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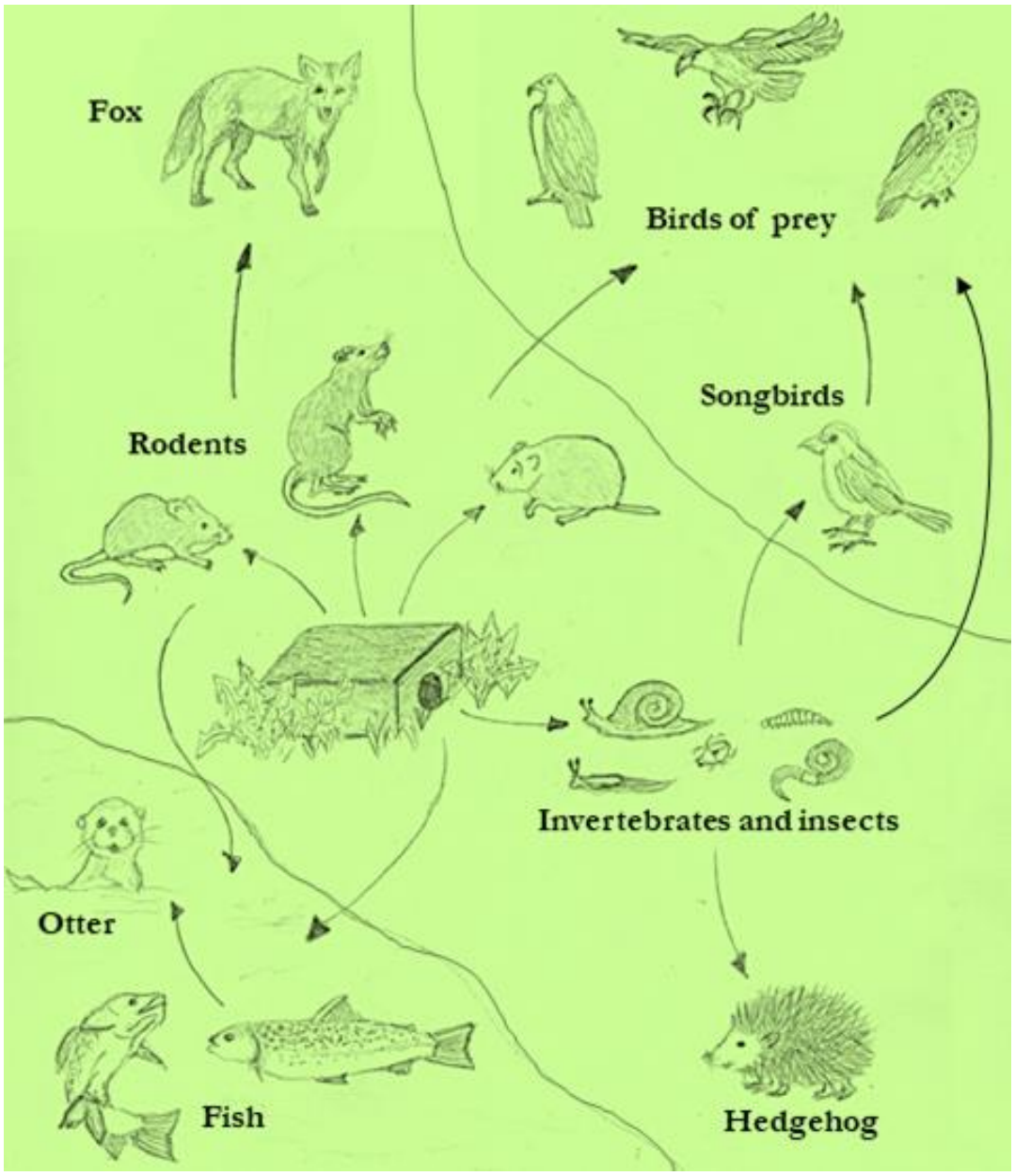
Anticoagulant rodenticides – Swiss situation analysis

Final Report [19.12.2022]



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Summary

This report provides a background on the application of anticoagulant rodenticides (AR) – including second generation AR (SGAR) – to combat damage caused by rodents and the occurrence of AR in the Swiss environment. Three main aspects are covered: 1) a review of the literature on the topic; 2) development and validation of an analytical method for AR and the generation of limited screening data on liver AR concentrations in biota from the Swiss terrestric and aquatic environments; 3) surveys targeting professionals and experts concerning the application of AR in Switzerland and their possible effects.

Anticoagulant rodenticides are a group of compounds that inhibit blood coagulation. They are used in rodent baits with the aim to cause (delayed) death in rodents as a way to control rodent infestation. Due to their intrinsic properties, these compounds typically bioconcentrate and bioaccumulate along the food chain and the environment.

In Switzerland ARs are regulated in the Ordinance on Biocidal Products and the Ordinance on Plant Protection Products. Given the toxicity of ARs, their application has been restricted, for example, application in higher concentrations by professional users only and reduced AR concentrations in products for non-professional users.

Since the introduction of AR-containing products on the market to fight against rodents, residues of anticoagulants have been increasingly detected worldwide in non-target organisms and the environment. This ranges from insects and snails to top predators such as otters, foxes and birds of prey. In vertebrates, AR concentrations are mainly reported for liver. However, there is no well-defined concentration of concern of AR in liver samples, a range of 20 to 200 ng/g is given. In general, 100 ng/g is often used as benchmark, for birds of prey also 20 ng/g has been suggested.

For AR screening, a sensitive and robust analytical method was developed. The screening was made possible thanks to the support of various organizations and individuals who provided us with liver samples from foxes, birds of prey, hedgehogs and fishes. Foxes and fish did not die as a result of poisoning but were either hunted or live caught. Birds and hedgehogs were sick or weak on arrival at animal care centres and died during care, for these species it is unknown if AR contributed to mortality. In most samples, one or more AR were detected. Summed AR concentrations exceeded 100 ng/g in 24% of foxes and 14% of birds of prey.

Surveys conducted with professionals and experts in the public and private pest control sector revealed that of eight ARs, brodifacoum, bromadiolone and difenacoum were dominant active ingredients in rodenticide products used in Switzerland. The surveys further showed that mainly solid products such as blocks are used rather than grains or pellets. In addition, professional users reported low recovery rates of dead rodents, typically below 5%, leaving ample opportunity for ARs to be dispersing into the environment and accumulate in non-target organisms.

This report shows that the occurrence of AR in the Swiss terrestric and aquatic environment is widespread and that high concentrations are found in biota: A broader monitoring in Switzerland is therefore advised to substantiate results from the present, limited screening and to allow for determining a baseline exposure. Such a set of baseline data can then be used to support regulatory measures and to evaluate effects of possible future measures of AR regulation on exposure of wildlife.

Content

1. Problem definition

Rodents cause damages worldwide to human health and materials. As a preventive and damage controlling measure, anticoagulant rodenticides (AR) are applied to control rodent pests and keep damage as low as possible. Due to the intelligent nature of rodents, the lethal effect of the applied and consumed AR compounds has to be delayed to prevent bait avoidance. Delayed effects often go in line with persistent, toxic and bioaccumulative compound properties which in turn pose a risk to the environment. This negative impact can be seen, for example, in secondary poisoning of non-target organisms.

In Switzerland, the use of ARs is regulated in the Swiss biocidal products ordinance (Biozidprodukteverordnung (VBP 2022)) and in the Swiss plant protection product ordinance (Pflanzenschutzmittelverordnung (PSMV 2022)). The regulatory context depends on whether the products are used as biocides for the protection of human health or materials or as plant protection product for crop protection on the field. Although reports exist of suspected secondary poisonings in Switzerland (e.g., Stalder, Vogler et al. (2021)), so far, no screening or widespread monitoring of AR was performed in Switzerland. Nevertheless, studies performed worldwide indicate that widespread environmental pollution by ARs is also conceivable in Switzerland. Recently, the use of ARs was identified as a “high-risk” use in the implementation of the parliamentary initiative on risk reduction in the use of pesticides (Pa. Iv. 19.475).

The aim of this study is to gain insights into the use of ARs and their occurrence in environmental compartments and non-target biota in Switzerland and their potential impact on the environment. This should clarify, which samples are most promising for monitoring due to a high probability of AR residues in samples. Furthermore, we tried to determine, how many and which sampling points are useful for screening to investigate a potential AR-contamination of the Swiss environment. To provide information on where the greatest environmental impacts are expected to occur and if poisonings of pets/wild animals/birds are known or suspected, we consulted several Swiss experts on their assessment of the exposure situation of non-target organisms with ARs. Furthermore, a survey was conducted with members of the Association of Swiss Pest Controllers (VSS) and authorities of cantonal capitals. To obtain an overview of the potential exposure of non-target biota to AR, we developed an LC-MS/MS method to analyse AR and conducted a screening with liver samples from different non-target organisms.

The following literature research lays the fundament for setting up an AR screening taking into account different selected environmental compartments across Switzerland.

2. Introduction to anticoagulant rodenticides

2.1. A short history on rodenticide development

Rodents such as rats, mice and sometimes voles are hygiene pests, which can act as carriers and transmitters of various diseases to humans as well as to domesticated animals and wildlife. The transmission takes place through their bites, faeces and urine carrying several bacteria, ecto- and endoparasites as well as viruses (Battersby 2015). As they feed on crops as well as on stored food, pest rodents might cause contamination of food supplies but also of objects and materials (Buckle and Smith 2015). In addition, insulation, building material, furniture, doors and electrical cables can be damaged by rodents due to their urge of gnawing (Shumake, Sterner et al. 2000). Furthermore, pest rodents are predators that feed on wildlife and local vegetation, potentially reducing biodiversity in an existing ecosystem, e.g., on islands after accidental introduction (Amori and Clout 2003). Especially of concern are house mice (*Mus musculus*), roof rats (*Rattus rattus*) and brown rats of the species *Rattus norvegicus* originating from Asia, which spread in Europe since the 18th century. Mainly living in sewers, these animals can colonize and multiply greatly above ground in case of food availability (Macdonald, Mathews et al. 1999). Another important target of rodenticides are common voles (*Microtus arvalis*) and water voles (*Arvicola amphibius*), which are associated with agricultural losses by causing damage to fields and affecting fodder production (Abi Khalil, Barbier et al. 2021).

After trying to control these pest rodents with the help of traps and predators such as cats or ferrets (Van den Brink, Elliott et al. 2018), chemical pest control was introduced to protect human health, avoid material damages and food contamination as well as for biodiversity conservation on islands (Masuda, Fisher et al. 2015, Kotthoff, Rüdel et al. 2019).

In the past, botanicals such as strychnine and products containing arsenic compounds, endrin, barium carbonate or white phosphorus were used (Van den Brink, Elliott et al. 2018). Furthermore, products such as toxaphen (worldwide banned since 2004), scilliroside and thallium(I) sulphate (Tl_2SO_4, thallous sulphate) were applied (Heinisch and Klein 1994, Van den Brink, Elliott et al. 2018). In the 1940s, rodenticides composed of anticoagulants as active substances were developed and commercialized, so called first generation anticoagulant rodenticides (FGARs). These are based on the mode of action of dicoumarol, which is naturally occurring in plants. Among FGARs, warfarin was the first FGAR to be recommended for rodent control (Link 1959). Initially working well, pest rodents soon became resistant to these chemicals possibly due to mutations leading to decreased binding affinities at the receptor site (Buckle and Smith 2015). Thus, similar but more potent and persistent rodenticides, so called second generation anticoagulant rodenticides (SGARs), were introduced in the 1970s. But some resistance cases to these SGARs, especially bromadiolone and difenacoum, have also been lately observed (Buckle, Jones et al. 2020), including in Swiss populations of mice (Pelz, Rost et al. 2012). SGARs are generally more prone to unintentional poisoning of non-target organisms due to higher persistence, potency and their increased bioaccumulation. However, only a limited number of similarly effective but less critical AR-alternatives have been identified or approved so far. Authorized AR-alternatives in Switzerland encompass carbon dioxide, alpha-chloralose reducing essential metabolic processes leading to (lethal) hypothermic reactions, as well as cholecalciferol (vitamin D_3) inducing hypercalcemia (vitamin D overdose) leading to circulatory blockage, heart and renal failure (ECHA 2021).

2.2. Mode of action and use of anticoagulant rodenticides

Most rodenticide products commercialized and authorized in Switzerland are anticoagulant rodenticides that share the same, slow acting mode of action. Anticoagulant rodenticides block the vitamin K cycle, which inhibits the vitamin K epoxide reductase enzyme and prevents the regeneration of vitamin K. Vitamin K is essential for biosynthesis of clotting factors and thus the blood coagulation process (Silverman 1980). Eventually, death is induced by uncontrolled internal and

external haemorrhages caused by increased permeability of blood vessels and the loss of blood's clotting ability (Kotthoff, Rüdel et al. 2019). Exposed rodents are subjected to a delayed death occurring 3 to 11 days after ingestion with males dying on average after 5.8 days and females after 8.2 days (Cox and Smith 1992). This delay prevents bait aversion by intelligent animals and increases the success of the pest control measure. Multiple AR may have synergistic effects (Lohr 2018). Moreover, AR act on all vertebrates, which increases the danger for unintentional poisoning of non-target organisms.

Symptoms of AR in wildlife include subcutaneous haemorrhage, haemorrhage into the thoracic-, abdominal cavities or into the gastrointestinal tract as well as unclotted blood in heart or major blood vessels in case of fresh carcasses (Hosea 2000). Furthermore, physical signs of bleeding and blue coloured mesenteric or subcutaneous fat deposits can be detected due to added marker dyes to AR products (Hosea 2000). In addition, sublethal effects such as increase of embryo mortality, teratogenic effects, behavioural effects and increase in susceptibility to bacteria and parasites were suggested to be related to AR exposure (Munday and Thompson 2003, Brakes and Smith 2005, Vidal, Alzaga et al. 2009, Serieys, Foley et al. 2013).

Formerly used as plant protection products on the field, ARs are nowadays mainly applied as biocides in livestock farming, urban areas (residential and commercial), and sewer systems as well as in the food industry. Main users are professional pest controllers, but also agribusinesses, local authorities, and private users (Regnery, Friesen et al. 2019). A positive correlation between AR occurrence in predatory wildlife and human population density was found by López-Perea, Camarero et al. (2015). Furthermore, AR residuals in red foxes and the local livestock density (e.g., high pig density) as well as the percentage of urban area on administrative district level were positively correlated in a study performed in Germany (Geduhn, Jacob et al. 2015).

Anticoagulant rodenticides are mainly available/sold as loose powder, paste, foam and solid bait formulations (ECHA 2018). Often cereals or wheat are impregnated with ARs. Furthermore, bait formulations are supplemented with dyes and bittering agents such as denatonium benzoate to reduce an inadvertent ingestion by, e.g., humans and birds (eCA 2016d)

To protect direct consumption by non-target species, tamper-resistant bait stations adjusted to target species are often used to reduce access for non-target species. However, direct bait consumption by non-target species still occurs and biomagnification cannot be prevented this way (Lettoof, Lohr et al. 2020).

Besides their use in pest control, some anticoagulant compounds were also used in the past in human anticoagulation therapy, e.g., warfarin or diphacinone (Field, Goldfarb et al. 1952). Nowadays, the pharmaceutical vitamin K antagonists phenprocoumon and acenocoumarol are prescribed, showing the same mode of action as ARs used for rodent control (Regnery, Friesen et al. 2019).

2.3. Physico-chemical properties, fate, and behaviour of anticoagulant rodenticides

As already mentioned above, ARs are classified into FGARs and SGARs based on chemical structure and development period (Lettoof, Lohr et al. 2020). Based on their core molecular structure ARs belong to the classes of indandiones (FGARs), 4-hydroxycoumarins (F- and SGARs), or thiocoumarins (SGARS) (King and Tran 2015). FGARs encompass the active substances chlorophacinone, coumatetralyl and warfarin. For FGARs, several ingestions are needed to be effective. The active compounds bromadiolone, brodifacoum, difenacoum, difethialone and flocoumafen belong to the SGARs. SGARs are more potent compounds than FGARs and were developed to be more tissue persistent to react to emergence of reduced effectiveness (Fourel, Sage et al. 2018). Hence, a one-time application of SGAR is enough to provoke lethal effects in pest rodents (Kotthoff, Rüdel et al. 2019). Chlorophacinone, coumatetralyl and warfarin are present as two enantiomers and often sold as racemic mixture of R and S enantiomers (Regnery, Friesen et al. 2019). In contrast to FGARs, SGARs exist as two diastereomeric forms (*cis*- and

trans-isomers). The mixing ratio in available products differs by active substance with bromadiolone being generally composed of 70-90% *trans*-isomer, difethialone consists of <70% *cis*-isomer and the three remaining SGARs contain around 50-80% *cis*-isomer (Regnery, Friesen et al. 2019). In general, AR are characterized by low water solubility and low vapour pressure. In addition, short photolytic half-lives in water, strong adsorption to organic matter, high lipophilicity and a high bioaccumulation potential were predicted or reported for SGARs (eCA 2016a, eCA 2016b, eCA 2016c, eCA 2016d, eCA 2016e, eCA 2016f, eCA 2016g, eCA 2016h), see Table 1.

Table 1. Physicochemical properties of first (FG) and second generation (SG) anticoagulant rodenticides (AR), adapted from (Regnery, Friesen et al. 2019)).

Compound [F]=FGAR [S]=SGAR	Water solubility (mg/L) at 20°C and pH 7	Log P_{OW} at pH 7	Log K_{OC}	Photolytic half-live in water (h)	Degradation in soil DT50[$] (d) at 12°C	Measured BCF_{fish} (L/kg)	Reference
Chlorophacinone[F]	344	2.4	5.0	24-48	128	-	(eCA 2016c)
Coumatetralyl[F]	460	1.5	2.2-2.4	8	13.1-19.4	11.4	(eCA 2016d)
Warfarin[F]	267	0.7	2.4	≥54 days	53	≤21.6	(eCA 2016h)
Brodifacoum[S]	0.06-0.2	4.9-8.5	4.0-4.7	<24	298	-	(eCA 2016a)
Bromadiolone[S]	18.4	3.8-4.1	3.2-4.2	0.2	n.d.	460	(eCA 2016b)
Difenacoum[S]	1.7	4.8	5.2	<8	833	1'100	(eCA 2016e)
Difethialone[S]	0.4	6.3	3.2-8.0	0.4-1	635*	-	(eCA 2016f)
Flocoumafen[S]	0.1	6.1	5.0	38	213*	24'300	(eCA 2016g)

[$]DT50: Time to 50% compound degradation in soil at 12°C extrapolated from 20-25°C; *at 20°C

FGARs are mainly excreted via urine (e.g., 80% for Warfarin), on the contrary, SGARs are mainly excreted via faeces (partly as metabolites, e.g., 15% bromadiolone, 25% difenacoum, 36% chlorophacinone) (Prat-Mairet, Fourel et al. 2017). The metabolisation route and potency of the enantiomers can differ as reported for warfarin (Regnery, Friesen et al. 2019). Also difference in persistence, half-lives and toxicity were reported for SGAR diastereomers (Fourel, Damin-Pernik et al. 2017, Fourel, Damin-Pernik et al. 2017, Fourel, Sage et al. 2018). Due to the highest expression of vitamin K epoxide reductase enzyme in the liver, SGARs show a very high persistence and accumulation potential especially in liver tissue (Lettoof, Lohr et al. 2020, Rattner and Harvey 2021). Whereas ARs are eliminated in mice plasma between 0.5 days (coumatetralyl) and 92 days (brodifacoum), the liver elimination half-lives of ARs in mice range from 16 days (coumatetralyl) up to 307 days (brodifacoum) (Vandenbroucke, Bousquet-Melou et al. 2008). Degradation in soil differs per compound but in general is relatively slow, especially under anaerobic conditions, see Table 1 (eCA 2016a, eCA 2016b, eCA 2016c, eCA 2016d, eCA 2016e, eCA 2016f, eCA 2016g, eCA 2016h). Bioconcentration factors estimated or measured in fish differed between FGARs and SGARs, ranging between 1.0-492 L/kg and 108-40'000 L/kg, respectively. Investigating fox faeces, Prat-Mairet, Fourel et al. (2017) found that AR persistence is not linked to the log K_{OW}, but possibly an association to organic materials may exist. This is also supported by André, Guyon et al. (2005), who reported an association of ARs and humic acids.

Based on these characteristics, SGAR residuals and their metabolites are predicted to persist mainly in biological tissue of organisms, in suspended particulate matter as well as in (organic-rich) soils and sediments rather than in the water column or air (Regnery, Friesen et al. 2019).

Regnery, Brinke et al. (2020) investigated weathering conditions of baits and reported that even solid bait formulations composed of kerosene wax, which are considered as sewage and weather resistant, are prone to dissolve or disintegrate if in prolonged or repeated contact with sewage or rainwater. This might lead to active substances leaching into an adjacent medium such as water. Besides, also high humidity can affect the condition of the bait material. Less than 24h water

contact was needed to release 3.8 ± 1.0 % (n=21) bromadiolone and 0.6 ± 0.4 % (n=15) brodifacoum (based on the amount of active substance used) into the water phase. Baits placed directly on river sediment with a water content of 18-34% led to 0.3-6.5% of brodifacoum leaching to the sediment (Regnery, Brinke et al. 2020).

2.4. Toxicity of anticoagulant rodenticides

Nakayama, Morita et al. (2018) list median lethal doses (LD50) of ARs in different animals. In general, SGARs showed higher toxicity compared to FGARs (Table 2). Furthermore, there is a large variability in LD50 values established for different animals, and data are generally limited. In particular, liver threshold values for different animals and compounds are lacking (see Section 4).

Table 2. Overview of median lethal dose (LD50 in mg/kg) of first (FG) and second generation (SG) anticoagulant rodenticides (AR) in selected animals. (Adapted from (Rammell, Hoogenboom et al. 1984, Jackson and Ashton 1992, Howald, Mineau et al. 1999, Erickson and Urban 2004, Vandenbroucke, Bousquet-Melou et al. 2008, Nakayama, Morita et al. 2018)).

	FGAR			SGAR				
	Chloro-phaci-none	Couma-tetralyl	Warfa-rin	Brodi-facoum	Bromadi-olone	Dife-nacoum	Difethi-alone	Flocou-mafen
Mouse	374	<1000	20.5	0.4	1.75	0.8	1.29	0.8
Rat	14-323	-	11	0.35-0.5	0.56-0.84	-	0.55	-
Dog	20-50	-	-	0.25-1.0	8.1	-	-	-
Cat	2.5-20	-	-	<25	<25	-	-	-
Chicken	942	-	-	3.15	-	-	-	-
Northern bobwhite	>2150	-	258	-	138	-	0.26	-
Ring-necked pheasant	-	-	<100	10	-	-	-	-
Mallard	620	-	-	4.6	-	-	-	-
Australasian harrier	-	-	-	10	-	-	-	-
Red fox	-	-	-	5	-	-	-	-
Hawks	-	-	-	10	-	-	-	-
Mink	-	-	-	9.2	-	-	-	-

A summary of toxicity data in aquatic species can be found in Regnery, Friesen et al. (2019). Briefly, observed acute toxicity in fish ranged from a LC50 (*Oncorhynchus mykiss*) of 0.04 mg/L for brodifacoum to LC50 (*Salmo gairdniri*) 65 mg/L for bromadiolone. EC50-values in the *Daphnia magna* immobilisation assay were reported between 0.0044 mg/L (difethialone) and >105 mg/L (warfarin). Growth inhibition in algae was highest by exposure to brodifacoum with an EC50 of 0.04 mg/L conducted with *Selenastrum capricornutum* and lowest for warfarin with an EC50 of >83 mg/L performed with *Scenedesmus subspicatus*. Furthermore, teratogenicity and embryo lethality in zebrafish (*Danio rerio*) at LC50 of 305 mg/L and EC50 of 60 mg/L warfarin (Weigt, Huebler et al. 2012) as well as lethal and sublethal effects were reported by Fernández, Santos et al. (2014). In addition, bromadiolone was found to induce embryo teratogenicity at 350 µg/L in the African clawed frog *Xenopus laevis* (Ondracek, Bandouchova et al. 2015). In general, a high level of AR exposure leads to overt toxicosis and lethality (Rattner and Harvey 2021), whereas low level AR exposure is suggested to affect fitness, immune function and other sublethal endpoints (Fraser, Mouton et al. 2018).

To our knowledge, Environmental Quality Standards (EQS) for marine and freshwater only exist for brodifacoum with an Annual Average-EQS (AA-EQS) of 1 pg/L and a Maximum Acceptable Concentration-EQS (MAC-EQS) of 20 ng/L for marine water and an AA-EQS of 1 pg/L and a MAC-EQS of 200 ng/L for freshwater (RIVM 2021). EQS for the other ARs could not be found. As ARs show a high bioaccumulation potential, see above, the partition will be directly in sediment and organisms, thus high concentrations in water are not expected.

2.5. Exposure pathways

ARs can enter the environment during manufacturing and production of active ingredients and products, inadequate application or disposal of products as well as through carcasses, faeces and urine of exposed animals (Regnery, Friesen et al. 2019). Island rodent eradications performed by broadcast aerial application also constitute a way of ARs to reach the environment (Masuda, Fisher et al. 2015). However, this exposure pathway is not relevant for Switzerland. Further pathways to the environment via wastewater treatment plants (WWTPs) include run-off after application in agriculture, livestock or other urban infrastructure sites, sewer baiting or application of anticoagulant pharmaceuticals for medical treatment (Gómez-Canela, Barata et al. 2014).

Exposure of target animals is based on voluntary ingestion of baits placed. However, this is also a reason for unintentional exposure of non-target animals. There are three known routes for accidental poisoning of non-target species by ARs. These encompass i) primary exposure through direct ingestion or contact with bait, ii) secondary (or tertiary) exposure by uptake of primarily (or secondary) exposed individuals and iii) secondary poisoning through consumption of organisms exposed via emissions to the environment (Regnery, Friesen et al. 2019).

Primary exposure is suggested to occur in small mammals, reptiles, invertebrates, marine biota, and birds (Nakayama, Morita et al. 2018). Regarding most predators and scavengers, pathway ii) seems most relevant as those mainly feed on rodents and other small mammal species or scavenge the carcasses of poisoned animals (Elliott, Hindmarch et al. 2014, Geduhn, Jacob et al. 2015) (Figure 1). For example, an analysis of fox stomach contents of urban foxes living in the City of Zurich revealed that 26% contained remains from rodents such as water voles, common voles and mice (Contesse, Hegglin et al. 2004). Besides, also remains from birds, invertebrates as well as wild fruits were found (Contesse, Hegglin et al. 2004). Moreover, raptors were reported to predominantly prey on small mammals or birds (Nakayama, Morita et al. 2018).

Exposure of humans to AR is mainly due to their use in human anticoagulation therapy. However, occasionally, exposure through oral uptake of rodenticide bait can occur, e.g., mainly due to accidental uptake by young children (King and Tran 2015). Treatment regimens encompass fresh-frozen plasma (FFP) and vitamin K_1 (phylloquinone/phytonadione) (King and Tran 2015).

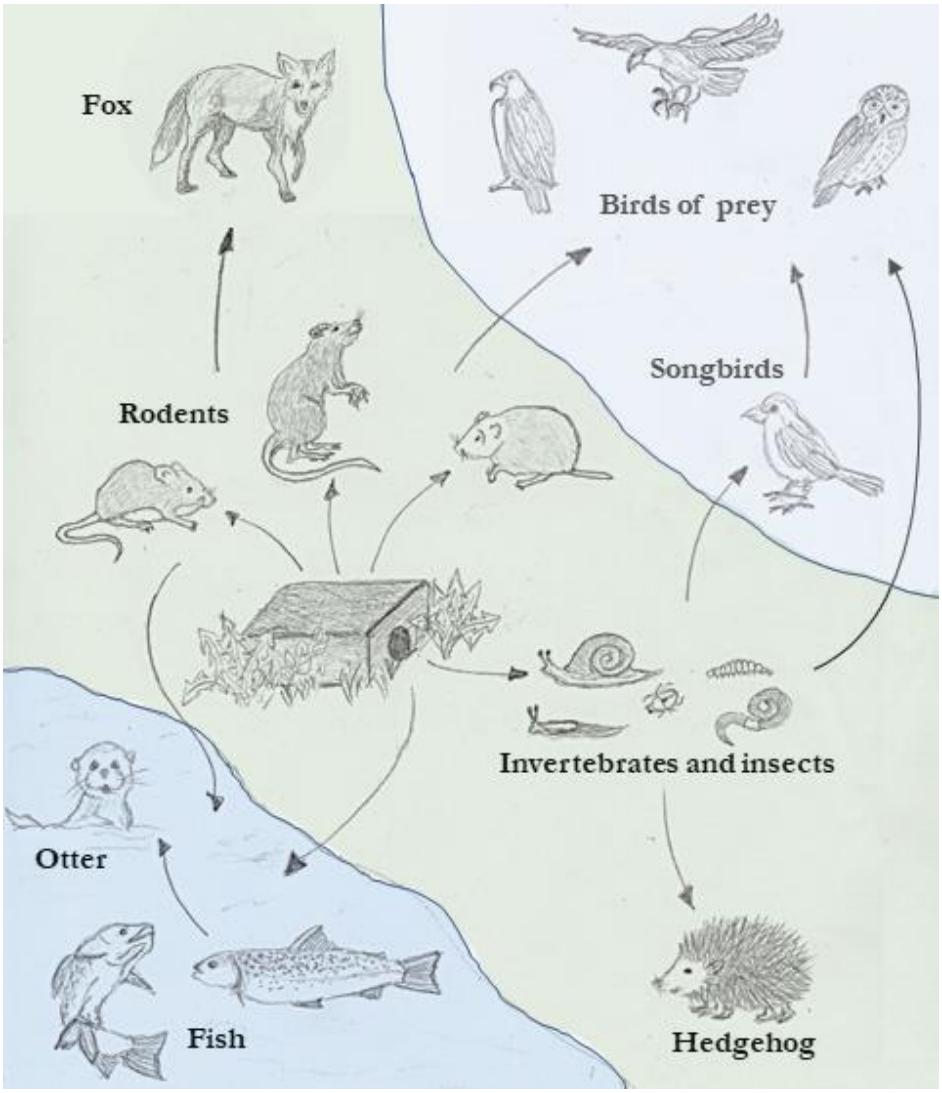

Figure 1. Possible pathways for anticoagulant rodenticide secondary poisoning of non-target animals in Switzerland.

2.6. Authorization of anticoagulant rodenticides in Switzerland

In Switzerland, eight ARs are currently approved as biocides to be used for rodent control (BAG 2020) for the protection of human health or materials. These comply with the EU approved ARs according to the database on biocidal active substances of the European Chemicals Agency (ECHA) (assessed on August 5, 2021) for biocidal application and encompass the FGARs chlorophacinone, coumatetralyl and warfarin as well as the SGARs bromadiolone, brodifacoum, difenacoum, difethialone and flocoumafen (ECHA 2021). Furthermore, bromadiolone is also approved in Switzerland as plant protection product (PPP) for crop protection on fields with an application volume of 5 g per colony of voles or water voles (BLW 2021). However, the sell-out deadline was the 30.11.2021 and the usage deadline ends on the 30.11.2022. Besides bromadiolone, no other ARs are authorized as PPP. Alternatives for bromadiolone as PPP encompass sulphur, aluminium phosphide and calcium phosphide (Van den Brink, Elliott et al. 2018).

All ARs meet the exclusion criteria laid down in the Biocidal Products Regulation; however, as alternatives are scarce, the use of ARs is still approved to control pest rodents. Currently, 69 rodenticide products of the product type RT14-rodenticides are authorized in Switzerland (Table 3) (ECHA 2021).

Table 3. Summary of authorized products containing rodenticidal active substances in the market area of Switzerland (ECHA 2021). *Anticoagulant rodenticides.

Active substance	Number of authorized products	%
Alphachloralose	5	7.3
Brodifacoum*	22	32
Bromadiolone*	11	16
Carbon dioxide	1	1.5
Cholecalciferol	2	2.9
Coumatetralyl*	1	1.5
Difenacoum*	21	30
Difethialone*	3	4.4
Flocoumafen*	3	4.4
Total	69	100
FGAR	1	1
SGAR	60	87
non-ARs	8	12

Recently, the teratogenicity of warfarin was shown, which led to a classification of all products with concentrations of ARs above 0.003% as toxic for reproduction (Pieper, Holthenrich et al. 2014, EC 2016). Thus, ARs were given a substance-specific limit of 0.003% in products sold to non-professionals in Switzerland on 01.03.2018 (BAG 2018). No reduction of effectiveness is expected applying <0.003% active ingredient compared to the previously used 0.005% for products containing difethialone, brodifacoum and flocoumafen. However, reducing the concentration of the active ingredients difenacoum, bromadiolone and FGARs might result in an efficiency reduction due to resistance occurrence observed in some regions (Buckle and Smith 2015, Regnery, Friesen et al. 2019).

Non-professional users (general public, private individuals) are authorized to apply ARs for biocidal use exclusively in tamper-proof and attachable bait boxes, which need to be placed inside buildings. The sale of loose baits is only permitted in sachets and no pulse or permanent baiting is allowed (BAG 2020). Concentrations of active substances above 0.003% are not allowed to be used by the public/private as they are classified as teratogenic. Package sizes are restricted for non-professional users depending on the formulation type and AR classification. Formulations as grain, pellet, paste as well as wax blocks in amounts of 50-1500 g, depending on the substance and the targeted rodents, may be used.

Professional users without a professional permit (e.g., farmers) are still authorized to use concentrations of active substances above 0.003% and no minimum package size is defined. The use in and around buildings exclusively in tamper-proof and attachable bait boxes is permitted. However, no pulse or permanent baiting is allowed (BAG 2020). Professional users with a professional permit for general pest control (VFB-S, SR 814.812.32) are authorized to apply ARs for rodent control in tamper-resistant and attachable bait boxes as well as at concealed, inaccessible, and secured bait sites installed in and around buildings, outdoors, in landfills, and in sewers. For this group of users, active substances concentrations are also allowed to exceed 0.003% with no limit regarding the package size. Pulse baiting is allowed with formulations, which contain brodifacoum, flocoumafen, or difethialone. Moreover, permanent baiting with bromadiolone or difenacoum containing products is allowed in places subjected to a high potential for reinvasion or if other control measures have not been effective (BAG 2020).

3. Anticoagulant rodenticides in the environment

According to Nakayama, Morita et al. (2018), 30 papers were published between 1998 and 2015 regarding AR exposure of non-target organisms in the US, Canada, UK, France, Spain, Denmark, Norway and New Zealand. To get a broader overview of research activity in the area we looked in Scopus[1] and in titles and abstracts of published works for the following keywords: “environment” and “anticoagulant and rodenticide” or “FGAR” or “SGAR” or “bromadiolone” or “brodifacoum” or “difenacoum” or “difethialone” or “flocoumafen”. The search in Scopus provided 490 hits for the period from 1990 until 2021. The number of papers per year are plotted in Figure 2 and indicate a steady increase in research output over time.

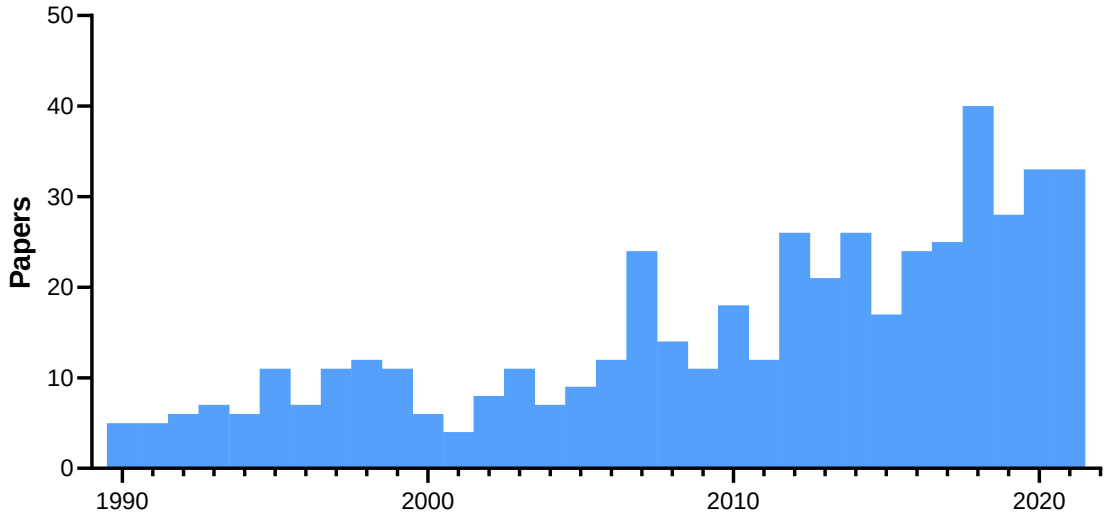

Figure 2. Development of the broadly defined research area “anticoagulant rodenticides and environment” as indicated by published papers over the years.

The last years, focus on AR exposure kept increasing, involving studies regarding AR occurrence from more countries and in different matrices such as aquatic biota and surface water. Recently, Regnery, Friesen et al. (2019) published a detailed description of recent publications regarding the occurrence of ARs in different matrices. In the following, a summary of *inter alia* these works is intended to give an overview about the general occurrence of AR residuals in the environment and serve as a basis to estimate a possible contamination of Swiss environments.

In general, different limits of quantification (LOQs) reported by different publications seem to determine the number of AR residues found in the respective investigated environmental samples, likely explaining the publication dependent heterogeneous distribution of relative AR positives versus total samples analysed. The lack of standardized extraction protocols and analytical methods, which vary among different publications, constitutes a major drawback for inter-publication comparison. Unfortunately, multiple publications fail to provide information on the use of internal standards and a coherent sampling strategy. To conclude, the comparison of AR positives versus total samples (due to varying LOQs) and AR concentrations (due to partially lacking internal standards and lacking method details) reported in the various publications remains a challenge. Furthermore, concerning aquatic exposure and a proposed (low) AA-EQS for brodifacoum of 1 pg/L, it is important to recognize that reported LOQs in water are sometimes more than 1000-fold this AA-EQS.

[1] www.scopus.com

3.1. Airborne contamination

Information about the contamination of air by ARs is scarce. However, due to the low vapour pressure of ARs (see Section 2.3), a high risk of airborne contamination is very unlikely. An exception could be shortly after broadcast aerial application, which is mainly used for rodent eradications on islands and therefore does not apply to Switzerland.

3.2. Contamination of water bodies

In general, ARs are known for their low water solubility (see Section 2.3). Thus, concentration in water bodies are expected to be low. This is in accordance with surface water assessments performed by several studies. Steffen (2014) reported no detection of warfarin, bromadiolone and difenacoum in German surface waters (LOQ_{AR}=5 ng/L). Chen, Zhu et al. (2014) found brodifacoum in only one out of 15 Chinese river waters, wastewater and well water samples and no bromadiolone. The exception was traced back to an illegal discharge of a local AR factory. Ten analysed Spanish groundwater samples showed no concentrations of ARs above the detection limit of 80 to 500 ng/L (LOD varying per AR) after intense bait application with chlorophacinone or bromadiolone (Hernández, Bernal et al. 2013). In addition, accidental discharge of bait pellets containing brodifacoum into a freshwater lake in New Zealand led to no detection of this active substance in the water column two days (17) and two weeks (n=10) after the spill (Fisher, Funnell et al. 2012). Norström, Remberger et al. (2009) analysed *inter alia* Swedish surface water and stormwater runoff and found no AR residuals above the limit of detection (LOD_{water}=5 ng/L). Warfarin was detected by Watkins, Winemiller et al. (2014) in three out of five sites downstream of US suburban WWTP discharges, whereas US groundwater (Barnes, Kolpin et al. 2008) and untreated sources of drinking water monitoring campaigns (Focazio, Kolpin et al. 2008) revealed no detects of warfarin.

However, sewer baiting presents a substantial contributor of AR release to the aquatic environment especially with heavy and prolonged rainfall (Regnery, Schulz et al. 2020). Baits in sewers are usually made out of wax or fat, where active compounds are not chemically bound. This might constitute a danger of bait disintegration in moist or wet conditions followed by the release of active substances into the environment (Regnery, Friesen et al. 2019, Regnery, Brinke et al. 2020). In addition, baits are often placed near water courses as rodents prefer to make their burrows at water-related sites. In consequence, baits are vulnerable to wash-off and run-off. Furthermore, Regnery, Schulz et al. (2020) found that ARs are insufficiently removed by conventional WWTPs. Even though one would expect ARs to stick to sludge based on their physico-chemical properties (Table 1), Regnery, Schulz et al. (2020) did not find any in activated sludge. In addition, Gómez-Canela, Barata et al. (2014) reported AR-residuals with a daily discharge of a few grams per day in WWTP effluents. This is especially of concern, as also pharmaceutical vitamin K antagonists prescribed, e.g., for thromboembolic disease treatment such as phenprocoumon and acenocoumarol add to the load of ARs reaching WWTPs and thus possibly also receiving water bodies (Regnery, Friesen et al. 2019). For example, Wode, van Baar et al. (2015) found phenprocoumon in groundwater (seven out of 14 samples) and surface water (seven out of 11 samples) at a former wastewater infiltration site in Germany. Furthermore, there is only limited information available regarding the transformation of ARs. Thus, transformation products of ARs might pass WWTPs and end up in the aquatic environment.

To conclude, elevated concentrations of AR residuals in the water phase of water bodies is not expected due to their low water solubility and high lipophilicity. In addition, dilution effects and irregular entry present a challenge for AR detection in water samples. Short-term higher concentrations could occur in close vicinity to WWTP effluents and stormwater overflow basins as well as in surface waters close to application areas such as densely populated areas, agricultural sites or known rodent burrows in relation to recent baiting events. However, a single proposed AA-EQS for an SGAR (i.e., brodifacoum) is very low (1 pg/L) and current method detection limits do not allow for an adequate monitoring of aqueous concentrations of AR in surface waters.

3.3. Occurrence of anticoagulant rodenticides in sediments and soils

In general, studies regarding the presence of ARs in soils and sediments indicated a low risk of contamination. For example, Cavanagh and Ward (2014) detected no ARs at New Zealand riverine sites (LOD ranged between 5 and 100 ng/g). However, flocoumafen was found in two (n=21) estuarine sediments (Cavanagh and Ward 2014). Kotthoff, Rüdel et al. (2019) investigated the presence of eight ARs in nine suspended particulate matter samples and detected only bromadiolone at levels up to 9.24 ng/g. In addition, accidental discharge of bait pellets containing brodifacoum into a freshwater lake in New Zealand led to no detection of this active substance in the lake-bottom sediment two days (n=7) and two weeks (n=9) after the spill (Fisher, Funnell et al. 2012). Soil samples from city parks as well as sediments from urban areas collected during a Swedish AR-monitoring program revealed no AR-concentrations above the limit of detection (LOD_{soil}=1 ng/g and $LOD_{sediment}$=1 ng/g) (Norström, Remberger et al. 2009).

Hernández, Bernal et al. (2013) detected residues of bromadiolone in three and chlorophacinone in two soil samples (n=60) after intense bait application. Furthermore, broadcast application of pellet baits composed of brodifacoum resulted in 33% (n=21) of investigated soil samples containing residuals of brodifacoum in levels up to 56 ng/g (Pitt, Berentsen et al. 2015). Interestingly, prior to baiting, two out of seven investigated soil samples already showed residuals of brodifacoum in concentrations up to 3 ng/g, possibly remains from prior baiting events (Pitt, Berentsen et al. 2015). This indicates a possible long term immobility and persistence of brodifacoum in soil compartments (Pitt, Berentsen et al. 2015).

To conclude, ARs can occur in sediments and soil after bait application and direct application of rodenticides into burrows could lead to local contamination. As ARs are applied as baits, it is possible that they will be consumed by fish or other aquatic organisms before they reach the sediments.

3.4. Exposure of non-target species with anticoagulant rodenticides

Many studies showed the prevalence of ARs in non-target wildlife. This was observed for a vast variety of species including different birds of prey, mammals, invertebrates, and reptiles. Differences in species sensitivities, toxicokinetics, bioaccumulation, season, and geography of the different ARs in non-target biota were observed (Elmeros, Lassen et al. 2018, Seljetun, Eliassen et al. 2019, Rattner and Harvey 2021). In the past, the focus was mainly on terrestrial wildlife exposure, however recent studies also show exposure to aquatic wildlife. Depending on detection limits and investigated animals, Nakayama, Morita et al. (2018) reported in their review rodenticide residuals in animal livers between 23% (France, 42 of 181 animals) and 93% (Denmark, 523 of 560 animals), suggesting that AR residuals in non-target animals can be found in all countries (Nakayama, Morita et al. 2018). In total, 55% of investigated non-target organisms published in several articles between 1998 and 2015 contained AR residuals (n=2694). Mostly brodifacoum (31%), bromadiolone (30%) and difenacoum (26%) were found, other AR were present in less than 10% of the investigated animals. Among the investigated animals, AR residuals were mainly found in animals of higher trophic levels such as in predators (57%) and in raptors (57%) indicating biomagnification. Anticoagulant rodenticides occurred also in 50% of the investigated reptiles, possibly through consumption of affected invertebrates (Nakayama, Morita et al. 2018). Koivisto, Santangeli et al. (2018) detected AR residuals, mainly bromadiolone, in livers of 82% predators and scavengers from 17 species in Finland (n=131). Sánchez-Barbudo, Camarero et al. (2012) found AR residues in 39% (n=401) wild and domestic animals found dead in Spain. According to the authors, 35% of the investigated animals may have died by AR poisoning. Exposure to AR was determined as sublethal at a total AR-liver concentration of 5 (3-7) ng/g wet weight and as lethal at 706 (473-1'054) ng/g wet weight (Sánchez-Barbudo, Camarero et al. 2012).

3.4.1. Exposure of aquatic biota to anticoagulant rodenticides

Baits containing AR are often placed near water courses such as river shores as rodents prefer to make their burrows at water-related sites. In consequence, baits can reach the aquatic environment via wash-off and run-off processes. Furthermore, according to Regnery, Parrhysius et al. (2019), WWTP effluents are a source of ARs entering the aquatic environment, where they can accumulate in aquatic biota such as fish. Also stormwater overflow can release untreated but diluted wastewater potentially containing baits as well as poisoned rodent carcasses, which might be flushed out from sewers and directly released into the aquatic ecosystem (Regnery, Friesen et al. 2019). Regnery, Parrhysius et al. (2019) found SGAR residuals in 83% (n=12) of fish liver samples collected from seven Bavarian streams receiving treated WWTP effluent as well as in 59% (n=32) of fish liver samples collected from 25 bioaccumulation ponds of municipal WWTPs. Also Kotthoff, Rüdel et al. (2019) detected brodifacoum in concentrations up to 12.5 ng/g in 88% of liver samples from bream (*Abramis brama)* collected in 2015 at 16 riverine sites and two lakes and stored at the German Environmental Specimen Bank. Some samples also contained residuals of difenacoum, bromadiolone, difethialone, and flocoumafen above LOQ (Kotthoff, Rüdel et al. 2019). Cavanagh and Ward (2014) detected bromadiolone and coumatetralyl in concentrations up to 34 ng/g and 24 ng/g, respectively, in liver of brown trout (five out of seven), eel (two out of 17) and yellow-eye mullet (one out of three). However, no residuals of warfarin, brodifacoum and flocoumafen were detected. In the eight samples with detects in livers, none of the muscle samples showed residuals of the five investigated AR. This corresponds with findings by Norström, Remberger et al. (2009), where no AR residuals were detected in fish muscle tissue during a national screening program in Sweden. Whole-body fish tissue samples from several coastal marine species collected at the Wake Atoll after a rodent eradication attempt on Wake Island with brodifacoum showed no AR residuals above (an unspecified) LOQ (Siers, Shiels et al. 2016). Riegerix, Tanner et al. (2020) determined LD50s of different representatives of saltwater (black triggerfish and red-toothed triggerfish) and freshwater fishes (fathead minnow and largemouth bass) to diphacinone (LD50=90-303 µg/g), chlorophacinone (LD50=125-402 µg/g), and brodifacoum (LD50=36-96 µg/g). According to the authors, fish have a lower sensitivity to the examined ARs compared to other taxa such as mammals or birds. Thus, fish exposed to ARs might be a possible AR-source for predator species with a predominantly fish-eating diet.

No warfarin was detected in caged freshwater mussels analysed after four weeks of exposure in the Grand River, Ontario, upstream and downstream of a WWTP (de Solla, Gilroy et al. 2016). Wild mussels collected upstream the same WWTP contained up to 1.15 ng/g ww warfarin (mean method detection limit=0.69 ng/g ww).

Pitt, Berentsen et al. (2015) reported brodifacoum residuals in 44% of fiddler crabs (n=16) and in 75% of hermit crabs (n=20) sampled within four weeks after broadcast application of pellet baits composed of brodifacoum on Palmyra Atoll, tropical Pacific. Also, nine out of ten black-spot sergeant fish showed concentrations up to 315 ng/g brodifacoum. Moreover, Masuda, Fisher et al. (2015) found low concentrations of brodifacoum residuals in some blue cod liver (two out of 24, 26 and 92 ng/g), in four out of 24 limpets (range=1-16 µg/g) and in four out of 24 mussels (range=1-22 ng/g) collected 43-176 days after broadcast application of brodifacoum bait on Ulva Island, New Zealand.

To conclude, there is a risk of exposure of aquatic organisms to ARs, especially in water bodies downstream of WWTP effluents, in streams that run through urban areas, in water courses where baits are placed on shores or after broadcast application of AR. Although the sensitivity of fish to AR appears lower compared to other non-target organisms and thus the risk of mortality seems to be low for individual exposed aquatic organisms, bioaccumulation may occur, and aquatic animals could act as toxic vectors for predator species at higher trophic levels. Furthermore, AR were mainly found in fish liver instead of whole muscle tissues (Kotthoff, Rüdel et al. 2019). Thus, the investigation of fish liver samples is recommended.

3.4.2. Occurrence of anticoagulant rodenticides in terrestrial non-target organisms

Many publications report the occurrence of ARs in terrestrial biota. Especially predators such as foxes were examined, but also cases of AR residuals in invertebrates and reptiles are known. In the following, a non-exhaustive list of examples for AR occurrence in different terrestrial non-target animals is given.

Fourel, Sage et al. (2018) reported the occurrence of bromadiolone in 81% (n=48) investigated red fox livers from France after bromadiolone application for vole outbreak control. Interestingly, concentrations of up to 2060 ng/g were found composed of mainly *trans*-bromadiolone and rarely *cis*-bromadiolone. As foxes specialize on voles, if available, they might get temporarily settled, which increases the risk of bioaccumulation (Fourel, Sage et al. 2018). A monitoring study conducted in Austria found AR residuals in 60% of collected fox liver samples with high concentrations of brodifacoum (up to 750 ng/g), bromadiolone (up to 700 ng/g) and difethialone (up to 480 ng/g) (Hauzenberger, Lenz et al. 2020). Also, difenacoum, chlorophacinone, flocoumafen and coumatetralyl were found but in lower concentrations, suggesting that FGARs play a minor role in comparison to SGARs. Geduhn, Jacob et al. (2015) reported that 60% (n=331) of red foxes (*Vulpes vulpes*) liver samples collected in Germany contained at least one of the eight investigated ARs, predominantly the SGARs brodifacoum and bromadiolone with LOQs between 1 and 5 ng/g ($LOQ_{coumatetralyl=}$1 ng/g, $LOQ_{warfarin,difenacoum=}$2 ng/g, $LOQ_{brodifacoum,bromadiolone}$=3 ng/g and $LOQ_{difethialone,flocoumafen,chlorophacinone=}$5 ng/g). In 20% of the samples, concentrations were high enough to possibly induce biological effects. Seljetun, Eliassen et al. (2019) found AR residuals in 54% (n=139) investigated faecal samples of red foxes, which were shot during the regular hunting season in Norway. Forty 40% of the samples contained more than one AR and 7% contained even four different AR. Mainly brodifacoum was found, but also residuals of coumatetralyl (17%), bromadiolone (16%), difenacoum (5%), difethialone (1%), and flocoumafen (1%) were detected. Differences in geographical region were found but no correlation of AR occurrence with seasonality, age or gender was discovered (Seljetun, Eliassen et al. 2019). In another study by Seljetun, Sandvik et al. (2020), a comparison of faecal and liver samples of red foxes collected in Norway revealed that AR occurred in 53% and 83% of the samples, respectively, suggesting that liver samples are more suitable for AR residual analysis in non-target organisms. However, fecal samples could be used as non-lethal means for assessing AR exposure. Also Prat-Mairet, Fourel et al. (2017) investigated scats as a non-invasive alternative in contrast to liver samples. However, the authors point out that SGAR concentrations decreased rapidly after excretion due to weathering exposure (degradation $t_{1/2}$ ranged from 5.3 days for chlorophacinone to 8.0 days for bromadiolone). Thus, it was concluded that concentrations observed in faeces do not represent exposure due to different transformation processes, absorption and excretion of different ARs (Prat-Mairet, Fourel et al. 2017). In Denmark, Elmeros, Lassen et al. (2018) investigated AR prevalence in 31 stone martens (*Martes foina*) and 29 polecats (*Mustela putorius*) and found residuals of mostly bromadiolone in 100% and 97% of examined liver tissues samples, respectively. On average, concentrations of bromadiolone were 957 ng/g ww (max.: 2083 ng/g ww) in stone martens and 272 ng/g ww (max.: 1026 ng/g ww) in polecats. Although regulatory restrictions with regards to their use away from buildings were made the year prior to the study, bromadiolone occurrence in investigated animal classes remained the same. Prevalence of AR residuals was positively correlated to the urban area and concentrations were lower in autumn compared to other seasons. Also Koivisto, Santangeli et al. (2018) detected ARs in livers of *inter alia* foxes, raccoon dogs and mustelids collected in Finland, mainly SGARs dominated by bromadiolone.

In the Greater Cape Town region of South Africa, Serieys, Bishop et al. (2019) found AR residuals in 92% of caracal livers (*Caracal caracal*, n = 28) and in 44% opportunistically sampled Cape Clawless otter livers (*Aonyx capensis*, n = 9). The proximity to vineyards was suggested to be the most important risk factor for caracals to AR exposure (Serieys, Bishop et al. 2019). Lemarchand, Rosoux et al. (2010) found only bromadiolone in livers of two out of 20 Eurasian otter (*Lutra lutra*) killed by road traffic in France in concentrations of 400 and 850 ng/g fresh weight. This corresponds with findings in France by Fournier-Chambrillon, Berny et al. (2004), who reported AR

prevalence in two out of eleven European otters (*Lutra lutra*) as well as occasional findings in European mink (*Mustela lutreola*), American mink (*Mustela vison*) and polecats (*Mustela putorius*).

Dowding, Shore et al. (2010) found AR residuals (mostly SGARs) in 67% of 120 investigated European hedgehogs (*Erinaceus europaeus*) from throughout Britain. Twenty-three % of hedgehogs showed residuals of more than one AR. This corresponds with findings by López-Perea, Camarero et al. (2015) who reported a 57% prevalence of ARs in Algerian hedgehogs (*Atelerix algirus*; n=104) and 58% in European hedgehogs (*Erinaceus europaeus*; n=48), which were collected throughout Majorca Island and Catalonia, respectively. Mainly brodifacoum (40%), bromadiolone (35%) and difenacoum (26%) were found, but also flocoumafen (8.7%), difethialone (7%) and warfarin (0.3%) were present in some samples. Thirty-five % of analysed hedgehogs had up to five different ARs in their liver.

Alomar, Chabert et al. (2018) investigated residuals of chlorophacinone, bromadiolone and brodifacoum in slugs and observed an accumulation of these compounds with no sign of mortality after a five-day exposure. This corresponds with Shirer (1992), suggesting that invertebrates are unlikely to be affected by ARs due to their differences in the blood clotting system. Also Shore and Coeurdassier (2018) assume that invertebrates are most likely affected via a different pathway compared to vertebrates. However, slugs examined during a field study conducted by Alomar, Chabert et al. (2018) revealed that over 90% contained brodifacoum after baiting with brodifacoum. Therefore, the authors suggest that slugs are susceptible to primary exposure and could serve as a potential transmission route for ARs to their predators.

Pitt, Berentsen et al. (2015) found brodifacoum residuals in 100% of ants (n=15) and in 88% of cockroaches (n=18) collected within four weeks after broadcast application of pellet baits composed of brodifacoum on Palmyra Atoll, tropical Pacific. Furthermore, brodifacoum residuals were detected in 52% of analysed geckos (n=21) (Pitt, Berentsen et al. 2015). Lettoof, Lohr et al. (2020) investigated AR residuals in urban reptiles in Australia and found SGARs in livers of three investigated species: 91% in dugites (rodent predator), 60% in bobtails (omnivore) and 45% in tiger snakes (frog predator). The authors report a three-to-five-fold higher tolerance towards ARs in comparison to birds or mammals and suggest that reptiles can retain ARs for years without showing effects themselves. Thus, reptiles can act as toxic vectors to higher trophic levels leading to a widespread AR contamination in the food web (Lettoof, Lohr et al. 2020).

In conclusion, ARs are widespread in terrestrial non-target organisms. Concentrations found in mammals were partly high enough to possibly induce biological effects. Anticoagulant rodenticide residuals in invertebrates were regularly observed, although no effects on invertebrates were assumed due to their differences in the blood clotting system. However, invertebrates can serve as a potential transmission route for ARs to other trophic levels. Livers provide the best sample material for monitoring and low LOQs can be obtained.

3.4.3. Prevalence of anticoagulant rodenticides in avian non-target organisms

Based on research conducted with wild Norway rats laboratory enclosure trials, 67% rodents killed by a lethal dose of brodifacoum died above ground, where they would be potentially available for avian scavengers (Cox and Smith 1992). Furthermore, all poisoned rodents were out in the open 24h prior to death already showing symptoms (Cox and Smith 1992). According to Howald, Mineau et al. (1999), 13.4% of radio-tagged rats died above ground after a baiting campaign on Langara Island. Thus, rodents killed by AR would be an easy prey for avian scavengers and a possible route for secondary exposure. Besides target organisms, non-target small mammals represent an important route for AR exposure to avian non-target organisms such as barn owls (Geduhn, Esther et al. 2016).

Geduhn, Esther et al. (2016) found AR residues in 55% (n=11) barn owl (*Tyto alba*) liver samples collected in Germany. The prevalent AR were brodifacoum, bromadiolone and flocoumafen detected in concentrations over 200 ng/g. A monitoring study conducted in Austria found AR residuals in 86% and 75% of collected tawny owls and hawk owls liver samples, respectively, dominated by brodifacoum present in concentrations up to 77 ng/g and 28 ng/g, respectively (Hauzenberger, Lenz et al. 2020). Lohr (2018) found higher AR concentrations in owl carcasses in winter compared to in summer. Thus, the authors suggested that a seasonal variability in AR poisoning might occur due to possibly increased baiting or shift in diet and thus higher risk of exposure in winter. Similar findings were reported by Christensen, Lassen et al. (2012), who found a strong tendency for seasonal variations with lowest AR liver concentrations reported in autumn. It has been suggested that this finding is because birds migrating from northern Scandinavia in the fall were exposed to lower levels of ARs. The authors reported AR residuals in 84-100% (n=430) birds, depending on the species, dominated by the SGARs difenacoum, bromadiolone and brodifacoum (Christensen, Lassen et al. 2012). In Norway, Langford, Reid et al. (2013) reported that 70% of the investigated golden eagles and 50% of the examined eagle owls contained a total SGAR liver concentration of 11 to 255 ng/g.

In the US, livers from four species of birds of prey (red-tailed hawks (*Buteo jamaicensis*), barred owls (*Strix varia*), eastern screech owls (*Megascops asio*) and great horned owls (*Bubo virginianus*)) were analysed for AR residuals. From 2006 to 2010, 86% (n=161) contained AR residuals, predominantly brodifacoum, and 6% of birds died from AR toxicosis (Murray 2011). Despite the implementation of restrictions on the sale of SGAR products through general consumer outlets in 2011, 96% (n=94) of liver samples collected from 2012-2016 still contained AR residuals (Murray 2017). Brodifacoum was found in almost all positive birds (95%), while more than two SGARs were detected in 66% of all bird livers (Murray 2017). In the UK, research by Walker, Chaplow et al. (2013) reported SGARs in livers of 85% (n=58) barn owls (*Tyto alba*), 100% (n=20) kestrel (*Falco tinnunculus*) and 94% (n=18) red kites (*Milvus milvus*). Mainly difenacoum and bromadiolone were detected, but multiple AR residuals were frequently observed. However, liver concentrations were generally low and probably not the cause of mortality (Walker, Chaplow et al. 2013). Difenacoum and bromadiolone were also the predominant substances found in raptors during Scottish monitoring schemes conducted in the period 2000-2010 (Hughes, Sharp et al. 2013). Bird livers from 773 individuals comprising seven different species were analysed containing detectable AR residuals between 29% (Peregrine falcon, *Falco peregrinus*) and 69% (Red kite, *Milvus milvus*). In France, poisoning by bromadiolone was suggested to be the cause of mortality for 28 red kites (*Milvus milvus*) and 16 common buzzards (*Buteo buteo*) after intensive bromadiolone application (Coeurdassier, Riols et al. 2014). Moreover, a potential impact of bromadiolone on the breeding population of red kites was assumed (Coeurdassier, Riols et al. 2014). Moriceau, Lefebvre et al. 2022 reported AR residues (mainly SGARs) in 50% of 156 liver samples from dead wild raptors collected over 12 years (2008-2019) in south-eastern France. Detected AR concentrations were higher in predators than in scavengers with 83% and 39% positive AR detects, respectively. Also research conducted in Taiwan by Hong, Morrissey et al. (2019) between 2010 and 2018 reported AR residuals of up to 89% in different raptor species (n=221, species=21) pointing out that AR residuals in non-target organisms might be a worldwide problem. Especially common rodent- and snake-eating birds frequently contained AR residuals, mainly brodifacoum followed by flocoumafen and bromadiolone (Hong, Morrissey et al. 2019). Furthermore, the authors reported a correlation between AR occurrence frequency and supply of free ARs to farmers by the Taiwanese government (Hong, Morrissey et al. 2019). Geographic differences in AR residual occurrence were reported by López-Perea, Camarero et al. (2015), who detected AR residuals in livers of 58% (n=26) of a resident population of Eurasian scops owl (*Otus scops*) from Majorca in contrast to 14% (n=7) of a migratory population from Catalonia.

4. Screening of environmental samples for anticoagulant rodenticides residues

4.1. Method development

For the analysis of ARs in liver, an ESI-LC-MS/MS method was developed to quantify seven SGARs. A detailed description of the sample preparation, clean-up and analysis can be found in Annex A. Briefly, 3-4 g frozen liver is homogenized with nanopure water in a 50:50 w:w ratio. Ca. 5 g of homogenate is then weighed into a PP-tube to which internal standard, a ceramic homogenizer and acetonitrile are added after which the homogenate is vortexed. Subsequently, a centrifugation step is performed before freezing the supernatant for 5 to 30 h at -22°C to support phase separation. After another centrifugation step, an aliquot of the supernatant is vortexed with a sorbent to remove additional fat and matrix. Subsequently, sorbent is removed by centrifugation and 0.4 mL of the supernatant layer is mixed with nanopure water and measured by ESI-LC-MS/MS. Chromatographic parameters such as gradient, flow and column were taken from Regnery, Parrhysius et al. (2019) with slight modification. No chromatographic separation of individual AR stereoisomers was intended to obtain a sum-peak. Limits of quantification were mostly in the range of 0.05 to 0.1 ng/g (see Annex A for an LOQ example).

Figure 3 shows the chromatographic separation of the seven analysed ARs. Initially, chlorophacinone was included in the method. However, ionization did not work well (slight elevation visible at 3.6 min) and there is no approved product with this active ingredient available on the Swiss market. Thus, chlorophacinone was excluded from the final method.

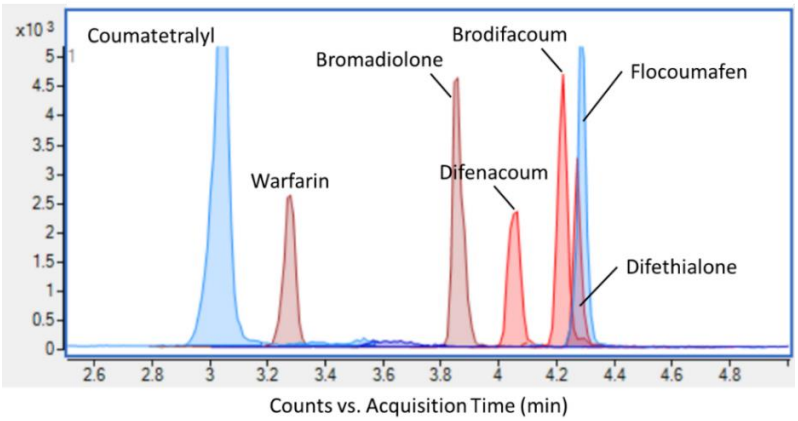

Figure 3. Chromatographic separation of AR-standards; brodifacoum and flocoumafen can be separated based on ion mass.

As the extraction method involves aliquots of liver between 3 to 4 g, there can be concerns that an inhomogeneous distribution of ARs in a larger liver from a fox or a large fish could affect results obtained from individual liver sections. A high variability in AR concentrations across aliquots would preclude the use of homogenates of a single aliquot per liver and would warrant analysis or the homogenization of complete livers – a challenging and costly task for e.g., foxes and large fish.

To investigate homogeneity of AR concentrations in larger livers we divided seven livers into three to eight aliquots of ca. 3 to 4 g and analysed pieces individually. Livers from foxes were not available as complete livers but rather two lobes cut from the whole liver. Bird livers were always whole livers. The coefficient of variation (CV) of AR concentrations was on average 10% (n=25 quantified AR series; four examples are shown in Figure 4). A CV of 10% is typically within analytical LC-MS/MS accuracy (see Annex A for a detailed data overview). This low CV seen across liver aliquots and across various AR supports the analysis of a single subsection of a larger liver rather than having to collect and homogenize entire livers.

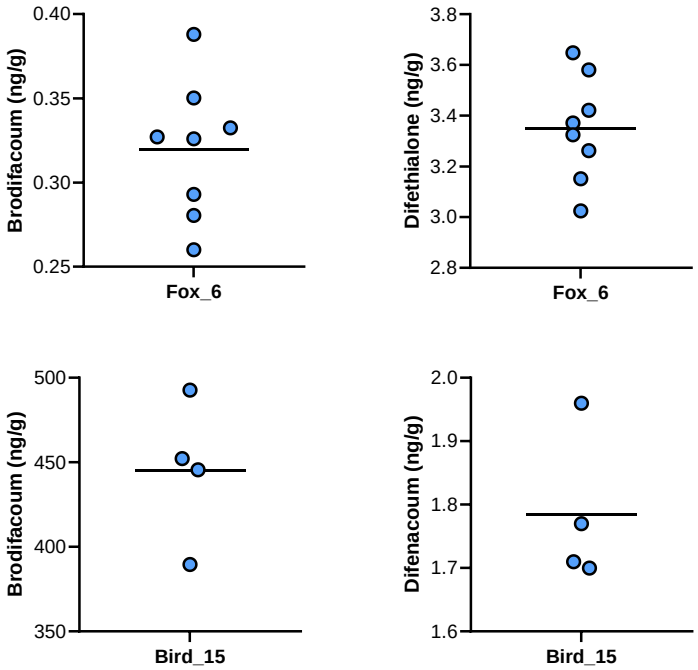

Figure 4. Four examples out of 25 cases where a compound was quantified in multiple aliquots from the same liver. In the case of Fox 6, eight pieces were analysed (comprising part of the total liver) and in the case of Bird 15 four pieces were analysed (the complete liver included a fifth piece which was analysed by Julia Regnery at BfG). Lines indicate mean values of the individual data (dots). See Annex A for full data set and all compounds and analysed samples (n=25 combinations).

4.2. Method validation

We validated our chemical analytical method by analysing ten fish liver homogenates from a study published by Regnery, Schulz et al. (2020). These fish liver samples were collected and analysed by the Federal Institute of Hydrology (BfG) in 2018/2019. We achieved comparable results to those reported by Regnery, Schulz et al. (2020). Exceptions were brodifacoum, bromadiolone and difenacoum concentrations in Sample 1 L and 48 L (sample numbers from Regnery, Schulz et al. (2020)) which showed a difference of more than factor 2 (see Annex A). A possible reason for the difference is that the samples were already three to four years old and stored, not vacuum sealed, in aluminium cups (Annex A). It is likely that liquids evaporated with time and compounds became more concentrated leading to higher concentrations measured with our Ecotox Centre method. The difference between BfG and Ecotox Centre results was largest for Sample 48L. A reanalysis of the sample by the Ecotox Centre confirmed the observed discrepancy.

In addition to the analysis of ca. 3.5 year old fish samples by the Ecotox Centre, Julia Regnery (BfG, Koblenz, Germany) analysed aliquots from ten liver samples from our screening set: six fox livers and four livers from birds of prey. Figure 5 shows results for three compounds, additional graphs and data can be found in Annex A. Using fresh samples, comparability between both analyses was improved over the first validation round and all results were within a factor of 2 difference. As the second validation round also included an additional source of variability – the liver homogenization step (i.e., the first comparison was done with already homogenized samples) – this indicates good comparability between analytical methods serving as a mutual validation.

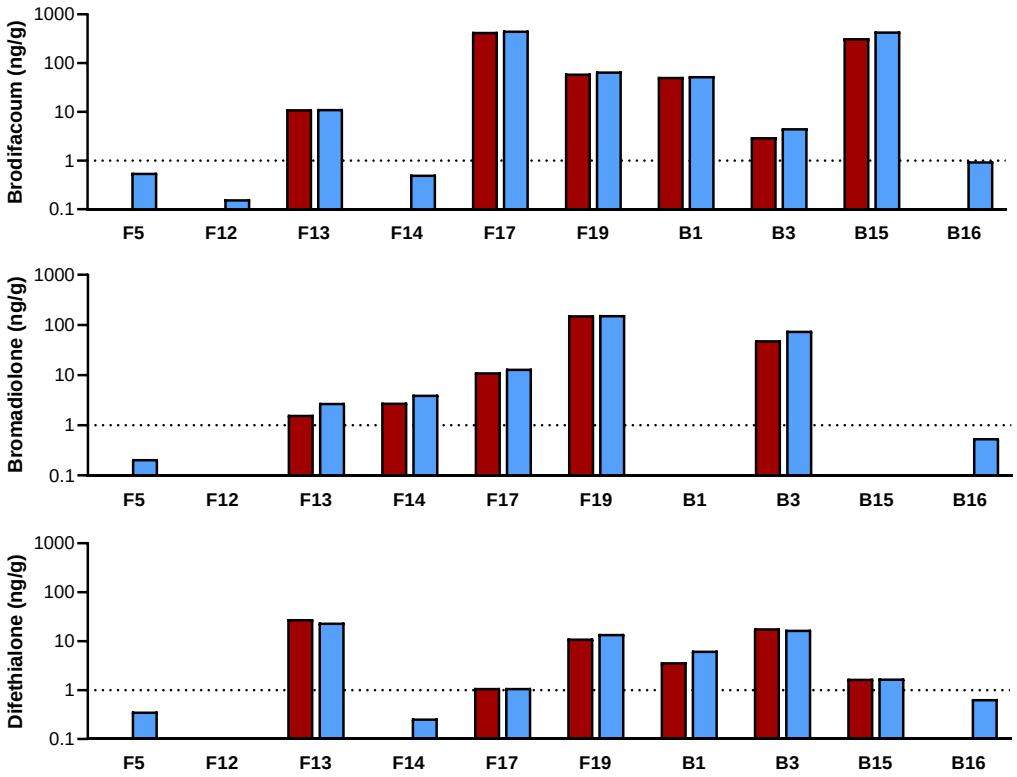

Figure 5. Comparison of AR concentrations in six fox (F) and four bird (B) liver samples quantified by Julia Regnery at the Federal Institute of Hydrology (BfG; data shown in red) and quantified by the Ecotox Centre (blue). Concentrations vary a lot across samples and are thus plotted along a log axis. Dotted lines are drawn at 1 ng/g, this was roughly the LOQ for this particular analysis by BfG. Further details on these samples can be found in Annex A.

4.3. Anticoagulant rodenticide screening of 76 liver samples

To obtain an overview of the potential exposure of non-target biota to AR, a screening was conducted with different liver samples. Liver samples were selected based on the literature review, expert survey (Section 5) and availability.

4.3.1. Sample details

A total of 25 fox livers were obtained via the Institute of Parasitology (University of Zurich). The investigated foxes were shot by game wardens and hunters in the framework of their fox hunting activities for the management of local fox populations. The carcasses were collected and dissected at the Institute of Parasitology within the framework of a parasite screening program. The foxes originated from four different hunting districts: Winterthur Hegiberg, Elsau Geitberg, Dübendorf and Dürnten. Foxes were classified as female (n=11) and male (n=14) as well as younger than one year (n=11) and older than one year (n=14).

Livers from 21 different birds of prey (n_{female}=8, n_{male}=13) could be obtained: 18 common buzzards, two tawny owls and one common kestrel. Birds were randomly collected by the Greifvogelstation Berg am Irchel from raptors that died during care at the station. The cause of dead was mostly unknown, however, animals had a trauma, were lean and weak at administration. Animals were stored at -20 °C and dissected at the University of Zurich (Vetsuisse Faculty, Institute for Food Safety and Hygiene, Section of Poultry and Rabbit Diseases).

Four hedgehog liver samples (n_{female}=3, n_{male}=1) were provided by SWILD (independent research and consulting association of wildlife biologists) and the Hedgehog Centre of Zurich. Three of the hedgehogs died during care, the fourth was already dead on arrival.

In three cantons (Sankt Gallen, Thurgau and Aargau), 41 fish liver samples were collected by cantonal fisheries inspectorates or by hobby fisher. Samples from smaller fish caught at the same location were pooled to obtain a larger liver mass and thus maintain low LOQ values. The final fish sample set numbered 30.

Sample identifiers of all samples including descriptions of the individual samples such as sampling location, sampling dates and sample weights can be found in Annex A.

4.3.2. Results of anticoagulant rodenticide screening

Fox liver samples contained mainly brodifacoum and bromadiolone (see Figure 6) but also difenacoum, difethialone and flocoumafen were detected (see Annex A). In 23 out of 25 fox liver samples, up to four ARs were found above LOQ (Table 4 and Annex A). The sum of AR concentrations was above 100 ng/g in six out of 25 samples (Table 4). This concentration of 100 ng/g is regarded as a concentration of concern (Section 6). The highest single detected single compound concentration was 1100 ng/g of brodifacoum in Fox 8, an older female fox from outside the city of Winterthur. Given our small sample set, this value can be viewed as high in relation to a large study from Germany. In the German study, a maximum of 2433 ng/g of brodifacoum was found in one of 331 foxes (Geduhn, Jacob et al. 2015).

Livers from foxes younger than 1 year had on average 1.6 different AR compounds, 2.9 compounds were found in those older than one year. This is expected, when considering increasing opportunities of exposure with age and the long retention times of AR following ingestion. Mean concentrations were also higher in older foxes (187 ng/g) compared to younger foxes (5.4 ng/g).

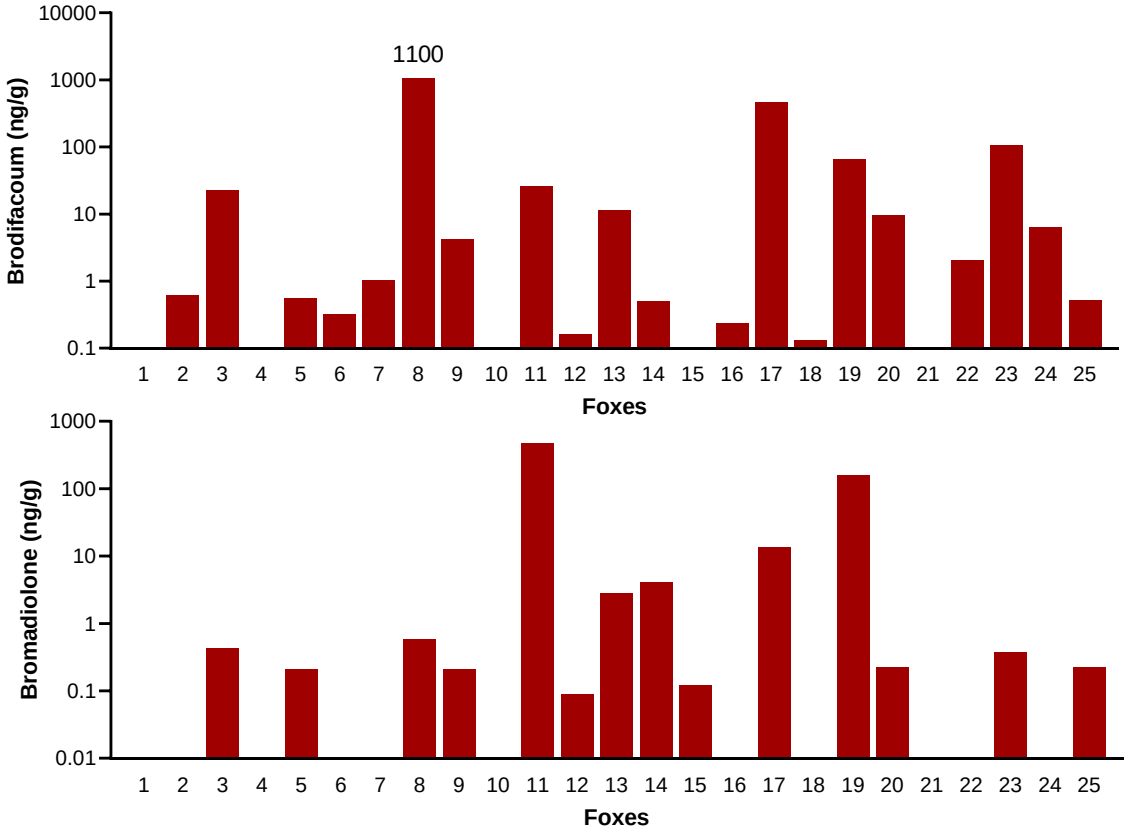

Figure 6. Concentrations of brodifacoum (top) and bromadiolone (bottom) in fox liver samples. Fox 8 had the highest single compound concentration in the sample set (i.e., 1100 ng/g of brodifacoum).

Brodifacoum, bromadiolone, difenacoum and difethialone were found in birds of prey (Annex A). Only one sample, Bird 11, had all seven analysed AR concentrations below LOQ. The other 20 samples contained up to four different ARs (Figure 7). Three out of 21 birds of prey (i.e., 14%) had an AR sum above 100 ng/g, a concentration of concern (Section 6). However, for birds of prey also a critical concentration as low as 20 ng/g has been suggested (Thomas, Mineau et al. 2011). Ten out of 21 (48%) birds of prey exceeded this threshold of 20 ng/g. Although our sample size is too small to allow for comparative analyses, the highest detected sums of AR in buzzards of 440 and 450 ng/g (Bird 4 and Bird 15) is close to the maximum concentration of 721 ng/g detected in 141 buzzards investigated in Denmark (Christensen, Lassen et al. (2012); >20% of buzzards with >100 ng/g). Also Moriceau, Lefebvre et al. (2022) observed an incidence of raptors with summed second-generation AR concentrations of >100 ng/g close to 14% which matches our observation (i.e., 14% also).

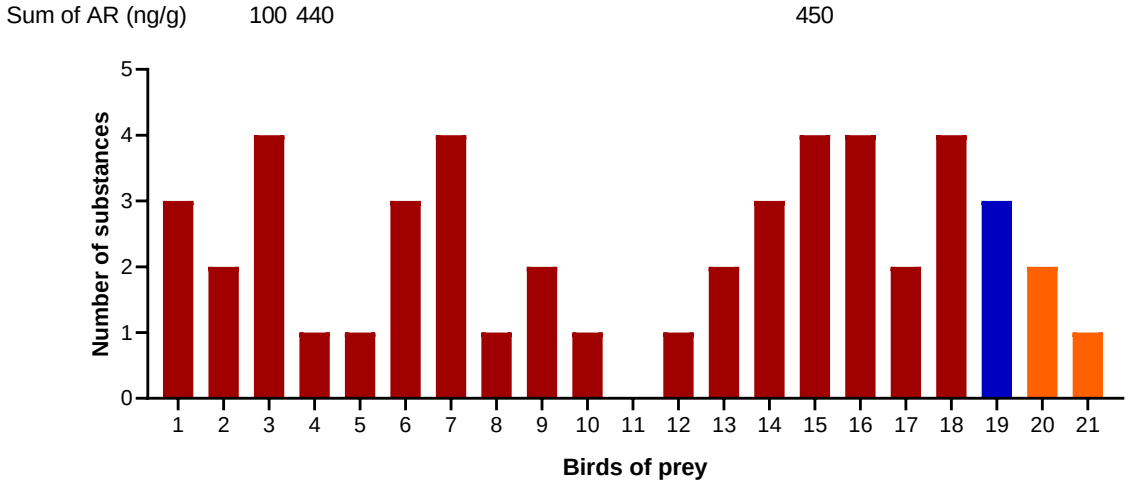

Figure 7. Number of different anticoagulant rodenticides (AR) found in livers from birds of prey. Liver samples with a summed AR concentration of 100 ng/g are indicated with the sum AR shown above the bar. Red: common buzzard; blue: common kestrel; orange: tawny owl.

All four hedgehog livers contained up to four different ARs (brodifacoum, bromadiolone, difenacoum and coumatetralyl (Table 1; Annex A). The highest concentration of an individual compound found was brodifacoum with 0.85 ng/g in Hedgehog 4. Although the sample size is extremely small, this observation hints at least at a general AR background exposure of terrestric wildlife living in urban settings. It has to be noted that much higher AR concentrations were observed in a study from the UK with a large sample size (n=120, >10% hedgehogs with >100 ng/g; Dowding, Shore et al. 2010).

AR were detected in 22 of 30 fish samples and several fish samples contained three AR. Summed AR concentrations in most samples were below 0.5 ng/g (Table 4, Figure 8). In eight samples, summed AR concentrations were between 0.5 and 1.0 ng/g. The liver of Fish 18 contained 1.5 ng/g of total AR and the highest sum concentration of 36 ng/g occurred in Fish 19, a brown trout from a purely agricultural influenced catchment without treated sewage effluent (Eschelisbach, TG). The origin of the samples was diverse in terms of species, habitat, sizes and ages. Although AR were not always detected, these data indicate a general background exposure of aquatic wildlife to AR, even in Lake Constance (Sample 6, pooled lake whitefish: sum AR 0.83 ng/g; Sample 7, a European perch, sum AR 0.26 ng/g). However, one very high detect (i.e., Fish 19) indicates that also in the aquatic environment high AR concentrations are no exception and requires further investigation and monitoring.

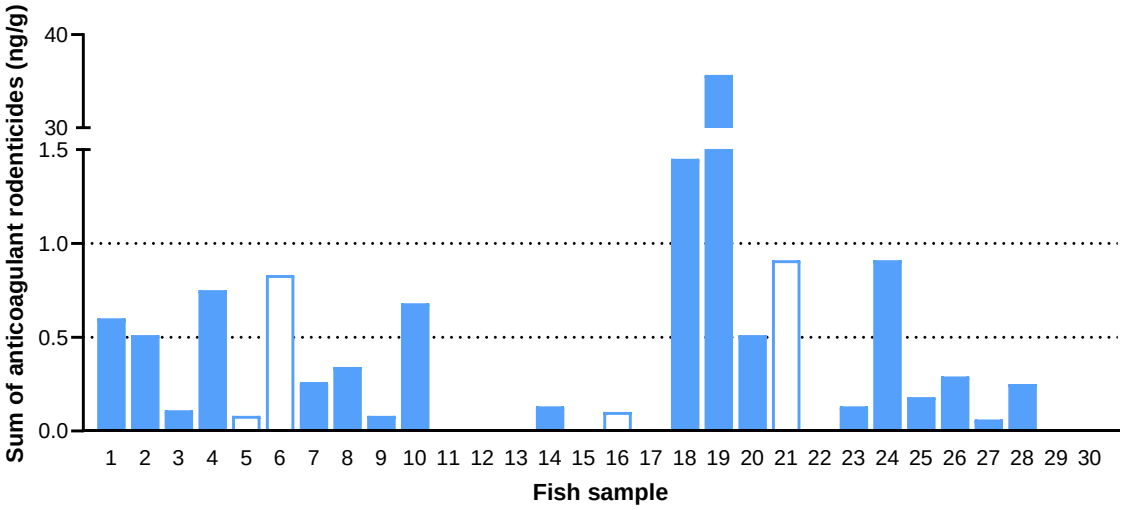

Figure 8. Summed anticoagulant rodenticide (AR) concentrations in fish liver samples (ng/g). Data from individual fish are shown as filled bars (blue), data from pooled samples as open bars (white); no bar indicates all AR concentrations below LOQ. Dotted lines denote concentrations of 0.5 and 1.0 ng/g.

Table 4. Number of anticoagulant rodenticides (#AR) detected above LOQ and sum of ARs (ng/g; rounded) detected in liver samples from foxes, birds of prey, fish and hedgehogs.

Foxes			**Birds of prey**			**Fish**			**Hedgehogs**		
ID	#AR	Sum (ng/g)	ID	#AR	Sum (ng/g)	ID	#AR	Sum (ng/g)	ID	#AR	Sum (ng/g)
1	1	1.8	1	3	60	1	3	0.60	1	1	0.18
2	1	0.61	2	2	9.8	2	2	0.51	2	3	0.31
3	3	180	3	4	100	3	1	0.11	3	1	0.05
4	0		4	1	440	4	1	0.75	4*	4	1.8
5	4	1.2	5	1	0.06	5	1	0.08			
6	2	3.7	6	3	23	6	2	0.83			
7	1	1.0	7	4	68	7	1	0.26			
8	4	1100	8	1	0.29	8	1	0.34			
9	4	4.8	9	2	30	9	1	0.08			
10	1	8.8	10	1	0.09	10	2	0.68			
11	4	510	11	0		11	0				
12	3	0.52	12	1	0.65	12	0				
13	4	39	13	2	2.9	13	0				
14	4	6.1	14	3	1.3	14	1	0.13			
15	1	0.12	15	4	450	15	0				
16	2	1.3	16	4	2.2	16	1	0.10			
17	4	480	17	2	0.92	17	0				
18	2	0.36	18	4	72	18	3	1.5			
19	4	240	19	3	89	19	4	36			
20	2	9.8	20	2	40	20	3	0.51			
21	0		21	1	0.37	21	3	0.91			
22	1	2.1				22	0				
23	4	110				23	1	0.13			
24	1	6.4				24	2	0.91			
25	2	0.73				25	1	0.18			
						26	2	0.29			
						27	1	0.06			
						28	1	0.25			
						29	0				
						30	0				

* Only one sample contained the first generation AR coumatetralyl.

Supplementary information in Annex A

A1. “Background information on liver samples”
Sampling of foxes, birds of prey and fish including sample identifiers and sampling locations.

A2. “Sample homogenization, extraction and ESI-LC-MS/MS analysis”
Details and photos of the preparation of liver samples and details on chemical analysis.

A3. “Raw data and comprehensive plots”
Data on all analysed AR in table format, including interlaboratory comparison with BfG. Uniform plots for all individual AR, sum of AR and number of AR grouped for foxes, birds of prey and fish.

5. Consultation of experts on the potential exposure situation in Switzerland

Several Swiss experts were consulted on their assessment of the exposure situation of non-target organisms with ARs. These included experts from the Institute of Veterinary Pharmacology and Toxicology (University of Zurich), the Vetsuisse faculty (University of Zurich), ornithologists at bird-of-prey rehabilitation centres, wildlife rangers and SWILD - Urban Ecology & Wildlife Research (independent research and consulting association of wildlife biologists). The experts agreed that exposure of non-target organisms with AR would be possible and also cases of AR poisoning of wildlife and pets are known or suspected (Stalder, Vogler et al. 2021), see also Section 6.

Besides results obtained by expert consultation, a survey with members of the association of Swiss Pest Controllers (VSS) and cantonal capitals was conducted. The results of these surveys can be found in the sections below.

5.1. Survey of members of the Association of Swiss Pest Controllers

A survey with 16 open and closed questions on the use of AR by pest controllers was created in two languages (German and French). Information was collected on usage, control and documentation of AR-baiting. The questionnaires were distributed to the ca. 50 members of the Association of Swiss Pest Controllers (VSS) by the VSS executive board.

In total, eight VSS members returned the questionnaire. Due to the small sample size, it is not possible to make any nationwide statements regarding the use of ARs by professional pest controllers. It is also not possible to determine how much the responding VSS-members contribute to the total volume of ARs applied in Switzerland. One cantonal capital reported data that are more fitting for a professional pest controller, these data were integrated here as “Company 9” (see also Section 5.2).

Nevertheless, some general insights into the use of ARs by professional pest controllers could be gained. In the following, the obtained results are summarized and a detailed listing of the results can be found in Annex B.

According to the survey, rodent control is mainly performed with ARs, however, also physical methods such as snap traps are used. Non-tox baits are applied between 0 and 40%. Baiting takes place both indoors and outdoors, usually one of the areas dominates depending on the company. Anticoagulant rodenticides are applied in different areas such as domestic, industrial and agricultural environments, food processing plants, grocery stores and sewers with amounts of up to 1’500 to 5’000 kg per area per year (industrial areas and Companies 5 and 9, Annex B). Rodent control is often regularly or permanently conducted at the same locations. Amount and place of bait application are always documented, either handwritten or digital. Three out of nine respondents maintain a database regarding rodent control. Solid baits such as wax blocks were the most common product type, followed by paste. Grain or pellets are rarely employed. Baiting boxes are almost always used that are protected from water entry and inaccessible to most non-target organisms. Baits are always controlled after application between every 5 days up to three to four times per month depending on the location. Bait residues are collected and disposed of in the company, e.g., in hazardous waste containers, which are regularly disposed of at a disposal site. In general, rodent carcasses are explicitly searched for and removed. However, rodent carcasses are rarely found between <1% and 5% except for one respondent, who reported 40% of found carcasses. In cases where rodent carcasses are found, these are either deposited at animal carcass collection points, disposed with the help of waste disposal companies or disposed with municipal waste in waste incineration plants. Two respondents observed AR-resistance, one to bromadiolone and the other to difenacoum.

In Figure 9, the average amount of active ingredient applied per year per respondent is shown.

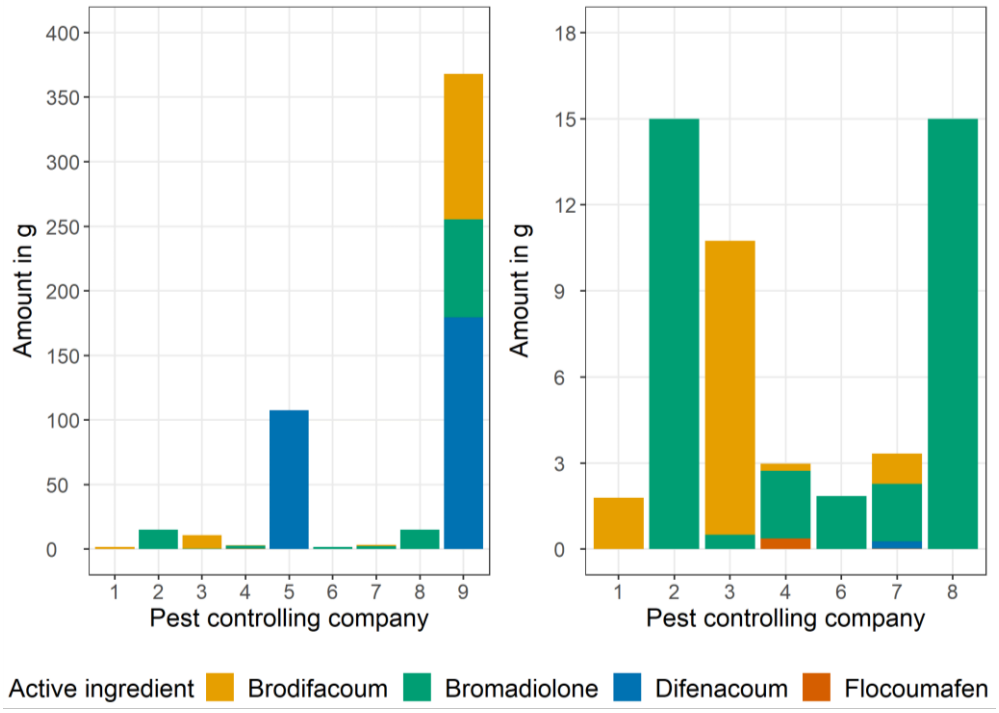

Figure 9. Amount of active ingredients in products containing anticoagulant rodenticides applied by responding VSS-members. The y-axis showing the amount of active ingredient is scaled differently on the left, showing all pest controlling companies and right, without pest controlling Companies 5 and 9. Data for Company 9 was originally reported for a cantonal capital but moved to this data set.

A total of approximately 10'500 kg of AR-containing products were applied, representing 540 g of active AR ingredients for nine pest control companies. Fifteen different products with AR were applied containing 0.0025% (difenacoum and flocoumafen), 0.005% (brodifacoum, bromadiolone and difenacoum) and 0.4% (coumatetralyl) active ingredient. Products containing bromadiolone were mentioned most often. Products with difenacoum were named most frequently in terms of quantity amounting to approximately 53% of the total applied active ingredients, followed by brodifacoum (23%) and then bromadiolone (21%) and non-ARs (3%), see Figure 10. Two non-AR products with cholecalciferol and alpha-chloralose were applied by two pest control companies. Flocoumafen was only applied in small quantities (<<1%).

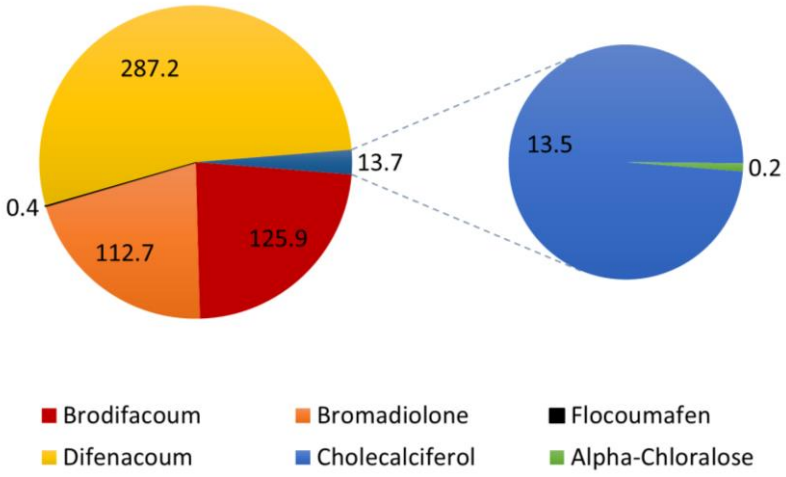

Figure 10. Composition of products applied by responding pest controlling companies. Depicted numbers represent the sum amount of active ingredient in products used in g.

5.2. Survey of cantonal capitals

All 26 canton capitals were contacted by phone or email to obtain information on the use of AR in these cities. The focus was on a few questions regarding the use of AR, documentation and contact data of the contracted pest controlling companies, if applicable. Four cities completed the long questionnaire originally created for VSS members, Annex B. However, data from one city appeared more indicative of a commercial user (i.e., very large amounts of active ingredients and products) and were moved to the survey of VSS members.

In general, the answers of these cities regarding documentation corresponded well with those by VSS-members. Mainly solid baits such as wax blocks are used that are protected from water entry and inaccessible to most non-target organisms. One city also uses direct baiting of rat burrows by means of special shovels. Baits are always controlled after application between every 6 days up to every four weeks depending on the location. Bait residues are collected and disposed of by the company conducting the baiting or on site. However, some hotspots need to be baited continuously in the form of a long-term intervention. Rodent carcasses are rarely found. Anticoagulant rodenticides are applied in public areas in the city such as parks or lakefronts. One main application area of one city was the sewage system, where baiting was conducted at 550 locations. Dead animals are flushed away in the sewage system, transported to the WWTP, removed there, and disposed of properly. Products and active ingredients used differed from those reported by responding VSS-members. One city applied coumatetralyl (40 g active ingredient, 10 kg of product) and difenacoum (0.4 g active ingredient, 8 kg of products). Another city used difenacoum (0.96 g active ingredient[2], 23.8 kg of product) on the surface and difethialone (1 g active ingredient, 40 kg of product) in the sewage system. The remaining two cities used the same active ingredient as those reported by responding VSS-members, see Table 5.

Table 5. Amount of active ingredients in products used by three cantonal capitals (A-C[3]).

Capital	Difenacoum (g)	Coumatetra-lyl (g)	Difethialone (g)	Brodifacoum (g)	Bromadiolone (g)	Flocoumafen (g)
A	0.4	40				
B	0.96		1			
C				0.1	0.1	0.1

Little information is available on the other 22 capitals. On five websites, information about rodent control is available. However, the survey revealed that in most cantonal capitals, contact or responsible persons are unknown or do not exist. Often external, private pest controlling companies are requested to deal with a case of rodent outbreak, e.g., by the municipal police. Rodent control data are not collected by the cities, but by the contracted companies. Furthermore, the interviewees responded that rats are not a known problem in smaller cities.

Supplementary Information in Annex B

B1. "Long questionnaire to VSS-members in German, French and Italian"
B2. "Short questionnaire for cantonal capitals"
B3. "Summary of survey answers from VSS-members"
B4. "Summary of survey answers from cantonal capitals"

[2] The reported value was 96 g, but was corrected to 0.96 g on the basis of the product weight (23.8 kg) and the formulation with 0.005% active ingredient.
[3] A fourth capital "D" also reported data, however, given the amount of active ingredient (>100 g) and product (>5'000 kg) declared, these were interpreted as the amount used by a professional user and added to the VSS survey (labelled there as "Company 9").

6. Potential challenges, open questions and outlook concerning future anticoagulant rodenticide monitoring

6.1. Potential challenges in evaluating the environmental impact of anticoagulant rodenticides

Several challenges exist for evaluating the environmental impact of ARs. In this section, some of these challenges are explored.

Sensitive methods are needed to detect anticoagulant rodenticides in environmental compartments

Limits of quantification vary widely among the used analytical methods, which could lead to an underestimation of the AR prevalence in non-target organisms (Thomas, Mineau et al. 2011). Isotopically labelled analogues of high purity are needed to take into account matrix effects, incomplete extraction and ion suppression (Regnery, Friesen et al. 2019). However, the purchase is quite costly and the majority of studies did not include those (Regnery, Friesen et al. 2019). Walker, Chaplow et al. (2013) suggests using matrix-matched samples to increase recoveries and repeatability leading to the reporting of higher liver SGAR concentrations. Furthermore, chirality of racemates for some AR might complicate the detection of ARs in environmental compartments (Regnery, Friesen et al. 2019).

Concerning the aqueous compartment, we were able to quantify AR to low levels in fish livers and our chemical analytical results compared well with those from experts in this field. However, investigating AR residuals directly in water samples, e.g., in surface water after heavy rainfall events is challenging. As only contaminant peaks are expected and these require targeted sampling procedures.

Sample choices can bias the outcome

Studies of ARs in non-target animals often examine animals found dead. These animals may have been directly poisoned by ARs (Stalder, Vogler et al. 2021) or involved in accidents. Anticoagulant rodenticides may contribute to accidents due to potential changes in physiology and behaviour (Brakes and Smith 2005, López-Perea, Camarero et al. 2015, Lettoof, Lohr et al. 2020). The birds and hedgehogs we analysed had a sampling bias, as they were brought in sick. Foxes and fish were hunted or caught and less prone to sampling bias. Investigating effects of AR on captive instead of free-ranging birds could also bias the results. Captive birds are exposed to fewer environmental stressors and other compounds as well as tend to have fewer injuries and are thus less susceptible to AR-toxicity (Rattner and Harvey 2021).

Anticoagulant rodenticide potency differs depending on the species under investigation

Rattner and Harvey (2021) reported differences in structure, activity and inhibition of the vitamin K epoxide reductase (VKOR) enzyme eventually leading to differences in species sensitivity to AR toxicity. This might explain that raptors such as kestrels and owls were found to be far more sensitive to AR exposure compared to other avian species. Furthermore, it is challenging to extrapolate acute toxicity data to assess the hazard among avian species due to different scaling factors for the different compounds (Rattner and Harvey 2021). Moreover, variations in toxicokinetics of the different ARs was observed showing differences in AR metabolism and hepatic binding capacity (Rattner and Harvey 2021). In addition, it is difficult to draw a simple cause-effect relationship between liver residual concentration and toxicosis as ARs can also bind to other enzymes or can be stored in liver fat and thus be less toxicologically active (Rattner and Harvey 2021).

External factors influence anticoagulant rodenticide toxicity

Animals previously exposed to ARs were found to be more affected due to lingering effects despite restoration of clotting time compared to animals without previous contact with ARs (Rattner

and Harvey 2021). Bioaccumulation also leads to older animals being more likely to develop effects in comparison with younger animals, as seen for foxes in our screening. Besides bioaccumulation and long lasting effects of residues, also weather events, reduced food availability and malnutrition can affect toxicity and thus probably redistribution of ingested ARs (Rattner and Harvey 2021).

Establishing robust threshold values for anticoagulant rodenticide in liver is challenging

Several threshold values of AR in liver linked to toxicosis were proposed (Rattner and Harvey 2021). These range over two orders of magnitude (Thomas, Mineau et al. 2011). The lowest suggested threshold found in literature was 10 ng/g liver wet weight, established based on a toxicosis observed in one examined great horned owl (*Bubo virginianus*) (Stone, Okoniewski et al. 1999). Other proposed thresholds ranged from >100-200 ng/g liver wet weight established for brodifacoum and difenacoum based on studies with free-ranging and captive barn owls to 700 ng/g liver wet weight proposed for brodifacoum in barn owls (Rattner and Harvey 2021). However, using a probabilistic model, a threshold value for lethal liver exposure of about 100-200 ng/g for raptors would result in toxicity probabilities of 11% and 22%, respectively, for barn owls (Thomas, Mineau et al. 2011). According to Thomas, Mineau et al. (2011) based on pooled data, SGAR liver residues of 20 ng/g would lead to 5% and 80 ng/g results in 20% of birds becoming symptomatic. However, the authors indicate that AR-metabolisation and over-estimation of AR residuals due to repetitive ingestion after uptake of the lethal dose might influence the probabilistic model and thus lead to a flattening of the probability curve (Thomas, Mineau et al. 2011). Thus, further research is needed to establish robust threshold values of AR in non-target organisms (Rached, Moriceau et al. 2020). Consequently, although we quantified AR well over 20 ng/g in livers of foxes, birds of prey and one fish (Section 4), we have no robust framework to determine risks for these non-target animals.

Effects of anticoagulant rodenticide on the population level are difficult to assess

Anticoagulant rodenticides might not kill non-target species or add to natural mortality, hence it is challenging to assess the direct impact of ARs on populations (Van den Brink, Elliott et al. 2018). However, embryo mortality and teratogenicity as well as potential impact on breeding population due to AR exposure indicate that effects on population level are likely (Munday and Thompson 2003, Weigt, Huebler et al. 2012, Coeurdassier, Riols et al. 2014, Ondracek, Bandouchova et al. 2015).

6.2. Suggestions for improving the current anticoagulant rodenticide exposure situation

In studies investigating bromadiolone exposure of red fox (*Vulpes vulpes*) (Fourel, Sage et al. 2018) and red kite (*Milvus milvus*) (Fourel, Damin-Pernik et al. 2017), *trans*-bromadiolone was found to be more persistent in the food chain compared to *cis*-bromadiolone. Thus, the authors suggest changing the ratio of diastereoisomers, e.g., more *cis*-bromadiolone compared to *trans*-bromadiolone, to reduce the risk of secondary poisoning while keeping the effectiveness towards rodent controls.

Furthermore, Walther, Geduhn et al. (2021) found that brodifacoum concentration in liver tissue of non-target small mammals was approximately 50% lower in case baiting in bait boxes was restricted to indoors only. Thus, the authors suggest that exposure of non-target species can be reduced by restricting the use of AR baits to the inside of buildings in comparison to bait application in as well as around buildings.

In France, the use of mechanical traps, chemical treatment with aluminium phosphide or the fostering of predators to control mole populations was suggested (Coeurdassier, Riols et al. 2014). Furthermore, chemical control was strictly prohibited above a regulatory threshold of vole density determined by surface indicators. In addition, the authors suggest that landscape and agricultural

practices should be modified aiming at the destruction of vole and mole tunnels (Coeurdassier, Riols et al. 2014).

To reduce the current AR exposure situation in sewers, a poison-free rat management can be applied. Friesen, Behrendt et al. (2022) reported on a rat management pursued in Erfurt without the deployment of ARs. The method is based on preventive measures such as reducing food availability through safe food storage and avoiding accessible waste and improperly constructed compost piles. Furthermore, defective pipes in the sewer system are regularly inspected and maintained. Unused sewer junctions are closed using robot technology to reduce available retreats for rat reproduction. These measures led to a reduction of rat infestations in the city of Erfurt to maximal six per year (Friesen, Behrendt et al. 2022). Also, the city of Zurich successfully stopped the systematic and widespread application of ARs by remediating and repairing sewage pipes especially defective and unused house connections as well as by flushing sewers regularly (Friesen, Behrendt et al. 2022). In Hamburg, an EDP- and GIS-assisted rat management is pursued. Traps and baiting boxes are equipped with sensors and remote data transfer to enable a fast response to rat infestation in sewers (Friesen, Behrendt et al. 2022).

6.3. Remaining questions and outlook for future monitoring

Are poisonings of pets/wild animals/birds known or suspected beyond the current screening? (Veterinarians? Ornithologists? Foresters?)

Already prior to results from the current screening, poisoning of wildlife and pets were known according to Swiss experts. Prof. Nägeli (Institute of Veterinary Pharmacology and Toxicology, University of Zurich) reported that a hazard for wildlife and pets is undisputed. They already published occurrences and poisoning of foxes with AR (Kupper, Grobosch et al. 2006). Furthermore, they also record cases of poisoning with AR every year, especially in dogs. However, no statistics are available because poisoning cases are not required to be reported.

Curti, Kupper et al. (2009) reported 864 poisonings of dogs (7% died or needed to be euthanized) based on feedback from the veterinary community through the free of charge Tox Info Suisse in the period of 1997-2006. Pest control agents such as rodenticides, insecticides and molluscicides were reported to be the main cause of poisoning amounting to 308 cases. Among rodenticides, alpha-chloralose was the main reason for poisoning with 67 cases compared to 45 cases related to poisoning with other rodenticides mainly SGARs. Also, cat poisoning by rodenticides, mainly alpha-chloralose, were reported. However, the authors state that the results do not allow conclusions about the actual incidence, causality, or mortality of animals due to AR poisonings. Furthermore, results might also be biased especially because feedback from the veterinary community is obtained in case of unknown hazard potential of the toxicant or due to uncertainty about the therapy or prognosis. Thus, actual cases of poisoning caused by ARs are expected to be higher than reported as therapy using vitamin K1 is well known among the veterinary community in comparison to therapy after alpha-chloralose ingestion (Curti, Kupper et al. 2009). In 2015, it was still possible to conduct a (non-representative) statistical evaluation based on feedback from the veterinary community. According to this study by Schediwy, Mevissen et al. (2015), pest control substances such as rodenticides and insecticides were the second leading cause of poisoning in dogs (246 cases). Furthermore, surveys of hedgehog populations performed in 2016 and 2017 in Zurich found a sharp decline over the last 25 years. The reasons for the decline are still unclear, but rodenticides are discussed every now and then as a possible reason (Taucher, Gloor et al. 2020).

According to ornithologists, poisoning of birds by ARs are suspected but could in most cases not be confirmed due to a lack of possibilities and capacities to prove the occurrence of ARs. Some of the birds of prey showed symptoms related to poisoning by ARs or were found with a mouse in the mouth showing respective symptoms (personal communication: Vreni Mattmann, Vogelwarte Sempach). According to Swiss experts at the Vetsuisse faculty at the University of Zurich, AR can be responsible for secondary poisoning of birds of prey in Switzerland. For example,

brodifacoum poisoning was found to be the cause of death of a kestrel (*Falco tinnunculus*) due to ingestion of poisoned prey (Stalder, Vogler et al. 2021).

Wildlife rangers from Zurich reported that in the 2019/20 hunting year (1.4.2019 to 31.3.2020), 389 foxes, 10 badger and 14 beech marten were found dead, but the cause of death was not related to road traffic, accidents with dogs or because they were hunted. Rather, the cause of death was disease-related (especially scabies in foxes) or due to rail traffic[4].

Affected non-target organisms such as foxes, owls and fish were found in Canada, Germany, US, UK, Germany, Denmark, France, Spain, Finland, Taiwan, New Zealand among others, which mainly showed residuals of SGARs, e.g., brodifacoum, bromadiolone and difenacoum. Most AR-containing-products authorized on the Swiss market contain brodifacoum, bromadiolone and difenacoum (see Table 3). Results from our screening and survey confirmed that the mentioned SGAR are the main ones found in non-target organisms and the main ones applied by practitioners working in pest control.

Which samples are most promising for further monitoring (high probability of anticoagulant rodenticide residues in samples). Sediment/water and/or biota (liver or faecal samples?).

Based on the literature study, AR residues were found in all kinds of matrices. No literature studies regarding AR residuals in air were found. The presence of AR in soils and sediments was scarce and mainly related to intense and broadcast baiting events. Reports of detects in surface waters are also scarce, but hampered by LOQs that are too high for its purpose. Exceptions could be after WWTP effluent discharges (due to medical prescriptions of ARs) or stormwater overflow basins in relation to recent sewer baiting events and heavy, prolonged rainfalls. Most AR residues were found in livers of biota. Publications report of AR residues in aquatic, terrestrial as well as avian non-target organisms. Residues were most often reported for foxes and birds of prey, although this reporting might be biased due to the fact that most studies primarily focused on terrestrial biota samples from predators. Using scats (faecal samples) instead of livers would present a non-invasive way to investigate potential AR uptake by non-target organisms. However, FGARs are mainly excreted via urine and a rapid decrease of SGARSs concentrations in scats after excretion due to weathering exposure cannot be excluded (Prat-Mairet, Fourel et al. 2017). Furthermore, accumulation and metabolism of SGARs in organisms varies; hence it is challenging to estimate uptake and fate of active substances in organisms based on AR residuals in scats.

Future developments for AR residues screening may encompass novel (bio-)analytical techniques such as blood clotting assays (Hindmarch, Rattner et al. 2019) and other biomarkers recently reviewed by Rached, Moriceau et al. (2020). Given the development of improved toxicokinetic understanding in AR exposed specimens, non-invasive blood sampling in combination with AR screening may be used to obtain time-resolved information on AR blood levels. In addition, hair was also suggested as sample type to investigate AR residues (Zhu, Yan et al. 2013). However, AR concentrations in hair were low (around three-fold above LOQ) and not correlated to AR blood levels. Furthermore, the stability and extractability of AR from hair remains to be investigated.

Based on the present state of knowledge – summarised above – liver samples of aquatic and terrestrial animals prone to secondary poisoning or at the end of the food chain are best suited for screening. This is also why liver samples were selected for the current screening. In general, adults are more affected than juveniles because of the longer time available for AR residue accumulation. This we confirmed in our screening by finding fewer ARs in younger compared to older foxes (Section 4.3.2). Thus, adult non-target organisms might be more suitable for an AR-screening; however, a bias in results could occur by leaving out juvenile non-target organisms. Non-target organisms of interest would be fish, e.g., brown trout (*Salmo trutta*), European chub *(Squalius cephalus)*, zander (*Sander lucioperca*) or common bream (*Abramis brama)*, terrestrial

[4] https://www.stadt-zuerich.ch/ted/de/index/gsz/beratung-und-wissen/tier-und-mensch/wildhut.html

animals, e.g., foxes, least weasel (*Mustela nivalis*), stoat (*Mustela erminea*) or European hedgehog (*Erinaceus europaeus*) and avian species, e.g., common buzzard (*Buteo buteo*), common kestrel (*Falco tinnunculus*), red kite (*Milvus milvus*), northern goshawk (*Accipiter gentilis*), western barn owl (*Tyto alba*), long-eared owl (*Asio otus*), Eurasian eagle-owl (*Bubo bubo*) or tawny owl (*Strix aluco*). In our screening we managed to capture a few of the species mentioned above – albeit in very low numbers. According to Swiss experts at the University of Zurich, urban pigeons could also be of interest for screening, as they are representative of direct ingestion of AR baits by non-target organisms.

How many and which sampling points are useful for a more comprehensive screening of anticoagulant rodenticide contamination of the Swiss environment?

Killing of terrestrial and avian animals exclusively for the purpose of this project was not performed and is also not advised for future monitoring studies. Thus, sampling locations for non-target animals will also in future depend on the availability of found and collected carcasses or required kills from authorized individuals such as wildlife officers. Carcasses should be frozen as soon as possible, and livers of these dead animals should be intact for investigating AR occurrence and amounts. In case liver samples of non-target animals are already available in sample databases such as, e.g., at the Institute of Parasitology (University of Zurich) or at the Swiss Rabies Centre, these could be used to consolidate sampling efforts. This aspect will require coordination prior to launching future studies.

Aqueous non-target organisms such as fish are recommended to be caught by cantonal fisheries inspectorates or by members of the Swiss Fishing Association. To investigate areas of possible higher risks, fish could be samples after WWTP effluents or stormwater overflow basins. Here, sampling points in rivers that pass urban areas and with higher percentages of WWTP effluents also come into consideration. However, it should be noted that the fish with highest AR concentrations in the liver came from a stream that is not impacted by treated sewage effluents. Furthermore, fish in Lake Constance, with very high effluent dilution potential were also shown to be exposed to AR.

For the pilot AR-screening, we investigated 76 liver samples (see Section 4) from raptors (collected by the Greifvogelstation Berg am Irchel), foxes (collected by wildlife rangers in the greater Zurich area and provided to us by the Institute of Parasitology at University of Zurich) and fish (collected by cantonal fisheries inspectorates and by members of the Swiss Fishing Association). A comprehensive monitoring study regarding AR occurrence in non-target organisms should also take into account seasonal variations. In addition, a larger geographical distribution (e.g., five larger regions of Switzerland) together with a significantly larger sample size as well as differential land use (urban versus rural) should be explored. Concerning the investigated non-target organism, the available samples from foxes, birds of prey and fish is appropriate. Hedgehogs can be used to complement the sample set. To evaluate effects of measures as well as temporal trends, a repeated monitoring, at least at selected locations, is advised.

7. Concluding bullets

- Internationally, AR are found across the terrestric environment and exposure also occurs in the aquatic environment.
- A limited screening of terrestric biota in Switzerland provides a first indication that AR exposure likely occurs to a similar degree as in adjacent European countries, at least for foxes and birds of prey. A survey of fish also indicates widespread contamination of the aquatic environment with AR.
- Robust thresholds for acceptable AR concentrations in liver are lacking and hamper risk assessment. A level of 100 to 200 ng of AR per g of liver is often applied as a “general” concentration of concern for foxes and birds of prey; no thresholds were found for fish.
- Future monitoring should significantly expand sampled regions and also sample numbers. At least to confirm observations from the current screening study.
- Future monitoring should provide a baseline so that subsequent AR trends can be determined, at least at selected locations. This will allow for assessments of the effectiveness of future measures or regulations concerning the use and application of AR.

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Annexes

A1. Background information on liver samples – Sampling of foxes, birds of prey and fish including sample identifiers and sampling locations.

A2. Sample homogenization, extraction and ESI-LC-MS/MS analysis – Details and photos of the preparation of liver samples and details on chemical analysis.

A3. Raw data and comprehensive plots – Data on all analysed anticoagulant rodenticides (AR) in table format, including interlaboratory comparison with BfG. Uniform plots for all individual AR, sum of AR and number of AR grouped for foxes, birds of prey and fish.

B1. Long questionnaire to VSS-members in German, French and Italian.

B2. Short questionnaires for cantonal capitals.

B3. Summary of survey answers from VSS-members.

B4. Summary of survey answers from cantonal capitals.

Annex A1 – Background information on liver samples

Foxes

Fox liver samples were provided by Professor Manuela Schnyder and Dr. Daniel Hegglin of the Institute of Parasitology of University Zurich. Foxes were hunted by four different hunters in four hunting districts and transported to the University of Zurich (Tierspital) the next day. There they were weighed and dissected as part of a parasite monitoring programme. Livers were removed and two liver lobes were cut off, weighed and stored frozen in 50 mL Falcon tubes until further processing by homogenization and extraction (Annex A.2). Details of the fox samples are shown in Table A1.1 and images of the hunting districts, giving an impression of rural/urbanized areas, are shown in Figure A1.1.

Table A1.1. Fox sampling, origin and basic details of liver samples.

No.	Location	Hunting District	Hunting Day	Sex	Age	Weight of Liver Subsample (g)
1	Hegiberg	168	15.12.21	female	<1	6.3
2	Geitberg	149	16.12.21	male	>1	28.3
3	Geitberg	149	16.12.21	female	>1	21.4
4	Geitberg	149	16.12.21	male	<1	23.5
5	Geitberg	149	16.12.21	male	<1	25.1
6	Geitberg	149	16.12.21	male	>1	33.7
7	Hegiberg	168	16.12.21	male	<1	20.2
8	Hegiberg	168	16.12.21	female	>1	18.0
9	Hegiberg	168	16.12.21	male	>1	21.3
10	Hegiberg	168	16.12.21	female	<1	31.5
11	Hegiberg	168	16.12.21	female	>1	23.2
12	Geitberg	149	16.12.21	male	<1	17.4
13	Geitberg	149	16.12.21	male	<1	17.6
14	Geitberg	149	16.12.21	female	>1	8.8
15	Geitberg	149	16.12.21	female	<1	17.4
16	Hegiberg	168	16.12.21	male	>1	18.6
17	Dübendorf	101	05.01.22	male	>1	17.2
18	Hegiberg	168	07.01.22	female	>1	19.5
19	Hegiberg	168	07.01.22	male	>1	18.5
20	Hegiberg	168	07.01.22	female	>1	22.7
21	Hegiberg	168	07.01.22	male	<1	24.7
22	Dürnten	84	17.01.22	female	>1	14.9
23	Dürnten	84	17.01.22	female	>1	15.5
24	Dübendorf	101	17.01.22	male	<1	20.7
25	Dübendorf	101	18.01.22	male	<1	22.9

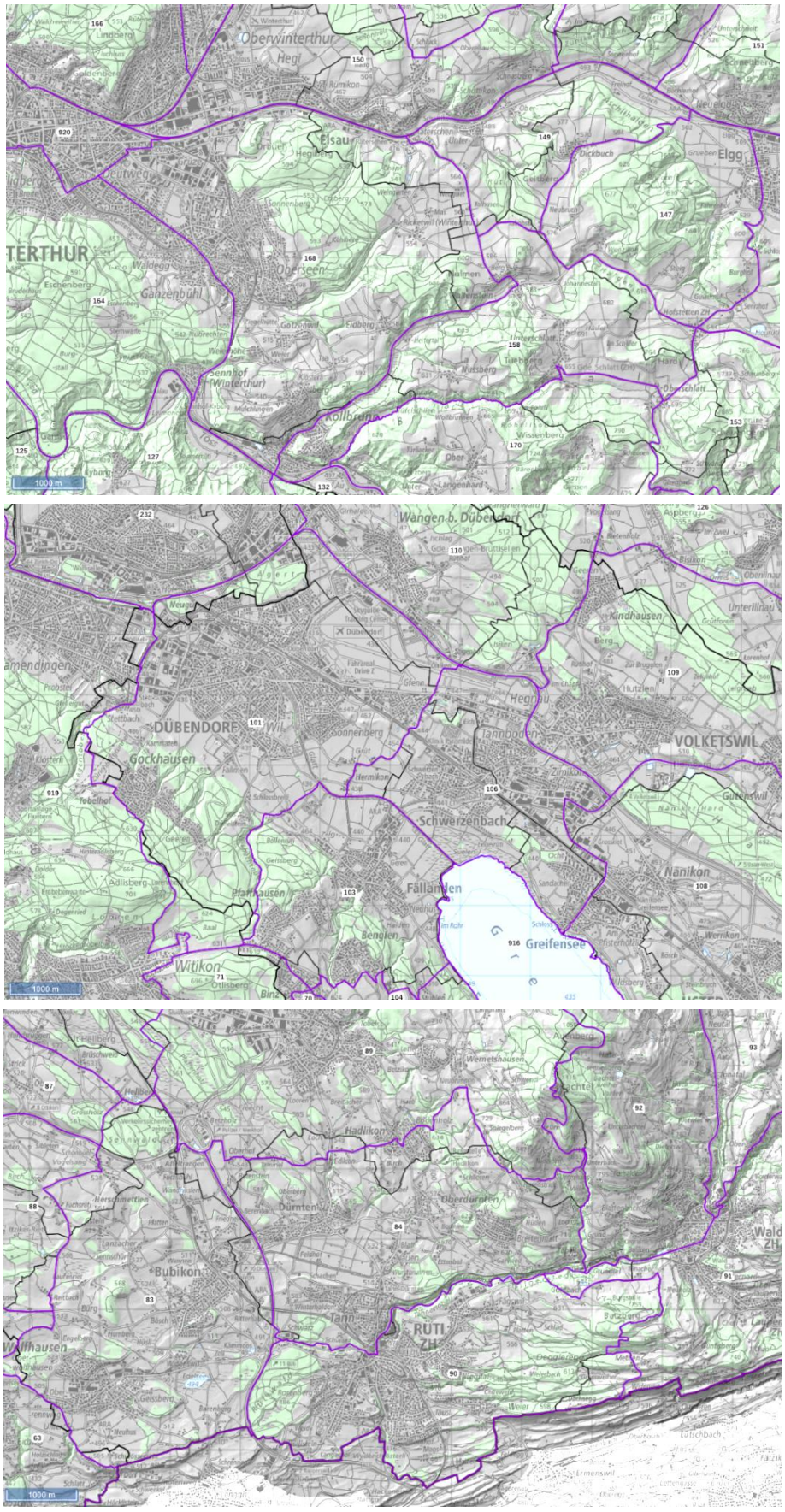


Figure A1.1. Foxes came from four hunting districts in canton Zurich: top (149, Geitberg; 168, Hegiberg); middle (101, Dübendorf), bottom (84, Dürnten). See Table A1.1 for further details. Maps taken from https://maps.zh.ch/.

Birds of prey

Frozen birds of prey specimens were provided by Andreas Lischke from the Bird of Prey Station (Greifvogelstation) Berg am Irchel. The station takes in and cares for sick birds. In the first months of 2022, birds that died during care were frozen and stored. These frozen birds were transported to the University of Zurich (Vetsuisse Faculty, Institute for Food Safety and Hygiene, Section of Poultry and Rabbit Diseases) and left to thaw overnight. The next day, with the assistance of Dr Sarah Albini and Dr Barbara Vogler, livers were dissected and stored frozen in Falcon tubes until further processing by homogenization and extraction (Annex A.2). Details of the 21 birds and samples are provided in Table A1.2. For 16 birds the location where they were found is known and listed in Table A1.2 and shown in Figure A1.2.

Table A1.2: Bird of prey sampling, origin and basic details of liver samples (- = not recorded).

No.	Species	Location	Arrival Day	Sex	Weight at Arrival (g)	Weight Thawed (g)	Liver Weight (g)
1	CB	Trüllikon	14.03.22	f	515	655	9.4
2	CB	Flaach	05.03.22	m	490	416	7.8
3	CB	Wangen bei Dübendorf	12.02.22	f	815	569	16.4
4	CB	-	18.02.22	f	nr	553	12
5	CB	Stein am Rhein	03.02.22	m	610	504	24.1
6	CB	Winterthur	20.01.22	m	575	513	10.5
7	CB	Diessenhofen	02.02.22	m	525	415	7.2
8	CB	Bülach	04.03.22	m	510	499	8.9
9	CB	Effretikon	04.02.22	m	470	466	5.2
10	CB	Zufikon	04.02.22	m	610	485	12.9
11	CB	Mammern	07.02.22	f	495	492	12.2
12	CB	-	-	m	nr	511	18.1
13	CB	-	-	m	nr	486	6.2
14	CB	-	-	f	nr	1088	17.9
15	CB	Zufikon	04.02.22	m	510	547	15.9
16	CB	Adliswil	24.01.22	m	465	461	16
17	CB	Küsnacht	11.02.22	m	420	519	17.4
18	CB	Oberweningen	10.02.22	f	580	577	8.6
19	CK	Dettighofen	07.02.22	f	215	196	3.6
20	TO	Langnau am Albis	18.02.22	f	490	554	15.3
21	TO	-	-	m	nr	336	11.2

CB: common buzzard (*Buteo buteo*); CK: common kestrel (*Falco tinnunculus*); TO: tawny owl (*Strix aluco*).

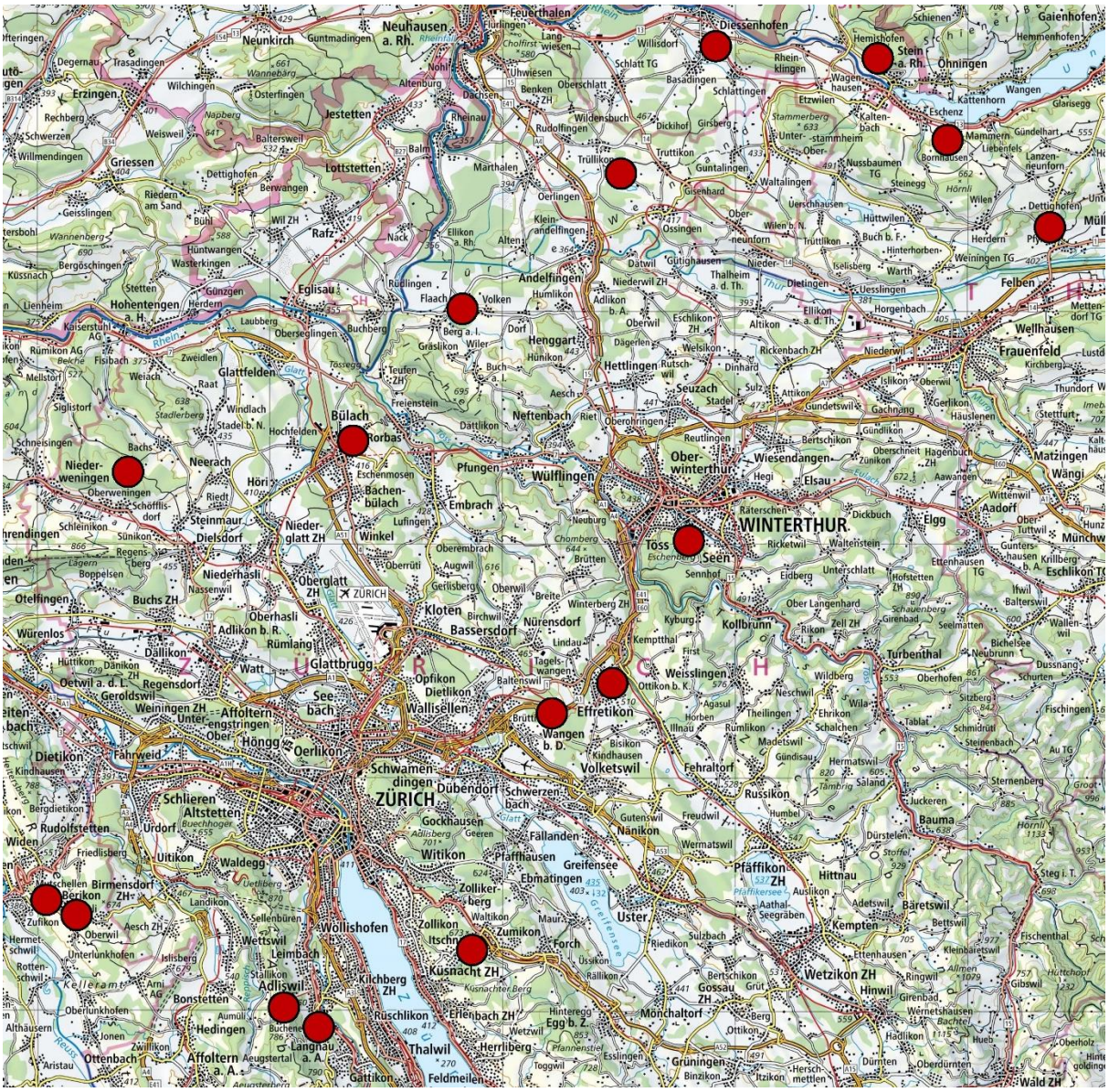


Figure A1.2. Overview of the approximate locations where 16 of the 21 birds were found, see Table A1.2 for further details. Background map © Swisstopo (https://map.geo.admin.ch/).

Hedgehogs

Four frozen hedgehog liver samples were provided by Anouk Taucher from SWILD – Urban Ecology & Wildlife Research. Animals were brought to SWILD by members of the public. One animal was dead on arrival (No. 1 in Table A1.3) the other three died during care. Details of animals and the locations they were found at are provided in Table A1.3. Locations are also shown on the maps in Figure A1.3 and indicate the degree of urbanized areas around the place animals were found.

Table A1.3. Details of hedgehogs provided by Anouk Taucher (SWILD; - = not recorded).

No.	Location	Date	Sex	Weight (g)	Liver Weight (g)
1	Dorfstrasse 35, Urdorf	04.08.21	f	-	1.7
2	Mattenhof 20a, Zürich	02.01.22	f	-	6.3
3	Haumühlestrasse, Embrach	06.01.22	f	-	7.6
4	Hardhof 48, Zürich	10.01.22	m	-	8.4

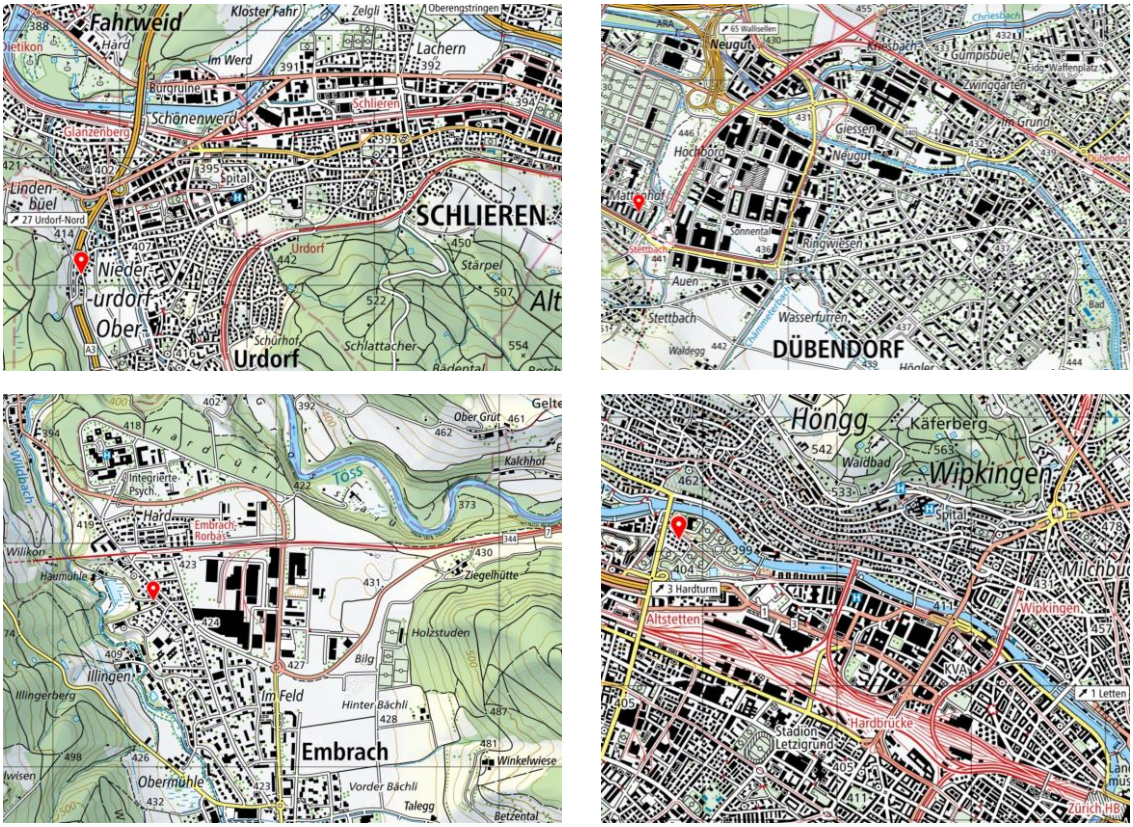


Figure A1.3. Red marks indicate locations where the four hedgehogs listed in Table A1.3 were found, all in built up areas. Background maps © Swisstopo (https://map.geo.admin.ch/).

Fish

Fish samples were collected either by cantonal authorities (TG and SG) or hobby fisher (AG). Following catch by means of electro fishing or angling (or netting in case of sample numbers 5-7, Table A1.4), livers were removed from the fish and stored frozen in Falcon tubes (SG) or plastic bags or containers (SG, TG and AG) until further processing. Catch details are listed in Tables A1.4-A1.6 and maps of catch locations are shown in Figures A1.4-A1.6.

Table A1.4. Details of fish caught in canton Sankt Gallen provided by Michael Kugler (Volkswirtschaftsdepartement, Amt für Natur, Jagd und Fischerei; - = not recorded). Samples within a box and with grey shading were pooled after weighing and before homogenization.

No.	Species	Location	Catch Date	Sex	Length (cm)	Weight (g)	Liver Weight (g)
1	CH	Glatt, Flawil	30.03.22	f	ca. 40	-	6.71
2	CH	Glatt, Flawil	30.03.22	f	ca. 40	-	4.83
3	CH	Glatt, Flawil	30.03.22	m	ca. 30	-	1.79
4	BT	Glatt, Flawil	30.03.22	f	ca. 25	-	0.79
5_1	GL	Thur, Bazenheid	01.05.22	-	35.6	331	2.26
5_2	GL	Thur, Bazenheid	01.05.22	-	31.5	290	1.55
5_3	GL	Thur, Bazenheid	01.05.22	-	23.1	82	0.81
6_1	LW	Lake Constance	21.04.22	f	34.2	259	1.28
6_2	LW	Lake Constance	21.04.22	m	30.5	206	1.11
7	EP	Lake Constance	nr	m	30.5	361	3.89
8	BT	Goldacher Dorfbach	22.04.22	f	23.0	130	2.03
9	CH	Simmi	22.04.22	f	46	1200	17.98
10	CH	Rietach	22.04.22	f	44	1060	10.32
11	BT	Aatalweiher	10.05.22	-	37	-	7.36
12	BT	Aatalweiher	10.05.22	-	38	-	5.68
13	BT	Aatalweiher	10.05.22	-	38	-	4.12

CH: chub (*Squalius cephalus*); BT: brown trout (*Salmo trutta*); GL: grayling (*Thymallus thymallus*); LW: lake whitefish (*Coregonus sp.*); Euopean perch (*Perca fluviatilis*).

Table A1.5. Details of fish caught in canton Thurgau provided by Margie Koster (Amt für Umwelt, Abteilung Gewässerqualität und –nutzung; - = not recorded). Samples within a box and with grey shading were pooled after weighing and before homogenization.

No.	Species	Location	Catch Date	Sex	Length (cm)	Weight (g)	Liver Weight (g)
14	BA	Salmsacher Aach	19.05.22	-	30.0	236	1.01
15	CH	S. Aach	19.05.22	-	29.0	242	3.14
16_1	CH	S. Aach	19.05.22	-	24.5	162	1.89
16_2	CH	S. Aach	19.05.22	-	21.5	96	0.82
16_3	CH	S. Aach	19.05.22	-	21.5	108	0.93
17_1	CH	Murg, Auenpark, Frauenfeld	20.05.22	-	12.0	-	0.03
17_2	CH	Murg, A.Park, F.feld	20.05.22	-	15.0	-	0.12
17_3	CH	Murg, A.Park, F.feld	20.05.22	-	12.5	-	0.16
17_4	CH	Murg, A.Park, F.feld	20.05.22	-	22.0	-	0.95
17_5	CH	Murg, A.Park, F.feld	20.05.22	-	19.0	-	0.32
18	BT	Hornbach, Güttingen	19.04.22	-	34.1	365	3.00
19	BT	Eschelisbach, Güttingen	19.04.22	-	30.3	293	1.90
20	BT	Stichbach, Langrickenbach	19.04.22	-	22.1	116	1.29

BA: barbel (*Barbus barbus*); CH: chub (*Squalius cephalus*); BT: brown trout (*Salmo trutta*).

Table A1.6. Details of fish caught in canton Aargau provided by Thomas Stucki (Departement Bau Verkehr und Umwelt, Abteilung Wald, Sektion Jagd und Fischerei). Samples within a box and with grey shading were pooled after weighing and before homogenization.

No.	Species	Location	Catch Date	Sex	Length (cm)	Weight (g)	Liver Weight (g)
21_1	BT	Pfaffnern (Rev. 129)	31.03.22	-	26	-	1.50
21_2	BT	Pfaffnern (Rev. 130)	14.04.22	-	29	-	2.28
21_3	BT	Pfaffnern (Rev. 130)	14.04.22	-	30	-	1.62
22	CH	Aare (Rev. 17/3)	17.05.22	-	44	-	12.75
23	BT	Aare (Rev. 17/3)	06.05.22	-	30	-	3.72
24	BT	Wigger	10.04.22	-	58	2150	22.74
25	BT	Wigger	14.04.22	-	37	451	8.42
26	BT	Wigger	20.05.22	-	46	915	12.63
27	CH	Aare	06.05.22	-	53	2160	40.52
28	WC	Limmat (Rev. 640/2)	15.04.22	-	102	-	85.10
29	CH	Limmat (Rev. 640/1)	29.05.22	-	38	-	11.18
30	CH	Limmat (Rev. 640/1)	29.05.22	-	40	-	11.04

BT: brown trout (*Salmo trutta*); CH: chub (*Squalius cephalus*); WC: wels catfish (*Silurus glanis*).

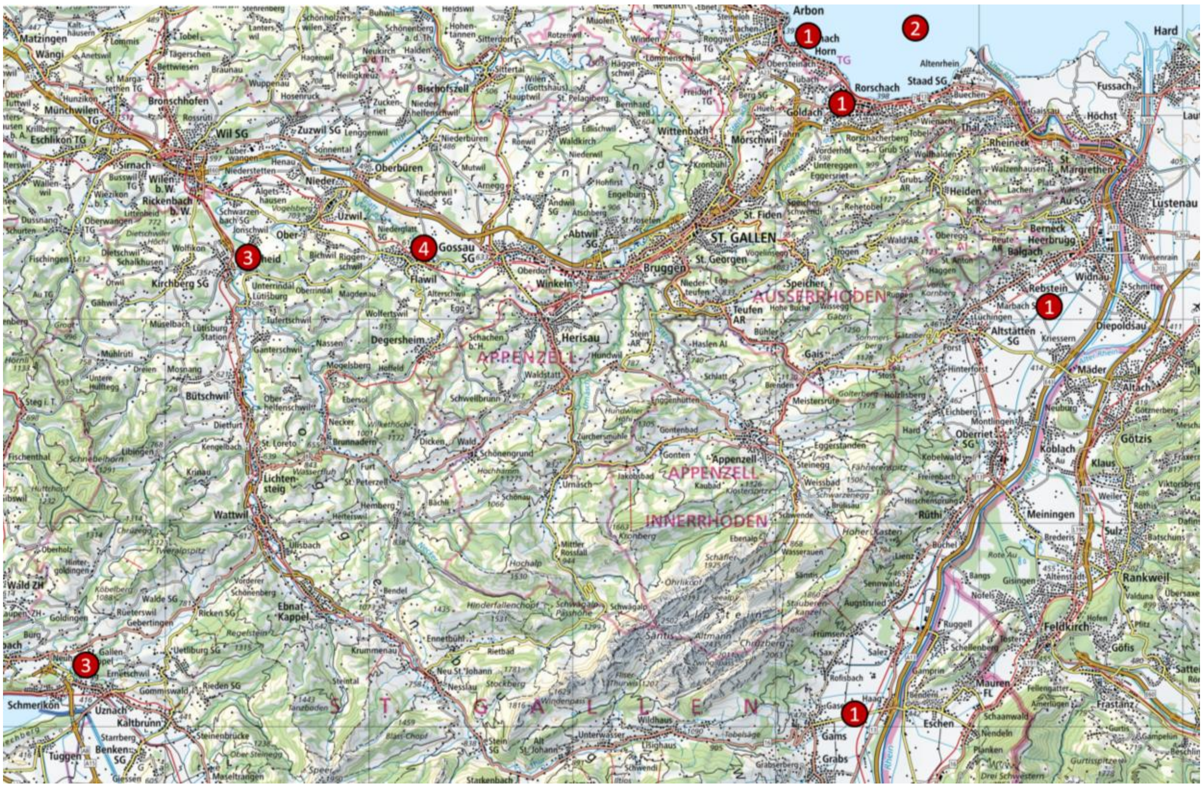


Figure A1.4. Approximate catch locations of fish from canton Sankt Gallen and number of fish caught near the marked location. Background map © Swisstopo (https://map.geo.admin.ch/).

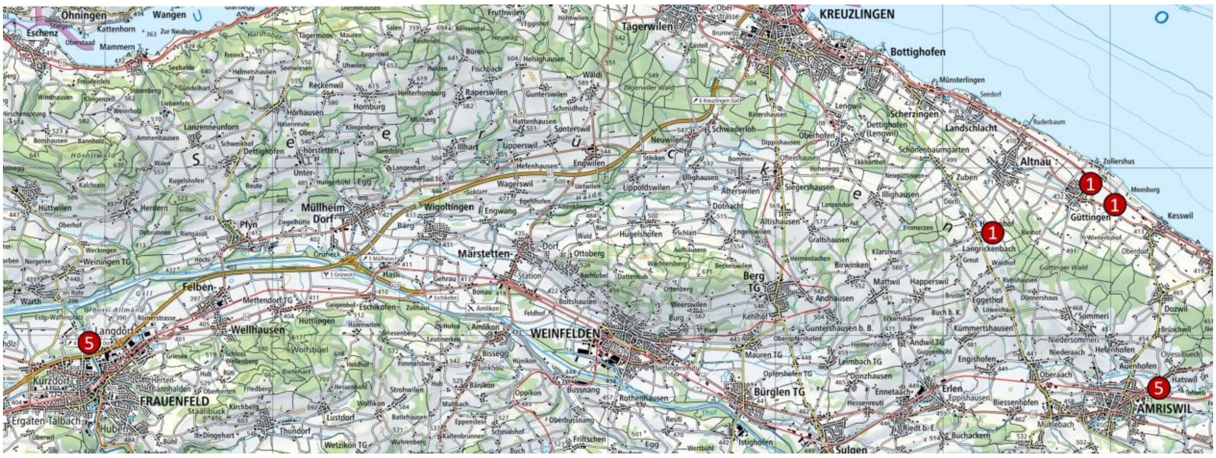


Figure A1.5. Approximate catch locations of fish from canton Thurgau and number of fish caught near the marked location. Background map © Swisstopo (https://map.geo.admin.ch/).

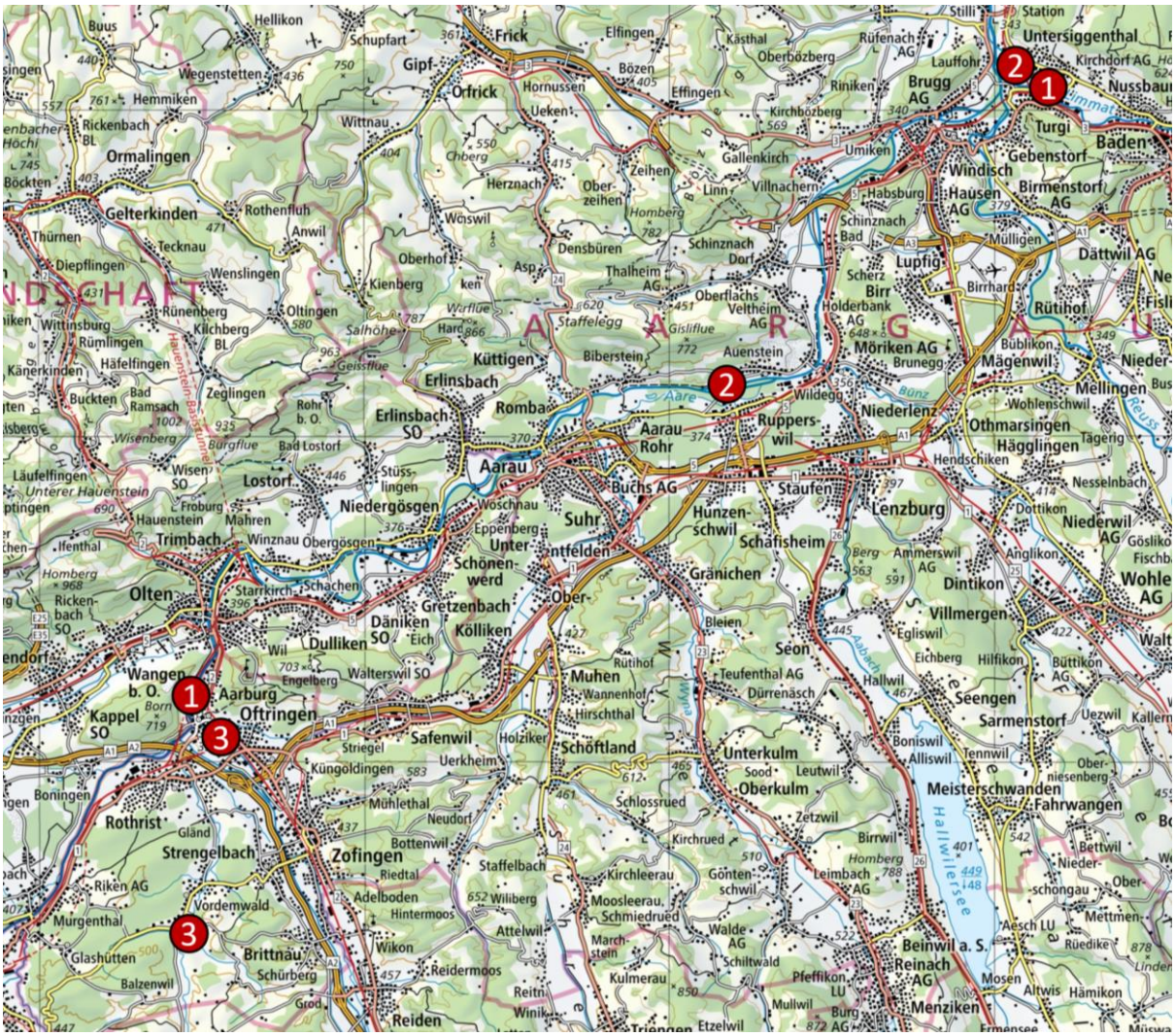


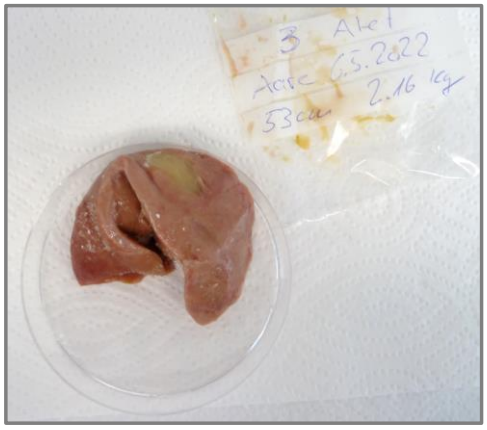
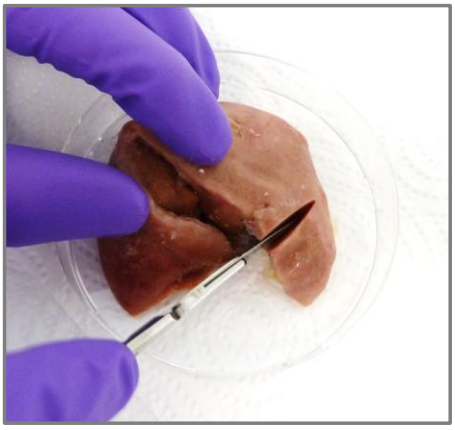
Figure A1.6. Approximate catch locations of fish from canton Aargau and number of fish caught near the marked location. Background map © Swisstopo (https://map.geo.admin.ch/).

Annex A2 – Sample homogenization, extraction and ESI-LC-MS/MS analysis

1. Sample homogenization and extraction

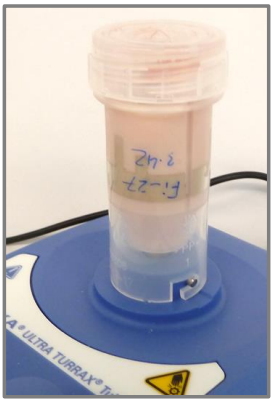
Step 1: sample splitting

Frozen liver samples were half or fully thawed and livers weighing less than 5 g were transferred to a dispersing tube (DT-20 tube). Larger liver samples were cut into pieces within an intended range of 3 to 4 g. One piece was transferred to a DT-20 tube and the other pieces were placed, individually, in 50 mL PP-centrifuge tubes and frozen. From two very large fish livers (40 and 88 g), only a single 3 to 4 g piece was removed and put into a DT-20 tube and the remaining liver sample was frozen again in one piece. Samples in DT-20 tubes were either directly processed or frozen for later processing.

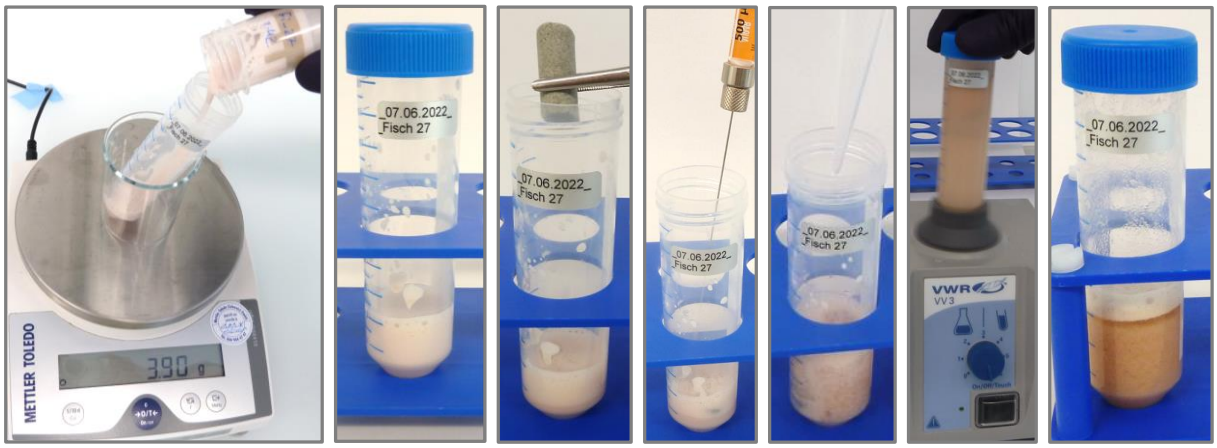

Step 2: homogenization

To a partially thawed liver sample in a DT-20 tube the same weight of nanopure water was added. Then the liver was homogenized with an Ultra-Turrax Tube Drive (UTTD) until foamy.

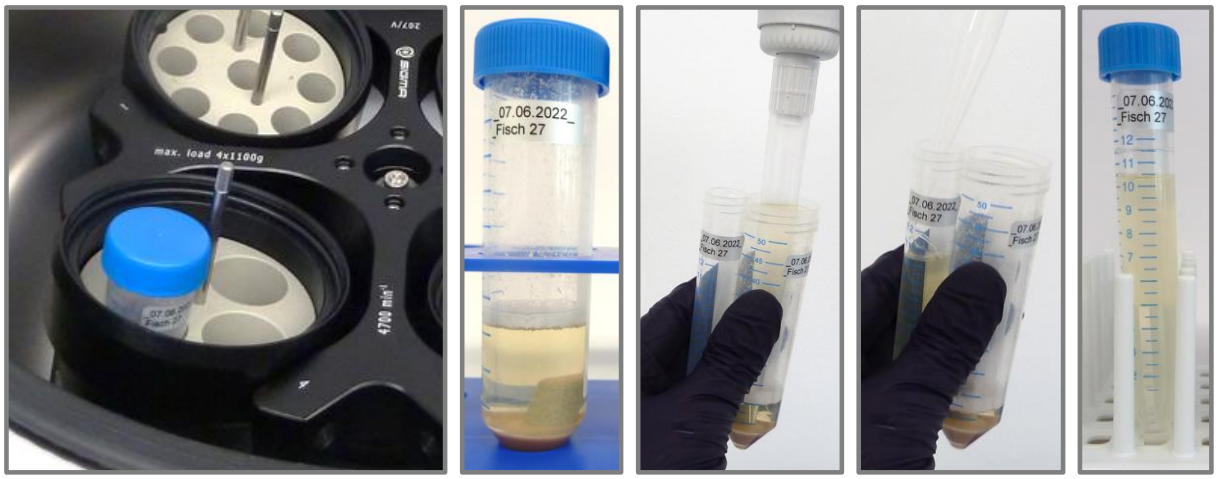

Step 3: extraction

Around 5 g of the homogenate was weighted into a 50 mL PP-centrifuge tube to which a ceramic homogenizer, a mix of internal standards (50 µL of 200 ng/mL = 10ng per internal standard) and 10 mL of acetonitrile were added, with acetonitrile causing precipitation of proteins in the sample. Subsequently, the tube was vortexed for 1 min.



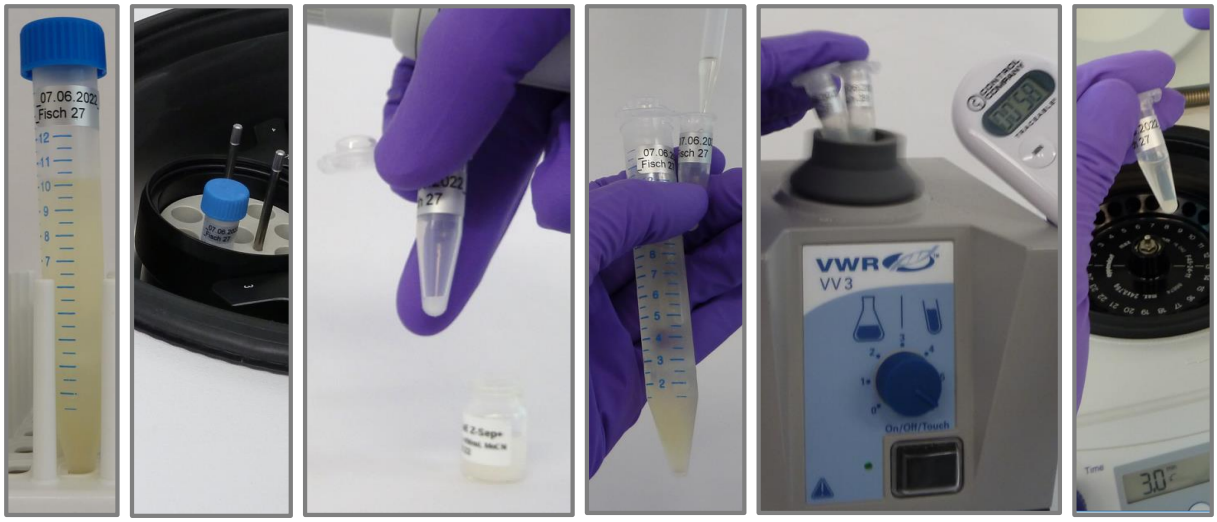
Step 4: centrifugation

Samples were centrifuged for 4 min at 4000 g. Then, 10 mL of supernatant was transferred to a 15 mL centrifugation tube and stored at -22 °C for 5-30 h. The freeze out step removes most fat and also some amount of water.



Step 5: clean up

Frozen extracts were then centrifuged in a cooled centrifuge. Forty mg of Z-Sep+ (silica gel base and zircon-based phase), added as a 20 µL slurry in acetonitrile, was pipetted into a 1.5 mL PP-Eppendorf Safe-Lock tube. Subsequently, 0.5 mL of the supernatant (representing about 100 mg of liver) was added and vortexed for 1 min and then centrifuged for 3 min at 13'200 RPM to remove additional fat and matrix[1]. Finally, 0.4 mL of upper layer is transferred to a LC/MS sample vial after which 0.2 mL of nanopure water in added and the vial briefly vortexed.



The final image above shows the slightly colored Z-Sep+ sorbent and the finished "Fish 27" sample in an LC-MS vial. Part of the materials and equipment used for processing samples is listed in Table A2.3.

2. LC-MS/MS measurement

Chemical analyses were performed using electrospray ionization (ESI) in positive mode on an Agilent G6495A Triple Quadrupole (QQQ) mass spectrometer (for parameter settings see Table A2.1). Chromatographic parameters such as eluents, gradient, flow and column were taken from Regnery, Parrhysius et al. 2019 (except for the buffer where ammonium formate showed better performance than ammonium acetate). Briefly, a Phenomenex 50 x 2 mm Luna PFP column with 3 mm particle size was used within a column compartment maintained at 40 °C and with an upstream security guard

[1] The supernatant should not have an intense colour, otherwise more sorbent has to be added to the tube followed by vortexing and centrifugation. Too much sorbent can deplete the amount of internal standard.

cartridge and an eluent flow rate of 0.6 mL/min. Eluent A comprised of 4 mM ammonium formate in water (from a stock of 2 g ammonium formate per 8 mL water, 1 mL was added to 1 L of water). Eluent B comprised of 100% LCMS grade methanol. A sample run lasted 7 min and started with 80% A and 20% B and this condition was maintained for 0.5 min. For the next 3.5 min Eluent A was reduced to 10% and this condition was maintained for a further 0.55 min. At 4.55 min into the run, the initial condition (80% A and 20% B) was reintroduced and maintained until minute 7, subsequently, the next run starts. To prevent and monitor cross contamination, one or two double blanks (just solvent and no analyte or internal standard) were measured between samples.

Table A2.1. Parameter settings of the Agilent G6495A Triple Quadrupole (QQQ) mass spectrometer used in positive mode.

Parameter	Value
Gas Temp (°C)	200
Gas Flow (l/min)	17
Nebulizer (psi)	25
SheathGasHeater	325
SheathGasFlow	12
Capillary (V)	3500
VCharging	500

Ion Funnel Parameters	Value
Pos High Pressure RF	200
Pos Low Pressure RF	100

Calibration was performed over 10 to 16 points – depending on the expected sample concentration – covering the range of 15 to 40’000 ng/L (see Figure A2.1 for an example). The monitored mass transitions and compound specific tuning parameters of target analytes and their isotope-labeled analogs in ESI+ ionization mode are shown in Table A2.2. The source of the analytes and matching internal standards are listed in Table A2.3.

Table A2.2. Retention time and mass transitions of analyzed compounds. * = [M-H2O+H]+

Compound [M+H]+	**Retention Time (min)**	**Quantifier MRM** precursor (m/z)/ product ion (m/z)/ collision energie [V]	**1. Qualifier MRM** precursor (m/z)/ product ion (m/z)/ collision energie [V]	**2. Qualifier MRM** precursor (m/z)/ product ion (m/z)/ collision energie [V]
Coumatetralyl	3.11	293.1/175/26	293.1/91/64	
Coumatetralyl-d4	3.11	297.2/179/26	297.2/91/40	
Warfarin	3.32	309.1/163/14	309.1/251/22	309.1/147/14
Warfarin-d5	3.31	314.2/163/14	314.2/256/22	314.2/152/14
Bromadiolone*	3.82	509/251/20	511/251/20	
Bromadiolone-d5*	3.84	516/256/20	514/256/20	
Difenacoum	4.03	445.2/257/20	445.2/179.1/40	
Difenacoum-d4	4.02	449.2/257/20	449.2/179.1/40	
Brodifacoum	4.19	525.1/337/24	523.1/335.1/24	
Brodifacoum-d4	4.18	529.1/337/24	527.1/335.1/24	
Flocoumafen	4.23	543.2/355.2/24	543.2/523/16	543.2/159/44
Flocoumafen-d4	4.23	547.2/355.2/24	547.2/527/16	547.2/159/44
Difethialone	4.23	539.1/256/46	539.1/335/26	539.1/178/40
Difethialone-d4	4.24	545.1/256/46	545.1/337/26	545.1/178/40

Table A2.3. List of part of the materials and equipment used for processing and analyzing anticoagulant rodenticides in liver samples.

Name	Product Number	Vendor
Brodifacoum	DRE-C10667500	LGC
Bromadiolone	DRE-C10680000	LGC
Chlorophacinone	DRE-C11460000	LGC
Coumatetralyl	DRE-C11740000	LGC
Difenacoum	DRE-C12608000	LGC
Difethialone	DRE-C12625000	LGC
Flocoumafen	DRE-C13662000	LGC
Warfarin	DRE-C17940000	LGC
Brodifacoum-d4	B677902	TRC-Canada
Bromadiolone-d5 (Mixture of Diasteromers)	B678202	TRC-Canada
Coumatetralyl-d4	C765602	TRC-Canada
Difenacoum-d4	D445352	TRC-Canada
Difethialone-d4	D445453	TRC-Canada
Flocoumafen-d4	F401502	TRC-Canada
Warfarin-d5	W498502	TRC-Canada
(±)-Chlorophacinone-d4 (indanedione-d4)	TRC-C375251	TRC-Canada
Rodenticides Mixture 248 100 µg/mL in Acetonitrile	DRE-GS09000248AL	LGC
Acetonitrile, Optima™ LC/MS Grade, Fisher Chemical	A955-212	Fisher
Methanol (optima LC/MS) Fisher	A456-212	Fisher
DT-20 tube	3703100	IKA
Ultra-Turrax Tube Drive	3646000	IKA
Ceramic Homogenizers, 50 mL tubes	5982-9313	Agilent
Supel™ QuE Z-Sep+	55296-U /55486-U	Merck
Centrifuge tubes Polypropylene CELLSTAR Greiner Bio-One, 50mL	7.227 261	Huberlab
Centrifuge tubes Polypropylene CELLSTAR Greiner Bio-One, 15mL	7.188 271	Huberlab
ms-Pure septum non-pigmented multiple injection HPblack 100er	G004-HP-CS-FKSKFK10	infochroma
Agilent compatible 2 mL Screw Vial amber 100er	G004-HP-H	infochroma
Eppendorf Safe-Lock Tubes, 1.5 mL	7.400 585	Faust
Luna® 3 µm PFP(2) 100 Å, LC Column 50 x 2 mm	00B-4447-B0	Phenomenex
SecurityGuard™ ULTRA Cartridges UHPLC PFP 2.1mm ID Columns	AJ0-8787	Phenomenex
SecurityGuard ULTRA Holder, for UHPLC Columns 2.1 to 4.6mm ID	AJ0-9000	Phenomenex

3. Calibration and limits of quantification (LOQ)

Figure A2.1 shows an example of a calibration curve that was linear over a long concentration range. Most of the sample peaks fell in between 0 and 10'000 ng/L.

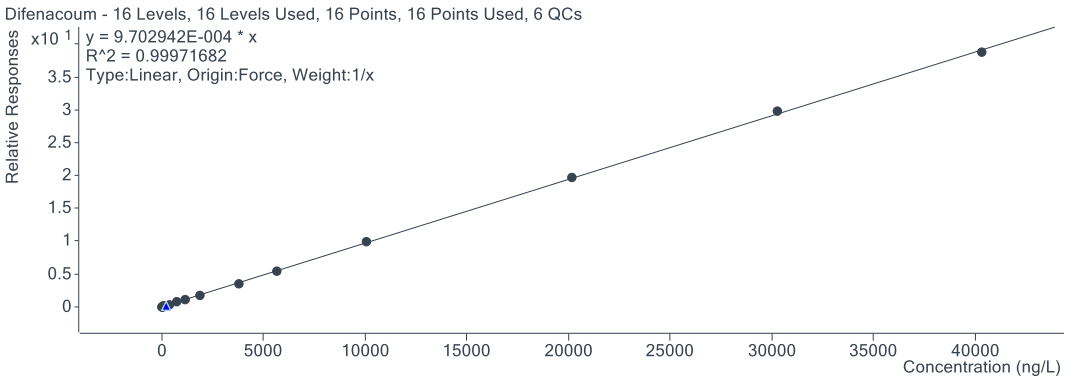


Figure A2.1. Calibration curve of difenacoum.

Figure A2.2 shows an example of a brodifacoum peak with low intensity that was still adequately quantifiable. The signal to noise ratio (S/N) of the quantifier was 29 and that of the qualifier 26. This is above the acceptable S/N ratio of 10. The ratio between quantifier and qualifier was 108%, this is also within the acceptable range (70-130%). The peak was also above the lowest calibration standard. The peak was quantified at 0.09 ng brodifacoum per g of liver from Bird 10, a common buzzard. In this sample, a theoretical LOQ of 0.03 ng/g is possible. This LOQ is calculated by dividing 0.09 by 2.6, with 2.6 being the ratio of the lowest S/N in the sample (S/N=26) and the LOQ S/N benchmark (S/N=10). However, 0.03 ng would fall below the lowest calibration standard and an LOQ of 0.1 or slightly lower is realistic for most samples and most compounds.

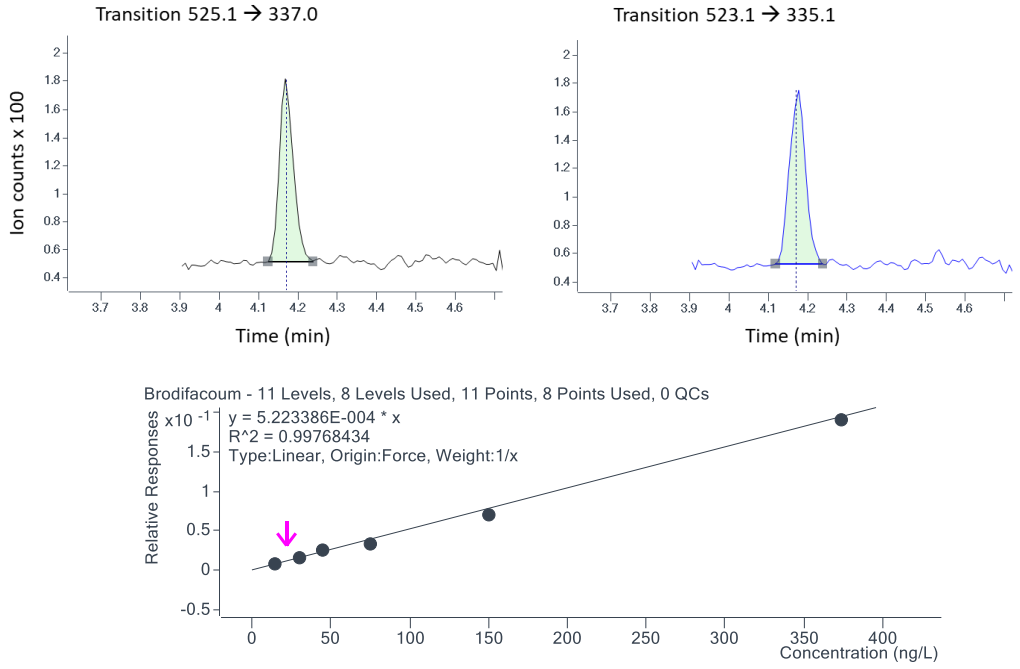


Figure A2.2. Top panel, left: peak of the quantifier of brodifacoum in sample "Bird 10" with a signal to noise ratio (S/N) of 29. Top panel, right: peak of the qualifier of brodifacoum in sample "Bird 10" with an S/N ratio of 26. Lower panel: position of the quantifier peak within the lower part of the brodifacoum calibration range.

Annex A3 – Raw data and comprehensive plots

Table A3.1. Unrounded concentrations (ng/g) of anticoagulant rodenticides in fox liver samples.

Sample	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen	Sum	Age
1				1.78		1.78	<1
2	0.61					0.61	>1
3	22.79	0.42			152.31	175.52	>1
4							<1
5	0.56	0.21		0.36	0.08	1.21	<1
6	0.32		3.35			3.67	>1
7	1.03					1.03	<1
8	1073.99	0.59	0.11	11.11		1085.80	>1
9	4.26	0.21	0.06	0.22		4.75	>1
10				8.84		8.84	<1
11	25.80	481.14	0.06	0.57		507.57	>1
12	0.16	0.09			0.28	0.52	<1
13	11.34	2.78		23.69	0.78	38.59	<1
14	0.51	4.03		0.26	1.33	6.13	>1
15		0.12				0.12	<1
16	0.24			1.03		1.27	>1
17	461.06	13.37	2.14	1.10		477.67	>1
18	0.13			0.22		0.36	>1
19	67.01	157.04		13.99		238.06	>1
20	9.57	0.22				9.79	>1
21							<1
22	2.05					2.05	>1
23	105.02	0.38	0.08	0.13		105.61	>1
24	6.44					6.44	<1
25	0.52	0.22				0.73	<1

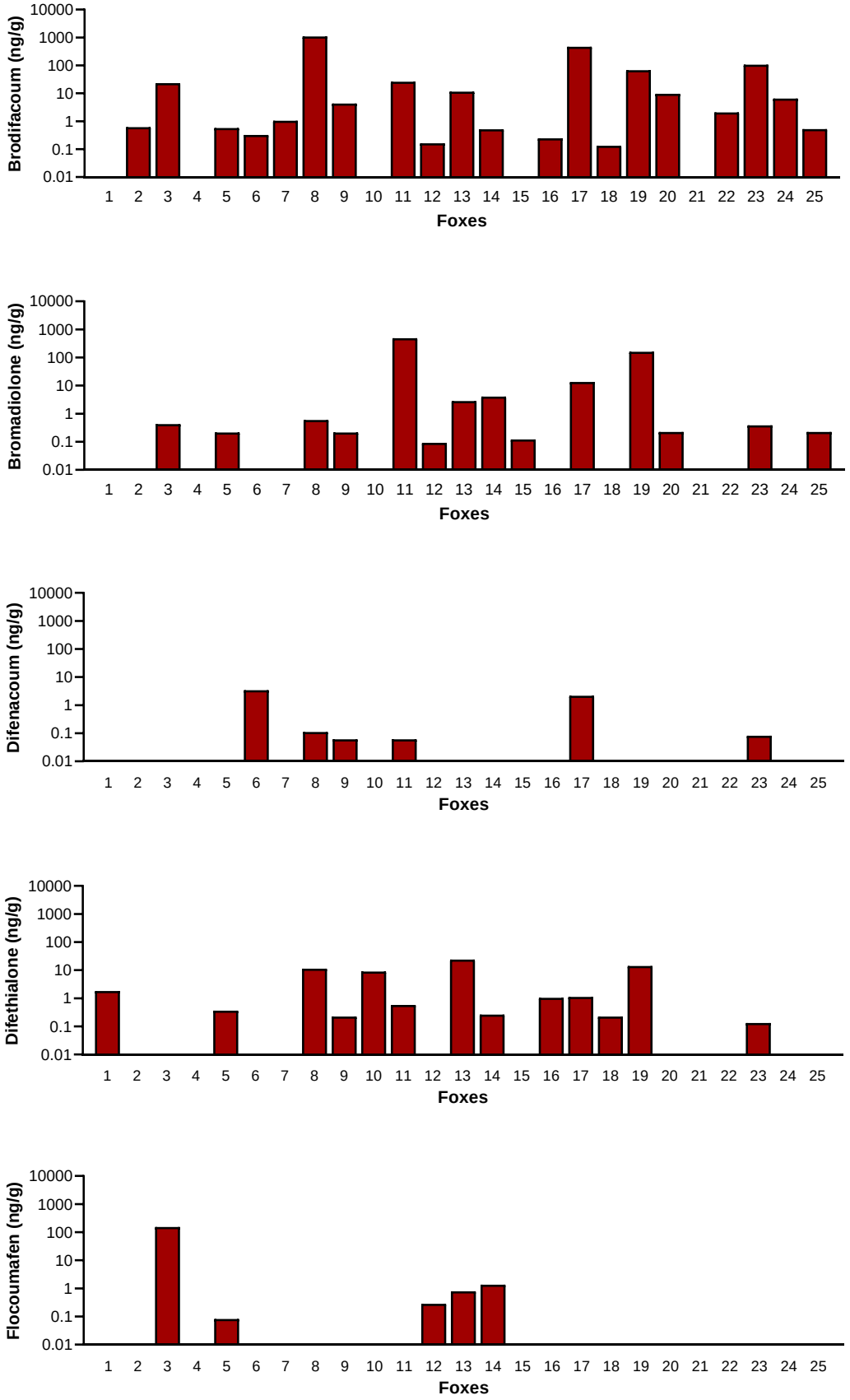


Figure A3.1. Concentrations of anticoagulant rodenticides in fox liver samples.

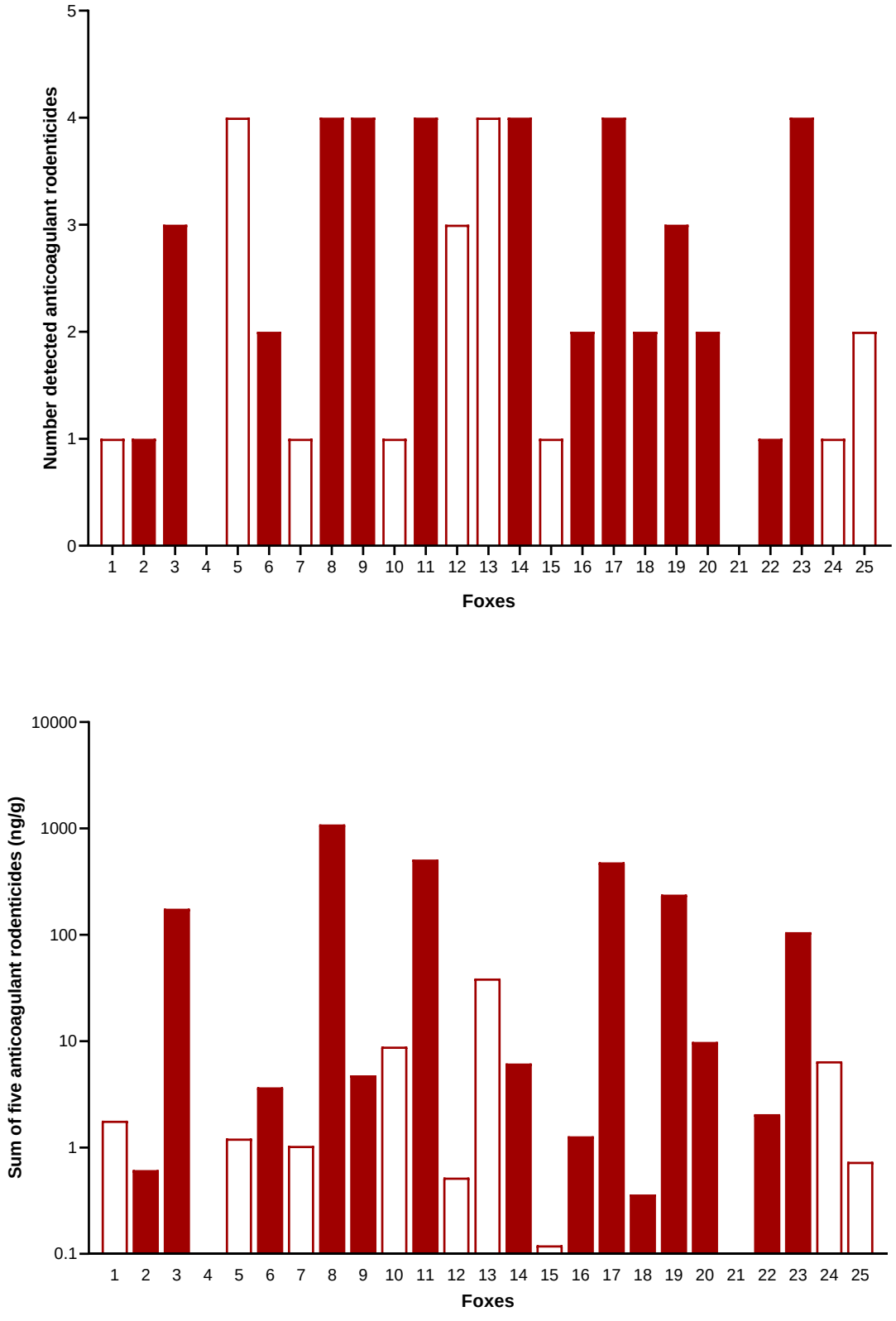


Figure A3.2. Number of anticoagulant rodenticides detected above LOQ in fox liver samples (top) and summed concentrations of anticoagulants in liver samples (bottom). Foxes younger than 1 year old are shown with open bars, foxes older than 1 year are shown with filled bars. Foxes 4 and 21 with no detects are younger than 1 year old.

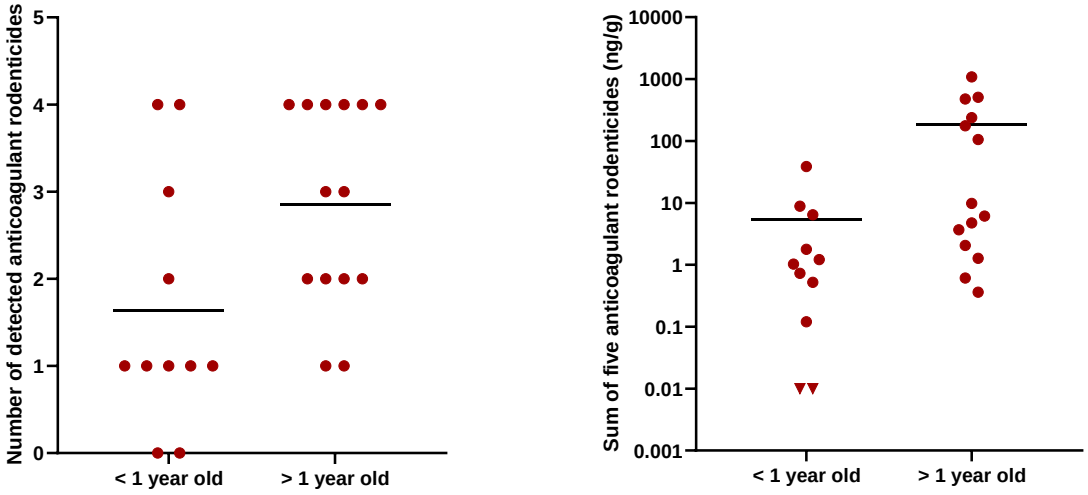


Figure A3.3. Scatter plot of number of detected anticoagulant rodenticides (left) and sum of concentrations of anticoagulant rodenticides in livers from foxes younger or older than one year (lines show averages; triangles show two samples below LOQ that were attributed a value of 0.01 ng/g).

Table A3.2. Unrounded concentrations (ng/g) of anticoagulant rodenticides in liver samples from birds of prey.

Sample	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen	Sum
1	53.87		0.11	6.36		60.34
2	7.31			2.56		9.87
3	4.59	75.96	6.54	16.96		104.05
4	441.20					441.20
5	0.06					0.06
6	21.58		0.81	1.05		23.44
7	7.46	60.41	0.21	0.27		68.34
8			0.29			0.29
9	22.53			7.69		30.22
10	0.09					0.09
11						0.00
12	0.65					0.65
13	1.75		1.09			2.85
14	0.24		0.36	0.70		1.30
15	444.98		1.79	1.71	0.20*	448.67
16	0.96	0.55	0.06	0.65		2.21
17	0.32		0.60			0.92
18	70.90	0.66	0.18	0.26		72.00
19	0.40	5.92		82.32		88.64
20	40.21		0.17			40.38
21	0.37					0.37

* The ratio between quantifier and qualifier was strongly reduced (below the acceptable range of 70-130%). The data were closely inspected and deemed acceptable.

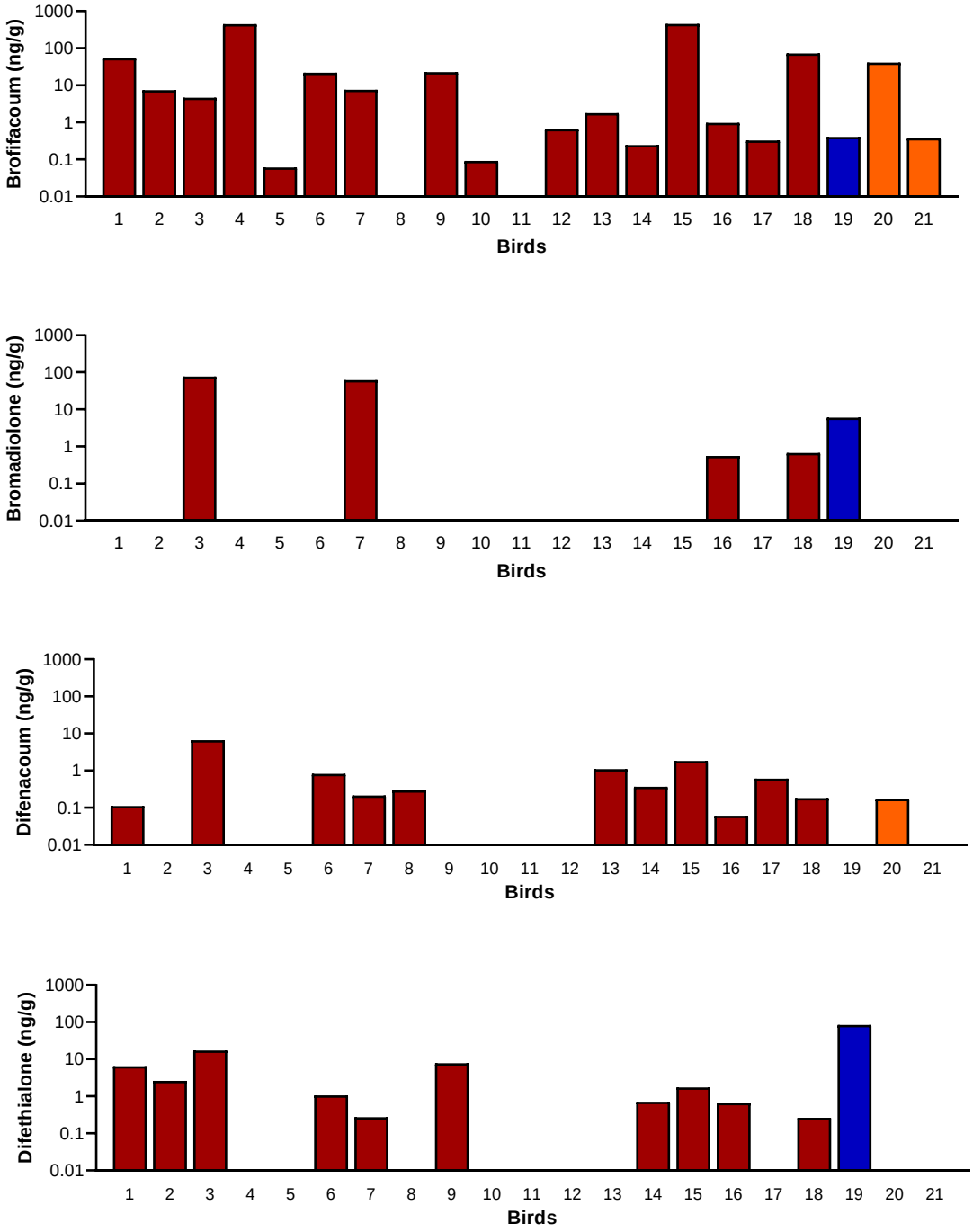


Figure A3.4. Concentrations of anticoagulant rodenticides in liver samples from birds of prey: common buzzard (red), common kestrel (blue), and tawny owl (orange). Flocoumafen was only found in Bird 15 (0.2 ng/g), data not plotted.

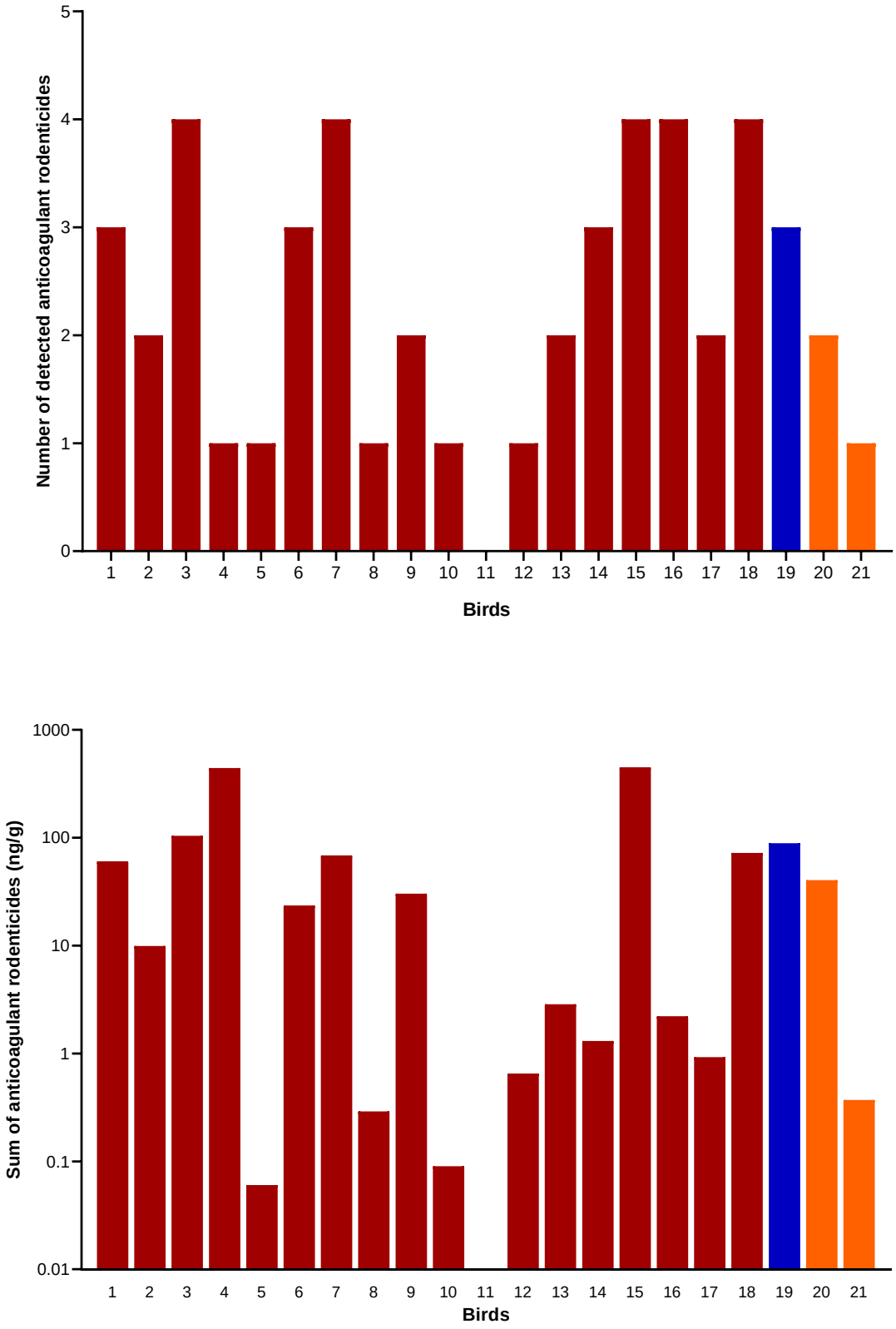


Figure A3.5. Number of anticoagulant rodenticides detected above LOQ in liver samples from birds of prey (top) and summed concentrations of anticoagulants in liver samples (bottom). Common buzzard (red), common kestrel (blue), and tawny owl (orange).

Table A3.3. Unrounded concentrations (ng/g) of anticoagulant rodenticides in individual fish liver samples or pools (_P) of fish liver samples.

Sample	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen	Sum
1	0.25	0.28		0.07		0.60
2	0.31	0.21				0.51
3	0.11					0.11
4	0.75					0.75
5_P	0.08					0.08
6_P	0.37		0.46			0.83
7				0.26		0.26
8	0.34					0.34
9	0.08					0.08
10	0.21		0.47			0.68
11						0.00
12						0.00
13						0.00
14	0.13					0.13
15						0.13
16_P	0.10					0.10
17_P						0.00
18	1.37	0.07				1.45
19	29.54	3.17	0.07	2.88		35.66
20	0.30			0.21		0.51
21_P	0.56			0.07	0.29	0.91
22						0.00
23	0.13					0.13
24	0.86		0.05			0.91
25	0.18					0.18
26	0.29					0.29
27	0.06					0.06
28	0.25					0.25
29						0.00
30						0.00

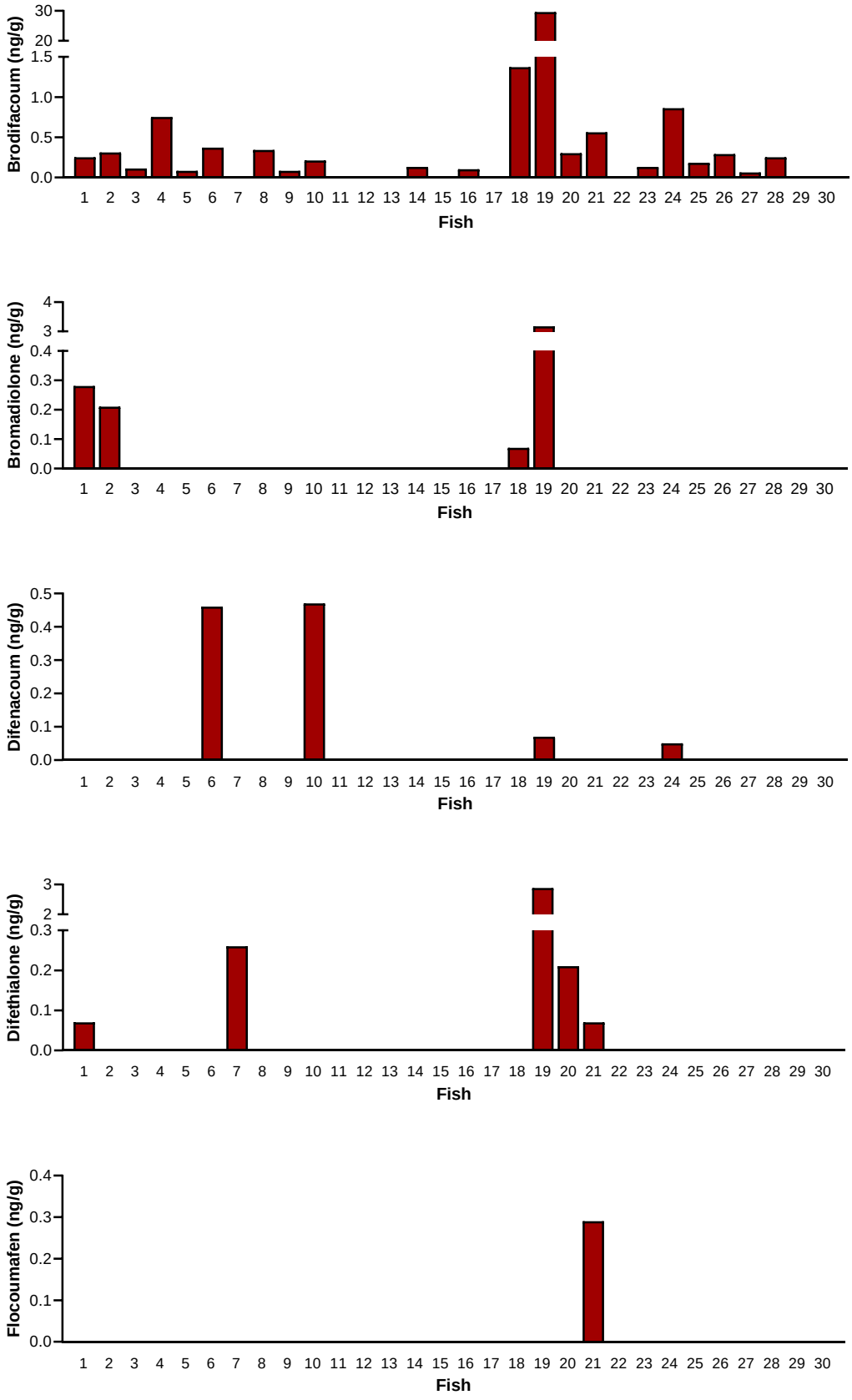


Figure A3.6. Concentrations of anticoagulant rodenticides in fish liver samples.

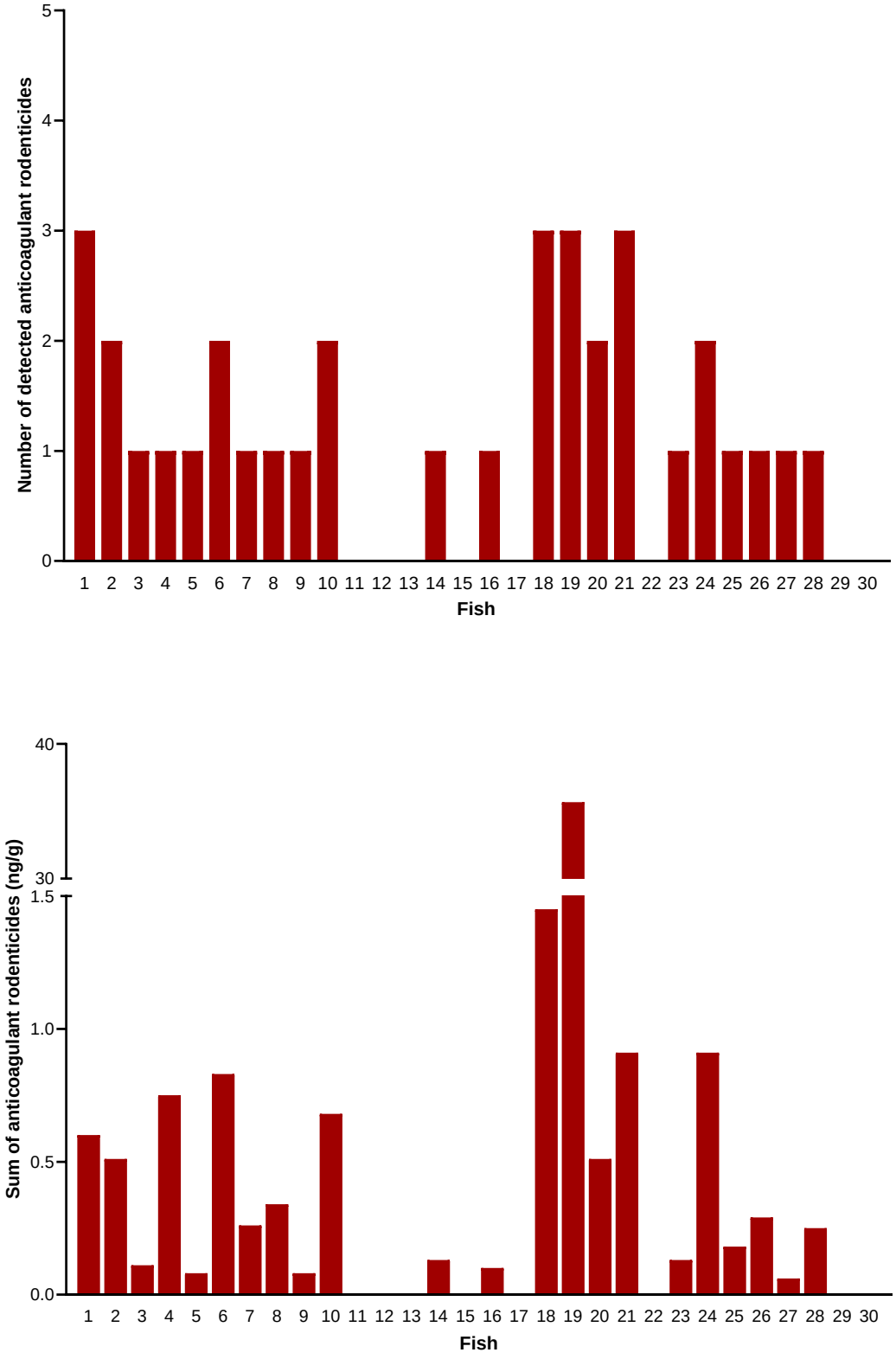


Figure A3.7. Number of anticoagulant rodenticides detected above LOQ in fish liver samples (top) and summed concentrations of anticoagulants in liver samples (bottom).

Table A3.4. Unrounded concentrations (ng/g) of anticoagulant rodenticides in four hedgehog liver samples.

Sample	Brodifacoum	Bromadiolone	Coumatetralyl	Difenacoum	Sum
1	0.18				0.18
2	0.11	0.05		0.15	0.31
3	0.05				0.05
4	0.85	0.18	0.74	0.07	1.83

Table A3.5. Unrounded concentrations (ng/g) of anticoagulant rodenticides in ca. 3 to 4 g aliquots of fox and bird liver samples analysed by the Ecotox Centre and Julia Regnery from BfG (BFG data are shown with grey background). BfG data marked with * are between LOD and LOQ.

Sample	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen
Fox_5/1	0.49	0.20		0.39	0.09
Fox_5/2	0.53	0.19		0.35	0.09
Fox_5/3	0.55	0.20		0.44	0.07
Fox_5/4	0.68	0.21		0.37	0.10
Fox_5/5	0.51	0.21		0.40	0.08
Fox_5/6	0.56	0.23		0.25	0.07
Fox_5/8	0.58	0.21		0.33	0.09
Average	0.56	0.21		0.36	0.08
CV	11.2%	6.8%		17.1%	11.9%
Fox_5/7	0.8*				0.09
Fox_6/1	0.33		3.02		
Fox_6/2	0.33		3.42		
Fox_6/3	0.39		3.65		
Fox_6/4	0.28		3.37		
Fox_6/5	0.26		3.15		
Fox_6/6	0.33		3.26		
Fox_6/7	0.29		3.32		
Fox_6/8	0.35		3.58		
Average	0.32		3.35		
CV	12.8%		6.2%		
Fox_12/3	0.16	0.09			0.28
Fox_12/2					0.47
Fox_13/1	11.42	2.93		17.47	0.81
Fox_13/2	12.01	2.86		23.37	0.82
Fox_13/4	12.14	2.67		27.33	0.75
Fox_13/5	9.81	2.67		26.59	0.73
Average	11.34	2.78		23.69	0.78
CV	9.4%	4.8%		19.0%	5.9%
Fox_13/3	11.2	1.6		28.1	0.85
Fox_14/1	0.51	4.03		0.26	1.33
Fox_14/2	0.7*	2.8			0.89
Fox_17/1	496.73	13.52	2.58	1.33	
Fox_17/2	502.03	13.40	2.08	1.31	
Fox_17/3	431.96	13.29	2.15	0.90	
Fox_17/5	413.53	13.26	1.74	0.85	
Average	461.06	13.37	2.14	1.10	
CV	9.7%	0.9%	16.1%	23.4%	
Fox_17/4	436	11.3	1.6	1.1	
Fox_19/3	70.01	165.28		16.13	
Fox_19/4	64.01	148.80		11.85	
Average	67.01	157.04		13.99	
CV	6.3%	7.4%		21.7%	
Fox_19/2	60.4	156		11.3	

Bird_1/3	53.87		0.11	6.36	
Bird_1/2	51.6			3.7	
Bird_3/1	5.24	71.16	6.64	17.08	
Bird_3/2	4.58	80.05	6.50	14.88	
Bird_3/3	3.96	79.17	6.33	19.04	
Bird_3/4	4.58	73.44	6.68	16.84	
Average	4.59	75.96	6.54	16.96	
CV	11.3%	5.7%	2.4%	10.0%	
Bird_3/5	3.0	49.6	7.4	18.3	
Bird_15/1	445.57		1.70	1.63	0.18
Bird_15/2	389.50		1.71	1.66	0.19
Bird_15/3	492.66		1.96	1.91	0.23
Bird_15/4	452.20		1.77	1.62	0.20
Average	444.98		1.79	1.71	0.20
CV	9.5%		6.8%	8.2%	10.8%
Bird_15/5	323		2.3	1.7	0.19
Bird_16/2	0.96	0.55	0.06	0.65	
Bird_16/3	0.8*	0.2*			

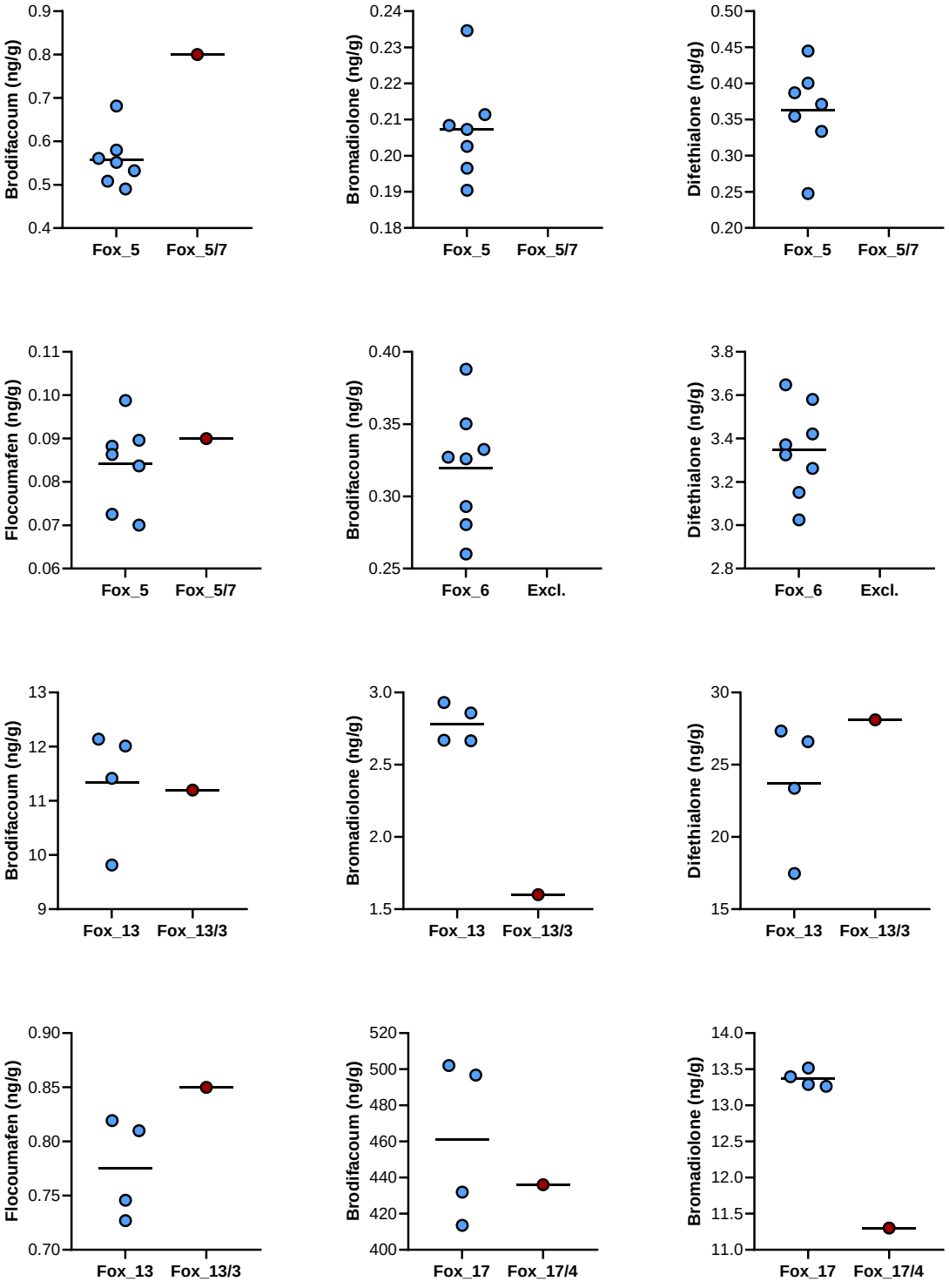


Figure A3.8. Concentrations of anticoagulant rodenticides in ca. 3 to 4 g aliquots of fox livers analysed by the Ecotox Centre (light blue) or BfG (red). Fox 6 was not analysed by BfG and is shown as excluded (excl.). A missing red dot indicates a result below LOQ.

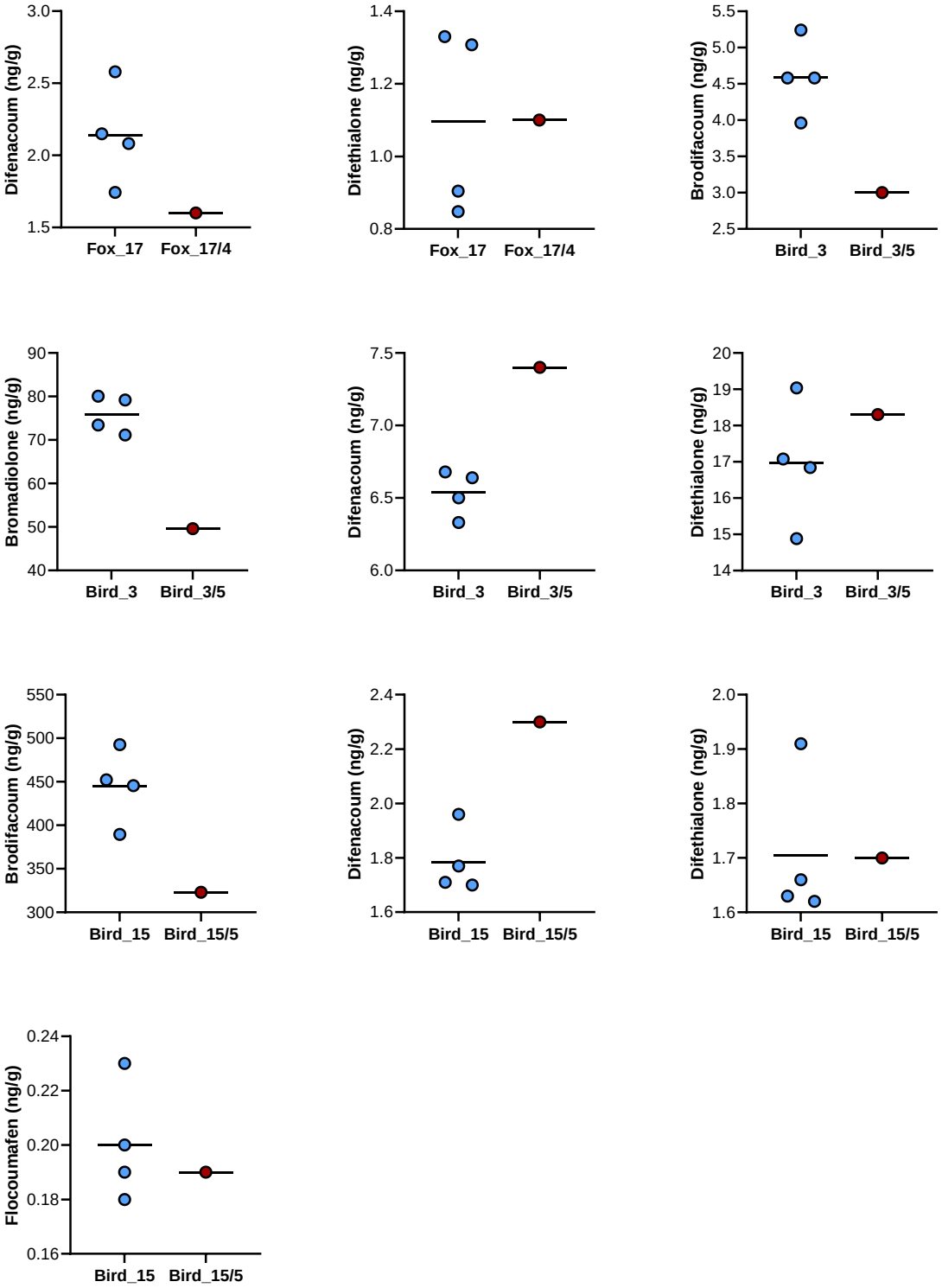


Figure A3.9. Concentrations of anticoagulant rodenticides in ca. 3 to 4 g aliquots of fox and bird livers analysed by the Ecotox Centre (light blue) or BfG (red).

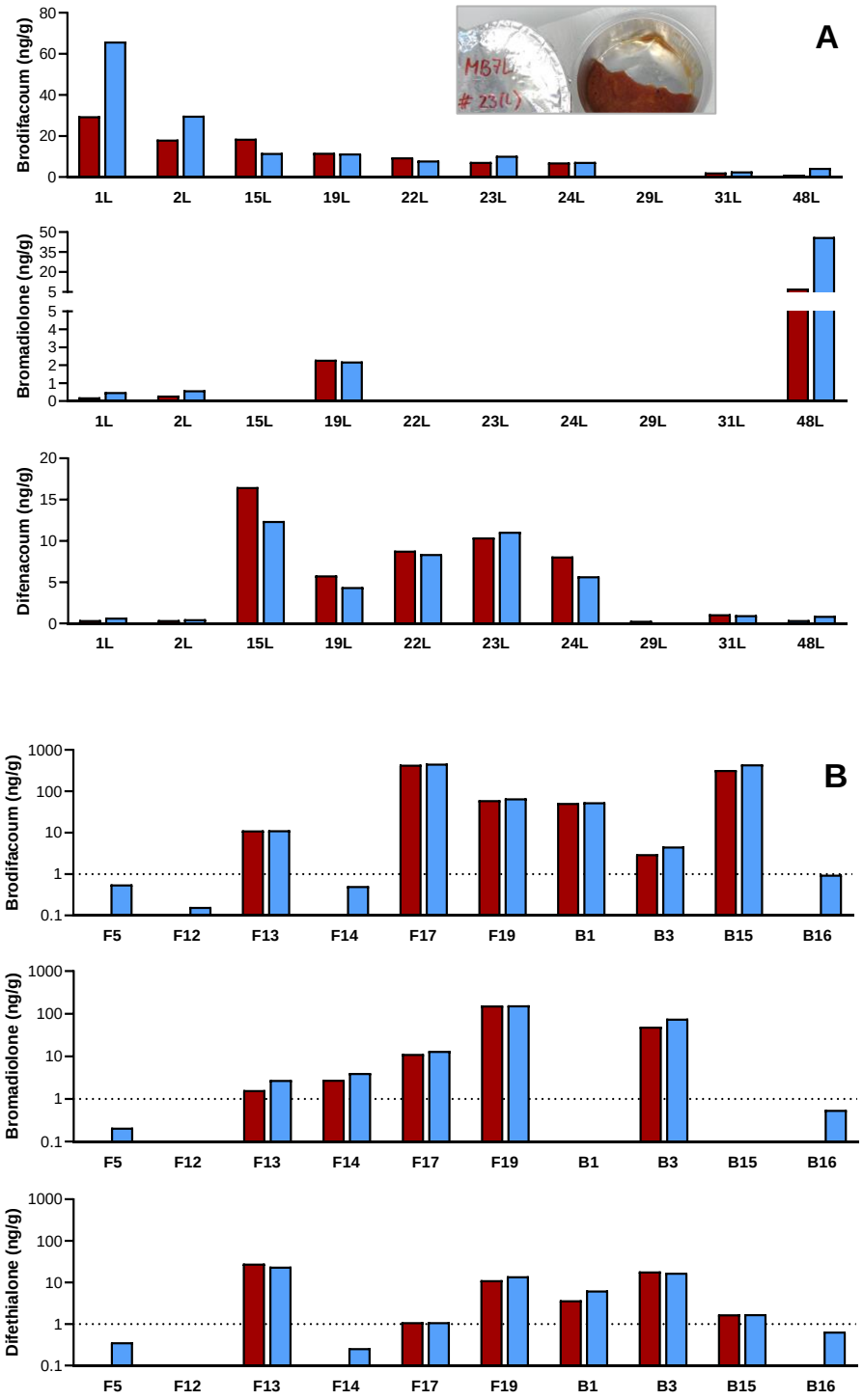


Figure A3.10. Panel A: Comparison of concentrations of three anticoagulant rodenticides in ten fish liver samples collected and homogenised by the Federal Institute of Hydrology (BfG) and quantified by BfG (red) in 2018/2019 and Ecotox Centre (blue) in 2022. Sample labels correspond to exact sample identifiers published by Regnery, Schulz et al. (2020); the image shows how samples were stored frozen for ca. 3.5 years in aluminium cups. Panel B: Comparison of concentrations of three anticoagulant rodenticides in 3-4 g aliquots of fox (F) and bird (B) liver samples quantified by BfG (red) and Ecotox Centre (blue).

Note: as data ranges in Panel A are small, linear y-axes are used; data ranges in Panel B are large and thus log scales are used.

Annex B1 – Long questionnaires to VSS-members in German, French and Italian

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Fragebogen zur Schadnagerbekämpfung

Rodentizide werden zur Bekämpfung von Schadnagern in Form von Ködern eingesetzt, um die menschliche Hygiene und Materialien zu schützen. Als Wirkstoffe werden meist Blutgerinnungshemmer, sogenannte Antikoagulanzien verwendet, die eine hohe Wirksamkeit aufweisen. Nachteile sind jedoch ihre Toxizität für Menschen und Tiere, ihre teilweise schlechte Abbaubarkeit sowie ihre Anreicherung in der Nahrungskette.

Mit dem folgenden Fragebogen möchten wir im Auftrag des Bundesamtes für Umwelt (BAFU) einen Überblick über den generellen Einsatz von Rodentiziden in der Schweiz gewinnen, um darauf basierend eine Einschätzung der möglichen Umweltbelastung durch den Einsatz von Rodentiziden vornehmen zu können.

Vielen Dank für Ihre Mithilfe. Ihre Informationen werden einen signifikanten Beitrag zur Abschätzung der möglichen Umweltbelastung an Rodentiziden in der Schweiz leisten.

1. **Welche Rodentizide und Methoden setzen Sie zur Schadnagerbekämpfung ein?**
   _____% Antikoagulanzien
   _____% Chemische Mittel
   _____% Physische Methoden z.B. Schlagfalle
   ☐ Bei den angegebenen Prozent handelt es sich um eine Schätzung

2. **Werden Menge und Ausbringungsort der Köder dokumentiert?**
   ☐ Ja
      ☐ Handschriftliche Dokumentation
      ☐ Digitale Dokumentation
      ☐ Zugang zu Datenbanken, die eine leichte statistische Auswertung ermöglichen
         ☐ Lokale Ablage
   ☐ Nein

3. **Wieviel Prozent an Antikoagulanzien-Rodentiziden werden von Ihnen in Innen- bzw- Aussenräumen eingesetzt?**
   Innenräume: _____%
   Aussenräume: _____%
   ☐ Bei den angegebenen Prozent handelt es sich um eine Schätzung

4. Was sind die Zielorte der Schadnagerbekämpfungsmassnahme und wieviele Schadnagerbekämpfungsmassnahmen führen Sie durch?

Wenn bekannt, bitte durchschnittlich verwendete Mengen an eingesetzten Produkten pro Jahr (kg) auf die einzelnen Bereiche verteilen.

Zielorte	Anzahl Kunden	Durchschnittliche Menge an eingesetzten Produkten pro Jahr (kg)
Häusliche Umgebungen		
Industrielle Umgebungen		
Landwirtschaftliche Umgebungen		
Lebendsmittelverarbeitende Betriebe		
Lebensmittelgeschäfte		
Abwasserkanäle		
Sonstiges		

☐ Bei den angegebenen Werten handelt es sich um eine Schätzung

Bitte spezifizieren Sie Sonstiges:

_____
_____
_____
_____

5. Wie häufig werden Schadnagerbekämpfungsmassnahmen an den gleichen Standorten veranlasst?

(Mehrere Antworten sind möglich)

☐ Regelmässig (_____ pro Jahr)
☐ Permanente Beköderung (durchschnittlich während _____ Monaten pro Jahr)
☐ Selten an den gleichen Standorten
☐ Andere Antwort (bitte spezifizieren):

_____
_____

☐ Bei den angegebenen Werten handelt es sich um eine Schätzung

6. Wie wird die Beköderung durchgeführt?

Direkte Beköderung (z.B. loser Köder) _____%
Beköderung in Boxen _____%
Sonstiges _____%

Bitte spezifizieren Sie Sonstiges:

_____
_____
_____

☐ Bei den angegebenen Prozent handelt es sich um eine Schätzung

7. Welche Ködertypen kommen zum Einsatz?

(Mehrere Antworten sind möglich)

☐ Formköder z.B. Wachsblock
☐ Paste oder abgepackte Wurfbeutel (z.B. mit Körner, Pellets oder Granulat)
☐ Schüttfähiger/loser Köder wie Körner, Pellets oder Granulat
☐ Schaum oder Gel
☐ Flüssige Köder
☐ Sonstige (bitte spezifizieren):

_____

_____

_____

8. Sind Köder immer vor Nicht-Zielorganismen geschützt?

☐ Ja (bitte spezifizieren Sie wie):

_____

_____

_____

_____

☐ Nein

9. Welche Produkte und in welcher Menge pro Jahr werden von Ihnen eingesetzt?

Bitte geben Sie die durchschnittliche Menge an eingesetztem Produkt pro Jahr (kg) unter Nennung des Produktnamens an.

Name des Produkts (Antikoagulanzien)	**Durchschnittlich verwendete Menge an Produkt pro Jahr (kg)**

Name des Produkts (nicht Antikoagulanzien z.B. Alphachloralose oder Cholecalciferol)	**Durchschnittlich verwendete Menge an Produkt pro Jahr (kg)**

☐ Bei den angegebenen Mengen handelt es sich um eine Schätzung

10. Von welchen Herstellern werden die eingesetzten Rodentizide bezogen?

_____

_____

_____

_____

11. Wie häufig werden die Köder bei Einzelaufträgen nach deren Ausbringung kontrolliert?

☐ Alle _____ Tage

☐ Alle _____ Wochen

☐ Alle _____ Monate

☐ Selten (z.B. 1x nach Ausbringung)

☐ Nie

☐ Bei den angegebenen Zahlen handelt es sich um eine Schätzung

12. **Was passiert mit den restlichen Rodentiziden nach der Beköderungsperiode?**
(Mehrere Antworten sind möglich)
☐ Permanente Beköderung
☐ Köder werden in der Natur zurückgelassen
☐ Köderrückstände werden vor Ort (z.B. im Hauskehricht) entsorgt
☐ Köderrückstände werden eingesammelt und in der Firma entsorgt
☐ Sonstiges (bitte spezifizieren):

_____
_____
_____
_____

13. **Was passiert mit den Schadnager-Kadavern?**
Im Falle, dass die Kadaver entfernt werden, bitte spezifizieren Sie die weitere Vorgehensweise.
☐ Schadnager-Kadaver werden der Natur überlassen
☐ Schadnager-Kadaver werden explizit gesucht und entfernt
    ☐ Abgeben bei Tierkadaversammelstellen
    ☐ Vergraben der Kadaver
    ☐ Beseitigung mithilfe von Abfallentsorgungsunternehmen
    ☐ Beseitigung mit dem Siedlungsabfall in KVA
    ☐ Sonstiges (bitte spezifizieren): _____
☐ Sonstiges (bitte spezifizieren):

_____
_____
_____
_____

14. **Wie viele der durch die Schadnagerbekämpfungsmassnahme getöteten Schadnager werden schätzungsweise gefunden?**
_____%

15. **Wurden Resistenzen zu bestimmten Rodentiziden beobachtet?**
☐ Ja (bitte spezifizieren Sie bei welchen Produkten und an welchem Standort die Resistenz beobachtet wurde):

_____
_____
_____
_____
☐ Nein

16. **Wie häufig kommen Non-Tox-Köder zum Einsatz?**
Prozent: _____%
oder Anzahl: _____.
☐ Bei den angegebenen Prozent handelt es sich um eine Schätzung

Vielen Dank für Ihre Mithilfe!

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Questionnaire sur la lutte contre les rongeurs nuisibles

Les rodenticides sont utilisés pour lutter contre les rongeurs nuisibles sous forme d'appâts afin de protéger l'hygiène humaine et les matériaux. Les substances actives utilisées sont généralement des inhibiteurs de la coagulation sanguine, appelés aussi anticoagulants, qui présentent une grande efficacité. Ils présentent toutefois des inconvénients : leur toxicité pour l'homme et les animaux, leur dégradation parfois difficile et leur accumulation dans la chaîne alimentaire.

Avec le questionnaire suivant, nous souhaitons obtenir, sur mandat de l'Office fédéral de l'environnement (OFEV), une vue d'ensemble de l'utilisation générale des rodenticides en Suisse, afin de pouvoir procéder, sur cette base, à une évaluation de l'impact environnemental potentiel de l'utilisation des rodenticides.

Nous vous remercions de votre aide. Vos informations contribueront de manière significative à l'estimation de la charge environnementale potentielle des rodenticides en Suisse.

1. **Quels rodenticides et méthodes utilisez-vous pour lutter contre les rongeurs nuisibles?**
   _____% Anticoagulants
   _____% Agents chimiques
   _____% Méthodes physiques, par exemple piège à déclic
   ☐ Les pourcentages indiqués sont des estimations.

2. **La quantité et le lieu d'application des appâts sont-ils documentés ?**
   ☐ Oui
      ☐ Documentation manuscrite
      ☐ Documentation digitale
      ☐ Accès à des bases de données permettant une analyse statistique facile
      ☐ Archivage local
   ☐ Non

3. **Quel est le pourcentage de rodenticides anticoagulants que vous utilisez à l'intérieur et à l'extérieur ?**
   Intérieur: _____%
   Extérieur : _____%
   ☐ Les pourcentages indiqués sont des estimations.

4. **Quels sont les lieux ciblés par la mesure de lutte contre les rongeurs nuisibles et combien de mesures de lutte contre les rongeurs nuisibles mettez-vous en œuvre ?**
Si vous le savez, veuillez répartir les quantités moyennes de produits utilisés par an (kg) entre les différents secteurs.

Localisations	**Nombre de clients**	**Quantité moyenne de produits utilisés par an (kg)**
Environnements domestiques		
Environnements industriels		
Environnements agricoles		
Entreprises de transformation de denrées alimentaires		
Magasins d'alimentation		
Égouts		
Autres		

☐ Les valeurs indiquées sont des estimations.

Veuillez spécifier autre: _____

5. **A quelle fréquence des mesures de lutte contre les rongeurs nuisibles sont-elles ordonnées sur les mêmes sites ?**
(Plusieurs réponses possibles)
☐ Régulièrement (4 par an)
☐ Appâtage permanent (en moyenne pendant de 12 mois par an)
☐ Rarement sur les mêmes sites
☐ Autre réponse (à préciser):
_____
_____

☐ Les valeurs indiquées sont une estimation.

6. **Comment l'appâtage est-il effectué ?**
Appâtage direct (p.ex. appât en vrac dans les égouts) _____%
Appâtage dans des boîtes _____%
Autres _____%
Veuillez spécifier autre :
_____
_____
_____
_____
☐ Les pourcentages indiqués sont une estimation.

7. **Quels types d'appâts sont utilisés ?**
(Plusieurs réponses sont possibles)
☐ Appât moulé, par ex. bloc de cire
☐ Pâte ou sachets à lancer emballés (par ex. avec des grains, des pellets ou des granulés)
☐ Appât en vrac/sans appât comme des grains, des pellets ou des granulés
☐ Mousse ou gel
☐ Appât liquide
☐ Autres (à préciser) :
_____
_____

_____

_____

8. **Les appâts sont-ils toujours protégés contre les organismes non ciblés ?**

☐ Oui (veuillez spécifier comment) :

_____

_____

_____

_____

☐ Non

9. **Quels sont les produits que vous utilisez et en quelle quantité par an ?**

Veuillez indiquer la quantité moyenne de produit utilisée par an (kg) en citant le nom du produit.

Nom du produit (anticoagulants)	**Quantité moyenne de produit utilisée par an (kg)**

Nom du produit (non anticoagulants p.ex. alphachloralose ou cholécalciférol)	**Quantité moyenne de produit utilisée par an (kg)**

☐ Les quantités indiquées sont une estimation

10. **Auprès de quels fabricants les rodenticides utilisés sont-ils achetés ?**

_____

_____

_____

_____

11. **Pour les commandes individuelles, à quelle fréquence les appâts sont-ils contrôlés après leur épandage ?**

☐ Tous les _____ jours
☐ Tous les _____ semaines
☐ Tous les _____ mois
☐ Rarement (par ex. 1x après épandage)
☐ Jamais

☐ Les chiffres indiqués sont une estimation

12. Que se passe-t-il avec les rodenticides restants après la période d'appâtage ?
(Plusieurs réponses possibles)
☐ Appâtage permanent
☐ Les appâts sont laissés dans la nature
☐ Les résidus d'appâts sont éliminés sur place (par ex. dans les ordures ménagères)
☐ Les résidus d'appâts sont collectés et éliminés dans l'entreprise
☐ Autre (à préciser) :

_____
_____
_____
_____

13. Que se passe-t-il avec les cadavres de rongeurs nuisibles ?
Dans le cas où les cadavres sont enlevés, veuillez spécifier la marche à suivre.
☐ Les cadavres de rongeurs nuisibles sont laissés dans la nature
☐ Les cadavres de rongeurs nuisibles sont explicitement recherchés et enlevés
- ☐ Déposer dans des centres de collecte de cadavres d'animaux
- ☐ Enfouissement des cadavres
- ☐ Élimination à l'aide d'entreprises de gestion des déchets
- ☐ Élimination avec les déchets urbains dans les UIOM
- ☐ Autre (à préciser) :

_____
_____
_____
_____
_____

☐ Autre (à préciser) :

_____
_____
_____
_____

14. Combien de rongeurs nuisibles tués par la mesure de lutte contre les rongeurs nuisibles sont retrouvés, selon les estimations ?
_____ %

15. Des résistances à certains rodenticides ont-elles été observées ?
☐ Oui (veuillez préciser pour quels produits et à quel endroit la résistance a été observée) :

_____
_____
_____
_____

☐ Non

16. Quelle est la fréquence d'utilisation des appâts non toxiques ?
Pourcentage : _____.%
ou Nombre: _____.
☐ Les nombres indiqués sont des estimations.

Merci beaucoup pour votre aide !

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Questionario sul controllo dei roditori

I rodenticidi sono usati per controllare i roditori nocivi sotto forma di esche per proteggere l'igiene umana e i materiali. I principi attivi utilizzati sono per lo più anticoagulanti, che sono molto efficaci. Gli svantaggi, tuttavia, sono la loro tossicità per gli esseri umani e gli animali, la loro degradabilità in parte scarsa e il loro accumulo nella catena alimentare.

Con il seguente questionario, su incarico dell'Ufficio federale dell'ambiente (UFAM), vorremmo ottenere una panoramica dell'uso generale dei rodenticidi in Svizzera, per poter fare su questa base una valutazione del possibile impatto ambientale dell'uso dei rodenticidi.

Grazie mille per la vostra collaborazione. Le vostre informazioni daranno un contributo significativo alla stima del possibile impatto ambientale dei rodenticidi in Svizzera.

1. **Quali rodenticidi e metodi usate per il controllo dei roditori?**
   _____% Anticoagulanti
   _____% Agenti chimici
   _____% Metodi fisici, ad esempio trappola a scatto
   ☐ Le percentuali indicate sono una stima.

2. **La quantità e il luogo di applicazione delle esche sono documentati?**
   ☐ Sì
      ☐ Documentazione scritta a mano
      ☐ Documentazione digitale
         ☐ Accesso a banche dati che permettono una facile analisi statistica
         ☐ Archiviazione locale
   ☐ No

3. **Che percentuale di rodenticidi anticoagulanti usate rispettivamente all'interno e all'esterno?**
   all'interno: _____%
   all'esterno: _____%
   ☐ Le percentuali indicate sono una stima.

4. **Quali sono i luoghi di destinazione della misura di controllo dei roditori e quante misure di controllo dei roditori eseguite?**
Se noto, si prega di distribuire le quantità medie di prodotti utilizzati per anno (kg) nelle singole aree.

Destinazioni	Numero di clienti	Quantità media di prodotti usati all'anno (kg)
Ambienti domestici		
Ambienti industriali		
Ambienti agricoli		
Impianti di trasformazione alimentare		
Negozi di alimentari		
Fogne		
Altro		

☐ I valori indicati sono una stima.

Si prega di specificare altro:
_____
_____
_____
_____

5. **Quanto spesso vengono avviate misure di controllo dei roditori negli stessi siti?**
(Sono possibili più risposte)
☐ Regolarmente (_____ all'anno)
☐ Esca permanente (in media durante _____ mesi all'anno)
☐ Raramente negli stessi siti
☐ Altra risposta (specificare):
_____
_____
☐ I valori indicati sono una stima.

6. **Come si effettua l'adescamento?**
Esca diretta (ad esempio, esca sfusa) _____%
Esche nelle scatole _____%
Altro _____%
Si prega di specificare altro:
_____
_____
_____
☐ Le percentuali indicate sono una stima

7. **Quali tipi di esche sono usate?**
(Sono possibili diverse risposte)
☐ Esca sagomata, per esempio blocco di cera
☐ Sacchetti in pasta o preconfezionati (ad esempio con grani, pellet o granuli)
☐ Esche sfusesciolte come grani, pellet o granulato
☐ Schiuma o gel
☐ Esca liquida
☐ Altro (specificare):
_____
_____
_____

8. Le esche sono sempre protette dagli organismi non bersaglio?

☐ Sì (specificare come):

_____
_____
_____
_____

☐ No

9. Quali prodotti e in che quantità usate all'anno?

Si prega di indicare la quantità media di prodotto utilizzato all'anno (kg), citando il nome del prodotto.

Nome del prodotto (anticoagulanti)	**Quantità media di prodotto usato all'anno (kg)**

Nome del prodotto (non anticoagulanti come l'alfacloralosio o il colecalciferolo)	**Quantità media di prodotto usato all'anno (kg)**

☐ Le quantità indicate sono una stima.

10. Da quali produttori vengono acquistati i rodenticidi utilizzati?

_____
_____
_____
_____

11. Con quale frequenza vengono controllate le esche dopo la loro applicazione nel caso di ordini individuali?

☐ Ogni _____ giorni
☐ Ogni _____ settimane
☐ Ogni _____ mesi
☐ Raramente (ad esempio 1x dopo l'applicazione)
☐ Mai

☐ Le cifre indicate sono una stima.

12. Cosa succede ai rodenticidi rimanenti dopo il periodo di adescamento?

(Sono possibili diverse risposte)

☐ Esche permanenti
☐ L'esca viene lasciata in natura
☐ I residui delle esche vengono smaltiti sul posto (ad esempio nei rifiuti domestici)
☐ I residui delle esche sono raccolti e smaltiti nell'azienda

☐ Altro (specificare):

_____
_____
_____
_____

13. Cosa succede alle carcasse dei roditori?

Nel caso in cui le carcasse vengano rimosse, si prega di specificare la procedura successiva.

☐ Le carcasse dei roditori sono lasciate nella natura

☐ Le carcasse di roditori sono espressamente cercate e rimosse.

- ☐ Consegna a mano nei punti di raccolta delle carcasse di animali
- ☐ Le carcasse sono seppellite
- ☐ Smaltimento con l'aiuto di aziende di smaltimento rifiuti
- ☐ Smaltimento con rifiuti urbani in impianto di incenerimento dei rifiuti

Altro (specificare): _____

☐ Altro (specificare):

_____
_____
_____
_____

14. Quanti dei roditori uccisi dalla misura di disinfestazione vengono ritrovati? (stimato)

_____%

15. È stata osservata una resistenza a certi rodenticidi?

☐ Sì (Si prega di specificare in quali prodotti e in quale posizione è stata osservata la resistenza):

_____
_____
_____
_____

☐ No

16. Quanto spesso vengono usate esche non tossiche?

Percentuale: _____%

o numero: _____

☐ Le percentuali indicate sono una stima.

Grazie mille per il vostro aiuto!

Annex B2 – Short questionnaires for cantonal capitals

German:

1.) Werden in Ihrer Stadt Antikoagulanzien-Rodentizide (AR) zur Schadnagerbekämpfung eingesetzt?
2.) Werden die Daten zur Schädlingsbekämpfung (z.B. eingesetzte Mittel, Menge und Häufigkeit) von Ihnen erfasst, z.B. in einer Datenbank?
3.) Wenn ja, könnten Sie uns diese Daten zur Verfügung stellen?
4.) Falls die Schädlingsbekämpfung extern vergeben wird, könnten Sie uns sagen, an welche Schädlingsbekämpfer?

French:

1.) Des rodenticides anticoagulants (RA) sont-ils utilisés dans votre ville pour lutter contre les rongeurs nuisibles ?
2.) Les données relatives à la lutte contre les rongeurs nuisibles (p. ex. produits utilisés, quantité et fréquence) sont-elles enregistrées par vos soins, p. ex. dans une base de données ?
3.) Si oui, pourriez-vous mettre ces données à notre disposition ?
4.) Si la lutte contre les nuisibles est confiée à un tiers, pourriez-vous nous dire à quels professionnels de la lutte contre les nuisibles ?

Italian:

1.) La Sua città ricorre all'uso di rodenticidi anticoagulanti (RA) per il controllo dei roditori?
2.) I dati sul controllo dei roditori nocivi (per esempio metodi usati, quantità e frequenza) sono registrati da voi, per esempio in un database?
3.) Se sì, potrebbe mettere a disposizione questi dati per noi?
4.) Se il controllo dei roditori è affidato a terzi, potrebbe dirci a quali disinfestatori?

Annex B3 – Summary of survey answers from VSS-members

Table B3.1. Summary of survey filled in by several members of the Association of Swiss Pest Controllers (A-J). Estimated numbers are written in blue. Data in Column J were originally reported for Cantonal Capital D. Information on products and suppliers is kept confidential (conf.).

	A	B	D	E	F	G	H	I	J (Cant. D)
Method for rodent control (%)									
anticoagulants	50	80	75	70	70	30	70		88
Chemical agents									
Physical methods	50	20	25	30	30	70	30	80	12
Documentation of rodent control								5	
Handwritten	x		x	x				15	
Digital		x	x		x	x	x		x
- Database					x		x	x	
- Local storage	x	x		x		x			
Application area (%)									
Interior	40	20	95	90	50	1	10	x	80
Exterior	60	80	5	10	50	99	90		20
Target location (customers and Ø quantity of products used per year)	Customers kg	Customers kg	Customers kg	Customers 15	Customers kg	Customers kg	Customers kg	Customers kg	Customers kg
Domestic environments	x 8	x In	120 25	10-50 2-10	30 2	190 35	50 6	300	1000 0.5[j]
Industrial environments	x 4	x total		1-4 3-5	100 500	2 1	40 8	150	2000 2.5[j]
Agricultural environments	x 2	x 300	ca. 8 20	2 10-15	0 0	1 1	0-5 3	2	
Food processing plants		x	150-200 If needed	5 1-5	300 1500		20 6		See industrial environments
Grocery stores	x 2			3 1-4	100 10		40 6	9	2000 0.5[j]
Sewers				0 0	0 0		5-10 1	9	
Other								100[a]	
Frequency of rodent control at same locations									
Regularly (per year)		Monitoring			4-12		2-3	100a	2
Permanent (average x months per year)			12	12	12		4		12
Rarely at the same locations	x		private	private		x		4	
Other		Local controls		Service contracts				12	
Baiting									
Direct							1		
Baiting boxes	100	100	100	98	100	100	99		100
Other				2[b]					

Baiting types								100	
Solid bait e.g. wax blocks	x	x	x	x	x		x		x
Paste		x	x	Paste		x	x		
Grain or pellets							x		
Foam or gel								x	
Fluid baits									
Other				Non-Tox blocks					
Protection of non-target organisms	Baiting boxes with key	Baiting boxes	In boxes	Baiting boxes, cavities in the building, Shafts closed with a door/hatch	In locked tamper-proof boxes	In steal baiting boxes	In steal or plastic boxes	In steal or plastic boxes	yes
Products and avg. amounts of rodenticides used per year	Product kg	Product kg	Product kg	Product	Product kg	Product kg	Product kg	Product kg	Product kg
Anti-coagulant	Conf. 18 - 36	Conf. 300	Conf. 100-200	Conf. 10-30	Conf. 1665	Conf. 37	Conf. 30	Conf. 300	Conf. 1520
Anti-coagulant	-	-	Conf. 10	Conf. 5-15	Conf. 484		Conf. 20		Conf. 2250
Anti-coagulant	-	-	Conf. 5	Conf. 1-5			Conf. 1		Conf. 2450
Anti-coagulant	-		-	Conf. 1-5			Conf. 1		Conf. 350
Anti-coagulant	-			Conf. 7-12			Conf. 10		Conf. 790
Anti-coagulant	-						Conf. 10		
Non AR	-			Conf. 0-2			Conf. 3		
Non AR	-						Conf. 10	Conf. 8	
Manufacturers of applied products	Conf.	Conf.	Conf.	Conf.	Conf.	Conf.	Conf.	Conf.	Conf.
Frequency of bait control									
Every x days	5		7-14						
Every x weeks		1-2	1-2		2		1	4-6	2
Every x months			3-4	3[c]					
Rarely e.g. 1x						x			
Never									
Fate of baits after application									
Permanente baiting		x	x	x	x	x		x	x
Baits are left in nature									
Bait residues are disposed of on site (e.g. in household garbage)	x	x	x	x	x	x	x[d]	x	x
Other									
Fait of rodent carcasses									
Rodent carcasses are left to nature			x						

Rodent carcasses are explicitly searched for and removed	x	x		x	x		x	x	x
Depositing at animal carcass collection points			x				x	x	
Burial of the carcasses									
Disposal with the help of waste disposal companies								x	x
Disposal with municipal waste in waste incineration plants		x	X (mice)	x	x			x	
Others				x[e]		x[f]			
Carcasses found	40	-[g]	< 1%	ca. 1 %	5 %	0.5 %	2	3	20%
Observed AR resistance	no	no	Yes (Bromadiolone)	no	Yes (Difenacoum)	no	no	no	no
Non-Tox baits									
Percent (%)	20	40	10[h]	1-3	20	0	20-30[i]	0	90[j]

[a] Sewers with specific box against water entry

[b] inaccessible areas: cavities, closed shafts

[c] Service contracts: 1.) After installation 4-6 days; 2.) Depending on infestation level: third date 1-4 weeks; 3.) Depending on infestation level: fourth date 2-5 weeks

[d] Baits are taken back to the company and properly disposed of in hazardous waste containers. These containers are regularly disposed of at a disposal site. Everything is documented.

[e] In case of larger quantities, the nearest carcass collection point is searched for - the isolated carcasses are collected and deposited in an airtight container in the car and disposed of promptly

[f] Since baiting takes place only outdoors, carcasses are never found

[g] not recorded and thus no statement possible

[h] service contracts

[i] tendency to rise

[j] this appears to be an erroneous entry.

Annex B4 – Summary of survey answers from cantonal capitals

Table B4.1. Survey results of cantonal capitals obtained by call or mail.

	Mentioning on website	Survey by mail	Survey by call	VSS-questionnaire	No reply	Information
1	x		x			After notification, ARs are very selectively applied in accessible canals where main rat populations were sighted. Baits (Sorkil-Bloc) are installed with no water contact by hanging them with wires in the shafts and baits are controlled 3 days after installation. Rodent control by the sewage network operators is restricted exclusively to public areas in the canalization; on surfaces, private pest controllers are hired. The control perimeter as well as order quantity are recorded, but the quantity used differs as bait stations are stocked according to demand. Reasons for rodent outbreaks are often untidiness and improper waste disposal. Sewage network operators are aware of the problem of AR for non-target organisms and efficient alternatives to AR would be gladly accepted
2	x	x		x		See Table B4.2
3	x	x	x	x		See Table B4.2
4	x		x			Rodent control is outsourced to private pest controllers, no information about applied products, quantity or frequency of AR use is available to them
5		x			x	No respective contact person found on website
6			x			Contact person unknown and thus no information available
7		x			x	No respective contact person found on website
8			x			Contact person unknown and thus no information available
9		x			x	No respective contact person found on website
10			x			No contact person assigned for pest control in the municipality
11			x			No information available, contact person unknown
12		x			x	No respective contact person found on website
13			x			When rodents are sighted, private pest controllers are hired. The city has a framework contract with the pest controlling company and data is only collected by them. Thus, no other information is available.
14		x		x		See Table B4.2
15			x			No information available, contact person unknown
16		x			x	No respective contact person found on website

17			x			There was a small rodent outbreak some years ago, which was brought under control with setting traps by city police and wildlife rangers. No database available due to very few incidents. Sometimes private pest controllers are hired. However, no information about applied products, quantity or frequency of AR use is available to them.
18			x			No information available, contact person unknown
19		x	x			Rodent control is outsourced to private pest controllers. City maintains no database regarding pest control and no information about applied products, quantity or frequency of AR use is available to them.
20		x	x			No information available, contact person unknown
21		x	x	x		See Table B4.2
22		x				City has been entrusting the pest control interventions for several years to a private pest controller, which will be able to provide more information.
23			x			No information available, contact person unknown
24		x				ARs are used by three sections: the division of waste management, the division of cleanliness of the public and the division of recovery and treatment of waste. Data about rodent control is not recorded in a database. Pest control as well as installing and managing of baits is mandated by two private pest controllers.
25		x				The city does not carry out rodent control campaigns in its municipal sewage systems. On a case by case basis, they mandate specialized companies. Over the last 5 years, they had only conducted 2 control campaigns by placing bait on some communal shafts. The rodent control for the City are entrusted to two private pest controllers.
26	x	x	x		x	The city offers free assistance in the event of rat infestations in the city area. No further information could be obtained.

Table B4.2. Summary of survey filled in by three cantonal capitals. Estimated numbers are written in blue.

	A	**B**	**C**
Method for rodent control (%)		n.d.	
anticoagulants	99		100
Chemical agents			
Physical methods	1		

Documentation of rodent control Handwritten Digital - Database - Local storage			n.d.			
					X	
	X X					
Application area (%) Interior Exterior			n.d.			
					90 10	
	100					
Target location (customers and Ø quantity of products used per year)	Customers	kg	Customers	kg	Customers	kg
Domestic environments			5-6	Unknown		
Industrial environments			17-25[a]	22.55 (2019) 23.8 (2020)		
Agricultural environments						
Food processing plants						
Grocery stores						
Sewers			550	40		
Other		18[b]				8-15[c]
Frequency of rodent control at same locations			n.d.			
Regularly (per year)	1-3				4	
Permanent (average x months per year)						
Rarely at the same locations						
Other	There may be additional new locations each year					
Baiting			n.d.			
Direct	40[d]					
Baiting boxes	60				100	
Other						
Baiting types						
Solid bait e.g. wax blocks	X		X		X	
Paste	X					
Grain or pellets	X					

Foam or gel Fluid baits Other						
Protection of non-target organisms	yes		yes		yes	
Products and avg. amounts of rodenticides used per year	Product	kg	Product	kg	Product	kg
Anti-coagulant	Confidential (Coumatetralyl)	10	Confidential (Difenacoum…)	2.52 (2019) 0 (2020)	Confidential	2
Anti-coagulant	Confidential (Difenacoum)	6	Confidential (Difenacoum)	0.8 (2019) 4.6 (2020	Confidential	2
Anti-coagulant	Confidential (Difenacoum)	2	Confidential (Difenacoum)	18.6 (2019) 19.2 (2020)	Confidential	2
Anti-coagulant			Confidential (Difenacoum)	0.625 (2019) 0 (2020)		
Anti-coagulant			Confidential (Difethialone)	40		
Non AR	Confidential starting in 2022					
Manufacturers of applied products	Confidential		Confidential		Confidential	
Frequency of bait control						
Every x days	6-7					
Every x weeks			2-4		1	
Every x months			2-3			
Rarely e.g. 1x						
Never						
Fate of baits after application						
Permanente baiting			X[e]			
Baits are left in nature						
Bait residues are disposed of on site (e.g. in household garbage)					X	

Bait residues are collected and disposed of in the company Other	X	X	
Fait of rodent carcasses Rodent carcasses are left to nature Rodent carcasses are explicitly searched for and removed Depositing at animal carcass collection points Burial of the carcasses Disposal with the help of waste disposal companies Disposal with municipal waste in waste incineration plants Others	X X[f]	 X X Dead animals are flushed away in the sewage system, transported to the wastewater treatment plant, removed there and disposed of properly.	X
Carcasses found	5-10%	Unknown	0%
Observed AR resistance	-	-	-
Non-Tox baits Percent (%)	0	0	0

[a] The occurrence of rats is always subject to fluctuations. Pest control is only initiated where rats are sighted or have already settled in inhabited zones or recreational facilities. The use of pesticides is based on the principle "as little as possible and as much as necessary".

[b] Average over last 10 years, public areas in the city such as parks.

[c] Lake shores

[d] e.g. Direct baiting of rat burrows by means of special shovels.

[e] Some hotspots need to be baited continuously in the form of a long-term intervention.

[f] Rodent carcasses are rarely found.