2018

Pharmacokinetics of Anticoagulant Rodenticides in Target and Non-target Organisms

Katherine Horak
U.S. Department of Agriculture, katherine.e.horak@aphis.usda.gov

Penny M. Fisher
Landcare Research

Brian M. Hopkins
Landcare Research

Chapter 4
Pharmacokinetics of Anticoagulant Rodenticides in Target and Non-target Organisms

Katherine E. Horak, Penny M. Fisher, and Brian Hopkins

1 Introduction

The concentration of a compound at the site of action is a determinant of its toxicity. This principle is affected by a variety of factors including the chemical properties of the compound (pKa, lipophilicity, molecular size), receptor binding affinity, route of exposure, and physiological properties of the organism. Many compounds have to undergo chemical changes, biotransformation, into more toxic or less toxic forms. Because of all of these variables, predicting toxic effects and performing risk assessments of compounds based solely on dose are less accurate than those that include data on absorption, distribution, metabolism (biotransformation), and excretion of the compound. These factors are commonly referred to as ADME. The quantitative study of these properties is called pharmacokinetics and often encompasses the determination of compound concentrations in tissues of interest including blood and the time course of absorption, metabolism, and excretion. A goal of pharmacokinetics is an understanding of the relationship between dose and the concentration of the active compound at the target site. Toxicokinetics is a "unique expansion of pharmacokinetics", with doses being much greater than those in pharmacokinetic studies (Welling 1995). This is a complicated task, especially for anticoagulant rodenticides (ARs), as exposure (dose) frequently occurs over multiple days and can result from consumption of poisoned animals containing varying concentration of ARs and their metabolites, not simply a toxic bait.

K.E. Horak, PhD (✉)
U.S. Department of Agriculture, National Wildlife Research Center,
4101 LaPorte Avenue, Fort Collins, CO 80521, USA
e-mail: katherine.e.horak@aphis.usda.gov

P.M. Fisher • B. Hopkins
Landcare Research, Lincoln, New Zealand

N.W. van den Brink et al. (eds.), *Anticoagulant Rodenticides and Wildlife*,
Emerging Topics in Ecotoxicology 5, DOI 10.1007/978-3-319-64377-9_4

Although ARs have been in use for decades, there are limited data on the ADME of these compounds for non-target animals. Most of the data have been derived from laboratory strains of rodents as a traditional model system. Current understanding of the pharmacokinetics (how the exposed animal affects the compound?) and pharmacodynamics (how the compound affects the exposed animal?) of ARs tend to focus on how their toxic effects are mediated in a particular species (e.g., Littin et al. 2002). Such information is essential to predicting the efficacy of ARs in rodent management and adverse effects of their use on non-target species, and allows for a more accurate comparison of various classes of ARs (first-, intermediate- and second-generation). When information about the ADME properties of an AR are combined with data such as binding affinities and physiological/ecological characteristics of an organism, more accurate risk assessments can be made. These hazard and risk assessments are important as the US EPA and other regulatory agencies include pharmacokinetic data and models in their work plans and formal ecological risk assessments.

2 Formulations

All ARs currently on the market are formulated into palatable baits for oral delivery to targeted rodent pests. Typically, bait formulations are based on cereal grain made into compressed blocks or pellets along with smaller portions of binding, flavoring, non-target deterrents and coloring (blue or green) agents. A wax or water proofing ingredient is included in some formulations. Such bait formulations impart low production costs plus relative safety and ease of application, usually in a bait station. Other types of specialized formulations do exist but have more limited applications in particular environmental conditions, for example liquid concentrates that can be diluted for use in areas where an abundant food source is present, or in areas where there is little or no water supply. Similarly, concentrated anticoagulant tracking powders are available for use when conventional baits may not be appropriate or accepted (e.g., dusting into rat burrows that are likely to lead into buildings and into wall voids which are not accessible for normal bait placement).

3 Routes of Exposure

While current bait formulations rely on voluntary ingestion of sufficient quantities for efficacy against target animals, they also present the potential for unwanted primary exposure of other animals that access and eat bait. Secondary oral exposure to ARs can also occur via ingestion of animal tissues that contain AR residues; this exposure pathway has emerged as a particular concern for wildlife species that prey on rodents or scavenge the carcasses of poisoned animals.

Besides their use as rodenticides some anticoagulant compounds, particularly warfarin (2001) and historically diphacinone (e.g. Field et al. (1952)), have wide application in human anticoagulation therapy. Human exposure to anticoagulants through rodenticide bait can occur (Tran and King 2015; Watt et al. 2005) and is most typically oral, whether accidental (e.g., exploratory ingestion by very young children) or deliberate (e.g., attempts at self-harm) (e.g. Bruno et al. 2000). Less commonly dermal (Spiller et al. 2003) or inhalation (Lee et al. 2014) exposure of humans has been documented in the context of occupational exposure during manufacture or inappropriate application of bait for rodent control. Development and refinement of human therapies and treatments for anticoagulant poisoning have substantially driven research and clinical evaluations of pharmacokinetics and responses of blood coagulation indices (e.g., prothrombin time following treatment regimens with Vitamin K_1) (Hanslik and Prinseau 2004).

4 Absorption, Distribution, Metabolism and Excretion

A study by Subota et al. (2016) examined the absorption and physiological effects of a dermally applied solution of sodium warfarin in laboratory rats (*Rattus norvegicus*). They found that dermal applications of 10 μg and 100 μg of warfarin significantly increased prothrombin time (PT) when compared to controls indicating that the compound is absorbed through the skin in quantities sufficient to exert its pharmacological effect. The dermal absorption of the coumarin backbone of the class of anticoagulants has also been studied. The absorption of coumarin in both ethanol and oil:water emulsion was tested using both human and rat skin (Yourick and Bronaugh 1997). In both human abdominal and rat dorsal skin, absorption was greater in the emulsion vehicle and the majority of the absorption occurred within six hours of application. No coumarin metabolism was found in these experiments, suggesting that transdermal exposure to coumarins will result in a higher systemic exposure to the active parent compound than oral exposure prior to first pass metabolism. Additional studies of skin absorption of coumarin also found that it was extensively absorbed through human, rat, and mouse skin which was attributed to the compound's lipophilicity (Beckley-Kartey et al. 1997).

Active concentrations in bait are typically 0.02–0.05 g/kg for second-generation ARs (SGARs) and 0.25–0.5 for the less potent first-generation ARs (FGARs). Here we focus on oral exposure resulting from ingestion of bait containing an AR and also ingestion of animal tissue that contains residual anticoagulant concentrations. Anticoagulants are generally considered to be well-absorbed through the gastrointestinal tract (Watt et al. 2005), presumably within a few hours. Wright and Hayden (1955) suggested that the efficacy of absorption of a coumarin derivative in rats could be reduced by the presence of high fat levels in gut contents. Berny et al. (2006) indicated that anticoagulants (warfarin, chlorophacinone and bromadiolone) underwent very little, if any, degradation by microflora in sheep rumen.

After absorption across the intestinal epithelium, anticoagulant compounds enter the hepatic circulation prior to being distributed throughout the whole body of mammals. In birds, a fraction of the blood from the gastrointestinal tract is directed to the kidneys through the coccygeomesenteric vein. This can dramatically decrease the anticoagulant concentration in the systemic circulation as the compounds are metabolized by the liver largely through the action of cytochrome P450 isozymes (CYPs). Ring hydroxylation is thought to be an important biotransformation step for the coumarin-based anticoagulant compounds, although this is less well described for the indandione compounds. Hydroxylated metabolites can further undergo conjugation with glucuronic acid prior to entering the systemic circulation, and potentially undergoing enterohepatic recirculation. This can affect the amount of compound that reaches target locations. Enterohepatic recirculation of unmetabolized parent compound, may partly explain the higher toxicity of some of the second-generation anticoagulants (Bachmann and Sullivan 1983).

Metabolism, distribution, and excretion of ARs are affected by numerous factors. Hepatic metabolism of ARs exhibits a biphasic profile. Initial metabolism is rapid, often clearing a large percentage of the compound within the first days following exposure. In the terminal phase of hepatic metabolism, clearance of anticoagulant compound occurs more slowly leading to the low residual levels of SGARs often found for long periods of time post-exposure. Anticoagulant rodenticides, particularly warfarin, are highly protein-bound to serum albumin (Watt et al. 2005). This affects their distribution to active sites and therefore necessitates higher bait concentrations to ensure adequate unbound compound is available to interact with target proteins. Some second-generation ARs, such as brodifacoum and flocoumafen, appear to undergo relatively little biotransformation being excreted largely as unchanged parent compound in feces. This is in contrast to first-generation compounds that appear to undergo more extensive metabolism, with a range of metabolite compounds excreted in urine.

In addition to providing information about effects to the primary target animal, a firm understanding of the ADME characteristics of ARs will help in assessing the potential risks they pose to non-target species. The information provided below serves as a summary of studies of the pharmacokinetics of AR in non-target species. When examined together, these studies give a general understand of the ADME characteristics of ARs in non-target species. However, the paucity of data on some compounds and species indicate that additional studies should be undertaken to improve our understanding of the potential effects across species.

4.1 Warfarin

Warfarin is a racemic mixture of the R and S stereoisomers with the (S)-isomer having approximately seven-fold greater activity than the (R)-isomer (Breckenridge and L'E Orme 1972). Cytochrome P450 mediated warfarin metabolism is well established in mammalian species and has been employed as a determinant of

Table 4.1 Mean metabolic activities (pmol/min/nmol P450) of each of the hydroxylated forms of warfarin in rat, chicken, ostrich and mallard (*Anas platyrhynchos*) owl includes snowy owl and great horned owl

Species	4′-OH	6′-OH	7′-OH	8′-OH	10′-OH	Total
Rat	92.8	48.5	14.9	2.3	37.4	196.0
Chicken	1204.0	294.5	113.7	43.4	4.5	1660.2
Ostrich	523.4	68.0	17.2	30.4	ND	638.0
Mallard	96.8	18.2	10.5	ND	9.1	134.6
Owl	25.9	2.6	ND	1.5	ND	32.0

Adapted from Watanabe et al. (2010)

warfarin sensitivity. The stereoisomers are metabolized by different phase 1 enzymes; the predominant metabolism of the S isomer in humans is via CYP2C9 whereas metabolism of R-warfarin is mainly via CYP3A4 with involvement of CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C18 and CYP2C19 (Rettie et al. 1992; Ngui et al. 2001; Zhang et al. 1995; Wienkers et al. 1996). In rats, CYP1A, CYP2B, CYP2C, and CYP3A isoforms are known to metabolize warfarin (Guengerich et al. 1982). This phase 1 metabolism is achieved through the production of relatively inactive hydroxywarfarins – 6-, 7-, 8-, 10-, and 4′-hydroxywarfarin (Kaminsky and Zhang 1997; Jones and Miller 2011). It has been reported that one of the mechanisms that induces warfarin-resistance in rats (*Rattus rattus*) is elevated CYP-mediated warfarin metabolism (Ishizuka et al. 2007). Phase II metabolism of warfarin has not been well studied, although it is known that sulfated and glucuronyl conjugates (Sutcliffe et al. 1987) can be formed and that the primary metabolites are made more water-soluble by phase II enzymes (Jansing et al. 1992). It has been suggested that the glucuronide metabolites are principally formed in the kidney in man (Kaminsky and Zhang 1997). Elimination of warfarin is predominantly renal; however, salivary excretion has also been described in rabbits (Sakai et al. 1983).

In contrast to rats, it appears that the major CYP isoforms of bird species are different to the predominant isoforms in mammals. The dominant CYP isoforms of bird species are thought to be CYP2H1/2 (resembles rat CYP2B1/2), CYP1A4/5 (resembles rat CYP1A1/2) or an inducible form that resembles rat CYP2E (Louis et al. 1993). Some bird species demonstrate a warfarin metabolic activity that is significantly greater than in rats (e.g., in chickens, *Gallus domesticus*, warfarin metabolic activity to hydroxylated forms of warfarin was observed to be >5-fold greater than rats). However, such activity is quite variable among avian species (Table 4.1). This difference in activity between mammals and birds and among avian species corresponds directly to the rank order of warfarin susceptibility, as demonstrated in owls *(*snowy owl, *Bubo scandiacus* and great horned owl, *Bubo virginianus)* which show very little warfarin metabolism compared with rodents and other bird species. This low metabolism may also slow the elimination rate of warfarin which suggests that owls possess a low ability to detoxify anticoagulant rodenticides in general, a contributing factor perhaps to the high frequency of reported poisoning accidents in these species. In addition to the usual five major hydroxylated metabolites identified in mammals, birds also appear to produce a unique warfarin alcohol

metabolite (Table 4.1) (Watanabe et al. 2010). This suggests that birds have an ability to uniquely metabolize warfarin, which differs from that of rats and human (Rettie et al. 1992; Ngui et al. 2001). Saengtienchai et al. (2011) propose that in chickens, this is through activity of aldehyde oxidase in cytosol rather than being a CYP-mediated pathway.

Interestingly, another study by the same group (Watanabe et al. 2015) found that the half-life of warfarin in chicken was longer than in most mammalian species that possess greater warfarin metabolic ability. This suggests that protein binding of warfarin in the chicken (primarily to albumin in the plasma) may be greater in birds which results in the longer half-life and less toxicity despite the high metabolic ability. Watanabe and coworkers postulated that since the albumin content in birds is less than in mammals, it might have greater warfarin-binding capacity. In regards to other bird species, Watanabe et al. (2015) also characterized warfarin metabolism in liver microsomes of the crow (*Corvus macrorhynchos*), mallard and ostrich (*Struthio camelus*). Microsomes from chickens and crows had higher activity than those from mallard or ostrich, further suggesting that the half-life of warfarin may be variable among avian species.

Warfarin has been shown to interact with the ABCB1 transporter in human liver (Wadelius et al. 2004). In modelling human pharmacodynamics, Holford (1986) determined that warfarin is essentially completely absorbed, reaching a maximum plasma concentration between 2 and 6 h. It distributes into a small volume of distribution (10 L/70 kg) and is eliminated with a very low clearance (0.2 L/h/70 kg) and elimination half-life is about 35 h (Holford 1986). Warfarin also undergoes extensive biliary (enterohepatic) recycling with approximately 10% of administered dose of ^{14}C–warfarin excreted by rats within 5 h after intraperitoneal injection (Powell et al. 1977). This latter study found relatively little radioactivity in the feces, indicating that considerable enterohepatic recycling takes place with extensive gut flora-mediated hydrolysis of warfarin conjugates occurring. This was confirmed by Remmel and coworkers (1981) who identified that there was a significant decrease in the volume of distribution of warfarin in antibiotic-treated rats.

The time course of warfarin concentrations in the serum, liver, kidneys, muscle, and abdominal fat of male Sprague-Dawley rats after intravenous injection of a 0.25 mg/kg or 1.0 mg/kg body weight has been determined (Levy et al. 2003). Warfarin concentrations in serum declined exponentially by over one order of magnitude between 80 and 240 h after intravenous injection of a 0.25 mg/kg or 1.0 mg/kg dose. During this time, concentrations in other tissues declined more slowly. In another study, S-warfarin concentrations in serum and liver were followed for approximately 50 days after intravenous injection of 1 mg/kg dose. This revealed a very slow terminal elimination phase in serum nearly parallel to the decline in liver warfarin concentrations. The residues of warfarin in liver of rats over time after a single sublethal dose declined from 1.26 μg/g at 1 week, to below the limit of detection (<0.1 mg/g) at 24 weeks after dosing (combined results of 2 trials), with an estimated liver-elimination half-life of 26.2 days (Fisher et al. 2003) (Table 4.2).

Although an effective anticoagulant, because of its low molecular weight, warfarin can readily cross the placenta; therefore, administration during pregnancy has

Table 4.2 Half-life values reported for warfarin in blood and liver

Species	Plasma t ½	Liver t ½ (days)	Reference
Norway rat		26.2	Fisher et al. (2003)
House mouse	14.9 days*	66.8	Vandenbroucke et al. (2008)
Cat	26.2 h		Smith et al. (2000b)
Human	42 h		O'Reilly et al. (1963)
Human	35 h		Holford (1986)
Human (R isomer)	20–60 h		Breckenridge et al. (1974); Hewick and
Human (S isomer)	18–35 h		McEwen (1973); O'Reilly (1973, 1976;
Rats	6–41 h		O'Reilly et al. (1971); Orme and
Rats (ex-germ free)	18.2 h		Breckenridge (1976)
			Yacobi et al. (1975)
			Remmel et al. (1981)
Chicken	27.4 h (male)		Watanabe et al. (2015)
	34.0 h (female)		

Plasma half-life significantly longer than that reported in other species (perhaps an artifact of high dose levels).

consequences in regards to reduced mean birth weight (Sareli et al. 1989) and increased percentage of abortion, stillbirth, and neonatal death incidences (Sareli et al. 1989; Ginsberg and Hirsh 1989). The majority of the information regarding coumarin anticoagulants in pregnancy has been derived from the use of warfarin to prevent pulmonary embolism in pregnant women (Ginsberg and Hirsh 1989; Greaves 1993). Howe and Webster (1992) showed that neonatal rats administered subcutaneous warfarin and Vitamin K_1 for up to 12 weeks developed marked maxillonasal hypoplasia. In humans, warfarin is a known teratogen that induces nasal hypoplasia that can lead to neonatal respiratory distress and upper airway obstruction; stippled epiphyses marked by multiple ossification centers that severely deform the long bone that could lead to dwarfism, and central nervous system defects such as microcephaly, hydrocephalus, agenesis of corpus callosum, and Dandy-Walker malformation have also been associated with warfarin exposure (Ginsberg and Hirsh 1989). In addition, eye anomalies are also evidenced (optic atrophy, microphthalmia, and Peter anomaly of eye) (Hall 1990).

Pharmacokinetic studies of warfarin enantiomers indicate that significant differences exist in clearance of S- and R-warfarin in humans, rats and rabbits (Hignite et al. 1980). These findings have also been confirmed in cats (Smith et al. 2000a), with the enantiomers being highly protein-bound (>99%) at all concentrations evaluated, as described in other species (Brown et al. 1979). However, the binding affinity for both enantiomers to human and rat albumin appears to be significantly greater than other species evaluated (Panjehshahin et al. 1992), and is enantioselective appearing to have higher binding affinity for S-warfarin (Park 1988; Brown et al. 1979). Warfarin has also been detected in the albumen and yolk of hens eggs after warfarin ingestion (Kammerer et al. 1998). The study suggests that the kinetics involved are two fold – the first a direct diffusion of warfarin across the magnum

wall of the chicken into the egg's albumen one day after ingestion, and the second a later accumulation at 3–4 days which appears to arise from liver depots. Although appearing to be an effective elimination route, the amount of excretion into the egg was minor compared to that excreted in urine and feces. However, as only the levels of the parent warfarin were measured, the elimination efficiency and accumulated levels of any metabolites remains unknown.

4.2 Coumatetralyl

The Danish Environmental Protection Agency (2011) produced a comprehensive regulatory data dossier on coumatetralyl from which much of the following toxicokinetic information was summarized. As with other anticoagulants, in rats coumatetralyl is absorbed quickly after oral dosing. Maximum blood concentrations in rats occurred at 3 h after single dosing in males and between 8 and 24 h in females, with absorbed fraction estimates of 75% and 86% of total dose for males and females, respectively. After single oral dosage of radiolabeled coumatetralyl, 49–56% of administered radioactivity was retained in the body (excluding the gastrointestinal tract). The largest fractions were found in liver (21–25%) and skin (7–16%). All other organs retained less than 1% of the dose at sacrifice (Danish Environmental Protection Agency 2011). Reported persistence and half-lives of coumatetralyl in blood and liver are summarized in Table 4.3. Coumatetralyl has an intermediate hepatic persistence in comparison to the SGARs.

The primary route of coumatetralyl excretion in rats is via urine and to a smaller extent via feces, with respiratory (exhaled) excretion negligible. The rate of excretion was sex-dependent, where single-dosed males excreted about 20% of an administered dose in urine and about 20% in feces until sacrifice at 7 days. In comparison, single-dosed females excreted about 37% in urine and about 12% in feces. With repeated oral dosing, the ratio shifted towards 44% renal and 33% fecal excretion until sacrifice, which was possibly due to enzyme induction over a 14 day period (Danish Environmental Protection Agency 2011).

Table 4.3 Half-life values reported for coumatetralyl in blood and liver of rodents

Species	Plasma t ½ (hours)	Liver t ½ (days)	Reference
Norway rat		55	Parmar et al. (1987)
Norway rat		62	Eason et al. (2003)
Norway rat	71 (male) 46 (female)		Danish Environmental Protection Agency (2011)
House mouse (*Mus musculus*)	12.5	15.8	Vandenbroucke et al. (2008)
Red deer (*Cervus elaphus scoticus*)		18.9	Crowell et al. (2013)

Coumatetralyl is metabolized by hydroxylation, with four metabolites identified in urine and feces. In rats, sex and dose did not have major effects on the metabolite profiles. One main metabolite comprised 27% of the administered oral dose, with three isomers of this main metabolite making up less than 10% of the dose. Two further minor metabolites identified in urine (2% of the dose), with only traces of unchanged coumatetralyl excreted in urine (Danish Environmental Protection Agency 2011).

4.3 Chlorophacinone

As for other first- or intermediate-generation anticoagulants, chlorophacinone is most efficacious when consumed in numerous doses over multiple days, i.e., a lethal single dose requires a far greater quantity than the additive quantities that constitue a lethal multiple/chronic dose (e.g., Jackson and Ashton 1992). In the rat, the main route of chlorophacinone elimination is fecal (>99%). Belleville (1977) reported that in a study of rats dosed with C^{14}-labelled chlorophacinone, 90% was recovered in the feces within 48 h of oral administration and 100% within 4 days. Biliary excretion was investigated in this study, and it was found that 8 h after intraduodenal chlorophacinone administration, 26% of the radioactivity was found in the bile, providing further support that excretion in the feces is the main route of elimination. Four hours after a single oral dose, concentration of chlorophacinone in the blood reached its peak, and tissue to blood ratios were 4.2 for liver:blood, 0.9 for kidney:blood and 0.6 for lung:blood (Belleville 1977). Berny et al. (2006) found the oral bioavailability of chlorophacinone to be 92% in sheep with little degradation in the rumen. Vein et al. (2013) found that rats from wild populations with known resistance to ARs did not show any differences in profiles of hepatic accumulation of chlorophacinone over 4 days following bait ingestion. In the same study, these authors described extensive metabolism of chlorophacinone, with similar proportions of three hydroxylated metabolite compounds produced by each strain of rat, with one dominant metabolite accounting for approximately 50% of residual concentration in liver.

Two studies have examined chlorophacinone half-life in tissue (Table 4.4). Sheep dosed intraruminally with 1 mg chlorophacinone/kg body weight had a time to maximum plasma concentration of 30 h in contrast to intravenous dosing with peaked values at the time of dosing (Berny et al. 2006). Half-lives of chlorophacinone in blood and liver are shown in Table 4.4.

Table 4.4 Half-life values for chlorophacinone in blood and liver

Species	Plasma t½ (hours)	Liver t½ (days)	Reference
Sheep	30.1		Berny et al. (2006)
Mouse	11.7 days	35.4	Vandenbroucke et al. (2008)

4.4 Diphacinone

Like chlorophacinone, diphacinone is more toxic when ingested as consecutive, multiple doses over a number of days (i.e., a lethal single dose requires a far greater quantity than the additive quantities that constitue a lethal multiple/chronic dose). For rodents, there are highly variable chronic oral toxicity estimates with apparent differences in the susceptibility of male and female animals (e.g. Kusano 1974). Reported persistence and half-lives of diphacinone in blood and liver are summarised in Table 4.5.

Yu et al. (1982) reported that diphacinone was not extensively metabolised by rats following oral administration of C^{14}-labelled diphacinone at 0.2 mg/kg or 1.5 mg/kg, with more than 60% of the dose excreted in feces and 10% in urine over 8 days, with the same elimination pattern observed in mice. Continued elimination in feces over 4–8 days indicated that biliary excretion rather than incomplete absorption was the major route of elimination in both rodent species. At 8 days after dosing, rat tissues retained about 20% of radiocarbon (tracer in administered dose), with highest concentration in the liver, substantial residues in kidney and lung, and lower residues in brain, fat, blood and muscle (Yu et al. 1982). Another study, of radio-labelled diphacinone administered orally to mice (Cahill and Crowder 1979) reported similar results, with radioactivity reaching highest levels in liver and lungs, with maximum liver concentrations at 3.0–7.5 h after administration. In an additional study of the excretion of radio-labelled diphacinone in mice, Cahill (1977) did not detect unchanged diphacinone in feces, indicating the compound was likely to be efficiently absorbed in the gastrointestinal tract and extensively metabolised, prior to excretion of as-yet undefined metabolites in urine.

In contrast to findings in rodents, Bullard et al. (1976) reported that cows dosed with 1 mg/kg diphacinone by intraruminal injection had almost constant liver residues of up to 0.15 mg/g over 30–90 days after dosing. Another study by the same group found mammillary transfer of diphacinone to milk of cows dosed intraruminally with 2.75 mg/kg. The milk from these cows contained a maximum concentration of 0.021 ppm diphacinone. They did not detect any diphacinone in the

Table 4.5 Half-life values reported for diphacinone in liver. Liver retention is expressed as the time period for which residues are reported to persist in the liver unless the value is preceded by t½

Species	Liver t½ (days)	Reference
Norway rat	t½ 3	Fisher et al. (2003)
Cattle (*Bos taurus*)	>90	Bullard et al. (1976)
Deer (*Cervus elaphus scoticus*)	t½ 6 (mean)	Crowell et al. (2013)
Pig (*Sus scrofa*)	t½ 5.43–14.12 t½ 12.4	Fisher (2006) Crowell et al. (2013)
American kestrel (*Falco sparverius*) Eastern screech-owl (*Megascops asio)*	t½ 0.325 (initial) t½ 2.49 (terminal) t½ 0.88 (initial) t½ 29.2 (terminal)	Rattner et al. (2011, 2014)

milk of cows dosed with 1 mg/kg diphacinone, leading to the hypothesis that transfer of diphacinone to the milk is dependent on plasma concentration (Bullard et al. 1977). Recent research (Crowell et al. 2013) confirms that diphacinone is metabolised and distributed quite differently in cattle compared with in other mammals, including exhibiting longer hepatic persistence.

In two studies investigating the effects of anticoagulants to non-target raptors, Rattner and colleagues exposed both American kestrels (*Falco sparverius*) and eastern screech owls (*Megascops asio*) to diphacinone. Kestrels were dosed with diphacinone in a gelatin capsule at a range of 35.1–675 mg/kg body weight. In this study, birds that succumbed within 24 h had greater liver residue levels than those that died 27–47 h post dose, (32.8–56.3 μg/g liver and 13.4–19.4 μg/g liver, respectively). From these data, the clearance of diphacinone from the liver was determined to be biphasic with an initial half-life of 0.88 days (7.8 h) and a terminal half-life of 29.2 days (Rattner et al. 2011). In a separate study, eastern screech owls were provided diphacinone mixed into their diet for three or seven days for a cumulative average dose of 4.74 mg/kg or 7.98 mg/kg body weight. During the dosing period, kidney residue levels were greater than those found in the liver with the concentration of diphacinone in both tissues decreasing rapidly in the post-dose period being <0.1 μg/ g liver after three weeks. Clearance of diphacinone was again found to be biphasic and cleared more rapidly from the kidney than the liver. Initial half-life was found to be 0.88 days in liver and 0.14 days in kidney while terminal half-life (day 4–21 post-dose) in liver of 29.2 days and kidney 10.2 days (Rattner et al. 2014).

4.5 *Pindone*

Reported persistence and half-lives of pindone in blood and liver are summarised in Table 4.6. In mammals, pindone is far less persistent than SGARs and also less persistent in liver than other FGARs. In dogs, pindone was fairly well absorbed (67%) but tissue distribution appeared to be slightly different from other anticoagulants, as plasma concentrations remained higher than tissue concentrations for 8 days, while concentrations in liver and kidney were comparable (Fitzek 1978).

Table 4.6 Half-life estimates for pindone in blood and liver

Species	Plasma t½ (hours)	Liver t½ (days)	Reference
Norway rat		2.1	Fisher et al. (2003)
Dog	120		Fitzek (1978)
Dog	45.6		Martin et al. (1991)
Sheep		8–16	Nelson and Hickling (1994)
Sheep	96–120		Robinson et al. (2005)
Cattle	74.4		Martin et al. (1991)
Chicken	67.2		Martin et al. (1991)

In sheep, residues were detected in the liver and fat of dosed animals for 8 days, but after 2 weeks no residues were detectable (Nelson and Hickling 1994). No information was found about the excretion profile of pindone.

4.6 *Brodifacoum*

After ingestion and absorption of brodifacoum by mammals, high concentrations are bound in the liver and remain stable, being slowly metabolised and excreted (Erickson and Urban 2004). The persistence of brodifacoum in mammalian liver is well documented and is in contrast with its more rapid elimination from blood (Table 4.7). While no hepatic half-life values were found for avian species, limited data suggests that birds have similarly prolonged persistence of brodifacoum in liver. Brodifacoum concentrations in chicken livers showed little variation (mean 0.66, range 0.45–1.00 μg/g) over 14 days following sublethal oral exposure (Fisher 2009). Similarly, in Japanese quail (*Coturnix japonica*) orally administered brodifacoum (0.8 and 2.5 mg/kg body weight), there was no substantial decline in hepatic concentrations over the following 5 days (Webster et al. 2015).

Table 4.7 Half-life values reported for brodifacoum in blood plasma/serum and liver following a range of exposure levels and routes of administration. Liver retention is expressed as the time period for which residues are reported to persist in the liver unless the value is preceded by t½. Plasma is t½ unless otherwise specified

Species	Plasma t½ (hours)	Liver retention (days)	Reference
Rat		t½ 350	Batten and Bratt (1990)[a]
Rat		t½ 128 (terminal)	Batten & Bratt (1990)[a]
Rat		t½ 282	Hawkins et al. (1991)[a]
Rat		t½ 150–200	Bratt & Hudson (1979)[a]
Rat		t½ 136	Belleville (1991)[a]
Rat	156 (serum)	>80	Bachmann and Sullivan (1983)
Rat		t½ 130	Parmar et al. (1987)
Rat		t½ 113.5	Fisher et al. (2003)
Mouse	2200.8	t½ 307.4	Vandenbroucke et al. (2008)
Rabbit	60.8 ± 1.9 (terminal)		Breckenridge et al. (1985)
Dog	144		Woody et al. (1992)
Dog	21.6–112.8		Robben et al. (1998)
Dog	1.4 (initial) 8.7 (terminal)		Murphy et al. (1985)
Possum	480–720	>252	Eason et al. (1996)
Sheep		>250	Laas et al. (1985)
Horse	28.8		Boermans et al. (1991)
Chicken	27.36		Fisher (2009)

[a]Unpublished reports cited by Erickson and Urban (2004)

Bachmann and Sullivan (1983) suggested that serum and liver concentrations of brodifacoum were likely to accumulate with repeated daily exposures, based on simulations of the data they obtained following a single 0.2 mg/kg dose to laboratory rats. The major route of excretion of unbound brodifacoum in mammals is through feces (Sutcliffe et al. 1987). Field observations of rodent feces colored to different extents by the green or blue dyes used in anticoagulant bait formulations are common - an estimated 14–21.6% of ingested brodifacoum is excreted unchanged in rat feces in the period between ingestion of a lethal amount of bait and death (Fisher 2009).

To better understand the pharmacokinetic properties of brodifacoum, Bachmann and Sullivan (1983) gavaged male Sprague Dawley rats with varying doses of brodifacoum in polyethylene glycol 400. They found that metabolic processes play a significant role in the efficacy of brodifacoum as pretreatment with SKF525A, a non-selective cytochrome P450 inhibitor, increased the anticoagulant effect whereas phenobarbital, cytochrome P450 inducer, decreased the anticoagulant effects (Bachmann and Sullivan 1983).

4.7 Bromadiolone

Absorption of bromadiolone in laboratory rats was >70% of the administered dose, based on carcass, bile and urinary excretion measurements (European Food Safety Authority 2010). In rats given an oral dose of radiolabelled bromadiolone, within 48 h approximately 53% of was excreted in feces and <1% in urine (European Food Safety Authority 2010). It seems that biliary elimination plays a major role, with about 30% of an administered bromadiolone dose excreted in bile in the 8 h after dosing (Nahas 1987). Bromadiolone is extensively metabolised to at least 10 minor metabolites and 1 major metabolite, tentatively identified as a benzylic carbon hydroxylated analogue of the parent compound. This metabolite is conjugated with glucuronic acid before excretion in bile and hydrolysis in the gastrointestinal tract (European Food Safety Authority 2010).

After oral administration to rats, bromadiolone was eliminated relatively quickly from blood, resulting in high concentrations in liver tissues (Kamil 1987). Elimination of radiolabelled bromadiolone from rat liver is reported to be biphasic, consisting of a rapid initial phase lasting from 2 to 8 days after dosing and a slower terminal phase (Anonymous 2010). Reported persistence and half-lives of bromadiolone in blood and liver are summarised in Table 4.8, noting an unusually long half-life in blood of mice (Vandenbroucke et al. 2008). In a study using pigs, Enouri et al. (2015) dosed two treatment groups with either 0.05 or 0.5 mg/kg bromadiolone and euthanized sub-groups at time points up to 9 weeks. Detectable levels of bromadiolone were found in liver, skin and adherent fat, feces, and plasma three week post-dosing. As expected**,** liver tissue had the highest bromadiolone residue concentrations. Liver values for pigs dosed with 0.05 mg/kg were 51.8 μg/g at six weeks while pigs dosed with 0.5 mg/kg bromadiolone had liver residues of

Table 4.8 Half-life values reported for bromadiolone in blood and liver following a range of exposure levels and routes of administration. Liver retention is expressed as the time period for which residues are reported to persist in the liver unless the value is preceded by t½

Species	Blood t½ (hours)	Liver retention (days)	Reference
Norway rat (*Rattus norvegicus*)	26–57		Kamil (1987)
Norway rat	25–26	t½ 170	Parmar et al. (1987)
Rat		318	European Food Safety Authority (2010)
Sheep		256	Nelson and Hickling (1994)
Mouse	33.3 days	28.1	Vandenbroucke et al. (2008)

213.9 μg/g at nine weeks post dosing. The authors also reported a biphasic depletion of bromadiolone in the feces and that clearance was not dose-dependent in liver and muscle tissues as the slope of the concentration vs time graphs were the same regardless of dose administered (Enouri et al. 2015).

4.8 Difenacoum and Difethialone

There is limited information about the pharmacokinetics of difenacoum or difethialone. Vandenbroucke et al. (2008) cite an unpublished report (Bratt 1987) where an oral dose of radiolabelled difenacoum (1.2 mg⁄ kg) administered to rats produced the highest concentration of radioactivity in liver after 24 h, indicating a similar timeframe of absorption as other anticoagulant compounds. Biphasic elimination of difenacoum from liver and a terminal half-life of elimination of 118 days is also cited (Vandenbroucke et al. 2008). In a 1985 study by Breckenridge and coworkers, male New Zealand rabbits were intravenously dosed with difenacoum dissolved in polyethylene glycol 200. Difenacoum exhibited a biphasic decay in the plasma with a terminal half-life of 83.1 h. This terminal half-life is much great than that for warfarin, which was attributed to difenacoum's greater volume of distribution relative to warfarin (Breckenridge et al. 1985). In their assessment of multiple anticoagulant compounds in mice, Vandenbroucke et al. (2008) estimated plasma elimination half-lives of 20.4 and 38.9 days for difenacoum and difethialone, respectively, and liver elimination half-lives of 61.8 and 28.5 days, respectively. In a study of dogs administered a range of anticoagulant compounds, Robben et al. (1998) estimated plasma half-life of difethialone in two animals of 2.2 and 3.2 days. More recently, Damin-Pernik et al. (2016a) showed that while cis- and trans-isomers of difenacoum were similarly absorbed after oral administration to laboratory rats, in liver the half-life of the trans-isomers were 24.2 h compared to 706 h for the cis-isomers.

Table 4.9 Half-life values reported for flocoumafen in liver following a range of exposure levels and routes of administration. Liver retention is expressed as the period for which residues are reported to persist in the liver unless the value is preceded by t½

Species	Liver retention (days)	Reference
Norway rat (*Rattus norvegicus*)	$t^1/_2$ 220	Huckle et al. (1988)
Sheep	>256	Nelson and Hickling (1994)
Quail (*Coturnix coturnix*)	$t^1/_2 > 100$	Huckle et al. (1989b)
Dog	>300	Veenstra et al. (1991)
Mouse (*Mus musculus*)	93.8	Vandenbroucke et al. (2008)

4.9 *Flocoumafen*

In rats, absorption of orally-administered flocoumafen is rapid, reaching a maximum concentration in blood after 4 h and falling to half-maximum by 8 h (Huckle et al. 1989a). The persistence of flocoumafen in liver is of a similar extent to that of brodifacoum (Table 4.9). A plasma half-life of flocoumafen in mice was estimated as 26.6 d (Vandenbroucke et al. 2008), consistent with the overall shorter retention time of anticoagulants in blood compared to liver.

Following administration of flocoumafen, liver residues in rats consisted mainly of unchanged flocoumafen, although in a repeat-dose study a polar metabolite was also detected. Eight urinary metabolites were detected after percutaneous exposure to ^{14}C–flocoumafen but represented a small proportion of the total dose, with most excretion occurring in the feces as unchanged flocoumafen (Huckle and Warburton 1986 cited by Eason and Wickstrom (2001)). Veenstra et al. (1991) found retention of about 8% of an administered flocoumafen dose of 0.4 mg/kg in the liver of beagle dogs 300 days after dosing. When oral ^{14}C–flocoumafen doses of 0.02 mg/kg or 0.1 mg/kg body weight were given to rats, once weekly for up to 14 weeks, approximately one-third of each weekly low dose was eliminated through the feces within 3 days, mostly within the first 24 h. At the higher dose, the fecal excretion ranged from 18% after the first dose to 59% after the 10th dose (Huckle et al. 1988). This study indicated the potential of flocoumafen to bioaccumulate in rat liver, as hepatic residues increased throughout the 14 week repeat-dosing period.

Japanese quail metabolize flocoumafen more rapidly than rats, with a proportion of an administered dose retained in the liver and an elimination half-life of 155 days. In quail, fecal excretion of radio-labelled flocoumafen following an oral dose of 0.14 mg/kg body weight accounted for 23–26% of the dose over the 7-day period; approximately half of this was recovered within the first 24 h with less than 0.5% of the dose excreted in urine within 7 days (Huckle et al. 1989b).

In a dietary toxicity study, 30-week-old laying hens were fed a diet designed to produce different exposures to flocoumafen (1.5, 5, 10 and 50 mg/kg) over five consecutive days (Eadsforth et al. 1993). The LC_{50} at the end of a 28-day observation period was 16.4 mg/kg. Livers of birds administered 5–50 mg/kg had concentrations of flocoumafen (1.5 nmol/g) that were independent of dose, indicating a

saturable high-affinity binding site in liver. While residual flocoumafen in the corresponding muscle samples was at relatively low concentrations in all treatment groups, higher dose-related residues were found in samples of abdominal and skin-associated fat and there was clear evidence of the transfer of dose-related residues into eggs. Eadsforth and colleagues also describe another study in which hens were dosed with [^{14}C]flocoumafen for five consecutive days at a daily rate of 1 mg/kg or 4 mg/kg body weight, where 68% of the daily radioactive dose was eliminated over the following 24 h via excreta (Eadsforth et al. 1993). Residues in liver at death through poisoning or at euthanasia accounted for <1% of the cumulative administered radioactivity. Residues in eggs at 10 days after first exposure were primarily in the yolk with maximum concentrations of 1.0 mg/kg (0.18% of the low dose) and 2.1 mg/kg (0.06% of the high dose), with 40% of the total activity in the yolk comprising unchanged flocoumafen (measured as ^{14}C- flocoumafen equivalents).

5 Toxicokinetics of Anticoagulant Rodenticides

While the mechanism of action of warfarin (i.e., coumadin) and its time course of therapeutic effect (i.e., prolonged prothrombin time) have been thoroughly investigated in man (e.g., Holford 1986; Sutcliffe et al. 1987), remarkably few studies have examined the time course of AR toxicity (i.e., coagulopathy) and clotting time recovery that accompanies exposure in non-target animals. Following administration of a therapeutic dose or a toxic dose(s) and the inhibition of vitamin K epoxide reductase, there is a lag period of several days prior to the appearance of prolonged clotting time. This lag period (2–3 days) reflects the clearance half-life of fully-functional coagulation factors, for example, half-life of factors II, VII, IX and X in man are 120 h, 6 h, 18 h and 65 h, respectively(Lee et al. 2006) accompanied by the appearance of increasing quantities of *des*-carboxy dysfunctional factors that are unable to assemble on cell surfaces to form active coagulation complexes. A detailed study in which domestic cats received a single intravenous bolus dose of racemic warfarin (0.5 mg/kg) describes maximum prothrombinopenic effects within 24–48 h, and recovery of prothrombin complex activity to pre-exposure baseline levels within 70 h (Smith et al. 2000b). However, in this study inter-individual variation among cats was substantial, as has been noted in man. A study in domestic dogs administered warfarin (5 mg/kg) or diphacinone (2.5 mg/kg) as divided doses for 3 days demonstrated prolonged clotting time within 2–3 days (Mount and Feldman 1983). While administration of a single dose of vitamin K_1 (5 mg/kg) restored clotting function in the warfarin-treated dog within 24–48 h, multiple vitamin K_1 doses were required in diphacinone-dosed dogs as they exhibited impaired clotting function for as long as 30 days.

Few studies have examined toxicokinetics of ARs in non-target wildlife. Dietary exposure of golden eagles (*Aquila chrysaetos*) to 2.7 μg diphacinone/g ww resulted in prolonged clotting time within 5 days with coagulopathy apparent at least 5 days

post-exposure (Savarie et al. 1979). Similarly, Eastern screech-owls (*Megascops asio*) fed a diet containing 10 μg diphacinone/g ww exhibited coagulopathy within 3 days, and upon termination of exposure clotting function was restored within 4 days (Rattner et al. 2014). As alluded to in Chap. 3 of the text, latent episodes of hemorrhage, occurring days to weeks following termination of AR exposure, have been observed in some trials in non-target wildlife.

6 Conclusions

Although ARs have been in use for decades, there are relatively little data on the ADME properties and binding properties of these compounds. These data are challenging to obtain but are invaluable for many reasons. ADME data is important for the basic understanding of anticoagulant compounds enabling scientists to compare anticoagulants to other classes of contaminants and therapeutics. These data are also used to evaluate the comparative efficacy of anticoagulants and to develop hazard and risk assessments. These risk assessments are used by the US EPA and comparative international agencies to evaluate registration and use of these products. ADME data is often used in risk assessment models; therefore, the better the pool of data from which to draw, the more robust the risk assessment.

Numerous areas of research of anticoagulants may need further study to determine what, if any, effect role they play in non-target risk. These include placental and in ovo transfer of residues. Both of these may represent significant routes of exposure to anticoagulant compounds, and surprisingly have not been well-characterized. Also, fecal excretion of anticoagulants has not been well studied. Fecal excretion may contribute significantly to the transfer of anticoagulant compounds across the environment. From the few available reports, excretion of parent compound or metabolite is compound dependent with some anticoagulants being excreted in the feces as metabolites while others are excreted in high percentages as parent compound.

Additional studies investigating basic ADME properties of anticoagulants need to be conducted. These include competitive binding studies to determine the active binding sites of these compounds. This information would both improve risk assessments when exposure to multiple compounds is being considered and to assess the potential for cumulative toxicities. Recently published studies have examined the pharmacokinetic properties of various SGAR diastereoisomers and their ability to inhibit vitamin K epoxide reductase (VKOR) (Damin-Pernik et al. 2016a, b). It was demonstrated that *cis*- and *trans*- SGAR isomers exhibit similar potency in their ability to inhibit VKOR, but their pharmacokinetic properties differ considerably. This research team has suggested that it might be possible to create eco-friendly baits that are efficacious in controlling pest species, but with the shorter tissue half-life SGAR isomer that would pose less risk to non-target predators consuming poisoned prey.

References

Anonymous (2010) Directive 98/8/EC concerning the placing of biocidal products on the market , Inclusion of active substances in Annex I or IA to Directive 98/8/EC Assessment Report – Bromadiolone

Bachmann KJ, Sullivan TJ (1983) Dispositional and pharmacodynamic characteristics of brodifacoum in warfarin-sensitive rats. Pharmacology 27:281–288

Beckley-Kartey SAJ, Hotchkiss SAM, Capel M (1997) Comparative in vitro skin absorption and metabolism of coumarin (1,2-benzopyrone) in human, rat, and mouse. Toxicol Appl Pharmacol 145 (1):34–42. doi:10.1006/taap.1997.8154

Belleville MJ (1977) Absorption, distribution, metabolism and excretion studies in the rat using 14C–labeled chlorophacinone. Unpublished report submitted to U.S. EPA by Lipha Research Center Lyon, France

Berny PJ, de Oliveira LA, Videmann B, Rossi S (2006) Assessment of ruminal degradation, oral bioavailability, and toxic effects of anticoagulant rodenticides in sheep. Am J Vet Res 67(2):363–371. doi:10.2460/ajvr.67.2.363

Boermans HJ, Johnstone I, Black WD, Murphy M (1991) Clinical signs, laboratory changes and toxicokinetics of brodifacoum in the horse. Can J Vet Res 55(1):21–27

Breckenridge A, L'E Orme M (1972) The plasma half lives and the pharmacological effect of the enantiomers of warfarin in rats. Life Sci 11(7):337–345. doi:10.1016/0024-3205(72)90073-2

Breckenridge A, Orme M, Wesseling H, Lewis R, Gibbons R (1974) Pharmacokinetics and pharmacodynamics of the enantiomers of warfarin in man. Clin Pharmacol Ther 15(4):424–430

Breckenridge AM, Cholerton S, Hart JA, Park BK, Scott AK (1985) A study of the relationship between the pharmacokinetics and the pharmacodynamics of the 4-hydroxycoumarin anticoagulants warfarin, difenacoum and brodifacoum in the rabbit. Br J Pharmacol 84(1):81–91

Brown NA, Jähnchen E, Müller WE, Schmidt W, Wollert U (1979) Species differences of the stereoselective binding of phenprocoumon and warfarin enantiomers to serum albumins. Comp Biochem Physiol Part C Comp Pharmacol 62(1):101–105

Bruno GR, Howland MA, McMeeking A, Hoffman RS (2000) Long-acting anticoagulant overdose: brodifacoum kinetics and optimal vitamin K dosing. Ann Emerg Med 36 (3):262–267. doi:10.1067/mem.2000.108317

Bullard RW, Thompson RD, Holguin G (1976) Diphenadione residues in tissues of cattle. J Agric Food Chem 24(2):261–263

Bullard RW, Thompson RD, Kilburn SR (1977) Diphenadione residues in milk of cattle. J Agric Food Chem 25(1):79–81. doi:10.1021/jf60209a042

Cahill WP (1977) Body burden determination and metabolite identification of diphacinone in the mouse. Unpublished thesis, The University of Arizona 140 p

Cahill WP, Crowder LA (1979) Tissue distribution and excretion of diphacinone in the mouse. Pestic Biochem Physiol 10:259–267

Crowell M, Eason C, Hix S, Broome K, Fairweather A, Moltchanova E, Ross J, Murphy E (2013) First generation anticoagulant rodenticide persistence in large mammals and implications for wildlife management. New Zealand J Zool 40(3):205–216. doi:10.1080/03014223.2012.746234

Damin-Pernik M, Espana B, Besse S, Fourel I, Caruel H, Popowycz F, Benoit E, Lattard V (2016a) Development of an ecofriendly anticoagulant rodenticide based on the stereochemistry of difenacoum. Drug Metab Dispos 44(12):1872–1880

Damin-Pernik M, Espana B, Lefebvre S, Fourel I, Caruel H, Benoit E, Lattard V (2016b) Management of rodent populations by anticoagulant rodenticides: toward third-generation anticoagulant rodenticides. Drug Metab Dispos 45:160–165

Danish Environmental Protection Agency (2011) Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: Coumatetralyl

Eadsforth CV, Gray A, Huckle KR, Inglesfield C (1993) The dietary toxicity of flocoumafen to hens: elimination and accumulation following repeated oral administration. Pestic Sci 38(1):17–25

Eason C, Wickstrom M (2001) Vertebrate Pesticide Toxicology Manual (Poisons). Department of Conservation, PO Box 10–420, Wellington, New Zealand

Eason C, Wright G, Batcheler D (1996) Anticoagulant effects and persistence of brodifacoum in possums (Trichosurus vulpecula). N Z J Agric Res 39:397–400

Eason CT, Fisher P, O'Connor C, Murphy E, Endepols S (2003) Environmental health implications of the different toxicokinetics of coumarins and indandiones (abstract). Paper presented at the Solutions to Pollution Christchurch Convention Centre September 28–October 1

Enouri S, Dekroon K, Friendship R, Schrier N, Dowling PM, Johnson R (2015) Depletion of bromadiolone in tissues of hogs following oral exposure. J Swine Health Prod 23(6):298–305

Erickson W, Urban D (2004) Potential risks of nine rodenticides to birds and nontarget mammals: a comparative approach. United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, DC

European Food Safety Authority (2010) Conclusion on the peer review of the pesticide risk assessment of the active substance bromadiolone. European Food Safety Authority J 8(10). doi:10.2903/j.efsa.2010.1783

Field JB, Goldfarb MS, Ware AG, Griffith GC (1952) Effect in man of a new indandione anticoagulant. Exp Biol Med 81(3):678–681. doi:10.3181/00379727-81-19984

Fisher PM (2006) Diphacinone in pigs: sublethal exposure and residual persistence in tissues. Paper presented at the Proceedings Twenty-Second Vertebrate Pest Conference, Berkeley

Fisher P (2009) Residual concentrations and persistence of the anticoagulant rodenticides brodifacoum and diphacinone in fauna. PhD, Lincoln University, New Zealand

Fisher P, O'Connor C, Wright G, Eason C (2003) Persistence of four anticoagulant rodenticides in the liver of laboratory rats. New Zealand Department of Conservation, Wellington

Fitzek A (1978) Pharmacokinetics of 2-pivalylindan-1,3-dione in dogs. Acta Pharmacol et Toxicol 42(2):81–87

Ginsberg JS, Hirsh J (1989) Anticoagulants during pregnancy. Annu Rev Med 40:79–86. doi:10.1146/annurev.me.40.020189.000455

Greaves M (1993) Anticoagulants in pregnancy. Pharmacol Ther 59(3):311–327

Guengerich FP, Dannan GA, Wright ST, Martin MV, Kaminsky LS (1982) Purification and characterization of liver microsomal cytochromes p-450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or beta-naphthoflavone. Biochemistry 21(23):6019–6030

Hall JG (1990) Fetal warfarin syndrome. In: Birth defects encyclopedia. Blackwell, Cambridge, pp 731–732

Hanslik T, Prinseau J (2004) The use of vitamin K in patients on anticoagulant therapy: a practical guide. Am J Cardiovasc Drugs (Drugs, Devices, and Other Interventions) 4(1):43–55

Hewick DS, McEwen J (1973) Plasma half-lives, plasma metabolites and anticoagulant efficacies of the enantiomers of warfarin in man. J Pharm Pharmacol 25:458–465

Hignite C, Uetrecht J, Tschanz C, Azarnoff D (1980) Kinetics of R andS warfarin enantiomers. Clin Pharmacol Ther 28(1):99–105

Holford NH (1986) Clinical pharmacokinetics and pharmacodynamics of warfarin. Understanding the dose-effect relationship. Clin Pharmacokinet 11(6):483–504

Howe AM, Webster WS (1992) The warfarin embryopathy: a rat model showing maxillonasal hypoplasia and other skeletal disturbances. Teratology 46(4):379–390. doi:10.1002/tera.1420460408

Huckle KR, Hutson DH, Warburton PA (1988) Elimination and accumulation of the rodenticide flocoumafen in rats following repeated oral administration. Xenobiotica 18(12):1465–1479

Huckle KR, Hutson DH, Logan CJ, Morrison BJ, Warburton PA (1989a) The fate of the rodenticide flocoumafen in the rat: retention and elimination of a single oral dose. Pestic Sci 25(3):297–312

Huckle KR, Warburton PA, Forbes S, Logan CJ (1989b) Studies on the fate of flocoumafen in the Japanese quail (Coturnix coturnix japonica). Xenobiotica 19(1):51–62

Ishizuka M, Okajima F, Tanikawa T, Min H, Tanaka KD, Sakamoto KQ, Fujita S (2007) Elevated warfarin metabolism in warfarin-resistant roof rats (Rattus rattus) in Tokyo. Drug Metab Dispos 35(1):62–66. doi:10.1124/dmd.106.011775

Jackson WB, Ashton AD (1992) A review of available anticoagulants and their use in the United States. In: University of California, Davis, 1992. Proceedings of the 15th Vertebrate Pest Conference, pp 156–160

Jansing RL, Chao ES, Kaminsky LS (1992) Phase II metabolism of warfarin in primary culture of adult rat hepatocytes. Mol Pharmacol 41(1):209–215

Jones DR, Miller GP (2011) Assays and applications in warfarin metabolism: what we know, how we know it and what we need to know. Expert Opin Drug Metab Toxicol 7(7):857–874. doi:10.1517/17425255.2011.576247

Kamil N (1987) Kinetics of bromadiolone, anticoagulant rodenticide, in the Norway rat (Rattus norvegicus). Pharmacol Res Commun 19(11):767–775

Kaminsky LS, Zhang ZY (1997) Human P450 metabolism of warfarin. Pharmacol Ther 73(1):67–74

Kammerer M, Pouliquen H, Pinault L, Loyau M (1998) Residues depletion in egg after warfarin ingestion by laying hens. Vet Hum Toxicol 40(5):273–275

Kusano T (1974) The toxicity of diphacinone to laboratory rats and mice. Japan J Sanit Zool 24(3):207–213

Laas FY, Forss DA, Godfrey MER (1985) Retention of brodifacoum in sheep and excretion in faeces. N Z J Agric Res 28:357–359

Lee M, Morfini M, Negrier C, Chamouard V (2006) The pharmacokinetics of coagulation factors. Haemophilia 12(s3):1–7

Lee H-J, You M-R, Moon W-R, Sul H, Chung C-H, Park C-Y, Park S-G (2014) Evaluation of risk factors in patients with vitamin K-dependent coagulopathy presumed to be caused by exposure to brodifacoum. Korean J Intern Med 29(4):498–508. doi:10.3904/kjim.2014.29.4.498

Levy G, Mager DE, Cheung WK, Jusko WJ (2003) Comparative pharmacokinetics of coumarin anticoagulants L: physiologic modeling of S-warfarin in rats and pharmacologic target-mediated warfarin disposition in man. J Pharm Sci 92(5):985–994. doi:10.1002/jps.10345

Littin KE, O'Connor CE, Gregory NG, Mellor DJ, Eason CT (2002) Behaviour, coagulopathy and pathology of brushtail possums (Trichosurus vulpecula) poisoned with brodifacoum. Wildl Res 29:259–267

Louis CA, Sinclair JF, Wood SG, Lambrecht LK, Sinclair PR, Smith EL (1993) Synergistic induction of cytochrome P450 by ethanol and isopentanol in cultures of chick embryo and rat hepatocytes. Toxicol Appl Pharmacol 118(2):169–176. doi:10.1006/taap.1993.1022

Martin GR, Sutherland RJ, Robertson ID, Kirkpatrick WE, King DR, Hood PJ (1991) Assessment of the potential toxicity of a poison for rabbits, pindone (2-pivalyl 1, 3 indandione), to domestic animals. Aust Vet J 68(7):241–243

Mount ME, Feldman BF (1983) Mechanism of diphacinone rodenticide toxicosis in the dog and its therapeutic implications. Am J Vet Res 44:2009–2017

Murphy MJ, Ray AC, Woody B, Reagor JC (1985) Serum brodifacoum concentrations and coagulopathic effects in anticoagulant poisoned dogs treated with Vitamin K1. Toxicologist 5:88

Nahas K (1987) Kinetics of bromadiolone, anticoagulant rodenticide in the Norway rat Rattus norvegicus. Pharmacol Res Commun 19(11):767–775

Nelson PC, Hickling GJ (1994) Pindone for rabbit control: efficacy, residues and cost. In: Halverson WS, Crabb AC (eds) Proceedings of 16th Vertebrate Pest Conference. University of Davis, California, pp 217–222

Ngui JS, Chen Q, Shou M, Wang RW, Stearns RA, Baillie TA, Tang W (2001) In vitro stimulation of warfarin metabolism by quinidine: increases in the formation of 4′- and 10-hydroxywarfarin. Drug Metab Dispos 29(6):877–886

O'Reilly RA, Aggeler PM, Leong LS (1963) Studies on the coumarin anticoagulant drugs: The pharmacodynamics of warfarin in man. J Clin Investig 42(10):1542–1551

O'Reilly RA, Welling PG, Wagner JG (1971) Pharmacokinetics of warfarin following intravenous administration to man. Thromb Diath Haemorrh 25(1):178–186

O'Reilly RA (1973) Interaction of sodium warfarin and disulfiram (Antabuse®) in man. Ann Intern Med 78(1):73–76

O'Reilly RA (1976) The stereoselective interaction of warfarin and metronidazole in man. N Engl J Med 295(7):354–357

Orme M, Breckenridge A (1976) Enantiomers of warfarin and phenobarbital. N Engl J Med. 295(26):1482–1483

Panjehshahin MR, Yates MS, Bowmer CJ (1992) A comparison of drug binding sites on mammalian albumins. Biochem Pharmacol 44(5):873–879

Park BK (1988) Warfarin: metabolism and mode of action. Biochem Pharmacol 37 (1):19–27. doi:10.1016/0006-2952(88)90750-2

Parmar GH, Bratt H, Moore R, Batten PL (1987) Evidence for a common binding site in vivo for the retention of anticoagulants in rat liver. Hum Toxicol 6:431–432

Powell ML, Pope B, Elmer GW, Trager WF (1977) Biliary excretion of warfarin metabolites and their metabolism by rat gut flora. Life Sci 20 (1):171–177. doi:10.1016/0024-3205(77)90145-X

Rattner BA, Horak KE, Warner SE, Day DD, Meteyer CU, Volker SF, Eisemann JD, Johnston JJ (2011) Acute toxicity, histopathology, and coagulopathy in American kestrels (Falco sparverius) following administration of the rodenticide diphacinone. Environ Toxicol Chem 30(5):1213–1222. doi:10.1002/etc.490

Rattner BA, Horak KE, Lazarus RS, Goldade DA, Johnston JJ (2014) Toxicokinetics and coagulopathy threshold of the rodenticide diphacinone in eastern screech-owls (Megascops asio). Environ Toxicol Chem 33(1):74–81

Rettie AE, Korzekwa KR, Kunze KL, Lawrence RF, Eddy AC, Aoyama T, Gelboin HV, Gonzalez FJ, Trager WF (1992) Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in the etiology of (S)-warfarin-drug interactions. Chem Res Toxicol 5(1):54–59. doi:10.1021/tx00025a009

Remmel RP, Pohl LR, Elmer GW (1981) Influence of the intestinal microflora on the elimination of warfarin in the rat. Drug Metab Dispos 9(5):410–414

Robben JH, Kujpers EA, Mout HC (1998) Plasma superwarfarin levels and Vitamin K1 treatment in dogs with anticoagulant rodenticide poisoning. Vet Q 20:24–27

Robinson MH, Twigg LE, Wheeler SH, Martin GR (2005) Effect of the anticoagulant, pindone, on the breeding performance and survival of merino sheep Ovis aries. Comp Biochem Physiol Part B 140:465–473

Saengtienchai A, Ikenaka Y, Watanabe K, Ishida T, Ishizuka M (2011) Comparative metabolism of warfarin in rats and chickens. Poult Sci 90(12):2775–2781

Sakai K, Nishiura A, Fujihara Y, Kutsana T, Nishino T (1983) Salivary excretion of warfarin in rabbits: relationship between pharmacological effect and salivary pharmacokinetics of warfarin in rabbits. J Pharm Dyn 6:991–999

Sareli P, England MJ, Berk MR, Marcus RH, Epstein M, Driscoll J, Meyer T, McIntyre J, van Gelderen C (1989) Maternal and fetal sequelae of anticoagulation during pregnancy in patients with mechanical heart valve prostheses. Am J Cardiol 63(20):1462–1465

Savarie PJ, Hayes DJ, McBride RT, Roberts JD (1979) Efficacy and safety of diphacinone as a predacide. In: Kenaga EE (ed) Avian and mammalian wildlife toxicology, vol ASTM Special Technical Publication 693. ASTM STP, American Society for Testing and Materials, pp 69–79

Smith SA, Kraft S, Lewis D, Freeman L (2000a) Plasma pharmacokinetics of warfarin enantiomers in cats. J Vet Pharmacol Ther 23(6):329–337

Smith SA, Kraft S, Lewis D, Melethil S, Freeman L (2000b) Pharmacodynamics of warfarin in cats. J Vet Pharmacol Ther 23(6):339–344

Spiller HA, Gallenstein GL, Murphy MJ (2003) Dermal absorption of a liquid diphacinone rodenticide causing coagulaopathy. Vet Hum Toxicol 45(6):313–314

Subota V, Mirkov I, Demenesku J, Popov Aleksandrov A, Ninkov M, Mileusnic D, Kataranovski D, Kataranovski M (2016) Transdermal toxicity of topically applied anticoagulant rodenticide warfarin in rats. Environ Toxicol Pharmacol 41:232–240. doi:10.1016/j.etap.2015.12.006

Sutcliffe FA, Macnicoll AD, Gibson GG (1987) Aspects of anticoagulant action: a review of the pharmacology, metabolism, and toxicology of warfarin and congeners. Rev Drug Metab Drug Interact 5(4):226–261

Tran M-H, King NC (2015) Epidemiology and symptomatology of long acting anticoagulant rodenticide poisoning. J Epidemiol Res 2(2):p1

Vandenbroucke V, Bousquet-Melou A, De BP, Croubels S (2008) Pharmacokinetics of eight anticoagulant rodenticides in mice after single oral administration. J Vet Pharmacol Ther 31(5):437–445

Veenstra GE, Owen DE, Huckle KR (1991) Metabolic and toxicological studies on the anticoagulant rodenticide, flocoumafen. Arch Toxicol Suppl 14:160–165

Vein J, Vey D, Fourel I, Berny P (2013) Bioaccumulation of chlorophacinone in strains of rats resistant to anticoagulants. Pest Manage Sci 69(3):397–402

Wadelius M, Sorlin K, Wallerman O, Karlsson J, Yue QY, Magnusson PK, Wadelius C, Melhus H (2004) Warfarin sensitivity related to CYP2C9, CYP3A5, ABCB1 (MDR1) and other factors. Pharmacogenomics J 4(1):40–48. doi:10.1038/sj.tpj.6500220

Watanabe KP, Saengtienchai A, Tanaka KD, Ikenaka Y, Ishizuka M (2010) Comparison of warfarin sensitivity between rat and bird species. Comp Biochem Physiol Toxicol Pharmacol 152(1):114–119. doi:10.1016/j.cbpc.2010.03.006

Watanabe KP, Kawata M, Ikenaka Y, Nakayama SM, Ishii C, Darwish WS, Saengtienchai A, Mizukawa H, Ishizuka M (2015) Cytochrome P450-mediated warfarin metabolic ability is not a critical determinant of warfarin sensitivity in avian species: In vitro assays in several birds and in vivo assays in chicken. Environ Toxicol Chem 34(10):2328–2334. doi:10.1002/etc.3062

Watt BE, Proudfoot AT, Bradberry SM, Vale JA (2005) Anticoagulant rodenticides. Toxicol Rev 24(4):259–269

Webster K, Harr K, Bennett D, Williams T, Cheng K, Maisonneuve F, Elliott J (2015) Assessment of toxicity and coagulopathy of brodifacoum in Japanese quail and testing in wild owls. Ecotoxicology 24:1087–1101. doi:10.1007/s10646-015-1449-1

Welling PG (1995) Differences between pharmacokinetics and toxicokinetics. Toxicol Pathol 23(2):143–147

Wienkers LC, Wurden CJ, Storch E, Kunze KL, Rettie AE, Trager WF (1996) Formation of (R)-8-hydroxywarfarin in human liver microsomes. A new metabolic marker for the (S)-mephenytoin hydroxylase, P4502C19. Drug Metab Dispos 24(5):610–614

Woody BJ, Murphy MJ, Ray AC, Green RA (1992) Coagulopathic effects and therapy of brodifacoum toxicosis in dogs. J Vet Intern Med 6(1):23–28

Wright HP, Hayden M (1955) The effect of diet upon the response to oral anticoagulants. J Clin Pathol 8(1):65–68

Yacobi A, Lai CM, Levy G (1975) Comparative pharmacokinetics of coumarin anticoagulants XV: Relationship between pharmacokinetics of dicumarol and warfarin in rats. J Pharm Sci 64(12):1995–1998

Yourick JJ, Bronaugh RL (1997) Percutaneous absorption and metabolism of coumarin in human and rat skin. J Appl Toxicol 17(3):153–158. doi:10.1002/(SICI)1099-1263(199705)17:3<153::AID-JAT426>3.0.CO;2-E

Yu CC, Atallah YH, Whitacre DM (1982) Metabolism and disposition of diphacinone in rats and mice. Drug Metab Dispos 10(6):645–648

Zhang Z, Fasco MJ, Huang Z, Guengerich FP, Kaminsky LS (1995) Human cytochromes P4501A1 and P4501A2: R-warfarin metabolism as a probe. Drug Metab Dispos 23(12):1339–1346