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PUBLIC COMMENTS

1. Comment from Douglas Deitch, dated 10/30/2020
2. Comment from Poison Free Malibu, dated 10/30/2020
3. Comment from Member of the Public that would like the CZ to be redrawn as a smaller area, dated 10/30/2020
4. Comment from Member of the Public wanting mention of coastal railroad issues, dated 11/2/2020
5. Comment from Beach Cities Preservation Alliance, dated 11/3/2020
6. Comment from County of Marin Board of Supervisors Dennis Rodoni, dated 11/4/2020
7. Comment from Alliance of Coastal Marin Villages, dated 11/4/2020
8. Comment from League of California Cities, dated 11/4/2020
9. Comment from Member of the Public regarding Environmental Justice, dated 11/4/2020
10. Comment from Attorney/Government Relations Consulting, dated 10/30/2020

2. Comment from Poison Free Malibu, dated 10/30/2020

Continued from Addendum 1

Heavy rainfall provokes anticoagulant rodenticides' release from baited sewer systems and outdoor surfaces into receiving streams

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Abstract

Prevalent findings of anticoagulant rodenticide (AR) residues in liver tissue of freshwater fish recently emphasized the existence of aquatic exposure pathways. Thus, a comprehensive wastewater treatment plant and surface water monitoring campaign was conducted at two urban catchments in Germany in 2018 and 2019 to investigate potential emission sources of ARs into the aquatic environment. Over several months, the occurrence and fate of all eight ARs authorized in the European Union as well as two pharmaceutical anticoagulants was monitored in a variety of aqueous, solid, and biological environmental matrices during and after widespread sewer baiting with AR-containing bait. As a result, sewer baiting in combined sewer systems, besides outdoor rodent control at the surface, was identified as a substantial contributor of these biocidal active ingredients in the aquatic environment. In conjunction with heavy or prolonged precipitation during bait application in combined sewer systems, a direct link between sewer baiting and AR residues in wastewater treatment plant influent, effluent, and the liver of freshwater fish was established. Moreover, study results confirmed insufficient removal of anticoagulants during conventional wastewater treatment and thus indirect exposure of aquatic organisms in receiving streams via tertiary treated effluents and combined sewer overflows. Nevertheless, further research is required to determine the ecological implications and risks for aquatic organisms as well as fish-eating predators from chronic AR exposure at environmentally relevant concentrations.

Keywords: Biocides, combined sewer overflow, PBT, rat management, sewer baiting, wastewater treatment

1. Introduction

In recent years, European Union (EU)-wide application of anticoagulant rodenticides (ARs) to control commensal rodents for hygienic and public health reasons has been increasingly restrained because of human and environmental risks. Second-generation ARs are classified as (very) persistent, (very) bioaccumulative, and toxic (PBT or vPvB, respectively) substances (Regnery et al. 2019a, van den Brink et al. 2018). In order to minimize environmental exposure due to the toxicological relevance of anticoagulants at trace concentrations, national best practice guidelines and mandatory instructions for use of ARs were implemented in Germany from 2012 (Umweltbundesamt 2019). Nevertheless, prevalent findings of AR residues in liver tissue of freshwater fish (Kotthoff et al. 2019, Regnery et al. 2019b) from streams in Germany recently highlighted the emergence of aquatic exposure pathways. So far, worldwide monitoring of AR residues mainly focused on terrestrial and avian non-target species and their routes of exposure (Elmeros et al. 2018, Koivisto et al. 2018, Serieys et al. 2019, van den Brink et al. 2018). As discussed in detail in a review by Regnery et al. (2019a), AR residue screening in aquatic compartments is challenging, and accordingly little is known about direct and indirect exposure routes as well as anticoagulants' distribution and fate in the aquatic environment.

Three first-generation (i.e., warfarin, coumatetralyl, and chlorophacinone) and five second-generation ARs (i.e., difenacoum, bromadiolone, brodifacoum, flocoumafen, and difethialone) are currently approved in the EU for biocidal use under the EU Biocidal Products Regulation (BPR) No. 528/2012 (European Union 2012) with maximum permissible concentrations in bait formulations in the range of 0.0025% (difethialone) and 0.079% (warfarin). While pest control professionals are presumed to be among the main users of biocidal ARs in Germany, agribusinesses, local authorities, and household consumers also represent important user groups (Regnery et al. 2019a). Considering the lack of detailed market data (Regnery et al.

2019a) as well as manifold applications of ARs in urban and suburban areas (Meyer and Kaukeinen 2015), evidence of specific emission sources and aquatic exposure pathways is not straight forward. Several potential rodenticide emission scenarios for different environmental compartments have been described by the European Chemicals Agency. For example deployment of baits in the immediate vicinity of watercourses represents a likely direct emission source of ARs into the aquatic environment (e.g., due to wash off from bank slopes, aboveground bait stations, or rodent burrows, respectively, as well as contaminated run-off, ECHA 2018b). Rodent control in and around municipal sewer systems by local authorities and commissioned pest control professionals is assumed to be another important emission source of ARs into the aquatic environment in urban and suburban settings (Gómez-Canela et al. 2014a, Kotthoff et al. 2019, Regnery et al. 2019b). In a recent survey in Germany (Regnery et al. 2020), the annual domestic use of ARs in municipal sewer baiting scenarios in 2017 was estimated at approximately 225 metric tons of bait material and thus 32 kg of active ingredients (thereof 21.4 kg of warfarin, 5.6 kg of difenacoum, 3.1 kg of brodifacoum, and 1.8 kg of bromadiolone). From the sewers, exposure of the aquatic environment presumably occurs directly via baited storm drains or combined sewer overflows (CSOs) that discharge highly diluted but untreated sewage directly into receiving surface waters when stormwater runoff causes an increase within the system, or indirectly via wastewater treatment plant (WWTP) effluents (ECHA 2018b). In their retrospective biological monitoring study, Regnery et al. (2019b) confirmed exposure of aquatic organisms via municipal effluents and thus incomplete removal of anticoagulants during conventional wastewater treatment. A study in Spain also reported incomplete removal of ARs during activated sludge treatment and their discharges into receiving streams at trace level (Gómez-Canela et al. 2014a, 2014b, Gómez-Canela and Lacorte 2016). Nevertheless, comprehensive monitoring data on the occurrence and fate of ARs in WWTPs and receiving surface waters during or shortly after widespread chemical rodent control in and around municipal sewer systems are not available. Such data are required to establish robust relationships and causative associations as previously shown for the unintended poisoning of terrestrial non-target organisms (Geduhn et al. 2015).

To further investigate the above-mentioned potential emission pathways, a comprehensive WWTP and surface water monitoring campaign was conducted at two urban catchments in Germany in 2018 and 2019. At both monitoring sites, common sewer baiting schemes (ECHA 2018b, Regnery et al. 2020) such as bi-annual, annual, or biennial preventive rodent control measures using second-generation ARs had been used for years. However, the receiving streams Queich and Moselle differ in size and thus effluent load at studied WWTP outfalls. Over several months, the occurrence and fate of all eight authorized biocidal anticoagulants was monitored in a variety of aqueous, solid, and biological environmental matrices during and after widespread sewer baiting with AR-containing bait. This study contributes valuable information to future risk assessments of ARs and assists in developing more effective and practical risk mitigation measures to protect the aquatic environment.

2. Experimental

2.1. Monitoring site A at River Queich

A schematic map providing an overview of all WWTP and surface water sampling locations at monitoring site A is shown in Figure 1. The studied WWTP at monitoring site A (hereafter referred to as WWTP A) serves the medium-sized town of Landau in der Pfalz, Rhineland-Palatinate, Germany and employs conventional treatment (i.e., mechanical, biological, chemical) with a treatment capacity of 90,000 person equivalent. WWTP A discharges into the small stream Queich after it flows through the urban center of Landau. The small stream's mean discharge is in the range of 1.75 m^3/s with an estimated 7% effluent contribution of WWTP A near its outfall under dry weather and average flow conditions.

Annual preventive rat control measures in the WWTP's associated sewer system (i.e., mostly combined, with a total length of approximately 230 km and 4900 sewer manholes) were carried out during a three-week period in May 2018 through commissioned and trained pest control professionals. A total of approximately 2000 brodifacoum-containing bait blocks (i.e., 225 g each containing 0.005% of active ingredient) were deployed in the town's combined sewer system. As a benchmark, approximately 2500 brodifacoum-containing bait blocks had been applied in 2017, whereas no widespread sewer baiting occurred in 2019. Baits were lowered into the manhole to (short above) the berm and were attached to the manhole's gully trap or step irons by wire to prevent direct wastewater contact during normal sewer operation as well as dragging off by rats or being flushed away. According to provided information, remaining baits were generally not collected for disposal after the baiting campaign ended. In addition, a total of approximately 60 bromadiolone-containing baits (i.e., 200 g each containing 0.005% of active ingredient) were deployed above ground in tamper-resistant bait stations near watercourses throughout the urban center.

During the monitoring campaign a total of 10 samplings were carried out between March and July 2018. Activated sludge grab samples and 24-hour composite samples of raw wastewater and corresponding treated effluent as well as operational and water quality parameters were kindly provided by WWTP staff. Deposited solids from the bottom of two different CSO structures were also provided by WWTP staff. All of the riverine sampling sites were situated in a rural setting downstream of the town's urban center and were chosen based on their ease of access and their position in relation to the outfall of WWTP A (Figure 1). Sediment grab samples of Queich were obtained from 0 – 5 cm depth near the bank using a small stainless-steel shovel whereas surface water grab samples were scooped midstream by lowering a bucket from adjacent pedestrian bridges. Surface water quality parameters such as temperature, dissolved oxygen concentration, pH, and electrical conductivity were measured *in situ* with sensors using a Multi 3630 IDS handheld (WTW, Weilheim, Germany). Other water quality parameters such as dissolved organic carbon and nutrient levels as well as characteristics of solid samples were determined in laboratory according to Standard Methods (Wasserchemische Gesellschaft and Normenausschuss Wasserwesen im DIN 2020). Suspended particulate matter (SPM) in the stream was collected over defined time intervals (generally 14 days) using passive sedimentation boxes (Schulze et al. 2007). Upstream and downstream of the WWTP A outfall, two sedimentation boxes were suspended in the water from overhanging structures using steel cables (i.e., bankside, approximately 0.2 m above ground). Fish liver and filet samples (frozen at -20°C) from the investigated stream were kindly provided by the Upper Fisheries Authority, Structural and Approval Directorate South, Rhineland-Palatinate. A total of 15 individuals of brown trout (*Salmo trutta fario*), perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), chub (*Squalius cephalus*), and common gudgeon (*Gobio gobio*) had been caught at the same time from the same river stretch downstream of the WWTP A outfall in July 2019, approximately one year after the initial WWTP and surface water monitoring campaign ended (Table S1).

2.2. Monitoring site B at River Moselle

The studied WWTP at monitoring site B (hereafter referred to as WWTP B) serves the city of Trier, Rhineland-Palatinate, Germany and employs conventional treatment (i.e., mechanical, biological, chemical) with a treatment capacity of 170,000 person equivalent. WWTP B discharges into Moselle, a tributary of River Rhine. The mean discharge of Moselle at gauge Trier is 277 m^3/s. Under average flow conditions the overall effluent contribution of Moselle is less than 5% (Karakurt et al. 2019), with an estimated 0.1% effluent contribution of WWTP B near its outfall during dry weather conditions.

Until 2017, city-wide preventive rat control measures in the sewers had been carried out through commissioned and trained pest control professionals once a year during an eight-week period in spring or fall using second-generation AR-containing bait (e.g., approximately 3500 bait blocks with 0.005% brodifacoum in fall 2017). Since then, rat control in sewers had been switched to a more targeted approach based upon reported sightings of rats. A total of approximately 1055 bait blocks (i.e., 200 g each containing 0.0029% of brodifacoum) were successively deployed in different urban districts of the city's combined sewer system (i.e., approximately 550 km total length with 15,000 sewer manholes) between January and May 2019 by public services through trained municipal staff. Similar to monitoring site A, bait blocks were attached to the manhole's gully trap or step irons by wire. However, remaining baits were now removed from the manholes and collected for appropriate disposal at the end of baiting campaigns according to personal communication. Moreover, baits were not applied in stormwater channels.

A total of 7 samplings were carried out at the WWTP between April and June 2019. To study the fate of ARs during wastewater treatment, grab samples included activated sludge, biosolids, sludge liquor, prewashed mineral material from the grit chamber, and grit chamber solids washing water. Twenty-four-hour composite samples of raw wastewater and corresponding treated effluent as well as operational and water quality parameters were kindly provided by WWTP staff. Sediment grab samples from sand traps of two different stormwater retention basins discharging into small tributary creeks were also provided by public services. Due to expected substantial dilution of effluent discharges in the receiving stream, surface water grab samples upstream and downstream of WWTP B were collected less frequently than WWTP samples. As described earlier, surface water quality parameters and characteristics of solid samples were either measured *in situ* with sensors or determined in laboratory according to Standard Methods (Wasserchemische Gesellschaft and

Normenausschuss Wasserwesen im DIN 2020). Downstream of WWTP B, two sedimentation boxes were suspended in the river from overhanging structures or buoys near river kilometer (rkm) 167.0 and 186.1, respectively, using steel cables. SPM samples were retrieved after defined time intervals (i.e., 14 days) throughout the sampling campaign. In addition, monthly composites of SPM were obtained from five permanent water quality monitoring stations along Moselle upstream (Perl at rkm 241.9, Palzem at rkm 229.8, and Trier at rkm 196.0) and downstream (Fankel at rkm 59.4 and Koblenz at rkm 2.0) of WWTP B over the course of six months (January – July 2019) by the Federal Institute of Hydrology's Radiology and Monitoring Department (refer to https://geoportal-wasser.rlp-umwelt.de for details). Aliquots of sediment grab samples that had been collected within a permanent state monitoring program at locations upstream (at rkm 196.0) and downstream (at rkm 184.0) of WWTP B during this period were also kindly provided. An overview of all WWTP and surface water sampling locations at monitoring site B is shown in Figure 2. Due to the lengthy spatial distance, monthly composite SPM sampling locations (with the exemption of Trier at rkm 196.0) are not illustrated in Figure 2. Fish liver (n = 35) and filet (n = 6) samples of chub (*S. cephalus*), perch (*P. fluviatilis*), European eel (*Anguilla anguilla*), European catfish (*Silurus glanis*), pike-perch (*Sander lucioperca*), and round goby (*Neogobius melanostomus*) were received between 2017 – 2020 from three different sites along a 6 km stretch of Moselle approximately 25 km downstream of WWTP B (Table S1). Fish had been caught for food consumption by local fishermen in compliance with German fishing regulation. Handling of fish samples followed a standardized protocol (Bayerisches Landesamt für Umwelt 2012). Tissue samples were individually wrapped in aluminum foil and immediately frozen at -20°C. Sampling, measuring, and dissection of quagga mussels (*Dreissena bugensis*) followed general procedures described in Schäfer et al. (2012). In general, ten individuals of mussels were pooled per sample to provide enough soft body sample material for subsequent analyses.

One additional sampling site for mussels at rkm 1.0 (i.e., near the confluence of Moselle and Rhine in the city of Koblenz; Table S1) is not depicted in Figure 2.

2.3. Analysis of anticoagulants in environmental matrices

Overall, all eight active ingredients used in biocidal ARs in Germany (i.e., warfarin, chlorophacinone, coumatetralyl, bromadiolone, difenacoum, brodifacoum, difethialone, and flocoumafen) as well as two pharmaceutical anticoagulants (i.e., phenprocoumon, acenocoumarol) were targeted in this monitoring study. Aqueous samples were extracted by solid phase extraction with hydrophilic-lipophilic balanced sorbent material using a modified method by Gómez-Canela et al. (2014b) as summarized in the Supplementary Material (SM). Ultra-sound assisted solvent extraction and dispersive solid phase extraction (dSPE) were used for the extraction and clean-up of WWTP solids (e.g., activated sludge, biosolids, and prewashed mineral material from grit chamber). Details can be found in the SM. Extraction of SPM and sediment samples followed the same procedure but omitted further clean-up by dSPE. Mean recoveries and standard deviations for each analyte are provided in Tables S2 – S3 for selected matrices. Ultra-sound assisted solvent extraction and dSPE cleanup procedures for all biological tissue samples (i.e., soft body mussel, fish liver, fish filet) are described in detail in Regnery et al. (2019b). Total lipid content in homogenized tissue samples was determined according to Smedes (1999). Additional information about all biological samples analyzed in this study is provided in Table S1 in the SM.

All sample extracts were analyzed by liquid chromatography – tandem mass spectrometry (LC-MS/MS) in negative electro-spray ionization mode using an Agilent 1260 Infinity LC (Waldbronn, Germany) coupled with a Sciex 4500 QTrap MS/MS system (Darmstadt, Germany). Instrument specifications and details of the analytical method are provided elsewhere (Regnery et al. 2019b). Individual deuterated internal standards were used for quantification of target analytes, namely difenacoum-d4, brodifacoum-d4, flocoumafen-d4, phenprocoumon-d5 (all Toronto Research Chemicals, North York, Ontario, Canada), bromadiolone-d5, warfarin-d5, chlorophacinone-d4 (all C/D/N Isotopes Inc., Pointe-Claire, Quebec, Canada), and difethialone-d4 (TLC, Aurora, Ontario, Canada). Coumatetralyl and acenocoumarol were quantified based on warfarin-d5. Analyte peaks with a signal-to-noise ratio of less than 10 or 3 of the mass transitions used for quantification and confirmation, respectively, or shifted retention time compared to their respective isotope-labeled analogs were discarded from further data evaluation. Samples with residual AR concentrations outside the calibration standard range (i.e., 0.01 – 5 ng/mL) were diluted accordingly and reanalyzed. Reported analyte concentrations in biological tissues are based on wet weight, whereas those in all other solids are based on dry weight. Method quantification limits (MQL) were in the low ng/L and ng/g range respectively for all analytes and are summarized in Table S4 in the SM. Values above the method detection limit (MDL) but below the respective MQL are denoted (i.e., parenthesized) when provided in Tables 1 and 2 and Table S1 in the SM.

2.4. Statistical analyses

Statistical analyses were performed using Origin 2017G, version b9.4.0.220 (OriginLab Corporation, Northampton, MA, USA). The significance of the differences of total hepatic AR concentrations in fish between groups was assessed through one-way ANOVA followed by Tukey-Kramer post-hoc test or through student's t-test when two groups were compared. Statistical differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Occurrence and fate of anticoagulants in WWTPs and receiving streams

Overall, a total of 242 environmental samples were screened for residues of eight ARs and two pharmaceuticals. Given the multitude of different sample matrices and analytes, Figures 3 and 4 illustrate the detection frequencies of all anticoagulant residues above their MQL in all samples collected at monitoring sites A and B over the course of this study. A summary of average operational and water quality parameters at the investigated WWTP A and WWTP B during respective monitoring campaigns is provided in Table S5. In addition, their daily raw wastewater inflow rates and total organic carbon loads, individual sampling dates, and daily total precipitation in the area recorded at the nearest weather station are depicted in Figures 5 and S1, respectively. Additional water quality parameters of Moselle and Queich as well as characteristics of select solid samples are summarized in Tables S6 – S8 in the SM.

Phenprocoumon was the only target substance that was frequently detected above its MQL in raw and treated wastewater at both WWTP (Table 1). Phenprocoumon is extensively metabolized in humans by hepatic microsomal enzymes (e.g., cytochrome P450 2C9) and is excreted almost entirely as a glucuronide conjugate, with less than 10% of the dose as parent compound (Kasprzyk-Hordern 2010). Higher concentrations of phenprocoumon in effluent compared to corresponding influent samples at WWTP A might be explained by cleavage of glucuronide conjugates during biological treatment. Similar findings were reported by Du et al. (2014) for warfarin. Activated sludge samples revealed no residues of phenprocoumon above MQL at WWTP A. At WWTP B, phenprocoumon was frequently detected at very low levels in samples of activated sludge (0.3 ± 0.1 ng/g dry weight) and biosolids (0.2 ± 0.1 ng/g dry weight). It was also present in sludge liquor (46.8 ± 21.8 ng/L) and grit chamber solids washing water (4.8 ± 1.7 ng/L) (Figure 4). Based on its physicochemical properties (i.e., water solubility of 12.9 mg/L at 20ºC and *n*-octanol-water partition coefficient log P_{OW} of 3.6 at neutral pH), phenprocoumon presumably ranks between first-generation and second-generation ARs. Their water solubility and estimated log P_{OW} at neutral pH are in the range of 267 – 460 mg/L and 0.7 – 2.4 (first-generation) and 0.1 – 18.4 mg/L and 3.8 – 8.5 (second-generation), respectively (Regnery et al. 2019a). Although a slight reduction (i.e., 12 ± 8%) of phenprocoumon concentrations in wastewater was observed after treatment at WWTP B (Table 1), results indicate poor biodegradability. This is in good agreement with predicted values (i.e., not readily biodegradable according to EPI Suite^{TM} (US EPA 2012)) and previous observations (Regnery et al. 2019b, Wode et al. 2015). Most likely because of dilution effects, its concentrations in surface water samples from Queich and Moselle were five- and fortyfold lower, respectively compared to discharged effluent concentrations (Table 1). Despite short predicted photolytic half-lives, anticoagulants were shown to be hydrolytically stable in water under environmentally relevant conditions and are not expected to partition to the atmosphere (Regnery et al. 2019a).

While warfarin was frequently detected in wastewater and surface water samples at trace level, its concentrations rarely exceeded MQL at both monitoring sites (Figures 3 and 4 and Table 1). The maximum concentration measured in raw wastewater and sludge liquor at WWTP B was 1.5 ng/L. Acenocoumarol was not detected above MQL at all. As warfarin is the only anticoagulant that is concurrently authorized for biocidal and pharmaceutical use, its presence in wastewater and receiving surface waters can be linked to rodent control measures (Regnery et al. 2019b, 2020) as well as consumption of blood-thinning medication by resident population (Ajo et al. 2018, Regnery et al. 2019a, Santos et al. 2013). Like phenprocoumon, warfarin is extensively metabolized in humans and only about 2% of the typical daily prescription dose is excreted as unchanged active ingredient (Crouse et al. 2012, Park 1988). According to IQVIA MIDAS, the annual domestic pharmaceutical use of these blood-thinning agents accounted for 801 kg of phenprocoumon and 26 kg of warfarin in Germany in 2018 (IQVIA 2019). While acenocoumarol is commonly used in neighboring European countries (e.g., The Netherlands, Italy, Switzerland), no product with the active ingredient acenocoumarol has been marketed in Germany since 1993 (DIMDI 2020).

The active ingredients brodifacoum and bromadiolone, which had been applied for rodent control by the municipality, were sporadically detected in samples from various WWTP and environmental compartments at monitoring site A, but concentrations rarely exceeded MQL (Figure 3). After several rain events caused elevated water levels due to runoff (Figure S2), bromadiolone was detected above MQL in a surface water grab sample (4.3 ng/L) from Queich during sampling S8. In addition, it was detected in one SPM sample at sampling S9 just under the MQL of 1.0 ng/g dry weight. At WWTP A, brodifacoum was detected in one 24-hour composite sample of raw wastewater characterized by high proportions of stormwater runoff during sampling S5 (2.3 ng/L) as well as three 24-hour composite samples of treated effluent during samplings S5, S6, and S9 (max. 2.7 ng/L, Figure 5). Brodifacoum was also detected in sandy material obtained from the bottom of a CSO structure (0.5 ng/g dry weight) as well as two grab samples of raw wastewater (max. 2.5 ng/L) that had been directly collected from the main sewer of a baited district at sampling S9. Despite such few quantifiable detections of both ARs in aqueous and solid samples, brodifacoum and bromadiolone were prevalent in liver tissue samples of fish caught in the small stream Queich in 2019 (Figure 3). Residues of difethialone and coumatetralyl were also detected in these fish liver samples, whereas difenacoum, flocoumafen, and chlorophacinone were not detected above MQL in any of the samples at monitoring site A (Figure 3). In contrast, corresponding filet samples contained no anticoagulant residues (Table S1).

At WWTP B, solely chlorophacinone occurred in one biosolids sample (3.2 ng/g dry weight). With the exemption of warfarin, none of the other biocidal anticoagulants were detected above MQL in any of the samples collected at WWTP B. However, brodifacoum (0.5 ng/g dry weight) was detected in sandy material that had been removed from the sand trap of a baited district's stormwater retention basin during maintenance operations. This stormwater retention structure generally discharges into one of Moselle's small tributary creeks. Although none of the analyzed surface water, SPM, sediment, and mussel samples from Moselle revealed quantifiable AR concentrations, residues of second-generation ARs were frequently observed in the liver of indigenous fish, first and foremost brodifacoum and difenacoum. Analyzed corresponding filet samples revealed no residues (Figure 4).

3.2. Evidence of anticoagulant rodenticide emission sources and pathways

Brodifacoum-containing bait blocks were exclusively used during sewer baiting at both communities in the respective year of monitoring (as well as the previous baiting campaign). Applying the calculations of the recently revised emission scenario document for biocides used as rodenticides for combined sewer systems (ECHA 2018b), the worst-case predicted direct and indirect emissions of active ingredient to WWTPs during sewer baiting (i.e., in the time period between initial bait placement and first inspection) amount to approximately 0.1 kg brodifacoum/day at WWTP A and less than 0.03 kg brodifacoum/day at WWTP B (based on provided information such as bait amount and product specifics, refer to sections 2.1 and 2.2). Hence, worst-case predicted brodifacoum concentrations in raw wastewater influent were expected to be in the range of 5.2 ng/L at WWTP A and 1.1 ng/L at WWTP B according to mean daily discharge rates (Table S5). Notably, worst-case predicted brodifacoum concentrations remained below its MQL in wastewater at WWTP B (Table S4).

Few detections of brodifacoum in the 2 ng/L concentration range in raw wastewater and treated effluent at WWTP A confirmed that sewer baiting can lead to indirect (e.g., via rat carcasses, urine and feces) and direct (e.g., via scouring and spillage) release of active ingredients into wastewater. A previous study provided crucial evidence that anticoagulants are not completely removed during conventional wastewater treatment and will enter the aquatic environment by way of effluent discharges (Regnery et al. 2019b). Nonetheless, information about the fate of ARs during conventional or advanced wastewater treatment is scarce. Baits deployed in the combined sewer system connected to WWTP A faced an increased risk of scouring (e.g., when precipitation causes a sudden increase within the combined sewer system) as remaining baits were usually not removed from the sewers at the end of annual preventive baiting campaigns according to provided information. Active ingredients are not chemically bound to the bait material and can be released upon disintegration of baits, e.g., during prolonged exposure to moist or wet conditions. As depicted in Figure 5 for the monitoring period in 2018, stormwater runoff repeatedly caused a surcharge in the combined sewer system and increased discharge rates at WWTP A (e.g., during sampling S5 when brodifacoum was detected in the influent). Thus, a substantial number of deployed baits were repeatedly immersed in wastewater in the combined sewer due to elevated wastewater levels or backwater. Diffuse release of active ingredients into stormwater runoff from rodent control measures at the surface by residents or pest control professionals (e.g., around buildings) were likely additional emission sources throughout the urban area (Spahr et al. 2020).

Notably, a short-duration extreme precipitation event occurred at monitoring site A on June 11, 2018 (i.e., between samplings S8 and S9) and caused severe flooding of the downtown area due to backwater in the combined sewer system and stormwater channels (Figure 5). Capacities were greatly exceeded at WWTP A and several intermittent CSO retention structures, which resulted in confirmed CSO discharges into Queich. Moreover, WWTP staff reported a large number of dead rats and mounting wires from disintegrated baits that were retained at the screen during this event. It was assumed that the majority of baits in the sewer was affected by scouring (‘worst-case-scenario’) at this event and thus emitted brodifacoum. Obviously, the flash flood also immersed the tamper-resistant bait stations used for surface rat control with bromadiolone that had been deployed near watercourses throughout the urban center. In good agreement, brodifacoum (0.5 ng/g dry weight) as well as traces of bromadiolone (i.e., just under the MQL) were detected in deposited solids from the bottom of two different CSO structures after this precipitation extreme.

Due to extensive impervious surfaces, densely populated urban and suburban areas are prone to flash floods during short-duration (i.e., hourly) precipitation extremes. A recent study estimated the extent and frequency of environmental impacts due to such heavy rainfall events in Germany between 2005 and 2017. CSOs and thus discharge of highly diluted but untreated sewage directly into receiving surface waters occurred in 65% of the investigated heavy rainfall events (Kind et al. 2019). A substantial increase in short-duration precipitation extremes as a consequence of a changing climate has been predicted (Lenderink and van Meijgaard 2008). This is even more critical for the application of AR-containing baits in stormwater channels that are not connected to retention basins or WWTP but discharge directly into natural water bodies. According to representative survey results, about 30% of German municipalities that applied rat control in their sewer systems in 2017 also applied AR-containing baits in stormwater channels (Regnery et al. 2020).

As implied by low predicted worst-case concentrations (ECHA 2018b) at WWTP B, ARs were not detected above MQL in WWTP sample matrices throughout the treatment train during normal operation of the associated combined sewer system. Nevertheless, brodifacoum was detected in deposits from the sand trap of one of the city's stormwater retention basins. Emissions likely resulted from diffuse release of active ingredients into stormwater runoff during outdoor rodent control as AR-containing bait was generally not applied in or near the city's stormwater infrastructure according to personal communication. Though no samples of deposits from CSO retention structures became available for analysis at monitoring site B during the sampling period, CSO discharges at WWTP B after heavy or prolonged rainfall most likely contributed to emissions of ARs into Moselle. Similar to what had been observed at monitoring site A in 2018, heavy rainfall at site B (i.e., 37 mm rainfall in one hour) caused flash flooding in several baited city districts shortly after the end of the monitoring campaign (Figure S1). As illustrated in Figure 2, several smaller conventional municipal WWTP (mostly cluster WWTP) discharge into Moselle downstream of WWTP B. The neighboring association of municipalities located North-East of Trier (approximately 28,200 inhabitants total) is connected to the next smaller WWTP along this stretch of Moselle. When surveyed via telephone, their municipal pest control official confirmed the annual use of approximately 300 bait blocks (i.e., 200 g each containing 0.005% of difenacoum) for sewer baiting among all 19 municipalities in 2019. After reported rat sightings above ground by residents, trained municipal workers applied baits in the combined sewer system of the respective municipality. According to this communication, difenacoum-containing baits had been used for sewer baiting since 2013. Hence, municipal sewer baiting activities can explain the observed hepatic brodifacoum and difenacoum residues in fish from monitoring site B (Table 2). In good agreement, mainly baits containing brodifacoum, difenacoum, or bromadiolone were used for sewer baiting in Germany in 2017 (Regnery et al. 2020), whereas difethialone and coumatetralyl are preferably used in agriculture (Koivisto et al. 2018, Regnery et al. 2019b).

Previous studies suggested that unlike high-volume pharmaceutical anticoagulants, biocidal anticoagulants are difficult to capture in routine surface water or WWTP monitoring schemes due to the transient character of AR input rates as well as elevated detection limits compared to predicted environmental concentrations for these compartments. In Germany, difenacoum, brodifacoum, and bromadiolone were estimated to exhibit the highest market shares of AR active ingredients based on registered commercial biocidal products. Still, actual quantities of these active ingredients applied as rodenticides appear minor compared to other high-volume chemicals (Regnery et al. 2019a). Results summarized in Figures 2 and 3 on the one hand corroborate the assumption that actual AR concentrations in WWTP influent, effluent, and receiving surface waters are generally either too low or/and sporadic to be routinely monitored using current analytical methods, even with extensive sample enrichment and cleanup. On the other hand, they demonstrate that analyses of biological tissue samples provide crucial information regarding the burden of the aquatic environment with these PBT/vPvB substances as fish reflect an average exposure to AR emissions over time.

3.3. Hepatic residues of anticoagulant rodenticides in fish

Recent research demonstrated that second-generation ARs bioaccumulate in fish liver under environmentally realistic conditions and exposure scenarios (Kotthoff et al. 2019, Regnery et al. 2019b). Anticoagulants' high protein binding capacity and the persistence of specifically second-generation ARs in liver tissues of terrestrial wildlife is well documented (Horak et al. 2018). So although it is generally difficult to link hepatic AR residues in wild fish to distinct exposure events, there is an undeniable relationship between flushed away brodifacoum-containing baits during sewer baiting in 2018 at site A and elevated brodifacoum residues (5.2 – 29.9 ng/g wet weight) in the liver of all analyzed fish from the small stream Queich in 2019 (Table 2). As mentioned earlier, the effluent contribution of WWTP A under dry weather condition is approximately 7%. Moreover, frequent detections of hepatic bromadiolone residues (max. 1.0 ng/g wet weight) confirmed the assumption that outdoor surface baiting in the vicinity of watercourses also represents a prominent emission source of ARs into the aquatic environment. All fish from Queich were captured at the same time and location approximately 2 km downstream of WWTP A's discharge point one year after the flash flood incident (Figure 1). Distinct hepatic phenprocoumon residues (0.03 – 0.22 ng/g wet weight) due to the high-volume use and release of phenprocoumon indicated their frequent exposure to high WWTP effluent contributions in the small stream. Yet, the median concentration of phenprocoumon in these samples is < 1% that of the concentration of brodifacoum (Table 2). Unlike second-generation ARs, previous findings and an estimated bioconcentration factor

(BCF) of 122.3 L/kg in fish (BCFWIN v2.17, US Environmental Protection Agency) already pointed towards marginal bioaccumulation potential of phenprocoumon in native aquatic organisms (Regnery et al. 2019b). In contrast, the estimated BCF for brodifacoum in fish is 35,648 L/kg (Regnery et al. 2019a). In order to provide catches for recreational fishing, the Queich is stocked with hatchery-reared brown trout (i.e., individuals between 30 – 35 cm total length and 0.3 – 0.5 kg total weight) each year in late February/early March by the local fishing association. The analyzed brown trout individuals with total hepatic anticoagulant residues of 19.0 and 32.6 ng/g wet weight had likely been released in spring 2018 based on their total length of 37 and 40 cm, respectively (Table S1). Notably, no anticoagulant residues were detected in liver samples of unexposed hatchery-reared fish analyzed as reference material (Regnery et al. 2019b).

Elevated hepatic residues of brodifacoum (max. 19.8 ng/g wet weight) and difenacoum (max. 16.5 ng/g wet weight) were also detected in fish from the considerably larger stream Moselle that had been caught between 2017 and 2019 about 25 – 30 km downstream of WWTP B (Table 2), presumably as a consequence of sewer baiting as mentioned above. Available experimental (1100 L/kg) and estimated (451, 9010, and 35,645 L/kg) BCF of difenacoum in fish are similar to those of brodifacoum (Regnery et al. 2019a). Interestingly, no AR residues were detected above MDL in pooled mussel samples from five different Moselle sampling sites. Two sites thereof corresponded with sampling locations of fish with hepatic AR residues. This is in good agreement with findings from Main River (Regnery et al. 2019b) and suggests that AR bioaccumulation processes fundamentally differ among these aquatic organisms and are not just driven by lipophilicity (Table S1).

Unfortunately, substantial data gaps exist regarding the understanding of AR uptake routes (e.g., aqueous uptake of water-borne chemicals, dietary uptake by ingestion of contaminated food, prey, or particles) in freshwater environments. Overall, hepatic AR residues were found in fish species from all sections of the investigated surface water bodies: sub-surface (e.g., chub), mid-water (e.g., perch, roach, brown trout), and benthic (e.g., gudgeon, round goby). It has been assumed that the abundance of ARs in liver of fish species is likely correlated with their feeding habits (Regnery et al. 2019a). At the small stream Queich, highest brodifacoum concentrations of 25.1 and 29.9 ng/g wet weight liver were observed in common gudgeons (Table S1), which inhabit freshwater habitats with sandy or gravelly bottoms and predominantly feed on benthic invertebrates (e.g., worms, aquatic insects and larvae, small mollusks). Gammarids exposed to wastewater effluents (Miller et al. 2019, Munz et al. 2018) or terrestrial invertebrates feeding on AR-containing bait may function as vector in the environment (Masuda et al. 2014, Pitt et al. 2015). Based on their physicochemical properties (e.g., not readily biodegradable, low water solubility, high lipophilicity, and photolytic instability) at ambient environmental conditions, second-generation ARs might occur particle-bound in wastewater effluents rather than freely dissolved. The potential formation of non-extractable residues of second-generation ARs in organic-rich matrices such as activated sludge under environmentally realistic exposure is thus another aspect that should be considered. Irreversible binding of phenolic compounds to humic substances and activated carbon is well documented (Burgos et al. 1996). Interactions of natural organic matter and AR can involve numerous mechanisms depending on functional groups (e.g., phenolic hydroxyl group) and environmental conditions (e.g., pH, ionic strength). In addition to hydrophobic interactions, complexation may result from ion exchange reactions, ion bridging, and hydrogen bonds (Andre et al. 2005, Delle Site 2001). Covalent binding reactions such as biologically-mediated or mineral surface-catalyzed oxidative coupling may account for irreversible binding of ARs to natural organic matter after adequate exposure (Burgos et al. 1996); bindings that might be reversed through metabolic processes in fish after ingestion (Tao et al. 2011). Two primary classes of enzymes involved in these biological coupling reactions, peroxidase and phenol oxidase, for example belong to the microbial community functional structure of activated sludge in municipal WWTPs (Wang et al. 2014). Bioavailability of non-extractable pesticide residues and their potential transfer along the food chain was already suggested for soil-dwelling organisms such as earthworms (Barois et al. 1993, Gevao et al. 2001). If this assumption extrapolates to fish, benthivorous fish potentially play a key role in the transfer of second-generation ARs through the trophic levels of the aquatic food web.

Highest total AR concentrations measured in omnivorous feeders such as roach and chub from Queich (11.3 and 10.2 ng/g wet weight, respectively) and Moselle (9.2 ng/g wet weight) were similar to those observed in the liver of chub individuals from other German streams with comparable effluent contributions (Regnery et al. 2019b). Fish with a predominantly piscivorous diet (i.e., pike-perch and perch with a total length exceeding 15 cm) experienced on average significantly higher total hepatic AR concentrations (20.4 ± 7.8 ng/ng wet weight, n = 13) compared to omnivorous feeders (5.8 ± 4.0 ng/g wet weight, n = 13) in this study, $t(18) = 6.04$, $p < 0.001$. A one-way analysis of variance showed that total hepatic AR concentrations differed significantly among different fish species caught at the same monitoring site (Figure 6), $F(4, 29) = 30.46$, $p < 0.001$. In Moselle, adult perch primarily prey on round goby, an overabundant invasive species utilizing benthic habitats (Borcherding and Gertzen 2016). Post-hoc comparisons using the Tukey-Kramer test indicated significant differences between both fish species ($p < 0.001$) as shown in Figure 6 and illustrate the biomagnification potential of very persistent second-generation ARs such as brodifacoum in the aquatic food chain when released into the aquatic environment.

3.4. Potential risks for fish and fish-eating predators

During environmental risk assessment of ARs under the BPR, no unacceptable risks had been identified for the aquatic compartment despite considerable acute toxicity of several ARs to aquatic species. The predicted environmental concentration (PEC) of the active substances in surface water calculated for the sewer baiting scenario (ECHA 2018b) was below the corresponding predicted no-effect concentration (PNEC), above which adverse effects to aquatic organisms were to be expected (Umweltbundesamt 2019). Experimental monitoring data for the aquatic environment and WWTP effluent (this study and reviewed in Regnery et al. (2019a)) corroborate these minor surface water PEC. Concomitantly, biota monitoring demonstrates the widespread emergence of ARs in liver tissue of freshwater fish.

AR's toxic mode of action in warm-blooded vertebrates (mammals, birds) is caused by the inhibition of vitamin K epoxide reductase, which results in the disruption of the carboxylation of clotting factors and, subsequently, the clotting cascade (Rattner et al. 2014). In terrestrial non-target species, hepatic AR residue levels of > 100 to 200 ng/g wet weight were associated with lethality (Fourel et al. 2017). In comparison, highest total hepatic AR levels measured in fish were 35 ng/g wet weight (Figure 6 and Table S1). While species-specific differences between fish and mammals regarding their sensitivity towards AR likely exist (Riegerix et al. 2020), sub-lethal effects may impair the fitness of individuals (i.e., anticoagulants could act as stressors). To date, direct links to physiological effects at environmentally relevant concentrations other than those directly caused by impaired blood coagulation are unknown (Rattner et al. 2014, 2020) especially with regard to chronic exposure with multiple active ingredients. According to Rattner et al. (2014), adverse effects associated with AR exposure of non-target wildlife are manifold, including factors such as impaired body condition and reproduction, increased susceptibility to disease, reduced resilience to extreme weather conditions, sensitivity to other contaminants, and disturbance of population dynamics.

Anticoagulants entering the aquatic environment and accumulating in indigenous freshwater fish are likely to be transferred in the food chain, potentially affecting the health of fish-eating predators (e.g., protected species such as European otter and common kingfisher). While the European otter diet is dominated by fish (85% of consumed biomass), the common kingfisher is an almost exclusive fish-eater. Similar to other fish-eating birds swallowing their prey as a whole, the upper size limit of consumed fish is clearly dependent on its body shape. Total hepatic AR residues detected in fish from Moselle and Queich illustrate not only a substantial AR burden in large predatory fish but also small-growing fish species with a shorter life span (i.e., less than 5 years) such as round goby and gudgeon (Figure 6 and Table S1). The gudgeon for instance is a common prey of many fish-eating predators like European otter or common kingfisher (Čech and Čech 2015, Lyach and Čech 2017). A case study from a lowland trout stream in the Czech Republic revealed that otters mostly preyed upon small-growing fish species. The authors reported that gudgeon was the most important otter prey and represented 38% of consumed biomass (Lyach and Čech 2017). So far, several monitoring studies reported residues of ARs in the livers of avian and mammalian predators with a fish-eating diet, e.g., in European otter (Serieys et al. 2019), American mink (Ruiz-Suarez et al. 2016), white-tailed sea eagle, and osprey (Hughes et al. 2013).

The high bioaccumulation potential of second-generation ARs via the aquatic food web therefore may pose an increased threat to (higher) aquatic organisms and fish-eating predators, which is also reflected in the environment risk assessment of secondary poisoning via the aquatic food chain. The corresponding PEC/PNEC ratios for rodenticide product formulations containing 0.0029% brodifacoum were 532.5 and 968, respectively, indicating high unacceptable risks of secondary poisoning for fish-eating mammals and birds (ECHA 2018a). However, these risk calculations are mostly based upon generic equations and default assumptions as experimental data for the determination of BCF and biomagnification factors as well as chronic oral toxicity tests covering most sensitive aquatic focal predators for the derivation of $PNEC_{oral,predator}$ are lacking. Dedicated research to identify focal species of the aquatic food chain is required to address these uncertainties and to evaluate the extent to which apex predators are affected by ARs. Moreover, ecotoxicological consequences of chronic rodenticide exposure to indigenous freshwater fish at concentrations relevant for surface water bodies have not been identified yet.

3.5. Risk mitigation measures for the aquatic environment

Although mandatory instructions for use and risk mitigation measures for ARs were stipulated at EU-level under the BPR and best practice guidelines were stipulated during national biocidal product authorizations to minimize the risks of environmental exposure (Umweltbundesamt 2019), the extent of compliance with these provisions in Germany is largely unknown. Furthermore, weaknesses exist due to missing national legal provisions on the sale of biocides (Regnery et al. 2019a). Several studies noticed that the typical use of ARs commonly violates respective use and disposal instructions (Koivisto et al. 2016, Regnery et al. 2019b, 2020). While almost 80% of 322 municipalities participating in a nationwide survey applied chemical rat control in their sewer systems in 2017 in Germany, only 31% of municipalities thereof conducted a monitoring prior application of rodenticides to confirm the presence of rats in their sewer systems. Even less (i.e., about 26%) collected remaining baits for appropriate disposal and efficiency control at the end of sewer baiting campaigns (Regnery et al. 2020). Considering that bait intake during preventive rodent control measures in sewers might not exceed 2% of baited manholes (Gras et al. 2012), it becomes apparent that large amounts of bait face an unknown fate in the sewers. Yet, the implementation of legally binding instructions for use and risk mitigation measures for ARs starting from 2012 already led to a substantial decrease in bait amounts used for sewer baiting over the last decade in Germany (Regnery et al. 2020). However, temporal trend analysis (1992 – 2015) of residual brodifacoum concentrations in fish liver samples from two German streams (Saar and Elbe) revealed no decreasing trend until 2015 (Kotthoff et al. 2019). Concordantly, regulatory restrictions and stewardship programs failed to prove an impact on AR residue abundances in terrestrial non-target predators in urbanized areas in Denmark (Elmeros et al. 2018) and the United Kingdom of Great Britain (CRRU UK 2020) until now. Recently, regulation (EU) 2016/1179 resulted in the market launch of second-generation ARs with reduced concentrations of active ingredient (i.e., below 0.003%) to circumvent limitations of use in the biocidal sector due to the classification of products as toxic for reproduction (Regnery et al. 2019a). The application of commercial products with lower doses that are still efficient in controlling rodents, e.g. as shown for brodifacoum (Frankova et al. 2019), may assist in reducing the ecological impacts and environmental residues of ARs.

The risk of active ingredient release during chemical rodent control measures in sewer systems can be minimized if contact of bait material with water and wastewater is strictly excluded (e.g., by use of devices that keep the bait dry, deployment of baits exclusively in manholes free from backing-up/runoff pouring in). Commercial paraffin-type rodent bait blocks are expected to have a long shelf life and thus efficacy (toxicity) of the active ingredient, even after extended placement in sewers (Papini et al. 2019). However, they disintegrate over time under common sewer conditions such as permanently high humidity or frequent contact with wastewater, resulting in unintended emissions to the WWTPs and receiving surface waters (ECHA 2018b). Hence, immediate collection and appropriate disposal of remaining bait at the end of baiting campaigns is crucial to prevent unintended direct emissions of the active ingredient into wastewater. As discussed earlier, unforeseen short-duration precipitation extremes will likely occur more often in the future (Lenderink and van Meijgaard 2008). Though mandated by the instructions for use, reactive approaches such as timely removal of all baits deployed by wire in combined sewers during widespread baiting campaigns to prevent scouring are not feasible under such circumstances. In order to confine emissions solely to release from carcasses, urine, and feces of poisoned rats, proactive measures are advised to avoid any bait contact with water (ECHA 2018b). Such measures may involve the use of waterproof bait protection stations designated for the safe application of ARs in sewer systems, or advanced trap systems, which can be operated low maintenance for several months without using toxins. Traps and bait stations for controlling rats in sewers are often equipped with sensors and allow transmission of monitoring data to remote computers or smartphones, making manual inspections of baiting points unnecessary. This is a clear advantage as it substantially reduces time, labor, and human exposure, and provides a near real-time documentation of rat activity. Nevertheless, there is an urgent need for integrated, rat ecology based approaches (Byers et al. 2019, Traweger et al. 2006) alongside those technical innovations to achieve a major shift in rodent control practice and minimize the use of ARs in urban rat management.

4. Conclusions

This comprehensive monitoring study identified sewer baiting in combined sewer systems as substantial contributor of ARs in the aquatic environment. In conjunction with heavy or prolonged precipitation during bait application in combined sewer systems, a direct link between sewer baiting and AR residues in WWTP influent, effluent, and the liver of freshwater fish was demonstrated. Moreover, results confirmed insufficient removal of anticoagulants such as phenprocoumon, warfarin, and brodifacoum during conventional wastewater treatment and thus indirect exposure of aquatic organisms in receiving streams via WWTP effluents and CSOs. Outdoor surface baiting in the vicinity of watercourses and untreated storm drain in the presence of ARs also contributed to AR emissions in streams.

The high detection frequency of second-generation ARs in fish liver samples of up to 100% contrasted the low detection frequency of ARs above MQL in environmental samples from WWTP effluent, surface water, sediment, or SPM and clearly illustrates that environmental exposure involving PBT and vPvB substances such as second-generation ARs may be independent from the time and place of application of biocidal products. However, for substances where possible adverse effects are not correlated to immediate exposure, the PEC/PNEC approach as an indicator for environmental risks within the regulatory assessment of biocides reveals shortcomings. Thus, hazard assessments of PBT/vPvB substances are an appropriate and necessary instrument of the foresightful European chemical regulation. Although several criteria for exclusion are fulfilled to bar ARs from approval as biocidal active substances under the BPR, the European Commission decided that their non-authorization would have disproportionate adverse health impacts for human society in comparison to the predicted environmental risks arising from their use. Further research should investigate the potential risks and hazards of ARs in the aquatic environment in order to pave the way for scientific-based, targeted, and effective regulatory decisions. Until then, the ecological implications for aquatic organisms as well as fish-eating predators remain largely unknown.

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Figure 1. Generalized map of monitoring site A illustrating all sampling locations (orange arrows). Grey shapes indicate urban and suburban settlements. Abbreviations: CSO = combined sewer overflow; PE = person equivalent; SPM = suspended particulate matter; WWTP = wastewater treatment plant. Refer to Table S1 for further information about biological samples.

Figure 2. Generalized map of monitoring site B showing sampling locations (orange arrows) in the vicinity of the studied wastewater treatment plant (WWTP B). Grey shapes indicate urban and suburban settlements. Abbreviations: PE = person equivalent; rkm = river kilometer; SPM = suspended particulate matter. Refer to Table S1 for further information about biological samples.

Figure 3. Detection frequency of anticoagulant residues above their respective method quantification limits (MQL) in samples (total n = 90) from different WWTP and aquatic compartments collected at monitoring site A.

Figure 4. Detection frequency of anticoagulant residues above their respective method quantification limits (MQL) in samples (total n = 152) from different WWTP and aquatic compartments collected at monitoring site B.

Figure 5. Mean daily discharge (blue squares) and total organic carbon (TOC) load (black spheres) at WWTP A as well as individual sampling dates (S1 – S10). The green-shaded area illustrates the duration of the sewer baiting campaign. Daily total precipitation (grey bars) recorded at a nearby weather station was obtained from Agrar-Meteorologie Rhineland-Palatinate.

Figure 6. Box plots of total hepatic anticoagulant residue concentrations in individual fish (blue diamonds) at monitoring site B grouped by fish species and predominant diet (refer to Table S1 for details). The letters indicate statistical differences (Tukey-Kramer post-hoc test, $p < 0.05$) between the mean concentrations (grey squares) of perch (24.2 ± 7.3 ng/g), pike-perch (14.2 ± 4.9 ng/g), European eel (3.9 ± 2.6 ng/g), round goby (4.7 ± 3.1 ng/g), and chub (3.6 ± 3.5 ng/g).

Table 1. Mean concentrations and standard deviations of warfarin and phenprocoumon in corresponding 24-hour composite samples of raw and treated wastewater as well as surface water grab samples of receiving streams at monitoring sites A and B. '<' indicates values below the respective method detection limits, values denoted in parenthesis are below method quantification limits. ND = not detected.

Monitoring site	*n*	Warfarin (ng/L)	Phenprocoumon (ng/L)
A			
WWTP influent	10	(0.5 ± 0.2)	20.0 ± 10.4
WWTP effluent	10	<0.3	28.0 ± 15.9
Queich prior WWTP outfall	10	(0.1 ± 0.1)	1.6 ± 1.1
Queich after WWTP outfall	10	(0.1 ± 0.1)	5.8 ± 3.2
B			
WWTP influent	7	(0.8 ± 0.4)	24.2 ± 11.4
WWTP effluent	7	<0.3	21.1 ± 8.8
Moselle prior WWTP outfall	4	ND	0.4 ± 0.1
Moselle after WWTP outfall	4	(0.1 ± 0.0)	0.5 ± 0.1

Table 2. Analyte detection frequencies, median, 95th percentile, and maximum concentrations of anticoagulants in fish liver samples from two different streams. Analyte concentrations are reported in ng/g relating to wet weight. '<' indicates values below the respective method detection limits, values denoted in parenthesis are below method quantification limits. ND = not detected.

Analyte	Liver tissues from Queich, n = 11				Liver tissues from Moselle, n = 35[b]			
	Frequency	Median	95th percentile	Maximum	Frequency	Median	95th percentile	Maximum
Rodenticides	(%)	(ng/g)	(ng/g)	(ng/g)	(%)	(ng/g)	(ng/g)	(ng/g)
Brodifacoum	100	12.2	29.7	29.9	100	3.3	15.1	19.8
Bromadiolone	90.9	0.5	0.8	1.0	25.7	<0.1	2.3	7.5
Difenacoum	36.4	<0.3	(0.5)	(0.5)	82.9	1.1	13.8	16.5
Flocoumafen	ND	ND	ND	ND	48.6	<0.01	0.35	1.7
Difethialone	45.5	<0.1	1.8	2.3	31.4	<0.1	1.5	4.0
Chlorophacinone	ND	ND	ND	ND	2.9	<0.1	<0.1	(0.2)
Coumatetralyl	81.8	0.04	0.06	0.06	51.4	(0.01)	0.03	0.06
Warfarin	36.4	<0.01	(0.02)	(0.02)	22.9	<0.01	(0.02)	(0.02)
Σ Rodenticides [a]	100	12.7	32.4	32.6	100	5.9	28.1	35.2
Pharmaceuticals								
Phenprocoumon	100	0.10	0.20	0.22	60.0	(0.02)	0.05	0.09
Acenocoumarol	45.5	<0.01	(0.02)	(0.02)	ND	ND	ND	ND

[a] At least one of eight ARs detected. ARs were summed for each specimen, with median presenting the rank order 6th value for Queich River and the rank order 18th value for Moselle River

[b] Reduced number of samples (n = 30) analyzed for pharmaceutical anticoagulants

CRediT author statement

Julia Regnery: Conceptualization, methodology, validation, formal analysis, investigation, visualization, writing- original draft, project administration

Robert S. Schulz: Investigation

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Julia Bachtin: Investigation

Marvin Brinke: Conceptualization, writing - review & editing, project administration

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Georg Reifferscheid: Supervision

Anton Friesen: Conceptualization, writing - review & editing

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

Graphical abstract

Highlights

- Anticoagulant rodenticides (AR) are emitted to the aquatic environment
- Chemical rodent control in combined sewer systems contributes to AR emissions
- AR are not sufficiently removed during conventional wastewater treatment
- Untreated storm drain in the presence of AR also contributes to emissions
- Second-generation AR accumulate in the liver of wild freshwater fish

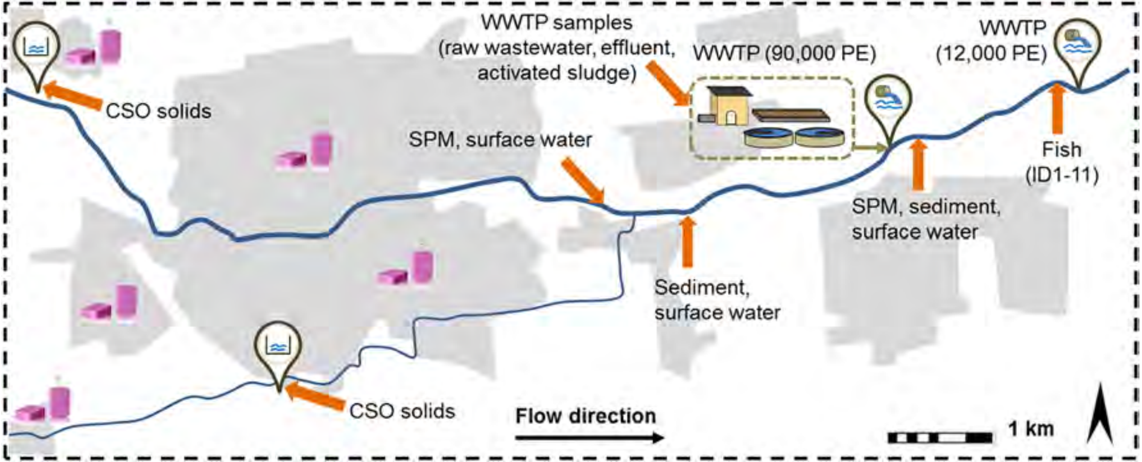

WWTP samples
(raw wastewater, effluent,
activated sludge)
WWTP (90,000 PE)
WWTP
(12,000 PE)
CSO solids
SPM, surface water
Fish
(ID1-11)
SPM, sediment,
surface water
Sediment,
surface water
CSO solids
Flow direction
1 km

Figure 1

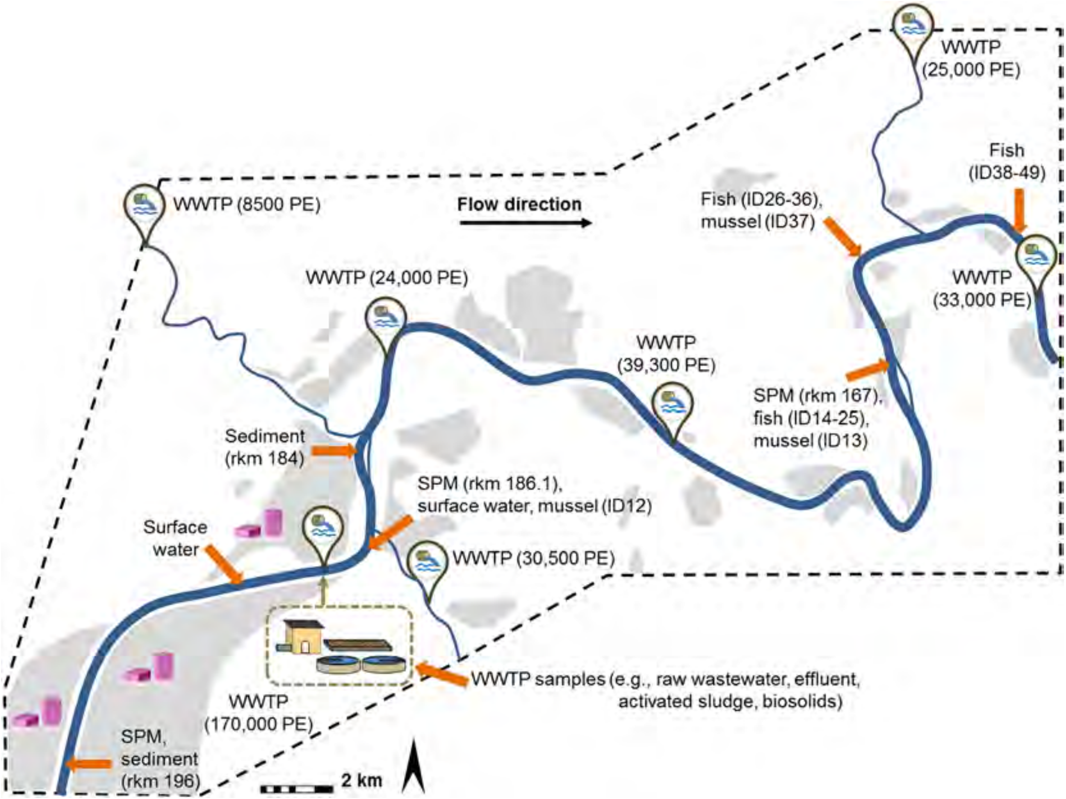
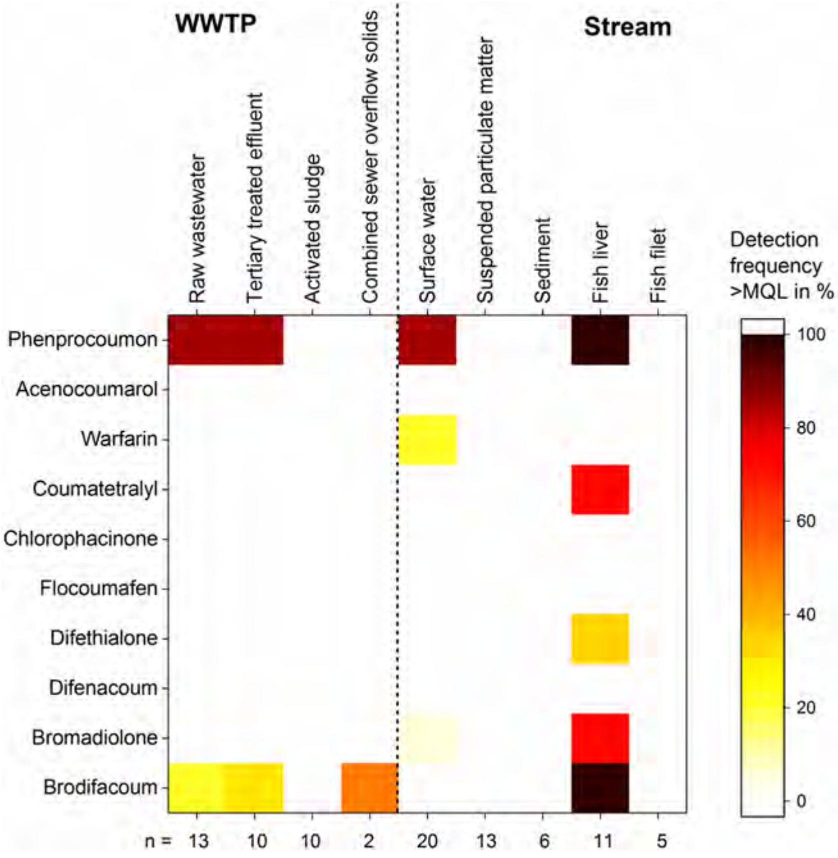

WWTP (25,000 PE)
Fish (ID38-49)
WWTP (8500 PE)
Flow direction
Fish (ID26-36), mussel (ID37)
WWTP (24,000 PE)
WWTP (33,000 PE)
WWTP (39,300 PE)
SPM (rkm 167), fish (ID14-25), mussel (ID13)
Sediment (rkm 184)
SPM (rkm 186.1), surface water, mussel (ID12)
Surface water
WWTP (30,500 PE)
WWTP samples (e.g., raw wastewater, effluent, activated sludge, biosolids)
WWTP (170,000 PE)
SPM, sediment (rkm 196)
2 km

Figure 2



WWTP
Stream
Raw wastewater
Tertiary treated effluent
Activated sludge
Combined sewer overflow solids
Surface water
Suspended particulate matter
Sediment
Fish liver
Fish filet
Detection frequency >MQL in %
Phenprocoumon
Acenocoumarol
Warfarin
Coumatetralyl
Chlorophacinone
Flocoumafen
Difethialone
Difenacoum
Bromadiolone
Brodifacoum
n = 13 10 10 2 20 13 6 11 5
100
80
60
40
20
0

Figure 3

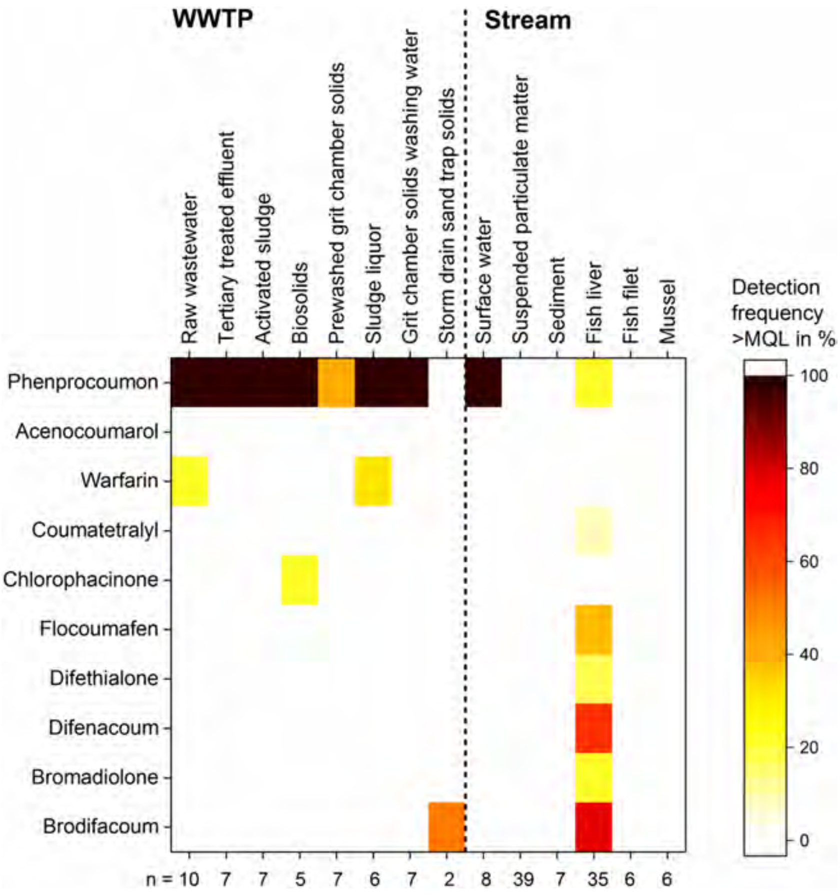

WWTP
Stream
Raw wastewater
Tertiary treated effluent
Activated sludge
Biosolids
Prewashed grit chamber solids
Sludge liquor
Grit chamber solids washing water
Storm drain sand trap solids
Surface water
Suspended particulate matter
Sediment
Fish liver
Fish filet
Mussel
Detection frequency >MQL in %
Phenprocoumon
Acenocoumarol
Warfarin
Coumatetralyl
Chlorophacinone
Flocoumafen
Difethialone
Difenacoum
Bromadiolone
Brodifacoum
100
80
60
40
20
0
n = 10 7 7 5 7 6 7 2 8 39 7 35 6 6

Figure 4

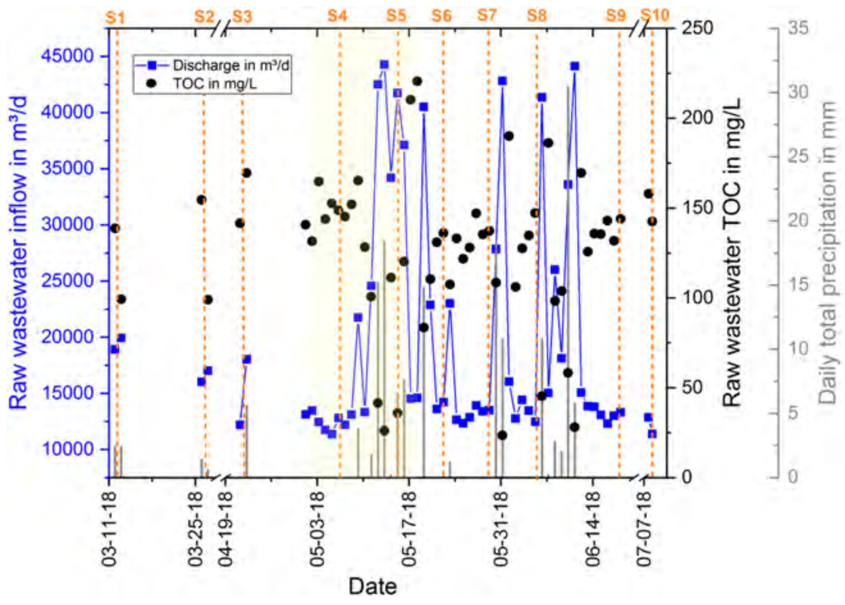
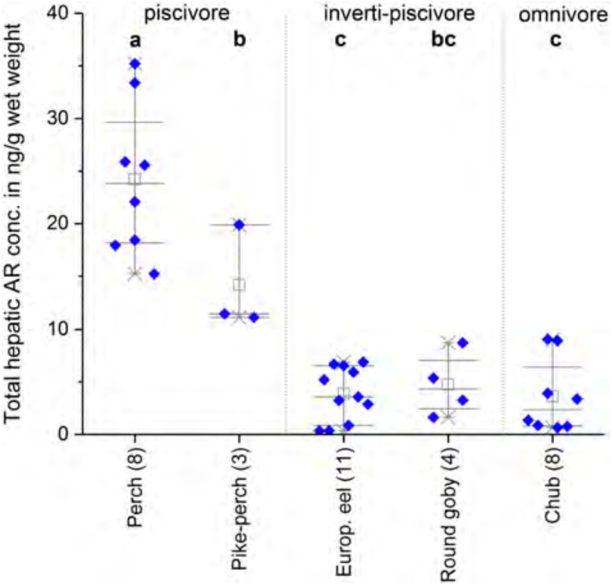

S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
Discharge in m³/d
TOC in mg/L
Raw wastewater inflow in m³/d
45000
40000
35000
30000
25000
20000
15000
10000
Raw wastewater TOC in mg/L
250
200
150
100
50
0
Daily total precipitation in mm
35
30
25
20
15
10
5
0
03-11-18
03-25-18
04-19-18
05-03-18
05-17-18
05-31-18
06-14-18
07-07-18
Date

Figure 5



Total hepatic AR conc. in ng/g wet weight
40
30
20
10
0
piscivore
inverti-piscivore
omnivore
a
b
c
bc
c
Perch (8)
Pike-perch (3)
Europ. eel (11)
Round goby (4)
Chub (8)

Figure 6

CAPITOL OFFICE
STATE CAPITOL
SACRAMENTO, CA 95814
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California State Senate

SENATOR
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TWENTY-SEVENTH SENATE DISTRICT

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December 6, 2019

The Honorable Karen Farrer, Mayor
The Honorable Mikke Pierson, Mayor Pro Tempore
The Honorable Rick Mullen, Councilmember
The Honorable Skylar Peak, Councilmember
The Honorable Jefferson Wagner, Councilmember
City of Malibu
23825 Stuart Ranch Road
Malibu, CA 90265

RE: Item 4.A. – Local Coastal Program Amendment No. 14-001

Dear Mayor, Mayor Pro Tempore, and Councilmembers:

As the City of Malibu continues its leadership in protecting native wildlife and sensitive coastal environment, I write to offer my support for a ban on the use of anti-coagulant rodenticides in the coastal zone.

Specifically, I support going beyond the staff proposal before you and enacting a ban on all pesticides, including herbicides, insecticides, rodenticides, and toxic chemical species. Anti-coagulant rodenticides are just one element of the larger problem of long-lasting poisons introduced to our coastal environment that place biological resources and sensitive habitats at risk.

I appreciate the complexity of the legal issues at hand, specifically whether state law precludes a city like Malibu from taking any action to ban the use of pesticides.

After consultation with numerous authorities, including in-house legal counsel, the County of Los Angeles, California Coastal Commission (Commission) staff and multiple non-governmental organizations, I believe that nothing precludes the City from acting on this issue because of how Malibu has structured this proposal – as an amendment to its Local Coastal Plan that is subject to approval by the Commission, which is itself a state agency. If the Commission determines the amendment does not comply with state law, then it will reject the proposal. If, however, the Commission approves it, then the state's Department of Pesticide Regulation (DPR), whose legal counsel decided to weigh in on this issue, can choose whether to challenge the Commission's decision.

As DPR's counsel acknowledges in its e-mail to the City Attorney, a Superior Court recently upheld a pesticide ban that the Commission approved as part of an LCP modified by Los Angeles County involving an area in the Santa Monica Mountains. DPR believes the facts in that case are different than the situation in Malibu, but for the purposes of what is before the City, that is an argument that is both specious and irrelevant.

The question before you is whether to adopt an LCP amendment to ban the use of certain pesticides and submit that amendment to the Commission, a state agency, for review and approval.

As a son of Malibu, I have the utmost respect for city officials and the process they undergo to make critical decisions like these. I look forward to our continued partnership to defend our community's extraordinary biodiversity and encourage you to take the necessary steps to protect our cherished natural habitats and wildlife.

Sincerely,

Henry Stern
Senator, 27th District
(D-Calabasas)

Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-018-1385-8

ADVANCEMENTS IN CHEMICAL METHODS FOR ENVIRONMENTAL RESEARCH

First evidence of anticoagulant rodenticides in fish and suspended particulate matter: spatial and temporal distribution in German freshwater aquatic systems

Matthias Kotthoff[1] · Heinz Rüdel[2] · Heinrich Jürling[1] · Kevin Severin[1] · Stephan Hennecke[1] · Anton Friesen[3] · Jan Koschorreck[3]

Received: 27 September 2017 / Accepted: 24 January 2018

Abstract

Anticoagulant rodenticides (ARs) have been used for decades for rodent control worldwide. Research on the exposure of the environment and accumulation of these active substances in biota has been focused on terrestrial food webs, but few data are available on the impact of ARs on aquatic systems and water organisms. To fill this gap, we analyzed liver samples of bream (*Abramis brama*) and co-located suspended particulate matter (SPM) from the German Environmental Specimen Bank (ESB). An appropriate method was developed for the determination of eight different ARs, including first- and second-generation ARs, in fish liver and SPM. Applying this method to bream liver samples from 17 and 18 sampling locations of the years 2011 and 2015, respectively, five ARs were found at levels above limits of quantifications (LOQs, 0.2 to 2 μg kg^{-1}). For 2015, brodifacoum was detected in 88% of the samples with a maximum concentration of 12.5 μg kg^{-1}. Moreover, difenacoum, bromadiolone, difethialone, and flocoumafen were detected in some samples above LOQ. In contrast, no first generation AR was detected in the ESB samples. In SPM, only bromadiolone could be detected in 56% of the samples at levels up to 9.24 μg kg^{-1}. A temporal trend analysis of bream liver from two sampling locations over a period of up to 23 years revealed a significant trend for brodifacoum at one of the sampling locations.

Keywords Anticoagulant rodenticides · Environmental monitoring · High-resolution mass spectrometry · Bream · Suspended particulate matter · Environmental Specimen Bank · Biocides

Highlights

- For the first time, anticoagulant rodenticides were identified in freshwater fish and SPM.
- A multi-method was developed to capture eight different anticoagulant rodenticides.
- Second generation anticoagulant rodenticides were found at levels > 10 μg kg^{-1}.
- A differing distribution of rodenticides between fish and SPM was found.
- At one site, the temporal trend of brodifacoum increased significantly in bream liver.

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Introduction

Since the introduction of warfarin as the first anticoagulant rodenticide on the US market in the late 1940s, rodent control worldwide has relied increasingly upon the use of these chemicals. As of 2017, anticoagulant rodenticides constitute more than 95% of the authorized rodenticides as biocides in the European Union (ECHA 2017b). The discovery of anticoagulant rodenticides (ARs) is today recognized as the most important step towards safer and more effective rodent control (Buckle and Eason 2015).

ARs comprise active substances belonging either to the class of 4-hydroxycoumarines such as warfarin or to 1,3-indandione derivatives such as chlorophacinone. Without regard to their chemical structure, ARs are grouped by their ability to prevent blood clotting (coagulation) by the inhibition of vitamin K which is essential for the production of several blood clotting factors such as prothrombin. Typical symptoms of AR intoxication, i.e., internal and external hemorrhages due to the increased permeability of blood vessels, occur several days after consumption of the rodenticide bait. This delayed mode of action is key to the effectiveness of ARs as it overcomes the bait shyness of rats.

Anticoagulant rodenticides are usually divided into first- and second-generation anticoagulant rodenticides (FGARs/SGARs) depending on the date of their introduction on the market. FGARs (i.e., warfarin, chlorophacinon, coumatetralyl) were firstly used in the late 1940s, 1950s, and 1960s, while SGARs (i.e., bromadiolone, difenacoum, brodifacoum, difethialone, flocoumafen) were developed in the 1970s and 1980s following an increasing concern about warfarin-resistant rodents. Ever since, ARs have been extensively used as pesticides to reduce human and animal infections by rodent-borne diseases, for crop protection against voles, or for species conservation on oceanic islands (Masuda et al. 2015). They are nowadays regulated in the European Union (EU) under the Biocidal Products Regulation (EU) No. 528/2012 (BPR) and the Plant Protection Products Regulation (EC) No. 1107/2009 (PPPR), depending on their intended use to either protect human health, animal health and materials or plants and plant products. Both regulations foresee that ARs need to be authorized prior to being made available on the European market. Under the PPPR, difenacoum (Reg. (EU) No. 540/2011) and bromadiolone (Reg. (EU) No. 540/2011) are the only anticoagulant active substances which are approved for the use in plant protection products in the EU. Under the BPR, the approval of eight anticoagulants, i.e., warfarin, chlorophacinone, coumatetralyl, bromadiolone, difenacoum, brodifacoum, difethialone, and flocoumafen as active substances for the use in rodenticides, have just recently been renewed. While the last authorization of an anticoagulant rodenticide as a plant protection product in Germany has expired in 2015, their authorizations as biocides in Germany have recently been prolonged (BVL 2017). As of September 2017, 704 rodenticide products were authorized in Germany under the BPR, of which about 91% contained an anticoagulant active substance, of these 12.2% FGAR and 79.0% SGAR (compare Table 1) (BAuA 2017).

The environmental risk assessment of ARs under the BPR authorization in the EU revealed high risks of primary and secondary poisoning for non-target organisms, which either feed directly on the bait or consume poisoned rodents. Moreover, all SGARs have been identified as being either persistent, bioaccumulative, and toxic (PBT-substances) or very persistent and very toxic (vPvB-substances). These inherent substance properties in combination with the given exposure of non-target organisms via primary and secondary poisoning and the extensive and widespread use of ARs are significant drivers for the likewise widespread contamination of various wildlife species worldwide. It is thus not surprising that residues of anticoagulant rodenticides, especially of the second-generation compounds, have been detected in a large variety of species. Residues of rodenticides were detected for example, in barn owls (Geduhn et al. 2016, Newton et al. 1990), tawny owls (Walker et al. 2008), common buzzards (Berny et al. 1997), golden eagles (Langford et al. 2013), polecats/mink (Elmeros et al. 2018, Fournier-Chambrillon et al. 2004, Ruiz-Suarez et al. 2014, 2016, Shore et al. 2003), weasels (McDonald et al. 1998), stoats (Elmeros et al. 2011), foxes (Berny et al. 1997, Geduhn et al. 2015, McMillin et al. 2008, Tosh et al. 2011), hedgehogs (Dowding et al. 2010), and snails (Alomar et al. 2018).

Most of these environmental monitoring studies focused on the terrestrial compartment, e.g., predatory birds (Gomez-

Table 1 Current numbers of registered biocidal products in Germany

Active substance	Number of registered products	%
Aluminum phosphide	9	1.3
Brodifacoum	196	27.8
Bromadiolone	127	18.0
Chloralose	51	7.2
Chlorophacinone	14	2.0
Coumatetralyl	14	2.0
Difenacoum	199	28.3
Difenacoum; bromadiolone	4	0.6
Difethialone	26	3.7
Flocoumafen	4	0.6
Hydrogen cyanide	1	0.1
Carbon dioxide	1	0.1
Warfarin	58	8.2
Total	704	100
FGARs	86	12.2
SGARs	556	79.0
Non-ARs	62	8.8

Ramirez et al. 2014, Ruiz-Suarez et al. 2014, Stansley et al. 2014, Thomas et al. 2011) and mammals (Quinn et al. 2012), as well as various non-target rodents (Elliott et al. 2014, Geduhn et al. 2014). However, little to nothing is known so far, about the exposure of aquatic life to ARs and the accumulation of ARs in aquatic food webs.

The environmental exposure assessment within the authorization of anticoagulant rodenticides under the BPR is based on the Emission Scenario Document (ESD) (Larsen 2003) which considers four main scenarios for the application of ARs, i.e., the application in and around buildings, in open areas (in rate holes), at waste dumps, and in the sewer system. Significant releases to surface water bodies are only assumed to occur from the application of ARs in the latter area of use, i.e., in sewer systems. It has been shown that AR can enter sewage treatment plants (STPs) and thereafter contribute to the loads of anticoagulants to receiving surface waters with effluents (Gomez-Canela et al. 2014). A maximum release to the sewerage system and consequently to surface water could result directly from the application of rodent bait into manholes of the sewer system and indirectly from the target animals' urine, feces, and dead bodies. The application of rodenticides in rainwater sewers which as a rule are not connected to a sewage treatment plant and discharge directly into receiving waters can be considered another release pathway.

Environmental monitoring of AR provides some specific challenges to the investigator. AR can enter the environment via different exposure routes where they have been shown to exhibit acute toxic effects at concentrations in the ppm and ppb range (e.g., bromadiolone (Eason et al. 2002, Thomas et al. 2011): LC_{50} of 2.86 mg L^{-1} for fish, *Lepomis macrochirus*; LD_{50} of 0.56 mg kg^{-1} in rat (oral) (ECHA 2010), or difethialone (ECHA 2007): EC_{50} of 4.4 μg L^{-1} for *Daphnia magna* acute, or LC_{50} of 51 μg L^{-1} for *Oncorhynchus mykiss*. SGARs in particular exhibit a high lipophilicity and environmental persistence and may thus enrich in predator tissues with high fat contents, e.g., mammalian liver (Eason et al. 2002, Thomas et al. 2011), which are complex matrices and thus require elaborate and challenging sample preparation. Furthermore, there are numerous AR substances that may enter the environment and so a comprehensive assessment of the presence of AR requires very sensitive and accurate multi-methods, covering a wide range of different ARs. Several analytical approaches for multi-methods for the quantitative determination of AR in biological samples have been developed, such as liquid chromatography (LC) and also ion chromatography (IC) coupled to tandem mass spectrometry (MS/MS) (Bidny et al. 2015, Chen et al. 2009, Jin et al. 2009, Jin et al. 2008, Marek and Koskinen 2007), two-dimensional LC coupled to MS/MS (Marsalek et al. 2015), IC coupled to fluorescence detection (Jin et al. 2007), methods using high resolution MS (Schaff and Montgomery 2013), and some other strategies that are presented in a review by Imran et al. (2015). Available analytical methods are so far hampered by the number of captured AR, as well as high limits of detection caused by complex biological and environmental matrices that are in contrast to low relevant environmental concentrations. However, the method we apply here is in good agreement (Hernandez et al. 2013) or better (Vandenbroucke et al. 2008a, Vandenbroucke et al. 2008b, Zhu et al. 2013) in terms of number of analytes covered and sensitivity with other LC-MS/MS-based methods for solid biological tissues such as liver and hair.

Good insight is available on risks of AR towards non-target mammals as well as exposure and associated risks of various predators (Christensen et al. 2012, Geduhn et al. 2016, Gomez-Ramirez et al. 2014, Hughes et al. 2013, Langford et al. 2013, Nogeire et al. 2015, Proulx and MacKenzie 2012, Rattner et al. 2014, 2015, Ruiz-Suarez et al. 2014, Thomas et al. 2011).

Even if concentrations are assumed to be low after systematic or accidental exposure of aquatic systems (Fisher et al. 2012, Primus et al. 2005), the environmental impact may yet be relevant due to the high bioaccumulation potential, especially of SGARs (Masuda et al. 2015). So far, no studies are available on AR residues and accumulation in fish or distribution of AR in natural aquatic systems. The aim of this study was to assess the exposure of freshwater fish to anticoagulant rodenticides by analyzing levels of anticoagulants in fish tissues. For this purpose, a highly sensitive and specific multimethod was developed to determine eight anticoagulants, which have been approved under the BPR for the use in rodenticides within the EU (cf. Table 2). We the applied this method in a spatial monitoring study for two time points for fish liver and one for suspended particulate matter (SPM) samples of the German Environmental Specimen Bank (ESB). Finally, retrospective analysis was performed for SPM and fish samples from selected sites to detect time trends. In addition, selected liver samples from otters (*Lutra lutra*) were analyzed to characterize the bioaccumulation potential of ARs in fish-eating mammal species.

Materials and methods

Collection and storage of samples

All samples were retrieved from the archive of the German Environmental Specimen Bank.

Bream (*Abramis brama*) samples were analyzed from 17 and 18 sampling locations for 2011 and 2015, respectively, and from 10 sampling years for two specific sampling locations. Sampling locations included 16 riverine sites and one (2011) and two (2015) lakes. Samples were processed and stored according to a dedicated ESB standard operating procedures (SOP) by Klein et al. (2012). SPM was analyzed from

Table 2 List of AR covered in this study used for quantification and information on used reference standards

AR	AR-generation	Chemical class (derivative)	Purchased from	Purity (%)
Brodifacoum	2	Hydroxycoumarine	Sigma-Aldrich	99.4
Bromadiolone	2	Hydroxycoumarine	Sigma-Aldrich	93.6
Chlorophacinone	1	Indandione	Sigma-Aldrich	98.9
Coumatetralyl	1	Hydroxycoumarine	Sigma-Aldrich	99.9
Difenacoum	2	Hydroxycoumarine	Sigma-Aldrich	98.9
Difethialone	2	Thiocoumarine	Dr. Ehrenstorfer	99.0
Flocoumafen	2	Hydroxycoumarine	Dr. Ehrenstorfer	98.0
Warfarin	1	Hydroxycoumarine	Sigma-Aldrich	≥ 98

the 16 riverine sampling sites sampled in 2015. SPM was collected and processed according to a specific SOP by Ricking et al. (2012).

Otter samples originate from the Upper Lusatia area in the east of Germany (partly Elbe catchment) and represent individuals that died as a result of traffic accidents or lethal diseases. The liver samples of five otter individuals were prepared and analyzed according to the protocol for bream liver samples.

Method summary

In order to optimize and merge available methods, and to secure the specificity of the method, the rodenticide analysis was performed on a UHPLC-chromatographic unit coupled to a high-resolution mass spectrometer operating at a resolution of 35,000. The adopted method mainly based on Thomas et al. (2011) for fish liver could be used to determine a total of eight different target molecules, as given in Table 2 and Table 3. The specificity of the method is assured by measuring the accurate mass of the analytes in tandem mass spectrometric mode (MS/MS) (see Table 3).

Table 2 harbors information on the reference substances used for quantification. Stable isotopically labeled (deuterated) internal standards (IS) were only available for bromadiolone (as D5; Campro Scientific, Germany, 99% D, 95% chemical, Lot # AB126P2), warfarin (as D5; Campro Scientific, Germany, 99% D, 99% chemical, Lot # E305P28), and chlorophacinone (as D4; Chiron AS, Norway, 99.4% D, 99% chemical Lot # 14266). The IS were added to the samples, but not used for evaluation in the final method.

A sample of about 0.5 g fish matrix (liver or muscle; frozen, cryo-milled ESB material) is mixed with roughly 3.5 g Na_2SO_4 (ratio 1:7), 100 μL IS solution (three IS, each 100 ng mL^{-1}), and 5 mL acetone in a 15-mL polypropylene test tube. This mixture is treated for 30 min in an ultra-sonic bath and for the same time on a vortex shaker. Subsequently, the test tube is centrifuged at 4000 rpm for 5 min. The clear supernatant is forwarded to a fresh test tube, whereas the pellet is extracted with an additional 4 mL volume of fresh acetone. The combined extracts were mixed with 1 mL of diethyl ether and evaporated in a N_2-stream at 50 °C to dryness. The remaining extract was then dissolved in 1 mL methanol and homogeneously mixed by treating for 5 min in an ultra-sonic bath. The slightly turbid suspension was forwarded to a 1.5-mL tube and centrifuged at 15,000 rpm for 2 min. The cleared supernatant was finally filtrated through a 25-mm diameter, 0.45-μm regenerated cellulose (RC) type membrane filter, before filling into a UHPLC (ultra-high performance liquid chromatography) vial for analysis.

A minimum of two solvent-based blank samples (up to four) were analyzed in every measurement series. Matrix-

Table 3 Accurate masses of ion transitions of rodenticides as used for the multiple monitoring method. The Q-Exactive instrument was run at a resolution of 35,000 ± 10 ppm

Substance	Theoretical mass of precursor [m/z]	Captured mass of product 1 [m/z]	Captured mass of product 2 [m/z]
Flocoumafen	541.16322	161.02353	289.08545
Bromadiolone	525.0707	250.06194	n.d.
Brodifacoum	521.07578	135.04408	187.03854
Difenacoum	443.16527	135.04442	293.13202
Warfarin	307.09758	161.02234	250.06195
Chlorophacinone	373.0637	145.02859	201.04637
Coumatetralyl	291.10267	141.07021	247.11263
Difethialone	537.05294	151.02104	n.d.

based quality control samples containing the rodenticides in defined concentrations (adapted to the expected concentration range in the samples: here 1.4 and 14 μg kg^{-1} for otter and SPM, and 1.0 and 10 μg kg^{-1} for bream liver) were measured about every 15 samples. Suitable rodenticide free matrices were identified in a preliminary screening. All samples were measured at least in duplicate, as specified in the respective table captions.

Instrumental parameters

A UHPLC Acquity (Waters), coupled to an Orbitrap Q-Exactive Plus (Thermo Scientific) high-resolution mass spectrometer, run in the multiple reaction monitoring mode with electrospray negative (ES-) ionization was used for all chemical analyses. Accurate masses of parent and daughter ions, as well as MS-parameters, were according to Table 3. The used column was 100 × 2 mm BEH C18, 1.7 μm (Waters), the column temperature was 55 °C, and 20 μL sample volume was injected and run with a flow of 0.35 mL min^{-1}. The solvents used were A: methanol +2 mM ammonium acetate in water (5 + 95, *v*/*v*) and B: methanol containing 2 mM ammonium acetate. The used UHPLC gradient program was 0 min 100% A → 10 min 100% B → 13 min 100% B → 15 min 100% A. Under the given conditions, of bromadiolone, two diastereomeric partners elute, which are reported here as a sum.

Method development and method validation

Initially, a comparison of AR concentrations in bream liver and fillet was performed by applying a crude preliminary method that had not been optimized. For difethialone and brodifacoum, we found 100- or 80-fold higher concentrations in liver, respectively. So, it was decided to focus on bream liver samples for further method development and subsequent analysis of environmental samples.

Commercial stable isotope labeled standards were purchased to improve the method. After repeated measurement cycles and calibrations, however, it was found that the analytical parameters are much better when using an external matrix matched calibration. So, the final method does not use the signals for the IS, but an external matrix calibration.

Calibration and validation of the method were performed by standard addition techniques using matrix calibrations in the range from 0.02 to 20.0 μg kg^{-1} and were evaluated to the lowest calibration level within the linear range of a calibration. Each calibration solution contained 100 μL of IS solution which were spiked with 25 μg L^{-1}, resulting in 5 μg kg^{-1} of each IS. The handling and measurement of the calibration and validation samples were identical to the treatment of the test samples. For validation of the method, six bream liver and SPM samples of 0.5 g each were fortified with defined AR at individual limit of quantification (LOQ) concentrations to prove for accuracy, repeatability, and precision at the LOQ level (standard addition technique), according to Table 4. Each sample was fortified with 100 μL of a solution containing all rodenticides in the respective concentrations ranging from 0.1 to 100 μg L^{-1} and with 100 μL of the IS solution. Otter liver was used to generate a respective matrix calibration, but due to limited sample material, no separate otter liver validation could be performed.

Due to varying sensitivities of individual AR, the dynamic ranges of the calibrations are different, but none of them showed an exponential behavior. To keep the procedure constant, even after the decision to omit using the IS, their addition to the samples was continued. For the given calibration ranges, all functions were linear and show coefficients of determination (r^2) of at least 0.99. The validated limits of quantification (LOQ) and standard deviations (SD) are given in Table 4. All data are reported on a wet weight basis.

Both matrices, bream liver and SPM, could be successfully validated at the indicated LOQ levels. These levels range in a substance, but also in a matrix-dependent manner from 0.2 to 2.0 μg kg^{-1}, and reflect the lowest achievable values according to observations derived from the matrix calibration functions shown in Fig. S1 of the Electronic supplementary material (ESM). The recoveries are within 90–110% and the relative standard deviation (RSD) is ≤ 10% ($n = 6$). The only exception is chlorophacinone whose mean recovery is 116% and RSD 26.3% in SPM. This seems acceptable since no quantitative data are being reported for chlorophacinone in this study. The achieved LOQs are similar or lower than recently published LC-MS/MS-based multi-methods for AR in tissues, ranging from 0.9 to 250 μg kg^{-1} (Fourel et al. 2017a, Hernandez et al. 2013, Jin et al. 2009, Marek and Koskinen 2007, Marsalek et al. 2015, Smith et al. 2017, Vandenbroucke et al. 2008b).

Analysis of temporal trends

Temporal trends for brodifacoum in fish tissue (wet weight data) were analyzed by applying a software tool from the German Environment Agency (LOESS-Trend, Version 1.1, based on Microsoft Excel). The application fits a locally weighted scatterplot smoother (LOESS) with a fixed window width of 7 years through the annual rodenticide levels. Then, tests on the significance of linear and non-linear trend components are conducted by means of an analysis of variance (ANOVA) following the procedure of Fryer and Nicholson (1999). For years with analytical results less than the LOQ, the data gaps were treated as ½ LOQ values.

Table 4 Studied AR and respective analytical parameters of method validation by fortification of respective matrix, $n = 6$

Substance	Bream liver			SPM		
	LOQ level [μg kg^{-1}]	Recovery [%]	RSD [%]	LOQ level [μg kg^{-1}]	Recovery [%]	RSD [%]
Flocoumafen	0.2	100	5.4	1.0	98	6.9
Bromadiolone	2.0	95	8.1	1.0	96	4.8
Brodifacoum	1.0	93	6.6	2.0	98	5.9
Difenacoum	0.2	96	9.7	1.0	98	10.3
Warfarin	0.2	103	6.9	0.2	102	7.5
Chlorophacinone	1.0	93	7.2	2.0	116	26.3
Coumatetralyl	0.2	110	4.1	0.2	106	5.4
Difethialone	1.0	95	3.3	1.4	103	4.6

Results and discussion

Results of environmental analysis

Spatial comparison: measurement of samples from different ESB sampling sites of the years 2011 and 2015

Results of the spatial monitoring exercise are presented in Table S1 (2011) and Table S6 (2015) in the electronic supplemental material and are summarized in Fig. 1 (for 2015) along with the spatial distribution of the sampling sites across Germany. Within all bream liver samples, only SGARs were found above the LOQ. For the year 2015, brodifacoum was the major AR found. It was detected in 88% of the samples with a maximum concentration of 12.5 μg kg^{-1} (average (Ø) 3.4 μg kg^{-1} and median 2.1 μg kg^{-1}). Difenacoum was found in 44% of the samples at comparably lower concentrations of up to 0.7 μg kg^{-1} (Ø 0.1 μg kg^{-1}). Bromadiolone was found in 17% of the samples at peaks of 7.1 μg kg^{-1} with Ø of 0.6 μg kg^{-1}, difethialone in 6% with highest levels at 6.3 μg kg^{-1}, and flocoumafen in 12% at highest levels of 0.3 μg kg^{-1}. For 2011, quite different substance and concentration patterns were found, as presented in Table S1, which may be due to the seasonal character of substance usage and varying intervals between application and sampling. To our knowledge, this is the first evidence of AR in freshwater fish tissue.

In addition, a set of co-located suspended particulate matter (SPM) samples from the year 2015 were analyzed. The results are presented in Table S3 and also included in Fig. 1. In contrast to results of the bream liver samples, in SPM, only bromadiolone was found above LOQ in 56% of the 16 samples with highest values of 9.2 μg kg^{-1} (Ø 4.9 μg kg^{-1}; median 4.3 μg kg^{-1}).

No ARs were detected > the LOQ in the five otter livers that were analyzed in addition to bream and SPM to exemplary include a fish-eating mammal as a top predator in the food web in this study. In contrast, in a study using French otter samples from 2010, 10% of the tested otter samples were contaminated with bromadiolone at levels of 400 and 850 μg kg^{-1} fresh weight (Lemarchand et al. 2010).

Temporal trend analysis: retrospective monitoring for rodenticides in fish liver samples from Saar River/Rehlingen and Elbe River/Prossen

Based on the results of the 2011 and 2015 spatial analysis, the sampling sites in Rehlingen at the Saar River and Prossen at the Elbe River were chosen for the temporal analysis.

The results of the temporal analysis are summarized in S4 (Saar/Rehlingen) and Table S5 (Elbe/Prossen).

From these temporal data, a significant time trend could be drawn only for brodifacoum at Saar/Rehlingen (Fig. 2). This trend indicated an average increase of brodifacoum at 0.3 μg kg^{-1} per year for the observed period and 1.3 μg kg^{-1} per year for the last 7 years.

Notably, brodifacoum was the most abundant AR measured in fish from both locations. Concentrations ranged between about 1 and 13 μg kg^{-1} in fish from Rehlingen and between 4 and 12 μg kg^{-1} in fish from Prossen, where it was found below LOQ only in the years 1992 and 2009. At Rehlingen also, bromadiolone, difenacoum, flocoumafen, and difethialone were found occasionally and at comparably low levels. Interestingly, for both sampling sites, the diversity of detected AR was higher in 2015 than in the years before. SPM was not subject to a retrospective analysis.

Assessment of relevance of rodenticide residues in fish and SPM

The analysis of fish samples at the different ESB sampling sites revealed the detectable occurrence in the order brodifacoum, difenacoum, bromadiolone, difethialone, and flocoumafen at levels above the LOQ. In contrast, in SPM, only bromadiolone was detectable.

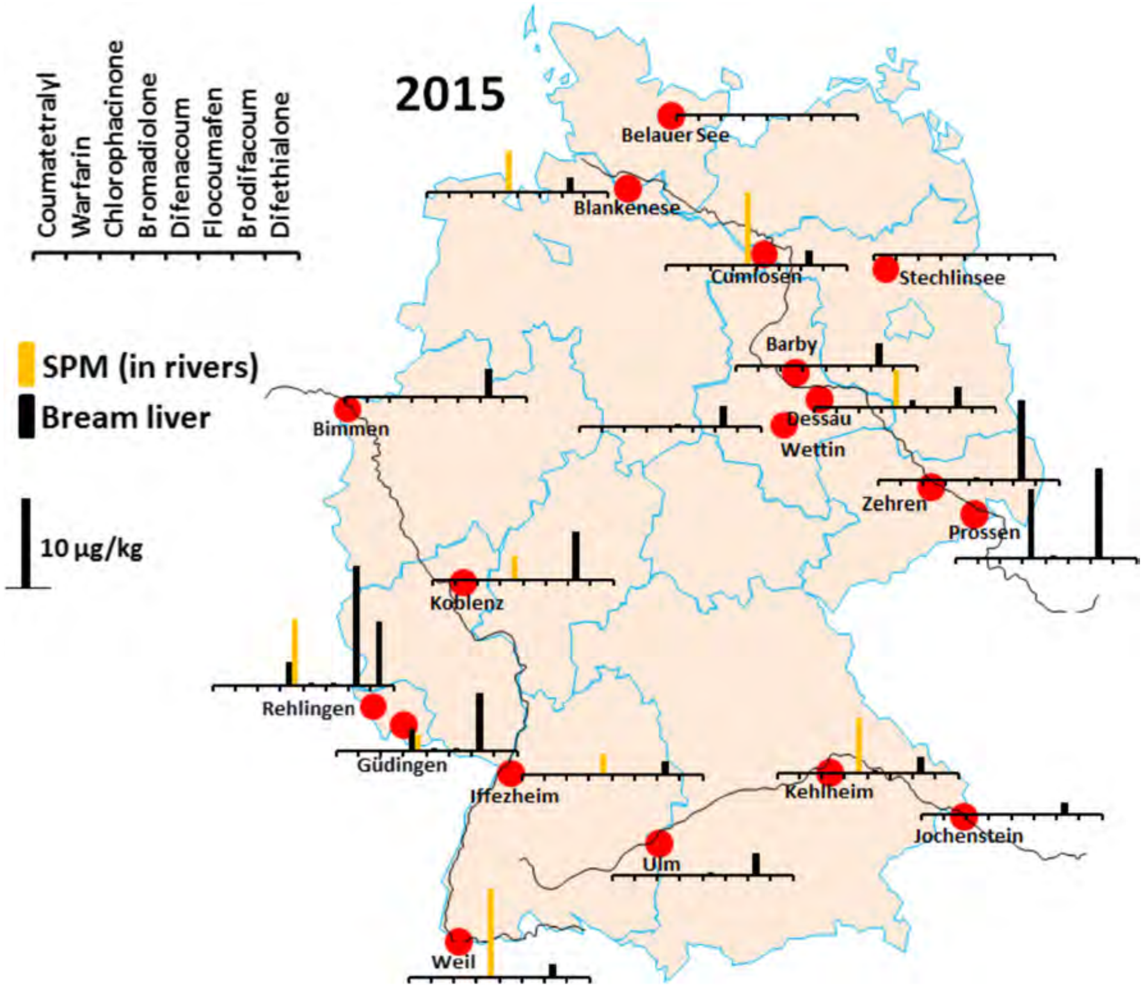

Fig. 1 Overview of 18 bream and 16 SPM sampling sites. Results of the spatial analysis for eight ARs in bream liver and SPM are displayed as black and yellow bars, respectively. For detailed results, see Table S1 and S3

Only SGARs were found above LOQ of the ARs measured in this study. This could be related to the higher persistency and potential for bioaccumulation of SGAR in comparison to FGAR. The partition coefficients n-octanol/water (log K_{ow}), as a measure for lipophilicity and bioaccumulation potential, for FGARs are < 5 (at environmentally relevant pH) while the respective values for SGARs are all > 5 (at environmentally relevant pH). Also, available toxicity studies with rats show much shorter half-lives of FGAR in livers when compared to SGAR which may indicate faster elimination rates in target and non-target organisms (Daniels 2013). Another plausible reason for the lack of FGARs in the analyzed fish samples might be that FGARs are generally used less frequently than SGARs, especially for the control of rats in sewer systems, which is assumed to be the source of emissions to surface water bodies. A survey of 508 local municipal authorities in Germany responsible for the rat control in sewers (Krüger and Solas 2010) indicated that bromadiolone followed by difenacoum and brodifacoum were used most often by local authorities for the control of brown rats in sewer systems.

Comparison with bioconcentration factors, adsorption coefficients, and use patterns

The bioconcentration factors (BCF, the ratio of a substance concentration in water and in fish tissue and expressed as L kg^{-1}) of fish as stated in the respective public Assessment Reports for their approval under BPR decrease in the following order: difethialone (39,974; estimated), brodifacoum (35,645; estimated), flocoumafen (24,300; measured), bromadiolone (460; measured), chlorophacinone (22.75; estimated), warfarin (≤ 21.6; measured), coumatetralyl (11.4; measured) (ECHA 2017a). The BCF values may explain why SGARs were detectable in the ESB fish samples, while FGARs were not. The organic carbon adsorption coefficients K_{oc} [L kg^{-1}], as given in the respective Assessment Reports for each of the active substances, increase in the order of warfarin (174), coumatetralyl (258), brodifacoum (9155), bromadiolone (14,770), chlorophacinone (75,800), flocoumafen (101,648), difenacoum (1.8* 10^6), difethialone (about 10^8) (ECHA 2017a). According to this, other highly

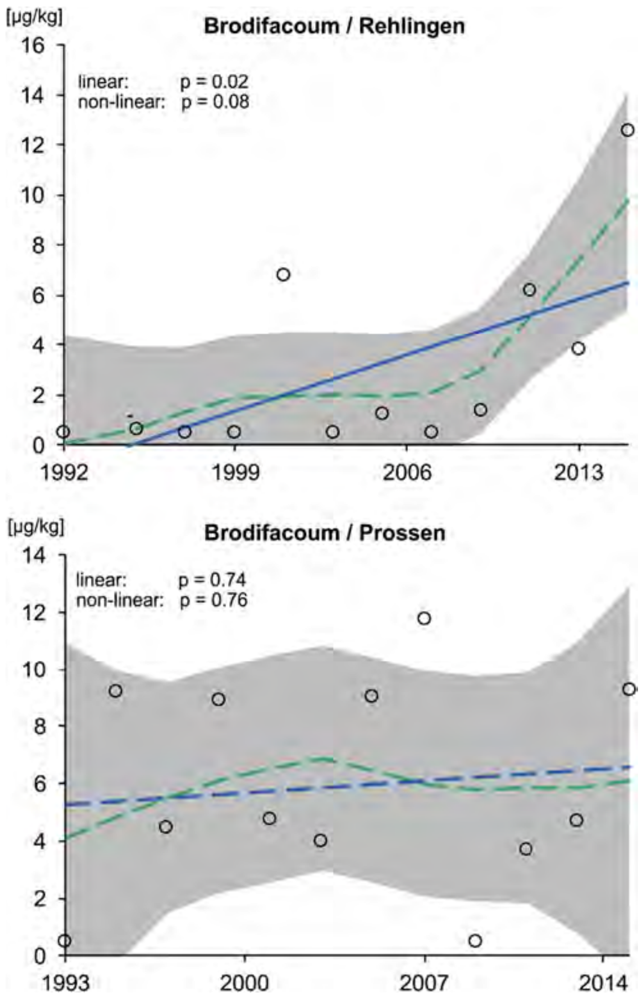

Fig. 2 Time trend analysis of both sampling sites for brodifacoum using the LOESS-Trend tool (compare "Materials and methods" section). Circles reflect actual results (mean values of replicates of pooled fish samples), while the blue solid or dashed line reflects the linear fit, the green dashed line the dynamic fit, and the gray area the confidence interval ($\alpha = 0.05$). For mean value calculations, data below the LOQ were substituted by a concentration of 50% of the LOQ (LOQ = 1.0 µg kg^{-1} for brodifacoum; compare Table S4 and S5 in the ESM). "-" indicates values results below LOQ

adsorptive anticoagulants such as difenacoum, which is according to Krüger and Solas (2010) commonly used for sewer baiting in Germany, should also be expected to absorb to SPM (assumed that comparable amounts are emitted). There may be several reasons why this is not the case: SPM samples in the ESB archive were pooled samples of 12 monthly subsamples, whereas only one ESB fish sample was collected per year after spawning at each of the riverine sampling sites. Depending on a seasonal exposure, higher or lower findings, compared to the concentrations actually found in this study, may be expected in SPM (e.g., when exposed in spring after treatment campaigns in municipal rodent control, AR can be expected in SPM), but the occurrence in fish that are sampled in a different season compared to the treatment might be unlikely, especially for FGAR with a low bioaccumulation potential. However, this cannot fully explain the exclusive presence of bromadiolone and we are unclear why other ARs were not detected in this matrix.

The varying treatment lengths, intervals, and substance patterns of AR treatment campaigns in Germany may also help to explain the occasional detections of other SGAR, as their presence may reflect the major AR applied in the catchment that year. Data on the amounts of AR that were used are unfortunately rarely available (Pohl et al. 2015). Rodenticides, which have been used most often by municipal authorities for sewer baiting (Krüger and Solas 2010), were those found most frequently in fish (difenacoum, brodifacoum, and bromadiolone) and bromadiolone in SPM.

Rough estimations suggest that the AR concentrations detected in fish are plausible given the available data on concentrations in sewage treatment plant (STP) effluent (Gomez-Canela et al. 2014) and the known BCF of the detected compounds in fish (For details, see ESM).

An important aspect of rodenticides was recently identified to be the metabolism by Fourel et al. (2017b). They found a high abundance of trans-bromadiolone in red kite, indicating individual metabolic rates for the two bromadiolone enantiomers. If fish could also metabolize bromadiolone isomers selectively, this could explain why we found bromadiolone more frequently in SPM compared to fish liver. This does, in turn, not help to understand why other ARs were not found in SPM.

Synopsis

In summary, our findings demonstrate that contamination of wildlife with anticoagulant rodenticides, especially SGARs, also involves aquatic species and is not confined to predatory birds or mammals of the terrestrial food web. We detected residues of SGARs in fish samples from almost every ESB sampling site, including the rivers Rhine, Elbe, and Danube. The ubiquitous exposure of fish is in contrast to the rather low concentrations of SGARs in biocidal products which ranged from 25 mg kg^{-1} (difethialone) to 75 mg kg^{-1} (difenacoum). An amount of approximately 50 kg of anticoagulant rodenticide active substance is used annually for rat control in sewers and above ground by municipal authorities in Germany, with approximately 75% were used exclusively for sewer baiting (Krüger and Solas 2010). Given this relatively moderate amount of use, the prevalence of detectable rodenticide residues in fish samples appears surprisingly high. Whether this is entirely accounted for by the persistent and bioaccumulative properties of the SGARs requires investigation. In general, there remains a lack of understanding about both the impacts of rodenticides on aquatic life and the pathways by which these compounds enter the environment. There are few published data on rodenticide levels in waste water (Gomez-

Canela et al. 2014) or surface water and no information on what specific substances or amounts are used. Experimentally derived BCF values for ARs are not always available and modeled BCF value may not enable a sound assessment of the potential for bioaccumulation in fish. Therefore, it is important to generate a better overview on the temporal spatial occurrence of AR in freshwater environments and to identify relevant sources and entry pathways. Further research is needed to unravel the exposure of freshwater environments to rodenticides. This may involve environmental fate studies as well as additional spatial and temporal monitoring activities. Monitoring of AR can thereby provide additional key information for their environmental risk assessment and the need to set appropriate risk mitigation measures within their authorization as biocides in the European Union.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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September 28, 2015

VIA E-MAIL AND U.S. MAIL

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Re: Validity of Santa Monica Mountains Local Coastal Program, Including Regulation of Pesticide Use

Dear Ms. Hogin:

As discussed this morning, my office respectfully disagrees with your conclusion that provisions of our Santa Monica Mountains Local Coastal Program (LCP) that regulate pesticide use are "not legally sound."

We are aware that questions have arisen as to whether California Food and Agricultural section 11501.1(a), which prohibits pesticide regulation by local governments, preempted the California Coastal Commission ("Coastal Commission") from certifying the LCP, insofar as such LCP included provisions regulating pesticide use.

We looked at this issue closely when assisting the County Department of Regional Planning in the preparation of its LCP. We have also reviewed the issue again when the recent inquiry arose. It is our opinion that the preemption language in section 11501.1(a) does not apply to the LCP because the LCP does not constitute a local regulation for purposes of that statute. Rather, the LCP was prepared and certified pursuant to the California Coastal Act, Public Resources Code ("PRC") section 30000 et seq. Based on the regulatory layout of the California Coastal Act, case law has consistently rejected the contention that an LCP, such as ours, is a local regulation. Thus, we believe section 11501.(a) does not apply to the LCP.

Moreover, section 11501.1, subsection (c), of the California Food and Agricultural states: "Neither this division nor Division 7 (commencing with section 12501) is a limitation on the authority of a state agency or department to enforce or administer any law that the agency or department is authorized or required to enforce or administer." This subsection explicitly recognizes that regulation of pesticides can be within the purview of other state agencies, such as the Coastal Commission. This provides further support that the certification of the LCP was legally proper, notwithstanding the provisions regulating pesticide use.

If you have any further questions concerning this matter, please contact me, Deputy County Counsel Jill M. Jones at (213) 974-1927.

Very truly yours,

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Interim County Counsel

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December 6, 2019

Via email

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Re: Local Coastal Program Amendment No. 14-001, December 9, 2019 Agenda Item No. 4.A.

Honorable Councilmembers,

We submit these comments on behalf of Poison Free Malibu, a community organization dedicated to eliminating pesticide threats to Malibu's wildlife. Poison Free Malibu wholeheartedly supports the City's adoption of a Local Coastal Program (LCP) amendment that would prohibit the use of herbicides, insecticides, and rodenticides known to harm wildlife. If adopted as part of an LCP amendment and certified by the California Coastal Commission, the pesticide prohibition would constitute state action that is permitted by Food and Agriculture Code section 11501.1. More importantly, combined with the protections of the County's LCP for the Santa Monica Mountains, an amendment to Malibu's LCP banning the use of pesticides would provide much-needed protection to mountain lions, bobcats, and raptors throughout their Santa Monica Mountains ranges. This would be consistent with the County of Los Angeles's LCP for the Santa Monica Mountains, which also prohibits harmful pesticides including rodenticides. Importantly, the amendment would implement California Coastal Act section 30240 which requires avoidance of significant disruption of habitat values in environmentally sensitive habitat areas (ESHA). Southern California mountain lion populations are already on the edge of extirpation, in large part due to stresses associated with rodenticide exposure. Herbicides, insecticides, and *all* rodenticides cause significant disruption.

Chatten-Brown, Carstens & Minteer LLP agrees with the conclusions of the Natural Resources Defense Council, the County of Los Angeles, the California Coastal

Commission, and the California Office of the Attorney General regarding the legality of amending an LCP to ban pesticides and other chemicals that harm wildlife. This position has been upheld by the Superior Court of Los Angeles. (*Mountainlands Conservancy, LLC v. California Coastal Commission*, Los Angeles Superior Court Case No. BS 149063.)

We have reviewed the November 27, 2019 email from the Chief Counsel of the Department of Pesticide Regulation. On its face, the email states that it was actively solicited by the City Attorney in support of her position. The email talks about the City's adoption of a "proposed ordinance." We don't have access to the initial email the City Attorney sent to Mr. Rubin, so we don't know if her correspondence asked specifically about the Coastal Commission's adoption of a prohibition in the Malibu LCP, or if she merely asked about the City's adoption of a rodenticide prohibition. The latter would be preempted by state legislation. The former would not. Food and Agricultural Code section 11501.1 specifically prohibits "local government" action. It does not apply to state agencies. Thus, state action in which the Coastal Commission adopts an LCP amendment prohibiting rodenticides in Malibu, pursuant to its authority to carry out Public Resources Code section 30240, is permitted by the Food and Agriculture Code. The Department of Pesticide Regulation attempts to distinguish from the County of Los Angeles decision because Malibu would be amending the LCP on its own initiative and not at the request of the Commission. But the City would be amending the LCP to satisfy Coastal Act requirements to stop ongoing significant disruption of ESHA habitat values. While this superior court case is not citable in court for most purposes, the facts align.

We have also reviewed the November 18, 2019 Memorandum provided to the City Council by the City Attorney. In this Memorandum, the City Attorney does not state that Malibu's adoption of a rodenticide ban would be any different than the County's adoption of a rodenticide ban, which was upheld by the court and not appealed. Instead, the City Attorney states that the Superior Court decided the case wrongly, asserting that the Coastal Commission lacks the authority to certify a Local Coastal Program Amendment that bans harmful chemicals. This should not be a basis for failing to protect Malibu's wildlife and environmentally sensitive habitat areas, especially when state law explicitly requires that LCPs be consistent with the policies contained in Chapter 3 of the Coastal Act. These policies, which constitute controlling state law, include Public Resources Code section 30240. Subdivision (a) states, "Environmentally sensitive habitat areas shall be protected against any significant disruption of habitat values, and only uses dependent on those resources shall be allowed within those areas." The protection of ESHA is not geographically limited to threats arising within the ESHA. Subdivision (b) provides, "Development in areas adjacent to environmentally sensitive habitat areas and parks and recreation areas…shall be compatible with the continuance of those habitat and recreation areas." The City Attorney's Memorandum has not explained how the Commission's certification of a Local Coastal Program amendment designed to protect wildlife inhabiting ESHA would conflict with Public Resources Code section 30240, a provision of state law that is central to the purpose for which the Commission was created.

Malibu's LCP currently prohibits insecticides and herbicides in and adjacent to ESHA. Wildlife and habitat protection consistent with section 30240 of the Coastal Act requires more. Poison Free Malibu's proposed language recognizes the interconnectedness of the City with the surrounding ESHA. Application of herbicides, insecticides, and other toxic chemicals outside of ESHA causes environmental harm within ESHA designated in both the Malibu and Santa Monica Mountains LCPs. Raptors that eat contaminated rodents within Malibu can fly distances and die in ESHA before being consumed by other species that then fall prey to rodenticides. Large predators such as bobcats, coyotes, and mountain lions have ranges that include both ESHA and non-ESHA areas. A rat or mouse poisoned within the City can poison mountain lions or bobcats in near or faraway ESHA, or contribute to mange and other conditions that lead to reduced reproduction and premature death.

Poison Free Malibu supports LCP amendment language prohibiting harmful chemicals beyond anticoagulant rodenticides. As the City's LCP already prohibits "[t]he use of insecticides, herbicides, or any toxic chemical substance which has the potential to significantly degrade Environmentally Sensitive Habitat Areas (ESHA)," the inclusion of this language in an LCP amendment is hardly novel. (LCP Land Use Plan, Policy 3.18.) The use of herbicides within the City eradicates food sources for butterflies and other plant-dependent species in Malibu and Santa Monica Mountains ESHA. It is also important to include *all* rodenticides in the LCP amendment. Due to the brutality inherent in anticoagulant rodenticides, there has been increased reliance on alternative chemicals, such as bromethalin, cholecalciferol, and even strychnine. These poisons act quickly, often without available antidotes, and commonly poison non-target wildlife and pets. They, too, pose dire threats to ESHA.

Thank you for the consideration of these comments, and we look forward to the City's action to protect wildlife on December 9, 2019.

Sincerely,

Michelle N. Black, on behalf of
Poison Free Malibu

From:	Poison Free Malibu
To:	Coastal Strategic Plan Comments
Subject:	November Item F5: Strategic Plan - Include Pesticide Prohibitions
Date:	Friday, October 30, 2020 1:53:40 PM
Attachments:	Malibu LCP-A PFM comments.docx AttachmentsToCoastalEmailFromPFM.zip

Dear Coastal Staff,

The city of Malibu has submitted an LCP amendment to prohibit toxic pesticides in the Malibu Coastal Zone to be considered at a hearing in the near future.
Los Angeles County has already done this in the unincorporated Los Angeles County Santa Monica Mountains LCP in 2014.
Several other LCPs also include prohibitions, possibly starting with Santa Cruz County in 1994.

Prohibitions are necessary due to the growing recognition of the threat to the coastal wildlife and ecosystem from pesticides.
Please consider adding the goal of encouraging LCPs and LCP amendments that encourage similar prohibitions in the Strategic Plan.

For example, the following could be added to Section 3.1.1 or as a separate item under 3.1:
"Encourage LCPs to incorporate prohibitions on toxic pesticides to protect wildlife and marine resources."

Attached are documents we submitted in support of the Malibu LCP amendment which go into detail supporting pesticide prohibitions.

Malibu LCP-A PFM comments.docx is the email to Coastal Staff on July 30, 2020.
AttachmentsToCoastalEmailFromPFM.zip are supporting documents referred to in the email

Thank you,

Joel
--
Joel Schulman PhD
Poison Free Malibu
Email: PoisonFreeMalibu@gmail.com
Website: PoisonFreeMalibu.org
Facebook: Poison Free Malibu
Phone: 310-456-0654

United States Department of the Interior

NATIONAL PARK SERVICE
Santa Monica Mountains National Recreation Area
401 West Hillcrest Drive
Thousand Oaks, California 91360-4207

In reply refer to:

December 6, 2019

Honorable Karen Farrer, Mayor
Honorable Council Members
City of Malibu
23825 Stuart Ranch Road
Malibu, CA 90265

Dear Mayor Farrer and Councilmembers:

Thank you for the invitation to comment on the proposed Local Coastal Program Amendment No. 14-001. In general, the National Park Service does not testify in support or opposition to local measures, but does provide subject matter expertise and comments to assist local governments in their evaluation of proposed actions, when invited to do so.

National Park Service scientists have been studying carnivores in the Santa Monica Mountains for more than two decades, since 1996. Our studies include observations and data collection on bobcats, coyotes, and mountain lions, predominantly. In these studies we have found widespread exposure to and large impacts of anticoagulant rodenticides on all three of these carnivores. The interaction between anticoagulant rodenticide exposure and death from mange resulted in the complete loss of bobcats from open space areas in the Conejo Valley.

Our studies have found anticoagulant rodenticide poisoing to be a leading cause of death for many carnivores. Specifically, we found over a nine-year study that 27% of coyotes were directly killed by anticoagulant rodenticide poisoning (Riley et al. 2003, Gehrt and Riley 2010), making it the second leading cause of death for these animals after vehicles. For bobcats, the interaction between rodenticide exposure and serious mange disease led to an epizootic of mange in bobcats in our study area, the first such epizootic that had ever been reported in the scientific literature (Riley et al. 2007). This epizootic had amajor impact on our study population: 19 bobcats collared bobcats died from mange disease over a three-year period from 2002-2004, and all of the study animals were lost in one habitat fragment in Oak Park, with little evidence of bobcat activity there for many years. Although bobcats eventually returned to that area by 2009 and 2010, including females that successfully raised kittens, we have been seeing more mange disease again in recent years. Population genetic studies with our colleagues at UCLA indicated that the mange epizootic was severe enough to create a genetic bottleneck (Serieys et al. 2015a). From the beginning, severe mange disease showed a very strong statistical association with anticoagulant rodenticide exposure (Riley et al. 2007), which was even more evident as our studies continued (Serieys et al. 2015b). Importantly, however, work with our colleagues at UCLA revealed significant and widespread immune system impacts of rodenticide exposure in bobcats, both inflammatory and immune suppressive effects (Serieys et al. 2018). These immune effects could then be leading to the development of severe mange disease in bobcats, and potentially mountain lions as well (see below). Finally, even more recent work has shown that gene expression in bobcats is profoundly affected by anticoagulant exposure, including for genes related to the immune system and the skin (Fraser and Mouton et al. 2018). So toxicants are affecting wildlife at fundamental physiological and genetic levels.

In addition, five mountain lions have now died directly from anticoagulant rodenticide poisoining during our long-term study of the behavior and ecology of this species, the last remaining large carnivore in the region. The first two died in 2004, but then a subadult female died in 2015, and two large, healthy adult males died this year, in March and August of 2019. In a recent analysis of survival and mortality causes across the 17 years of our study since 2002, death from anticoagulant poisoing has become an important cause of death for mountain lions, approaching intraspecific conflict and vehicles strikes (Benson et al. 2019). Finally, we have also documented notoedric mange in multiple mountain lions, including the first two that died of anticoagulant toxicosis and later P22 in Griffith Park. All of these mange-infected animals were also exposed to rodenticides, contributing to the link between this disease and the toxicants.

Overall, our studies have shown widespread exposure to these chemicals across the carnivores in our region that we have studied. We found a greater than 90% exposure rate of bobcats to anticoagulant rodenticides (Riley et al. 2007, Riley et al. 2010, Serieys et al. 2015b), a 96% exposure rate in mountain lions (23 of 24 have tested positive), and an 83% exposure rate in coyotes (Gehrt and Riley 2010). Moreover, for all of these species, 2/3 or more of the exposed animals had evidence of multiple different rodenticide compunds and sometimes in large amounts, indicating multiple exposure events. In recent years, we have documented three mountain lions that were exposed to 6 different compounds, the most that we have ever found.

We have seen widespread exposure in the three species that we have studied intensively, but we also know of exposure and effects in other species. We have found exposure in species as varied as raccoons, gray foxes, and a gopher snake, and we have documented death from rodenticide poisoning both in a collared gray fox and in a GPS-collared raccoon, as part of a road study in 2017. We know from colleagues at local wildlife rehabilitation facilities that raptors (e.g., owls, hawks) are often exposed to these toxicants, although no survival studies have been done locally.

These studies suggest that these compounds are having impacts on the wildlife of the Santa Monica Mountains and surrounding areas. We hope this information will be useful to you as you consider management of the use of anticoagulant rodenticides within the City of Malibu. Thank you for your consideration.

Sincerely,

David M. Szymanski

David Szymanski
Superintendent

cc: Reva Feldman, City Manager, City of Malibu
Bonnie Blue, Planner, City of Malibu

United States Department of the Interior

NATIONAL PARK SERVICE
Santa Monica Mountains National Recreation Area
401 West Hillcrest Drive
Thousand Oaks, California 91360-4207

In reply refer to:

July 8, 2013

Honorable Joan House, Mayor
Honorable Council Members
City of Malibu
23825 Stuart Ranch Road
Malibu, CA 90265

Dear Mayor House and Council Members:

Unfortunately due to other commitments, we will not be able to send a National Park Service representative to this evening's city council meeting. In lieu of our attendance, we are sending this statement regarding the proposed rodenticide ban that is on your agenda this evening.

National Park Service scientists have been studying carnivores in the Santa Monica Mountains for almost two decades. Our studies include observations and data collection on bobcats, coyotes, and mountain lions. In these studies we have found widespread exposure to and large impacts of anti-coagulant rodenticides on all three of these carnivores. Our research suggests an interaction between anti-coagulant rodenticide exposure and death from mange and mange deaths resulted in the complete loss of bobcats from many open space areas in the Conejo Valley.

Our studies have found anti-coagulant rodenticide poisoing to be a leading cause of death for many carnivores. Specifically, we found over a nine-year study that 27% of coyotes were directly killed by anti-coagulant rodenticide poisoning (Riley et al. 2003, Gehrt and Riley 2010), making it the second leading cause of death for these animals after vehicles. For bobcats, the interaction between rodenticide exposure and serious mange disease is a large impact with a total of 19 bobcats dying from mange disease over a three-year period and this severe mange disease showing a strong association with anti-coagulant rodenticide exposure (Riley et al. 2007). In addition, two mountain lions died directly from anti-coagulant rodenticide poisoining in 2004, and in our ongoing mountain lion study we find death from anti-coagulant poisoing to be the third leading cause of death for mountain lions.

Our studies also show widespread exposure to these chemicals across carnivores in our region. We found a 90% exposure rate of bobcats to anti-coagulant rodenticides (Riley et al. 2007, Riley et al. 2010), 7 of 8 mountain lions tested had been exposed (Beier et al. 2010), and 83% of coyotes had been exposed (Gehrt and Riley 2010). Moreover, for all of these species, 2/3 of the exposed animals had evidence of 2-5 different rodenticide compunds and sometimes in large amounts, indicating multiple exposure events.

These studies suggest that these compounds are having impacts on the wildlife of the Santa Monica Mountains and surrounding areas. We hope this information will be useful to you as you consider whether to implement a ban on anticoagulant rodenticides within the City of Malibu.

Thank you for your consideration.

Sincerely,

David M. Szymanski

David Szymanski
Superintendent

cc: Jim Thorsen, City Manager, City of Malibu

NRDC

November 26, 2019

Mayor Karen Farrer (KFarrer@malibucity.org)
Mayor Pro Tem Mikke Pierson (MPierson@malibucity.org)
Councilmember Rick Mullen (RMullen@malibucity.org)
Councilmember Skylar Peak (SPeak@malibucity.org)
Councilmember Jefferson Wagner (JWagner@malibucity.org)
City Clerk Heather Glaser (HGlaser@malibucity.org)
City Attorney Christi Hogin (Christi.Hogin@bbklaw.com)

Re: Proposed City of Malibu LCP amendment re rodenticides

Dear Mayor Farrer; Mayor Pro Tem Pierson; Councilmembers Mullen, Peak, Wagner; City Clerk Glaser; City Attorney Hogin:

NRDC has been asked to consider the legality of a proposal on the Malibu City Council's December 9, 2019 agenda to ask the California Coastal Commission to amend the City's Local Coastal Program (LCP) to ban the use of anticoagulant rodenticides within the Coastal Zone portions of the City. In our opinion, this would be legal and would generate very little litigation risk for the City. We have reviewed City Attorney Christi Hogin's November 18, 2019 memorandum on this issue, and are puzzled by her analysis and disagree with her conclusion to the contrary.

There is no question that anticoagulant rodenticides are harmful, and sometimes fatal, to mountain lions and other large predators, and that mountain lions have a significant presence in the Malibu area. See, e.g., the map at: https://www.nps.gov/samo/learn/nature/pumapage.htm. In that same document, the National Park Service states:

> Another major threat to the species is the widespread presence of anticoagulant rodenticides, commonly known as rat poisons, in the environment. Twenty-two out of 23 mountain lions tested in the study have tested positive for one or more anticoagulant compounds and three have died of intoxicant poisoning.

The protection of biological resources and environmentally sensitive habitat areas in the Coastal Zone is part of the Coastal Commission's job under Section 30240 of the Coastal Act. Absent other legal impediments, it is clear that the Coastal Commission has the power to ban anticoagulant rodenticides in the Coastal Zone.

An argument made by Ms. Hogin is that an amendment to the LCP to ban anticoagulant rodenticides would be barred by California Food and Agricultural Code Section 11501.1(a), which provides that:

> This division and Division 7 (commencing with Section 12501) are of statewide concern and occupy the whole field of regulation regarding the registration, sale, transportation, or use of pesticides to the exclusion of all local regulation. Except as otherwise specifically provided in this code, no ordinance or regulation of local government, including, but not limited to, an action by a local governmental agency or department, a county board of supervisors or a city council, or a local regulation adopted by the use of an initiative measure, may prohibit or in any way attempt to regulate any matter relating to the registration, sale, transportation, or use of pesticides, and any of these ordinances, laws, or regulations are void and of no force or effect.

In our view, Section 11501.1(a) does not forbid the Coastal Commission from adopting the LCP amendment now at issue. Preliminarily, we should note that adoption of an LCP amendment by the Coastal Commission brings little, if any, litigation risk to the City, because it is the Coastal Commission's action, rather than the City's, that would be challenged in litigation seeking to enforce state preemption over rodenticides. It is puzzling that Ms. Hogin's letter does not address this point.

On the merits, the second sentence of Section 11501.1(a) is not applicable here because it refers to local agencies, and the Coastal Commission is an agency of the State. The first sentence begins with text that appears to be rooted in the concept of field preemption – that the State has taken authority over the entire field of rodenticide control – but then explicitly qualifies itself by the phrase "to the exclusion of all local regulation." We read this provision to say that, consistent with the second sentence of the statute, the first sentence also applies only to local, and not statewide, regulation. It is well-settled that an LCP is fundamentally a creation of state, not local, law. *See, e.g., Charles A. Pratt Construction Company v. California Coastal Commission*, 162 Cal.App.4th 1068, 1075 (2008). If the State Legislature had intended to ban the Coastal Commission or any other state agency from acting in the area of rodenticide regulation, it could have done so, but it has not. Indeed, Food and Agricultural Code Section 11501.1(c) states:

> Neither this division nor Division 7 (commencing with Section 12501) is a limitation on the authority of a state agency or department to enforce or administer any law that the agency or department is authorized or required to enforce or administer.

In our view, Section 11501.(c) expressly allows the Coastal Commission to create and administer an LCP in any way that is lawful and appropriate under the Coastal Act, without restriction under Section 11501.1(a).

Our conclusion is reinforced by the recent decision of Los Angeles Superior Court Judge James Chalfant in the *Mountainlands Conservancy LLC v. California Coastal Commission* matter, Los Angeles County Superior Court Case No. BS 149063. In that case, Judge Chalfant – one of the best-respected judges in Los Angeles County on matters of land use -- rejected the same argument now being advanced by Ms. Hogin, explaining:

> The Commission found that a ban on the use of pesticides in the Santa Monica Mountains coastal region is necessary to avoid impacting the biological productivity and quality of coastal waters . . . In banning pesticide use in the certified LCP, the Commission is not compelling the county to exercise power that it does not have under state law. Instead, the commission is requiring a pesticide ban for the County's LCP, to be administered by the County, because the Commission has the authority to do so as part of its administration of the Coastal Act. *F&A Code section 11501.12(c) permits the commission to require the county to conform to this ban in administering the LCP*. (emphasis supplied)

Based on this analysis, it is our view that an LCP amendment banning anticoagulant rodenticides in the Coastal Zone area of Malibu is legal and poses little, if any, litigation risk to the City. We would be glad to discuss this if members of the City Council have questions or comments.

Sincerely,

David Pettit
Senior Attorney
Natural Resources Defense Council

Joel Reynolds
Western Director & Senior Attorney
Natural Resources Defense Council