Chapter 2

Poisoning by Anticoagulant Rodenticides in Humans and Animals: Causes and Consequences

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Abstract

Anticoagulant rodenticides (ARs) are a keystone of the management of rodent populations in the world. The widespread use of these molecules raises questions on exposure and intoxication risks, which define the safety of these products. Exposures and intoxications can affect humans, domestic animals and wildlife. Consequences are different for each group, from the simple issue of intoxication in humans to public health concern if farm animals are exposed. After a rapid presentation of the mechanism of action and the use of anticoagulant rodenticides, this chapter assesses the prominence of poisoning by anticoagulant rodenticides in humans, domestic animals and wildlife.

Keywords: anticoagulant, rodent, poisoning

1. Introduction

The management of rodents around the world is a great concern, in many aspects. Rodents are ubiquitous and opportunistic animals, some such as the brown rats (*Rattus norvegicus*) and the black rats (*Rattus rattus*) are present at all continents except Antarctic [1]. These rodent populations are an ecological and an economic issue in the islands where they are not indigenous [2]. Agriculture is also affected by rodents; in France, for instance, water voles (*Arvicola terrestris*) devastate some lands [3]. Finally, rodents are a major nuisance in cities where their proximity to and interactions with human populations and infrastructure can cause impairments and become a hazard for the public health as they are reservoirs of many diseases. In

China, rodents destroyed the rice stock that would be sufficient to feed 200 millions of people [4] and the estimation of the cost induced by rodents' damage is about 19 billions of dollars [5]. Many similar cases have been recorded around the world [1, 6].

To deal with these concerns, rodent populations have to be controlled. One of the most used management methods is the chemical method based on the use of anticoagulant rodenticides (ARs). Anticoagulant rodenticides (ARs) have been used since the 1940s to control rodent populations. Warfarin was the first molecule used. But after its use for more than one decade, resistant strains of rodents to ARs have emerged [7]. To deal with resistance, this first generation of ARs has been supplemented by a second generation. ARs of the second generation are frequently named 'superwarfarins' or long-acting anticoagulant rodenticides. Indeed, these molecules are more potent than the first generation due to their longer half-life, which implies longer tissue-persistence and better efficacy.

Indeed, the consequence of the widespread use of ARs and more specifically the second generation of ARs, that are more efficient and more persistent, has been an increase of the exposure risks and the intoxication risks for non-target species such as pets, wildlife as well as humans. Nevertheless, anticoagulant rodenticides are renowned as a safe method to manage rodent populations. This safety is due to their mechanism of action as well as on the implementation of good practices in their use and by the respect of related regulations. Beyond this renown, it is important to monitor the impact of using ARs regarding the risk of untargeted species poisoning and to discuss on the remaining grey area in our knowledge on anticoagulant rodenticides.

Hence, after a rapid presentation of the mechanism of action and the use of anticoagulant rodenticides, this chapter assesses the importance of the exposure and the intoxication by anticoagulant rodenticides.

2. Anticoagulant rodenticides

The current anticoagulant rodenticide molecules belong to the family of vitamin K antagonist (VKA) molecules. The effects of VKAs have been observed in the 'sweet clover' poisoning of bovines, which results in a haemorrhagic disease and often the death of the animal [8–10]. Clover (*Melilotus officinalis*), used as fodder, contains coumarin a precursor of dicoumarol which is a VKA (**Figure 1E**). If clover fodders are not stored under proper conditions, fermentations may occur. These fermentations change clover coumarin in dicoumarol. Thus, clover fodder become toxic [10]. Dicoumarol was synthesised by Paul and Stahmann in 1941, opening the opportunity of use VKA as medicine and rodenticide [11]. Then other VKA molecules have been synthesised, including the famous warfarin (**Figure 1D**) and all other products that are more potent than dicoumarol [12].

The main molecules used in the rodent population management are presented in **Figure 1**. VKA molecules are derived from a coumarin (**Figure 1A**), thiocoumarin (**Figure 1B**) or 1,3-indandione (**Figure 1C**) core. The distinction of the second generation of AR from the first generation is the radical. In second generation, radical includes three benzene structures,

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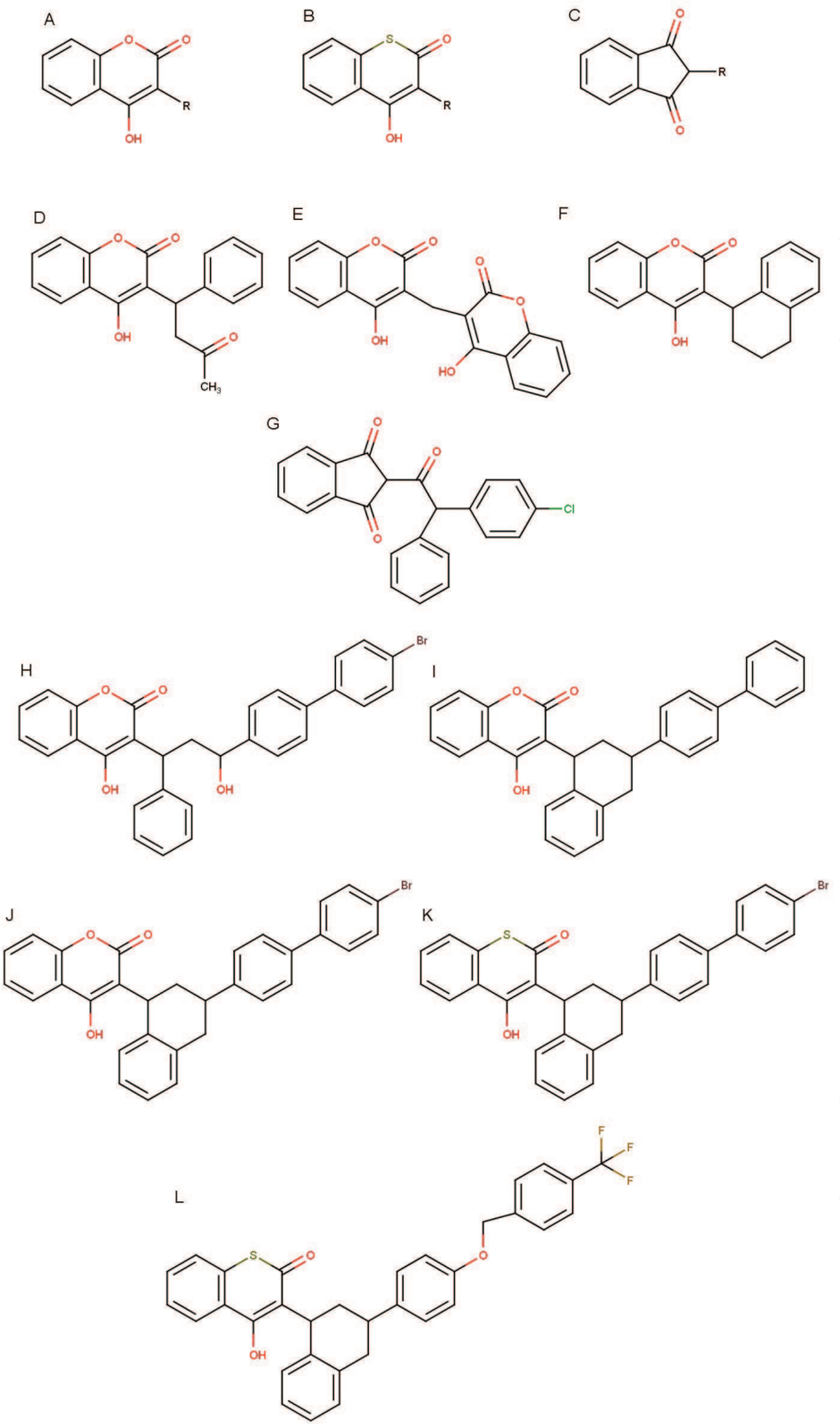

Figure 1. Chemical structure of: (A) coumarin core; (B) thiocoumarin core; (C) 1,3-indandione core; (D) warfarin; (E) dicoumarol; (F) coumatetralyl; (G) chlorophacinone; (H) bromadiolone; (I) difenacoum; (J) brodifacoum; (K) difethialone; and (L) flocoumafen.

which increase the fat solubility of the molecules and influence their pharmacokinetic properties. In order to understand the interest of VKAs and the key issue of their safety, it is important to present their mechanism of action and their pharmacokinetics.

2.1. Mechanism of action

Vitamin K antagonists (VKA) are non-competitive inhibitors of the vitamin K epoxide reductase enzyme (VKORC1) [13, 14]. This membrane enzyme of endoplasmic reticulum is responsible for the recycling of vitamin K. Vitamin K is a cofactor essential to many biotransformations of proteins and more specifically to obtain an active form of some clotting factors, the factors II, VII, IX and X. These factors, called vitamin K-dependent clotting factors, have to go through a post-translational gamma-carboxylation of their glutamate residues into gamma-carboxyglutamic acid to be able to chelate calcium and have their physiological activity [15, 16]. This reaction is done by gamma-glutamyl carboxylase (GGCX), which is another membrane enzyme of endoplasmic reticulum, and needs the oxidation of vitamin K hydroquinone to vitamin K epoxide to provide the required reducing power [17, 18]. Then VKORC1 recycles vitamin K epoxides to vitamin K hydroquinones (**Figure 2**) [19].

The amount of vitamin K provided by the majorities of food is not sufficient to offset the complete arrest of vitamin K cycle. Consequently, when VKORC1 is inhibited by VKA, a sufficient amount of vitamin K hydroquinone cannot be recycled from vitamin K epoxides to ensure the gamma-carboxylation of vitamin K-dependent proteins, and more especially the vitamin K-dependent clotting factors. Consequently, the blood concentrations of active vitamin K-dependent clotting factors decrease and lead to an increase of clotting times then, with time, to the death by haemorrhages.

2.2. Pharmacokinetics properties

Vitamin K antagonists are reputed to be highly and rapidly absorbed after *per os* administration. Then they are mainly stocked in liver. Their liver storage and their elimination are

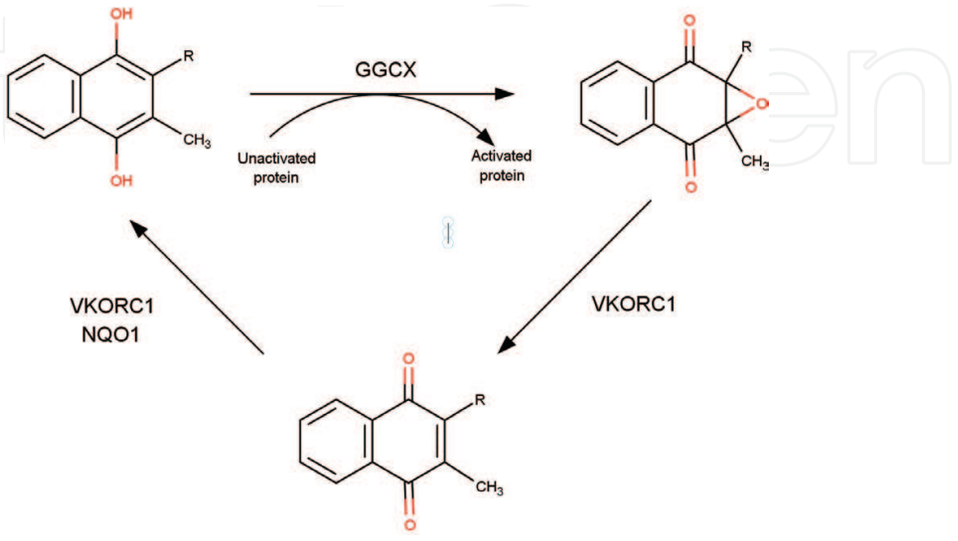

Figure 2. Vitamin K cycle.

key factors, which determine a part of their efficiency and their persistence. The elimination pathway depends on the molecule and on its enantiomeric form [20]. For example, enantiomers of warfarin are eliminated differently. The (S)-enantiomer is metabolised exclusively by the hepatic cytochrome P450 isoform 2C9 (CYP2C9) while (R)-enantiomer is metabolised by isoforms CYP1A2, CYP2C19, CYP3A and hepatic ketoreductase [21, 22]. Although the (R)-enantiomer has a longer half-life, it is less efficient and the modulation of its elimination does not have a significant impact on the coagulation [23–25]. There is a great discrepancy between tissue persistence of first generation and second generation of ARs. First-generation molecules have tissue persistence of few days while the second generation has tissue persistence of few weeks [26]. This point is a major concern for AR ecotoxicity.

Moreover, second-generation ARs (i.e. bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone) contain two asymmetric carbons systematically. Therefore, commercial second-generation ARs are a mixture of two diastereoisomeric forms (1R,3R)(1S,3S)-isomers and (1R,3S)(1S,3R)-isomers with different pharmacokinetic properties. For each second-generation AR, there is systematically one diastereoisomeric form with a shorter half-life than the other one (**Table 1**) [20, 27]. Proportion between stereoisomers in commercial baits is defined by regulatory documents. For example, bromadiolone must be a mixture of more than 70 of trans-isomers and fewer than 30% of cis-isomers. These differences in half-life between stereoisomers could be a fundamental point in the development of future more eco-friendly AR with modification of regulatory defined ratios.

2.3. Interest of anticoagulants in rodent population management

The first methods used to control rodent populations aim to kill them immediately. They were based on physical traps or on rapid killer molecules like strychnine. However, the neophobic behaviour of some rodents such as rats and their social organisation make these molecules ineffective. Indeed, the precocity of symptoms or death after a bait eating by congeners induces

Molecules	$T_{1/2}$ (h)
Brodifacoum cis	120.8
Brodifacoum trans	68.7
Bromadiolone cis	26.9
Bromadiolone trans	75.6
Difenacoum cis	78.3
Difenacoum trans	24.2
Difethialone cis	71.6
Difethialone trans	52.9
Flocoumafene cis	76.7
Flocoumafene trans	177.4

Table 1. Half-lives of some anticoagulant rodenticide enantiomers.

bait aversion in the rodent population [28, 29]. Conversely, the time to onset of anticoagulant action is sufficient to avoid that rodents link their symptoms and death to bait eating [29].

Moreover, the delay and the mechanism of action of ARs are the keystone of their safety of use comparatively to other rodenticides. Indeed, the delay allows the possibility to implement a treatment after an exposure to ARs and the mechanism of action can be easily bypassed which offers an efficient and safe antidotes, the vitamin K.

Nevertheless, some issues exist with anticoagulant rodenticides, first the resistance of some population to some AR molecules. This issue has led to the creation of the second generation of ARs, which are more efficient against resistant strains [30]. However, this generation is more persistent which involves other issues. This persistence extends the duration of antidote treatment after exposure. Moreover, it entails a greater concentration of AR molecules in rodents after its death; thus, it might increase the risk of secondary poisoning of predators or scavenger animals. Consequently, to prevent poisoning of humans and animals, many actions have been implemented in the use of ARs.

2.4. Prevention of poisoning

In Europe, an anticoagulant rodenticide product can be registered either as a plant protection product or as a biocide. According to the kind of registration, restriction and modality of use are defined in order to prevent human and animal intoxication. Nevertheless, there are important differences on the modality and restriction among the European Member States. Here, we present some member state (MS) actions to prevent poisoning.

The majority of MS distinguishes the individual use of ARs and the professional use. Professional users are mainly the pest control operator, they have to be trained. In some countries like France or Italy, the sale of ARs is restricted for the individual user, thus, in France, individuals cannot buy more than 1.5 kg of AR bait. In other countries, like Germany, only trained professionals are allowed to use the second generation of ARs. Moreover, some molecules can be allowed as biocides and be forbidden as plant protection products.

The presentation of ARs is also regulated. Baits are presented as poisoned seed, paste or foam. Previously concentred products like tracking powder and oil concentrate were used but they have been forbidden in many states. Thus, concentrations of the current AR baits are of the order of few dozen to few hundred milligrams of active product per kilogram of bait. The concentration depends on the efficiency of the active molecule. The main consequence of the use of products with low concentration is that it is difficult to reach the lethal dose at once for mammal heavier than rodents such as cats, dogs or humans. Nevertheless, the high half-life of some anticoagulants allows to reach this dose after a multi-exposure. The use of a bitter agent in bait is mandatory notably to avoid and limit exposure.

Finally, to avoid the exposure of untargeted species, in many states, baits have to be placed in secured bait stations. These stations have to be labelled to inform people on their content and on the action to perform in the case of exposure. Moreover, stations avoid the dispersion of baits which allows to control the consumption and they are waterproof, which prevent

water pollution. Nevertheless, some rodents are reluctant to enter in bait stations which might involve failing in pest control. In spite of all described elements to prevent exposures and intoxications of human and untargeted animals to ARs, many cases have been reported.

Therefore, some recent research makes effort to implement a third generation of ARs which is based on the stereochemistry concept, which would be efficient against resistant strains of rodents and be less persistent and thus less involved in secondary poisoning [20, 27].

3. Human exposures and intoxications

Intoxication with anticoagulant rodenticides is a major public health concern. The involvement of poison control centres is crucial in the record of poisoning cases in both rural and urban areas. Besides, emergency departments report rare cases of intoxication by suicide or homicide. Most of these poisonings occur following accidental exposure, especially ingestion in children. Bleeding severity is highly variable, depending on the rodenticide exposure and on the delay between the exposure and patient management. The diagnosis relies on simple coagulation tests. Emergency department physicians should be aware of anticoagulant poisonings since management differs according to the anticoagulant rodenticide including warfarin or long-acting superwarfarin types.

3.1. Epidemiology

The incidence of poisoning with anticoagulant rodenticides is difficult to assess, mostly based on national registries. In the literature, cases associated with bleeding are published as case reports or small series, probably corresponding to the most severe ones.

In the annual report based on the US National Poison Data System and published by the American Association of Poison Control Centers, data related to long-acting superwarfarin- or warfarin-type rodenticides intoxication are given separately. Over the last 5-year period (2011–2015), the cumulated number of exposures is 44,095 for long-acting superwarfarin-type and 1029 for warfarin-type drugs, with a single exposure in 97.3 and 95.6% of the cases, respectively [31–35]. Interestingly, the number of reported cases has slightly decreased since 2008 (**Figure 3**) [36–38]. The mean prevalence of exposure over the last 5 years is 3.4% for long-acting superwarfarin-type and 4.9% for warfarin-type drugs. The age distribution shows that children, especially those of less than 5 years old, are the most involved (**Figure 4**); only 9% of the reported cases are adults. Finally, clinical outcomes are reported (**Figure 5**). Remarkably, outcome is favourable in 93.6% of the cases, probably due to the limited ingested doses in relation to the bad taste of numerous rodenticides. The bitterness brought by the excipients in the currently marketed rodenticides considerably limits the ingested amounts, especially in young children. In cases associated with significant complications, severe bleedings are observed in less than 10% of cases, with fatal bleedings occurring in only eight patients among the 44,095 exposed patients during the last 5-year period in the USA. Overall, poisoning with rodenticides remains a rare cause of morbidities and fatalities [31–35].

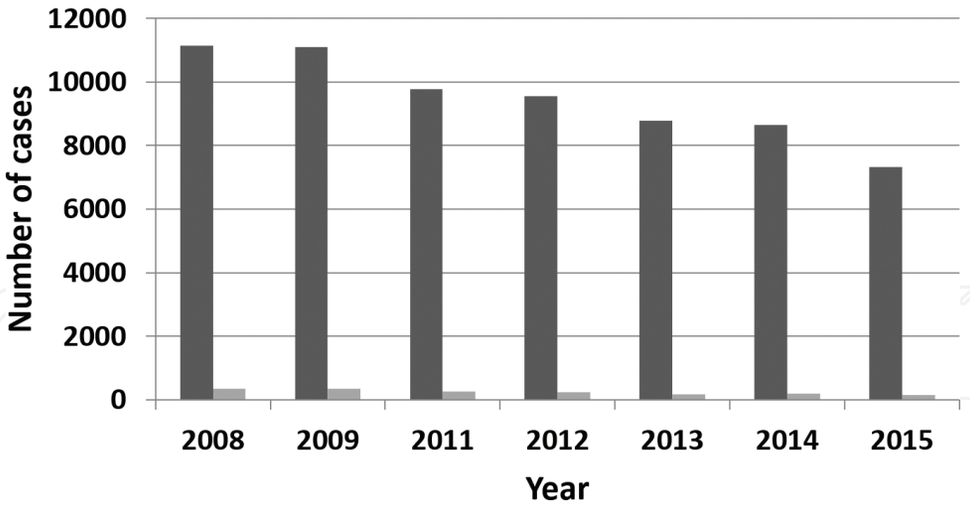

Figure 3. Number of poisonings with anticoagulant rodenticides reported by the American Association of Poison Control Centers from 2008 to 2015. Black bars: intoxications with long-acting anticoagulant-type rodenticides; grey bars: intoxications with warfarin-type rodenticides.

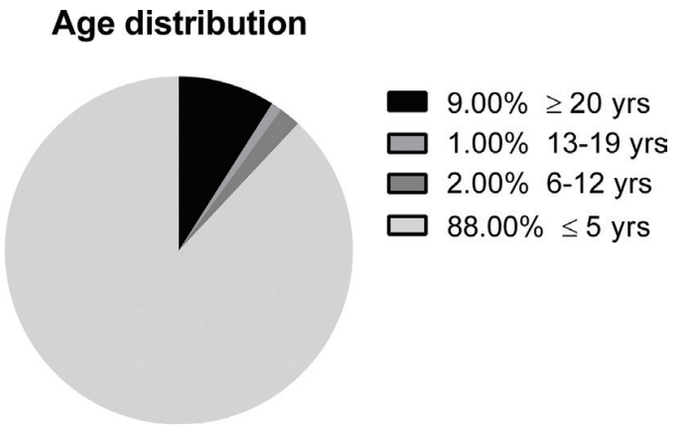

Figure 4. Distribution of the cases of poisoning with anticoagulant rodenticides reported by the American Association of Poison Control Centers in 2011–2015 according to the patient age.

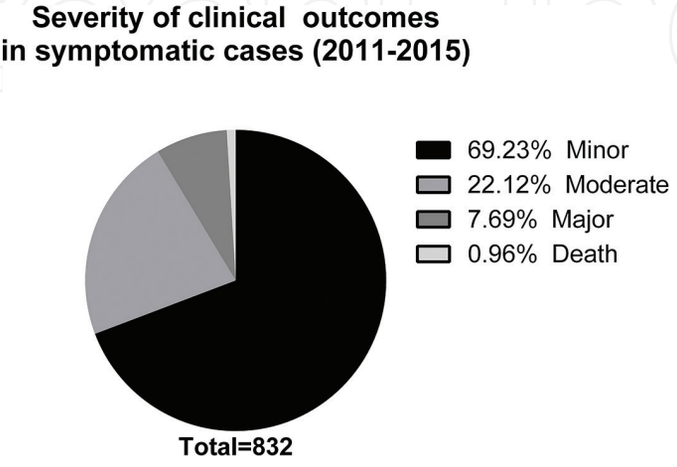

Figure 5. Distribution of the cases of poisoning with anticoagulant rodenticides reported by the American Association of Poison Control Centers in 2011–2015 according to the outcome.

3.2. Clinical outcomes and laboratory diagnosis

The threat in poisoning with rodenticides is the onset of severe bleeding. In humans like in rodents, anticoagulant rodenticides inhibit the enzyme vitamin K epoxide reductase complex (VKORC1) leading to the absence of vitamin K recycling, which is essential for the gamma-carboxylation of vitamin K-dependent proteins in the hepatocytes, especially clotting prothrombin, factors VII, IX and X. This leads to impaired functioning of gamma-carboxylated vitamin-K-dependent factors due to their inability to bind activated platelets. Given the half-lives of coagulation in humans, i.e. from 6 hours for FVII to ~60 hours for prothrombin, the onset of hypocoagulability and the risk of bleeding are delayed after the exposure to rodenticides. The risk of bleeding depends on the severity of the hypocoagulability state induced by rodenticides and on the duration of hypocoagulability. The spectrum of bleeding is wide: extended unexplained spontaneous ecchymosis, epistaxis, hematoma, bleeding from the gastro-intestinal or the genitourinary tract as well as intra-cerebral bleeding are reported [39–46].

The diagnosis of rodenticide intoxication has to be considered for any patient with prolonged prothrombin time (increased INR), prolonged activated partial prothrombin time; the vitamin K-dependent factor II, VII, X, IX coagulant activities are decreased while factor V coagulant activity and the fibrinogen level are normal [41, 43, 47, 48]. Liver dysfunction, cholestasis and severe starvation can be ruled out by normal liver enzymes and serum albumin concentration. Moderate to severe anaemia can be present, depending on the severity of bleeding. Special attention and high clinical suspicion are required in patients with apparent negative history of warfarin treatment. The diagnosis of rodenticide intoxication should be suspected when the international normalised ratio (INR) strongly fluctuates on vitamin K therapy, especially while high doses of vitamin K are required. The accessibility to anticoagulant rodenticides should be checked; monitoring of persons who deal with rodenticides in their home or workplace, especially those suffering from dementia or psychiatric disorders, is necessary [49]. The intoxication can be confirmed by the identification and measurement of the rodenticides in plasma by specific assays [42, 45, 50].

3.3. Principles of poisoning management

Acute life-threatening complications can be prevented with timely intervention. Immediate administration of high doses of phytomenadione (vitamin K1) and/or factor prothrombin complex concentrate (30 UI/kg FIX) can successfully reverse the anticoagulant effects of anticoagulant rodenticides. With tissue half-lives estimated at between 16 and 220 days, reversal of superwarfarin toxicity is a long-term issue. Therefore, long-term daily treatment for several weeks of phytomenadione is necessary. Treatment courses averaged 168 days. To avoid re-bleeding, close monitoring of INR is necessary. Adjunctive haemostatic therapy with recombinant factor VIIa and prothrombin complex concentrate has been used [50–54].

4. Overview on animal exposures and intoxications

To assess the importance of animal intoxications, it is important to discriminate two situations: the domestic animals and the wildlife. Concerning wildlife, the evaluations of exposures

and intoxications are often realised during focused scientific campaign and are often based on contamination studies or after an important mortality in wildlife. In domestic animal, besides the scientific campaign, there are, in some countries, animal specialised poison control centres, which can provide data on exposure, intoxications and linked symptoms.

In this part, it is important to take account of the differences between exposure and intoxication. Concerning exposure, it is the fact to take a dose of anticoagulants, it can be suspected by an owner who sees its animal eating baits, sometimes without knowing what the active substance is, or find in wildlife by pinpointing the presence of VKA in the sample. Intoxication is when the active substance induced clinical signs. This distinction is fundamental in the study of VKA toxicology. Indeed, to observe intoxication, the exposure dose and the delay of action has to be sufficient. This issue is discussed further concerning the wildlife exposure/intoxication studies.

4.1. Domestic animals

In France, two control poison centres are specialised in animals. The most important in terms of call number is the 'Centre National d' Informations Toxicologiques Vétérinaires (CNITV)' which responds to questions from owners or veterinarians on a 24-hour/7 day basis. We used this important database to assess the importance of VKA exposures and intoxications in domestic animals.

The data of the last 9 years have been analysed. During this period, the CNITV has received about 150,000 calls. Each month, 10.73% (CI 10.41-11.06) of solicitations are about VKA exposure or intoxication. Moreover, about whole VKA appeal is on domestic animals (99.2%). Appeals accrue from veterinary (69%) and owner (29%) mainly.

During the analysis of data, an important seasonality of the calls concerning VKAs has been pinpointed (**Figure 6**). Significant ($p < 0.05$) increases of the number of calls for VKA exposure are observed during the months of August, September and October followed by a significant decrease of appeal numbers from December to April, which is surprising. Indeed, based on our experience, the periods when people apply rodenticides in cities are at the beginning of winter (late November) and at the beginning of spring (March), when rodents are active and when the scarcity of food encourages rodents to eat baits. Conversely, during summer and the beginning of autumn, rodents can find many sources of food; consequently, they are less likely to eat baits, which increase the risk that baits are eaten by untargeted animals, notably dogs. However, summer is also the time when there is less human in cities and this element with a lenient weather encourages the presence of rodents outside where they are more visible. In response, cities and individuals might increase the number of baits, which is unfavourable for the rodent population management and rocket up the risk of pet exposures to VKA.

More generally, data pinpoint a trend reversal; before September 2013, the number of cases has significantly increased with a slope of 3.9% per annum ($p < 0.0001$) whereas after this date it has significantly decreased of 10.5% per annum ($p < 0.0001$). The increase trend has to be relativised as the total number of calls significantly increases during this period. However, after 2013, the total number of calls was stable ($p = 0.13$). Consequently, a significant decrease in the number of calls for VKAs after 2013 is confirmed. We hypothesise that the source of this diminution is the evolution of the regulation. Indeed, regulation has enforced the use of secured bait station since 2013, which seems to reduce the exposure of domestic species.

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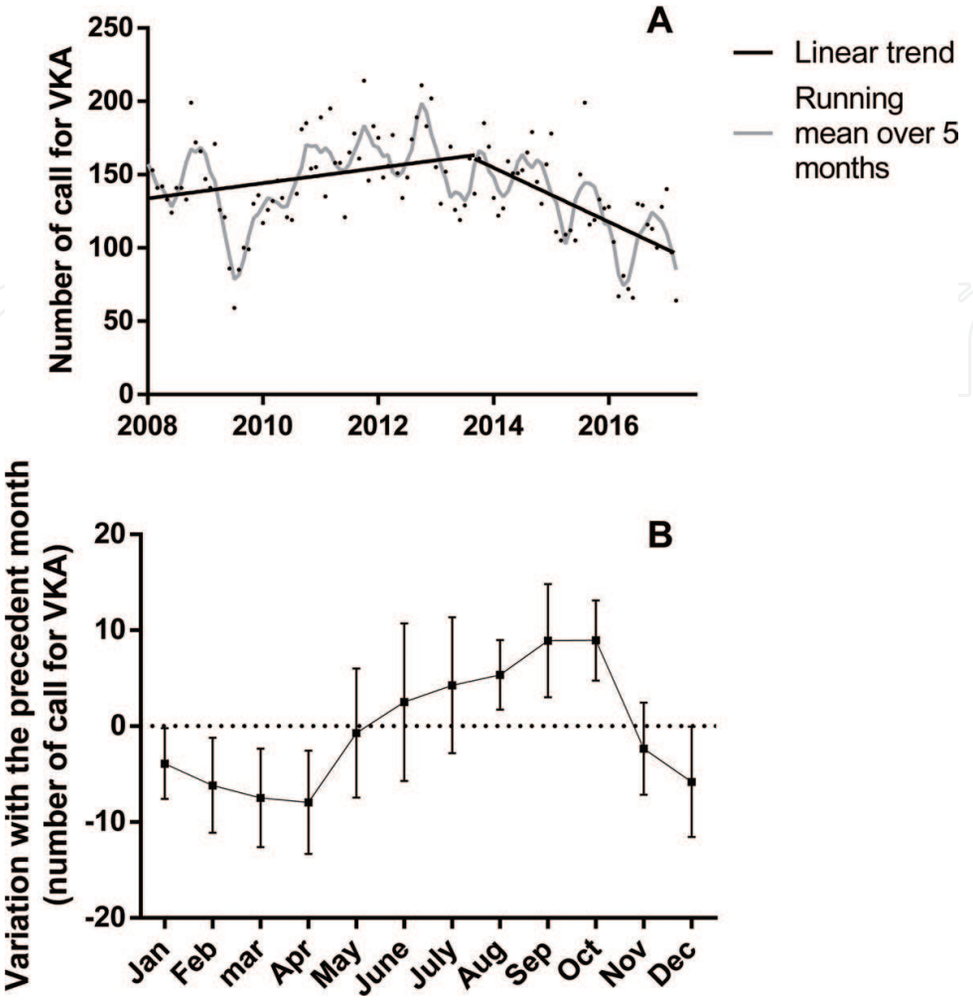

Figure 6. (A): Evolution of monthly calls for VKA exposure over time. Grey curve is the running means over 5 months. Dark lines are linear regression from January 2008 to September 2013 and from September 2013 to February 2017. (B) Variation of the number of calls for VKA exposure with the precedent month, values are represented as the mean of observed variations for the concerned month over the period 2008–2016 and its 95% confidence interval.

Pets and more specifically dogs are over represented (**Figure 7**). This might be explained by the lack of use of secured bait station by private individuals and by the behaviour of dogs. Poisoning is mainly accidental even if some malicious poisoning are reported (2.03%). The proportion of suspected malicious case concerning cats is significantly higher than for the general case. Indeed, cats and dogs represent, respectively, 19.14 and 63.64% of malicious reports. These uses of anticoagulant rodenticides with harmful intent against animals but also against humans have led to restrict the sale of rodenticides in some countries such as Italy [55].

Concerning molecules, in 22% of calls, the exact molecule is not identified. Nevertheless, exposures or intoxications with one of the six molecules authorised are reported, they are difenacoum, difethialone, brodifacoum, bromadiolone, chlorophacinone and coumatetralyl, which represent, respectively, 23, 18, 10, 9, 3 and 2% of the calls for AR. It is significant that the four main anticoagulants are second-generation ARs. This was predictable because first-generation ARs are less efficient on resistant strains of rodents consequently main ARs sold belong to the second generation.

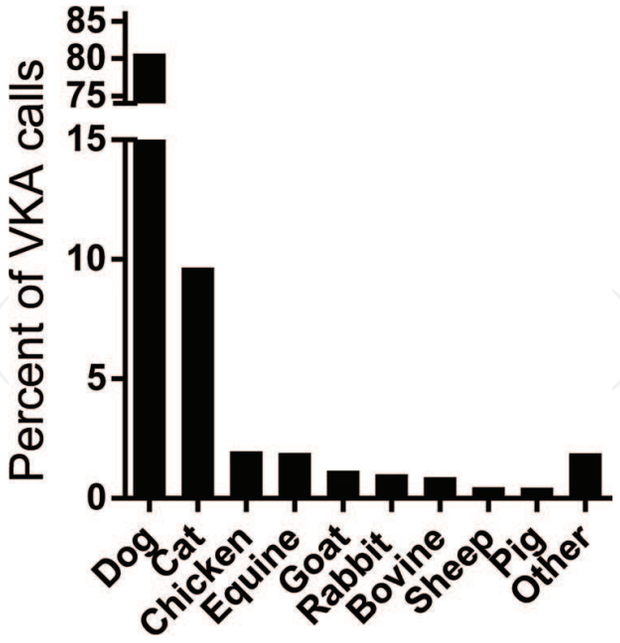

Figure 7. Percentage of species concerned by calls on VKA.

The consequences of an exposure without intoxication are completely different for pets and farm animals. Indeed, for pets when an exposure occurs, the aim is to prevent intoxication. In farm animals, more than intoxication prevention, the presence of anticoagulant molecules in products such as meat, eggs or milk has to be considered. Further, we discuss issues of ARs in pets then in farm animals.

In pets, depending on caller, the circumstances of appeal are different. Indeed, 90% of calls from individuals report exposure without intoxication, whereas the proportion of this circumstance drops significantly to 75% when it is a call from a veterinary ($p < 0.0001$). This can be easily understood, if an animal shows a symptom, the owner priority is to bring it to the veterinarian to be healed. Differences according to species are also pinpointed. In dogs, 81% of calls for AR exposure do not report symptoms versus 62% in cats ($p < 0.0001$). The source of this distinction may be the detection of the exposure, which is earlier in dogs. Moreover, cats may be more prone to be secondary exposed to ARs from intoxicated rodents and this kind of exposure is not visible by the owner. It should be noted that according to the low doses of bait and to the difference between the toxic doses for a rodent versus a cat, a large number of intoxicated rodents should be eaten to induce intoxication.

In dogs and cats, when an exposure is suspected and when it is possible, the best way to prevent intoxication is to induce vomiting in the first hour after the exposure. If medicines are not available, it is possible to use 10 volume hydrogen peroxide. Hydrogen peroxide solution can be given orally to animals at 1 mL for every 5 kg of weight. Be careful, salt must not be used to induce vomiting. Indeed, excess of salt can cause fatal hypernatremia [56].

Sometimes, even the exposure is uncertain or the absorption of VKA after vomiting is unknown, to confirm exposure, two means are currently tested by our team: the dosing of VKAs in plasma and their dosing in faeces. The difficulty of dosing in plasma is that for some VKAs, the presence in the plasma is temporary then VKAs are stored in liver. Therefore, dosing

in plasma is often associated with false-negatives. The dosing of VKA in faeces seems to be more reliable with an excretion that can be detected for several weeks.

As there is not any well-described toxicity dose, it is important to follow the possible effect of AR in order to prevent serious intoxication. Moreover, there is no correlation between the dose of VKA and the symptom severity [57]. Today, the gold standard to diagnose VKA intoxication is the prothrombin time [58]. It is advised realising a prothrombin time about 48 hours after the suspected exposure [59]. If the prothrombin time is elevated then a treatment has to be initiated. Other methods such as vitamin K clotting factor concentration measurement are explored to detect AR effects sooner. Factor VII seems to be a good candidate as its half-life after a VKA administration is the shorter [60].

If no treatment is given, symptoms may appear after 2–6 days [57, 58]. Symptoms are the classic signs of coagulopathy, which may be pinpointed by owner as bleeding, pale mucous membrane, haematomas, haematuria or haematemesis as well as their consequences on animal general condition as lameness, depression or lethargy [57, 58, 61]. Owing to intrathoracic bleeding, respiratory distress occurs frequently in anticoagulant intoxications [62–64].

Animals exposed with an elevation of prothrombin time after exposure or with AR-linked symptoms have to receive vitamin K_1 supplementation as long as the recycling mechanism is inhibited. Vitamin K_1 is given *per os* daily with a dose of 5 mg/kg of body weight. The duration of the VKA inhibition depends on the pharmacokinetics of AR molecule with huge differences between molecules. Thus, the duration of treatment after an exposure to the first-generation AR is estimated to 3 weeks versus 5–7 for the second generation. Nevertheless, there is a lack of studies to support these durations of treatment. Thus, a treatment can be initiated during at least 1 month then stopped during 48 hours. After 2 days of treatment discontinuation, the vitamin K regeneration mechanism can be assessed by measuring prothrombin time. If prothrombin time is elevated, treatment has to be replicated until a new assessment of regeneration mechanism else, the treatment can be arrested. Vitamin K_3 is inefficient to treat VKA intoxication. The treatment of symptomatic animals may require fresh plasma to reconstitute the pool of clotting factors urgently, moreover, in this case, it is recommended initiating the vitamin K treatment by an intravenous administration. If there is a proper compliance of the vitamin K treatment, the prognostic is excellent [57].

In farm animals, when an AR exposure occurs, the safety of the product has to be considered. Little information is available on the contamination of food following AR exposure. Nevertheless, many methods have been implemented to assess the residues in foodstuffs [65, 66]. Concerning meat, it has been shown that VKA molecules are present in muscle after exposure and that the cooking does not influence their activities [67]. Likewise, VKA molecules are also present in eggs after hen exposure, and are still detected in eggs 14 days after exposure [68]. Concerning the milk, it has been observed an excretion of VKA in human milk when a VKA is used as medication for the mother [69]. Consequently, it might be supposed that the same occurs in animals. Thus, when an animal is exposed, its litter should be separated of its mother and fed with relevant artificial milk. If separation is not possible or if diagnostic is late, litter should be supplemented with vitamin K_1. Concerning foodstuffs provided by an exposed animal, their management would be done in accordance with relevant authority.

4.2. Wildlife exposures and intoxication

Wildlife expositions or intoxications to ARs have been reported around the world for many mammals such as minks [70], bobcats [71], stoats and weasels [72], foxes [73, 74] and boars [67] and as well for many birds [75–77]. Exposition of fish was reported near an island where an eradication of rodent with brodifacoum was performed and the risk for human through the consumption appeared very low [78].

These intoxications may be primary when non-target species eat directly the bait. It is the case when baits are directly available without protection or when they are washed away and diluted in sea or river. In Spain, a study on water and soil samples revealed no imminent environmental risk in treated areas with chlorophacinone and brodifacoum [79]. However, the use of secured bait stations prevents this kind of exposition.

The secondary exposition occurs when a scavenger or a predator eats an exposed rodent. It is the most described exposition of wildlife to ARs and the most difficult to prevent. Many factors may influence the level of secondary exposition. First, due to the bait appetence, rodents can eat more AR than necessary to lead to their death, which might increase their concentration in AR. Moreover, if rodent is resistant to ARs, this phenomenon might be amplified. Indeed, a resistant rodent eats twice to fivefold more AR than susceptible rodent [1]. After the onset of symptoms in rodents, their behaviour evolves. They increase their activity during the day and stay longer in uncovered area, which enhances the risk to be hunted by predators [1]. The delayed action of ARs, inherent to its mechanism, allows rodents to eat several times the LD50 dose between the first bait intake and the death [1] and may as well increase the risk of secondary exposition. Pesticide usage has been correlated with non-target wildlife exposition [74, 75], and the intensity of treatment was related to incidence on local fox populations in France [80]. Finally, the diet is certainly going to influence secondary exposition and species like raptors, foxes and mustelids largely feeding on rodents when abundant are consequently the most at risk, as demonstrated for the red kite (Milvus milvus) [81]. The removal of visible rodent bodies helps to reduce the risk of secondary exposition [82] but is not always possible because of landscape limited access and in the case of aerial application [1]. Mitigation measures have been considered to protect predatory species but new approaches are still required [82].

Persistence and toxicity of the molecule are key factors. They depend on the used active ingredient [26, 83]. Historically, second-generation ARs had been designed to be more persistent and toxic on resistant strain. Thus, secondary poisonings of wildlife associated with the use of second generation are more often reported. But the development of new ARs recently proposed is based on the stereochemistry of second-generation ARs with reduced persistence but equivalent toxicity might greatly decrease the level of secondary exposition [20].

They are two types of consequences of the exposition of wildlife to ARs. First, if the species is eaten by human [67, 78], the consequences are comparable to those discussed for farm animals. Second, if the exposition is sufficiently important, it might lead to intoxication of the animals and to its death, which can be problematic mainly for endangered species. The rate of exposure of non-target species has often been evaluated, and summed liver concentrations above a limit of 0.2 mg/kg associated with clinical signs (i.e. macroscopic haemorrhages with no trauma) have been statistically characterised as representative of a high-risk toxic threshold

[84]. According to these criteria, hepatic concentrations above 0.2 mg/kg have been associated with mortalities in raptors and small mustelids from Denmark [85], in raptors and hedgehogs from Mediterranean region of Spain [86], in six raptor species from Canary Islands, Spain [77].

It is difficult to discriminate a simple exposition and intoxication in wildlife. Indeed, as well as in domestic animal, toxic doses are not well described for all species. Moreover, the majorities of exposition studies are performed on dead animal, and as the lesion induced by ARs is not specific, so it might be difficult to conclude to its implications. Less than 10% of exposed and dead birds have been confirmed to be intoxicated by ARs [1, 87]. Currently, there are no reports of a significant incidence of ARs on non-targeted species populations [82]. Nevertheless, impact of ARs on wildlife has to be more monitored in order to limit the impact of rodent population management. The probable future design of eco-friendly baits with new isomer ratio will change the need the way AR hepatic residues are monitored. The recently described multi-residue LC-MS/MS method [88] is an appropriate tool to start investigating second-generation AR diastereoisomer proportions in non-target wildlife and to evaluate their respective persistence in predators.

5. Conclusion

Anticoagulant rodenticides are a keystone of the rodent population management. Like other poisons, there is a risk of human or non-targeted species poisoning. The wide use of anticoagulant rodenticides near human living space and agriculture space involves an important exposure of humans and domestic animals. Nevertheless, since few years, many risk mitigation measures have been taken and the number of exposure in humans and domestic animals has decreased. Moreover, in contrast to the majority of chemical biocide, anticoagulant rodenticides have an effective antidote, the vitamin K. Consequently, anticoagulant poisoning is rarely fatal. However, the impact of anticoagulant rodenticides on wildlife is least well known and deserves more investigation.

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