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Anticoagulant rodenticides in Swiss birds of prey

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Cover photo (Common kestrels) by Andreas Lischke, Bird of Prey Sanctuary Berg am Irchel.

Summary

The use of anticoagulant rodenticides (ARs) is increasingly subject to criticism, as these are persistent, bioaccumulative, and toxic substances that are used regularly and occasionally end up in the environment. For this reason, the Federal Office for the Environment (FOEN) commissioned an initial study from the Ecotox Centre in 2021 to monitor ARs in the environment. Following this study, the FOEN commissioned us to conduct the present study to assess the background exposure of Swiss birds of prey. If possible, it should be determined whether there are certain thresholds of AR concentrations in the liver above which unacceptable impacts on birds of prey occur.

In addition to chemical analyses, postmortem examinations were also carried out to determine the cause of death. At total of 103 carcasses of common buzzards (*Buteo buteo*) and common falcons (*Falco tinninculus*) were examined. First, the birds were categorized into three groups based on the most likely cause of death: common trauma, suspicion of AR toxicosis, and other causes. Then, liver and blood samples were chemically analyzed for seven different ARs (Brodifacoum, Bromadiolone, Coumatetralyl, Difenacoum, Difethialone, Flocoumafen and Warfarin), as well as pentobarbital and alpha-chloralose. Since the two first-generation ARs Coumatetralyl (two cases) and Warfarin (no cases) could hardly be detected anymore, further evaluation was carried out only with the five second-generation ARs (SGARs).

The gathered data confirmed widespread background contamination of Swiss birds of prey by SGARs. The examined bird of prey livers showed concentrations above the limit of quantification (LOQ) between 0.1 to 582 ng/g in 92% of the cases. Depending on the threshold value used to determine the potential threat to birds of prey caused by ARs, 51% (lower range of ≥ 10 ng/g) or 16% (higher range of ≥ 100 ng/g) of the samples tested exceeded concentrations of concern. Macroscopically only 4% of the birds were suspected to have died from “AR toxicosis”, while 66% were diagnosed with “Other” and 30% with “Common trauma”. The comparatively high value of “Other” cases could be partly explained by an increased buzzard mortality likely caused by climatic conditions (frequent cold winds) and limited food supply between December 2024 and February 2025. An additional histopathological examination of formalin-fixed paraffin-embedded tissues from 10 selected carcasses did not yield any significant hemorrhages indicative of an AR toxicosis. The analysis of the blood samples showed a similar pattern to that of the liver samples with concentrations ranging between 0.1 and 9.7 ng/ml. However, blood samples showed considerably lower concentrations than liver samples. Above a total liver value of 50 ng/g, SGARs could be reliably detected in the corresponding blood samples. The modeling of threshold values planned for this study cannot be carried out with only four (presumed) cases of AR toxicity in the data set. Similarly, the current project can neither confirm nor refute the AR threshold values already proposed.

In summary, the widespread background contamination of Swiss birds of prey with AR, which was already established in our initial study, has been confirmed in the present study. Although direct AR toxicity appears to be low, a worryingly high proportion of animals are exposed to sublethal levels of ARs, which can have a negative impact on their fitness and reproductive capacity. This is particularly concerning for endangered species. In addition, combined contamination with other pollutants such as heavy metals and pesticides must also be assumed. Therefore, the efforts to reduce the release of SGARs into the environment should be increased. Initial steps in this direction have already been taken. For example, since 2025, ARs are no longer approved for private use in Switzerland. Similarly, no products containing ARs are approved as plant protection products.

Content

1 Introduction

1.1.1 Background and objective

As successful cultural followers, pest rodents have posed a problem since humans settled down and implemented systematic storage of food supplies. Since rodents transmit numerous pathogens and destroy both crops and food supplies, measures were soon taken to control them. Additional problems occur when rodents are introduced to isolated ecosystems as invasive species. Recently, the destruction of power cables and information and communication technology lines has also been added to the list of negative impacts. Initially, control measures mainly involved traps and natural predators such as cats. However, chemical control methods soon followed, using various poisonous plants (van den Brink, Elliott et al. 2018). In commercial rodent control, acute toxins such as strychnine, arsenic, or thallium were used in the first half of the twentieth century. The discovery of anticoagulant rodenticides (ARs) led to a drastic decrease in the use of acute poisons worldwide. ARs showed several benefits over the previous substances used and were attributed to being safe, sustainable, cheap, easy to apply, and effective. Since the mid-1950s, warfarin has been the primary compound used for rodent control worldwide (Jacob and Buckle 2018).

However, in addition to their advantages, it soon became apparent that ARs also had some serious disadvantages posing a significant risk to the environment and non-target organisms. First and foremost, their specific chemical characteristics, which classify them as persistent, bioaccumulative, and toxic (PBT), or even very persistent and very toxic (vPvB). As early as the 1970s, it was recognized that secondary poisoning with ARs could be fatal for non-target organisms, including mammals and birds of prey (Mendenhall and Pank 1980). The earliest studies documenting secondary poisoning of wild animals date back to the 1980s (Kaukeinen 1982, Rammell, Hoogenboom et al. 1984). Since then, numerous studies have detected residues of ARs in non-target organisms worldwide (Hohenberger, Friesen et al. 2022).

In 2021, the Federal Office for the Environment (FOEN) commissioned an initial Swiss monitoring project (Riegraf et al. 2022), which covered the use of ARs, their occurrence in environmental compartments and non-target biota in Switzerland, and their potential environmental impact. At this time, no screening or widespread monitoring of ARs had been performed in Switzerland. However, studies conducted worldwide suggested that widespread environmental pollution by ARs is possible in Switzerland as well. Additionally, the use of ARs was identified as a "high-risk" practice in the implementation of a parliamentary initiative on reducing the risk of pesticide use. Therefore, samples of biota from the greater Zurich area, such as foxes, birds of prey, fish (from multiple cantons) and hedgehogs, were tested for liver concentrations of ARs and showed similar residue concentrations to those observed in wildlife in surrounding countries (AT, DE, FR) (Geduhn, Jacob et al. 2015, Regnery, Schulz et al. 2020, Badri, Schenke et al. 2021). As the occurrence of ARs in the Swiss terrestrial and aquatic environment was shown to be widespread and high levels were frequently observed, a more extensive monitoring in Switzerland was initiated by FOEN to establish a baseline exposure resulting in the current project. This baseline enables the evaluation of changes resulting from modified AR use and the outcomes of potential regulations. In addition, a monitoring of AR concentrations in combination with pathological examinations was proposed to support the risk assessment by providing information on critical threshold levels in liver samples of birds of prey in Switzerland.

1.1.2 Anticoagulant rodenticides

Since their discovery in the 1920s and their following commercialization in the 1950s ARs have become the main rodenticides used (Shore 2018). This is due to their superior properties over alterative rodenticides such as delayed effect, high efficacy in target-organisms and the existence of an effective antidot for non-target organisms (Hadler and Buckle 1992). The majority of commercially available ARs belongs to the two groups indandiones (e.g. diphacinone,

chlorophacinone) and hydroxycoumarins (e.g. warfarin, brodifacoum, difenacoum). Based mainly on warfarin, a series of ARs were developed in the 1950s, all of which are classified as first generation ARs (FGARs). With the occurrence of resistance in various rat and mice populations as early as the 1960s, FGARs were soon replaced by analogue substances that were even more potent as well as effective against resistant strains, the second generation ARs (SGARs) (Jacob and Buckle 2018).

ARs have structural similarities to vitamin K and can therefore bind to the vitamin K epoxide reductase enzyme (VKOR) which is crucial for vitamin K regeneration. Consequently, vitamin K hydroquinone formation decreases, activation of clotting factors is reduced, and blood clotting is affected (Zimmermann and Matschiner 1974, Silverman 1980, Valverde, Espín et al. 2021). Eventually, death is induced by uncontrolled internal and / or external hemorrhages both as massive blood loss but also from small bleeds which can lead to disfunction of vital organs (Shore 2018). Exposed rodents are subjected to a delayed death which prevents bait aversion by intelligent animals and increases the success of the pest control measure. Due to the similar mode of action of ARs, effects of different ARs are expected to be additive (Rattner and Mastrota 2018). Moreover, ARs act on all vertebrates, which increases the danger for unintentional poisoning of non-target organisms (Hadler and Buckle 1992).

In living animals, AR poisoning is most clearly noticeable through apathy, weakness, and paleness of the mucous membranes. This is due to blood loss, which results in low blood pressure and oxygen deprivation (Murray 2018). Post-mortem findings of AR poisoning include subcutaneous and intramuscular hemorrhage, bleeding into the thoracic and abdominal cavities or into the gastrointestinal tract as well as unclotted blood in the heart or major blood vessels. Furthermore, internal bleeding affecting major organs like the brain and pallor of internal organs have been described (Mendenhall and Pank 1980, Stone, Okoniewski et al. 1999, Murray 2011). However, some of these diagnoses are rather unspecific and may also indicate trauma. In addition, sublethal effects such as increased embryo mortality, teratogenic effects, behavioral effects and increase in susceptibility to pathogens have been suggested to be AR exposure related (Munday and Thompson 2003, Vidal, Alzaga et al. 2009, Serieys, Foley et al. 2013, Chetot, Taufana et al. 2020).

1.1.3 Authorized substances and products in Switzerland

Anticoagulant rodenticides contain active substances that pose a particular risk to people, animals, and the environment. These substances are highly toxic, bioaccumulative, and persistent, which makes them candidates for substitution under the Biocidal Products Regulation (BPR). However, as alternatives are scarce, the use of ARs is still approved to control pest rodents. In its latest opinion on ARs in May 2025, ECHA's Biocidal Products Committee (BPC) stated that there are alternatives for only two out of 11 uses of ARs. For these uses (indoor control of mice by the general public as well as permanent baiting by professional users) it is not recommended to renew the approval of ARs. For all other uses the renewal of the approval is recommended due to the lack of sufficient alternatives. The following chemical alternatives are identified as eligible chemical alternatives for some AR uses: alpha-chloralose, carbon dioxide, cholecalciferol and hydrogen cyanide. Carbon dioxide is the only substance identified as having a significantly lower overall risk to human health, animal health, and the environment when used for permanent baiting of mice and brown and black rats by trained professionals, making it a suitable alternative. For indoor control of house mice by the general public and professionals, mechanical traps can be considered a suitable alternative. For all other uses (e.g. baiting outdoors or in sewers by professionals and trained professionals) it is concluded that there are no suitable chemical alternatives. Therefore, the BPC recommended renewing the approval of ARs for all other uses for seven years. The European Commission has not yet made a final decision on the recommendation.

Currently, 55 rodenticide products of the product type "PT14: Rodenticides" are authorized in Switzerland (Table 1) (ECHA 2025) containing seven authorized ARs: The FGARs chlorophacinone and coumatetralyl as well as the SGARs brodifacoum, bromadiolone, difenacoum,

difethialone and flocoumafen. These comply with the EU approved ARs according to the database on biocidal active substances of the European Chemicals Agency (ECHA) (assessed on September 02, 2025) for biocidal application. The Swiss and EU biocidal products regulations are harmonized, and the authorization of biocidal products is mutually recognized by Switzerland and EU countries. Beginning from 1 April 2025 authorizations for ARs against mice and rats for private users (general public, private individuals) in Switzerland are no longer granted. Until this ban, private users have been allowed indoor use of rodenticides with an active ingredient content of less than 0.003% (FOPH 2025).

Table 1 Overview of authorized products containing rodenticidal active substances in the market area of Switzerland for 2021 and 2025 (ECHA 2021, ECHA 2025).

Year	2021	**2025**	2021	**2025**
Active substance	Authorized products (n)		Authorized products (%)	
alpha-Chloralose	5	**7**	7	**13**
Brodifacoum*	22	**18**	32	**33**
Bromadiolone*	11	**9**	16	**16**
Carbon dioxide	1	**2**	2	**4**
Cholecalciferol	2	**2**	3	**4**
Coumatetralyl*	1	**1**	2	**2**
Difenacoum*	21	**13**	30	**24**
Difethialone*	3	**3**	4	**6**
Flocoumafen*	3	**0**	4	**0**
Total	69	**55**		
FGAR	1	**1**	1	**2**
SGAR	60	**43**	87	**77**
non-ARs	8	**10**	12	**21**

*Anticoagulant rodenticides

Authorization for carbon dioxide in Switzerland has expelled in 2019 but usage of the two products currently in use (Table 1) is still allowed until 2029 and 2023, respectively. Authorization for 10 of the above-mentioned products (five non-ARs and five SGARs) will expire at the end of this year (2025) (ECHA 2025). No ARs are currently registered as plant protection products (BLV 2025).

Another potentially problematic substance also used as a rodenticide is alpha-chloralose. Another potentially problematic substance used as a rodenticide is alpha-chloralose. Unlike ARS, however, its use as a biocide is restricted to indoor areas only. It is also more commonly used as an anaesthetic for wild, domestic, and laboratory animals with sedative-hypnotic properties (Silverman and Muir III 1993). In a recent review by Buij et al. (2025) focusing on deliberate poisoning, alpha-chloralose was identified as the third most common cause of (intended) poisoning in various European bird of prey species between 1996 and 2016. Additionally, barbiturates such as pentobarbital are another potentially problematic source of (intended) poisoning in wildlife. Pentobarbital is used to humanely euthanize different species of animals and has been increasingly detected in several wildlife species including birds of prey (Wolf 2019, Wells, Butterworth et al. 2020, Herrero-Villar, Sánchez-Barbudo et al. 2021). To obtain the most comprehensive

overview possible of the contaminants affecting Swiss birds of prey, the Vetsuisse partners proposed including these two substances in the analysis.

1.1.4 Current exposure situation of non-target organisms

ARs pose a significant risk to the environment because they can exert their effects not only in their target organisms but in all vertebrates. This is because the blood coagulation system they affect is conserved in all vertebrates (Shore 2018). Over the past decades, studies on the exposure of nontarget organisms to ARs have been repeatedly published. Exposure to ARs through direct ingestion (primary exposure) and over poisoned prey (secondary exposure) has been documented. The majority of primary exposure affect smaller animals such as small mammals, birds, reptiles and invertebrates. However, larger mammals such as wild boar or hares are also regularly affected (Shore and Coeurdassier 2018). Most studies have focused on secondary exposure of predators and scavengers because the substances (especially SGARs) are persistent and tend to accumulate in the food chain. These studies have also revealed that a broad spectrum of predatory and scavenging species is exposed to SGARs on a global scale (López-Perea and Mateo 2018, Shore 2018). In their review covering 30 studies between 1998 and 2015 Nakayama, et al. (2019) reported AR residuals in 55% (n=2694) of the investigated non-target organisms (n=4891). The proportion of animals with detected AR residues ranged from 23% (FR) over 58% (USA) and 59% (NZ) to a maximum of 93% (DK), suggesting that AR residuals can be found almost globally. Of the different ARs mostly brodifacoum (31%), bromadiolone (30%) and difenacoum (26%) were found, others were present in less than 10% of the investigated animals. Among the investigated samples, AR residuals were mainly found in animals of higher trophic levels such as predators (57%) and raptors (57%) indicating a trend to biomagnification (Nakayama, Morita et al. 2019). The reported concentrations cover the low n/g range up to the low ug/g range (López-Perea and Mateo 2018). Most studies monitor AR residues in the liver of deceased animals. However, residues are increasingly measured in blood (serum) samples from living animals to determine acute exposure or remobilization from fat (Murray 2020, Oliva-Vidal, Martínez et al. 2022, Spadetto, Gómez-Ramírez et al. 2024).

Our current investigations in Switzerland (Riegraf, Olbrich et al. 2022) revealed a similar exposure of non-target organisms to ARs. In 23 out of 25 fox liver samples, up to four ARs (brodifacoum, bromadiolone, difenacoum, difethialone and / or flocoumafen) were detected above the limit of quantification (LOQ). The sum of AR concentrations was above 100 ng/g in six of the tested samples. The highest single detected compound concentration was 1100 ng/g of brodifacoum in an older female fox from outside the city of Winterthur. In 20 out of 21 birds of prey up to four different ARs (brodifacoum, bromadiolone, difenacoum and / or difethialone) were detected above the LOQ. Only one sample, had all seven analyzed ARs below LOQ. Three birds of prey (14%) had an AR sum above 100 ng/g. Ten out of 21 (48%) birds of prey exceeded a sum concentration of 20 ng/g. The highest detected sums of AR were 440 and 450 ng/g (two buzzards). All four hedgehog livers contained up to four different ARs (brodifacoum, bromadiolone, difenacoum and / or coumatetralyl. The highest concentration of an individual compound found was brodifacoum with 0.85 ng/g. 22 out of 30 fish liver samples contained up to three ARs above the LOQ. Summed AR concentrations in most samples were below 0.5 ng/g. In eight samples, summed AR concentrations were between 0.5 and 1.0 ng/g. The highest sum concentration of 36 ng/g occurred in a brown trout from a purely agricultural influenced catchment (Eschelisbach, TG) without treated sewage effluent. These data indicate a general background exposure to AR even of aquatic wildlife.

1.1.5 Recent proposals for AR thresholds in birds of prey

As for now there is no scientifically consensus on threshold values in liver linked to AR toxicosis (Rattner and Harvey 2021). Proposed thresholds from different studies with both free-range and captive setups range over two orders of magnitude (Thomas, Mineau et al. 2011). The lowest suggested threshold based on a free-range study is as low as 10 ng/g liver wet weight,

established with one examined great horned owl (*Bubo virginianus*) (Stone, Okoniewski et al. 1999). Other proposed thresholds range from >150-180 ng/g liver wet weight established for brodifacoum based on studies with free-ranging and captive barn owls (Newton, Wyllie et al. 1990) to 700 ng/g liver wet weight proposed for brodifacoum in barn owls (Lohr 2018, Rattner and Harvey 2021). In order to account for the large differences caused by (amongst others) different exposure scenarios (field/laboratory) and different species studied probabilistic models were established for threshold determination. According to Thomas et al. (2011) a threshold value of about 100-200 ng/g would result in toxicity probabilities of 11% and 22%, respectively, for barn owls. Based on pooled data including barn owls, barred owls and great horned owls, SGAR liver residues of 20 ng/g would result in 5% of birds showing signs of AR toxicosis, whereas 80 ng/g would result in 20%. However, the authors indicate that AR metabolization and overestimation of AR residues due to repetitive ingestion after uptake of a lethal dose might influence the probabilistic model and thus lead to a flattening of the probability curve (Thomas, Mineau et al. 2011). In a more recent approach, Elliott et al. (2024) presented a probabilistic model that covered a large database including the family groups Accipitridae (hawks and eagles), Falconidae (falcons), Strigidae (common owls), and Tytonidae (barn owls) from North America. With this expanded data set, the authors obtain considerably lower threshold values. SGAR liver residues of approximately 10 ng/g would lead to 20% of birds showing signs of AR toxicosis on average for the first three groups. A liver concentration of 100 ng/g would even lead to a 50% probability for an AR toxicosis. Data for barn owls shows even lower thresholds with 0.3 ng/g. This could be caused by interspecific variabilities for example in metabolization or sensitivity of the VKOR enzymes. The most likely cause according to the authors is the reduced data availability for barn owls and the state a lower confidence in their threshold proposed for barn owls. These newest thresholds also still need to be confirmed and are therefore not yet widely recognized. Therefore, further research is needed to establish robust threshold values of AR in non-target organisms. However, in this study, we apply the threshold value of 10 ng/g proposed by Elliott et al. (2024) because the examined dataset is very comprehensive.

2 Material and Methods

2.1 Samples

The species selected for sampling in this project were chosen based on the number of each species of bird treated in 2023 at three Swiss bird sanctuary stations (Figure 1). This selection was made to ensure a sufficient amount of data for solid assessments and statistical analyses. Information about possible available numbers was provided by the Bird of prey station Berg am Irchel (ZH), Swiss Ornithological Institute (LU) and Wildlife station Landshut (BE).

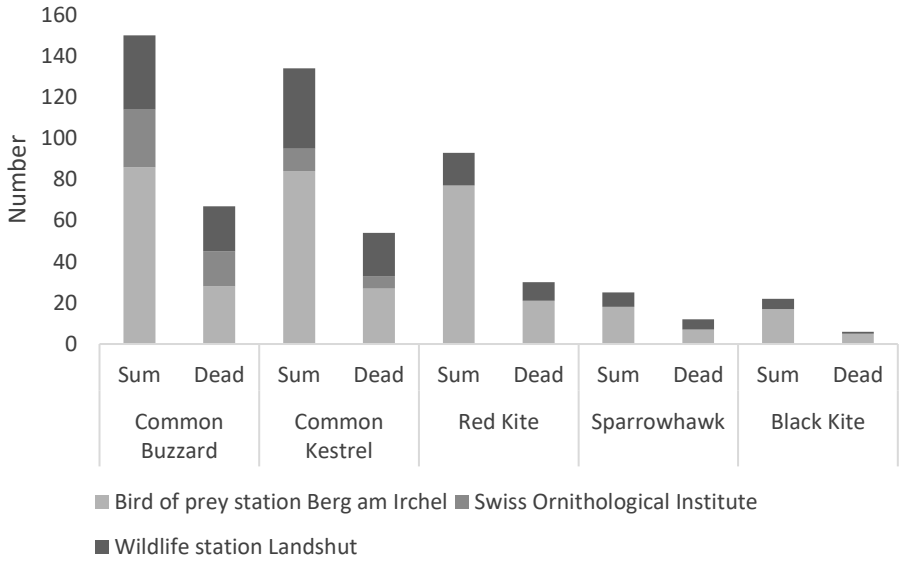

Figure 1 Different species and related numbers of birds treated in three different bird sanctuaries in the northern part of Switzerland in 2023. The sum refers to all birds delivered to the stations, "Dead" refers to all birds that were found dead, died at the station or had to be euthanized.

These numbers also reflect the distribution of birds of prey (excluding owls) in Switzerland. Common buzzards (*Buteo buteo*) are the most common, with up to 20.000 breeding pairs, followed by common kestrels (*Falco tinnunculus*) with up to 7.500 breeding pairs (Vogelwarte 2025). As a result, it was decided to sample buzzards as well as kestrels. 103 carcasses of both species were provided by the three bird sanctuaries within the sampling period.

2.1.1 Information on sampled species

Common buzzards as well as common kestrels are both generalist predators which are capable of taking a wide range of different prey species. In areas with markedly seasonal changes, they adapt their feeding preferences according to the abundance of possible prey. Buzzards primarily feed on small mammals, but they also prey on birds, reptiles, amphibians, large insects, and earthworms. They normally hunt actively, but occasionally scavenge as well. Kestrels feed primarily on small mammals, occasionally on birds, and rarely on insects and lizards. Unlike buzzards, which hunt by perching, soaring, and walking, kestrels hunt almost exclusively by hovering and attacking. However, kestrels have also been reported to scavenge when food is scarce. Both species can often be found in cultivated land. Buzzards are also common in sparse forests, while kestrels are found in hilly landscapes. Interestingly, kestrels also occur in residential areas, as long as there are sufficient open spaces. Both species are permanent residents in Switzerland

and only migrate south in extremely cold winters in exceptional cases (Gillmor, Hollom et al. 1980, Vogelwarte 2025).

2.1.2 Sampling and sample handling[1]

Only birds that were found dead, died during rehabilitation or were euthanized due to poor prognosis and poor prospects for reintroduction were used for this project. No birds were euthanized for the purpose of this study. Post-mortem examinations were conducted as soon as possible after death, to ensure good carcass condition. Therefore, birds were sent directly after confirmation of death to the Vetsuisse partners in Bern or Zurich via overnight delivery. Liver samples and, if possible, blood samples were stored at -20°C until sent to the Ecotox Centre for AR-residue analysis.

2.1.3 Information on sampled birds

The majority of samples (78) were provided by the Bird of prey station Berg am Irchel, followed by the Swiss Ornithological Institute (16) and the Wildlife station Landshut (9). Further details on the locations where the birds were found can be found in Appendix 1. Overall, 21 common kestrels and 82 common buzzards were sampled between April 2024 and February 2025. Basic data such as age, sex, site of location and body condition were already documented at the sanctuary stations (Table 2, Figure 2).

Table 2 Basic information on sampled birds.

Species		**Common buzzard (*Buteo buteo*)**		**Common kestrel (*Falco tinnunculus*)**	
Total number	**103**	**n**	**%**	**n**	**%**
		82	**80**	21	20
Sex	Male	39	48	13	62
	Female	43	52	8	38
Condition	Good	21	26	4	19
	Moderate	16	19	3	14
	Poor	45	**55**	14	**67**
Sampling period	Spring	3	4	4	19
	Summer	7	8	11	**52**
	Autumn	4	5	4	19
	Winter	68	**83**	2	10

[1] In addition to the examined bird of prey samples a set of 30 pigeon (*Columba livia forma domestica* (29), *Columba palumbus* (1)) liver samples provided by Vetsuisse Zurich as well as 13 wild boar (*Sus scrofa*) liver samples provided by a hunting colleague from the Ecotox Centre were included in the chemical analysis. Pigeons were sampled in different locations situated in cantons TI, BS, GE, and VD. The wild boars were hunted in Eptingen (BL) as well as Winterthur (ZH). Details are given in Appendix 5.

Sex was verified during necropsy, revealing a balanced distribution of 51 females and 52 males. The majority of samples (68%) was collected between December 2024 and February 2025 due to an increased buzzard mortality likely caused by climatic conditions (frequent cold winds) and limited food supply (personal information by Andreas Lischke, Bird of prey sanctuary Berg am Irchel). Consequently, 59% of all birds showed a poor and additional 19% a moderate body condition. Less than a quarter of the birds was in good condition. 70% of the birds examined in the current study died in the stations, 3% were found dead, and 27% had to be euthanized.

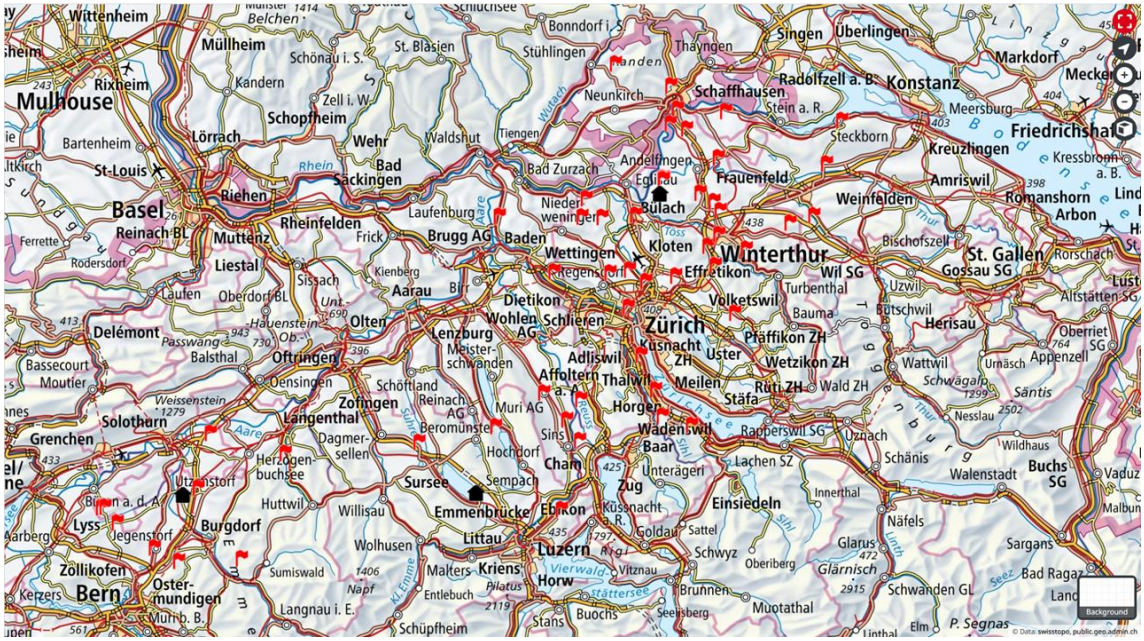

Figure 2 Sites of location of sampled birds. Each red flag represents a single sample location with several symbols overlapping. Black house symbols represent the locations of the three bird sanctuaries.

2.2 Pathological examination

The autopsy of the submitted animal carcasses was performed by board-certified pathologist at the two Vetsuisse facilities in Bern and Zurich. Post-mortem necropsy protocols followed the institutional standardized procedures of the Vetsuisse faculty. To prevent the decay process from obscuring the cause of death, examinations had to be carried out as quickly as possible. This was essential because the established diagnoses directly influence the identification of a possible threshold value. Focus was given on a detailed description of the external examination and the musculoskeletal system. For the project an adapted protocol, “Anticoagulant Rodenticide – Check-List Raptor Pathology” was created (Appendix 1). Carcasses were directly examined on the day of arrival and results were documented in a standardized excel sheet (Appendix 2) for following data analysis. Data included included: (1) case number, (2) bird number, (3) date of examination, (4) examinator, (5) species, (6) weight in g, (7) cause of death, (8) gender, (9) presence or absence of hemorrhages (externally and musculoskeletal system), (10) head, (11) internal examination, (12) sign for “common trauma”, (13) further findings, (14) samples taken, (15) body condition, (16) autolysis, (17) diagnosis and (18) out of scope examinations. Following necropsy, samples of brain, trachea, lung, heart, liver, kidney, spleen, oesophagus, proventriculus, ventriculus, intestine, skin, and skeletal musculature of every bird were fixed in 10% buffered formalin. For serum samples, cardiac blood was centrifuged for 4 min at 4000g, and serum was removed by pipetting. Fresh liver samples of approximately 3-4 g and serum or blood samples

were stored frozen until submitted to the Ecotox Centre (see Figure 3). Samples were always transported on ice.

Based on the anamnestic and macroscopic findings during the postmortem examination, the probable cause of death for each bird based on the presence and distribution of macroscopically observed hemorrhages was assigned from one of the three categories: Common trauma", "AR toxicosis" and "Other". The identification of probable AR toxicities was crucial in order to derive possible threshold values and/or implement the data set in a probabilistic model (see for example (Thomas, Mineau et al. 2011, Elliott, Silverthorn et al. 2024)).

Figure 3 Example of samples from birds of prey for chemical analysis at the Ecotoc Centre including liver and blood.

2.2.1 Histopathological examination

For additional histopathological examinations a set of samples of every bird was preserved in 10% buffered formalin (see 2.2). Histopathological examinations on ten selected samples were performed by a pathologist at Vetsuisse Bern. The individual birds selected were chosen to represent the full range of measured AR concentrations (from not detectable to high). Additionally, cases with the diagnosis "Other" were primarily selected. Specifically, the samples were screened under a microscope for extravascular erythrocytes indicative of hemorrhages that could be linked to an AR toxicosis.

2.3 Chemical analysis

In the previous project (Riegraf, Olbrich et al. 2022), an ESI-LC-MS/MS method was developed to quantify seven SGARs in liver samples of different species. A detailed description of the sample preparation, clean-up and analysis is provided in Appendix 3. Briefly, 3-4 g frozen liver is homogenized with an equal amount of ultrapure water. App. 5 g of the homogenate is then weighed into a PP-tube to which internal standard and a ceramic homogenizer are added before the mixture is shortly vortexed. 10 ml acetonitrile are added, and the mixture is vortexed for 60 seconds. Subsequently, a centrifugation step is performed before freezing the supernatant at-22°C for 5 to 30 h to support phase separation. After another centrifugation step 0.5 ml of the supernatant is mixed

with a zirconia-based sorbent (Zsep+; Supelco) to remove additional fat and matrix by vortexing. The sorbent as well as particles are removed by another centrifugation step. 0.4 ml of the supernatant is mixed with 0.2 ml ultrapure water in a LC/MS sample vial and transferred to the autosampler. The analysis is performed with an ESI-LC-MS/MS. Chromatographic parameters such as gradient, flow and column were adapted from Regnery et al. (2019). No chromatographic separation of individual AR stereoisomers was intended to obtain a sum peak. Limits of quantification (LOQs) depended on the amount of sample available for the analysis. With lower amounts of sample, the LOQ was higher. If a sufficient amount of sample (> 2.5 g) was available LOQs were mostly in the range of 0.05 to 0.1 ng/g (see Appendix 4). Figure 4 gives an example for the chromatographic separation of the analyzed ARs as well as alpha-chloralose and pentobarbital.

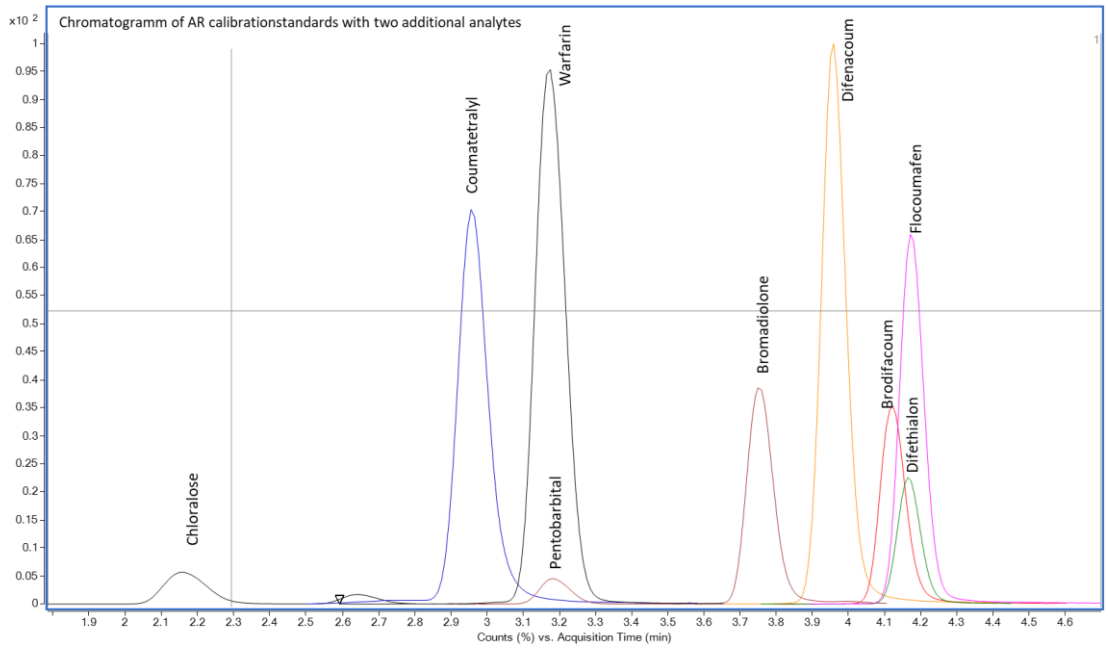

Figure 4 Example for the chromatographic separation of the analyzed ARs as well as alpha-chloralose and pentobarbital.

In addition to the seven analyzed ARs the method was expanded to the non-anticoagulant rodenticide alpha-chloralose as well as the anesthetic pentobarbital.

Furthermore, the method was adapted for blood (serum) samples. Blood samples are added up to 0.5 ml with ultrapure water and transferred into 2 ml centrifuge vials containing 800 µl acetonitrile and a small ceramic homogenizer. Internal standard is added, and the mixture is vortexed for 60 seconds. After centrifugation 1 ml of the supernatant is transferred into a fresh vial and stored for 5 to 30 h at -22°C to support phase separation. After another centrifugation step, 0.5 ml of the supernatant is vortexed with a zirconia-based sorbent (Zsep+; Supelco) to remove additional fat and matrix. The sorbent as well as particles are removed by another centrifugation step. 0.4 ml of the supernatant is mixed with 0.2 ml ultrapure water in a LC/MS sample vial and transferred to the autosampler for measurement.

2.3.1 Derivation of the limits of quantification

Limits of quantification were derived for each analyzed substance and sample. Therefore, up to five LOQs were derived for each sample. To simplify the interpretation, a sum LOQ (∑LOQ) was calculated that included only the LOQs of substances that showed detectable residues in the sample in question. More information is given in Appendix 3.

2.4 Statistical analyses

Statistical analysis was performed using the GraphPad Prism® statistical program (version 9.4.1). The data were first tested for normal distribution (Shapiro-Wilk test). Since all data sets were not normally distributed, the data for comparison were analyzed using either the Mann-Whitney U test (2 groups) or the Kruskal-Wallis test (> 2 groups). Differences were considered significant at $p < 0.05$. To test for a correlation between blood and liver concentrations the Spearman test was used.

3 Results and Discussion

3.1 Samples

Basic data such as age, sex, site of location, condition, and possible cause of death were already documented in section 2.1.3.

3.2 Pathological examination

Based on the macroscopic examination 66% of the examined birds did not show lesions indicative for “AR-Toxicosis” or “common trauma” and were therefore categorized as “other”. Only 4% of the birds were diagnosed with probable “AR toxicosis” and the remaining 30% with “common trauma”.

Between the two examined species there was a shift to a higher ratio of “Other” diagnosis for buzzards (see Figure 5) which could be explained with the high number of buzzards in poor body condition treated in the stations in winter 2024/2025 due to the bad environmental conditions mentioned earlier (2.1.3). On the other hand, overall, more kestrels were found in poor body condition than buzzards (Figure 5).

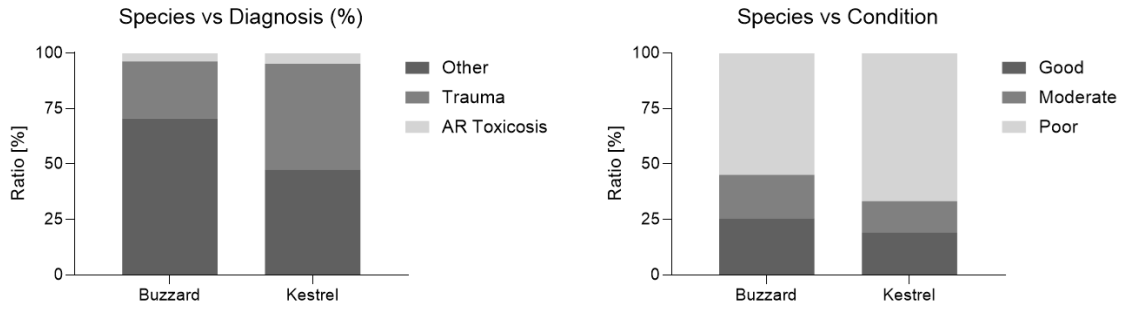

Figure 5 Ratio (%) of diagnoses (other, trauma, AR toxicosis) (left) and body condition (right) for examined buzzards and kestrels.

Regarding the diagnosis “AR toxicosis”, a 4% incidence of AR toxicity is low but still in line with previously published studies. For example, Murray (2011) documented a 5.6% incidence of AR toxicosis in a study with red tailed hawks and different owl species. Stone et al. (2003) found 7.2% of 12 species of birds of prey were positive for AR-Toxicosis. Albert et al. (2010) investigated different species of owls and found also a ratio of around 3.7% for AR toxicosis. In a more recent study from Murray (2017) the author documented a higher toxicosis ratio of 17% in four different species of birds of prey. In a broad study with data from different studies over more than a decade (1983 to 1996) in Great Britain Newton et al. (1999) detected 1.3% cases of AR toxicosis in 717 examined barn owls. On the contrary Elliott et al. (2024) summarized data from several north American studies ranging from 1989 till 2021 and including 24 species of bird of prey and found a percentage of 27.6% of birds with “SGAR poisoning as the cause or major contributing factor in the mortality”. The latter data set, however, differs significantly from the others mentioned because only samples with measurable residues of SGAR and at least one sign of rodenticide poisoning were included. If only the numbers with SGAR residues are considered in the actual study 3% were diagnosed with toxicosis. For the other studies mentioned, percentages relating to samples in which residues were detected are the following: 6.5%, 14.6%, 5.3%, 17.8%, 4.8% (in order of appearance). On the other hand, it hast to be kept in mind that some of the birds with the diagnosis “Other” also could have died of AR toxicosis or that mortality could have been triggered by effects of SGARs (Rattner, Lazarus et al. 2014). This is further discussed in section 3.3.2.

For diagnoses other than “AR toxicosis” Murray (2011, 2017) and Albert et al. (2010) diagnosed approximately 70% - 80% of the birds with “Common trauma” and 22 - 27% with “Other”. Newton et al. (1999) diagnosed 56% with “Common trauma” and 43% with “Other”. In comparison, the ratio of 30% trauma diagnosed in the current study seems therefore lower as usual. This could again be explained by the unusual high mortality rates of buzzards in the winter 2024/2025. Due to their enhanced flying speed, relatively more falcons than buzzards are treated for trauma at the stations in general (personal communication by Andreas Lischke, bird of prey sanctuary Berg am Irchel). This could also partly explain the difference in distribution of diagnoses between the two species with more traumata seen in kestrels than in buzzards.

The complete data set of the pathological examinations is provided as an online source on the projects homepage (link).[2]

3.2.1 Histopathological examination

Tissues of ten birds (6 kestrels and 4 buzzards) were examined by histopathology (Table 3). Overall, no severe internal bleeding as would be expected as a result of an AR toxicosis was found in any of the birds. Four birds with summed AR residues over 100 ng/g did not show more or more severe bleedings than the three birds with residues up to 100 ng/g or without any detectable residues.

Table 3 Histopathological examined samples with according sum of SGAR residues (∑SGAR), suspected diagnosis and location and number of hemorrhages detected.

Sample	Species	∑SGAR (ng/g)	Diagnosis	Location of hemorrhages (n)
ZAR_003	Common kestrel	359	Trauma	Lung (1)
BAR_004	Common kestrel	286	Trauma	Brain (1)
BAR_001	Common kestrel	231	Other	Brain, lung, heart (3)
ZAR_002	Common kestrel	106	Other	Brain, lung (2)
ZAR_015	Common buzzard	97	Other	Lung (1)
ZAR_004	Common buzzard	17	Other	Brain, lung (2)
ZAR_013	Common kestrel	11	Other	Lung (1)
ZAR_008	Common buzzard	0.5	Other	Lung, liver, spleen (3)
ZAR_012	Common buzzard	Not detected	AR Toxicosis	Liver, spleen (2)
ZAR_016	Common kestrel	Not detected	Other	Brain (1)

The fact that bleeding was most often found in the lungs (seven out of 10 samples) was unexpected but could be the cause of a brief increase in blood pressure through the process of dying. No hemorrhages in muscles were examined on the pectoralis major muscle, but in case of AR toxicosis, bleeding occurs more frequently in the joint areas. Thus, the sampling location has great effects on the detection of hemorrhages (personal information from Saskia Keller, Vetsuisse Bern). In comparison, Murray (2011) reported on six birds diagnosed with AR toxicosis, all of which had lesions indicative of severe hemorrhaging including pulmonary (n = 5), mesenteric (n = 3) and kidney (n = 1). It is noticeable that this study also identifies the lungs as the site with the most bleeding.

[2] https://www.oekotoxzentrum.ch/projekte/bodenoekotoxikologie/schwellenwerte-fuer-antikoagulanzien-in-der-leber-von-greifvoegeln

3.2.2 Association between pathological and histopathological examinations

No obvious correlations could be identified between the pathological and histopathological examinations of the ten birds examined. The brain held the second most frequently documented bleeding in tissue after the lungs. Two birds with no grossly remarkable hemorrhages and no signs of common trauma showed hemorrhages in brain tissue in the histopathological examination. As one of the birds also showed a torticollis (twisted head posture) at the sanctuary this could be an indication for a possible hidden head trauma. Three additional birds with histopathological hemorrhages in their brain tissue exhibited different types of head injuries. Two other birds with similar pathological observations – no hemorrhages, no sign of trauma - showed only hemorrhages in lung tissue of which one also showed a torticollis at the sanctuary. It should be noted, that all histopathological observed hemorrhages were generally mild, and not widespread as described by other studies (Murray 2018).

In a study with four birds of prey species presented to a wildlife clinic Murray (2011) reported on six AR toxicosis diagnoses where clinical and gross observations were clearly supported by histopathology.

3.3 Chemical analysis

3.3.1 Concentration and prevalence of ARs in liver samples

103 bird of prey liver samples were analyzed for residues of seven anticoagulant rodenticides as well as pentobarbital and alpha-chloralose (Appendix 4). AR residues with levels above the sample-specific LOQ were detected in 95 (92%) of the samples. The residues ranged from 0.1 to 582 ng/g ∑SGAR with a median of 7 ng/g and spanned three orders of magnitude (Figure 6).

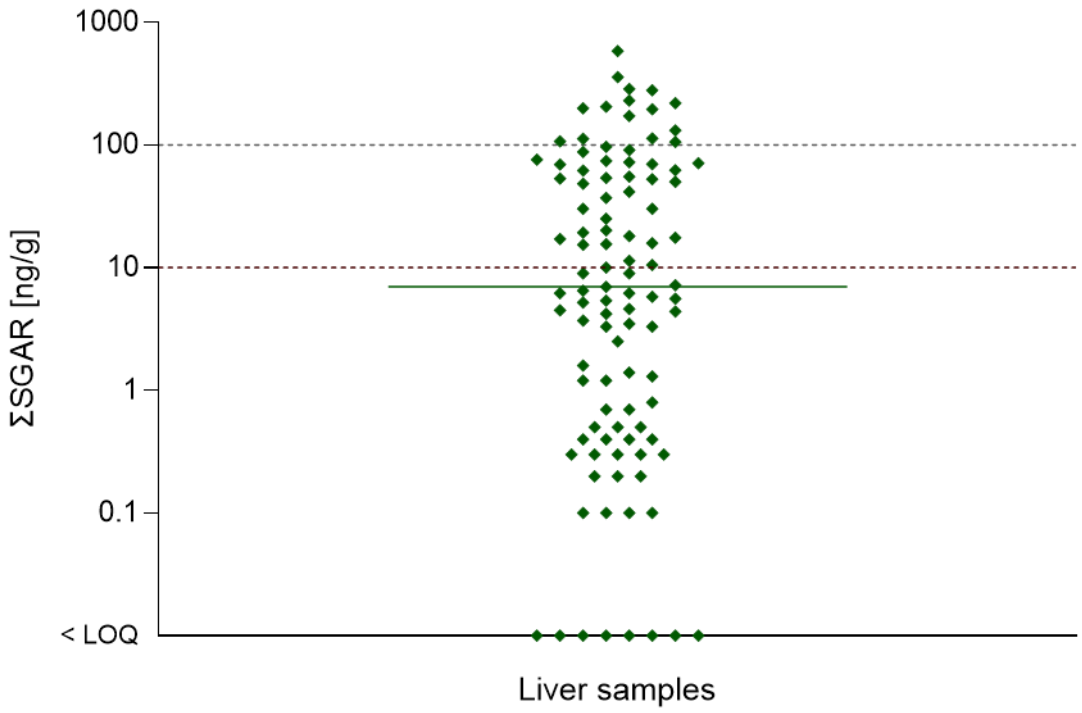

Figure 6 Summed concentration (∑SGAR) for five SGARS in examined bird liver samples. Every diamond refers to a single liver sample. Diamonds located directly on the x-axis show both not detectable values as well as values below the sample-specific limit of quantification (<LOQ). Dashed lines show the lower (red) and higher (grey) potential threshold levels (see also 1.1.5). The solid green line shows the median of all samples.

The concentrations of AR residues were similar to those observed in our previous study by Riegraf et al. (2022) and in wildlife in surrounding countries (AT, DE, and FR) (Geduhn, Jacob et al. 2015, Regnery, Schulz et al. 2020, Badry, Schenke et al. 2021). In general prevalence and concentration of ARs in birds of prey is reported to be high in both species of the genera Buteo and Falco in Europe and North America (López-Perea and Mateo 2018). In 48 of the samples (or 51%) concentrations were above the proposed threshold by Elliott et al. (2024) of 10 ng/g. 16% of the concentrations exceeded a higher threshold value of 100 ng/g (Newton, Shore et al. 1999). Overall, 92% of the examined birds were tested positive for AR residues.

Brodifacoum was detected in 96% of positive samples, either as the sole residue or in combination with one or more other ARs. In descending order of frequency, difenacoum (67%), difethialone (47%), bromadiolone (39%), and flocoumafen (23%) were detected (Figure 7).

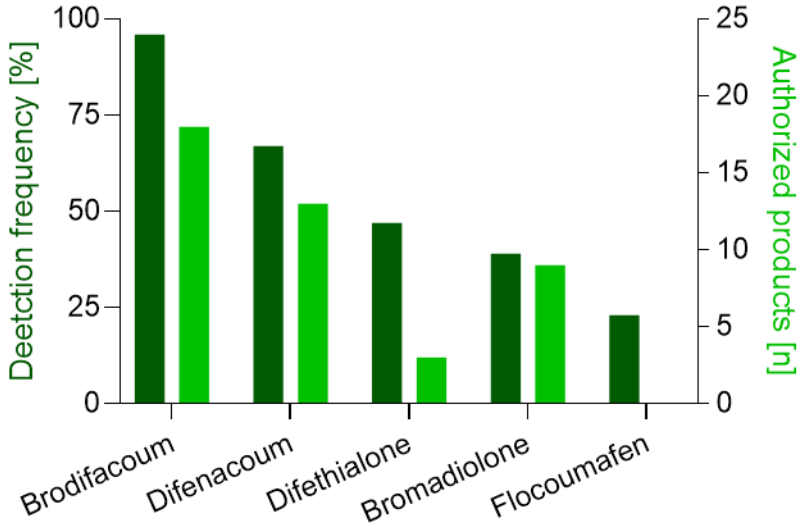

Figure 7 Proportion of individual substances in liver samples of birds of prey that tested positive compared with the number of substances approved in Switzerland.

This distribution initially reflects the products available in Switzerland, as most of them contain brodifacoum (33%), difenacoum (24%) or bromadiolone (16%) as active ingredients. Difethialone is only included in 6% of products, but this active ingredient is considered to be very persistent. This also applies to flocoumafen, which at the time of this study was no longer contained in any of the approved products (EU 2024).

Of the bird livers examined in this study, 71% contained two or more ARs, and 9% contained up to five (Figure 8). These findings correspond with findings from previous studies on AR residues in different raptor species. Elliott et al. (2022) examined over 700 birds of prey from western Canada and detected two or more ARs in 50% of all livers. Murray (2017) found two or more ARs in 66% of four species of birds of prey in the northeastern United States. Christensen et al. (2012) tested liver samples of six different bird of prey species in Denmark and detected more than one AR in 73% and five different substances in 3% of the samples.

No residues of alpha-chloralose were found in any of the samples tested. Pentobarbital was only found in birds which had been euthanized except one buzzard that had been found dead near Schleitheim (SH) with its oesophagus and gizzard full of meat pieces. In that case it seems to be possible that the bird was (intentional) poisoned.

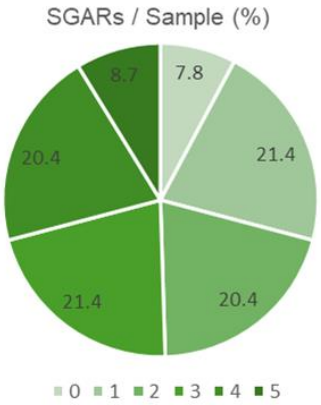

Figure 8 Percentage of the number of different SGARs found in liver samples of birds of prey.

3.3.2 Interpretation of data and possible correlations

It needs to be kept in mind when evaluating the data that only birds of prey that had to be taken to a bird santuary due to their poor condition were examined. Consequently, this is not a comprehensive overview of the total population, but only a specific sample. Therefore, conclusions for the entire population are difficult to draw.

There were no significant differences in the SGARs sums across the three diagnoses (Figure 9).

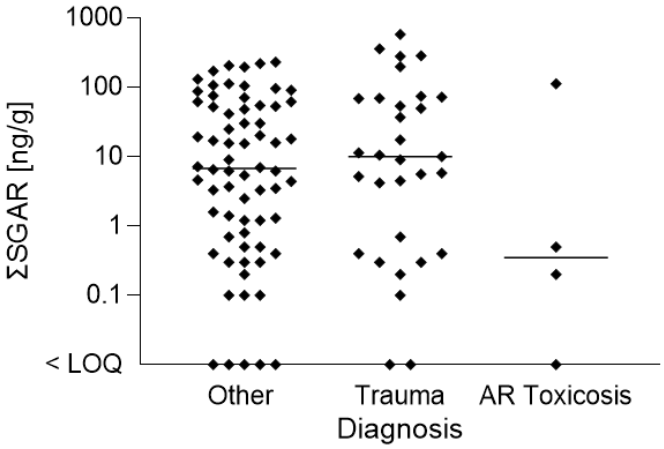

Figure 9 Sum of 5 SGARs (∑SGAR) in bird of prey liver samples in relation to the three different diagnoses. Diamonds located directly on the x-axis show both not detectable values as well as values below the sample-specific limit of quantification (<LOQ). The straight line shows the median of the concentrations.

When interpreting the results, it is important to keep in mind that the number of the three diagnoses was markedly unequal, which makes statistical analysis less reliable. Nevertheless, other studies also found no relationship between the concentrations of ARs and the cause of death (Albert, Wilson et al. 2010, Murray 2011) supporting the examinations in the current study. Of the 68 birds with no clear cause of death (classified as "Other"), 93% had AR residue levels above the sample-specific LOQ. Of these positive samples, 49% contained residues above 10 ng/g, and

14% contained residues that exceeded 100 ng/g of ∑SGAR. Therefore, it is possible that some of these cases may be connected with AR intoxication, for example, through sublethal effects (Rattner, Lazarus et al. 2014).

There was also no significant difference between the total AR concentration in the livers of the two species (Figure 10).

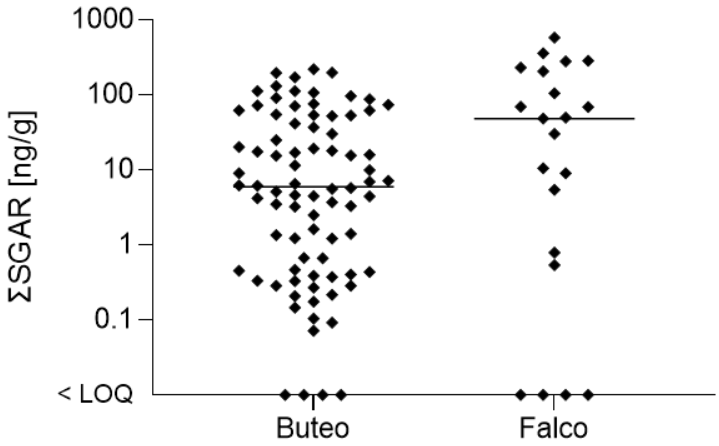

Figure 10 Sum of 5 SGARs (∑SGAR) in liver samples in relation to the two bird species. Diamonds located directly on the x-axis show both not detectable values as well as values below the sample-specific limit of quantification (<LOQ). The straight line shows the median of the concentrations.

However, the median concentration measured in kestrels was 48 ng/g, while it was only 6 ng/g in buzzards. This could be interpreted as a tendency for kestrels to have higher body burdens. This aligns with a study by Hughes et al. (2013) in which kestrels had the highest average AR content among seven bird of prey species, except for red kites. The increased concentrations could possibly be related to the fact that kestrels live more often in and near settlements than buzzards and the differences in the diet of the two species (see also 2.1.1).

There were also no significant differences between the concentrations for the two sexes. An analysis based on the age of the animals is not meaningful, as there are too few reliable data points available. In conclusion, no correlations were found between the different parameters of the sampled birds and the measured SGAR residues.

Furthermore, there was no relationship between AR residues and body condition (Figure 11). For all three documented conditions birds with low, medium and high liver concentrations were present. This was also observed in a study from Albert et al. (2010) where even owls with the highest residue levels as well as confirmed AR poisoning cases were sometimes in excellent body condition.

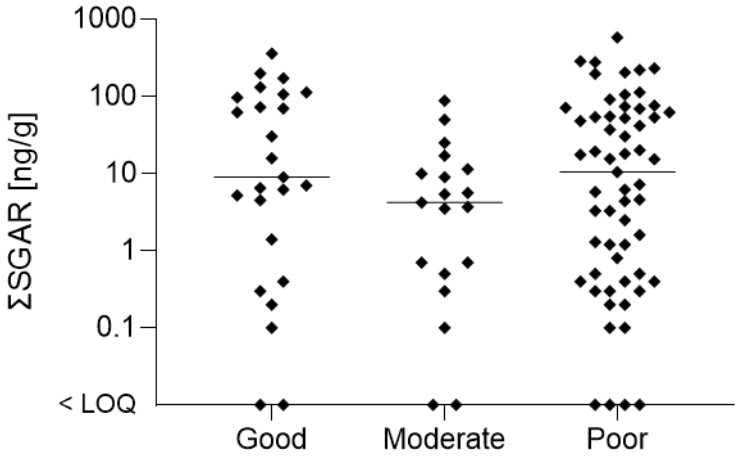

Figure 11 Sum of 5 SGARs (∑SGAR) in liver samples in relation to the birds' body condition. Diamonds located directly on the x-axis show both not detectable values as well as values below the sample-specific limit of quantification (<LOQ). The straight line shows the median of the concentrations.

Even if a probable AR toxicosis was only diagnosed in 3-4% of the cases, a negative impact of ARs on Swiss birds of prey cannot be excluded. The detected SGAR concentrations are again high (> 100 ng/g) in 16% of the examined birds. According to the latest study by Elliott et al. (2024), even at a contamination level of 10 ng/g SGAR, there is still a possibility of AR toxicosis or a serious negative impact on the fitness of at least 20% of birds of prey. This is particularly worrying in the case of endangered species (Knaus, Antoniazza et al. 2021) such as the little owl (*Athene noctua*) and the eagle owl (*Bubo bubo*) which also prey on target and non-target organisms for ARs. In addition, sublethal effects of AR exposure must also be taken into account, as these can lead to a reduction in general fitness and thus survival, as well as having negative effects on reproduction (Rattner, Lazarus et al. 2014, Spadetto, Gómez-Ramírez et al. 2024). In this context, the simultaneous exposure of wild animals to different pollutants should also be considered. Birds of prey are also affected by other PBT substances such as heavy metals (Mahat, Almasi et al. 2025) or brominated organic chemicals (Naert, Van Peteghem et al. 2007). Additionally, pharmaceuticals such as antibiotics and NSAIDs as well as plant protection products were detected parallel to ARs (Badry, Schenke et al. 2021).

As a consequence, it should be considered to replace at least some of the most problematic SGARs with less problematic substances. In this context, the European Commission conducted a recent assessment of the available rodenticides (see also section 1.1.3). Carbon dioxide, cholecalciferol, alpha-chloralose and hydrogen cyanide were identified as eligible chemical alternatives for some AR uses. Consequently, carbon dioxide was identified as having a significantly lower overall risk to human health, animal health, and the environment when used for permanent baiting by trained professionals, making it a suitable alternative. For indoor control of house mice, mechanical traps were considered a suitable alternative. In all other cases, the use of ARs was considered to be without alternative. Recently, the stereoisomeric structure of SGARs has been discussed as a possible solution for developing less toxic products (Fourel, Roque et al. 2024, Rattner, Erickson et al. 2024). SGARs consist of *cis*- and *trans*-diastereoisomers that have been shown to exhibit similar potency in inhibiting VKOR *in vitro*. *In vivo* studies with target rodents have shown that certain diastereoisomers are metabolized preferentially, resulting in a different residue pattern than that of the bait formulations used. These less persistent isomers could be used in bait formulations to reduce the risk of secondary poisoning of non-target organisms. Further proposals include combinations of different active substances (e.g., difenacoum with chlorophacinone), as these would have the same effects at lower doses as higher doses of individual active substances (Blažić, Stojnić et al. 2024).

In summary, it can be said that the impact of ARs on two widespread species of birds of prey in Switzerland, as confirmed in this study, should continue to be monitored critically. A medium-term switch to less problematic rodenticides should be considered, especially in view of the biodiversity that is already under severe pressure (Lindemann-Matthies and Bose 2008, Knaus, Antoniazza et al. 2021). Further low-threshold measures to prevent rodent infestations, such as adapted waste and wastewater management, should be implemented in the short term. The city of Zurich is a positive example for this approach (Schmidt and Müller 2024). Another positive example is Zurich Airport, which has an environmentally friendly and rodenticide-free concept for keeping rodents and thus birds of prey away while promoting the regional biodiversity. This includes, for example, the targeted promotion of all animals that eat mice and other small mammals, such as foxes, stoats, or weasels (Brandstetter 2023).

3.3.3 Blood analyses

In addition to the liver analysis 44 blood samples were analyzed for AR residues as well as alpha-chloralose and pentobarbital. In 28 (64%) of the samples up to three different SGARs were detected with total concentrations ranging between 0.1 and 9.7 ng/ml (Figure 12, Appendix 5).

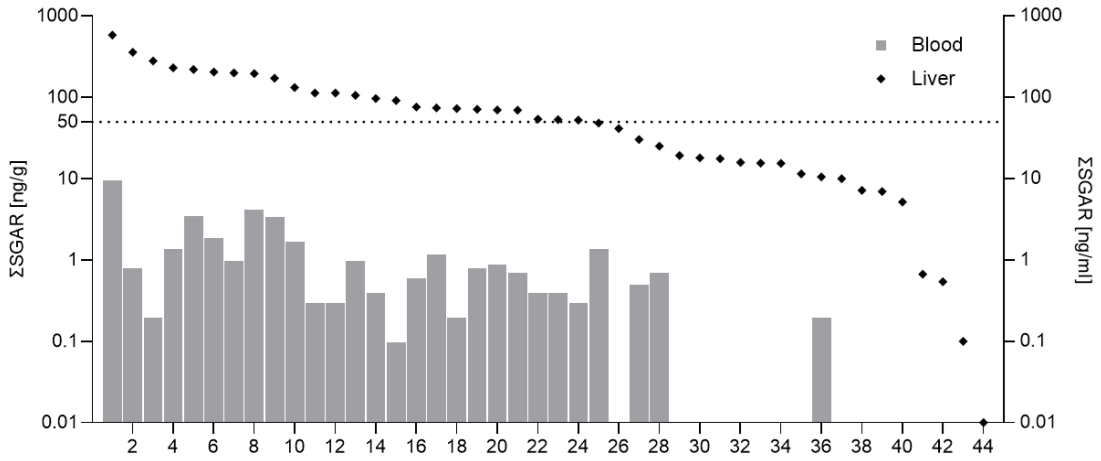

Figure 12 Total concentrations (∑SGAR) of the five SGARS in liver (diamonds; left x-axis) as well as blood (bars; right x-axis) samples of birds of prey. The dashed line indicates the threshold value above which SGARs can be reliably detected in a blood sample.

Additionally, there was a positive correlation (r = 0.8) between the sum of SGARs in liver and blood samples. Furthermore, when a medium-to-high sum concentration of SGARs (50 ng/g) was detected in a liver sample, SGARs were always detected in the blood sample as well.

Rates of appearance in descending order of frequency were, Brodifacoum (57%), bromadiolone (25%), difethialone (25%), difenacoum (21%), and flocoumafen (4%). Concentrations measured in blood samples were consistently lower than those measured in liver samples. This is in accordance with previously submitted data (Murray 2020) and probably due to the lipophilic nature of SGARS and the resulting tendency to accumulate in the liver. In contrast to accumulated AR concentrations in the liver, the residues measured in blood samples reflect a recent intoxication (Horak, Fisher et al. 2018). Yet, single SGARs detected in blood samples did mirror the SGARs detected in the related liver samples. All SGARs detected in a blood sample were also detected in the related liver sample. On the other hand, not all SGRAs detected in a liver sample were also detected in the related blood sample (Table 4).

Table 4 Number of individual substances detected in both the liver and blood samples of birds of prey, as well as the blood/liver ratio for these substances.

SGAR	Detects liver (n)	Detects blood (n)	Blood / Liver (%)
Number of samples			**44**
Brodifacoum	42	18	**43**
Difenacoum	34	9	**26**
Bromadiolone	25	7	**28**
Difethialone	20	7	**35**
Flocoumafen	13	4	**31**
Coumatetralyl	2	1	**50**

Therefore, blood analysis is a promising tool for a less-invasive and active monitoring of medium-to-high concentrations of SGARs in living animals which is already increasingly used for example in monitoring studies with nestlings of birds of prey (Spadetto, Gómez-Ramírez et al. 2024). However, the required amount of serum for a sufficient detection is 0.5 ml, (e.g. 1 ml whole blood) whereas only 1% of the total body weight (e.g. a minimum weight of 100 g) can be sampled without risking adverse health consequences (Oliva-Vidal, Martínez et al. 2022).

3.3.4 Threshold derivation

Initially the current project aimed to derivate a threshold for SGARS in birds of prey in Switzerland. This approach based on a study by Elliott et al. (2024) which used a modeling approach for this purpose. The first author was contacted and additionally an internal specialist on modeling (Andreas Scheidegger) was involved to discuss the approach as well as the data needed for a Swiss modeling approach or the possibility to implement the Swiss data into the existing model. For a successful modelling approach the combination of data from chemical analysis as well as pathological examinations is crucial. The data is then divided in two categories: data points are classified as 0 if there is no evidence of AR toxicosis and as 1 if there is evidence. Evidence is given if a sum concentration of SGARs above the LOQ is detected and pathological examinations show strong signs (e.g. pallor of mucous membranes, hemorrhages without trauma) of AR involvement in the circumstances of death.

From the 103 birds examined in the current study four (4%) showed strong macroscopic signs for a probable AR toxicosis. After chemical analysis of the livers three remained with measurable concentrations above the sample specific LOQ. One case with a relatively high concentration of 113 ng/g and two with low concentrations < 1 ng/g. Unfortunately, it is not possible to carry out a proper modeling approach with this data set. Figure 13 shows an ideal distribution of data points for this purpose and in contrast the actual data set for comparison.

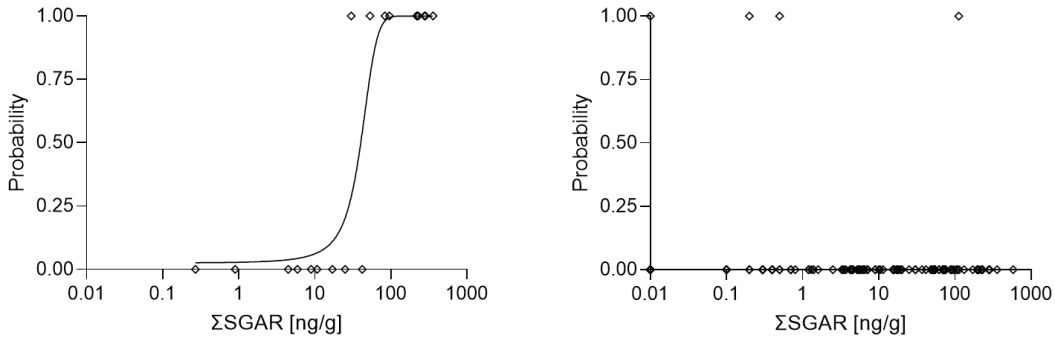

Figure 13 Example of ideal distribution of data points (left) and actual data set (right) for comparison. The probability of an AR toxicosis is modeled by classifying data points (∑SGAR concentrations on the x-axis) with strong evidence (probability) of toxicity as 1 and those without as 0.

As can be seen, the Swiss dataset does not exhibit the same distribution as that observed in the broad-based modelling study.

To gather enough data for a reliable derivation of thresholds a significantly higher amount of data points seems to be necessary. In a first modeling approach carried out by Thomas et al. (2011) 270 birds (data points) where included whereas the subsequent study of Elliott et al. (2024) included 951 birds. Another major difference to our study was, that both studies only included birds with detected SGAR concentrations for their models. Therefore, the initial datasets had to be even higher. In conclusion, if a threshold modeling approach for a Swiss situation analysis is requested, the data gathering had to be increased substantially.

In view of the difficulties in determining a general threshold value for the risk of probable AR toxicity, other evaluation criteria should also be considered. Biomarkers would be the obvious choice here. Since ARs influence blood coagulation, research is already being conducted into suitable biomarkers in this area. Measuring the time required for blood to coagulate outside the body, e.g., using prothrombin time (PT), is promising. However, there is currently no standard homologous (avian) thromboplastin available for the PT test, which is required to trigger coagulation in vitro. Other approaches that are still being researched include measuring VKOR activity and metabolomic and genomic analyses (Rattner, Lazarus et al. 2014, Rached, Moriceau et al. 2020).

4 Conclusions and Outlook

The present study confirmed the widespread and, in some cases, high background contamination of Swiss birds of prey with anticoagulant rodenticides.

- 92% of the buzzards and falcons examined showed AR liver concentrations above the LOQ ranging between 0.1 and 582 ng/g.
- 51% of all samples exceeded a lower threshold of 10 ng/g.
- 16% of all samples exceeded a higher threshold of 100 ng/g.
- Only 4% of the birds were macroscopically diagnosed with “AR toxicosis” as the most likely cause of death or reason for euthanasia. Including only samples with AR concentrations above the LOQ the diagnosis could be verified for 3% of the birds.
- 66% of all birds were diagnosed with “Other” as causes for death which could include hidden AR toxicoses or severe sublethal effects of ARs.
- Overall, the few cases with suspicion of AR toxicosis identified in the current project did not provide sufficient data for a threshold derivation.

Outlook

- Considering the low number of suspected AR toxicoses we have identified, a larger pan-European study would be needed to obtain enough bird samples for robust threshold derivations.
- The detected AR concentrations are again high (>100 ng/g) in 16% of the examined birds. Mixture toxicity may play a role in mortality and broader chemical analyses of liver samples may help substantiate this. For example, we aim to investigate PFAS in some of the bird livers using a CALUX bioassay (though the liver matrix is still a hurdle for this type of analyses).
- For a more comprehensive overview of the contamination not only birds that were admitted to bird sanctuaries could be examined. This would require a sensitive LC-MS/MS method so that only small volumes of blood samples would be needed.
- Further research into biomarkers, such as blood coagulation factors, for determining potentially critical exposure to ARs could supplement or even replace the threshold approach.
- The high background contamination with SGARs could pose a problem, especially with regard to endangered species, as sublethal effects can impair the overall fitness of a species. In this context, we are currently screening 36 liver samples from the near-threatened barn owl (*Tyto alba*), followed by 62 samples from the also near-threatened golden eagle (*Aquila chrysaetos*) as well as 24 samples from the vulnerable Eurasian eagle-owl (*Bubu bubo*). This will help to provide a broader picture of exposure for birds with different feeding habits in different Swiss regions.
- In conclusion, measures to reduce the introduction of ARs to the environment should be intensified and a medium-termed switch to less problematic rodenticides should be considered.

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6 Glossary

AR	Anticoagulant rodenticides
AT	Austria
BE	Berne
BL	Basel-Landschaft
BPC	Biocidal Products Committee
BPR	Biocidal Products Regulation
BS	Basel
DE	Germany
DK	Denmark
ECHA	European Chemicals Agency
EU	European Union
FGAR	First generation anticoagulant rodenticides
FR	France
GE	Geneva
LOQ	Limit of quantification
LU	Lucerne
NZ	New Zealand
PBT	Persistent, bioaccumulative, toxic
PT	product type
SGAR	Second generation anticoagulant rodenticides
SH	Schaffhausen
TG	Thurgau
TI	Ticino
VKOR	vitamin K epoxide reductase enzyme
vPvB	very persistent, very bioaccumulative
USA	United States of America
VD	Vaud
ZH	Zurich

7 Indexes

7.1 List of Figures

7.2 List of Tables

Appendix 1 Information on sampled birds

Table 5 Enlarged basic information on sampled birds.

Sample	Species	Location	Station	Date of examination	Sex	Body weight (g)	Liver weight (g)	Amount blood (ml)
BAR_001	CK	Deitingen	WSL	28.05.24	Male	140	1.67	0.10
BAR_002	CK	Koppigen	WSL	12.06.24	Male	123	2.49	-
BAR_003	CK	Jegenstorf	WSL	09.07.24	Male	151	2.49	-
BAR_004	CK	-	WSL	02.08.24	Male	148	2.50	-
BAR_005	CB	Hettiswil	WSL	12.08.24	Female	750	2.54	-
BAR_006	CB	Rüegsbach	WSL	21.08.24	Male	474	2.52	-
BAR_008	CB	Lotzwil	WSL	15.01.25	Male	580	2.51	-
BAR_009	CB	Schnottwil	WSL	21.01.25	Male	-	2.53	-
BAR_010	CB	Schnottwil	WSL	19.02.25	Female	723	2.52	-
ZAR_001	CK	Zürich	BSB	04.04.24	Male	133	2.50	-
ZAR_002	CK	Rüdlingen	BSB	26.04.24	Male	120	2.33	0.06
ZAR_003	CK	Hettlingen	BSB	30.04.24	Male	168	0.69	0.20
ZAR_004	CB	Hittnau	BSB	02.05.24	Male	516	0.95	-
ZAR_005	CB	Guntmadingen	BSB	07.05.24	Female	750	2.49	-
ZAR_006	CB	Buttisholz	SOI	22.05.24	Male	743	2.54	-
ZAR_007	CK	Schleitheim	BSB	04.06.24	Female	188	0.20	0.06
ZAR_008	CB	Volketswil	BSB	09.07.24	Female	622	0.48	-
ZAR_009	CB	Zürich	BSB	09.07.24	Female	634	2.51	0.35
ZAR_010	CB	Schöfflisdorf	BSB	09.07.24	Female	713	0.44	-
ZAR_011	CK	Wald	BSB	09.07.24	Female	167	2.55	-
ZAR_012	CB	Dübendorf	BSB	12.07.24	Female	761	2.48	-
ZAR_013	CK	Truttikon	BSB	12.07.24	Male	147	1.39	-
ZAR_014	CK	Brütten	BSB	12.07.24	Female	157	2.55	-
ZAR_015	CB	Otelfingen	BSB	16.07.24	Female	396	2.56	0.50
ZAR_016	CK	Eschenz	BSB	16.07.24	Female	226	2.50	-
ZAR_017	CK	Ebersecken	SOI	06.08.24	Female	97	1.79	0.10
ZAR_018	CK	Stans	SOI	07.08.24	Female	99	1.25	-
ZAR_019	CK	Wauwil	SOI	09.08.24	Male	143	0.55	0.05
ZAR_020	CB	Büron	SOI	04.09.24	Female	772	2.50	0.25
ZAR_021	CK	Gisikon	SOI	24.09.24	Male	177	2.55	0.05
ZAR_022	CK	-	BSB	25.09.24	Male	135	1.91	0.10
ZAR_023	CB	Siblingen	BSB	01.10.24	Female	750	2.55	0.13
ZAR_024	CK	Stans	SOI	15.10.24	Male	150	2.50	0.15
ZAR_025	CK	Beringen	BSB	21.10.24	Female	134	2.53	0.12
ZAR_026	CB	Andelfingen	BSB	29.10.24	Female	728	2.52	0.20
ZAR_027	CB	Hemishofen	BSB	01.11.24	Female	669	2.53	0.28
ZAR_028	CB	Hemishofen	BSB	02.12.24	Female	887	2.45	-

ZAR_029	CB	Wallisellen	BSB	13.12.24	Male	397	2.52	0.08
ZAR_030	CB	Dussnang	BSB	19.12.24	Male	559	2.52	-
ZAR_031	CB	Hinwil	BSB	27.12.24	Female	729	2.54	-
ZAR_032	CB	Littenheid	BSB	27.12.24	Female	713	2.54	0.20
ZAR_033	CB	Urdorf	BSB	27.12.24	Female	780	2.53	-
ZAR_034	CK	Neftenbach	BSB	30.12.24	Female	157	1.93	0.04
ZAR_035	CB	Wädenswil	BSB	30.12.24	Female	990	2.52	-
ZAR_036	CB	Steinmaur	BSB	30.12.24	Male	760	2.54	-
ZAR_037	CB	Bachenbü-lach	BSB	03.01.25	Female	695	2.51	-
ZAR_038	CB	Matzingen	BSB	03.01.25	Male	543	2.55	-
ZAR_039	CB	Kleinandelf-ingen	BSB	03.01.25	Male	445	2.54	-
ZAR_040	CB	Wettingen	BSB	03.01.25	Female	654	2.49	-
ZAR_041	CB	Thayngen	BSB	03.01.25	Female	623	2.49	-
ZAR_042	CB	Bussnang	BSB	06.01.25	Male	535	2.57	-
ZAR_043	CB	Zürich	BSB	06.01.25	Female	609	2.53	-
ZAR_044	CB	Wetzikon	BSB	06.01.25	Female	587	2.26	0.14
ZAR_045	CB	Würenlingen	BSB	06.01.25	Female	568	2.53	-
ZAR_046	CB	Winterthur	BSB	06.01.25	Male	591	2.55	-
ZAR_047	CB	Steckborn	BSB	06.01.25	Female	573	2.54	0.14
ZAR_048	CB	Würenlos	BSB	06.01.25	Female	623	2.55	0.20
ZAR_049	CB	-	BSB	06.01.25	Female	590	2.51	-
ZAR_050	CB	Bachs	BSB	07.01.25	Male	514	2.48	-
ZAR_051	CB	Winterthur	BSB	07.01.25	Female	971	2.50	-
ZAR_052	CB	Pfyn	BSB	08.01.25	Male	425	2.51	-
ZAR_053	CB	Flaach	BSB	08.01.25	Male	559	2.54	0.08
ZAR_054	CB	Bülach	BSB	08.01.25	Female	522	2.54	-
ZAR_055	CB	Fehraltorf	BSB	08.01.25	Male	456	2.51	0.25
ZAR_056	CB	Knutwil	SOI	10.01.25	Female	617	2.54	-
ZAR_057	CB	Hettlingen	BSB	10.01.25	Female	554	2.53	0.35
ZAR_058	CB	Rümlang	BSB	10.01.25	Female	545	2.51	-
ZAR_059	CB	Schleitheim	BSB	10.01.25	Male	678	2.49	0.15
ZAR_060	CB	Wädenswil	BSB	15.01.25	Male	456	2.48	-
ZAR_061	CB	Flaach	BSB	15.01.24	Male	472	2.52	-
ZAR_062	CB	Flaach	BSB	16.01.25	Female	529	2.55	-
ZAR_063	CB	Seuzach	BSB	20.01.25	Male	499	2.51	0.12
ZAR_064	CB	Hagenbuch	SOI	20.01.25	Male	620	2.54	0.05
ZAR_065	CB	Oberrieden	BSB	20.01.25	Male	470	2.49	0.39
ZAR_066	CB	Ossingen	BSB	20.01.25	Female	728	2.51	0.10
ZAR_067	CB	Winterthur	BSB)	20.01.25	Male	576	2.53	0.10
ZAR_068	CB	-	SOI	20.01.25	Male	477	2.55	0.22
ZAR_069	CB	Emmensee	SOI	21.01.25	Female	835	2.52	0.28
ZAR_070	CB	Langenberg	SOI	21.01.25	Female	798	2.54	-

ZAR_071	CB	Flurlingen	BSB	23.01.25	Male	483	2.50	0.26
ZAR_072	CB	Dachsen	BSB	23.01.25	Male	537	2.52	-
ZAR_073	CB	Stadel	BSB	23.01.25	Male	537	2.52	-
ZAR_074	CB	Willisdorf	BSB	24.01.25	Female	498	2.50	-
ZAR_075	CB	Weiach	BSB	27.01.25	Female	666	2.51	0.03
ZAR_076	CB	Kilchberg	BSB	27.01.25	Female	518	2.51	-
ZAR_077	CB	Horgen	BSB	27.01.25	Male	459	2.52	-
ZAR_078	CB	Bertschikon	BSB	27.01.25	Male	431	2.49	-
ZAR_079	CB	Zürich	BSB	27.01.25	Male	502	2.47	0.03
ZAR_080	CB	Matzingen	BSB	27.01.25	Male	482	2.50	-
ZAR_081	CB	Mühlau	SOI	28.01.25	Female	657	2.54	-
ZAR_082	CB	Obfelden	BSB	30.01.25	Female	522	2.49	0.02
ZAR_083	CB	-	BSB	05.02.25	Female	979	2.53	0.02
ZAR_084	CB	Glattbrugg	BSB	06.02.25	Male	583	2.53	0.32
ZAR_085	CB	Flaach	BSB	06.02.25	Female	790	2.51	0.20
ZAR_086	CB	Henggart	BSB	07.02.25	Male	720	2.51	-
ZAR_087	CB	Kemptthal	BSB	10.02.25	Female	637	2.51	-
ZAR_088	CB	Bassersdorf	BSB	10.02.25	Male	509	2.50	0.11
ZAR_089	CB	Hünenberg	SOI	11.02.25	Male	509	2.54	-
ZAR_090	CB	Schaffhau-sen	BSB	14.02.25	Male	472	2.54	0.13
ZAR_091	CB	Muri	SOI	18.02.25	Male	569	2.52	-
fZAR_092	CB	Root	SOI	18.02.25	Male	499	2.52	0.09
ZAR_093	CK	Watt	BSB	18.02.25	Male	140	2.19	0.01
ZAR_094	CB	Benken	BSB	21.02.25	Male	737	2.53	-

BAR: Pathology by Vetsuisse Bern; ZAR: Pathology by Vetsuisse Zurich; CB: Common buzzard (*Buteo buteo*); CK: Common kestrel (*Falco tinnunculus*); WSL: Wildlife station Landshut; BSB: Bird of prey station Berg am Irchel; SOI: Swiss Ornithological Institute; – no data (location) / no sample (blood).

Appendix 2 Templates

Anticoagulant Rodenticide – Check-List Raptor Pathology

sa / saK, Feb 2024

Preamble

Necropsy protocols will follow the institutional standardized procedure. Focus is given to a detailed description of the external examination and the musculoskeletal system.

If questions arise, please talk to your institutional supervisor:

- NRGK: Sarah Albini (salbini@vetbakt.uzh.ch)
- FIWI: Saskia Keller, Michelle Imlau (saskia.keller@unibe.ch)

General Information

Internal Case number: AR- **Bird number:**		**Date of examination:**	**Examinator:**
Species	☐ Common buzzard (Mäusebussard)	☐ Common Kestrel (Turmfalke)	☐ weight : ……..
Cause of death	☐ found dead / died	☐ killed	☐ euthanized with:……
Gender	☐ male	☐ female	☐ unknown

Lesions associated to potentional AR-Intoxication (e.g. Intramuscular haemorrhages)

Potential Lesions	**Checked for abnormali-ties**	**Description of abnormalities observed** *(in English)*	**Photos taken**
Haemorrhages (Externally and musculoskeletal)			
along leg/s	☐		☐
along wing/s	☐		☐
along the entirety of the pectoral muscles / sternum / keel	☐		☐
across the abdominal wall	☐		☐
other	☐		☐
Head			
mouth / glottis / choana / trachea	☐		☐
oesophagus			
superficial lacertions	☐		☐
nares	☐		☐
skull / calvarium / orbita	☐	*Please take photo*	☐
Internal Examination			

pallor of internal organs	☐		☐
pulmonary haemorrhage	☐		☐
internal bleeding into body cavity	☐		☐
other	☐		☐

Signs for «common trauma»

Potential Lesions	**Checked for abnormalities**	**Description of abnormalities observed** *(in English)*	**Photos taken**
generalized polytrauma (e.g. hit by car, collisions) without severe bleeding	☐		☐
fractures	☐		☐
severe degloving	☐		☐
puncture wounds	☐		☐
head trauma, ocular trauma	☐		☐
gunshot wounds	☐		☐
complete or partial amputation of a limb	☐		☐
other	☐		☐

Further findings

Potential Lesions	**Checked for abnormalities**	**Description of abnormalities observed** *(in English)*	**Photos taken**
feathers, beak, etc	☐		☐
head, incl. oral cavity	☐		☐
trachea, lung, air sacs	☐		☐
heart	☐		☐
oesophagus, proventriculus, gizzard	☐		☐
intestine, cloaca	☐		☐

liver	☐		☐
spleen	☐		☐
kidney	☐		☐
Other findings:	☐		☐

Samples taken for project

AR-examination	☐ blood (heart). Centrifuge approx.,4min/4000g, sample serum. → *Serum-Röhrchen* ☐ liver (5 g). Stored at -20°C until monthly shipment. → *Kleiner Histobecher mit grünem Deckel*	
Histology (only formalin-fixation, no trimming)	☐ brain ☐ oesophagus/ trachea ☐ lung ☐ heart (Längsschnitt) ☐ spleen	☐ liver ☐ proventriculus/ gizzard ☐ small intestine (jejunum) ☐ large intestine (caeca-bifurcation) ☐ skeletal musculature

DIAGNOSIS	Body condition	Freshness of cadaver / Autolysis
☐ AR toxicosis likely	☐ good	☐ fresh
☐ common trauma	☐ moderate	☐ mild autolysis
☐ other	☐ poor / emaciated	☐ moderate autolysis
	☐ ………………………..	☐ severe autolysis

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX XXXXX

[Other examinations performed (out of the scope of the study)]

parasitology	feathers ☐ tongue ☐ air sacs ☐ intestine ☐ Other:	☐
bacteriology		☐
mycology		☐
Staining (Gram ☐, ZN ☐)		☐
PCR		
Other		

Anticoagulant Rodenticite – Results Raptor Pathology

Fill in the Data = 0 for "absent" or 1 for "present" !!! No text in the coloured fields !!!

Case number	Bird number	Date of exam.	Examinator	Species	weight in g	Cause of death			Gender			Haemorrhages (externally and mucoskeletal system					Head					Internal examination			
pathology number	number of rehabilitation centre / ring if available			Buzz = Common Buzzard / Kest = Common Kestrel		found dead / died	killed	euthanized	male	female	unknown	along leg/s	along wing/s	along entire pectoral muscles / sternum / keel	across abdominal wall	other	mouth / glottis / choana / trachea	oesophagus	superficial lacerations	nares	skull / calvarium / orbita	pallor of internal organs	pulmonary haemorrhage	internal bleeding into body cavity	other
AR0001		xx.yy.2024		Buzz																					

sign for "common trauma"								Further findings										samples taken			Body condition	Autolysis	DIAGNOSIS	*out of scope examinatio*
generalized polytrauma (e.g. hit ba car) withour severe bleeding	fractures	severe degloving	puncture wounds	head trauma, ocular trauma	gunshot wounds	complete or partial amputation of a limb	other	feathers, beak etc.	Head, incl. oral cavity	Trachea, Lung, Air sacs	Heart	Oesophagus Proventriculus, Gizzard	Intestine, Cloaca	Liver	Spleen	Kidney	Other findings	heart blood	liver	histology set	good / moderate / poor, emaciated	fresh / mild /moderate / severe autolysis	AR toxicosis likely / common trauma / other	

Appendix 3 Extraction and chemical analysis

Liver

Detailed descriptions of the extraction method are provided in the final report on the first AR study (Riegraf, Olbrich et al. 2022). In brief, frozen liver samples were added up with the same weight of ultrapure water. Then the liver was homogenized until foamy. Around 5 g of the homogenate was mixed with internal standards and 10 mL of acetonitrile were added, with acetonitrile causing precipitation of proteins in the sample. Subsequently, the tube was vortexed for 1 min. Samples were centrifuged for 4 min at 4000 g. Then, 10 mL of supernatant was stored at -22 °C for 5-30 h to remove most of the fat and also some amount of water. Frozen extracts were then centrifuged in a cooled centrifuge. As a clean-up step 40 mg of Z-Sep+ (silica gel base and zircon-based phase; Supelco) was added to 0.5 mL of the supernatant (representing about 100 mg of liver) and vortexed and finally centrifuged to remove additional fat and matrix. For measurement, 0.4 mL of the supernatant mixed with 0.2 mL of ultrapure water is transferred to a LC/MS sample vial and briefly vortexed.

Blood

Blood (serum) samples were added up to 0.5 mL with ultrapure water and transferred into 2 mL centrifuge vials containing 800 µL acetonitrile and a small ceramic homogenizer. Internal standard (40 µL of 20 ng/mL) is added, and the mixture is vortexed for 60 seconds. After centrifugation 1 mL of the supernatant is transferred into a fresh vial and stored for 5 to 30 h at -22°C to support phase separation. After another centrifugation step, 0.5 mL of the supernatant is vortexed with Zsep+ to remove additional fat and matrix. The sorbent as well as particles are removed by another centrifugation step. 0.4 mL of the supernatant is mixed with 0.2 mL ultrapure water in a LC/MS sample vial and transferred to the autosampler for measurement.

LC-MS/MS

Detailed descriptions of the LC MS/MS method are provided in the final report on the first AR study (Riegraf, Olbrich et al. 2022). Briefly, 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 6). Chromatographic parameters such as eluents, gradient, flow and column were taken from Regnery et al. (2019).

Table 6 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 and covered the range of 15 to 40.000 ng/L. The monitored mass transitions and compound specific tuning parameters of target analytes as well as their isotope-labeled analogs in ESI+ ionization mode are given in Table 7. The source of the analytes and matching internal standards are listed in Table 8.

Table 7 Retention time and mass transitions of analyzed compounds.

Compound [M+H]+	Retention Time (min)	Quantifier MRM precursor (m/z)/ product ion (m/z)/ collision energy [V]	1. Qualifier MRM precursor (m/z)/ product ion (m/z)/ collision energy [V]	2. Qualifier MRM precursor (m/z)/ product ion (m/z)/ collision energy [V]
Chloralose (355 = formiatadduct)	2.40	355/308.7/10	355/161/16	309/161/10
Coumatetralyl	3.11	293.1/175/26	293.1/91/64	
Coumatetralyl-d4	3.11	297.2/179/26	297.2/91/40	
Pentobarbital	3.25	225.1/182/10	225/42.1/20	
Pentobarbital-d5	3.25	230.2/187.2/10	230.2/42.3/20	
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

* = [M-H2O+H]+

Table 8 Sources of the analytes and matching internal standards.

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 Diastereomers)	B678202	TRC-Canada
Chloralose	687779	HPC Standards GmbH
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
Pentobarbital sodium	LPM-PTB-537-NA	Lipomed
Pentobarbital-D5 in MeOH, 0.1 g/L	P-009-1ML	Sigma-Aldrich
Rodenticides Mixture 248 100 µg/mL in ACN	DRE-GS09000248AL	LGC

Limits of quantification

The LOQ was established from replicate injections of a matrix-free standard at a low concentration near the anticipated LOQ, using a signal-to-noise ratio (S/N) ≥ 10 as the acceptance criterion. For each analyte, S/N ratios were assessed for both quantifier and qualifier transitions, selecting the lower value to define the LOQ; values from replicate injections were then averaged. LOQ values for liver samples were subsequently calculated by incorporating the sample-specific dilution factor, with the internal standard response of samples compared to calibration standards to account for matrix effects via this factor.

LOQs were derived for each analyzed substance and sample. Therefore, up to five LOQs could be derived for each sample. A sum LOQ (∑LOQ) that included only the LOQs of substances that showed detectable residues in the sample in question was derived for every sample (see Table 9).

Appendix 4 Summary of results – liver

Table 9 Summary of unrounded results for measured liver residues and LOQs as well as information on probable diagnoses, species and condition of the sampled birds.

Sample	∑SGAR (ng/g)	∑LOQ (ng/g)	Diagnosis	Species	Condition
BAR_001	231.00	1.2	Other	Kestrel	Poor
BAR_002	0.00	0.0	Trauma	Kestrel	Poor
BAR_003	50.09	0.3	Trauma	Kestrel	Moderate
BAR_004	286.08	0.2	Trauma	Kestrel	Poor
BAR_005	0.27	0.05	Trauma	Buzzard	Good
BAR_006	0.22	0.06	Trauma	Buzzard	Poor
BAR_008	20.17	0.32	Other	Buzzard	Poor
BAR_009	3.70	0.12	Other	Buzzard	Moderate
BAR_010	55.12	0.12	Other	Buzzard	Poor
ZAR_001	0.05	0.00	Trauma	Kestrel	Poor
ZAR_002	105.69	0.5	Other	Kestrel	Poor
ZAR_003	358.61	0.8	Trauma	Kestrel	Good
ZAR_004	17.07	0.4	Other	Buzzard	Moderate
ZAR_005	9.05	0.2	Trauma	Buzzard	Good
ZAR_006	4.49	0.1	Trauma	Buzzard	Good
ZAR_007	279.19	7.4	Trauma	Kestrel	Poor
ZAR_008	0.43	0.23	Other	Buzzard	Moderate
ZAR_009	220.13	0.5	Other	Buzzard	Poor
ZAR_010	0.00	0.00	Other	Buzzard	Moderate
ZAR_011	30.29	0.5	Other	Kestrel	Good
ZAR_012	0.00	0.00	AR toxicosis	Buzzard	Good
ZAR_013	9.05	0.2	Other	Kestrel	Moderate
ZAR_014	5.41	0.4	Other	Kestrel	Moderate
ZAR_015	96.93	0.6	Other	Buzzard	Good
ZAR_016	0.00	0.0	Other	Kestrel	Good
ZAR_017	48.35	0.9	Other	Kestrel	Poor
ZAR_018	0.65	0.3	Other	Kestrel	Poor
ZAR_019	69.30	1.3	Trauma	Kestrel	Poor
ZAR_020	0.10	0.07	Other	Buzzard	Moderate
ZAR_021	69.84	0.22	Trauma	Kestrel	Good
ZAR_022	0.54	0.07	AR toxicosis	Kestrel	Poor
ZAR_023	0.67	0.08	Other	Buzzard	Moderate
ZAR_024	10.55	0.10	Trauma	Kestrel	Poor
ZAR_025	0.00	0.00	Other	Kestrel	Poor
ZAR_026	5.16	0.27	Trauma	Buzzard	Good
ZAR_027	15.88	0.11	Other	Buzzard	Good
ZAR_028	1.41	0.30	Other	Buzzard	Good
ZAR_029	196.36	0.37	Other	Buzzard	Poor
ZAR_030	5.78	0.21	Trauma	Buzzard	Poor
ZAR_031	6.18	0.24	Other	Buzzard	Good
ZAR_032	199.33	0.29	Trauma	Buzzard	Good

ZAR_033	106.96	0.30	Other	Buzzard	Good
ZAR_034	581.68	0.90	Trauma	Kestrel	Poor
ZAR_035	6.53	0.15	Other	Buzzard	Good
ZAR_036	0.37	0.17	Trauma	Buzzard	Good
ZAR_037	87.90	0.16	Other	Buzzard	Moderate
ZAR_038	3.25	0.26	Other	Buzzard	Poor
ZAR_039	1.35	0.23	Other	Buzzard	Poor
ZAR_040	0.33	0.14	Other	Buzzard	Poor
ZAR_041	6.24	0.24	Other	Buzzard	Poor
ZAR_042	0.05	0.11	Other	Buzzard	Moderate
ZAR_043	0.10	0.15	Other	Buzzard	Poor
ZAR_044	18.05	0.18	Other	Buzzard	Poor
ZAR_045	0.17	0.14	Other	Buzzard	Poor
ZAR_046	0.43	0.18	Other	Buzzard	Poor
ZAR_047	53.37	0.20	Other	Buzzard	Poor
ZAR_048	30.31	0.23	Other	Buzzard	Poor
ZAR_049	37.01	0.40	Trauma	Buzzard	Poor
ZAR_050	0.39	0.14	Other	Buzzard	Poor
ZAR_051	62.08	0.12	Other	Buzzard	Good
ZAR_052	0.40	0.13	Trauma	Buzzard	Poor
ZAR_053	74.26	0.34	Trauma	Buzzard	Poor
ZAR_054	1.63	0.12	Other	Buzzard	Poor
ZAR_055	15.45	0.29	Other	Buzzard	Poor
ZAR_056	3.31	0.29	Other	Buzzard	Poor
ZAR_057	52.54	0.72	Other	Buzzard	Poor
ZAR_058	62.16	0.17	Other	Buzzard	Poor
ZAR_059	172.17	0.18	Other	Buzzard	Good
ZAR_060	1.23	0.19	Other	Buzzard	Poor
ZAR_061	4.61	0.13	Other	Buzzard	Poor
ZAR_062	0.67	0.16	Trauma	Buzzard	Moderate
ZAR_063	19.27	0.14	Other	Buzzard	Poor
ZAR_064	25.06	0.13	Other	Buzzard	Moderate
ZAR_065	71.06	0.17	Other	Buzzard	Poor
ZAR_066	9.97	0.21	Trauma	Buzzard	Moderate
ZAR_067	11.45	0.22	Trauma	Buzzard	Moderate
ZAR_068	7.17	0.17	Other	Buzzard	Poor
ZAR_069	72.61	0.23	Trauma	Buzzard	Good
ZAR_070	0.29	0.09	Trauma	Buzzard	Moderate
ZAR_071	15.58	0.09	Other	Buzzard	Poor
ZAR_072	0.29	0.10	Other	Buzzard	Poor
ZAR_073	2.51	0.14	Other	Buzzard	Poor
ZAR_074	0.15	0.06	Other	Buzzard	Poor
ZAR_075	53.82	0.07	Trauma	Buzzard	Poor
ZAR_076	1.22	0.10	Other	Buzzard	Poor
ZAR_077	0.07	0.07	Other	Buzzard	Poor
ZAR_078	4.43	0.06	Other	Buzzard	Poor
ZAR_079	41.45	0.13	Other	Buzzard	Poor

ZAR_080	0.33	0.08	Other	Buzzard	Poor
ZAR_081	4.21	0.13	Trauma	Buzzard	Moderate
ZAR_082	17.58	0.23	Trauma	Buzzard	Poor
ZAR_083	131.71	0.23	Other	Buzzard	Good
ZAR_084	6.96	0.09	Other	Buzzard	Good
ZAR_085	113.01	0.10	Other	Buzzard	Good
ZAR_086	0.09	0.06	Trauma	Buzzard	Good
ZAR_087	5.61	0.22	Trauma	Buzzard	Moderate
ZAR_088	75.89	0.16	Other	Buzzard	Poor
ZAR_089	0.45	0.08	Other	Buzzard	Poor
ZAR_090	112.77	0.05	AR toxicosis	Buzzard	Poor
ZAR_091	3.49	0.06	Other	Buzzard	Moderate
ZAR_092	91.14	0.30	Other	Buzzard	Poor
ZAR_093	205.38	0.20	Other	Kestrel	Poor
ZAR_094	0.21	0.05	AR toxicosis	Buzzard	Good

Appendix 5 Summary of results – liver and blood

Table 10 Summary of unrounded results for measured residues in liver and blood as well as information on condition and probable diagnoses of sampled birds.

Sample	Condition	Diagnosis	∑SGAR (ng/g) liver	∑SGAR (ng/ml) blood
BAR_001	Poor	Other	231.00	1.4
ZAR_002	Poor	Other	105.69	1.0
ZAR_003	Good	Trauma	358.61	0.8
ZAR_007	Poor	Trauma	279.19	0.2
ZAR_009	Poor	Other	220.13	3.5
ZAR_015	Good	Other	96.93	0.4
ZAR_017	Poor	Other	48.35	1.4
ZAR_019	Poor	Trauma	69.30	0.7
ZAR_020	Moderate	Other	0.10	0.0
ZAR_021	Good	Trauma	69.84	0.9
ZAR_022	Poor	AR Toxicosis	0.54	0.0
ZAR_023	Moderate	Other	0.67	0.0
ZAR_024	Poor	Trauma	10.55	0.2
ZAR_025	Poor	Other	0.00	0.0
ZAR_026	Good	Trauma	5.16	0.0
ZAR_027	Good	Other	15.88	0.0
ZAR_029	Poor	Other	196.36	4.2
ZAR_032	Good	Trauma	199.33	1.0
ZAR_034	Poor	Trauma	581.68	9.7
ZAR_044	Poor	Other	18.05	0.0
ZAR_047	Poor	Other	53.37	0.4
ZAR_048	Poor	Other	30.31	0.5
ZAR_053	Poor	Trauma	74.26	1.2
ZAR_055	Poor	Other	15.45	0.0
ZAR_057	Poor	Other	52.54	0.3
ZAR_059	Good	Other	172.17	3.4
ZAR_063	Poor	Other	19.27	0.0
ZAR_064	Moderate	Other	25.06	0.7
ZAR_065	Poor	Other	71.06	0.8
ZAR_066	Moderate	Trauma	9.97	0.0
ZAR_067	Moderate	Trauma	11.45	0.0
ZAR_068	Poor	Other	7.17	0.0
ZAR_069	Good	Trauma	72.61	0.2
ZAR_071	Poor	Other	15.58	0.0
ZAR_075	Poor	Trauma	53.82	0.4
ZAR_079	Poor	Other	41.45	0.0
ZAR_082	Poor	Trauma	17.58	0.0
ZAR_083	Good	Other	131.71	1.7
ZAR_084	Good	Other	6.96	0.0

ZAR_085	Good	Other	113.01	0.3
ZAR_088	Poor	Other	75.89	0.6
ZAR_090	Poor	AR Toxicosis	112.77	0.3
ZAR_092	Poor	Other	91.14	0.1
ZAR_093	Poor	Other	205.38	1.9

Appendix 6 Additional samples

Pigeons

Of the 30 pigeon (*Columba livia forma domestica* (29), *Columba palumbus* (1)) liver samples examined 20 (67%) contained SGAR residues above the sample-specific LOQ (Figure 14). The median concentration was 1.1 ng/g and concentrations ranged between 0.2 and 654 ng/g. The pigeons may have ingested improperly placed rat poison, or they may have been deliberately poisoned with ARs. The five samples from TI contained low or undetectable concentrations of ARs, but all five contained alpha-chloralose residues with concentrations ranging from 1.2 to 4.7 µg/g. It is likely that these animals were deliberately poisoned.

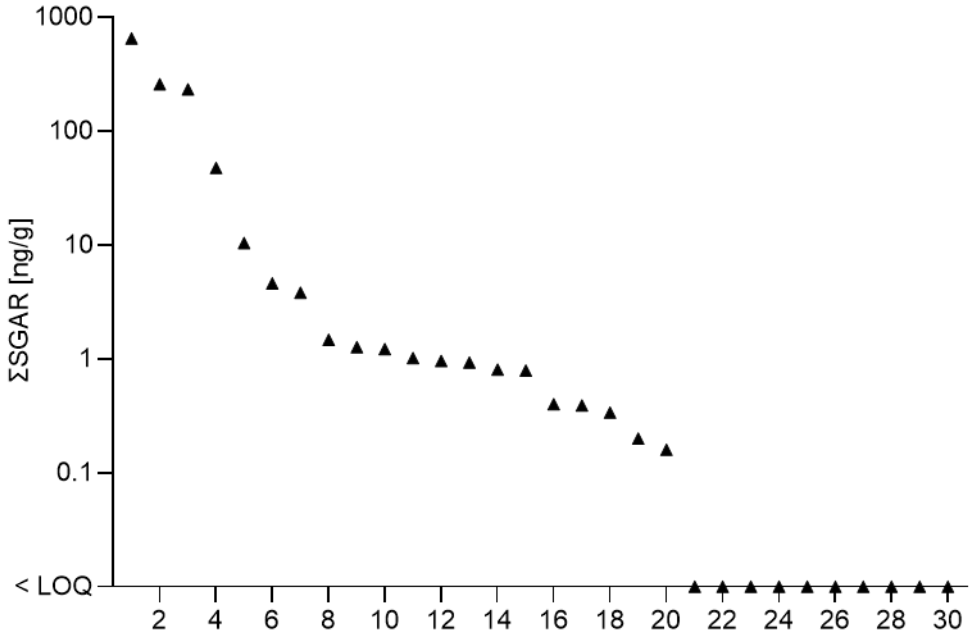

Figure 14 Total concentrations (∑SGAR) of the five SGARS in 18 pigeon liver samples from four different regions of Switzerland (TI, BS, GE, and VD).

Wild boars

Of the 11 wild boar (*Sus scrofa*) samples examined 2 (18%) contained SGAR residues above the sample-specific LOQ. One wild boar liver sample from the Eptingen area contained 15.29 ng/g SGAR residues, mainly Difenacoum. Another wild boar liver sample from the Winterthur area contained 1.03 ng/g Brodifacoum. Other studies on residues in wild boars found liver concentrations between 7 ng/g and 2400 ng/g and muscle concentrations between 10 ng/g and 163 ng/g (Eason, Wright et al. 2001, Alabau, Mentaberre et al. 2020). Alabau et al. (2020) found a positive correlation between liver and muscle concentration for brodifacoum. In both studies only brodifacoum was detected in muscle tissue if liver concentrations were higher than 150 ng/g (Alabau, Mentaberre et al. 2020) or 500 ng/g (Eason, Wright et al. 2001). Therefore, it seems unlikely that the concentrations detected in the current study could pose a risk for consumers of game meat. However, since wild boar liver is also frequently consumed, a risk cannot be completely ruled out if it is eaten regularly (Eason, Wright et al. 2001, Alabau, Mentaberre et al. 2020). In general, omnivorous game species are especially at risk of exposure to SGARs because they can be affected by both primary and secondary poisoning. There also seems to be a positive correlation between human population density and the prevalence of SGAR residues in wild boars, indicating a potential risk to consumers of game meat (Alabau, Mentaberre et al. 2020).