*t*BP 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 4*t*BP. 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 4*t*BP (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 4*t*BP 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 4*t*BP in the environment is reported so far. In the Elbe River, concentrations of 4*t*BP were reported in levels up to 78 ng/l in water and 75 µg/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 4*t*BP is mainly available for Asia ranging from about 0.01 µg/l in a Japanese river (Inoue et al. 2002) up to 1 µg/l in the Yangtze River (Liu et al. 2017). 4*t*BP was detected in fish bile up to 78.2 µg/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 µg/l (Luft et al. 2017). However, 4TSA was found to be present in the aquatic environments of Berlin with up to 1.15 µg/l and 38 µg/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 4*t*BP 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 4*t*BP, 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-mobilizable 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.

3.4.2 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 battery. 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 (Figure SI 3.7). The assessment criterion of DIBt was considerably exceeded (LID_{lb} > 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 (Figure SI 3.7).

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 mobilizable 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.

3.5 Conclusion

- 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 (4*t*BP) 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 4*t*BP 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.

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Chapter 4

UV AGED EPOXY COATINGS – ECOTOXICOLOGICAL EFFECTS AND RELEASED COMPOUNDS

Anna Maria Bell, Nils Keltsch, Peter Schweyen, Georg Reifferscheid, Thomas Ternes, Sebastian Buchinger

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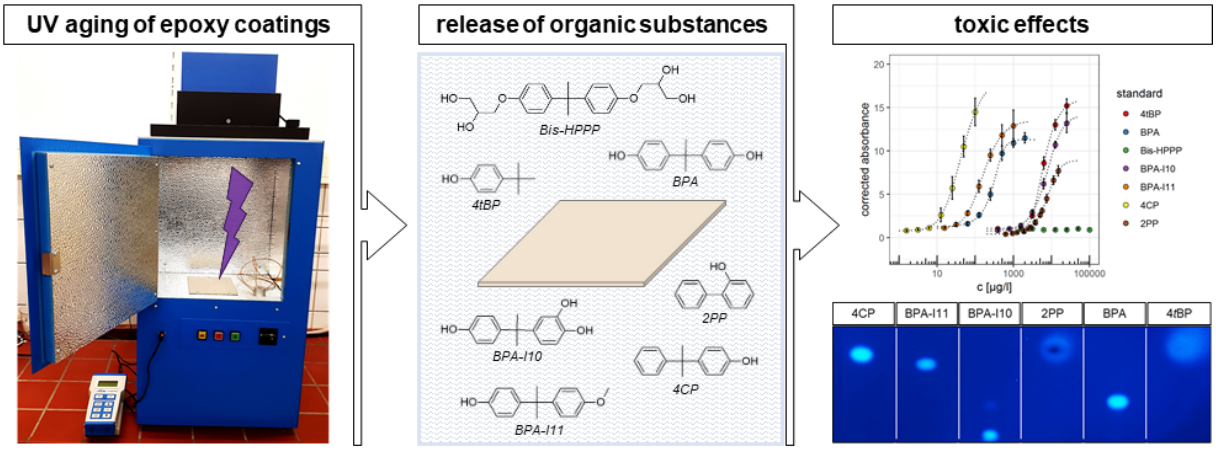


ABSTRACT

Organic coatings can guarantee long-term protection of steel structures due to causing a physical barrier against water and oxygen. Because of their mechanical properties and resistances to heat and chemicals, epoxy resin-based coatings are widely used for corrosion protection. Despite of the aromatic backbone and the resulting susceptibility to UV degradation, epoxy resins are frequently used as binding agent in top layers of anti-corrosion coating systems. Consequently, these organic polymers are directly exposed to sunlight and thus UV radiation. The present study was designed to investigate if toxic effects of epoxy resin-based-coatings are changed by UV-A irradiation. For this purpose, two epoxide-based top coatings were examined with and without UV aging for their bacterial toxicity and estrogenicity. In addition, chemical analyses were performed to identify released compounds as well as photolytic degradation products and to assign toxic effects to individual substances. UV-A irradiation of epoxy resin based top coatings resulted in an overall decrease of acute and specific ecotoxicological effects but as well to the formation of toxic transformation products. Both, in leachates of untreated and UV-A irradiated coatings, 4*t*BP was identified as the main driver of estrogenicity and toxicity to luminescent bacteria. BPA and structural analogs contributing to estrogenic effects in leachates were formed by UV-A irradiation. The combination of HPTLC coupled bioassays and LC-MS analyses supported the identification of bioactive compounds in terms of an effect-directed analysis. The present findings indicate that epoxide-based coatings are less suitable for the application as top coatings and more UV stable coatings like aliphatic polyurethanes should be preferred.

Keywords: epoxide, leaching, toxicity, UV-A, effect-directed analysis

4.1 INTRODUCTION

Alterations of metallic (construction) materials by the reaction with their environment are summarized under the term corrosion. To maintain relevant infrastructures, this natural process can be prevented by active and passive protection methods. Active corrosion protection is done by the negative polarization of the steel e. g. by galvanic anodes or impressed-current systems. In contrast, passive protection methods achieve their anti-corrosive properties by acting as barrier between the metal and the corrosive environment. An effective and frequently used passive method is the application of organic coatings systems since they can provide long-term protection of steel structures even under aggressive environmental conditions (e.g. in industrial or marine applications). Besides major steel structures such as bridges, (offshore) wind turbines and buildings, the bodyworks of vehicles are protected by organic coatings (Lyon et al. 2017). Protective paint systems are designed for protection periods up to over 25 years and are internationally standardized according to ISO 12944-1 (ISO 2018a). In perspective, systems even providing at least 50 years of corrosion protection are intended to be approved (Kuhlmann 2020).

For the fabrication of organic coatings, a wide range of binding agents can be applied, such as alkyd resins, acrylic resins, ethyl silicates, fluoropolymers, polyester resins, polysiloxanes, polyacrylates and polyaspartates (ISO 2018a). The most commonly used coatings are based on polyurethane or epoxy resins. A complete anti-corrosion coating system can consist of up to three different coating layers (priming, intermediate and top coating), which themselves consisting of one or two components and are applied usually in up to 360 µm film thickness. After application, the coating is continually exposed to weathering, especially to UV radiation. Because of their aromatic backbone, epoxide-based coatings have a decreased UV-stability compared to coatings based on polyurethane. Nevertheless, the former is frequently used as top layers in anti-corrosion coating systems. Consequently, these organic polymers are irradiated by sunlight and thus UV radiation. This might trigger the degradation of the chemical structure of the coating caused by the cleavage of chemical bonds due to photolysis and the attack of free radicals (autoxidation) (Hare 1992). Besides the negative effects on the durability of the coating by e.g. cracking or delamination from the coated surface, the light-induced degradation could possibly lead to the formation of transformation products with unwanted biological activities.

Depending on the corrosive category and protection period, the resistance of paints to weathering is examined as part of their performance testing under artificial (cycle test according to ISO 12944) and natural conditions (long-term exposure in nature according to BAW (2011)). The ISO standard asks for reduced emissions of volatile organic compounds (VOC) and prohibits the use of toxic and carcinogenic ingredients, but the assessment of possible environmental impacts is not part of the authorization of corrosion protection systems. However, coatings based on epoxy resins can contain a wide range of organic solvents, metallic pigments, UV stabilizers, biocides, and other potential hazardous substances as bisphenol A/F, alkylphenols and other phenolic substances and polyamines, which are added as curing agents (Jin et al. 2015, Verma et al. 2020). All these ingredients and reaction products formed during the hardening by polyaddition reactions potentially pose a risk to the aquatic environment if released from respective steel structures either by a direct contact with a water body or indirectly via the runoff from the structure after rainfall.

In previous studies, the leaching of bisphenol A, nonylphenol and 4-*tert*-butylphenol from various unweathered epoxy resin-based coatings was already reported. In this context, estrogenic activity and toxic effects to water fleas, luminescent bacteria and cypris larvae of barnacles were detected (Bell et al. 2020, Vermeirssen et al. 2017, Watermann et al. 2005). In contrast, investigations on UV aged coatings were mainly focused on topics of material and polymer sciences such as the

mechanisms of photodegradation or structural and performance changes. For instance, Liu et al. (2014) studied the microstructural alteration of a polyamide-cured epoxy coating and discovered the formation of micropores along with an increased water barrier function upon UV-A irradiation. Brand et al. (2020) recently investigated irradiated coating surfaces and elucidated degradation pathways leading to the delamination of polyurethane top layers. However, the toxicity of UV aged coatings to the (aquatic) environment has not been addressed so far.

Therefore, this study was designed to investigate if toxic effects of epoxy resin-based-coatings are changed by UV-A irradiation. For this purpose, two epoxide-based top coatings were examined with and without UV aging for their bacterial toxicity and estrogenicity. In addition, chemical analyses were performed to identify released compounds as well as photolytic products and to assign toxic effects to individual substances.

4.2 Materials & methods

4.2.1 Selection of coating systems and fabrication of test plates

The current study was performed with two different two-component epoxide-based top coatings for corrosion protection known for their elevated bacterial toxicity and estrogenic effects from previous investigations (Bell et al. 2020). The selected products are primarily used for the corrosion protection of hydraulic steel structures and are among the most relevant coatings for the federal transport infrastructure of Germany. The coating was performed by the Institute for Corrosion Protection (IKS, Dresden). The respective layers were applied on the front, back and edges of steel plates with a size of 150 × 160 × 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 and amounted to 200 µm and 500 µm for coating A and B, respectively.

The treatment with UV-A radiation was conducted in an indoor UV-chamber (UVA CUBE 400, Dr. Hönle AG, Germany) for 65 h only on the front of the coated plates with an average power of 230 W/m² and a total energy amount of 53.82 MJ/m². The UV doses were recorded using the corresponding UV-Meter equipped with an optical fiber sensor (340–405 nm). Using 215 MJ/m² as reference annual UV-radiation dose in central Europe (295–400 nm) (Atlas Material Testing Solutions 2001), the released radiation is equivalent to approximately three months of real time exposure in central Europe (230 W/m² × 65 h × 3600 s/h = 53.82×10^6 J/m²).

4.2.2 Leaching and sample preparation

The coated plates were attached on nylon strings and immersed in 3 l deionized water filled into all-glass aquariums (300 × 220 × 240 mm). The containers were covered by glass plates. Leaching water was sampled after 4 weeks and stored in closed, dark glass bottles at 2 to 8 °C. Samples that were not examined within 48 h after collection were kept frozen at ≤ -18 °C until further analysis. An aliquot of each sample was concentrated 1000-fold by solid phase extraction (SPE) using OASIS HLB 6cc (200mg) cartridges. The selected sorbent is designed for a universal application and can retain a wide range of acidic, basic, and neutral substances. The cartridges were conditioned with 2 ml n-heptane (Picograde, Promochem), 2 ml acetone (Picograde, Promochem), 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 and restored in ethanol (Optigrade, Promochem). Each coating was investigated as triplicate with and without UV aging. Two aquaria without plates served as controls.

4.2.3 BIOASSAYS

4.2.3.1 Luminescent bacteria assay

The bioluminescence of the marine bacterium *Aliivibrio fischeri* can be utilized to assess acute bacterial toxicity by quantifying the inhibition of the bacterial light emission after exposure to the test sample.

4.2.3.1.1 Luminescent bacteria assay in 96-well plates

The procedure of bioluminescence inhibition assay in 96-well plates was adapted from DIN EN ISO 11348-2 (ISO 2007) and performed with reconstituted liquid-dried bacteria (LCK 482, Hach Lange). Prior testing, ethanolic extracts were pre-diluted at least 1→1000 and simultaneously salinated with NaCl solution (2%, m/v). Each sample was tested in dilution series in a geometric sequence, threefold independently in white 96-well microtiter plates (µCLEAR, Greiner Bio-One) with three technical replicates each. Together with the three independent leaching experiments, nine biologically independent tests were performed in total for each coating and condition (with/without UV). A NaCl solution (2%, m/v) was used as negative control and 3,5-dichlorophenol (97%, Sigma; c = 4.5 mg/l) served as positive control. The half maximal effect concentration in terms of sample dilution (EC50) calculated from the tested dilution series was used as test result.

4.2.3.1.2 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 pre- and main cultures according to DIN EN ISO 11348-1 (ISO 2007b) in an Erlenmeyer flask with cap under constant agitation (350 ± 50 rpm) for 48 ± 2 h at room temperature. On the day of analysis, ethanolic extracts 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 × 10 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) (35:65, v/v) after focusing the samples with methanol. Before 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 BioLuminizer (Camag). Toxic fractions appear as dark zones.

4.2.3.2 Yeast Estrogen Screen (YES)

The reporter gene assay is based on the activation of the human estrogen receptor alpha (ERα) in case of the presence of ERα-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. The applied test strain according to McDonnell et al. (1991b) (1991a) is based on the strain *Saccharomyces cerevisiae* BJ3505 (protease deficient, MATα, PEP4::HIS3, prb-1-δ1.6R, HIS3-δ200, lys2-801, trp1-δ101, ura3-52gal2can1).

4.2.3.2.1 Yeast Estrogen Screen in 96-well plates

The estrogenicity was investigated in 96-well microtiter plates (Cellstar, Greiner Bio-One) according to ISO 19040-1 (ISO 2018b) by exposing the genetically modified yeast cells at 30 °C for 18 h to dilutions of the ethanolic extracts. Each sample was tested at least three-fold independently with four technical replicates each. Together with the three independent leaching experiments, nine biologically independent tests were performed for each coating and condition (with/without UV). Single compounds were investigated at least three times independently. Ethanol (1%) was used as negative control and a dilution series of 17*β*-estradiol (E2) (≥ 98%, Sigma-Aldrich) served as positive control (500 – 0.66 ng/l) and for calibration. The estrogenic potential of samples was quantified in terms of a 17*β*-estradiol equivalent concentration (EEQ).

4.2.3.2.2 HPTLC coupled Yeast Estrogen Screen (p-YES)

The investigation of estrogen-like effects on HPTLC-plates was performed according to Riegraf et al. (2019) and the chromatographic procedure was adopted from the luminescent bacteria assay (see 4.2.3.1.2). The yeast cells were exposed to the plates by spraying and incubated for 3 h under saturated humid atmosphere (NuAire CO_2-incubator with humidity control, NU-5820E). Estrogenic fractions appearing as fluorescent zones were documented using a TLC Visualizer 2 (CAMAG) at an excitation wavelength of 366 nm.

4.2.3.3 Data evaluation and statistics

The statistical analysis of bioassay data was performed using the open source software R (version 3.4.3). The fits of concentration-response relationships and estimates of EC50 were generated by a five-parameter log-logistic function (equation 4.1) by means of the extension package *drc* (version 3.0.1).

$$\boldsymbol{f(x) = c + \frac{d-c}{\left(1+\exp\left(b\times(\log(x)-\log(e))\right)\right)^{f}}} \qquad \textbf{(4.1)}$$

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.

Toxic units were calculated by dividing the mass concentrations of quantified standards (see Table 4.2) by their respective half maximal effective concentration (EC50) of the single compounds determined by the characterization of a complete concentration-response relationship in the YES and luminescent bacteria assay in 96-well plates.

4.2.4 CHEMICAL ANALYSES

4.2.4.1 Qualitative and quantitative analysis of extracts

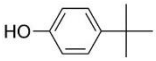
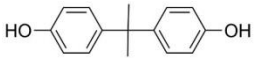
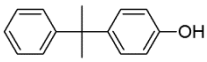
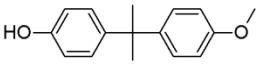
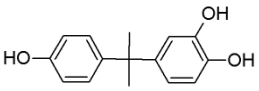
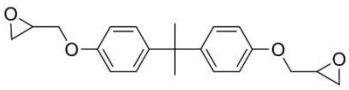
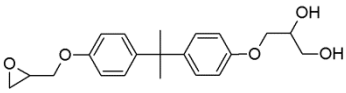
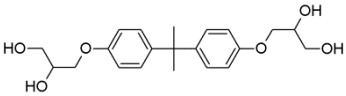
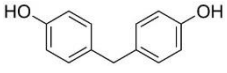
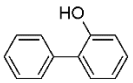
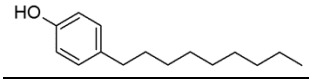
The identification and quantification of compounds released from untreated and UV-A aged coating systems were performed with an HPLC system (1260 Infinity series, Agilent Technology, Waldbronn, Germany) coupled to a high resolution mass spectrometer (TripleTOF, AB SCIEX). The QTOF system was equipped with a DuoSpray ion source and a TuboIon Spray probe for electro spray ionization (ESI) experiments. The parameter for positive and negative ionization were as follows (values for ESI(-) in parenthesis): ion source gas (GS) 1 and 2: 35 and 45 psi; curtain gas (CUR): 40 psi; source temperature (TEM): 550 °C; ion spray voltage floating (ISVF): 5500 eV (-4500 eV), declustering potential (DP): 60 V (-100 V); ion release delay (IRD): 67 ms; ion release width (IRW): 25 ms. Full scan experiments (100–1200 Da) were performed with an accumulation time of 0.2 s in the high

sensitivity mode. Eight independent data acquisition (IDA) experiments were acquired for MS^2 spectra accumulation (accumulation time: 0.05 s). The fragmentation conditions were as follows: mass range: 30–1200 Da; CE: 40 eV (-40 eV), collision energy spread (CES): 15 eV (-15 eV). The mass spectrometer was automatically re-calibrated after four runs using an automated calibrant delivery system (CDS) via atmospheric pressure chemical ionization (APCI). Chromatographic separation by HPLC was performed on a Zorbax Eclipse Plus C18 column (Narrow Bore RR, 2.1 × 150 mm, 5 µm) with a Zorbax Eclipse XDB-C8 Guard column (2.1 × 12.5 mm, 5 µm), both obtained from Agilent. The eluent depends on the polarity of the measurement. For positive ionization a gradient elution with Milli-Q water (A) and methanol (B), both with 0.1% formic acid, was performed. Because of an insufficient ionization for some analytes in the positive ionization mode, the detection was performed as well in the negative ionization mode using a gradient elution with Milli-Q water (A2) and methanol (B2) without adding formic acid. The following solvent gradient with a flow rate of the mobile phase of 0.3 ml/min was applied for both measurements: 0–2 min, 30% B (B2); 2–4 min, 80% B (B2); 4–12 min, 98% B (B2); 12–16 min, 98% B (B2); 16–20 min, 30% B (B2); 20–25 min, 30% B (B2). The injection volume was 10 µl and the column temperature was set to 50 °C. MS data acquisition was controlled with Analyst 1.6.2 (SCIEX). For the quantification of target compounds (Table 4.1) an external 9-point calibration was performed ranging from 10 to 250,000 ng/l in methanol. The identity of each analyte in the samples was checked by comparing retention time, high resolution MS^1 and high resolution MS^2 fragmentation of the corresponding $[M+H]^+$ or $[M-H]^-$ against the respective authentic standard. The response area of $[M+H]^+$ respectively $[M-H]^-$ at MS^1 level was used for quantification. Data processing was performed by the software MultiQuantTM 3.0.2 (SCIEX). The samples were measured after dilution with methanol by a dilution factor of 1×10^{-2}, 1×10^{-4} or 2×10^{-6} to reach the linear calibration range.

4.2.4.2 Effect-directed analysis of bioactive fractions

The identification of compounds contributing to bacterial toxicity and estrogen-like effects of the extracts was performed by thin layer chromatography. The extracts were developed according to the proceeding in HPTLC coupled assays. Based on the previously detected bio-active zones, toxic fractions were marked on the surface of the HPTLC plate with a pencil. The silica layer was scraped off the glass at relevant positions with a scalpel and placed in a reaction tube. The obtained fractions were extracted with 500 µl ethanol (Optigrade, Promochem) by thoroughly mixing for 30 s. After the centrifugation of the suspensions for 1 min at 14,000 g, the supernatants were filtered (PTFE, 0.45 µm) and subjected to LC-MS analysis as described in section 2.4.1.

Table 4.1: Analytical parameters of target compounds. Quantification limits (LOQ) are given at the level of the lowest analyte concentration of the calibration. Ionization mode used for quantification is indicated by underlining the respective precursor ion mass.

substance	CAS	retention time [min]	theoretical mass (m/z)		LOQ [mg/l]
			$[M+H]^+$	$[M-H]^-$	
4-*tert*-butylphenol (4*t*BP) 	98-54-4	7.40	151.112	<u>149.097</u>	1.25
bisphenol A (BPA) 	80-05-7	6.85	229.122	<u>227.108</u>	0.63
4-cumylphenol (4CP) 	599-64-4	6.53	245.117	<u>243.102</u>	0.03
4-[1-(4-methoxyphenyl)-1-methylethyl]phenol (BPA-I11) 	16530-58-8	7.86	243.138	<u>241.123</u>	0.13
5-hydroxybisphenol A (BPA-I10) 	79371-66-7	8.03	213.127	<u>211.112</u>	0.63
bisphenol A diglycidyl ether 	1675-54-3	7.97	<u>341.175</u>	339.160	1.25
bisphenol A (2,3-dihydroxypropyl) glycidyl ether 	76002-91-0	7.19	<u>359.185</u>	357.171	1.25
bisphenol A bis(2,3-dihydroxypropyl) ether (Bis-HPPP) 	5581-32-8	6.67	<u>377.196</u>	375.181	0.13
bisphenol F 	620-92-8	6.43	201.091	<u>199.076</u>	0.63
2-phenylphenol (2PP) 	90-43-7	7.22	171.080	<u>169.066</u>	0.13
4-nonylphenol 	104-40-5	11.04	221.190	<u>219.175</u>	1.25

4.3 RESULTS

4.3.1 ALTERATION OF TOXICITY BY UV IRRADIATION

In order to investigate, if ecotoxicological effects of epoxide-based coatings are changed by UV-A irradiation, two different top coatings were examined with and without UV-aging for their bacterial toxicity and estrogenicity.

All tested leachates caused high toxicity to bacteria and induced estrogenic effects. Interestingly, the UV-A irradiation of the coatings resulted in a significant decrease of toxicological effects (see Figure 4.1). In total, 185 ± 19 ng/l EEQ were determined in leachates of coating A without UV aging, whereas the mean EEQ concentration was 152 ± 13 ng/l in the corresponding leachates of UV aged coatings. The leachates of coating B contained 50.6 ± 7.2 ng/l EEQ without UV aging and 31.4 ± 4.5 ng/l EEQ after UV-irradiation. In the bioluminescence inhibition assay, the EC50-values in terms of percentage of the sample in the assay of coating A and B without UV aging were 0.190 ± 0.002% and 0.820 ± 0.011%, respectively. The UV-A irradiated plates resulted in EC50-values of 0.260 ± 0.002% in case of coating A and 1.860 ± 0.031% in case of coating B.

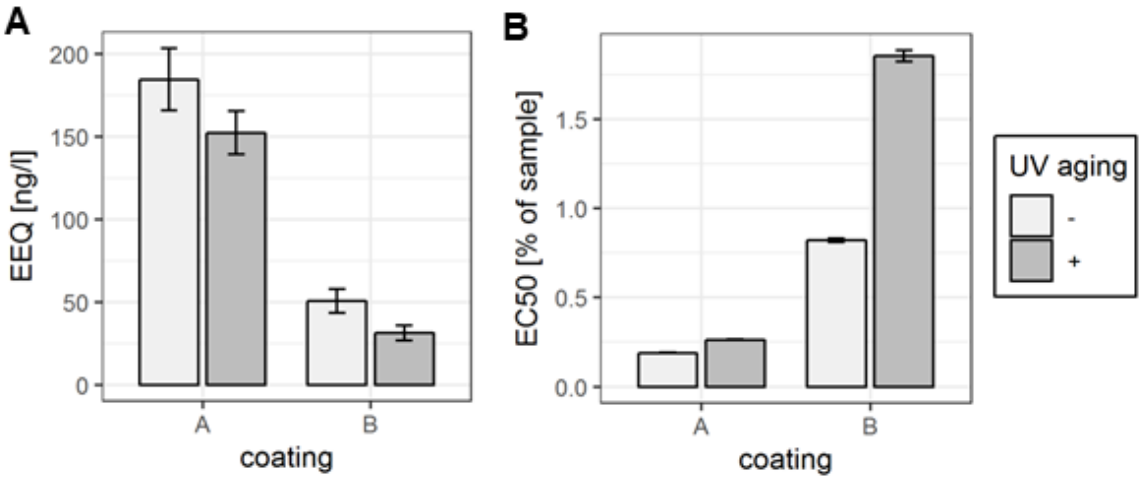


Figure 4.1: Toxic effects in leachates of untreated (-) and UV-A irradiated (+) coatings A and B (mean, n = 9, error bars indicate SE). Experiments were performed with concentrated samples. The presented results are calculated for the original aqueous leachates. Negative controls showed no effects. For the full dose-response data see Figure SI 4.1. *A*: Estrogenic activities detected as estradiol-equivalents (EEQ) with a recombinant yeast estrogen screen. *B*: Toxicity to luminescent bacteria is shown as EC50.

4.3.2 FORMATION OF TOXIC TRANSFORMATION PRODUCTS

Despite the observed reduced toxicity caused by the UV aging of coatings, it cannot be excluded that bioactive transformation products (TPs) are formed during the treatment and released into the environment. HPTLC coupled bioassays can assist in the identification of toxic compounds by the determination of toxic fractions in continuously separated samples. Therefore, leachates of untreated and UV-A irradiated coatings were investigated for differences in effect patterns by means of the HPTLC coupled luminescent bacteria assay as well as the planar Yeast Estrogen Screen (p-YES). The bioactive HPTLC fractions of respective standards and selected samples were subsequently analyzed by LC/MS. The phenols 4*t*BP and BPA were suspected targets to be present as bioactive

compounds due to their identification in previous studies investigating leachates of coatings for corrosion protection.

In all leachates of coatings without UV aging, bacterial toxicity was observed as only one, very intense signal at a Rf-value of 0.74 (see Figure 4.2A and Figure SI 4.2A). At the same height a signal with less intensity was detected in all leachates of coatings with UV aging. In case of the irradiated coating an additional inhibition zone occurred at a Rf-value of 0.32. All signals showed a dose dependent variation of intensity (see Figure SI 4.3). The same effect patterns were observed by the p-YES (see Figure 4.2B and Figure SI 4.2B) with the difference that minimal estrogenic activity already becomes visible at a Rf-value of 0.32 in leachates of coatings without UV aging. However, the increased signal intensity after UV-irradiation at Rf = 0.32 is clearly evident.

The Rf-values of observed effect signals matched well with the pattern of the suspected compounds 4*t*BP and BPA (not shown). The subsequent comparison of chromatograms revealed the presence 4*t*BP at a Rf-value of 0.74 and BPA at a Rf-value of 0.32 (Figure 4.3).

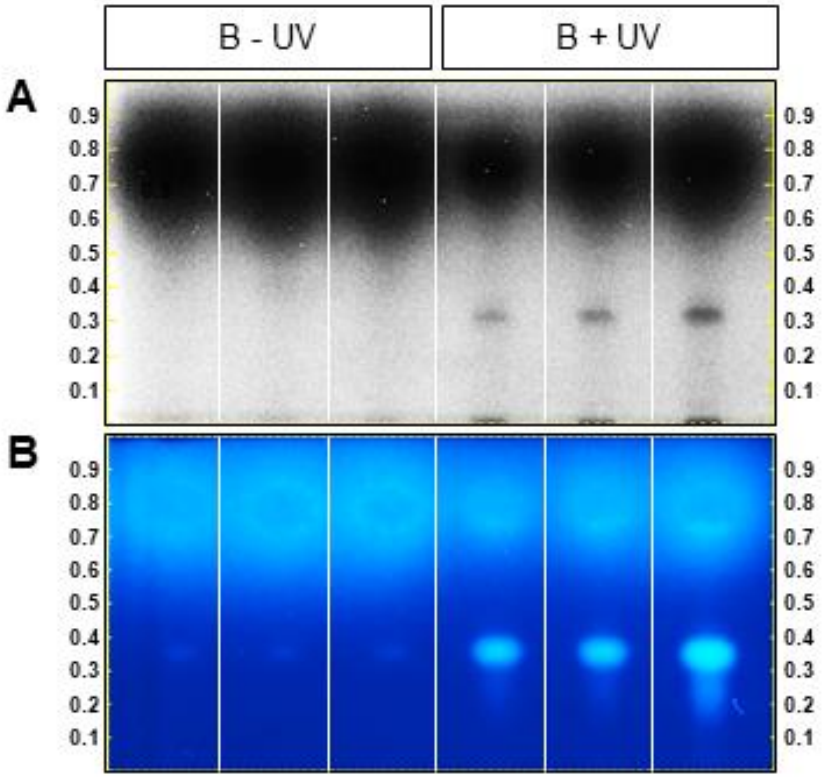


Figure 4.2: Toxicity in leachates of untreated (- UV) and UV-A irradiated (+ UV) coating B on HPTLC-plate. The ethanolic extracts (1000-fold concentrated) of all replicates were chromatographically developed with ethyl acetate / n-hexane (35:65). For a better visualization brightness and contrast were adjusted. *A*: Black and white image of luminescence signals after 11 min exposure of luminescent bacteria. The 1:10 diluted extracts were applied in a volume of 10 µl each. *B*: Fluorescence image of HPTLC coupled Yeast Estrogen Screen at an excitation wavelength of 366 nm. The 1:10 diluted extracts were applied in a volume of 5 µl each.

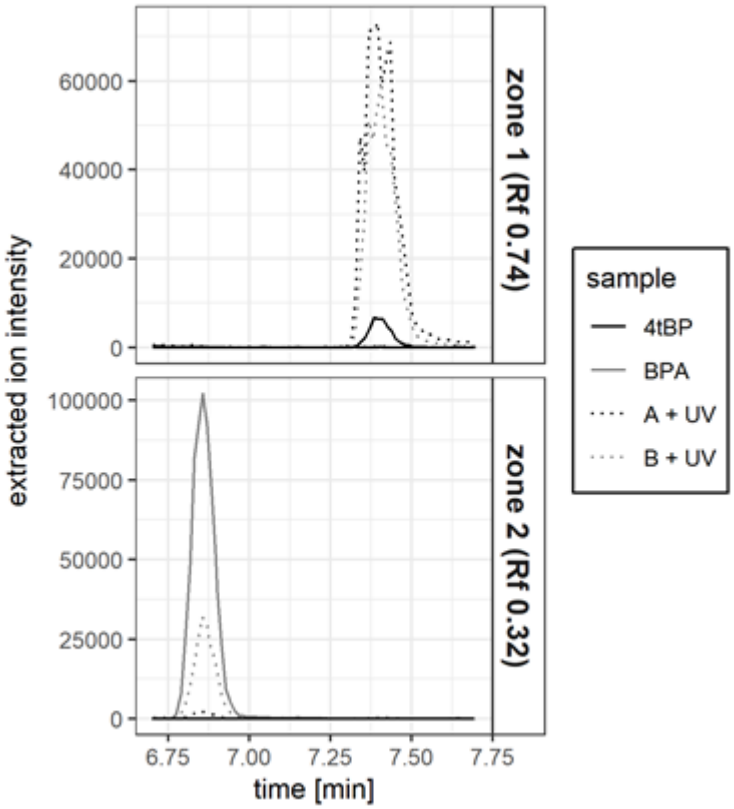


Figure 4.3: Extracted ion chromatograms of mass-to-charge ratio 227.108 (representing BPA) and 149.097 (representing 4*t*BP). Selected samples of UV-A irradiated coatings A and B as well as standard solutions of 4*t*BP and BPA were chromatographically developed on HPTLC plate with ethyl acetate and n-hexane (35:65). Toxic fractions were extracted and analyzed by LC-MS.

4.3.3 RELEASED SUBSTANCES

Following the initial identification, the samples were further analyzed for possible transformation products of BPA and other phenolic compounds by a targeted LC-tandem MS-approach. In addition to 4*t*BP and BPA, further four phenols, namely 4-cumylphenol (4CP), 4-[1-(4-methoxyphenyl)-1-methylethyl]phenol (BPA-I11), 5-hydroxybisphenol A (BPA-I10), bisphenol A bis(2,3-dihydroxypropyl) ether (Bis-HPPP), and 2-phenylphenol (2PP) were identified. These compounds were subsequently quantified by an external calibration (see Table 4.2). Three of these substances (4*t*BP, BPA and Bis-HPPP) were detected in all samples, further three (4CP, BPA-I11, BPA-I10) only in leachates of UV-irradiated coatings and one (2PP) just in leachates of untreated coating B. The contents of 4*t*BP and Bis-HPPP were significantly lower in leachates of UV-irradiated coatings compared to leachates of coating without UV treatment. The release of 4*t*BP was reduced by around 40% and 45% in leachates of coating A and B, respectively, leachates of UV-irradiated coating A showed a release of Bis-HPPP reduced on average by 72% compared to the untreated coating A. In contrast, in leachates of UV-irradiated coatings A and B the concentration of BPA increased about 6 and 19 times compared to leachates of coatings without UV-treatment.

Table 4.2: Quantified target compounds (n = 3, SE = standard error of the mean) in leachates of untreated (- UV) and UV-A irradiated (+ UV) coatings A and B. Compounds were measured after a 1000-fold enrichment, the results refer to the original aqueous samples under the assumption of a quantitative extraction of the compounds. Abbreviations of substances see Table 4.1. The measured concentrations in corresponding blank controls were below the quantification limit.

coating	4*t*BP [µg/l]		BPA [µg/l]		4CP [µg/l]		BPA-I11 [µg/l]		BPA-I10 [µg/l]		Bis-HPPP [µg/l]		2PP [µg/l]	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
A - UV	15000	1400	2.30	0.10	< LOQ		< LOQ		< LOQ		19.6	1.1	< LOQ	
A + UV	9400	2000	14.3	1.6	< LOQ		0.72	0.16	0.78	0.06	5.6	2.7	< LOQ	
B - UV	3320	110	0.90	0.01	< LOQ		< LOQ		< LOQ		1.04	0.40	0.67	0.23
B + UV	1840	410	16.9	3.7	0.23	0.17	1.37	0.48	1.20	0.23	0.95	0.20	< LOQ	

4.3.4 CHARACTERIZATION OF EFFECTS CAUSED BY IDENTIFIED COMPOUNDS

In addition to the quantification of the released substances, the individual compounds were characterized regarding to their toxic potency using the HPTLC-coupled and the microplate bioassays. On basis of the latter test approach, the relative contribution of individual substances to the bacterial toxicity and estrogen-like effects were estimated.

All the substances quantified in the leachates of the coatings were toxic to luminescent bacteria (Figure SI 4.5A). By far the highest toxicity was observed in the presence of 4*t*BP (EC50 = 14.0 ± 0.4 µg/l), which was also detected in the highest concentrations in the leachates. Therefore, the toxicity to luminescent bacteria was virtually completely driven by 4*t*BP and the contribution of the other quantified substances is negligible.

Six of the seven quantified substances showed estrogenic effects (Figure SI 4.5B). In this context, the most potent substance was 4CP (EC50 = 44.9 ± 5.9 µg/l) followed by BPA-I11 (EC50 = 161.3 ± 1.8 µg/l) and BPA (EC50 = 302 ± 24 µg/l). Although 4*t*BP induced the estrogenic effects less strong (EC50 = 6300 ± 220 µg/l), it could explain more than 99% of the estrogenicity of discovered compounds in leachates of untreated coatings (see Figure 4.4). Likewise, in leachates of UV irradiated coatings the estrogenic effects were driven by 4*t*BP, the mean contribution to estrogenicity of the quantified substances in leachates of coating A and B was 97% and 81%, respectively. On average, BPA explained 3% and 16% of the overall estrogenic potential caused by the identified compounds in samples of UV irradiated coatings A and B. In leachates of UV irradiated coating B, 2% and 1% of the estrogenicity can be assigned to BPA-I11 and 4CP, respectively. Although BPA-I10 and 2PP showed estrogenic potential in single testing, they did not contribute to the observed effects due to their low concentrations in the leachates. Bis-HPPP was not estrogenic far above the measured concentrations.

The investigation of single standards with the HPTLC coupled assays has shown that 4CP and 2PP are potentially contributing to the intense effect signals at Rf-value of 0.74 (Figure SI 4.4). Moreover, in appropriate concentrations the substances BPA-I11 and BPA-I10 become visible as additional signals at Rf-value of 0.62 and 0.05, respectively.

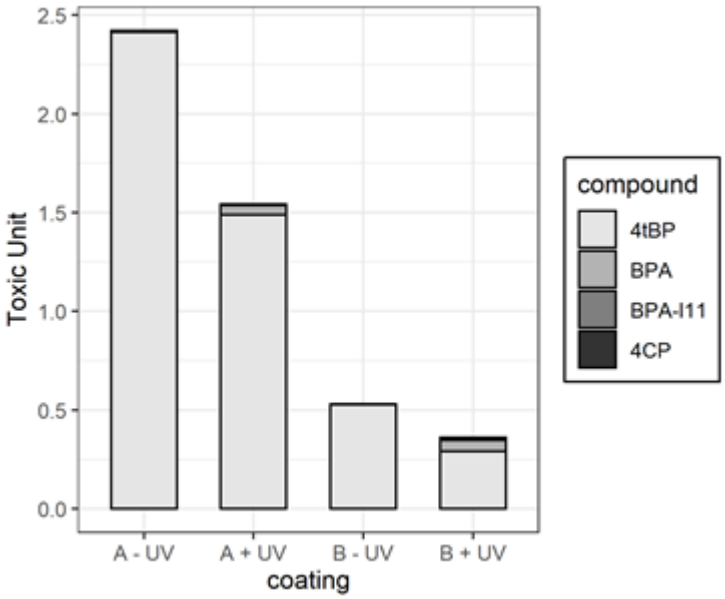


Figure 4.4: Toxic units (c/EC50) of main compounds contributing to estrogenic effects in leachates of untreated (- UV) and UV-A irradiated (+ UV) coatings A and B. The estimates of EC50 were generated by a five-parameter log-logistic function (equation 4.1). Bis-HPPP, BPA-I10 and 2PP had no significant contribution to the estrogenic potential caused by the identified compounds and are therefore not displayed.

4.4 Discussion

The investigation of two epoxy resin based top coatings revealed the potential release of hazardous substances into the environment along with elevated toxicity to luminescent bacteria and estrogen-like effects (see Table SI 4.1). Similar results were obtained in a study by Vermeirssen et al. (2017) who investigated leachates of anonymized epoxy resin based anti-corrosion coatings and detected up to 280 ng/l EEQ under worst case conditions. In contrast to the present observations, BPA and not 4*t*BP was identified as the main driver for the estrogenic effect in concentrations up to 10.4 mg/l BPA. In the luminescence inhibition assay, the lowest EC50 was determined by Vermeirssen et al. at a sample concentration of 0.4%. This finding is in the range of the results reported in the current study.

The observed decrease of ecotoxicological effects in leachates of UV-A irradiated coatings can be associated with the significant decrease of 4*t*BP concentrations. A volatilization of 4*t*BP during the fabrication of test plates could be responsible for this reduction. The treatment with UV-A radiation leads to increased temperatures of the investigated coating materials and thus an elevated evaporation of 4*t*BP is likely. Secondly, a possible degradation of 4*t*BP to less or non-toxic substances might explain reduced concentrations of 4*t*BP and lower toxic effects. Previous studies identified 4-(2-methyl-2-propanyl)-2-[4-(2-methyl-2-propanyl)phenoxy]phenol, 4,4'-di-*tert*-butyl-o,o'-biphenol, 4-*tert*-butylcatechol and 2-*tert*-butylphenol as TPs of 4*t*BP that can be generated by UV photolysis (Cirkva et al. 2005, Wu et al. 2016). To the best of our knowledge, for the two first mentioned TPs no analytical standards are available and ecotoxicological effects are not documented so far. In contrast, the 4*t*BP-degradation product 4-*tert*-butylcatechol is categorized as very toxic to aquatic life and the European Chemicals Agency (ECHA) proposes a predicted no effect concentration (PNEC) of 1.2 µg/l for freshwater. For 2-*tert*-butylphenol various endocrine effects were documented. For example, Tollefsen and Nilsen (2008) determined the binding affinity of 2-*tert*-butylphenol to the hepatic estrogen receptor from rainbow trout. Li et al. (2010) demonstrated antagonistic effects to the

androgen receptor and gamma inverse agonistic effects to an estrogen-related receptor using a set of recombined yeast strains.

In contrast to the reduced release of 4*t*BP, the release of BPA from both coatings was increased by the UV-A irradiation. BPA is the basis of the most common epoxy resins as it is a major compound for the production of the resin monomer, bisphenol A diglycidyl ether (BADGE). During the curing of coating components, the monomers are crosslinked by polyaddition reactions with polyfunctional amines added as hardener. Earlier studies demonstrated that the photooxidative change of epoxy polymers can be caused by various mechanisms. The UV radiation of epoxy resins e.g. can lead to a chain scission of C—N bonds and ring opening reactions of oxirane groups (Brand et al. 2020, Kim and Urban 2000, Liu et al. 2014). This indicates that respective parts of the polymeric matrix were degraded and/or released into the environment. BPA is one of the world's most important and commonly produced industrial chemicals. It primarily serves as intermediate in the production of polycarbonate and epoxy resins. Furthermore, it is used as additive e.g. in the manufacture of thermal paper, tires and flame retardants. Due to its widespread use, BPA has been found in waters since the late 1990s and is now detectable in almost all environmental compartments (Corrales et al. 2015, Michalowicz 2014). In particular, the input of BPA into the environment can be related to traffic and the release from construction materials. Lamprea et al. (2018) investigated a representative selection of building materials and automotive supplies and identified lacquered car bodyworks as important source of BPA contamination in urban runoff with a tested water emission of up to 360 ng/cm². On this basis, it can be assumed that the emission of BPA from weathered coatings plays a significant role in the overall exposure. However, investigations on a larger scale or, ideally, a substance flow analysis for BPA would be necessary for a robust estimation of the contribution to the overall pollution. In a European Union Risk Assessment Report dating from 2010, monitoring data for BPA in European water bodies were published; a mean concentration of 0.13 µg/l for freshwaters and 60 ng/g dry weight for freshwater sediments was reported (EC 2010). BPA is recognized as endocrine disruptor, can have adverse effects on the immune system and liver, promotes mutagenesis and carcinogenesis and is supposed to mediate neurotoxic and teratogenic effects (Michalowicz 2014). The ECHA proposes a PNEC of 18 µg/l in freshwaters and 1.2 µg/g dry weight in freshwater sediments.

Besides BPA, four related compounds could be identified via LC-MS analyses. Three of them (BPA-I10, 4CP and BPA-I11) were only detected in leachates of UV-irradiated coatings and were therefore assumed to be photolytic degradation products of BPA or the BPA based polymer. In aqueous solution, the degradation reaction of UV irradiated BPA followed a pseudo-first-order kinetic with a half-life time about 6 h (Kovacic et al. 2019). The light sensitivity of BPA and the formation of a variety of derivates by photochemical processes were already reported in former studies (Cardoso da Silva et al. 2014, Im and Loffler 2016, Kondrakov et al. 2014, Molkenthin et al. 2013). In this context, BPA-I10 was formerly identified as photo-transformation product of BPA. So far, to the best of our knowledge, the occurrence of BPA-I10 in environmental samples was not reported. The competitive binding of BPA-I10 to ERα was reported with a half maximal inhibition concentration (IC50) of 50 µM and at the same time this transformation product was identified as slightly less potent than BPA (IC50 = 10 µM) (Nakagawa and Suzuki 2001). In toxicity testing with MCF-7 and NIH3T3 cells, BPA-I10 exhibited estrogen-like and anti-androgenic effects with an EC50 of 1.8 µM and 14 µM, respectively (Kitamura et al. 2005). The present results of the yeast estrogen screen (EC50 = 7.5 mg/l or 31 µM) lie in the range of the previous reports. Moreover, Mutou et al. (2006) demonstrated a cytotoxicity of BPA-I10 on Jurkat cells in concentrations from 5 to 50 µM (1.2 to 12 mg/l). This amounts to the same order of magnitude as the present results of acute bacterial toxicity.

4CP is known as impurity in industrial grade BPA (Terasaki et al. 2004) and is used as an intermediate for the production of phenolic resins, insecticides and lubricants. In Polish surface waters, concentrations up to 6.2 ng/l were detected (Czarczynska-Goslinska et al. 2017). Further monitoring data for 4CP is mainly available for Asia ranging from about 2 ng/l in river estuaries around Dianchi Lake in China (Wang et al. 2013) up to 160 ng/l in river water of Nagoya city in Japan (Hasegawa et al. 2016). Moreover, investigations at the Panlong River in China revealed sediment concentrations up to 4.8 ng/g and a bioconcentration factor (BCF) of up to 10 by analyzing muscle, liver and gill tissue of wild fish (Wang et al. 2016). In one of six muscle samples of freshwater prawn from local supermarkets in USA, 4CP could also be detected with 1.96 ng/g wet weight (Zuo and Zhu 2014). Perez-Albaladejo et al. (2019) reported that 4CP induced cytotoxic effects (EC50 = 65 µM), led to the generation of reactive oxygen species (2–3-fold at 50 µM) and increased the P450 aromatase activity (1.3-fold at 20 µM) in human placental JEG-3 cells. In a yeast two-hybrid assay, 4CP showed 12 times higher estrogenic effects than BPA (Terasaki et al. 2005). This observation is consistent with the present data revealing a nearly 7 times lower EC50 of 4CP compared to BPA. Furthermore, a recent study reported that low concentrations of 4CP and BPA induce the proliferation of MCF-7 cells synergistically (Wang et al. 2020). Investigations of Rosenmai et al. (2014) indicate that 4CP act by several modes of action within the endocrine system. For example, the activation of estrogen receptor (EC50 = 0.10 µM) and the inhibition of the androgen receptor (EC50 = 5.1 µM) was determined by reporter gene assays. Furthermore, agonistic effects on the retinoic acid (RA) receptor (1.68 µM 4CP showed 20% of the activity of 0.1 µM all-trans RA) were shown (Kamata et al. 2008). A PNEC of 14.2 µg/l and 3.58 µg/g dry weight is proposed by the ECHA for freshwater and freshwater sediments.

BPA-I11 was previously mentioned as product of microbial transformation (McCormick et al. 2011) and of photodegradation catalyzed by nano TiO_2 (Jia et al. 2012). Information on the occurrence of BPA-I11 in environmental samples is very limited. Ashfaq et al. (2018) determined an average concentration of 2.75 ng/l in Jiulong River, China. Investigations on the competitive binding to ERα as well as the gene induction and cell proliferation with MCF-7 cells exhibited BPA-I11 as less estrogenic than BPA (Coleman et al. 2003). For instance, the relative effect on proliferation in response to BPA-I11 and BPA differed by one order of magnitude (2.63×10^{-4} vs. 2.00×10^{-3}, EC50 relative to estradiol). The opposite trend was observed during the present study, as compared by EC50 the effected estrogenicity of BPA-I11 was approximately twice as high as the effect induced by BPA. Furthermore, BPA-I11 was found to be responsible for the disruption of mitosis and cytokinesis in HeLa cells by inducing the formation of ectopic spindle poles (George et al. 2008). Even if the correlation of aneuploidy and tumorigenesis is not fully understood, this result indicates that BPA-I11 can exhibit carcinogenic effects. McCormick et al. (2011) reported a half maximal lethal concentration of up to 0.66 mg/l BPA-I11 to zebrafish embryos and thus an almost eight times higher toxicity as BPA.

The present study indicates that the UV aging of epoxy coatings could contribute to the emission of BPA and its transformation products into the environment. Due to the potentially incomplete enrichment of hazardous substances by SPE, the actual toxicity might even be underestimated. If a release may lead to locally elevated concentrations and pose an environmental risk, needs to be further investigated in field and laboratory studies. So far, known environmental concentrations are all below the proposed PNECs. However, the information on the environmental fate and effects of the TPs is very limited and PNECs were defined only for individual substances. As two of the identified photolytic products, i.e. 4CP and BPA-I11, are found to be more potent in the YES and the luminescent bacteria assay than BPA itself, they could be of more serious concern especially if continuously released and/or accumulated. The present study investigated changes in toxicity caused by

approximately three months of real time exposure to sunlight. To explore the underlying degradation and release kinetics and to predict the total environmental impact of photodegradation products, the individual protection period of coatings (up to 25 years) has to be considered. For a more complete picture also further weathering parameters (e.g. temperature, humidity and precipitation) and coating materials should be investigated.

Compared to other materials such as polyurethanes, acrylics, polyesters or alkyds, epoxy resins are well known for their susceptibility to UV degradation (Knudsen and Forsgren 2017). The absorption of UV radiation by aromatic moieties can lead to the breakdown of the polymeric structure along with the discoloration and chalking of the coating layer (Ghasemi-Kahrizsangi et al. 2015). To compensate this disadvantage, epoxy resin-based coatings are frequently covered by top coatings based on polyurethane. Furthermore, the UV resistance of polymers can be improved by different additives as light screens, UV absorbers, radical scavengers or quenchers (Hawkins 1984). Recent studies demonstrated that the UV resistance of epoxy coatings can for example be enhanced by polyaniline nanowires (Gao et al. 2021) or TiO_2 nanoparticles blended with poly-dimethylamino siloxane (Fadl et al. 2020). To extend the service life of steel coatings and to reduce their emissions in outdoor exposure to solar radiation, it is advisable to adjust the recipe of UV sensitive coatings accordingly or to use top layers with more photostable binding agents.

4.5 Conclusion

- The susceptibility of epoxy resin-based coatings to UV degradation was demonstrated by the alteration of acute and specific ecotoxicological effects and the release of toxic transformation products.
- Both, in leachates of untreated and UV-A irradiated coatings, 4*t*BP was identified as the main driver of estrogenicity and toxicity to luminescent bacteria. Additionally, BPA and close structural analogs contribute to estrogenic effects in leachates of UV-A irradiated materials.
- The combination of HPTLC coupled bioassays and LC-MS analyses supported the identification of bioactive compounds in terms of an effect-directed analysis.
- Due to their lower UV-stability compared to e.g. polyurethanes, epoxy resin-based coatings are less suitable for the application as top coatings.

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Chapter 5

FINAL CONCLUSION

The primary aim of the present thesis was to examine the individual ecotoxicological impact of emissions from selected construction products on the aquatic environment. Galvanic aluminum anodes and organic coatings used for corrosion protection were chosen as subjects of study owing to their high relevance for the transport infrastructure with the concurrent lack of necessary assessment procedures. To enhance the resistance and the environmental compatibility of respective buildings and infrastructure, the results of the experimental studies ought to contribute to the overarching objective of the associated research project - the development of simplified and standardized investigation and assessment procedures for construction materials.

The investigated aluminum anode only weakly but significantly inhibited algal growth in the worst-case exposure scenario. This acute effect could be fully ascribed to the main component aluminum. Beyond that, the anode material caused no acute toxicity, neither to the tested bacteria nor the sediment-dwelling amphipods. However, the content of aluminum and indium was elevated in the crustacea species after the exposition. This enrichment did not show a linear relationship to the applied test concentration but reflects the metal composition of the galvanic anode. Consequently, it can be assumed that the test organism does not have specific uptake mechanisms for any of the observed metals. Overall, the findings of this study do not indicate a direct environmental threat by the use of galvanic anodes for the cathodic protection of wind turbine support structures in the marine environment. However, the accumulation of metals in e.g. crustaceans might enhance their trophic transfer within the marine food web. In contrast, most of the investigated organic coating systems caused notable ecotoxicological effects. Only one top coating based on polyurethane showed no significant effects in any of the bioassays applied, whereas all epoxy resin based coating systems induced estrogen-like effects and acute toxicity to luminescent bacteria. Furthermore, the investigations revealed that the curing agent 4-*tert*-butylphenol acted as a main contributor to the observed adverse effects and was continuously released to the leaching medium. However, its total content in the polymerized product was not a robust indicator for the prediction of ecotoxic effects as the final amount released by the coating strongly depended on the respective formulation and the resulting release mechanism. In any case, 4-*tert*-butylphenol exceeded the predicted no effect concentration for freshwater in the laboratory experiments and potentially pose a risk to aquatic organisms when used in organic coating systems under environmental conditions. On the basis of the present findings, the tested epoxy coating systems would not pass the proposed assessment criteria of the DIBt as well and consequently would not be classified as environmentally compatible. The UV aging of epoxy coatings led to an overall decrease of estrogenic and bacterial-toxic effects and was associated with the significant decrease of 4-*tert*-butylphenol concentrations in the leachates. However, several transformation products were formed during the photolytic degradation of the coating material and contributed to the observed effects. In this context, especially bisphenol A as basis of most common epoxy resins but also structural analogs were identified in leachates of irradiated materials. As two of the identified photolytic products are found to be more potent than bisphenol A itself, they could be of more serious concern especially if continuously released to the aquatic environment and/or accumulated in biota.

The findings of this thesis lay the basis for a systematic assessment of the environmental compatibility of corrosion protection products and generally contribute to the development of standardized investigation methods for construction products. The adjustment of the investigation concept proposed for construction products as well as the adaption of single standard methods for the leaching of materials and the effect detection in leachates enabled the individual assessment of the ecotoxicological impact of galvanic aluminum anodes and organic coatings. The selected proceedings provided reproducible results and allowed the comparative evaluation of the environmental compatibility of products. This offers the opportunity for an easy selection of environmentally compatible

alternatives right from the planning stage of (public) construction projects without compromising on the intended function of a material. In this way, preference could be given to the most harmless product, or products emitting dangerous substances could be even fully excluded from use. Besides an objective decision basis, the results of ecotoxicological testing can offer information for planners and end users on the possible environmental risks posed by respective materials, e.g. when the application of the product in water protection or nature conservation areas is intended. Furthermore, the identification of hazardous ingredients and emitting substances can help to improve the individual product formulations. Firstly, where possible, toxic compounds could be replaced by harmless alternatives and secondly, their release could be prevented by other modifications of the composition. For example, polymeric materials can be protected against photodegradation by the addition of appropriate UV stabilizers. Furthermore, the formulation of the compounds might be optimized to achieve a more quantitative polymerization and thus lower amounts of monomeric compounds that are more likely to be released into the environment.

Beside suitable investigation methods, the verification of the environmental compatibility of construction products as a basic requirement according to the CPR necessitates valid assessment criteria. The DIBt proposed a concept for the classification of ecotoxicological effects related to construction works. However, legally binding limit values for emissions from construction products were not defined so far and the presence of harmonized European standards exclude any additional requirements on national level. The prediction of individual environmental concentrations based on the results from release experiments and the subsequent comparison with limit values for substances regulated by environmental legislation is one possible evaluation approach. This in turn would require the development of reliable models and transfer functions for different exposure scenarios. Irrespective of obligatory demands, a consistent evaluation of construction products is also possible by voluntary ecolabels. The most relevant logos for the labelling of low-emission building products include the Blue Angel, Natureplus and Emicode that are awarded to defined product groups under the fulfillment of various requirements and criteria. For the selection of environmentally friendly products in the infrastructure sector by ecolabels, corresponding requirements would still have to be defined for the most product categories. By requesting performance data on the ecotoxicity in this context, even more specific demands for the environmental compatibility of products could be made. A new emission-based assessment concept was recently implemented in the certification of ecologically beneficial construction products in Switzerland and could be considered as a blue-print for the product-specific evaluation in Europe. In contrast to ecolabels, environmental product declarations (EPDs) do not indicate the overall environmental preference of a product, but provide a neutral summary of environmental information basing on a life cycle assessment. For that more holistic view on the environmental impact, aspects like resource consumption, carbon emissions, contribution to the greenhouse effect or potential for eutrophication and acidification of water bodies are requested over all stages of product life. So far, the ecotoxicological impact of emissions from construction products is not taken into account in this concept, but could be a useful addition for a comprehensive product evaluation. For example, EPDs of construction products are utilized by the German Federal Institute for Research on Building, Urban Affairs and Spatial Development [Bundesinstitut für Bau-, Stadt- und Raumforschung, BBSR] for the certification of sustainable federal buildings circumventing the evaluation of single products.

While several important findings were gained by the present thesis, some questions remain unanswered and further research needs emerged. So far, the impact on organisms of higher trophic levels such as daphnids or fish and the observation of chronic endpoints were not part of the experimental studies. To develop a broader picture of the environmental impact of emissions from construction products, future investigations should be extended to the aforementioned areas. Furthermore, the

determination of biodegradability of organic constituents could be a useful addition to estimate the persistence of toxic compounds and the durability of the associated effects. The need for the identification of further bioactive ingredients and transformation products could be satisfied by the means of an effect directed analysis. The combination of HPTLC-coupled bioassays with chemical analyses has proven to be a promising approach and should be pursued further. The knowledge on the environmental fate and effects of single substances identified is very limited. To determine the actual environmental relevance of emissions and to transfer the results from the laboratory experiments to the field, the released compounds need to be monitored in the environment. Even though a harmonization of investigation methods is preferable for all construction products, individual adjustments are likely to be expedient, especially for polymeric and metallic materials. To derive further, necessary adaptions of the investigation strategy and to verify if recommendations made are applicable to similar product categories, a systematic assessment of all approved construction materials is advisable.

The detection of adverse effects without the necessary identification of causative substances, is the major strength of ecotoxicological methods. Especially the assessment of polymeric materials and construction products of unknown composition could benefit from this advantage. As shown by this thesis, in combination with chemical analysis, bioassays can be an important tool for the identification of potential toxic emissions from construction products. Therefore, the implementation of suitable ecotoxicological methods in a (legally binding) assessment concept is highly recommended. A current activity of the Network of Experts focuses on the development of investigation methods assessing the environmental compatibility of further relevant materials such as concrete, geotextiles and inflatable rubber dams. In addition, a survey is being prepared applying the developed examination concept to all approved anti-corrosion coating systems. This work builds on the present findings and is intended to contribute to the further characterization of emissions associated with buildings and infrastructure as well as to encourage the acceptance for the use of ecotoxicological methods evaluating the environmental compatibility of construction products.

Appendix

SUPPLEMENTARY DATA CHAPTER 2

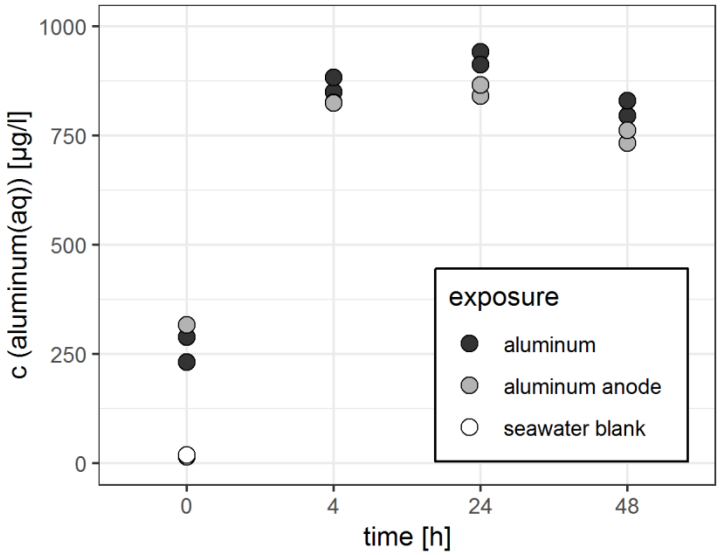

Fig SI 2.1: Maximum soluble Al concentration in natural seawater. Neutralized aluminum standard and aluminum anode stock solutions were added to natural seawater (ratio 1 to 1000) and the dissolved aluminum concentration was measured via ICP-MS immediately and after stirring for 4, 24 and 48 hours. Each experiment was repeated twice.

Table SI 2.1: Maximum soluble Al concentration in natural seawater. The experimental details are described in Fig SI 2.1 above.

	c (Al) [µg/l]					
exposure	seawater blank		aluminum standard		aluminum anode	
t [h]	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
0	15	18	232	288	317	317
4			851	883	826	825
24			942	912	840	866
48			796	830	733	763

Table SI 2.2: Physicochemical properties of sediment and seawater for exposure and control experiments (n.a. = data not available)

	sample 1	sample 2	sample 3	sample 4
	sediment			
elementary analysis	c [%]			
TOC	0.36	0.62	0.25	0.30
IC	0.11	0.17	0.15	0.02
C	0.47	0.79	0.40	0.31
N	< 0.2	0.14	< 0.1	<0.1
S	0.10	0.20	0.11	0.10
grain size [µm]	c [%]			
> 2000	n.a.	0.0	0.0	0.0
630–2000	n.a.	0.1	0.0	1.7
200–630	n.a.	45.5	9.5	50.8
63–200	n.a.	35.7	81.1	35.5
20–63	n.a.	2.4	3.2	0.5
< 20	n.a.	13.7	4.5	3.4
metal analysis	c [mg/kg] in fraction < 20 µm			
Al	48900	50900	55900	60500
As	27	29	19	40
Cd	0.5	0.5	0.4	0.3
Cr	82	75	101	100
Cu	21	40	33	55
Ni	39	73	53	52
Pb	42	42	59	64
Zn	150	152	173	221
Hg	0.18	0.18	0.24	0.19
pH	7.54	7.61	n.a.	n.a.
	seawater			
NH_4-N [mg/l]	< 0.015	< 0.015	< 0.015	< 0.015
NO_3-N [mg/l]	< 0.23	< 0.23	< 0.23	< 0.23
c (Al) [mg/l]	< 0.02	< 0.02	< 0.02	< 0.02
conductivity [mS/cm]	45.4	35.1	39.4	39.3
pH	7.73	7.85	8.09	8.00

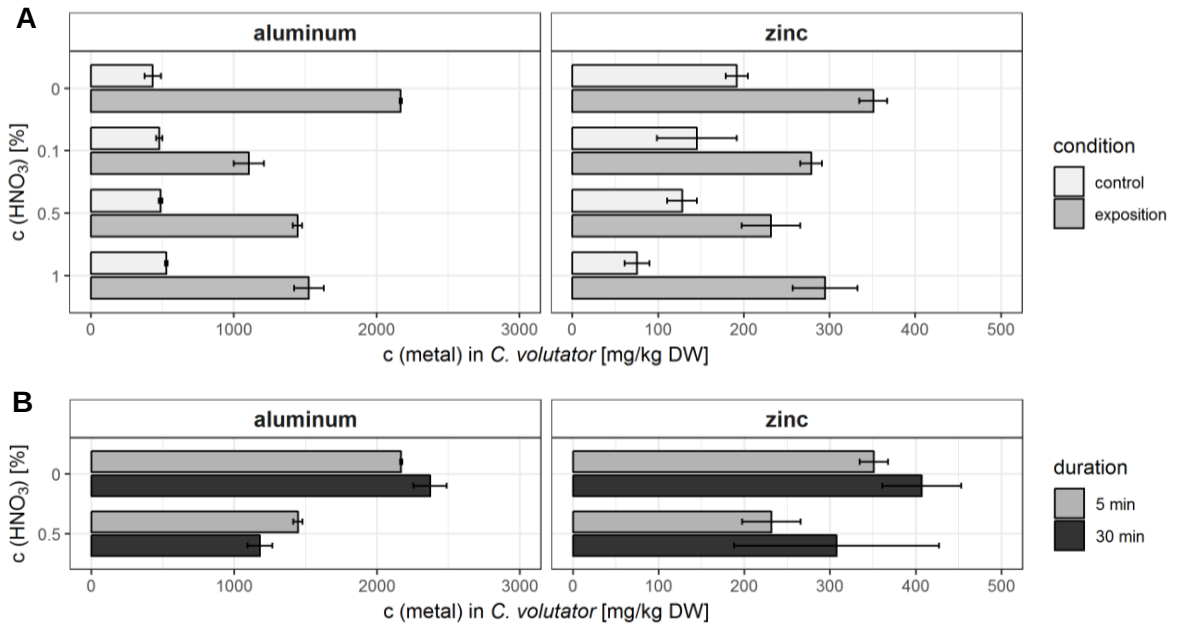

Fig SI 2.2: Average Al and Zn content (±SE) of *Corophium volutator* in a wash experiment to investigate the removal of adherent metal precipitates from the exoskeleton. Test organisms were simultaneously exposed to 100 mg/l Al and 10 mg/l Zn in natural seawater for three days. Afterwards each wash treatment was applied to 20 organisms (washing with demineralized water, 0.1 %, 0.5 % or 1 % HNO_3 under shaking at 250 rpm for 5 or 30 min). The same procedure was exerted to mud shrimps held in uncontaminated seawater as control. All samples were subjected to aqua regia digestion (n = 2) and analyzed via ICP-MS. *A:* Control and exposure experiments are compared by acid content of washing solution. *B:* Exposure experiments are compared by wash duration. Results of exerted control approaches are not presented but showed similar metal content as displayed in *A* (see table below).

Table SI2.3: Al and Zn content based on dry weight (DW) of *Corophium volutator* in a wash experiment to investigate the removal of adherent metal precipitates from exoskeleton (c = control, e = exposure). The experimental details are described in Fig 2 above.

HNO_3 [%]	0		0		0.1		0.5		0.5		1	
duration [min]	5		30		5		5		30		5	
condition	c	e	c	e	c	e	c	e	c	e	c	e
replicate	c (Al) [mg/kg DW] in *C. volutator*											
1	2162	376	2254	407	1002	458	1415	475	1269	563	1423	521
2	2176	492	2489	540	1211	501	1478	500	1095	577	1631	540
mean	2169	434	2371	474	1107	480	1447	488	1182	570	1527	530
replicate	c (Zn) [mg/kg DW] in *C. volutator*											
1	367	179	361	294	266	99	266	111	427	82	257	90
2	334	205	453	184	291	192	197	145	188	60	332	61
mean	351	192	407	239	278	145	231	128	308	71	295	75

Table SI 2.4: Microwave (turboWave) parameters for the digestion of freeze dried, ground and homogenized amphipods

step duration [min]	vessel temperature [°C]	max. power [W]	max. jacket temperature [°C]	max. pressure [bar]
5	80	500	60	80
8.0	160	850	60	100
4.0	220	1200	60	120
9.0	220	1200	60	120

Table SI 2.5: Average luminescence inhibition of the bacterium *Aliivibrio fischeri* in limit testing of dissolved aluminum anode and aluminum standard (maximal soluble Al concentrations in seawater under natural pH conditions)

	luminescence inhibition [%]	
replicate	aluminum anode	aluminum standard
1	-1.6	-3.0
2	-1.7	-2.9
3	-8.5	-9.3
mean	-3.9	-5.1
SE	2.3	2.1

Table SI 2.6: Average growth rate and growth inhibition of marine algae *Phaedactylum tricornutum* in limit testing of dissolved aluminum anode and aluminum standard (maximal soluble Al concentrations in seawater under natural pH conditions). The statistical results of two-tailed unpaired t-test are presented for control versus respective exposure experiments.

	growth rate [day^{-1}]			growth inhibition [%]	
replicate	control	aluminum anode	aluminum standard	aluminum anode	aluminum standard
1	1.20	0.99	0.94	17.4	21.9
2	1.08	0.78	0.81	28.2	25.2
3	1.14	0.69	0.79	39.3	30.9
mean	1.14	0.82	0.85	28.3	26.0
SE	0.04	0.09	0.05	6.3	2.6
t	-	3.35	5.0734		
df	-	4	4		
p	-	0.02857	0.007113		

Table SI 2.7: Maximum soluble and in algal growth inhibition test exposed Al concentrations in natural seawater. The contents were quantified by ICP-MS after Al equilibration (maximum), at the beginning (0 h) and the end of exposure (72 h); limit of quantification (LOQ) = 0.18 mg/l Al.

	measured Al concentration [mg/l]					
replicate	maximum		0 h		72 h	
	anode	aluminum	anode	aluminum	anode	aluminum
1	1.115	1.359	0.889	1.046	0.810	0.976
2	1.015	1.006	0.893	0.833	0.856	0.977
3	0.820	0.961	0.690	0.820	0.830	0.954
4					0.903	0.850
5					0.818	0.828
6					0.842	0.838
7					0.814	0.816
8					0.742	0.805
9					0.733	0.821
mean	0.983	1.108	0.824	0.899	0.817	0.874
SE	0.087	0.126	0.067	0.073	0.018	0.024

Table SI 2.8: Average growth inhibition of marine algae *Phaedactylum tricornutum* in dilution series of Zn in natural seawater (nominal test concentration 10 to 1 mg/l)

	growth inhibition [%]						
c (Zn^{2+}) [mg/l]	10	8.5	7	5.5	4	2.5	1
replicate							
1	80.3	72.5	49.7	30.4	19.5	8.0	0.4
2	93.0	83.7	51.2	35.4	26.8	13.4	11.6
3	83.1	79.1	62.2	44.9	35.6	20.1	7.1
mean	85.5	78.4	54.4	36.9	27.3	13.8	6.4
SE	3.8	3.3	3.9	4.3	4.7	3.5	3.2

Table SI 2.9: Nominal and determined Zn concentrations in natural seawater exposed in algal growth inhibition test. The contents were quantified by ICP-MS at the beginning (0 h) and the end of exposure (72 h); LOQ = 0.11 mg/l Zn.

	c (Zn^{2+}) $_{nominal}$ [mg/l]	10	8.5	7	5.5	4	2.5	1
		c (Zn^{2+})$_{measured}$ [mg/l]						
0 h	replicate							
	1	8.613	7.290	6.271	4.732	3.328	2.462	0.836
	2	8.754	7.373	6.125	4.835	3.421	2.098	0.849
	3	8.948	7.494	6.131	4.745	3.289	2.121	1.090
	mean	8.772	7.386	6.176	4.771	3.346	2.23	0.925
	SE	0.097	0.059	0.048	0.032	0.039	0.12	0.083
72 h	replicate							
	1	8.005	6.656	5.945	4.737	3.286	2.018	1.061
	2	7.407	6.509	5.795	4.728	3.202	2.031	0.830
	3	7.477	5.268	5.854	4.628	3.254	2.074	0.830
	4	7.751	7.043	6.211	4.762	3.336	2.079	0.834
	5	7.798	6.807	5.903	4.772	3.409	2.092	0.868
	6	7.673	5.788	5.945	5.128	3.430	2.067	0.893
	7	7.537	7.131	6.521	4.915	3.410	2.169	0.896
	8	7.833	7.082	6.088	4.696	4.585	2.175	0.908
	9	7.826	7.092	6.093	4.704	3.416	2.117	0.879
	mean	7.701	6.60	6.039	4.786	3.48	2.091	0.889
	SE	0.065	0.22	0.074	0.050	0.14	0.018	0.024

Table SI 2.10: Mortality of *Corophium volutator* caused by the dissolved galvanic anode and its main components aluminum and zinc. Test organisms were exposed to the different metal concentrations for ten days with (w/) sediment and three days without (w/o) sediment.

	mortality [%]																			
	control		aluminum anode						aluminum standard						zinc standard					
c (metal) [mg/kg H_2O]	0		1		10		100		1		10		100		0.1		1		10	
sediment	w/	w/o	w/	w/o	w/	w/o	w/	w/o	w/	w/o	w/	w/o	w/	w/o	w/	w/o	w/	w/o	w/	w/o
replicate																				
1	15	5	5	0	15	15	10	5	0	5	5	0	0	35	20	10	10	15	60	45
2	10	0	15	20	25	0	5	15	10	10	0	5	0	10	5	0	0	15	45	30
3	10	10	5	0	0	0	5	5	0	15	0	15	5	15	5	0	10	0		20
4	10	10	0	10	5	0	10	5	0	20	5	10	0	5	0	0	5	0		25
5	10	5				0		10				15		20				5		
6	0	5				0		10				0		20				20		
7	5	0																		
8	5	0																		
9	0	0																		
10	5	15																		
11	0	15																		
12	0	5																		
13		5																		
14		20																		
15		0																		
16		10																		
17		10																		
mean	5.8	6.8	6.3	7.5	11.3	2.5	7.5	8.3	2.5	12.5	2.5	7.5	1.3	17.5	7.5	2.5	6.3	9.2	52.5	30.0
SE	1.5	1.5	3.1	4.8	5.5	2.5	1.4	1.7	2.5	3.2	1.4	2.8	1.3	4.2	4.3	2.5	2.4	3.5	7.5	5.4

Table SI 2.11: Results of statistical analyses of bioassay data (n.a. = not available/applicable)

	c (metal) [mg/kg H_2O]	sediment	Shapiro-Wilk's test		F-test				t-test and Welch's t-test				Mann-Whitney U test	
			W	p	F	num df	denom df	p	t	df	p	power	W	p
control	0	w/	0.87042	0.06615	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		w/o	0.89229	0.05055	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
aluminum anode	1	w/	0.89495	0.4064	0.66986	11	3	0.5411	-0.1333	14	0.8959	n.a.	n.a.	n.a.
		w/o	0.86337	0.2725	0.40709	16	3	0.201	-0.1953	19	0.8472	n.a.	n.a.	n.a.
	10	w/	0.96307	0.7982	0.21572	11	3	0.04982	-0.9438	3.4421	0.4067	n.a.	n.a.	n.a.
		w/o	0.49609	2.07e-05	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	74.5	0.08991
	100	w/	0.72863	0.02386	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	19	0.5661
		w/o	0.82162	0.09114	2.239	16	5	0.3809	-0.5803	21	0.5679	n.a.	n.a.	n.a.
aluminum standard	1	w/	0.62978	0.00124	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	33	0.2725
		w/o	0.99291	0.9719	0.89559	16	3	0.7433	-1.6741	19	0.1105	n.a.	n.a.	n.a.
	10	w/	0.72863	0.02386	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	33	0.2784
		w/o	0.86059	0.1912	0.7856	16	5	0.6463	-0.2456	21	0.8084	n.a.	n.a.	n.a.
	100	w/	0.62978	0.00124	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	36.5	0.1226
		w/o	0.945	0.6997	0.34713	16	5	0.09692	-3.0757	21	0.00574	0.997742	n.a.	n.a.
zinc standard	0.1	w/	0.8397	0.1945	0.35354	11	3	0.1753	-0.4752	14	0.642	n.a.	n.a.	n.a.
		w/o	0.62978	0.00124	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	48.5	0.1908
	1	w/	0.86337	0.2725	1.157	11	3	0.9768	-0.1422	14	0.8889	n.a.	n.a.	n.a.
		w/o	0.86912	0.2227	0.50314	16	5	0.2709	-0.7451	21	0.4645	n.a.	n.a.	n.a.
	10	w/	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0	0.03098
		w/o	0.92708	0.5774	0.31985	16	3	0.1102	-5.9222	19	1.06e-05	0.9999998	n.a.	n.a.

Table SI 2.12: Al, Zn and In concentrations in *Corophium volutator* after exposure with the dissolved aluminum anode for ten days with (w/) sediment and three days without (w/o) sediment. At the end of the testing period, the organisms were washed with 0.5% HNO_3 for 30 min to remove adherent metal precipitates from exoskeleton, freeze-dried, homogenized, digested and subsequently analyzed by ICP-MS. N.a. = not available/applicable.

c (Al anode) [mg/kg H_2O]	metal	sediment	c (metal) in *C. volutator* [mg/kg DW]					enrichment factor
			replicate 1	replicate 2	replicate 3	mean	SE	
0	Al	w/	899	893	845	879	17	n.a.
		w/o	564	528	575	556	14	n.a.
	Zn	w/	88.4	84.5	77.5	83.5	3.2	n.a.
		w/o	101.2	94.5	101.7	99.2	2.3	n.a.
	In	w/	< LOQ	< LOQ	< LOQ	< LOQ	n.a.	n.a.
		w/o	0.005	0.001	0.001	0.002	0.002	n.a.
1	Al	w/	864	863	864	863	0.4	1.0
		w/o	715	710	703	709	3.7	1.3
	Zn	w/	89.6	93.8	93.4	92.3	1.3	1.1
		w/o	105.8	98.4	98.7	100.9	2.4	1.0
	In	w/	< LOQ	< LOQ	< LOQ	< LOQ	n.a.	n.a.
		w/o	0.028	0.022	0.027	0.026	0.002	11
10	Al	w/	971	907	973	950	22	1.1
		w/o	1940	1912	1961	1938	14	3.5
	Zn	w/	90.6	91.5	88.2	90.1	1.0	1.1
		w/o	121.6	122.2	123.7	122.5	0.6	1.2
	In	w/	< LOQ	0.001	< LOQ	< LOQ	n.a.	n.a.
		w/o	0.206	0.203	0.200	0.203	0.002	85.0
100	Al	w/	936	1047	1002	995	32	1.1
		w/o	2791	2625	2879	2765	74	5.0
	Zn	w/	85.3	89.1	95.5	90.0	3.0	1.1
		w/o	130.2	123.4	186.1	146.6	19.9	1.3
	In	w/	0.011	0.012	0.013	0.012	0.001	n.a.
		w/o	0.322	0.302	0.349	0.324	0.014	136.0

Table SI 2.13: Average Al, Zn and In content of investigated aluminum anode and non-exposed mud shrimp, ratio of this metal content to each other and enrichment factor in *Corophium volutator* after exposure to 100 mg dissolved aluminum anode per kg H_2O for three days without sediment.

	c(metal) [mg/kg]		metal content anode to mud shrimp	max. enrichment factor
	aluminum anode	mud shrimp		
Al	950,000	556	≈ 1,700	5.0
Zn	50,000	99.2	≈ 500	1.3
In	203	0.0024	≈ 85,000	136

SUPPLEMENTARY DATA CHAPTER 3

Table SI 3.1: Effective dry film thickness (EDFT) of investigated coating systems (mean, n = 3). The determination was conducted five times on front and back of each plate.

no. of coating system	primer coating	top coating	EDFT [µm]	SE
1	A	(I)	304	9
2	-	(I)	237	8
3	A	(II)	581	12
4	-	(II)	511	7
5	B	-	88	3
6	B	(III)	467	7
7	-	(III)	376	9

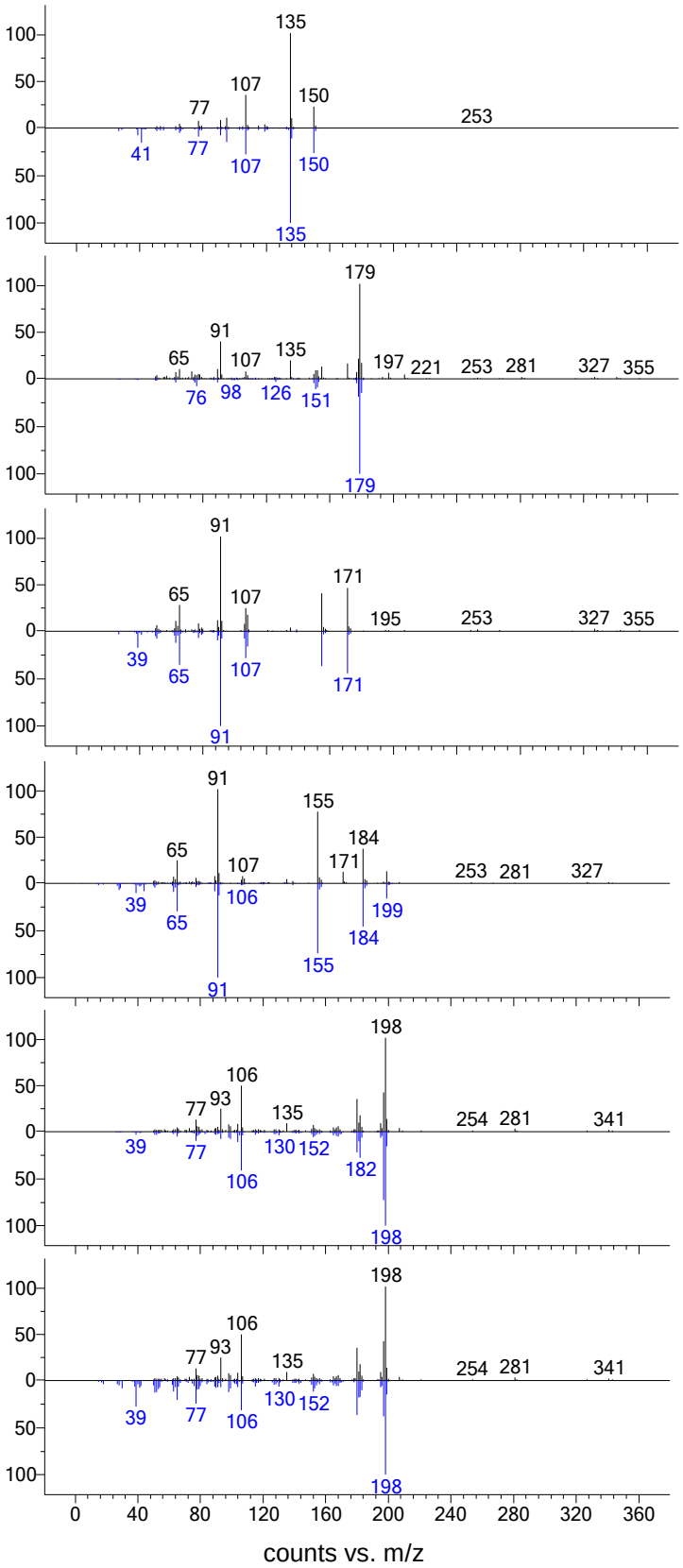

Figure SI 3.1: Comparison of representative MS spectra obtained from the samples (EI, 70 eV; black) and reference spectra of suspected target compounds (blue) from reference library (NIST08).

Table SI 3.2: Recovery of target compounds (mean, n = 3). Deionized water was spiked with 10 and 2.5 µg/l of each analyt, respectively, enriched by SPE and quantified by GC-MS (see material and methods section). N.d. = not detected.

substance	spike with 10 µg/l		spike with 2.5 µg/l	
	recovery [%]	SE	recovery [%]	SE
4-*tert*-butylphenol	89	4.7	90	1.2
4-toluenesulfonamide	98	3.7	100	4.4
4-tolueneethylsulfonamide	87	3.4	104	3.5
benzo[f]quinolone	75	5.3	94	2.3
3,3'-methylenedianiline	7	1.0	nd	nd
4,4'-methylenedianiline	3	0.7	nd	nd
bisphenol A	101	3.1	114	6.2
bisphenol A diglycidyl ether	59	11.4	nd	nd
bisphenol F	109	1.9	103	3.0

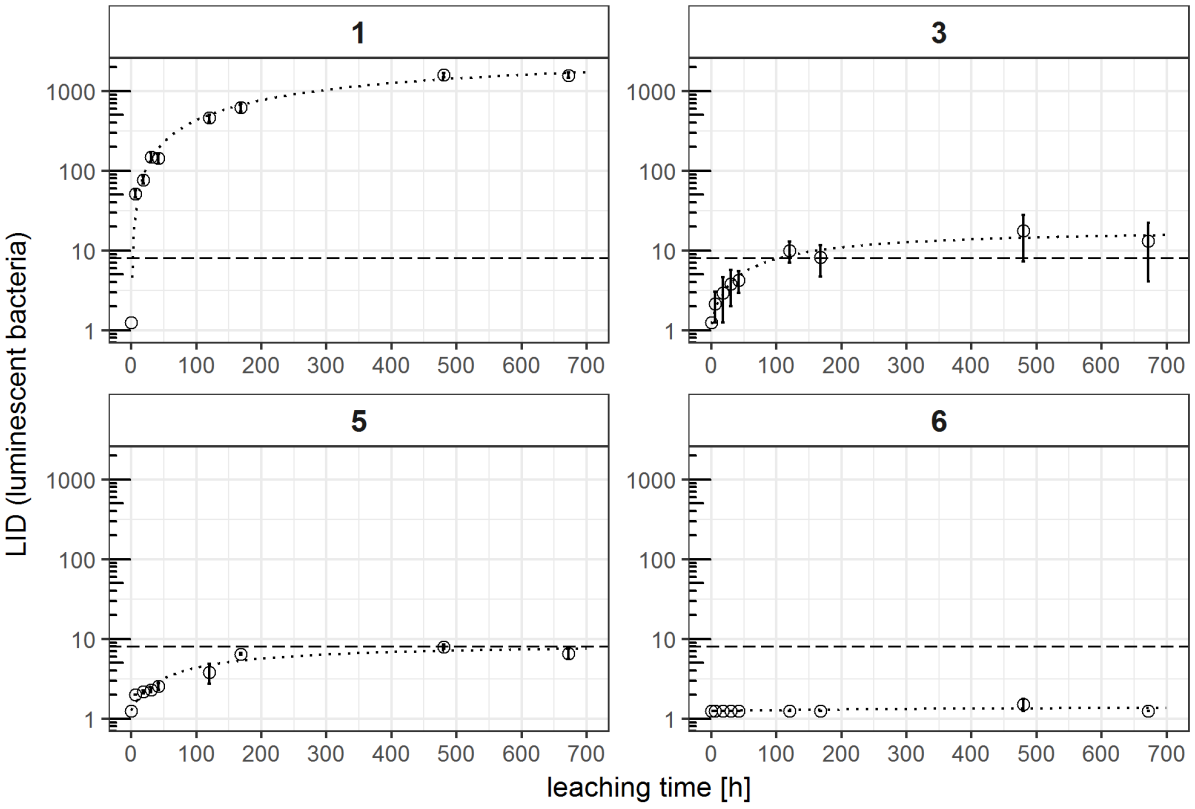

Figure SI 3.2: Time-dependent luminescence inhibition (mean, n = 3, error bars indicate SE) of anti-corrosion coating systems (no. are indicated on top of diagrams). Leaching time is displayed as duration of the specific step after renewal of leachant. Results calculated by linear interpolation are expressed as dilution stages causing 20% luminescence inhibition (lowest ineffective dilution, LID). The dashed lines indicate the assessment criterion set by DIBt, dotted lines show fits of data according to Michaelis-Menten equation (equation 3.1).

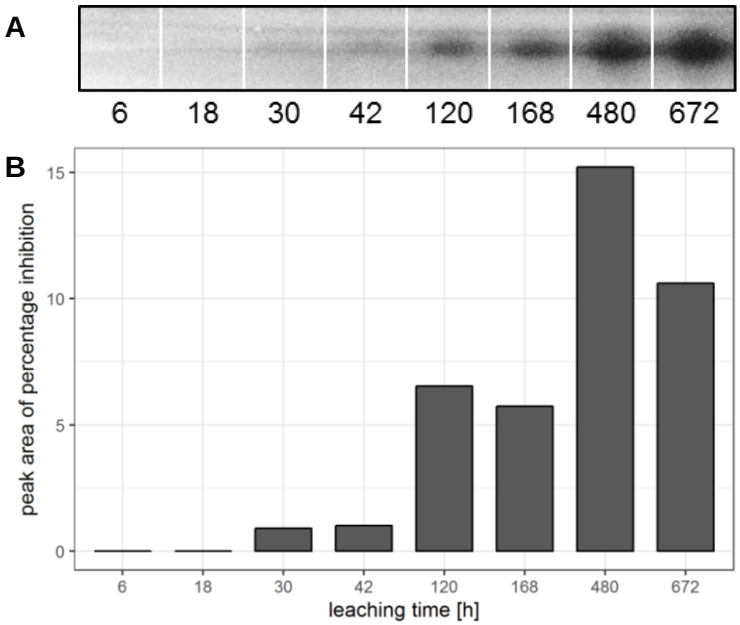

Figure SI 3.3: Time-dependent luminescence inhibition in aqueous leachates of anti-corrosion coating system no. 2 after chromatographic development and dipping into suspension of luminescent bacteria. Leaching time is displayed as duration of the specific step after renewal of leachant. Each aqueous sample was applied in 100 µl on the HPTLC plate (Silica gel 60 F_{254}). *A*: Black and white image of inhibition spots after approximately 10 min of exposition. For a better visualization brightness and contrast were adjusted, however, evaluation was performed on the original image. *B*: Corresponding peak area of luminescence inhibition after background correction according to Schulz et al. (2017).

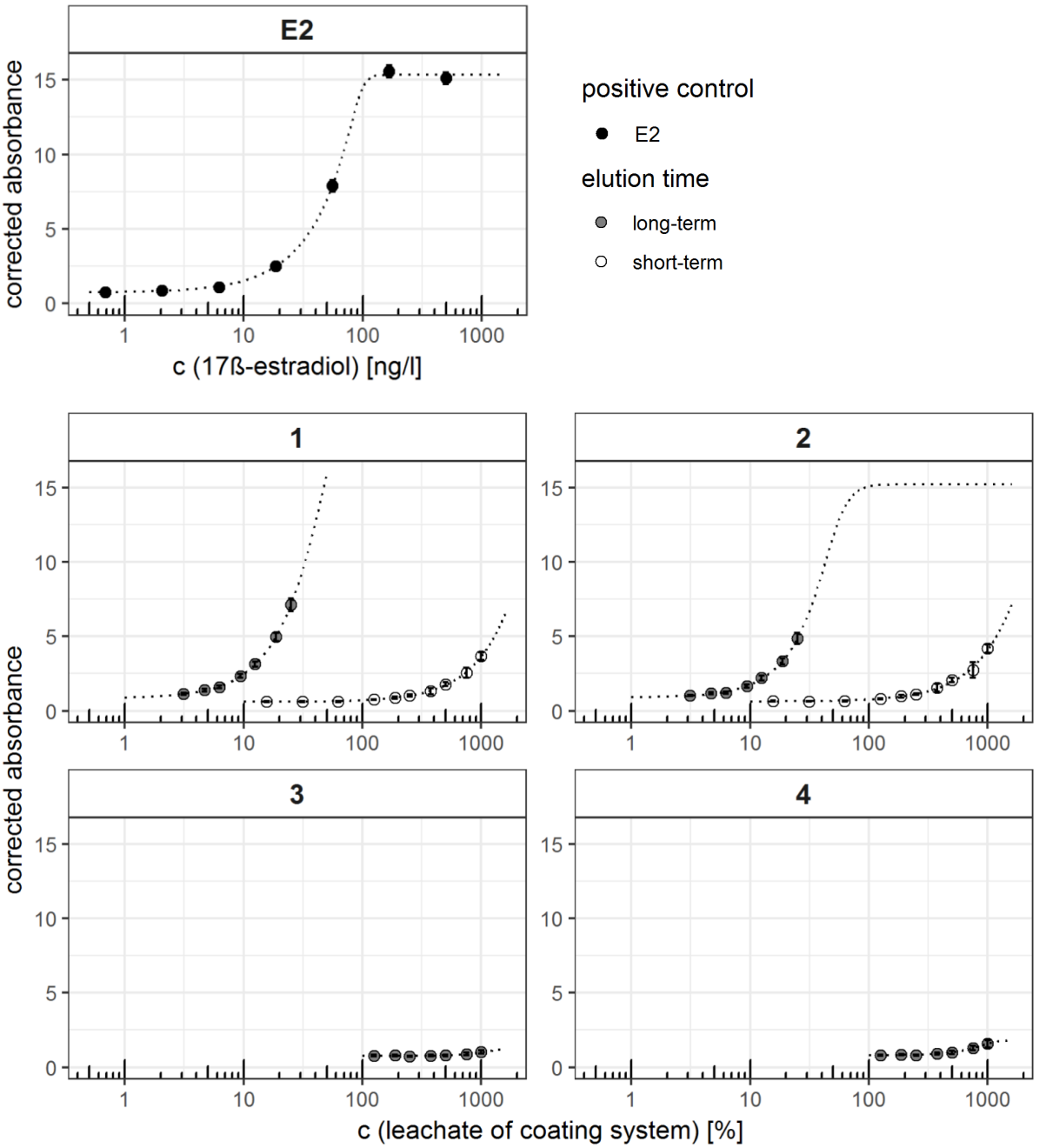

Figure SI 3.4: Concentration-dependent estrogenic activities in leachates of anti-corrosion coating systems (n = 9, no. of systems are indicated on top of diagrams) and dilution series of reference (n = 84). Effects were detected as corrected absorbance (mean, error bars indicate SE) in enriched, time-proportional 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 against 17*β*-estradiol dilution series. Dotted lines show the 5-parametric log-logistic fit of data (equation 3.2).

Table SI 3.3: GC-MS scan data of six unknown substances in leachates of anti-corrosion coating systems (sample is synonymous with no. of systems). The peaks that were detected in all replicates of one sample are listed with their retention time (RT), the five most intense fragments and their percentage abundance.

	Unknown 1											
sample	5						6					
replicate	1		2		3		1		2		3	
RT [min]	20.82		20.79		20.79		20.78		20.79		20.78	
	m/z	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*
	91.2	100	91.2	100	91.2	100	91.2	100	91.2	100	91.1	100
	155.1	52	155.1	52	155.1	51	155.1	52	155.1	52	155.1	53
	207.2	51	207.2	51	207.2	50	207.2	50	207.2	50	207.2	51
	108.1	38	108.1	38	108.1	38	108.1	39	108.1	38	108.1	39
	72.1	36	72.1	36	72.2	35	72.1	35	72.2	35	72.1	36

	Unknown 2						**Unknown 3**					
sample	5						5					
replicate	1		2		3		1		2		3	
RT [min]	23.82		23.76		23.78		26.21		26.2		26.16	
	m/z	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*
	91.2	100	135.1	100	91.2	100	197.2	100	198.1	100	198.2	100
	108.1	78	108.1	89	135.1	97	198.2	97	106.2	39	135.2	63
	155.1	64	91.2	83	108.1	76	207	67	197.1	39	65.1	53
	65.2	41	155	81	155.1	67	198.1	61	197.2	34	197.1	48
	78.2	33	197.2	61	65.1	67	106.1	40	155	23	77.2	43

	Unknown 4											
sample	6						7					
replicate	1		2		3		1		2		3	
RT [min]	24.51		24.54		24.58		24.55		24.54		24.68	
	m/z	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*
	198.2	100	198.2	100	198.2	100	198.2	100	198.2	100	198.2	100
	135.1	74	106.1	39	197.2	86	106.2	52	106.2	55	106.1	38
	106.2	57	180.2	38	106.2	78	197.2	40	197.2	45	180.2	35
	180.2	41	197.1	38	198.1	40	93.2	27	180.2	40	93.2	28
	197.2	41	93.1	19	180.1	36	180.1	26	93.2	27	197.2	22

	Unknown 5						**Unknown 6**					
sample	6						7					
replicate	1		2		3		1		2		3	
RT [min]	18.57		18.59		18.59		18.65		18.64		18.75	
	m/z	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*	*m/z*	*%*
	179.1	100	179.1	100	179.1	100	179.1	100	179.1	100	179.1	100
	178.1	21	178.2	24	179.2	21	178.2	25	178.2	24	178.1	21
	180.1	16	180.1	14	178.1	20	179.2	24	180.2	14	180.2	13
	89.2	12	107.1	14	180.1	18	403.1	16	59.2	8	208.1	9
	107.1	10	152.2	8	64.1	17	180.1	12	151	7	147.2	8

Table SI 3.4: Time-dependent release of 4-*tert*-butylphenol (mean, n = 3) in leachates of two selected top coatings for corrosion protection. Contents were measured in enriched samples, results refer to aqueous samples.

leaching time [h]	c (4*t*BP) [mg/l]			
	top coating (I) (coating system 2)		top coating (II) (coating system 4)	
	mean	SE	mean	SE
6	0.213	0.039	0.0221	0.0012
18	0.316	0.026	0.0363	0.0051
30	0.447	0.067	0.0433	0.0063
42	0.501	0.038	0.0551	0.0066
120	1.25	0.11	0.0952	0.0087
148	1.33	0.11	0.0828	0.0045
480	3.51	0.15	0.1693	0.0063
672	3.85	0.32	0.1514	0.0030

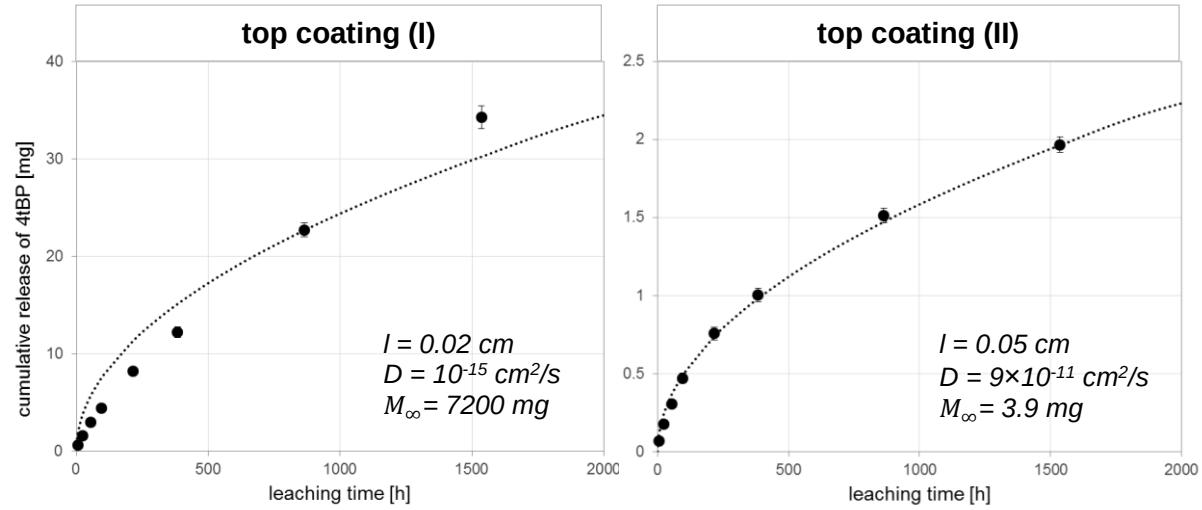

Figure SI 3.5: Cumulative release of 4*t*BP (mean, n=3, error bars indicate SE) in aqueous leachates of top coatings (I) and (II) as a function of time. Leaching time is displayed as duration from the start of the test. Dotted lines show fit of data according to diffusion release (equation 4).

Table SI 3.5: Cumulative release of 4*t*BP as measured (mean, n = 3) and modelled by diffusion equation. Resulting mean squared errors and their sums were used as indicator for the goodness of fit.

	cumulative leaching time [h]	measured cumulative release of 4*t*BP per plate [mg]	modelled cumulative release of 4*t*BP per plate [mg]	mean squared errors	
top coating (I) (coating system 2)	6	0.64	1.89	1.56	
	24	1.59	3.78	4.80	
	54	2.93	5.66	7.49	
	96	4.43	7.55	9.74	
	216	8.18	11.33	9.90	
	384	12.17	15.10	8.60	
	864	22.70	22.66	0.002	
	1536	34.25	30.21	16.38	∑= 58.5
top coating (II) (coating system 4)	6	0.066	0.12	0.0032	
	24	0.18	0.25	0.0049	
	54	0.31	0.37	0.0040	
	96	0.47	0.49	0.0004	
	216	0.76	0.74	0.0004	
	384	1.00	0.98	0.0005	
	864	1.51	1.47	0.0016	
	1536	1.97	1.96	0.00003	∑= 0.015

Table SI 3.6: Zinc concentrations in time proportional long-term samples (mean, n = 3). Properties of investigated materials are summarized in table 3.1.

no. of coating system	c (Zn) [mg/l]	
	mean	SE
1	0.0026	0.0003
2	0.0035	0.0006
3	0.0233	0.0049
4	0.0013	0.0001
5	2.909	0.048
6	0.0130	0.0031
7	0.0012	0.0001

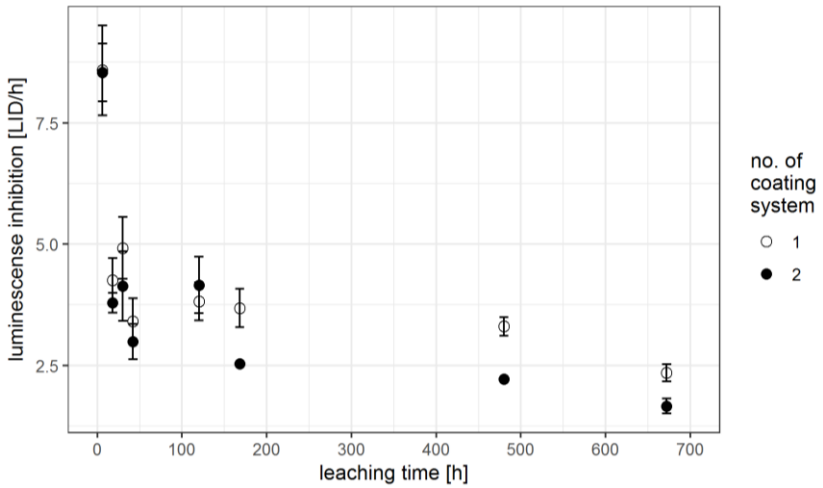

Figure SI 3.6: Luminescence inhibition of anti-corrosion coating systems (mean, n = 3, error bars indicate SE) normalized to one hour leaching time. Results calculated by linear interpolation are expressed as dilution stages causing 20% luminescence inhibition (lowest ineffective dilution, LID).

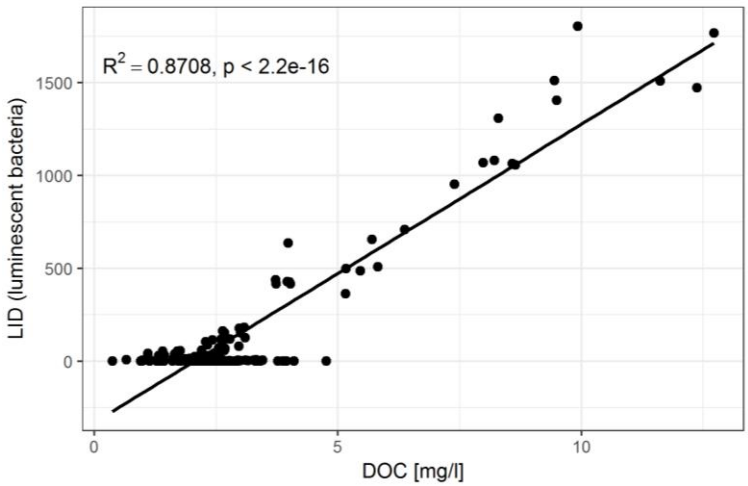

Figure SI 3.7: Correlation of DOC and luminescence inhibition in leachates of seven anti-corrosion coating systems (n = 168). Result of the regression test is shown as adjusted R^2 and associated p-value.

SUPPLEMENTARY DATA CHAPTER 4

Table SI 4.1: Average toxic effects and quantified target compounds in leachates of untreated (- UV) and UV-A irradiated (+ UV) coatings A and B. Experiments were performed with concentrated samples. The presented results are calculated for the original aqueous leachates under the assumption of a quantitative extraction of the compounds. Abbreviations of substances see Table 4.1.

	coating A		coating B	
	- UV	+ UV	- UV	+ UV
Estrogenic potential (EEQ [ng/l])	185	152	50.6	31.4
Toxicity to luminescent bacteria (EC50 [% of sample])	0.190	0.260	0.820	1.860
4*t*BP (c [µg/l])	15,000	9,400	3,320	1,840
BPA (c [µg/l])	2.30	14.3	0.90	16.9
4CP (c [µg/l])	< LOQ	< LOQ	< LOQ	0.23
BPA-I11 (c [µg/l])	< LOQ	0.72	< LOQ	1.37
BPA-I10 (c [µg/l])	< LOQ	0.78	< LOQ	1.20
Bis-HPPP (c [µg/l])	19.6	5.6	1.04	0.95
2PP (c [µg/l])	< LOQ	< LOQ	0.67	< LOQ

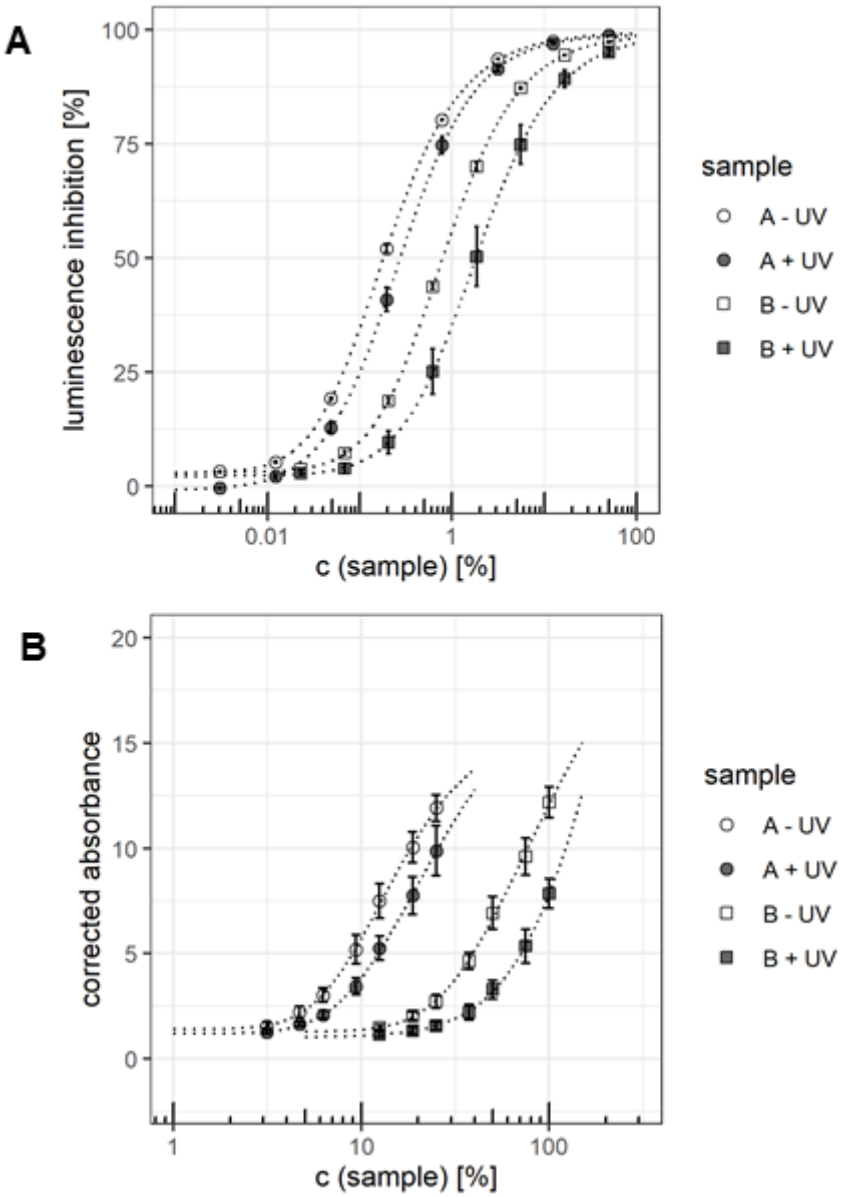

Figure SI 4.1: Concentration dependent toxicity in leachates of untreated (- UV) and UV-A irradiated (+ UV) coating A and B (mean ± SE). Dotted lines show the 5-parametric log-logistic fit of data (equation 4.1). Each coating was leached three times and each leachate was tested three times. Effects were measured in concentrated samples and the results shown are calculated for the original aqueous leachates. *A*: Toxicity to luminescent bacteria shown as luminescence inhibition compared to control. *B*: Estrogenic activity detected as corrected absorbance with a recombinant yeast estrogen screen.

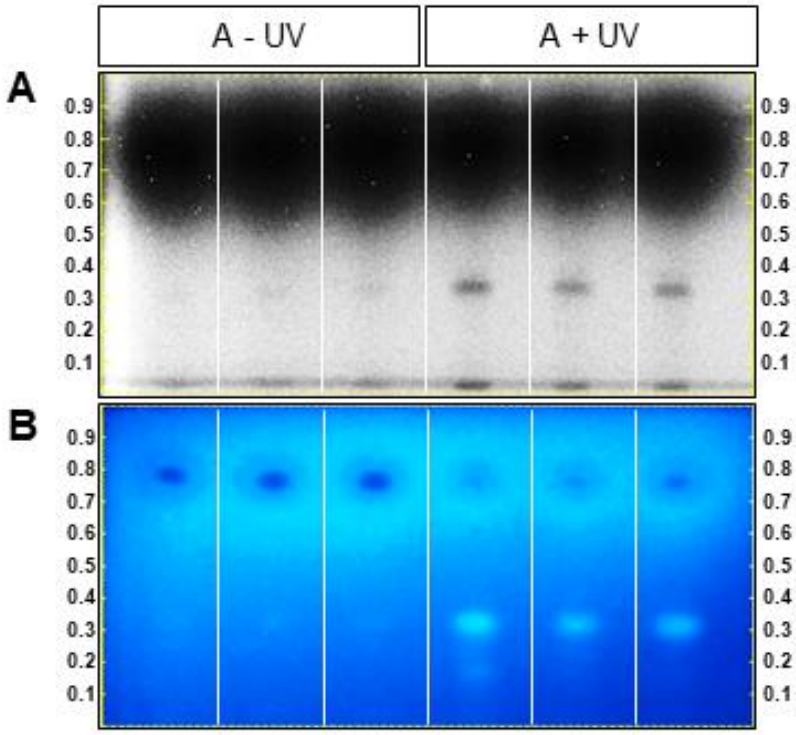

Figure SI 4.2: Toxicity in leachates of untreated (- UV) and UV-A irradiated (+ UV) coating A on HPTLC-plate. The ethanolic extracts (1000-fold concentrated) of all replicates were diluted 1:100, applicated in a volume of 25 µl each and chromatographically developed with ethyl acetate and n-hexane (35:65). For a better visualization brightness and contrast were adjusted. *A*: Black and white image of luminescence signals after approximately 11 min of exposition with luminescent bacteria. *B*: Fluorescence image of HPTLC coupled Yeast Estrogen Screen at an excitation wavelength of 366 nm.

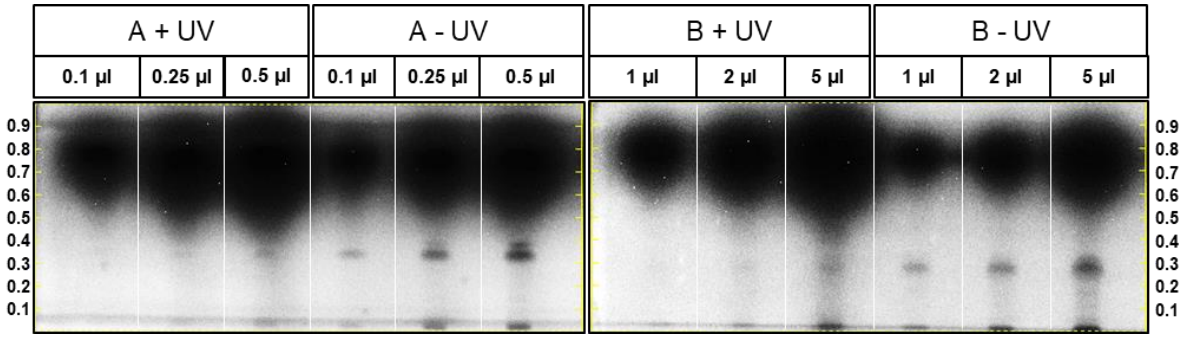

Figure SI 4.3: Dose dependent luminescence inhibition of selected samples of untreated (- UV) and UV-A irradiated (+ UV) coating A and B on HPTLC plates. The ethanolic extracts were diluted before application, the indicated volume corresponds to the absolute amount of original extracts (1000-fold concentrated). Black and white images were taken after chromatographic development with ethyl acetate and n-hexane (35:65) and exposition of luminescent bacteria for approximately 11 min. For a better visualization brightness and contrast were adjusted.

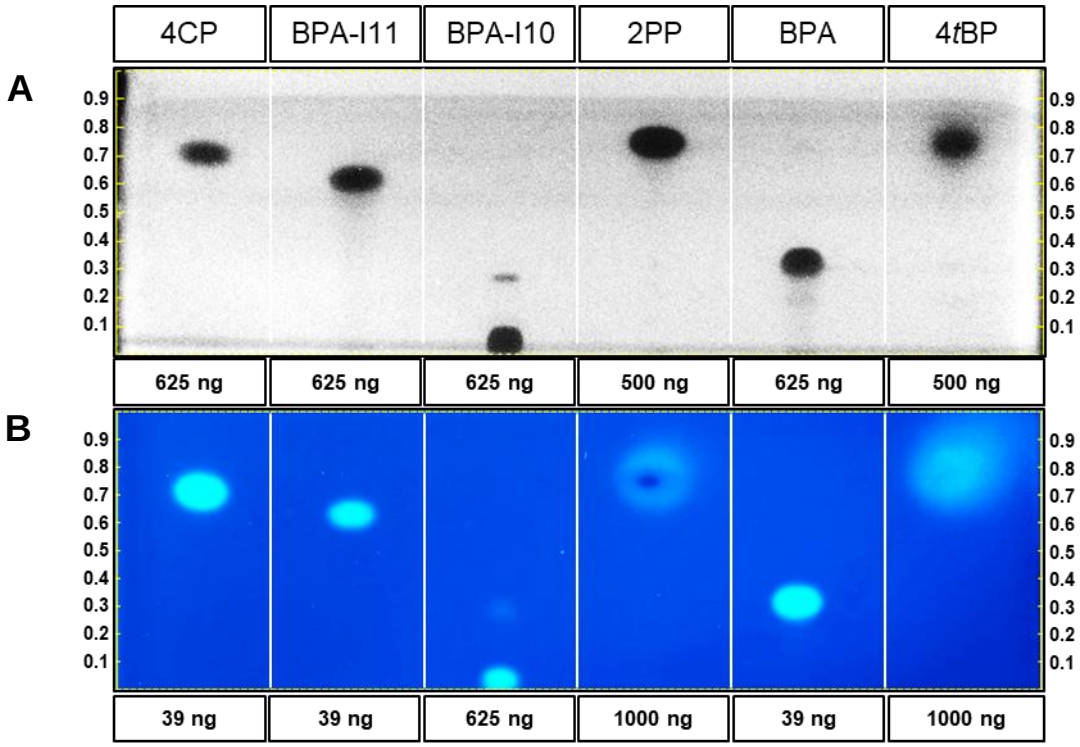

Figure SI 4.4: Toxicity of quantified compounds on HPTLC-plate. The standards were applicated as ethanolic solutions and chromatographically developed with ethyl acetate and n-hexane (35:65). For a better visualization brightness and contrast were adjusted. *A*: Black and white image of luminescence signals after approximately 11 min of exposition with luminescent bacteria. *B*: Fluorescence image of HPTLC coupled Yeast Estrogen Screen at an excitation wavelength of 366 nm.

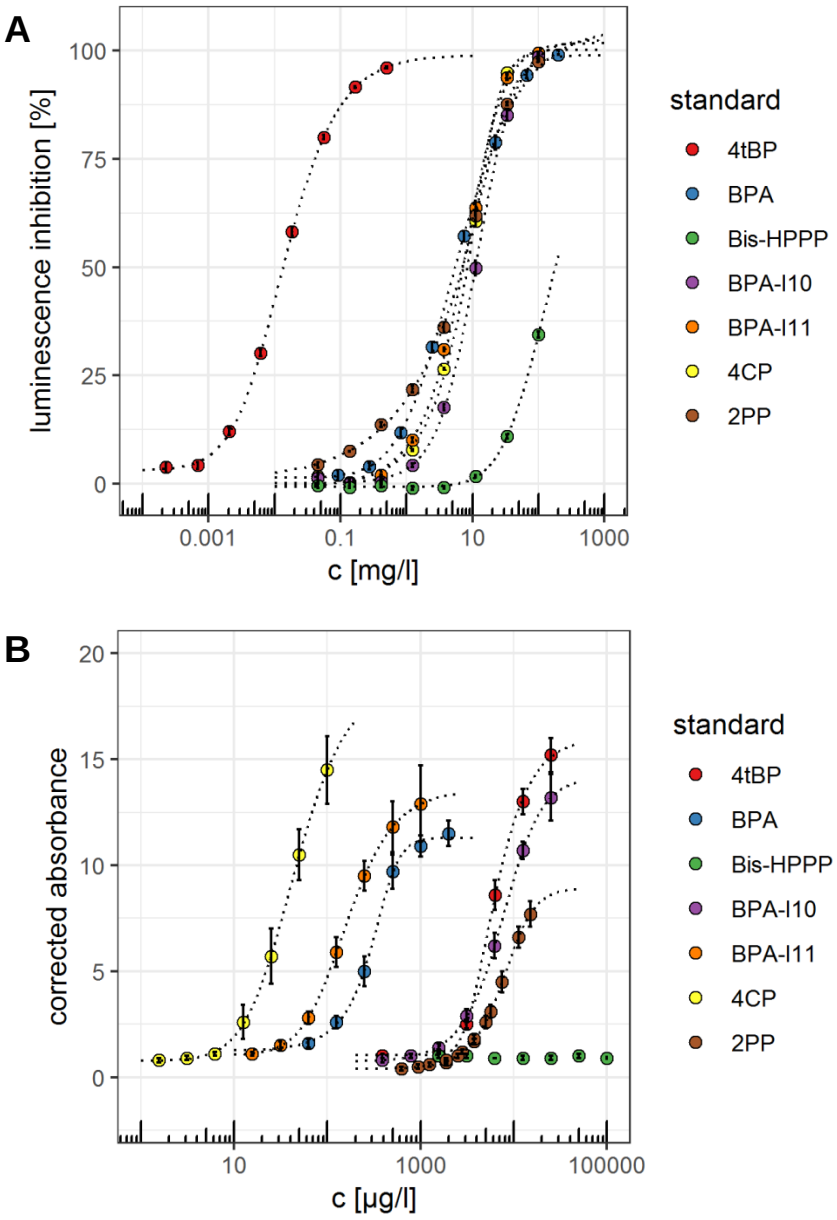

Figure SI 4.5: Concentration dependent toxicity (mean ± SE) of quantified compounds (Table 4.2) in bioassays on microplate. Dotted lines show the 5-parametric log-logistic fit of data (equation 1). Each standard substance was tested at least three times *A*: Toxicity to luminescent bacteria shown as luminescence inhibition compared to control. *B*: Estrogenic activity detected as corrected absorbance with a recombinant yeast estrogen screen.