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(54) Title: CONTROL METHOD

(57) Abstract: The present invention relates to vertebrate pesticide compositions for use in controlling pests such as rats and mice. The active substances in the vertebrate pesticide compositions comprise at least two components: a high concentration of low-toxicity anticoagulant and a low concentration of high-toxicity anticoagulant. The vertebrate pesticide compositions may also comprise various other components.

CONTROL METHOD

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The present invention relates to vertebrate pesticide compositions for use in controlling pests such as rats and mice. The active substances in the vertebrate pesticide compositions comprise at least two components: a high concentration of low-toxicity anticoagulant and a low concentration of high-toxicity anticoagulant. The vertebrate pesticide compositions may also comprise various other components required to deliver this mixture of anticoagulants to the target animals. The invention also relates to methods of making the vertebrate pesticide compositions and methods of controlling vertebrate pest populations using these compositions.

Vertebrate pests are damaging to the activities of Man and to the natural environment in a variety of ways (Lund, 1994). They consume various foods and feedstuffs intended for Man and his domesticated animals. Much of these food and feedstuffs that are not directly consumed by rodents are contaminated with faeces, urine and hair so as to render them either unfit for consumption or of substantially lower economic value. Vertebrate pests are also significant pests of growing agricultural crops such as, but not restricted to, wheat, oats, barley, maize, sorghum, sugarcane, rice, cacao, coconut and oil palm. By their various behaviours such as gnawing, burrowing, construction of nests and digging, vertebrate pests also damage installations, property and equipment so as to render them either unfit for their intended purpose or of lower economic worth. Vertebrate pests transmit a vast range of diseases to Man and his domesticated stock and companion animals (Webster and Macdonald, 1995). These include, but are not restricted to, leptospirosis, salmonellosis, cryptosporidiosis, bubonic plague, hantaan virus with pulmonary syndrome, leishmaniasis and toxoplasmosis. In the natural environment, vertebrate pests are predators of wildlife and, when inadvertently introduced by Man to regions where they did not previously exist, degrade habitats and predate vulnerable species so that they are a very significant detriment to global biodiversity (Moors, 1985). For all of these reasons, it is essential that methods exist that permit vertebrate pests to be controlled in ways that are cost-effective and do not, themselves, cause harm to the environment.

Anticoagulant rodenticides such as chlorophacinone, coumatetralyl, diphacinone and warfarin (generally known as "first-generation anticoagulants) were historically widely used as a means of controlling vertebrate pest populations (Buckle, 1994). These rodenticides are generally low toxicity anticoagulants.

However, the development of anticoagulant resistance in the 1950s and 1960s in Norway rat and House mouse populations, and in some other species of rodent pests, threatened the future of these effective compounds (Greaves, 1994). As a direct result, this stimulated research and resulted in the development of more potent anticoagulants that are now collectively referred to as the "second-generation anticoagulants" (Hadler and Shadbolt, 1975). These active substances include the compounds brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen.

The main advantage of these more potent, higher-toxicity, second-generation anticoagulants is that they control populations of rats and mice that are resistant to the earlier, first-generation anticoagulants. Their main disadvantage is that they are more persistent in the bodies of vertebrates than the first-generation anticoagulants and are therefore perceived to be a significant risk when they enter the environment (Burn *et al.*, 2002). Target rodents that consume rodenticide baits containing these persistent second-generation anticoagulants carry residues of them which may be passed on to predatory and scavenging species when they consume dying and dead target rodents (Brakes and Smith, 2005) . Therefore, second-generation anticoagulants are frequently the cause of death of individuals of a wide range of wildlife species, particularly predatory and scavenging birds of high conservation value (Newton *et al.*, 1997). Individuals that consume a sub-lethal dose of the active substances, both target and non-target species, carry long-lived residues in their bodies and this results in the widespread contamination of wildlife wherever second-generation anticoagulants are used (Walker *et al.*, 2010).

In order to appreciate the mechanisms of action of the anticoagulant rodenticides, an understanding of the process of mammalian blood clotting is necessary, in particular the role of vitamin K. Activated, or functional, blood clotting factors are required to

bind calcium ions to provide a substrate for the formation of blood clots. The reduced form of vitamin K, hydroxyquinone, is a cofactor for the enzyme gamma-glutamyl carboxylase, which catalyses the conversion of glutamate residues to gamma-carboxyglutamate (Gla), in the production of functional prothrombin (clotting factor II) and several other essential blood clotting factors (Factors VII, IX and X). Vitamin K is a micronutrient in mammalian diets and for viability molecules of the vitamin must be recycled (and thereby reactivated). This is achieved in two reduction steps: firstly vitamin K epoxide to vitamin K quinone and secondly the quinone to the hydroxyquinone, with dithiol as a dependent cofactor in both reactions. This recycling allows each vitamin K molecule to be utilised 1,000-10,000 times (Thijssen, 1995). Some authorities state that both reduction stages are catalysed by the enzyme vitamin K-2,3-epoxide reductase (VKOR) (e.g. Thijssen, 1995), though previously a second enzyme, vitamin K reductase, had been recognised (MacNicoll, 1985; Thijssen *et al.*, 1988; Thijssen *et al.*, 1989). Rost *et al.* (2004) refer to a 'vitamin K epoxide reductase multiprotein complex', all components of which may not yet have been identified.

Warfarin and its related substances, including both its congener first-generation anticoagulants and the second-generation anticoagulants, act by binding to the VKOR and inhibiting the two reduction stages of the vitamin K cycle. The precise mechanism of this interaction remains uncertain. The result of the anticoagulant-enzyme binding is the interruption of the vitamin K cycle and the production of non-functional (i.e. under-carboxylated) prothrombin and other clotting factors. Circulating, functional clotting factors degrade at differing rates and have different threshold levels of activity, and it takes some time before haemostasis is compromised, but eventually normal blood clotting fails and haemorrhage can occur. The depuration and regeneration of clotting factors II, VII and X in Norway rats are described in detail by Kerins and MacNicoll (1999).

Thus, all anticoagulants achieve their effect by blocking the same specific binding sites that are linked to enzymes involved in the activation of certain blood clotting factors. These binding sites are primarily located in the liver and pancreas of vertebrate animals. However, the fundamental difference between first- and second-

generation anticoagulants is their duration of residence at these binding sites, i.e. their pharmacokinetic persistence. The propensity of active substances to bind to substrates such as enzymes is called the binding coefficient. The first-generation anticoagulants have lower binding coefficients and short biological half-lives; for example in Norway rats warfarin has a half-life of elimination of approximately 10 days. The second-generation anticoagulants have higher binding coefficients and longer biological half-lives, which in Norway rats is generally greater than 100 days (W.H.O., 1995; Thijssen, 1995).

It has now been found that the levels of high toxicity and long persistence second-generation anticoagulants which are used in pesticide baits can be significantly reduced by combining such anticoagulants with higher levels of low toxicity, short persistence first-generation anticoagulants. One consequence of this is that new pesticides can be produced which retain high levels of efficacy, equivalent to those provided by pesticides which carry a high concentration of persistent second-generation anticoagulants, and yet have significantly reduced potential for adverse environmental impact.

The production of the new pesticides that are the subject of the invention has been facilitated by a deeper understanding of the mechanism of action of the anticoagulant pesticides, the reasons for their biological persistence and the inventor's insight into the interaction in vertebrate physiological systems between first- and second-generation anticoagulants and their common binding sites.

The invention relates to a vertebrate pesticide composition which is capable of being consumed by vertebrate pests, which contains a high concentration of a low toxicity (e.g. first-generation) anticoagulant and a low concentration of a high toxicity (e.g. second-generation) anticoagulant.

All anticoagulant active substances have two possible destinations in the bodies of animals that consume and digest them. First, after passing from the gut into the blood, they are bound tightly to specific binding sites found mainly in the liver and pancreas. When these binding sites are fully occupied, any anticoagulant taken in

excess of the quantity required to fill them comes to reside in other parts of the bodies of the animals, mainly in the blood and other tissues which do not posses specific binding sites. These are the second site of residence of anticoagulants in the bodies of poisoned animals. Anticoagulants are excreted from these two sites at different rates. They are excreted very slowly from the specific binding sites in the liver and pancreas, where their residues are tightly bound. They are excreted much more rapidly from the blood, and other tissues which do not possess specific binding sites, where they are not tightly bound. Thus, anticoagulants are said to undergo 'biphasic elimination' from the bodies of poisoned animals, with a fast phase and a slow phase.

For blood coagulation to be compromised, and therefore for anticoagulants to exert their toxic effects, an anticoagulant must be present in excess of that required fully to saturate all specific binding sites. Conversely, for blood coagulation to become re-established, the excess active ingredient must be excreted, and some active ingredient must be liberated from the specific binding sites, so that the vitamin K cycle may effectively resume.

For products currently on the market to be effective which contain only a single second-generation anticoagulant, all binding sites in the target animals must be occupied by the second-generation anticoagulant contained in them. The same is true for products currently on the market that contain mixtures of two different second-generation anticoagulants.

For example, EP 2 090 164 A1 (Zapi Industrie Chimiche SpA) discloses a two part rodenticide bait comprising a combination of two second-generation anticoagulants (i.e. bromadiolone and difenacoum) in an "effective disinfesting amount". Such amounts are said to be 0.0010% to 0.0040% each, by weight, which equates to a combined total concentration of 20 ppm to 80 ppm of second-generation anticoagulant per rodenticide bait. In fact, the Examples of EP 2 090 164 A1 all relate to combined concentrations of 40 ppm or higher. Given that bromadiolone and difenacoum are both second generation anticoagulants, and have higher binding coefficients and longer biological half-lives (Palmar *et al.*, 1987), the use of the compositions disclosed in EP 2 090 164 A1 results in long-term persistence of

significant levels of these anticoagulants in the environment, which is clearly undesirable.

In such cases, high residues of persistent anticoagulants come to reside in the bodies of target rodents and, subsequently if they consume dead and dying contaminated target rodents, in the bodies on non-target animals.

The invention involves mixtures of low doses of high toxicity anticoagulants (e.g. brodifacoum, bromadiolone, difenacoum, difethialone, flocoumafen and other compounds which may behave similarly to them in the bodies of target animals) and high doses of low toxicity anticoagulants (e.g. chlorophacinone, coumachlor, coumatetralyl, diphacinone, warfarin and other compounds which may behave similarly to them in the bodies of target animals). When products that contain these low-dose/high-dose mixtures are applied for pest control, a significant portion of the specific binding sites in the target animals are occupied by the less persistent and toxic first-generation anticoagulant, which is present in the product in high concentration. The product contains only a small quantity of the more toxic and persistent second-generation anticoagulant. However, because it is present in conjunction with bound residues of the low-toxicity low-persistence first-generation anticoagulant, it surprisingly exerts its full toxic effect as though it were present in much higher concentration. Thus, significantly less of the highly toxic and persistent second-generation anticoagulant are required for pest control and less is released into the environment.

The invention therefore provides a vertebrate pesticide composition comprising:

(i) a high concentration of low-toxicity anticoagulant, and

(ii) a low concentration of high-toxicity anticoagulant.

Preferably, the high-toxicity anticoagulant has high persistence.

Preferably, the low-toxicity anticoagulant has low persistence.

Preferably, the composition additionally comprises one or more flavour or flavour enhancers, human taste deterrents, preservatives, antioxidants, thickeners, binding

agents, colouring agents or carriers which are suitable for use with vertebrate pesticides or which enhance the attractiveness or palatability of vertebrate pesticides.

As used herein, the term "vertebrate pesticide" refers to a composition or kit which is capable of killing vertebrate pests. It may also be known either as a vertebrate toxicant or a rodenticide.

Preferably, the vertebrate pest is a mammal, most preferably a land mammal. The mammal may, for example, be a rodent or a marsupial. Examples of rodents include rats and mice. Examples of marsupials include possums.

The mammal may also be a rabbit, hare or mustelid (e.g. a stoat).

The vertebrate or mammal is not a human.

In other embodiments, the vertebrate pest is a bird. Examples of pest birds include pigeons, starlings, sparrows, quelea and crows.

In other embodiments, the vertebrate pest is a reptile.

As used herein, the term "anticoagulant" refers to a substance which delays or prevents the clotting of blood.

The clotting of blood may be measured by any standard means including by the partial thromboplastin time (PTT) or the prothrombin time (PT). Typically in the laboratory, using a rabbit brain thromboplastin, Norway rat blood will have a normal prothrombin time of 15-20s. Hence an anticoagulant will increase this time to greater than 30, 60 or 100 seconds. For example, 24 hours after the Norway rat has received a high dose of anticoagulant, prothrombin time will be greater than 300 seconds.

As used herein, the term "low concentration" refers to a concentration of 1-20 ppm of high-toxicity anticoagulant. In some embodiments, the concentration is preferably 1-5 ppm, 5-10 ppm, 10-15 ppm or 15-20 ppm, more preferably 2-10 ppm.

The low concentration may be made up of 1 or more, e.g. 2, 3, 4, or 5, high-toxicity anticoagulants provided that that the total concentration of all of the high-toxicity anticoagulants satisfies the above criteria.

As used herein, the term "high concentration" refers to a concentration of 100-1000 ppm of low-toxicity anticoagulant. In some embodiments, the concentration is preferably 100-200 ppm, 200-400 ppm, 400-600 ppm, 600-800 ppm or 800-1000pmm, more preferably 250-1000 ppm or 250-750 ppm.

As used herein, "ppm" refers to parts of the anticoagulant per million parts of the total vertebrate pesticide composition. For example, 50 ppm anticoagulant is equivalent to 0.005% of the total vertebrate pesticide composition by weight being the anticoagulant. As such, the concentrations given herein as ppm may readily be expressed either as ppm or the equivalent % weights (or indeed the equivalent mg anticoagulant/kg total vertebrate pesticide composition).

The high concentration may be made up of 1 or more, e.g. 2, 3, 4, or 5, low-toxicity anticoagulants provided that that the total concentration of all of the low-toxicity anticoagulants satisfies the above criteria.

The best concentration for each vertebrate pest may readily be determined (within the above ranges) by the skilled person, following the teachings disclosed herein.

Preferably, the vertebrate pesticide comprises only one type of low toxicity anticoagulant and/or only one type of high toxicity anticoagulant.

The toxicity of a substance is generally measured in terms of the amount of the substance which is capable of causing the death of defined percentage of animals of a particular species. For example, the LD_{50} of a substance indicates the amount of the substance ($mg.kg^{-1}$) that is capable of causing the death of 50% of animals of a particular species.

Another expression of the toxicity of an anticoagulant is the Lethal Feeding Period (LFP). This is the period, normally expressed in days, of unrestricted feeding before death on bait containing a given anticoagulant at a given concentration. For example, the LFP_{50} of a substance indicates the duration of time in days of bait consumption that is capable of causing the death of 50% of animals of a particular species.

As used herein, the term "high toxicity" refers to a second-generation anticoagulant, administered as a single dose by the oral route, which typically has an LD_{50} of less than 5.0 $mg.kg^{-1}$ in Norway rats and 10.0 $mg.kg^{-1}$ in house mice, preferably less than 2.0 $mg.kg^{-1}$ in Norway rats and 4.0 $mg.kg^{-1}$ in house mice.

As used herein, the term "low toxicity" refers to a first-generation anticoagulant, administered as a single dose by the oral route, which typically has an LD_{50} of greater than 15.0 $mg.kg^{-1}$ in Norway rats and 20 $mg.kg^{-1}$ in house mice, preferably greater than 20.0 $mg.kg^{-1}$ in Norway rats and 40 $mg.kg^{-1}$ in house mice

The anticoagulants achieve their effect by occupying specific binding sites that are linked to enzymes involved in the activation of certain blood clotting factors. These binding sites, which are primarily located in the liver and pancreas, bind second-generation anticoagulants much more persistently than the first-generation anticoagulants (WHO, 1995).

Therefore, as used herein, the term "persistence" refers to the duration of binding of anticoagulant in the specific binding sites. This duration is generally measured in experiments to determine the depuration half-life of the anticoagulant in liver tissue of the exposed animal, i.e. the period of time in days taken by an animal, usually a laboratory rat, to excrete half of an administered dose of an anticoagulant (for example Parmar *et al.* 1987).

Elsewhere in the field of ecotoxicology, ecological persistence is measured by certain other simple chemical criteria, such as the octanol-water partition coefficient. This is an indirect measure of lipophilicity, which is the inherent tendency of an active

substance to dissolve in and be retained by fats. These other expressions are related to measures of liver half-life but are different from them.

As used herein, the term "high persistence" refers to an anticoagulant which is released from the specific binding sites in Norway rats with a half-life of elimination typical of a second generation anticoagulant (e.g. greater than 50 days and preferably greater than 100 days) (WHO, 1995).

As used herein, the term "low persistence" refers to an anticoagulant which is released from the specific binding sites in Norway rats with a half-life of elimination typical of a first generation anticoagulant (e.g. less than 50 days and preferably less than 30 days) (WHO, 1995).

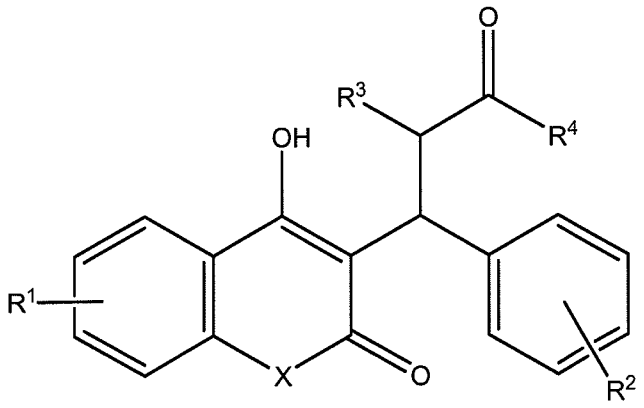
The anticoagulants referred to herein act by delaying or preventing blood coagulation by delaying or preventing the production of blood clotting factors, e.g. coagulation factors II (prothrombin) and/or VII (proconvertin) or Factors IX and X (Kerins and Macnicoll, 1999).

Preferably, the anticoagulants are vitamin K antagonists, i.e. they are inhibitors of one or more components of the vitamin K cycle, e.g. vitamin K epoxide reductase, vitamin K epoxide or gamma-glutamyl carboxylase, most preferably vitamin K epoxide reductase.

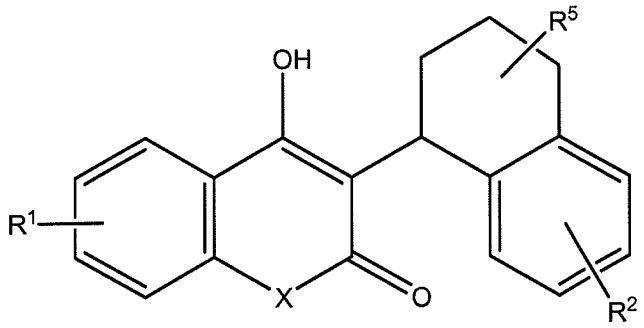
Warfarin and other coumarin-based drugs are known to block the action of vitamin K epoxide reductase.

In some preferred embodiments of the invention, the low toxicity anticoagulants are 4-hydroxy coumarin derivatives of Formula I:

Formula I



or Formula II:



wherein

X is a heteroatom selected from O, N and S, preferably O;

R1-R5 are independently selected from the group consisting of H, OH, halogen, C1-C5 alkyl and C1-C5 alkoxy.

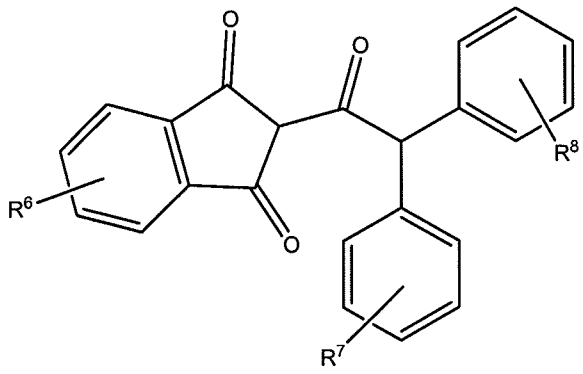
In some embodiments, R1, R2, R3 and R5 are H.

In other embodiments, R1, R3 and R5 are H; and R2 is Cl, preferably 4-Cl

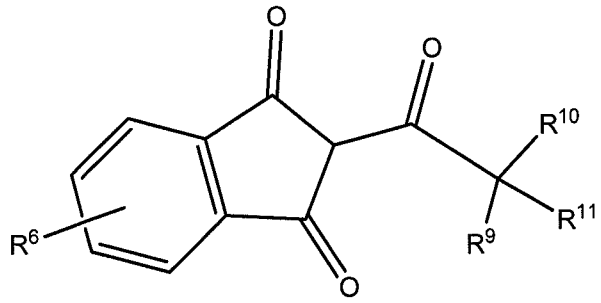
Preferably, R4 is methyl.

In other preferred embodiments of the invention, the low toxicity anticoagulants are 1,3-indandione derivatives of Formula III or IV or V:

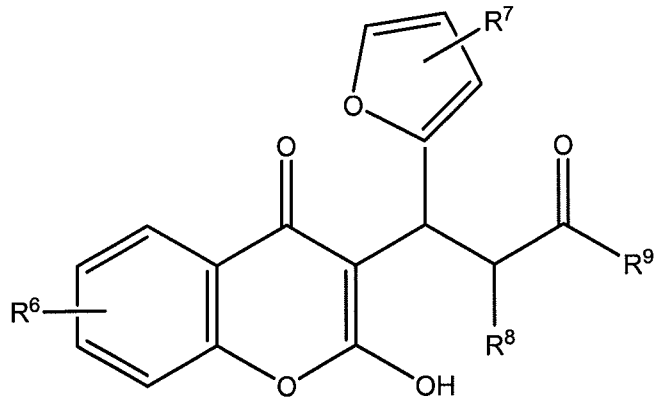
Formula III



Formula IV



Formula V



wherein

R6-R11 are independently selected from the group consisting of H, OH, halogen, C1-C5 alkyl and C1-C5 alkoxy.

Preferably, R6 and R7 are H.

Preferably, R8 is H or Cl, most preferably 4-Cl.

Preferably, R9-R11 are methyl.

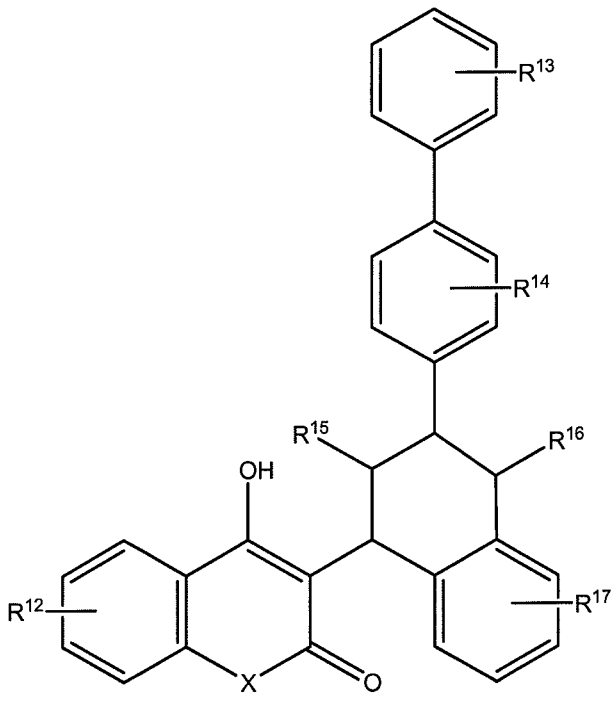
Preferably, the low-toxicity anticoagulant is a first generation anticoagulant.

Examples of first generation anticoagulants include warfarin, coumachlor, coumatetralyl, coumafuryl, pindone, diphacinone and chlorophacinone.

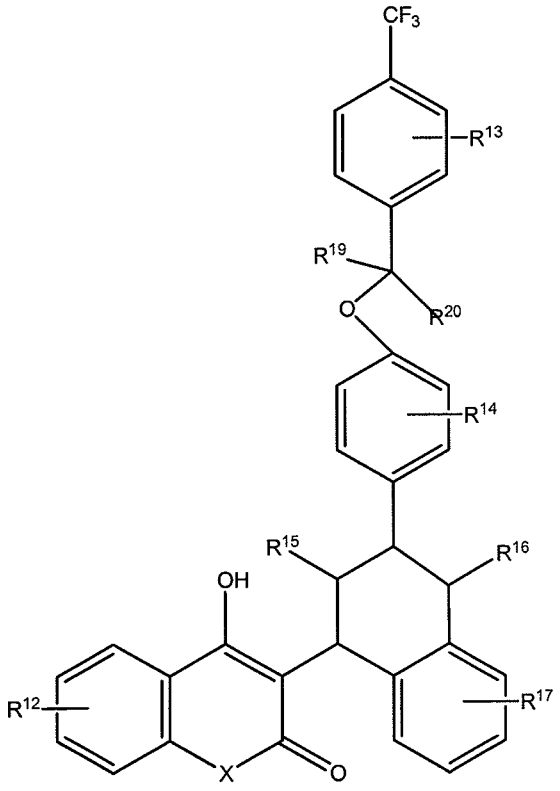
In some embodiments, warfarin is preferred. In other embodiments, coumatetralyl is preferred.

In some embodiments, the high-toxicity anticoagulant is a 4-hydroxycoumarin derivative of Formula VI or VII:

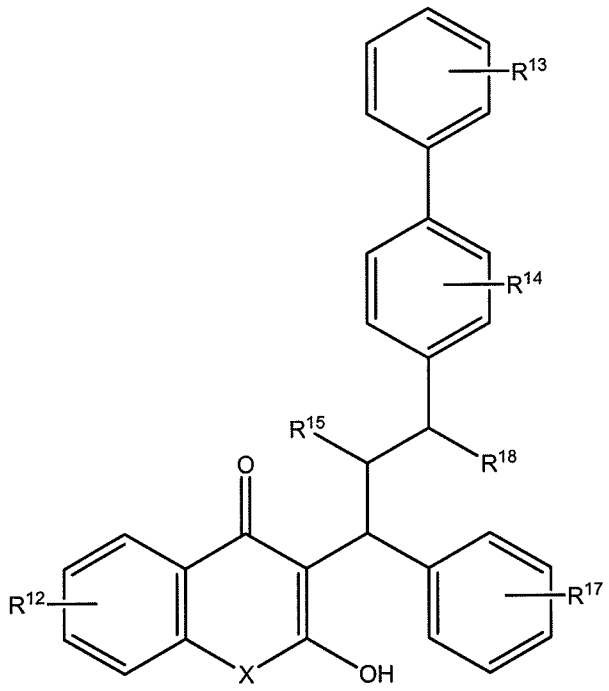
Formula VI



Formula VII



or Formula VIII:



wherein:

X is a heteroatom selected from O, N and S, preferably O;

R12-R20 are independently selected from the group consisting of H, OH, halogen, C1-C5 alkyl and C1-C5 alkoxy, preferably H or halogen.

Preferably R13 is Br, most preferably 4-Br.

Preferably R18 is OH.

Halogen may be fluoro, chloro, bromo or iodo, preferably chloro or bromo.

The anticoagulant may also be a tautomer, isomer, diastereomer or an enantiomer of a compound of Formula I-VIII, preferably a keto-enol tautomer.

Preferably, the high-toxicity anticoagulant is a second-generation anticoagulant.

Examples of second-generation anticoagulants include brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen. In some embodiments, brodifacoum is preferred.

In some embodiments, the low-toxicity anticoagulant is warfarin; and the high-toxicity anticoagulant is brodifacoum, bromadiolone, difenacoum, difethialone or flocoumafen.

In some embodiments, the low-toxicity anticoagulant is coumachlor; and the high-toxicity anticoagulant is brodifacoum, bromadiolone, difenacoum, difethialone or flocoumafen.

In some embodiments, the low-toxicity anticoagulant is coumatetralyl; and the high-toxicity anticoagulant is brodifacoum, bromadiolone, difenacoum, difethialone or flocoumafen.

In some embodiments, the low-toxicity anticoagulant is coumafuryl; and the high-toxicity anticoagulant is brodifacoum, bromadiolone, difenacoum, difethialone or flocoumafen.

In some embodiments, the low-toxicity anticoagulant is pindone; and the high-toxicity anticoagulant is brodifacoum, bromadiolone, difenacoum, difethialone or flocoumafen.

In some embodiments, the low-toxicity anticoagulant is diphacinone; and the high-toxicity anticoagulant is brodifacoum, bromadiolone, difenacoum, difethialone or flocoumafen.

In some embodiments, the low-toxicity anticoagulant is chlorophacinone; and the high-toxicity anticoagulant is brodifacoum, bromadiolone, difenacoum, difethialone or flocoumafen.

The following table lists some preferred combinations of low-toxicity and high-toxicity anticoagulants:

Table 1: Preferred combinations of low toxicity anticoagulant and high toxicity anticoagulant

	Low-toxicity anticoagulant	**High-toxicity anticoagulant**
1.	warfarin	brodifacoum
2.	chlorophacinone	brodifacoum
3.	diphacinone	brodifacoum
4.	coumatetralyl	brodifacoum
5.	warfarin	flocoumafen
6.	warfarin	difethialone
7.	warfarin	bromadiolone
8.	warfarin	difenacoum
9.	chlorophacinone	flocoumafen
10.	chlorophacinone	difethialone
11.	chlorophacinone	bromadiolone
12.	chlorophacinone	difenacoum

The vertebrate pesticide will in general be combined in a composition with one or more agents in order to increase the attractiveness of the pesticide composition to the vertebrate pest and to provide a suitable physical form, and hence to increase the effectiveness of the pesticide in killing the pest.

In particular, the vertebrate pesticide composition may include one or more of the following: a flavour or flavour enhancer; a preservative and/or antioxidant; a thickener or binding agent; a colouring agent; a human taste deterrent and a carrier. These additional components will be ones which are suitable for use with vertebrate pesticides or which enhance the attractiveness or palatability of vertebrate pesticides.

Flavours and flavour enhancers may include known food sources of the pest in question. Examples may include fruit, fruit extracts, vegetables, vegetable extracts, animal and/or vegetable proteins, vegetable oils and sugars. In some embodiments, sweeteners or bitter agents may be included.

Known food sources for rodents include cereals, e.g. whole, ground, broken, milled or flaked wheat, barley, oats, maize, rice, sorghum or sunflower seeds.

Preservatives include paraffin.

The vertebrate pesticide composition may be in any suitable form for administration to the pests. It may, for example, be a solid or a liquid, or a gas.

Suitable solids include grains, flakes, pastes, gels, fats, pellets, dusts and powders.

Particular examples include whole or pulverized cereals, flakes cereals and cereal pellets.

The pesticide composition may be contained in a wrapper or in covered form, e.g. in paper, card or plastic form.

In yet another aspect, the invention provides a process for the preparation of a vertebrate pesticide comprising the step of mixing:

(i) a high concentration of low-toxicity anticoagulant, and

(ii) a low concentration of high-toxicity anticoagulant.

Also provided is a process for the preparation of a vertebrate pesticide bait, comprising the step of mixing:

(i) a high concentration of low-toxicity anticoagulant, and

(ii) a low concentration of high-toxicity anticoagulant,

with one or more components selected from a flavour or flavour enhancer, a human taste deterrent, a preservative and/or antioxidant, a thickener or binding agent, a colouring agent, and a carrier.

The invention also provides a process for the preparation of a pest-specific bait, comprising the step of mixing:

(i) a high concentration of low-toxicity anticoagulant, and

(ii) a low concentration of high-toxicity anticoagulant,

with one or more carriers which are known to enhance uptake of bait by the pest species compared to a non-pest species.

The invention also provides a method of controlling vertebrate pests, wherein a vertebrate pesticide of the invention is applied to a target area or to a vertebrate pest's habitat.

Also provided is a method of killing a vertebrate pest population comprising the step of baiting, or some other form of appropriate application, an area which is inhabited by the pest population with a vertebrate pesticide of the invention.

The invention also provides the use of a vertebrate pesticide of the invention for controlling or killing vertebrate pests.

The skilled person will appreciate that the composition of the invention could be produced and/or sold as a concentrate and then diluted with an appropriate diluent (e.g. water) before use.

The invention therefore also provides a vertebrate pesticide concentrate which, when diluted, forms a vertebrate pesticide composition of the invention.

The invention also provides a concentrated formulation containing the aforementioned active ingredients such that when formulated as a bait, the active ingredients in the bait are within the ranges claimed in this invention.

The invention also provides a 2-100x concentrate of a vertebrate pesticide composition of the invention.

The invention further provides a method of producing a vertebrate pesticide composition, comprising the step of diluting a vertebrate pesticide concentrate with a diluent such as to produce a vertebrate pesticide composition of the invention.

In some embodiments, the concentrate is a 2-10x concentrate, a 2-20x concentrate, a 2-50x concentrate or a 2-100x concentrate. In some preferred embodiments, the concentrate is a 15-25x concentrate, most preferably about a 20x concentrate.

For example, a 10x concentrate may comprise 1000-10,000 ppm of the low-toxicity anticoagulant; and 10-200 ppm of high-toxicity anticoagulant.

For example, a 20x concentrate may comprise 2000-20,000 ppm of the low-toxicity anticoagulant; and 20-400 ppm of high-toxicity anticoagulant.

For example, a 30x concentrate may comprise 3000-30,000 ppm of the low-toxicity anticoagulant; and 30-600 ppm of high-toxicity anticoagulant.

The concentrate may also comprise a human taste deterrant or other additive, preferably at an appropriate concentration.

Suitable diluents and carriers for use in compositions of the invention include those that are used as diluents and/or carriers with known rodent control agents, for example waxes, and binding agents e.g. cellulose ethers, starch, polyvinyl alcohol, polyvinyl pyrrolidone, guar gum, carrageenan, gelatin, karaya gum, xanthum gum, acacia gum, locust bean gum, tragacanth, pectin and polyacrylates.

The invention further provides the use of:

(i) a high concentration of low-toxicity anticoagulant, and

(ii) a low concentration of high-toxicity anticoagulant,

as a combined preparation for simultaneous, separate or sequential use as a vertebrate pesticide.

The invention also provides a kit comprising:

(i) a high concentration of low-toxicity anticoagulant, and

(ii) a low concentration of high-toxicity anticoagulant,

as a combined preparation for simultaneous, separate or sequential use as a vertebrate pesticide, optionally together with instructions for admixing and/or for use as a vertebrate pesticide.

The kit may also comprise a face mask and/or gloves.

The invention further provides a bait box or a bait station comprising a vertebrate pesticide of the invention.

In yet a further embodiment, the invention provides a method of controlling or reducing vertebrate pests, the method comprising the steps of:

(a) applying a vertebrate pesticide composition comprising a high concentration of a low-toxicity anticoagulant to a target area or to a vertebrate pest's habitat; and

(b) applying a vertebrate pesticide composition comprising a low concentration of a high-toxicity anticoagulant to the same target area or to the same habitat.

The invention also provides a method of killing one or more pests in a vertebrate pest population, the method comprising the steps of:

(a) baiting an area which is inhabited or thought to be inhabited by the pest population with a vertebrate pesticide composition comprising a high concentration of a low-toxicity anticoagulant; and

(b) baiting the same area with a vertebrate pesticide composition comprising a low concentration of a high-toxicity anticoagulant.

Preferably the low-toxicity anticoagulant or the high-toxicity anticoagulant, or the concentrations thereof, are as defined herein.

Preferably one or both vertebrate pesticide compositions independently additionally comprise one or more flavour or flavour enhancers, human taste deterrents, preservatives, antioxidants, thickeners, binding agents, colouring agents or carriers.

Preferably steps (a) and (b) are performed within 21 days, preferably within 14 days and most preferably within 7 days of each other. In some embodiments, step (a) is performed first; in others, step (b) is performed first.

The invention further provides a kit comprising:

(a) one or more first vertebrate pesticide compositions comprising a high concentration of a low-toxicity anticoagulant, and

(b) one or more second vertebrate pesticide compositions comprising a low concentration of a high-toxicity anticoagulant,

wherein the low- and high-toxicity anticoagulants are as defined herein, preferably as a combined preparation for simultaneous, separate or sequential use, optionally together with instructions for use as a vertebrate pesticide.

Preferably, the first and/or second vertebrate pesticide compositions independently additionally comprise one or more flavour or flavour enhancers, human taste deterrents, preservatives, antioxidants, thickeners, binding agents, colouring agents or carriers.

Preferably the vertebrate pesticide compositions are in bait boxes or bait stations.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the elimination of warfarin from a male albino Norway rat that has received 170 $mg.kg^{-1}$ of warfarin, assuming a first phase half life of elimination of 15 hours.

Figure 2 shows the biphasic elimination of brodifacoum from a male albino Norway rat that has received 1 $mg.kg^{-1}$ brodifacoum, assuming a first phase half-life of elimination of 2 days and a second phase half life of elimination of 100 days.

EXAMPLES

The present invention is further illustrated by the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

Example 1: Mortality study using Y139C resistant house mice

This study used a resistant strain of house mouse that is homozygous for the VKORC1 mutation Tyr139Cys. They are known to be highly resistant to the anticoagulant warfarin, but can be effectively controlled with brodifacoum. Two groups of five female animals were used.

Table 2: Feeding regime

Test Day	Test animals	Control animals
1	warfarin	
2	brodifacoum + warfarin	brodifacoum
3	warfarin	
4	brodifacoum + warfarin	brodifacoum
5	warfarin	

Test animals were fed 250 ppm warfarin; Control animals were fed ground laboratory diet over a five day period. On Test day 2 and Test day 4, the Test animals and Control animals were given a limited feed of 50 ppm brodifacoum bait that would deliver 0.3 $mg.kg^{-1}$ of brodifacoum (i.e. 0.3 mg brodifacoum per kg animal body weight) each day (giving a total of 0.6 $mg.kg^{-1}$ of brodifacoum for each Test and Control animal). The animals were then maintained over a 14 day observation period.

The results were 5/5 mortality in the Test animals and 1/5 mortality in the Control animals.

In this study, an anticoagulant that is known to be totally ineffective against the target species was found to have a positive synergistic effect on a low dose of a second anticoagulant that is known to be effective against the target species when presented at higher formulation strength.

Example 2: Liver residue assay

This study used an albino susceptible strain of Norway rat. Such rats can usually effectively be controlled with both warfarin and brodifacoum. Test and Control groups of ten albino male Norway rats were used.

Over the 4 day test period and 14 day observation period, all animals were given vitamin K1 in the drinking water (which is the antidote for anticoagulants). Test animals were fed 250 ppm warfarin; and Control animals were fed ground laboratory diet the four day test period, under free feeding conditions:

On Test day 3, the Test group and the Control group were dosed with 1.0 $mg.kg^{-1}$ of brodifacoum (i.e. 1.0 mg brodifacoum per kg animal body weight, a rate over twice the LD_{99} dose, that would normally be lethal to the test animals). On completion of the observation period, animals were sacrificed and liver residue analysis was performed by the UK Food and Environment Research Agency.

The results are shown in Table 3 below.

Table 3: Analysis for residues in liver

	TEST - Test No. GB01-11-R022 10 males (warfarin 4 day no-choice)			CONTROL - Test No. GB01-11-R023 10 males (no warfarin)		
Animal	Brodifacoum (residue)	Warfarin (consumed)	Warfarin (residue)	Animal	Brodifacoum (residue)	Warfarin (residue)
1				1	1.31	0
2				2	1.17	0
3	0.83	138.7	0.08	3	0.82	0
4	0.79	119.9	0.12	4	0.79	0
5	0.49	139.5	0.07	5	0.86	0
6	0.92	172.2	<0.08	6	1.12	0
7	0.61	159.7	0.10	7	1.08	0
8	0.53	111.3	0.13	8	1.26	0
9	0.74	123.9	0.08	9	1.18	0
10	1.11	119.5	0.03	10	1.09	0
Mean	**0.75**		**0.09**	**Mean**	**1.07**	**0**

Residue levels in $mg.kg^{-1}$ wet weight liver; Consumed levels in $mg.kg^{-1}$ body weight.

The data given in Table 3 shows that co-administration with warfarin in the Test group resulted in a highly significant 30% reduction in the brodifacoum liver residue levels ($t=3.42$; $d.f.=16$; $p=0.004$). The very low warfarin liver residue levels in the Test group suggest that administration of warfarin was terminated prematurely. It is likely that continued administration of warfarin during the 14 day observation period would enhance the warfarin liver residue levels, and reduce the brodifacoum liver residue levels further.

In this second study, excretion of active ingredient will be predominantly by the first phase of elimination, with half-lives of elimination of 15 hours and 2 days for warfarin and brodifacoum respectively.

From consumption data of the 250 ppm warfarin formulation, the maximum warfarin intake was by Test animal number 6, which consumed 172 $mg.kg^{-1}$ of active ingredient. Warfarin would be expected to initially occupy all the specific binding sites, with the majority of active ingredient present in excess of the specific binding sites. The latter would be subject to a half life of elimination of 15 hours, with the majority eliminated by day 7. The bulk of the remaining active ingredient in the

specific binding sites would be subject to a half life of elimination of approximately 10 days.

On Test day 3, both Test animals and Control animals were dosed with 1 $mg.kg^{-1}$ of brodifacoum.

With a half life of elimination of approximately 2 day, the bulk of the active ingredient would be eliminated by day 11, after which most of the remaining active ingredient would be bound in the specific binding sites, and would be eliminated with a half life of elimination of approximately 100 days.

Specifically for the Test animals, between test day 7 and test day 11, warfarin, which initially occupied the majority of the specific binding sites, would have been eliminated and replaced by brodifacoum.

Thus for the Test animals, at the end of the experiment, a significant proportion of the specific binding sites would be occupied by warfarin, and there would be significantly less bound brodifacoum than in the control animals.

Example 3: Blood Clotting Response studies

This study used a resistant strain of house mouse that is homozygous for the VKORC1 mutation Tyr139Cys. Using groups of 5 male and 5 female animals, animals were either fed first generation anticoagulant rodenticide or ground laboratory diet over a 48 hour period.

After 24 hrs, some animals were dosed with 0.8 $mg.kg^{-1}$ brodifacoum.

After another 24 hrs, all animals were blood sampled under terminal anaesthesia, and coagulation times were determined.

First experiment

Using 200 ppm warfarin as the first generation anticoagulant, the data obtained below were obtained:

Table 4: Coagulation times using warfarin and brodifacoum

	48h no-choice feed	Brodifacoum at 24h	Coagulation Time (seconds)				Failed sample
	Warfarin		< 25s	100-200s	200-300s	>300s	
Test	200ppm	0.8mg.kg^{-1}		3M	1M+1F	1M+4F	
			<25s	30-45s	70-80S	100-200s	
Control 1	0ppm	0.8mg.kg^{-1}		1M (45s)	2M	2M+4F	1F
Control 2	200ppm	0.0mg.kg^{-1}	4M+4F	1M (30s)			1F

Second experiment

Using 200 ppm chlorophacinone as the first generation anticoagulant (with three groups of 3 female animals), the data obtained below were obtained:

Table 5: Coagulation times using chlorophacinone and brodifacoum

	48h no-choice feed	Brodifacoum at 24h	Coagulation Time (seconds)			Failed sample
	Chlorophacinone		**Animal 1**	**Animal 2**	**Animal 3**	
Test	200ppm	0.8mg.kg^{-1}	62.3	83.7	94.3	
Control 1	0ppm	0.8mg.kg^{-1}	42.1	44.1	46.0	
Control 2	200ppm	0.0mg.kg^{-1}	16.8	23.5	145.7	

The synergistic effect between first and second generation anticoagulant is demonstrated in the above tables for the two first generation anticoagulants, warfarin and chlorophacinone, when administered alongside the second generation anticoagulant brodifacoum.

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CLAIMS

1. A vertebrate pesticide composition comprising:
(i) a high concentration of low-toxicity anticoagulant, and
(ii) a low concentration of high-toxicity anticoagulant.

2. A vertebrate pesticide composition as claimed in claim 1, wherein the low-toxicity anticoagulant has low persistence.

3. A vertebrate pesticide composition as claimed in claim 1 or claim 2, wherein the high-toxicity anticoagulant has high persistence.

4. A vertebrate pesticide composition as claimed in any one of claims 1 to 3, wherein the low toxicity anticoagulant, when administered as a single dose by the oral route, has an LD_{50} of greater than 15.0 $mg.kg^{-1}$ in Norway rats and/or greater than 20 $mg.kg^{-1}$ in house mice, preferably greater than 20.0 $mg.kg^{-1}$ in Norway rats and/or greater than 40 $mg.kg^{-1}$ in house mice.

5. A vertebrate pesticide composition as claimed in any one of claims 1 to 4, wherein the high toxicity anticoagulant, when administered as a single dose by the oral route, has an LD_{50} of less than 5.0 $mg.kg^{-1}$ in Norway rats and/or less than 10.0 $mg.kg^{-1}$ in house mice, preferably less than 2.0 $mg.kg^{-1}$ in Norway rats and/or less than 4.0 $mg.kg^{-1}$ in house mice.

6. A vertebrate pesticide composition as claimed in any one of claims 1 to 5, wherein the low-toxicity anticoagulant and/or the high-toxicity anticoagulant is a vitamin K antagonist.

7. A vertebrate pesticide composition as claimed in any one of claims 1 to 6, wherein the low-toxicity anticoagulant is a first generation anticoagulant.

8. A vertebrate pesticide composition as claimed in any one of claims 1 to 7, wherein the low-toxicity anticoagulant is a 4-hydroxy coumarin derivative, preferably of Formula I or II; or a 1,3-indandione derivative, preferably of Formula III, IV or V.

9. A vertebrate pesticide composition as claimed in any one of claims 1 to 8, wherein the low-toxicity anticoagulant is selected from the group consisting of warfarin, coumachlor, coumatetralyl, coumafuryl, pindone, diphacinone, chlorophacinone, or a derivative or variant thereof.

10. A vertebrate pesticide composition as claimed in any one of claims 1 to 9, wherein the high-toxicity anticoagulant is a second generation anticoagulant.

11. A vertebrate pesticide composition as claimed in claim 10, wherein the second generation anticoagulant is a 4-hydroxycoumarin derivative, preferably of Formula VI, VII or VIII.

12. A vertebrate pesticide composition as claimed in claim 10, wherein the second generation anticoagulant is selected from the group consisting of brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen, or a derivative or variant thereof, preferably brodifacoum.

13. A vertebrate pesticide composition as claimed in any one of claims 1 to 12, wherein:
(a) the low toxicity anticoagulant is warfarin and the high toxicity anticoagulant is brodifacoum; or
(b) the low toxicity anticoagulant is chlorophacinone and the high toxicity anticoagulant is brodifacoum.

14. A vertebrate pesticide composition as claimed in any one of claims 1 to 13, wherein the concentration of the low-toxicity anticoagulant in the composition is 100-1000 ppm, preferably 100-200 ppm, 200-400 ppm, 400-600 ppm, 600-800 ppm or 800-1000 pmm, and more preferably 250-1000 ppm or 250-750 ppm.

15. A vertebrate pesticide composition as claimed in any one of claims 1 to 14, wherein the concentration of high-toxicity anticoagulant in the composition is 1-20 ppm, preferably 1-5 ppm, 5-10 ppm, 10-15 ppm or 15-20 ppm, more preferably 2-10 ppm.

16. A vertebrate pesticide composition as claimed in any one of claims 1 to 15, wherein the composition additionally comprises one or more flavour or flavour enhancers, human taste deterrents, preservatives, antioxidants, thickeners, binding agents, colouring agents or carriers.

17. A bait box or a bait station comprising a vertebrate pesticide composition as claimed in any one of claims 1 to 16.

18. A method of controlling or reducing vertebrate pests, wherein a vertebrate pesticide composition as defined in any one of claims 1 to 16 is applied to a target area or to a vertebrate pest's habitat.

19. A method of killing one or more pests in a vertebrate pest population comprising the step of baiting an area which is inhabited or thought to be inhabited by the pest population with a vertebrate pesticide composition as defined in any one of claims 1 to 16.

20. A method of controlling or reducing vertebrate pests, the method comprising the steps of:
(a) applying a vertebrate pesticide composition comprising a high concentration of a low-toxicity anticoagulant to a target area or to a vertebrate pest's habitat; and
(b) applying a vertebrate pesticide composition comprising a low concentration of a high-toxicity anticoagulant to the same target area or to the same habitat.

21. A method of killing one or more pests in a vertebrate pest population, the method comprising the steps of:

(a) baiting an area which is inhabited or thought to be inhabited by the pest population with a vertebrate pesticide composition comprising a high concentration of a low-toxicity anticoagulant; and

(b) baiting the same area with a vertebrate pesticide composition comprising a low concentration of a high-toxicity anticoagulant.

22. A method as claimed in claim 20 or claim 21, wherein the low-toxicity anticoagulant or the high-toxicity anticoagulant, or the concentrations thereof, are as defined in any one of claims 2 to 15.

23. A method as claimed in any one of claims 20 to 22, wherein one or both vertebrate pesticide compositions independently additionally comprise one or more flavour or flavour enhancers, human taste deterrents, preservatives, antioxidants, thickeners, binding agents, colouring agents or carriers.

24. A method as claimed in any one of claims 20 to 23, wherein steps (a) and (b) are performed within 21 days, preferably within 14 days and most preferably within 7 days of each other.

25. A method as claimed in any one of claims 20 to 24, wherein step (a) is performed first.

26. A method as claimed in any one of claims 20 to 24, wherein step (b) is performed first.

27. A kit comprising:

(a) one or more first vertebrate pesticide compositions comprising a high concentration of a low-toxicity anticoagulant, and

(b) one or more second vertebrate pesticide compositions comprising a low concentration of a high-toxicity anticoagulant,

wherein the low- and high-toxicity anticoagulants are as defined in any one of claims 2 to 15, preferably as a combined preparation for simultaneous, separate or sequential use, optionally together with instructions for use as a vertebrate pesticide.

28. A kit as claimed in claim 27, wherein the first and/or second vertebrate pesticide compositions independently additionally comprise one or more flavour or flavour enhancers, human taste deterrents, preservatives, antioxidants, thickeners, binding agents, colouring agents or carriers.

29. A kit as claimed in claim 27 or claim 28, wherein the vertebrate pesticide compositions are in bait boxes or bait stations.

30. A process for the preparation of a vertebrate pesticide comprising the step of mixing:
(i) a high concentration of low-toxicity anticoagulant, and
(ii) a low concentration of high-toxicity anticoagulant,
wherein the low- and high-toxicity anticoagulants are as defined in any one of claims 2 to 15.

31. A process for the preparation of a vertebrate pesticide bait, comprising the step of mixing:
(i) a high concentration of low-toxicity anticoagulant, and
(ii) a low concentration of high-toxicity anticoagulant,
wherein the low- and high-toxicity anticoagulants are as defined in any one of claims 2 to 15,
with one or more components selected from a flavour or flavour enhancer, a human taste deterrent, a preservative and/or antioxidant, a thickener or binding agent, a colouring agent, and a carrier.

32. A process for the preparation of a pest-specific bait, comprising the step of mixing:
(i) a high concentration of low-toxicity anticoagulant, and
(ii) a low concentration of high-toxicity anticoagulant,
wherein the low- and high-toxicity anticoagulants are as defined in any one of claims 2 to 15,

with one or more carriers which are known to enhance uptake of bait by the pest species compared to a non-pest species.

33. Use of a vertebrate pesticide composition as defined in any one of claims to 1 to 16 for controlling or killing vertebrate pests.

34. Use of:

(i) a high concentration of low-toxicity anticoagulant, and

(ii) a low concentration of high-toxicity anticoagulant,

wherein the low- and high-toxicity anticoagulants are as defined in any one of claims 2 to 15,

as a combined preparation for simultaneous, separate or sequential use as a vertebrate pesticide.

35. A vertebrate pesticide concentrate which, when diluted, forms a vertebrate pesticide composition as claimed in any one of claims 1 to 16.

36. A 2-100x concentrate of a vertebrate pesticide composition as claimed in any one of claims 1 to 16.

37. A concentrate as claimed in claim 35, wherein the concentrate is a 15-25x concentrate.

38. A method of producing a vertebrate pesticide composition, comprising the step of diluting a vertebrate pesticide concentrate with a diluent such as to produce a vertebrate pesticide composition as claimed in any one of claims 1 to 16.

. . .

Figure 1

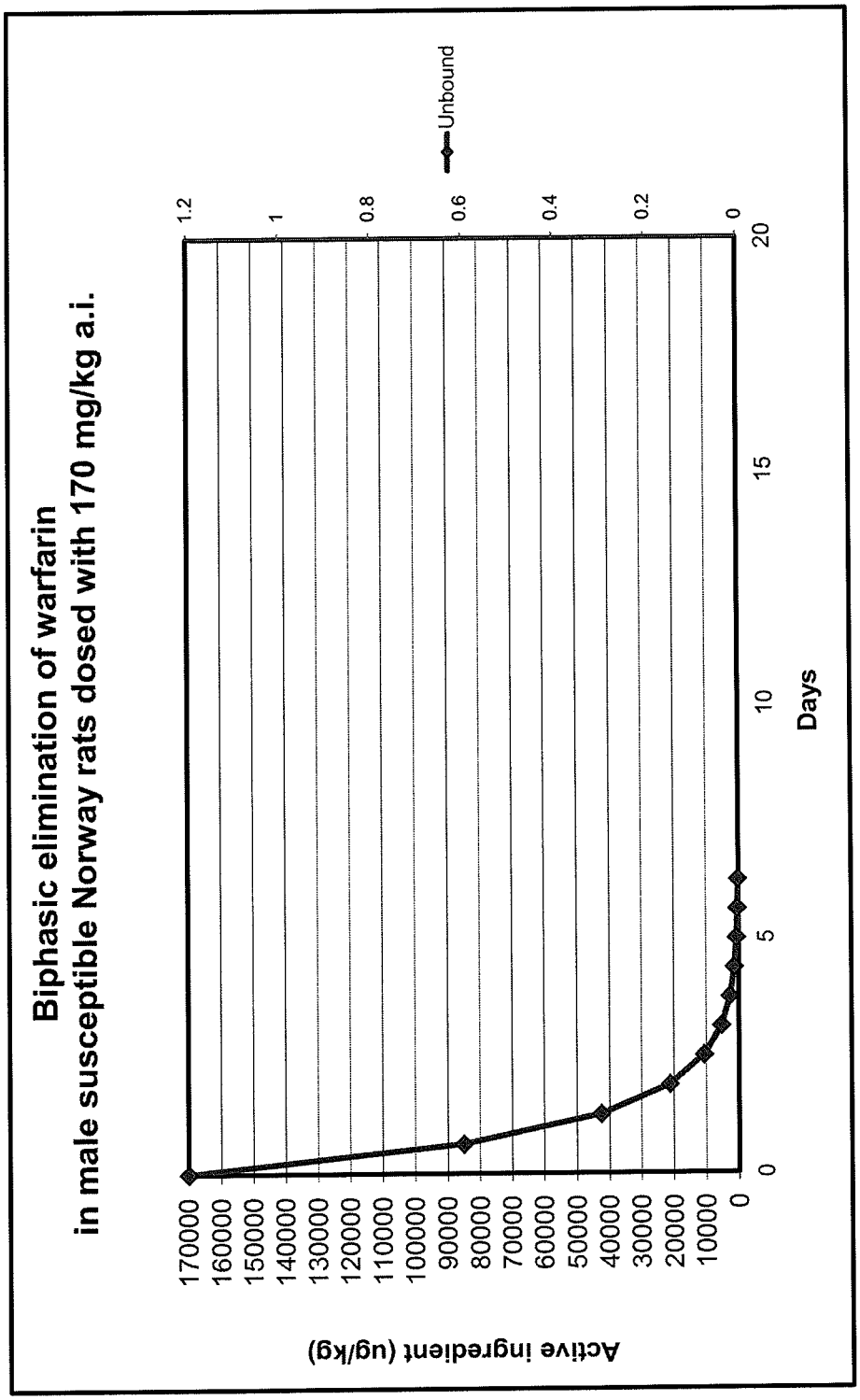
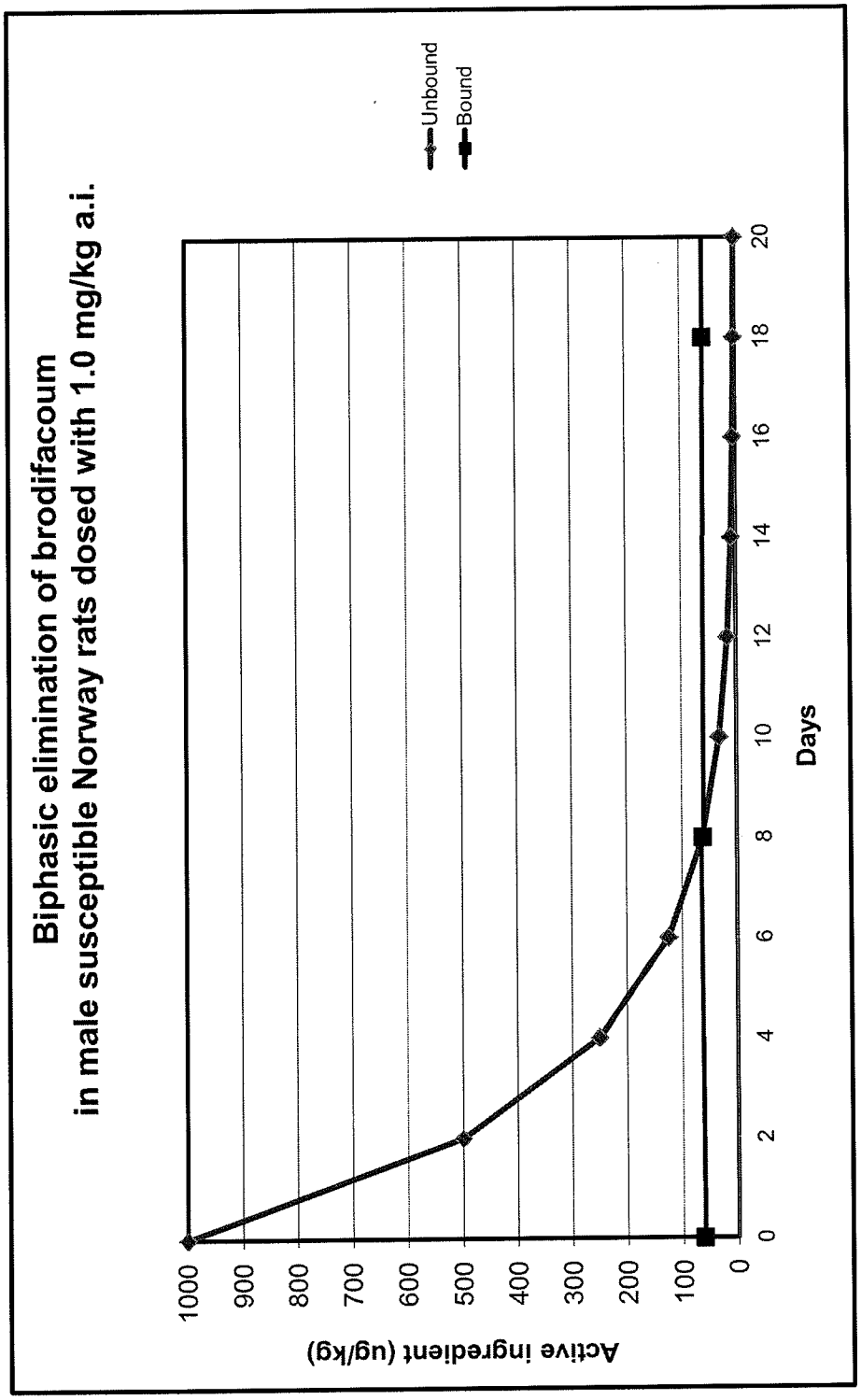

Biphasic elimination of warfarin
in male susceptible Norway rats dosed with 170 mg/kg a.i.
Unbound
Active ingredient (ug/kg)
Days
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20

Figure 2


Biphasic elimination of brodifacoum
in male susceptible Norway rats dosed with 1.0 mg/kg a.i.
Unbound
Bound
Active ingredient (ug/kg)
1000
900
800
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
16
18
20
Days

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/053088

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N43/16 A01N43/18 A01N25/00 A01N25/08
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/158892 A1 (CAYLEY PATRICIA JANE [GB] ET AL) 24 June 2010 (2010-06-24) paragraph [0072]; claims; examples	1-38
X	EP 2 090 164 A1 (ZAPI IND CHIMICHE S P A [IT]) 19 August 2009 (2009-08-19) paragraphs [0047], [0058] - [0061]; claims 10,15; examples	1-38
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/	Authorized officer
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X	CN 101 163 720 A (SYNGENTA LTD [GB]) 16 April 2008 (2008-04-16) the whole document	1-38
X,P	WO 2013/110962 A1 (BABOLNA KOERNYEZETBIOLOGIAI KOEZPONT KFT [HU]; BAJOMI DANIEL [HU]; DAR) 1 August 2013 (2013-08-01) page 6, line 24 - page 7, line 12; claims; example 9	1-38

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Information on patent family members

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