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2. Anticoagulant poisons

2.1 BRODIFACOUM (TALON®, PESTOFF®)

Chemical Name: 3-[3-(4'-bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one.

Synonyms: Brodifacoum is the approved common name. Talon® and PESTOFF® are trade names

Brodifacoum is one of the most widely used rodenticides world-wide. It has been used in New Zealand to control possums since the early 1990s. On islands, aerial application techniques are used. On mainland New Zealand it is used in cereal baits in bait stations. In January 2000 the Department of Conservation announced plans to reduce the field use of brodifacoum on the mainland.

It is essential that wildlife or livestock do not gain access to areas where brodifacoum is being used. Brodifacoum can persist (>1 year) in the liver and kidneys of sub-lethally poisoned wildlife or livestock. Hence it is important that the risk of contamination of wildlife or livestock is recognised and the product is used carefully to minimise non-target contamination.

2.1.1 Physical and chemical properties

The empirical formula for brodifacoum is $C_{31}H_{23}BrO_3$ and the molecular weight is 523.4. It is an off-white to fawn-coloured odourless powder with a melting point of 228–232°C. It is of very low solubility in water (less than 10 mg/L at 20°C and pH 7). Brodifacoum is slightly soluble in alcohols and benzene, and soluble in acetone. It is stable at room temperature. Commercial concentrated solutions of brodifacoum are available for bait manufacturers.

2.1.2 Historical development and use

Brodifacoum is a synthetic compound that was developed a few decades ago. It is structurally related to a naturally occurring coumarin that causes haemorrhagic syndrome in cattle eating improperly cured or mouldy sweet clover. The rodenticidal properties of brodifacoum were described in the early 1970s. It is a very potent anticoagulant active against rats and mice, including strains resistant to warfarin and other anticoagulants (Rennison & Hadler 1975). A single ingestion of 1 mg/kg is usually sufficient to kill. In New Zealand it is used principally to control possums and rats, though it has also been used for rabbits (Williams et al. 1986a, b). In January 2000 the Department of Conservation took steps to reduce the mainland field use of brodifacoum because of concerns relating to contamination of birds and game (Eason et al. 1999c). Because of the tendency for uncontrolled exposure of non-targets through secondary poisoning (Eason et al. 1999c), the suggested practice of secondary poisoning of stoats (Brown et al. 1998) is not recommended, particularly in areas where game may be hunted for human consumption.

Brodifacoum has been used successfully in recent rodent eradication programmes on New Zealand's offshore islands to protect populations of

endangered indigenous birds (Taylor & Thomas 1989, 1993; Buckle & Fenn 1992; Robertson et al. 1993; Towns et al. 1993). In addition to its use to control and eradicate rats, brodifacoum has been successfully used in ground-laid baits or in baits placed in bait stations to eradicate rabbits (Merton 1987; Towns et al. 1993), to control wallabies (D. Moore pers. comm.) and brushtail possums (Eason et al. 1993b). Field use of brodifacoum-containing baits for rabbit or wallaby control has been discontinued in New Zealand.

Cereal baits (Talon®, PESTOFF®) containing brodifacoum are used for rodent and possum control. For possum control the baits are best used to further reduce low possum numbers following use of fast-acting poisons (such as cyanide, 1080, or cholecalciferol) for the initial population reduction. The slow action of this poison overcomes the problems associated with bait shyness in areas where possum control has been sustained for many years.

2.1.3 Fate in the environment

Brodifacoum is most unlikely to be found in water even after aerial application of baits for rodent control on offshore islands. Brodifacoum is not mobile in soil and is extremely insoluble in water (<10 mg/L water at pH 7). When baits disintegrate, brodifacoum will be likely to remain in the soil, where it will be slowly degraded by soil micro-organisms. The half-life in soil varies from 12 to 25 weeks depending on the soil type. Microbial degradation will be dependent on climatic factors such as temperature, and the presence of species able to degrade brodifacoum. In leaching studies, 2% of brodifacoum added to soil leached more than 2 cm in four soil types tested (World Health Organisation 1995).

Since brodifacoum remains absorbed in soil when baits disintegrate, only the erosion of soil itself would see any brodifacoum reaching water, and even then brodifacoum would be likely to remain bound to organic material and settle out in the sediment. If baits were sown directly into streams or rivers, localised short-term contamination might occur.

2.1.4 Toxicology and pathology

Onset of symptoms

The latent period between the time of ingestion and the onset of clinical signs varies considerably and in possums may take as long as 1–4 weeks (Littin et al. 2000). In rats the onset of symptoms and death usually occur within a week. Clinical signs reflect some manifestations of haemorrhage. Onset of signs may occur suddenly; this is especially true when haemorrhage of the cerebral vasculature or pericardial sac occurs. Clinical signs commonly include anaemia and weakness. Haemorrhaging may be visible around the nose, mouth, eyes, and anus of mammals. When pulmonary haemorrhage has occurred, blood-tinged froth may be visible around the nose and mouth. Swollen, tender joints are common and if haemorrhage involves the brain or central nervous system, ataxia or convulsions can occur. Poisoned animals die of multiple causes related to anaemia or hypovolemic shock. Possums respond significantly more slowly with onset of toxicosis occurring between 2 and 3 weeks after dosing. In general, possums appear to be less sensitive to anticoagulants, which may be due to species differences in the ability to metabolise xenobiotics (Olkowski et

al. 1998) or difference in the half-lives of vitamin K-dependent clotting factors, or vitamin K epoxide reductase receptor binding.

Mode of action

Brodifacoum, like other anticoagulant toxicants, acts by interfering with the normal synthesis of vitamin K-dependent clotting factors in the liver of vertebrates (Hadler & Shadbolt 1975). In the liver cells the biologically inactive vitamin K1-2,3 epoxide is reduced by a microsomal enzyme into biologically active vitamin K, which is essential for the synthesis of prothrombin and other clotting factors (VII, IX, and X). Brodifacoum antagonism of the enzyme vitamin K1-epoxide reductase in the liver causes a gradual depletion of the active form of the vitamin, and consequently of vitamin K-dependent clotting factors, which results in an increase in blood-clotting time until the point where no clotting occurs.

The greater potency of second-generation anticoagulants such as brodifacoum compared to first-generation anticoagulants such as warfarin and pindone is likely to be related to their greater affinity for vitamin K-epoxide reductase and subsequent accumulation and persistence in the liver and kidneys after absorption (Huckle et al. 1988). Anticoagulants share this common binding site, but the second-generation anticoagulants have a greater binding affinity than the first-generation compounds (Parmar et al. 1987). All tissues that contain vitamin K-epoxide reductase (e.g. liver, kidney, and pancreas) are target organs for accumulating these toxicants.

Pathology and regulatory toxicology

Generalised haemorrhage is frequently evident at post-mortem. Areas commonly affected are the thoracic cavity, subcutaneous tissue, stomach, and intestine. The heart is sometimes rounded and flaccid with subepicardial and subendocardial haemorrhages. Histomorphological analysis of the liver may reveal centrilobular necrosis as a result of anaemia and hypoxia. In possums, post-mortem findings range from mild to moderate haemorrhage in some limbs and in the gastrointestinal tract, to extensive haemorrhage throughout the body and major organs.

Brodifacoum is a slight skin irritant and a mild eye irritant in the rabbit. Various *in vitro* and *in vivo* studies (including the *Salmonella* reverse mutation assay, the forward mutation assay using mouse lymphoma cells, and the micronucleus test in mice) have been undertaken to assess the genotoxic potential of brodifacoum. No mutagenic activity was detected. Brodifacoum, when given by oral gavage to female rats at daily dose levels of 0.001, 0.01, or 0.02 mg/kg body weight during days 6–15 of pregnancy, caused no evidence of adverse developmental effects on the foetuses. Higher daily doses (above 0.05 mg/kg) caused an anticoagulant effect in the dams, which resulted in a high incidence of abortion.

Pregnant female rabbits were given oral gavage doses of 0.001, 0.002, or 0.005 mg brodifacoum/kg body weight per day from days 6–18 of pregnancy. At the highest dose level a high proportion of maternal deaths occurred as a result of haemorrhage. Although the survivors showed signs of haemorrhage, there were no effects on the developing foetuses.

On the basis of these studies, brodifacoum can be classified as non-mutagenic and lacking in tetratogenic potential. In a 5-day study in rats, a no-observed-effect level for brodifacoum was 0.02 mg/kg/day (WHO 1995).

Fate in animals

Absorption, metabolism and excretion of brodifacoum compared with other anticoagulant toxicants[3]

Brodifacoum is absorbed through the gastrointestinal tract. It can also be absorbed through the skin (Table 9).

TABLE 9. ACUTE TOXICITY (LD_{50} mg/kg) OF BRODIFACOUM IN RATS (Hone & Mulligan 1982).

SPECIES ROUTE	LD_{50} (mg/kg)
Rat (oral)	0.27
Rat (dermal)	50.00

After absorption, high concentrations in the liver are rapidly established and remain relatively constant. Disappearance from serum is slow with a half-life in rats of 156 hours or longer. The slow disappearance from the plasma and liver and the large liver:serum ratio probably contribute to the higher toxicity of brodifacoum when compared with warfarin or pindone (Bachmann & Sullivan 1983). It is apparent that a proportion of any ingested dose of brodifacoum bound in the liver, kidney, or pancreas remains in a stable form for some time and is only very slowly excreted.

In contrast to brodifacoum, warfarin will undergo relatively extensive metabolism. The metabolites will be more polar (water soluble) than the parent compounds and therefore more readily excreted in the urine.

Brodifacoum, like other second-generation metabolites, is not readily metabolised and the major route of excretion of unbound compound is through the faeces. Enterohepatic recirculation, the process that allows drugs and pesticides that have been absorbed to return to the gastrointestinal tract from the liver via the biliary tract, undoubtedly plays an important role.

Tables 10 and 11 present comparative data on the persistence of anticoagulants. Kelly & O'Malley (1979) reported the mean half-life for disappearance of warfarin from the plasma of human volunteers given a single oral dose of 0.5–100 mg/kg body weight varied from 24 to 58 hours. No dose-level effect on half-life was apparent even over this large range of doses. Second-generation anticoagulants are much more slowly cleared from the bloodstream.

In a comparative study in rabbits Breckenridge et al. (1985) reported plasma elimination half-lives of 5.6 hours for warfarin, 83.1 hours for difenacoum, and

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[3] In this section the metabolism fate of brodifacoum in animals is compared with that for other anticoagulants to avoid repetition in the following sections. First-generation anticoagulants (developed c. 1950–70) are listed in Table 10, and second-generation anticoagulants (developed c. 1970–2000) in Table 11.

TABLE 10. PERSISTENCE OF FIRST-GENERATION ANTICOAGULANTS.

	SPECIES	BLOOD t½† (hours)	LIVER RETENTION‡ (days)	REFERENCE
Warfarin	Rat ♂, Rat ♀	18, 28	–	Pyrola 1968
	Rabbit	6	–	Breckenridge et al. 1985
	Possum	12	–	Eason et al. 1999b
	Human	15–58		O'Reilly et al. 1963
	Pig		30–40	O'Brien et al. 1987
Pindone	Dog	120	–	Fitzek 1978
	Sheep	–	8–16	Nelson & Hickling 1994
Coumatetralyl	Rat	–	t½ 55	Parmar et al. 1987
Diphacinone	Cattle	–	>90	Bullard et al. 1976

† t½ for plasma or liver is the elimination half-life. It is convention to report the elimination t½ (b-phase) rather than the a-phase.

‡ 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.

TABLE 11. PERSISTENCE OF SECOND-GENERATION ANTICOAGULANTS.

	SPECIES	BLOOD t½ (hours)† (except where specified)	LIVER RETENTION‡ (days)	REFERENCES
Difenacoum	Rat		t½ 118	Bratt 1987
	Rat	–	t½ 120	Parmar et al. 1987
	Rabbit	83	–	Breckenridge et al. 1985
Bromadiolone	Rat	26–57		Kamil 1987
	Rat	25–26	t½ 170	Parmar et al. 1987
	Sheep	–	256	Nelson & Hickling 1994
Flocoumafen	Rat	–	t½ 220	Huckle et al. 1989
	Sheep	–	>128	Nelson & Hickling 1994
	Quail	–	t½ 155	Huckle & Warburton 1989
	Barn owl	–	>100	Newton et al. 1990
	Dog	–	>300	Veenstra et al. 1991
Brodifacoum	Rat	156	>80	Bachmann & Sullivan 1983
	Rat	–	t½ 130	Parmar et al. 1987
	Rabbit	60	–	Breckenridge et al. 1985
	Dog	6 days	–	Woody et al. 1992
	Dog	0.9–4.7 days (mean 2.8)	–	Robben et al. 1998
	Possum	20–30 days	>252	Eason et al. 1996c, d
	Sheep	–	>250	Laas et al. 1985
	Human	16–36		Weitzel et al. 1990
Difethialone	Rat	2.3 days	t½ 108§	Lechevin & Poche 1988
	Dog	2.2–3.2 days		Robben et al. 1998

† t½ for plasma or liver is the elimination half-life. It is standard convention to report the elimination t½ (b-phase) rather than the a-phase.

‡ 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.

§ The half-life hepatic elimination for difethialone reported by Lechevin & Poche (1988) is unusually short for a second-generation anticoagulant, which suggests that difethialone may be unique.

60.8 hours for brodifacoum. There are very limited data on the influence of dosage on elimination. However, in the case of bromadiolone, a dose-dependent increase in plasma elimination half-life from 25.7 to 57.5 hours was reported after the oral dosage was increased from 0.8 to 3 mg/kg in rats (Kamil 1987).

Woody et al. (1992) observed an elimination half-life for brodifacoum in serum of 6 ± 4 days in four dogs. The plasma half-life of brodifacoum determined in three patients with severe bleeding disorders was found to be approximately 16–36 days (Weitzel et al. 1990).

There are very limited data on the persistence of warfarin or pindone in the liver of animals. Two studies in non-rodent species indicate comparatively rapid clearance from the liver. Warfarin concentrations declined in pigs to very low concentrations after approximately 30 days, and concentrations were declining in those that received a lethal dose and those that survived (O'Brien et al. 1987).

In sheep receiving sub-lethal doses of bromadiolone (2 mg/kg), flocoumafen (0.2 mg/kg), and pindone (10 mg/kg), bromadiolone was detectable in the liver for 256 days and flocoumafen for 128 days. In contrast pindone was undetectable in the liver after 16 days (Nelson & Hickling 1994). Diphacinone, which is a close relative to pindone, appears to have a hepatic persistence profile more akin to that of second-generation anticoagulants. In cattle receiving a single injection of 1 mg/kg, almost constant residue concentrations were found in liver and kidney, 30, 60, and 90 days after dosing (Bullard et al. 1976). It is noteworthy that in persistence studies, and in risk assessment, limited consideration has been given to organs other than the liver. This is surprising considering that quite high concentrations of anticoagulants are found in the kidneys and lungs relative to other tissues some time after dosing.

Brodifacoum was detected in the liver of sheep 128 days after oral administration (0.2 and 2.0 mg/kg body weight) in concentrations of 0.64 and 1.07 mg/kg dry weight (equivalent to 0.22 and 0.36 mg/kg wet weight), respectively. The peak levels which occurred at 2 days in the high-dose group and at 8 days in the low-dose group, were 6.50 and 1.87 mg/kg dry weight (2.21 and 0.64 mg/kg wet weight), respectively (Laas et al. 1985).

Parmar et al. (1987) found that elimination of radio-labelled brodifacoum, bromadiolone, and difenacoum from rat liver was biphasic, consisting of a rapid initial phase lasting from days 2 to 8 after dosing and a slower terminal phase when the elimination half-lives were 130, 170, and 120 days, respectively. Elimination of coumatetralyl was more rapid, with a half-life of 55 days.

Similar results for difenacoum were found by Bratt (1987). After a single oral ^{14}C-difenacoum dose of 1.2 mg/kg body weight, the highest concentration of radioactivity (41.5% of the dose) was found in the rat liver 24 hours after dosing. The elimination from the liver was biphasic. The half-life of elimination of the radioactivity during the first rapid phase was 3 days, and for the slower phase was 118 days. A similar biphasic elimination was also apparent in the kidney. In the pancreas the concentration declined more slowly than in any of the other tissues (182 days). The parent compound was the major component in the liver 24 hours after dosing (42%).

Unchanged flocoumafen comprised the major proportion of the hepatic radioactivity in rats and was eliminated with a half-life of 220 days (Huckle et al.

1989). 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. Despite the more rapid metabolism of flocoumafen in Japanese quail, a proportion of the administered dose is retained in the liver, with an elimination half-life of 155 days after oral dosing (Huckle & Warburton 1989).

There are limited data on the persistence of anticoagulants in New Zealand native species. In a study in weta, brodifacoum persisted for approximately 1 week after dosing (Morgan et al. 1996a).

Species variation in response to brodifacoum

For second-generation anticoagulants like brodifacoum only a single dose is needed to induce death, if sufficient toxicant is ingested, and brodifacoum is extremely toxic in a number of animal species. The toxicity of brodifacoum varies between mammal species (Table 12) and bird species (Table 13).

In most mammals LD_{50} values are 1 mg/kg or less. Some higher values are reported in sheep and dogs, but there is considerable variability in these reports (LD_{50} in sheep 5–25 mg/kg and in dogs 0.25–3.56 mg/kg).

TABLE 12. ACUTE ORAL TOXICITY (LD_{50}mg/kg) OF BRODIFACOUM FOR MAMMAL SPECIES (Godfrey 1985; Eason et al. 1994a, Eason & Spurr 1995).

SPECIES	LD_{50} (mg/kg)
Pig	0.1
Possum	0.17
Rabbit	0.2
Cat	0.25–25
Dog	0.25–3.56
Rat	0.27
Mouse	0.4
Bennett's wallaby	1.3
Sheep	5–25

TABLE 13. ACUTE ORAL TOXICITY (LD_{50}mg/kg) OF BRODIFACOUM FOR BIRD SPECIES (Godfrey 1985).

BIRD SPECIES	LD_{50} (mg/kg)
Southern black-backed gull	<0.75†
Canada goose	<0.75†
Pukeko	0.95
Blackbird	>3.0‡
Hedge sparrow	>3.0‡
California quail	3.3
Mallard duck	4.6
Black-billed gull	<5.0†
House sparrow	>6.0‡
Silvereye	>6.0‡
Australasian harrier	10.0
Ring-necked pheasant	10.0
Paradise shelduck	>20.0‡

† Lowest dose tested

‡ Highest dose tested

It has been suggested that anticoagulants are unlikely to affect invertebrates, which have different blood-clotting systems from vertebrates (Shirer 1992) and a New Zealand-based study has shown that brodifacoum lacks insecticidal properties in weta (Morgan et al. 1996a).

Small birds such as silvereyes, sparrows, blackbirds, and California quail are considered more resistant to brodifacoum than some larger birds such as southern black-backed gulls, Canada geese, and pukeko (Godfrey 1985). However, some large birds, including Australasian harriers, ring-necked pheasants, and paradise shelducks, are also relatively resistant.

Aquatic toxicology

There are limited data on the aquatic toxicology of brodifacoum. In the unlikely event of a significant amount of brodifacoum bait being applied directly to a small stream, poisoning of aquatic invertebrates and fish could result. The EC_{50} from *Daphnia magna* (first instar) was 1.0 mg/kg after 24 hours of exposure and 0.34 mg/kg after 48 hours using 50 ppm pelleted baits. The LC_{50} (24 hours) for rainbow trout is 0.155 mg/L. The LC_{50}s (96 hours) for rainbow trout and bluegill are 0.05 and 0.165 mg/L, respectively (World Health Organisation 1995.

2.1.5 Diagnosis and treatment of anticoagulant poisoning

Diagnosis of non-target poisoning in domestic animals

Diagnosis of anticoagulant toxicosis is based on exposure history, clinical signs, response to treatment, laboratory analyses, and in lethal cases, lesions. Differential diagnoses vary with the species involved, and include other causes of coagulopathy (clotting disorders) such as autoimmune thrombocytopenia (reduced platelet numbers), liver disease, and hereditary clotting factor deficiencies like Von Willebrand's disease or Haemophilia A (Beasley et al. 1997d).

Clinical signs

Although in some cases signs have been observed within 24 hours of ingestion, there is usually a lag period of 3–5 days between exposure and the onset of clinical signs of anticoagulant toxicosis. This delayed onset represents the time required to deplete hepatic stores of vitamin K, and reduce preformed vitamin K-dependent, clotting factor concentration in the plasma to the point of functional deficiency.

Initial clinical signs of anticoagulant poisoning are usually characterised by depression/lethargy and anorexia, followed shortly by anaemia with pale mucous membranes, dyspnoea, exercise intolerance, and haemorrhaging from numerous sites, as evidenced by haematemesis (vomiting blood), epistaxis (blood from the nose), haemoptysis (bronchial or pulmonary bleeding), melaena ('tarry' faeces), and haematomas in various locations. Periarticular or intraarticular haemorrhage causing swollen joints and lameness is especially common in pigs, and abortion induced by placental haemorrhaging has been reported in cattle. Convulsions indicate bleeding into the central nervous system. Animals experiencing prolonged toxicosis may be icteric (jaundiced). Similar clinical signs occur in humans and include haematuria, bleeding gums, and easy or spontaneous bruising (Park et al. 1986).

As blood loss continues, cardiac murmurs, irregular heart beat, weak peripheral pulses, ataxia, recumbency, and coma will be observed. Death due to hypoxia and hypovolemic shock may occur from 48 hours to several weeks after exposure. Animals may occasionally be found dead with no premonitory signs, especially if severe haemorrhage occurs in the cerebral vasculature, pericardial sac, abdominal cavity, mediastinum, or thorax (Murphy & Gerken 1989; Felice & Murphy 1995).

Laboratory diagnosis

Laboratory evaluation of suspect anticoagulant exposures in domestic animals includes measurement of packed cell volume (haematocrit), clotting parameters, and residue analysis.

The activity of vitamin K-dependent clotting factors (II, VII, IX, and X) is commonly measured using a suite of tests, including prothrombin time (PT), activated coagulation time (ACT), and activated partial thromboplastin time (APTT). Abnormal prolongation of PT is usually the earliest indicator of anticoagulant-induced coagulopathies, due to the involvement of factor VII in the coagulation pathway assessed by this clotting parameter. Factor VII has the shortest half-life of the vitamin K-dependent factors (6.2 hours in dogs), and is therefore the first to be depleted in plasma (Murphy & Gerken 1989). Elevations of PT from 2–6 times normal may occur within 24–48 hours of ingestion of a toxic dose. This is followed several hours later by elevation in APTT to 2–4 times normal values in cases of significant exposure. In general, changes in clotting parameter times are suggestive of anticoagulant exposure if they are prolonged beyond 25% of normal values. Assessment of coagulation parameters requires a sample of fresh, non-haemolysed blood collected in a sodium citrate (Blue Top) tube, stored at 4°C, and submitted immediately. The diagnostic laboratory may require submission of a parallel sample from a 'normal', unexposed animal of the same species to serve as a control.

The onset and severity of clinical signs of anticoagulant toxicosis are usually linked with declines in packed cell volume, except in cases of massive, acute haemorrhage. Therefore, regular assessment of this end point is a useful tool to determine the appropriate course of treatment and to monitor progress.

Suspect anticoagulant exposures can often be confirmed by laboratory identification of toxicant residues in vomitus (only in cases of very recent ingestion, prior to the onset of clinical signs) or tissue. The antemortem sample of choice is whole blood or serum (residues are protein-bound), while liver is the best post-mortem sample. Blood samples should be stored at 4°C. Liver specimens should be wrapped in foil, sealed in plastic, and shipped frozen.

Response to treatment

Anticoagulant-induced coagulopathies (clotting disorders) can be distinguished from other types of coagulopathies by clinical response to treatment with the specific antidote, vitamin K1. Tests used to assess coagulation time should indicate significant improvement in clotting ability within 12–24 hours of initiation of treatment, and should return to normal within 36–48 hours (Murphy 1999).

Lesions

Post-mortem lesions resulting from anticoagulant rodenticide exposure are characterised grossly by generalised haemorrhage, especially in the thoracic or abdominal cavities, mediastinal space, periarticular tissues, subcutaneous tissues, subdural space, and gastrointestinal tract. Sudden deaths are often marked by massive haemothorax, haemopericardium, and pulmonary oedema or haemorrhage. The heart is often flaccid, with subepicardial and subendocardial ecchymoses. Centrilobular hepatic necrosis secondary to anaemia and hepatocellular hypoxia may be observed histologically (Osweiler 1996b; Beasley et al. 1997d).

Treatment of anticoagulant toxicosis in domestic animals

Companion animals usually present with signs of haemorrhage or anaemia, or with a history of recent ingestion of anticoagulant bait but no clinical effects. In the latter cases, either the dose ingested is insufficient to cause significant inhibition of vitamin K-dependent clotting factor production, or insufficient time has elapsed to deplete pre-exposure plasma clotting factor concentrations to the point of deficiency. Because treatment of anticoagulant toxicosis can be expensive (especially with large dogs exposed to second-generation products requiring prolonged therapy), animals presenting with a history of exposure but no clinical signs should be assessed carefully before treatment is initiated.

Therapeutic goals for veterinarians in the treatment of anticoagulant poisoning are (1) to decrease toxicant absorption; (2) to correct low haematocrit and/or hypovolemia; and (3) to correct clotting factor deficiencies. Recommendations for the treatment of anticoagulant toxicosis in companion animals are as follows (Mount & Feldman 1983; Murphy & Gerken 1989; Osweiler1996b; Beasley et al. 1997d).

Animal is presented asymptomatic, within several hours of suspected/ confirmed oral exposure:

- Induce emesis with household salt solution or washing soda crystals (if <3 hours) or perform gastric lavage.
- Administer activated charcoal (1–2 g/kg) with a saline cathartic (magnesium sulphate at 250 mg/kg in 5–10 times as much water).
- Decision to initiate vitamin K therapy depends on potential exposure dose and effectiveness of emesis. If suspected dose ingested is low (<10% of LD_{50}), may elect to decontaminate and release with instructions to monitor for signs of haemorrhage. If potential exposure is more significant, measure PT at 24, 48 and 72 hours after ingestion. If results are normal, defer treatment but monitor for 10–30 days (depending on the compound involved).

Animal is presented with signs of haemorrhage and/or anaemia:

- Animals with a packed cell volume of <15% with severe bleeding, or with associated complications of anaemia, require clotting factor replacement immediately (Murphy 1999). Correct low haematocrit and/or hypovolemia, and provide clotting factors with IV transfusion of fresh whole blood or plasma at 10–20 mL/kg. The initial 25% of the volume is given relatively rapidly, and the remainder by slow drip.
- Handle affected animals with care. Sedate as needed (avoid protein-binding drugs like promazine that may displace toxicant residues and exacerbate signs). Maintain core body temperature. Oxygen may be beneficial with

severe dyspnoea. Give replacement IV fluids only after clotting factors are on board.

- Initiate antidotal therapy using vitamin K_1 (phytonadione, phylloquinone), which is the most effective form available. Vitamin K_3 is not recommended. Hepatic bioavailability of oral vitamin K_1 is greater than the parenteral form, so this route should be used unless contraindicated (e.g. in cases of vomiting, gastrointestinal haemorrhage, or concurrent administration of activated charcoal). If parenteral administration is required on initial presentation, approved vitamin K_1 formulations can be given IV (slowly, over 15–20 minutes, with a small-bore needle), although this route is associated with frequent anaphylactoid reactions. The subcutaneous route is safer, but absorption is slow in dehydrated animals.
- Recommended doses of vitamin K_1 for companion animals range from 1 mg/kg (once a day) for first-generation products such as warfarin and pindone, to 2.5 mg/kg for bromadiolone, and 2.5–5.0 mg/kg for potent, second-generation anticoagulants such as brodifacoum and diphacinone. Specific doses are not available for flocoumafen and coumatetralyl, but a starting dose of 2.5 mg/kg is reasonable (Felice & Murphy 1995). Oral bioavailability is enhanced by concurrent feeding of a small fatty meal.
- Oral vitamin K_1 therapy must be maintained for as long as the toxicant is active in inhibiting vitamin K epoxide recycling. Recommendations for duration of treatment in companion animals range from 7–14 days for warfarin and pindone, to 21 days for bromadiolone, and 30 days for brodifacoum and diphacinone (Felice & Murphy 1995; Beasley et al. 1997d). In all cases, premature termination of treatment should be avoided, and prothrombin time should be measured 5–7 days after the end of the treatment period.
- Avoid protein-bound drugs, elective surgery, strenuous exercise, and large volumes of fatty food during the convalescent period. Previously exposed animals may be more sensitive to subsequent anticoagulant exposure for weeks to months after recovery, due to biologically active residues in the liver.

2.1.6 Non-target effects

Brodifacoum has the potential to cause both primary and secondary poisoning of non-target species. However, as with the other vertebrate pesticides, the adverse effects of brodifacoum on wildlife are dependent more on how baits are used and the behaviour of non-target species than susceptibility of individual species to the toxin. Baits in bait stations are less accessible to non-target species than baits on the ground. Secondary poisoning of birds is likely where target species (e.g. rabbits and rats) are a major constituent of the diet (e.g. brown skua and harriers).

Despite these distinctions, a wide range of small and large birds have been found dead from primary or secondary poisoning after field use of brodifacoum in New Zealand: saddlebacks, blackbirds, chaffinches, house sparrows, hedge sparrows, silvereyes, song thrushes, paradise shelducks, Australian magpies, robins, western weka, Stewart Island weka and brown skuas (Towns et al. 1993; Williams et al. 1986a,b; Taylor & Thomas 1993; Taylor 1984; D. Brown pers. comm.; L. Chadderton pers. comm.).

These findings suggest that the reported differences in sensitivity (from published LD_{50} values; see Table 13) may be either inaccurate or irrelevant

predictors of susceptibility to brodifacoum, since species such as house sparrows, silvereyes, and paradise shelducks are reported to be moderately resistant.

The impacts of brodifacoum-poisoning operations on populations of non-target species that might have eaten baits have been monitored in several studies. Numbers of three indigenous bird species (western weka, Stewart Island weka, and pukeko) have been severely reduced in poison areas. For example, the entire western weka population on Tawhitinui Island was exterminated by direct consumption of Talon® 50WB intended for ship rats, which they obtained by reaching into bait stations, by eating baits dropped by rats, and by eating dead or dying rats (Taylor 1984). About 80–90% of the Stewart Island weka on Ulva Island were similarly killed by Talon® 50WB intended for Norway rats (L. Chadderton, pers. comm.), and 98% of the western weka on Inner Chetwode Island were killed after the aerial distribution of Talon® 7-20 (Wanganui No.7 cereal baits with 20 ppm brodifacoum) intended for kiore (D. Brown pers. comm.). More than 90% of pukeko on Tiritiri Matangi Island were killed after aerial distribution of Talon® 20P for eradication of kiore (C.R. Veitch pers. comm.). Some introduced ground-feeding bird species such as brown quail, blackbirds, house sparrows, and common mynahs on Tiritiri Matangi Island were also decimated (C.R. Veitch pers. comm.). However, despite deaths of some individuals, populations of other bird species have been less affected. For example, on Stanley Island 41 of 43 banded North Island saddlebacks were still alive more than 1 month after aerial distribution of Talon® 20P (Towns et al. 1993). On Red Mercury Island, all nine little spotted kiwi with radio transmitters were still alive 1 month after aerial distribution of Talon® 20P (Robertson et al. 1993). On Tiritiri Matangi Island, little spotted kiwi, North Island saddlebacks, and North Island robin populations were not detrimentally affected by aerial distribution of Talon® 20P (C.R. Veitch pers. comm.). The South Island robin population on Breaksea Island was not detrimentally affected by the use of Talon® 50WB in bait stations (Taylor & Thomas 1993), and all banded South Island robins on Inner Chetwode Island are thought to have survived aerial distribution of Talon® 20P (D. Brown pers. comm.). Brodifacoum residues have been detected in dead birds after aerial application of baits for rodent eradication (Morgan et al. 1996a), but the extent of wildlife contamination and impact after continued use has not been comprehensively studied.

Sub-lethal doses of brodifacoum have caused abortions and reduced lambing rates in sheep (Godfrey 1985), and concerns have been expressed about the adverse effects of small doses of anticoagulants on tawny owls (Townsend et al. 1981). However, there are no publications that elucidate any potential long-term effects of low-level brodifacoum exposure in birds.

There are no published LD_{50} data on the direct acute toxicity of brodifacoum to New Zealand bats. However, data from other anticoagulants suggest they may be susceptible if they were to consume the toxin.

There are no published LD_{50} data on the acute toxicity of brodifacoum to reptiles or amphibians. However, reptiles, at least, are known to be susceptible to brodifacoum. Telfair's skinks (*Leiolopisma telfairii*) were found dead after eating rain-softened Talon® 20P used for rabbit eradication on Round Island,

Mauritius, and post-mortem analyses revealed brodifacoum concentrations of 0.6 mg/kg in samples of liver (Merton 1987). Skink numbers have increased markedly since the removal of rabbits (North et al. 1994). In New Zealand, lizard numbers increased after use of Talon® 20P to eradicate rabbits and rats on Stanley Island (Towns et al. 1993) and rats on Tiritiri Matangi Island (C.R. Veitch pers. comm.).

Invertebrates have been seen eating baits containing brodifacoum, and residues of brodifacoum have been found in beetles (Coleoptera) collected from bait stations containing Talon® 50WB intended for rats on Stewart Island (G.R.G. Wright unpubl. data). It is considered that invertebrates are unlikely to be directly killed by brodifacoum (Shirer 1992; Morgan et al. 1996a). However, a number of unpublished observations suggest that brodifacoum may be toxic to molluscs (D. Merton pers. comm.). Contaminated invertebrates may pose a risk of secondary poisoning to insectivorous vertebrates. However, recent studies have shown that brodifacoum does not persist in weta. If there is a similar lack of persistence in other invertebrates, then the risk of secondary poisoning via invertebrates would be short-lived. However, at this time the persistence of brodifacoum in molluscs has not been elucidated. Molluscs have a hepato-pancreas; in mammals anticoagulant rodenticides binds to vitamin K epoxide reductase in both the pancreas and the liver. It is therefore conceivable that the hepato-pancreas is a target organ in molluscs.

Secondary poisoning

The risk of secondary poisoning to non-target species is far greater from second-generation anticoagulants such as brodifacoum than from first-generation anticoagulants such as warfarin, because second-generation compounds are not substantially metabolised and excreted before death. For example, five out of six owls died after feeding on rats killed by brodifacoum for 8–11 days (Mendenhall & Pank 1980).

The only confirmed report of secondary poisoning of insectivorous birds with brodifacoum was in a zoo, where avocets, rufous-throated ant pittas, golden plovers, honey creepers, finches, thrushes, warblers, and crakes died in an aviary after feeding on pavement ants and cockroaches that had eaten brodifacoum baits (Godfrey 1985). However, the potential for invertebrates to 'carry' poison to birds has been suggested (Stephenson et al. 1999).

In New Zealand, predator and scavenger populations have been monitored during five brodifacoum-poisoning operations. Comparable numbers of brown skuas and New Zealand falcons, the main avian predators at risk, were seen before and after use of Talon® 50WB in bait stations for eradication of Norway rats on Hawea Island (Taylor & Thomas 1989). There was no evidence of New Zealand falcons or moreporks being killed by use of Talon® 50WB in bait stations for eradication of Norway rats on Breaksea Island (Taylor & Thomas 1993). There was no evidence of a detrimental effect on populations of moreporks on Stanley Island (Towns et al. 1993) or Red Mercury Island (Robertson et al. 1993) after aerial distribution of Talon® 20P for eradication of kiore. Moreporks and Australasian harriers on Tiritiri Matangi Island decreased after aerial distribution of Talon® 20P, but it is not known whether this was induced by poisoning (C.R. Veitch pers. comm.) or the removal of their major food item, rats.

The perceived hazards of secondary poisoning to non-target wildlife have restricted second-generation anticoagulants such as brodifacoum from being registered for field use in the USA (Colvin et al. 1991). The detection of brodifacoum residues in a range of wildlife including native birds such as kiwi (*Apteryx* spp.) (Robertson et al. 1993), raises serious concerns about the long-term effects of broad-scale field use of brodifacoum in New Zealand. This is compounded by the recent detection of residues in a wide range of species: weka, morepork, Australian harrier, pukeko, grey duck, mallard, black-backed gull, robin, saddleback, chaffinch, mynah, magpie, and blackbird (Murphy et al. 1998; Dowding et al. 1999; G.R.G. Wright pers. comm.). Of far less concern was the detection of brodifacoum in cats and stoats, introduced species regarded as pests and largely responsible for the decline of native birds, such as kiwi. Nevertheless, because of the potential for uncontrolled contamination of wildlife (demonstrated by field survey data) broad-scale field use of brodifacoum in New Zealand (Eason et al. 1999c, 2000) is currently being restricted by the Department of Conservation.

Recent surveys of wildlife have indicated that extensive contamination has occurred where there has been sustained use of brodifacoum. Samples of liver were collected from feral pigs, feral red deer, feral cats, stoats, and weka that were shot, or trapped, except for one feral pig and six weka found dead (Eason & Murphy 2000). All the animals were killed in areas where brodifacoum was currently in use for possum and rat control. In all cases the method of application of baits followed label instruction and bait stations were used. Fourteen out of 35 pigs (40%) contained no residues. The remaining 21 pigs, including one which was found dead, contained residues of brodifacoum at concentrations ranging from 0.007 to 1.78 mg/kg. The pig found dead contained the highest liver residue. Eleven of 33 feral deer (33%) were contaminated but the concentration did not exceed 0.03 mg/kg. Clearly, in the case of deer, the most likely route of ingestion of brodifacoum is by feeding on baits that were not adequately contained in bait stations. (Possums are known to spill significant amounts of baits when feeding.) This being the case, it seems probable that at least some of the pigs may have ingested bait in the same way as deer, compounded by some ingestion of brodifacoum-poisoned target species. Fifty-seven out of 71 cats (80%), and 98 out of 115 stoats (85%) contained residues. Concentrations in cats ranged from 0.078 to 1.84 mg/kg and in stoats from 0.008 to 1.32 mg/kg. Six weka were found dead and contained residues of between 0.11 and 2.3 mg/kg. The other 12 weka were trapped; four contained no residues, and eight (67%) contained between 0.01 and 0.95 mg/kg.

These recently acquired residue results reinforce earlier recommendations that pigs and possums should not be hunted for human consumption, from areas where baits containing brodifacoum have been used for possum control, for at least 9 months after the application of the baits (Eason et al. 1996d).

In summary, indigenous New Zealand birds most at risk from feeding directly on cereal-based baits containing brodifacoum are those species that are naturally inquisitive and have an omnivorous diet (e.g. weka, pukeko, brown skua, and kea). The risk of secondary poisoning is probably greatest for predatory and scavenging birds (especially the weka, brown skua, Australasian harrier, morepork, and southern black-backed gull) that feed on target species (e.g. live

or dead rats, rabbits, and possums). Recently published surveys by Department of Conservation and Landcare Research staff clearly demonstrate widespread wildlife contamination that extends to native birds as well as game species (Murphy et al. 1999; Gillies & Pierce 1999; Dowding et al. 1999; Meenken et al. 1999; Eason et al. 1999c; Robertson et al. 1999a; Stephenson et al. 1999). This pattern is mirrored overseas where there is field use of second-generation anticoagulants (Young & de Lai 1997; Shore et al. 1999; Stone et al. 1999).

The risks of non-target mortality and contamination after pest control must be carefully balanced against the benefits. The eradication of rabbits using brodifacoum on Round Island, Mauritius, in 1986 illustrates this most clearly. Telfair's skinks and other lizards on the island were considered at risk from poisoning by eating poisoned insects and/or bait and some were killed (Merton 1987). Three years after eradication of the rabbits there has been a dramatic regeneration of vegetation and marked increases in the numbers of lizards, including Telfair's skink (North et al. 1994). In New Zealand, the benefits of using brodifacoum (or related compounds) to eradicate rats and/or rabbits from offshore islands are also becoming apparent. For example, eradication of rats from Korapuki Island (using bromadiolone, a second-generation anticoagulant related to brodifacoum) in 1986 resulted in a 10-fold increase in lizard numbers in 3 years (Towns 1991) and a 30-fold increase in 6 years (Towns 1994). Similarly, in 1996, the successful removal of rats from Kapiti Island has resulted in a significantly improved survival rate for stitchbirds and saddlebacks, and benefits to other taxa are expected (Empson & Miskelly 1999). However, on mainland sites where the persistence of brodifacoum raises concerns about the possible transfer of this compound through the food chain to humans, dogs, or wildlife, a precautionary approach is recommended. Because of this, the use of this poison has been under review. Nevertheless, its total removal from mainland use leaves a significant gap in the armoury of the conservationist, pest controller involved in endangered species protection (Stephenson 2000).

2.1.7 Summary

Advantages	Disadvantages
Generally available and no licence required.	High risk of secondary poisoning of non-target species
Effective against possums that have developed poison/bait shyness	Persistent (>9 months) in liver of vertebrates (can enter food chain and put meat for human consump -tion at risk)
Effective for rodent control	
Antidote available	Although an antidote (vitamin K) is available, long-term treatment is needed
	Expensive compared to 1080 or cyanide
	Possums can eat excessive amounts of bait (increase costs)
	Possums take 2–4 weeks to die

- Brodifacoum is a synthetic pesticide that was developed approximately 20 years ago.
- Brodifacoum is not readily soluble. It binds strongly to soil and is slowly degraded. It is most unlikely to significantly contaminate waterways unless large amounts of baits enter streams.

- It is a potent anticoagulant, which acts by interfering with the synthesis of vitamin K-dependent clotting factors. Brodifacoum is toxic to mammals, birds, and reptiles.
- Brodifacoum is extremely persistent in the livers of lethally poisoned, and to a lesser extent the meat of sub-lethally poisoned, animals, which heightens the risk of secondary poisoning of non-target species.
- Livestock must not be allowed access to brodifacoum baits as residues may persist in survivors of a sub-lethal dose for >9 months.
- Non-target effects on individual birds of a number of species have occurred after brodifacoum use for rodent control.
- Adverse effects on individual populations of a number of species of birds have been observed after brodifacoum use for rodent control. However, short-term losses are likely to be superseded by long-term gains once predators have been removed.

2.2 FLOCOUMAFEN (STORM®)

Chemical Name: 4-hydroxy-3-[1,2,3,4-tetrahydro-3-[4-(4-trifluoromethylbenzyloxy)=phenyl]-1-naphthyl] coumarin

Synonyms: Flocoumafen is the approved name, Storm® is the trade name.

Flocoumafen and brodifacoum are extremely similar in terms of their chemistry, biological activity, potency, persistence, and risk of secondary poisoning. For further details on the toxicology, mode of action, etc. of flocoumafen see the previous section on brodifacoum.

2.2.1 Physical and chemical properties

Flocoumafen is an off-white solid with a melting point *cis*-isomer of 181–191°C and a vaporisation point at 133 pPa (25°C). Flocoumafen's solubility is 1.1 mg/L in water and >10 g/L in acetone, alcohols, chloroform, and dichloromethane. It is stable to hydrolysis and does not undergo any detectable degradation when stored at pH7–9 for 28 days at 50°C.

2.2.2 Historical development and use

Flocoumafen is a second-generation anticoagulant that was developed by Shell Research in the early 1980s. Flocoumafen has been used against a wide range of rodent pests including the principal commensal species. It is also effective against rodents that have become resistant to other anticoagulant rodenticides. It is currently registered for use in New Zealand as a rodenticide under the trade name 'Storm'. Flocoumafen is not extensively used in the field in New Zealand.

2.2.3 Fate in the environment

Flocoumafen is not readily soluble in water. In physico-chemical terms flocoumafen is extremely similar to brodifacoum. Hence if flocoumafen-containing baits were to be used in the field, when these baits disintegrate flocoumafen is likely to remain in the soil where it will be slowly degraded by soil micro-organisms. Microbial degradation will be dependent on climatic

factors such as temperature, and the presence of species able to degrade flocoumafen.

2.2.4 Toxicology and pathology

Onset of symptoms

Flocoumafen is a potent second-generation anticoagulant similar to brodifacoum. Its symptoms, time to onset of poisoning, mode of action, and toxicity to birds and mammals are like those of brodifacoum (Table 14). For practical considerations, species such as dogs, cats, and pigs, the risk of poisoning from baits or secondary poisoning from eating contaminated rodents will be similar to that for brodifacoum.

TABLE 14. ACUTE ORAL TOXICITY (LD_{50}mg/kg) OF FLOCOUMAFEN (Hone & Mulligan 1982).

SPECIES	LD_{50} (mg/kg)
Dog	0.075–0.25
Gerbil	0.18
Rat	0.25
Rabbit	0.70
Sheep	>5.0
Cat	>10.0
Goat	>10.0
Pig	60.0

Mode of action

Like other anticoagulant toxins, flocoumafen acts by interfering with the normal synthesis of vitamin K-dependent clotting factors in the liver of vertebrates (Hadler & Shadbolt 1975). In the liver cells the biologically inactive vitamin K_1-2,3 epoxide is reduced by a microsomal enzyme into biologically active vitamin K, which is essential for the synthesis of prothrombin and other clotting factors. Flocoumafen antagonism of the enzyme vitamin K_1-epoxide reductase in the liver causes a gradual depletion of the vitamin and consequently of vitamin K-dependent factors, which results in an increase in blood-clotting time until the point where no clotting occurs.

Pathology and regulatory toxicology

Pathological lesions in animals poisoned with flocoumafen are similar to those for brodifacoum and other anticoagulants. In regulatory studies flocoumafen has been shown to lack genetoxicity in a range of *in vitro* and *in vivo* regulatory toxicology studies evaluating the potential of this toxicant to induce chromosomal damage or genetic mutation.

In a teratogenicity study in rats some deaths or signs of haemorrhaging were reported at 0.4 mg/kg/day in females, but there were no reports of teratogenicity in foetuses. Hence regulatory studies indicate that flocoumafen lacks mutagenic or teratogenic effects at the doses tested (WHO 1995).

Fate in animals

(see Section 2.1.4)

Absorption, metabolism, and excretion

The persistence of flocoumafen in sub-lethally exposed animals is as great, if not greater, than that of brodifacoum (see Table 11). In rats, absorption of flocoumafen is also rapid reaching a maximum concentration in blood after 4 hours (Huckle et al. 1989) (see Table 15). Similar rapid absorption occurs for other anticoagulants (Kamil 1987) (Table 15).

TABLE 15. OCCURRENCE OF PEAK PLASMA CONCENTRATIONS IN ANIMALS AFTER ORAL INGESTION OF ANTICOAGULANTS.

ANTICOAGULANT AND DOSE		SPECIES	T_{max} HOURS	REFERENCE
Warfarin	50 mg/kg	Possum	6	Eason et al. 1999a
Bromadiolone	0.8 mg/kg	Rat	6–9	Kamil 1987
Flocoumafen	0.14 mg/kg	Rat	4	Huckle et al. 1989

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, indicating some low level of metabolism is occurring (Warburton & Hutson 1985; Huckle & Warburton 1986).

In rats, eight urinary metabolites have been detected after percutaneous exposure to ^{14}C-flocoumafen (Huckle & Warburton 1986). However, they represented only a small proportion of the total dose, with most excretion occurring in the faeces as unchanged flocoumafen. Unchanged flocoumafen comprised the major proportion of the hepatic radioactivity in rats and was eliminated with a hepatic half-life of 220 days (Huckle et al. 1989). 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.

There are insufficient comparative data in different species to clarify whether or not there is a pattern of species variation in the metabolism of flocoumafen. However, it appears that quail are able to metabolise floucoumafen more effectively than rats (Huckle & Warburton 1989).

The metabolism of flocoumafen by Japanese quail may be partly responsible for the shorter liver retentions of this toxicant in quail (hepatic half-life 155 days; Huckle & Warburton 1989) versus rats (hepatic half-life 220 days; Huckle et al. 1988). Up to 12 radioactive components were detected in the excreta of quail (Huckle & Warburton 1989). Faecal 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 hours. Less than 0.5% of the dose appeared in the urine within 7 days (Huckle et al. 1989).

When oral ^{14}C-flocoumafen doses of 0.02 mg/kg body weight 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 faeces within 3 days, mostly within the first 24 hours. At the higher dose the faecal excretion ranged from 18% after the first dose to 59% after the tenth dose (Huckle et al. 1988).

Species variation in response to flocoumafen

For a number of species the LD50 is less than 1 mg/kg and this is similar to brodifacoum (see Tables 12 and 14). However, there are several species with surprisingly high LD50 values (e.g. pigs). No aquatic toxicity data was found.

2.2.5 Diagnosis and treatment of poisoning

As for brodifacoum (see Section 2.1.5).

2.2.6 Non-target effects

Flocoumafen has the potential to cause both primary and secondary poisoning of non-target species. However, the adverse effects of flocoumafen on wildlife are dependent more on how baits are used and the behaviour of non-target species than the susceptibility of individual species to the toxin. Baits in bait stations are less accessible to non-target species than baits on the ground. Secondary poisoning of birds (e.g. brown skua and harriers) is likely where target species (e.g. rabbits and rats) are a major constituent of the diet. Flocoumafen is extremely persistent in the livers of lethally and sub-lethally poisoned animals, which heightens the potential risk of secondary poisoning in non-target species. As the use of flocoumafen is largely restricted to commensal rodents, the risks of exposure of wildlife are lower, except when it is used around farm buildings.

Livestock must not be allowed access to flocoumafen baits as residues are likely to persist in their livers for up to 9 months or more. There is very little detailed information available on the non-target impacts of this toxin. However, as the properties of this toxin are very similar to those found in brodifacoum, the potential for non-target impacts are likely to be very similar.

2.2.7 Summary

Advantages	Disadvantages
Generally available and no licence required	High risk of secondary poisoning of non-target species if used widely in the field
Effective for rodents	Persistent (>9 months) in liver of vertebrates (can enter food chain and put meat for human consumption at risk) if used in the field
Antidote available	Although an antidote (vitamin K) is available, long-term treatment is needed.
	Expensive compared to 1080 or cyanide

- Flocoumafen has chemical and biological effects that are almost indistinguishable from brodifacoum.
- Flocoumafen is a synthetic pesticide that was first registered for use approximately 20 years ago.
- Flocoumafen is not readily soluble, it binds strongly to the soil, and is slowly degraded. It is most unlikely to contaminate waterways as it is used principally for controlling commensal rodents or in bait stations.
- It is a potent second-generation anticoagulant, which acts by interfering with the synthesis of vitamin K-dependent clotting factors. Flocoumafen is toxic to mammals, birds, and reptiles.
- When used near farms, livestock must not be allowed access to flocoumafen baits, as residues may persist in the survivors for >9 months.

2.3 BROMADIOLONE (RID RAT®, CONTRAC®, SUPERSQUEAK®)

Chemical Name: 3-[3-(4'- bromobiphenyl-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxycoumarin.

Synonyms: Bromadiolone is the approved name

Baits containing bromadiolone include Rid Rat®, Contrac®, Supersqueak®, and are targeted at rodents. Bromadiolone has chemical and biological effects that are similar to brodifacoum. However, it is slightly less potent than both brodifacoum and flocoumafen.

2.3.1 Physical and chemical properties

The empirical formula for bromadiolone is $C_{30}H_{23}BrO_4$ and the molecular weight is 527.4. Technical grade bromadiolone (97% pure) is a yellowish powder with a melting point of 200–210°C. Its solubility at 20°C is 19 mg/L in water, 730 g/L in dimethylformamide, 8.2 g/L in ethanol, and 25 g/L in ethyl acetate. Bromadiolone is stable at temperatures <200°C.

2.3.2 Historical development and use

Bromadiolone was synthesised and marketed by a French company in the mid-1970s, and has since been widely used to control commensal and field rodents in many countries. The toxicant was introduced into New Zealand for sale about 1980 and is registered as a rodenticide, and has on occasion been used in New Zealand for rabbit or rat control on islands. It is not widely used in the field in New Zealand. It is not registered in New Zealand for possum control, and is marketed principally for commensal rodent control.

In spite of bromadiolone belonging to a group of more potent second-generation anticoagulants, resistance problems have been encountered in rodents after repeated use overseas. It can, however, be effective, in cases where it has not been used before, against rodents that have become resistant to other anticoagulant rodenticides. In some countries, particularly in Europe, bromadiolone has increasingly been used for field control of rodents, which is leading to secondary contamination of non-target species, including mustelids and birds (Shore et al. 1999). This situation parallels the phenomena we have observed in New Zealand with brodifacoum. Advocates for the use of bromadiolone in the field suggest that there is a lower risk of secondary poisoning compared to brodifacoum because it is less potent.

2.3.3 Fate in the environment

Bromadiolone is a second-generation anticoagulant, and as such has many of the properties that are common to the other anticoagulants. It is only slightly insoluble in water, and binds strongly to the soil, and is slowly degraded. In a study carried out in four types of soil, bromadiolone scarcely moved in soil rich in organic matter, but could be shifted through soil with low clay or organic compounds (WHO 1995).

2.3.4 Toxicology and pathology

Onset of signs

As for other anticoagulants.

Mode of action

Bromadiolone, as a second-generation anticoagulant, interferes with the Vitamin K_1-dependent clotting factors when a lethal or sub-lethal dose is ingested. In the liver cells the biologically inactive vitamin K_1-2,3 epoxide is reduced by a microsomal enzyme into biologically active vitamin K, which is essential for the synthesis of prothrombin and other clotting factors. Bromadiolone antagonism of the enzyme vitamin K1-epoxide reductose in the liver causes a gradual depletion of the vitamin and consequently of vitamin K-dependent clotting factors, which results in an increase in blood-clotting time until the point where no clotting occurs. It is cumulative and will remain in the system in sub-lethal quantities for extended periods.

Pathology and regulatory toxicology

Generalised haemorrhage is frequently evident at post-mortem. As for other anticoagulants, areas commonly affected are the thoracic cavity, subcutaneous tissue, stomach, and intestine. The heart is sometimes rounded and flaccid with subepicardial and subendocardial haemorrhages. Histomorphological analysis of the liver may reveal centrilobular necrosis as a result of anaemia and hypoxia.

In regulatory toxicology studies, bromadiolone has been shown to lack mutagenicity in *in vitro* (the Chinese hamster ovary cells) and *in vivo* (mouse micronucleus) test symptoms, and teratogenic effects (WHO 1995).

Fate in animals

(See Section 2.1.4.) The persistence of bromadiolone is similar to that of brodifacoum (see Table 11). The half-life in the liver of rats is 170 days (Parmar et al. 1987) and residues have been detected in sheep liver after 256 days (Nelson & Hickling 1994).

Species variation in response to bromadiolone

The acute oral LD_{50} for various species is detailed in Table 16. No information was found for aquatic toxicology.

TABLE 16. ACUTE ORAL TOXICITY (LD_{50}mg/kg) OF BROMADIOLONE (Hone & Mulligan 1982).

SPECIES	LD_{50}(mg/kg)
Dog	c. 10.0
Rat	0.65 (acute)
Mouse	0.99
Rabbit	c. 1.0
Guinea pig	2.8
Pig	c. 3.0
Chicken	c. 5.0
Rat	0.06–0.14 × 5 (chronic)

2.3.5 Diagnosis and treatment of poisoning

As for brodifacoum (see Section 2.1.5).

2.3.6 Non-target effects

Bromadiolone is persistent in the livers of sub-lethally poisoned animals, which heightens the potential risk of accumulated secondary poisoning to non-target species.

Livestock must not be allowed access to bromadiolone baits as residues may persist in the liver for up to 9 months or more (WHO 1995).

There are no published LD_{50} data on the acute toxicity of bromadiolone to bats, reptiles, or amphibians. However, reptiles are known to be susceptible to brodifacoum, a similar anticoagulant toxicant.

In a laboratory study, only one out of six owls died following 10 days treatment with bromadiolone-poisoned rats, compared with five and six deaths in owls eating brodifacoum-poisoned rats (Mendenhall & Pank 1980). Nevertheless, there are increasing concerns overseas regarding non-target mortality and contamination of raptors where there is broad-scale field use of bromadiolone (Shore et al. 1999). The reduced risk of secondary poisoning from bromadiolone compared with brodifacoum that is suggested by Mendenhall & Pank (1980) may not imply limited risk if there is sufficient exposure to allow bromadiolone to accumulate to toxic levels.

2.3.7 Summary

Advantages	Disadvantages
No licence requirement	Very limited field data in New Zealand, marketed principally for commensal rodents
Antidote available	Persistent and likely to lead to secondary poisoning or contamination of non-target species, if widely used in the field
Effective for rodents	

- Bromadiolone is a synthetic pesticide that was first registered for use approximately 20–30 years ago.
- Bromadiolone is not readily soluble, it binds strongly to soils, where it is slowly degraded. It is most unlikely to contaminate waterways as it is used principally for controlling commensal rodents or in bait stations.
- It is a potent anticoagulant, which acts by interfering with the synthesis of vitamin K-dependent clotting factors.
- Bromadiolone is toxic to mammals, birds, and reptiles.
- Livestock must not be allowed access to baits containing bromadiolone as residues may persist in the survivors for >9 months.
- Bromadiolone is effective against rodents that have become resistant to other first-generation anticoagulant rodenticides.

2.4 COUMATETRALYL (RACUMIN®, NO RATS & MICE®)

Chemical Name: 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl) coumarin

Synonyms: Coumatetralyl is the approved common name

Coumatetralyl is classified as a first-generation anticoagulant. It is less potent than brodifacoum, flocoumafen, or bromadiolone, but more potent than warfarin and pindone. Internationally it is sold under the trade name Racumin®, No rats & mice®.

2.4.1 Physical and chemical properties

The empirical formula for coumatetralyl is $C_{19}H_{16}O_3$ and the molecular weight is 292.6. It is practically insoluble in water, slightly soluble in ether and benzene, soluble in alcohol and acetone, and readily soluble in dimethyl formamide.

2.4.2 Historical development and use

This rodenticide was developed in 1957 by scientists at Bayer, and is marketed world-wide. It is used as a tracking powder or as a cereal bait, wax block, and paste for rodent control.

2.4.3 Fate in the environment

No published information is available on the fate of this rodenticide in soil. It would be likely to be broken down slowly in soil by micro-organisms.

2.4.4 Toxicology and pathology

Onset of signs

Coumatetralyl baits containing 1 mg/kg will kill rats in 5–8 days. In general, the symptoms of poisoning do not appear suddenly.

Mode of action

As for other anticoagulant rodenticides (see brodifacoum), post-mortem examinations reveal extensive multiple haemorrhages throughout the body with considerable quantities of unclotted blood in the chest and abdominal cavities. Rats can withstand single doses of 50 mg/kg of this toxicant, but are unable to survive doses of 1 mg/kg when that dose is ingested over 5 successive days.

Pathology and regulatory toxicology

We could not find any regulatory toxicology studies in the published literature.

Fate in animals

Coumatetralyl is markedly less persistent (in sub-lethally poisoned animals) than brodifacoum (see Table 11). The hepatic half-life of sub-lethally exposed rats is 55 days (Parmar et al. 1987).

TABLE 17. ACUTE ORAL TOXICITY (LD_{50} mg/kg) OF COUMATETRALYL (Hone & Mulligan 1982; Worthing & Hance 1991).

SPECIES	LD_{50} (mg/kg)	
Rat	16.5	(single dose)
	0.3	(5 days)
Pig	1.0–2.0	(1–7 days)
Hen	50.0	(8 days)
Fish	1000.0	(96 hours)

Species variation in response to coumatetralyl

There are comparatively few acute toxicity data for coumatetralyl (Table 17).

2.4.5 Diagnosis and treatment of poisoning

As for brodifacoum (see Section 2.1.5).

2.4.6 Non-target effects

Other than pets gaining access to bait, there are few references to non-target deaths in other species. In recent studies coumatetralyl-poisoned rat carcasses were fed to weka and ferrets. One out of 10 ferrets died, but no weka were killed (O'Connor & Eason 1999).

2.4.7 Summary

Advantages	Disadvantages
No licence required	Not as potent as brodifacoum or other second-generation anticoagulants
Effective for rodent control	
Antidote	
Less persistent than brodifacoum, flocoumafen, and bromadiolone	

- This compound was first introduced in 1957 and is sold as Racumin®, and is used as a tracking powder or as a cereal bait for rodent control.
- This bait needs to be ingested over several consecutive days to be most effective.
- As for other anticoagulants, rodents die within 5–7 days after ingesting a lethal dose of the toxin.
- As for other anticoagulants, coumatetralyl interferes with the synthesis of vitamin K-dependent clotting factors. If ingested in large enough quantities, it is toxic to mammals, birds, and reptiles.

2.5 DIPHACINONE (DITRAC®, LIQUATOX®, PESTOFF® (FOR FERRETS))

Chemical Name: 2-(diphenylacetyl)-1,3-indandione

Synonyms: Diphacinone is the approved common name

Like coumatetralyl, diphacinone is classified as a first-generation anticoagulant.

2.5.1 Physical and chemical properties

The empirical formula for diphacinone sodium salt is $C_{23}H_{15}O_3$ Na and the molecular weight is 362.4. It is soluble in water; more soluble in ethyl alcohol, acetone and hot water; insoluble in benzene and toluene.

2.5.2 Historical development and use

Diphacinone is a first-generation anticoagulant of the indandione class, produced and primarily used in the USA, where it is used to control mice, rats, prairie dogs (*Cynomys* spp.), ground squirrels, voles, and other rodents (Hayes & Laws 1991); and in South America where cattle are treated with diphacinone to provide live baits for vampire bats (Mitchell 1986).

Diphacinone is more toxic than warfarin or pindone to most rodents. In New Zealand it is registered primarily for rodent control, and more recently it has been incorporated into a fish-based bait for ferret control (Ogilvie et al. 1995).

This anticoagulant was first introduced for use in New Zealand in the 1950s as a rodenticide. It is available in both a liquid concentrate (Liquatox®) on a limited-sale basis in 50-ml plastic envelopes that are mixed with a litre of water for use as a liquid rodent bait, and a Ditrac® All Weather Block.

2.5.3 Fate in the environment

Comparative soil absorption and mobility studies have shown diphacinone to be relatively immobile. When tested in the laboratory, the half-life of diphacinone in soil under aerobic conditions is about 30 days and under anaerobic conditions is about 60 days (WHO 1995).

2.5.4 Toxicology and pathology

Onset of signs

Diphacinone baits at 3 mg/kg will kill rodents in 5–8 days. Rats can withstand relatively high single doses of this toxicant, but are unable to survive doses of <1 mg/kg when that dose is ingested over 5 successive days.

Mode of action

Diphacinone, like other anticoagulants, inhibits the formation of vitamin K-dependent clotting factors. This inhibition is prolonged when compared with the relatively short effect of warfarin. This is consistent with its prolonged persistence (90 days) in the liver (Bullard et al. 1976).

Pathology and regulatory toxicology

Clinical and post-mortem signs of toxicosis are as for other anticoagulants. Post-mortem examinations have revealed extensive multiple haemorrhages throughout the body with considerable quantities of unclotted blood in the chest and abdominal cavities.

Multidose studies with diphacinone in rats have demonstrated the difficulty in establishing a clear NOEL with persistent bioaccumulative compounds (like most anticoagulant rodenticides). No clear NOELs were obtained in a 90-day study spanning doses of 1.7–27.0 µg/kg/day (Elias & Johns 1981).

Fate in animals

See brodifacoum (Section 2.1.4 and Table 11).

Absorption, metabolism, and persistence

When diphacinone was administered (orally) to rodents, as with other anticoagulants concentrations reached their highest levels in the liver. Eight days after the administration of the compound in rats and 4 days in mice, the liver had the highest level of residues, but kidneys and lungs also contained significant concentrations of diphacinone; brain, fat, and muscles had the lowest levels (Yu et al. 1982). This is the typical pattern observed in tissue distribution studies with all anticoagulant poisons (see Table 18).

TABLE 18. RADIOACTIVITY IN THE TISSUE OF FEMALE RATS 8 DAYS AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF ^{14}C-DIPHACINONE (0.4 mg/kg). Results are ppm equivalent expressed as a mean ± SE (Adapted from Yu et al. 1982).

TISSUE	CONCENTRATION
Liver	1.394 ± 0.072
Kidney	0.239 ± 0.050
Lung	0.110 ± 0.011
Gonad	0.081 ± 0.018
Spleen	0.075 ± 0.002
Blood	0.051 ± 0.007
Heart	0.031 ± 0.005
Fat	0.026 ± 0.009
Muscle	0.017 ± 0.004

Few data exist on the changing patterns of tissue distribution over time. However, by comparing two different publications on diphacinone distribution (in rats after 8 days: Yu et al. 1982 and cows after 90 days Bullard et al. 1976), it would appear that residue can readily be detected in a range of tissues within a week of ingestion (Table 18), but after 3 months the liver and kidney are the only organs containing significant concentrations (Table 19).

Metabolism of this compound in rats involves mainly hydroxylation and conjugation reactions (Hayes & Laws 1991). The persistence of diphacinone in the liver is more prolonged than of warfarin or pindone (see Section 2.1.4).

TABLE 19. DETECTABLE DIPHACINONE RESIDUES (MEAN ± S.D.) IN TISSUE OF CATTLE GIVEN A SINGLE INJECTION OF 1 mg/kg (Adapted from Bullard et al. 1976).

DAYS AFTER TREATMENT	RESIDUES FOUND (ppm ± S.D.)	
	LIVER	KIDNEY
30	0.15 ± 0.01	0.08 ± 0.01
60	0.14 ± 0.1	0.10 ± 0.02
90	0.15 ± 0.00	0.08 ± 0.00

Species variation in response to diphacinone

There is a marked species variation in the susceptibility of animals to the toxic effects of diphacinone (Table 20).

TABLE 20. ACUTE ORAL TOXICITY (LD_{50}mg/kg) OF DIPHACINONE.

SPECIES	ACUTE ORAL LD_{50} (mg/kg)
Rat (unspecified)	0.3–2.3
Dog	3.0–7.5
Cat	14.7
Rabbit	35.0
Pig	150.0
Mouse	340.0
Mallard duck	3158.0

2.5.5 Diagnosis and treatment of poisoning

As for brodifacoum (see Section 2.1.5).

2.5.6 Non-target effects

Other than pets gaining access to bait, there are no references to non-target deaths in other species in New Zealand. Birds have been shown to have been poisoned by eating carcasses. Both great-horned owls and saw-wet owls eating poisoned carcasses were affected, but not barn owls. In the USA golden eagles showed signs of haemorrhages after eating meat from animals poisoned with diphacinone (Savarie et al. 1979).

Bats have been shown to be susceptible to diphacinone. In Latin America, where paralytic bovine rabies is transmitted by the common vampire bat (*Desmodus rotundus*), cattle are given sub-lethal intramuscular doses of diphacinone. These cattle effectively act as live baits, and bats that suck blood from treated cattle are killed (Thompson et al. 1972; Mitchell 1986; Said Fernandez & Flores-Crespo 1991). Although information derived in vampire bats cannot be extrapolated to the susceptibility of New Zealand's short and long-tailed bats, it does suggest that they may be susceptible to anticoagulants via primary or secondary poisoning. Diphacinone is likely to have a slightly lower tendency to cause secondary poisoning when compared with bromadiolone, brodifacoum, or flocoumafen, because it is less potent.

2.5.7 Summary

Advantages	Disadvantages
No licence required	More persistent than coumatetralyl
Effective for rodent control	Less potent than brodifacoum
Antidote	
Less persistent than brodifacoum	

- Diphacinone is a first-generation anticoagulant.
- Diphacinone in bait formulations needs to be ingested over several days before a lethal dose is taken. Rodents will die within 5–8 days of ingesting a lethal dose.
- Like other anticoagulants diphacinone interferes with the synthesis of vitamin K-dependent clotting factors. If ingested in large enough quantities, it is toxic to mammals, birds, and reptiles.
- Diphacinone is not readily soluble, it binds strongly to the soil and is slowly degraded.

2.6 PINDONE

Chemical Name: 2-pivaloyl-1,3-indandione.

Synonyms: Pindone is the approved common name.

Pindone is one of the earliest first-generation anticoagulant rodenticides developed in the 1940s.

2.6.1 Physical and chemical properties

The empirical formula for pindone is $C_{14}H_{14}O_3$ and the molecular weight is 230.3. It is a yellow crystalline powder with a melting point of 108.5–110.5°C and low solubility in water (18 mg/L) at 25°C. A sodium salt ('Pival' or pindone-sodium) $C_{14}H_{13}NaO_3$ with a molecular weight 252.3 is readily water soluble.

2.6.2 Historical development and use

Pindone, like diphacinone, belongs to the indandione class of anticoagulants, which differ chemically from coumarin anticoagulants such as brodifacoum or warfarin.

Pindone was synthesised in 1937 (Beauregard et al. 1955) and developed as a pesticide in the early 1940s. It was originally evaluated as an alternative to pyrethrins because of its insecticidal properties (Kilgare et al. 1942). Subsequently it was selected for extended study as it possessed the strongest insecticidal and anticoagulant characteristics of a series of 1,3 indandiones (Crabtree & Robinson 1953). In 1948 pindone was shown to be an effective alternative to DDT for the treatment of body lice (Eddy & Bushland 1948), but it has not been widely used as an insecticide.

Pindone has been used world-wide to control rodents, though its use for the control of rats and mice has decreased following the introduction of more

potent anticoagulants such as brodifacoum. There are two other indandiones, diphacinone and chlorophacinone, which were synthesised in the 1950s and 1960s, and these two newer, more potent compounds have also contributed to a reduction in pindone use for rodent control.

In New Zealand pindone has been used to control wallabies and possums, but in both Australia and New Zealand it has proved most effective for rabbit control (Eason & Jolly 1993). Pindone is currently registered in New Zealand for the control of rabbits and possums. It is most effective for rabbit control.

2.6.3 Fate in the environment

There are no published data on the fate of pindone or its metabolites in soil and water. However, there are unpublished data that indicate that pindone is slowly degraded in soil and water (G. Wright pers. comm.).

In comparative studies, pindone is more slowly leached from oat grain bait for rabbits when compared with 1080. The authors attribute this to the lower solubility of pindone in water (Wheeler & Oliver 1978). This has recently been confirmed by New Zealand researchers (Booth et al. 1999a), which may in part explain the higher than expected non-target mortality observed after broad-scale use of pindone.

A small survey of water samples in a catchment area where pindone baits had been aerially sown for rabbit control was completed in 1994 (G.R.G. Wright pers. comm.). No pindone residues were detected.

2.6.4 Toxicology and pathology

Onset of signs

As for other anticoagulants.

Mode of action

Pindone acts like the other anticoagulant toxicants by interfering with the normal synthesis of vitamin K-dependent clotting factors in the liver. The weaker potency of first-generation anticoagulants such as pindone is related to a generally lower binding affinity when compared to second-generation compounds (Parmar et al. 1987; Huckle et al. 1988). The mechanism by which pindone exerts insecticidal and fungicidal activity has not been described in the literature.

Pathology and regulatory toxicology

As with all other anticoagulant compounds, clinical signs of toxicosis in animals will usually reflect some manifestation of haemorrhage. Onset of signs may occur suddenly; this is especially true when haemorrhage of the cerebral vasculature or pericardial sac occurs. Clinical signs commonly include anaemia and weakness. Haemorrhaging may be visible around the nose, mouth, eyes, and anus and animals may pass bloody faeces. When pulmonary haemorrhage has occurred, blood-tinged froth may be visible around the nose and mouth. Swollen, tender joints are common and, if haemorrhage involves the brain or central nervous system, ataxia or convulsions can occur. Poisoned animals will die usually of multiple causes associated with anaemia or hypovolemic shock. Some

possums receiving high doses of pindone have died without any signs of haemorrhaging, and necropsy has revealed liver damage (Jolly et al. 1994). The authors were unable to locate any regulatory toxicology data relating to pindone.

Fate in animals

Absorption, metabolism, and excretion

Pindone is absorbed through the gastrointestinal tract. Tissue distribution of pindone seems to be somewhat different from other anticoagulants. Plasma concentrations remain higher than tissue concentrations for 8 days and concentrations in the liver and kidney are comparable (Fitzek 1978). Pindone is far less persistent than second-generation anticoagulants such as brodifacoum, and is less persistent than diphacinone, which is consistent with our understanding of the mode of action and relative potencies of these compounds.

In dogs this compound is fairly well absorbed (67%) and the plasma elimination half-life is approximately 100 hours after administration of 3 mg/kg (Fitzek 1978). In sheep, residues were detected in the liver and fat of animals dosed with 20 mg/kg for 8 days, but at 2 weeks none was detected (Nelson & Hickling 1994).

Species variation in response to pindone

There are limited acute toxicity data available on pindone, but even these data show marked species variation. For first-generation anticoagulants such as pindone, either very large single doses or repeated smaller doses are generally needed to induce death. A single dose of approximately 18 mg/kg is, however, sufficient to kill rabbits (Table 21).

TABLE 21. ACUTE ORAL TOXICITY (LD_{50}mg/kg) OF PINDONE (Beauregard et al. 1955; Oliver & Wheeler 1978; Hone & Mulligan 1982; Eason & Jolly 1993).

SPECIES	LD_{50} (mg/kg)
Rabbit	6–18
Dog	50
Norway rat	75–100
Sheep	approx. 100
Possum	>100

In rabbits the repeat dose (7 days) LD_{50} is 0.52 mg/kg/day, while all rabbits receiving 1 mg/kg for 7 days died. By contrast, pindone doses of up to 12 mg/kg/day do not cause clinical or post-mortem haemorrhage in sheep (Oliver & Wheeler 1978). Possums appear to be even more resistant to pindone than sheep. None of 12 possums dosed at 8 and 16 mg/kg/day for 5 days died. One of 12 possums died when dosed with 32 mg/kg/day for 5 days, and 9 of 14 possums died when dosed with 64 mg/kg/day for 5 days. From these data an LD_{50} of 51 mg/kg/day for 5 days was calculated (Jolly et al. 1994).

Non-target research conducted in Australia provides information on the susceptibility of horses, cattle, goats, chickens, dogs, and cats. All these species were less susceptible than rabbits. Daily doses of pindone, ranging from 0.3 to 2.5 mg/kg, were administered for 5 days. No mortalities occurred and

susceptibility was assessed by using extension of prothrombin time as a biomarker of poisoning. In this study, cattle and cats appeared most susceptible out of the six species tested, and horses least susceptible to pindone toxicity (Martin et al. 1991). Nevertheless, the rabbit remains outstanding as the most susceptible mammalian species evaluated to date.

Aquatic toxicology

There are no published aquatic toxicity data for pindone.

2.6.5 Diagnosis and treatment of poisoning

As for brodifacoum (see Section 2.1.5).

2.6.6 Non-target effects

No systematic studies have been conducted to monitor the non-target impact of baits. During 1992–94 the aerial application of pindone baits to control rabbits increased. There have been numerous anecdotal reports (E.B. Spurr pers. comm.) of extensive bird kills from both primary and secondary poisoning following broad-scale rabbit control in New Zealand, but no monitoring to determine whether or not populations are being affected. Birds found killed included plovers, quails, rails, wrybills, silvereyes, grey warblers, black-back gulls, and Australian harriers (Sullivan 1994). Even less is known about the effects of pindone on invertebrates and reptiles.

In Australia similar rabbit poisoning operations have caused concern with wedge-tailed eagles, noted to be a species at risk. Doses as low as 1–4 mg/kg/day for 5–7 days have caused deaths in this species (D. King pers. comm.).

2.6.7 Summary

Advantages	Disadvantages
No licence required	Not as potent as second-generation anticoagulants or non-anticoagulant poisons such as 1080, cholecalciferol, or cyanide
Effective for rodent control	Not potent for possum control
Highly effective for rabbit control	
Antidote	
Less persistent than brodifacoum	

- Pindone is a synthetic pesticide that was first synthesised in 1937. Its insecticidal and rodenticidal properties were demonstrated in the 1940s.
- Pindone is not readily water soluble, but the sodium salt of pindone (pival) is readily water soluble and is sometimes used in New Zealand instead of pindone.
- Pindone is a first-generation anticoagulant with low potency compared to compounds like brodifacoum, a second-generation anticoagulant.
- Pindone is moderately toxic to a range of species. Rabbits are extremely susceptible; by contrast sheep, possums, and horses are comparatively resistant. There are anecdotal reports that raptors are particularly susceptible to secondary poisoning.
- Pindone is far more effective for rabbit control than it is for possums.

- Pindone is moderately persistent; far more persistent in animals than 1080, but considerably less persistent than brodifacoum.
- The toxicity and non-target impacts of pindone are poorly documented.

2.7 WARFARIN

Chemical Name: 3-(α-acetonylbenzyl)-4-hydroxycoumarin.

Synonyms: Warfarin.

Warfarin, like pindone, is one of the earliest first-generation anticoagulant rodenticides

2.7.1 Physical and chemical properties

The empirical formula for warfarin is $C_{19}H_{16}O_4$ and the molecular weight is 308.3. It is a colourless, odourless, and tasteless crystalline powder with a melting point of 161°C. It is insoluble in water and benzene, freely soluble in alkaline solution, readily soluble in acetone, and only moderately soluble in alcohols.

2.7.2 Historical development and use

Warfarin baits are registered in New Zealand for rodent control, but bromadiolone, brodifacoum, and flocoumafen are often preferred by pest managers because of their greater potency. Warfarin is a first-generation anticoagulant that has been used in a range of rodent baits since it was first introduced in 1947.

Cereal pig-baits containing warfarin are available from the Animal Control Products factory in Wanganui.

As warfarin is a first-generation anticoagulant, for most animals the baits will need to be ingested regularly over several days before any of the symptoms of poisoning will occur.

2.7.3 Fate in the environment

There are no published data on warfarin degradation. However, significant contamination of soil and water following the use of bait stations is extremely unlikely. Minor contamination is likely around the bait station, which should not be a major risk to non-target species. Degradation by soil micro-organisms and slow dispersal of warfarin in the soil is probable: this is based upon data on the degradation of similar anticoagulant toxicants.

2.7.4 Toxicology and pathology

Onset of signs and pathology

As for all anticoagulants, the onset of symptoms will depend on the dose, nature, and amount of bait consumed.

Rats

Warfarin baits administering 1 mg/kg will kill rats in 5–8 days. Rats can withstand single doses of 50 mg/kg, but are unable to survive doses of 1 mg/kg

bodyweight when that dose is ingested for 5 successive days. In general the symptoms of poisoning do not appear suddenly, and will culminate in death within 5–7 days of the initial ingestion of a lethal dose.

Pigs

Approximately 3 days after poisoning, some pigs will become lame, depressed, and lethargic. Food consumption decreases and blood is commonly observed in faeces. There is a great deal of individual variation in the time it takes for pigs to die from warfarin poisoning, with some pigs dying before or soon after they have shown the initial symptoms of poisoning and others living up to 31 days, progressively weakening over time.

Mode of action

Warfarin, like the other anticoagulants, inhibits the synthesis of vitamin K-dependent clotting factors. In addition, warfarin is reported to induce capillary damage. Two different metabolites are thought to be responsible for these effects: 4-hydroxycoumarin inhibits the formulation of prothrombin and reduces the clotting power of the blood, whereas there is some evidence that, at sufficient dosage, benzalacetone produces capillary damage that exacerbates bleeding.

Pathology and regulatory toxicology

Animals may be subjected to a hypovolemic crisis secondary to massive haemorrhage into body cavities, subcutaneous tissues, and the alimentary, respiratory, and urinary tracts in cases where large doses of the toxin have been ingested. Animals that receive a lower dose of the toxin may show signs of lethargy, anaemia, anorexia, bloody faeces, and abdominal pain. In pigs, extensive haemorrhages into the stomach and the small and large intestine are the most common signs of anticoagulant pathology, with skeletal muscle, peritoneum, and weight-bearing joints common sites of haemorrhage.

There are limited regulatory toxicology studies on warfarin and no data relating to potential mutagenic effects. While all anticoagulant rodenticides are likely to be embryotoxic if ingestion occurs at a sufficiently high dose, warfarin is unique in this class of compounds and was found to be both embryotoxic and teratogenic when administered to rats (WHO 1995), causing internal hydrocephalus and anomalies of skeletal ossification. In humans undergoing continuous drug treatment with warfarin, defects have in the past been classified as warfarin embryopathy and include both skeletal and non-skeletal abnormalities. No cases of embryopathy from anticoagulants in their use as rodenticides have been reported.

Fate in animals

Absorption, metabolism, and excretion

Warfarin is readily hydroxylated by rat microsomal enzymes to at least eight metabolites, including 6-,8- and especially 7-hydroxywarfarin (Sutcliffe et al. 1987). It is not persistent, and is readily excreted with an elimination half-life of about 18 hours in male rats and 28 hours in female rats (Pyrola 1968). The half-life in rat liver is reported to be 7–10 days for warfarin, which contrasts with half-lives exceeding 100 days for second-generation anticoagulants (Thijssen 1995) (see Tables 10 and 11).

Species variation in response to warfarin

The toxicity of warfarin varies according to species and whether exposure was a single or multiple dose (Table 22). For example, the single dose LD_{50} is 50–100 mg/kg in rats (species unspecified) versus 1 mg/kg for 5 days (Osweiler et al. 1985).

Values for the acute oral LD_{50} of warfarin for Norway rats vary between 1.5 and 3.75 mg/kg (Hone & Mulligan 1982). The strain and sex of the test animals and the carrier used in the administration probably affected the results obtained.

TABLE 22. ACUTE ORAL TOXICITY (LD_{50}mg/kg) OF WARFARIN (Osweiler et al. 1985).

SPECIES	SINGLE DOSE (mg/kg)	REPEATED DOSE (mg/kg)
Pig	3	0.5
Dog	50	5
Rat (unspecified)	50–100	1
Cat	50–100	1

Aquatic toxicology

There are no published data available for warfarin.

2.7.5 Diagnosis and treatment of poisoning

As for brodifacoum (see Section 2.1.5).

2.7.6 Non-target effects

Although less potent than 1080 or brodifacoum, warfarin still has the potential to cause primary poisoning of non-target species. Secondary poisoning is relatively uncommon (Osweiler et al. 1985; Prakash 1988).

If warfarin baits are used for control of pest species, it is important that the baits are not positioned where livestock may eat them.

2.7.7 Summary

Advantages	Disadvantages
No licence required	Not as potent as second-generation anticoagulants or non-anticoagulant poisons such as cholecalciferol and 1080
Effective for rodent control	
Antidote	
Less persistent than brodifacoum	

- Warfarin is a first-generation anticoagulant.
- In order for a lethal dose to be ingested, the target species needs to consume either one large single dose or a small dose for several days in a row.
- Because warfarin has a slow mode of action, bait shyness is not readily induced.
- It is not persistent when compared to brodifacoum, but is considerably less potent than second-generation anticoagulants.

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