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Evaluation of the Danish resistance strategy

Anticoagulant use in rat control

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Indhold

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1. Danish summary

Den brune rotte, (*Rattus norvegicus*), er en skadevoldende gnaver og som har et stort potentiale med hensyn til overførelse af smitte til os mennesker og vores husdyr. Siden 1907 har vi i Danmark haft en lov, som har påbudt kommunalbestyrelserne at bekæmpe rotter overalt. I starten af 1950'erne blev antikoagulanterne introduceret, og dermed havde man fået et effektivt bekæmpelsesmiddel i rottebekæmpelsen. Disse antikoagulanter er i dag de eneste tilladte kemiske bekæmpelsesmidler til rotter i Danmark.

Den første antikoagulant warfarin blev introduceret i 1950 og i de efterfølgende 30 år blev andre og stærkere antikoagulanter introduceret, hvoraf difethialon er den sidste ankommende antikoagulant fra 1986. I Danmark fandt man for første gang resistens mod warfarin i 1962 og resistens har i årene derefter spredt sig til mange dele af landet. Bromadiolon, som blev introduceret i 1979, er den mest anvendte antikoagulant til rottebekæmpelse i Danmark, men allerede et år efter dens introduktion kunne der konstateres resistens mod bromadiolon. Det samme skete for aktivstoffet difenacoum, som blev introduceret i 1976 og med resistens mod midlet i 1979. I Danmark har man i perioden 1962 og frem til 2008 jævnligt undersøgt for antikoagulant resistens og har skaffet sig viden om antikoagulant resistens, dens udbredelse og spredningspotentiale. Den seneste moniteringsperiode (2001 og frem til og med 2008) for antikoagulant resistens viste, at resistens mod bromadiolon og difenacoum var meget udbredt i store dele af landet.

Antikoagulanterne opdeles i to hovedkategorier:

- Første generations antikogulante rodenticider (FGARs), som omfatter aktivstofferne; warfarin (som siden 1990 ikke har været på det danske marked), coumatetralyl og siden 2015 også chlorophacinon.
- Anden generations antikoagulante rodenticider (SGARs), som omfatter aktivstofferne; bromadiolon, difenacoum, brodifacoum, flocoumafen og difethialon.

Alle antikoagulanter er blødningsforstyrrende stoffer, som bevirker, at blodet ikke kan størkne (koagulere) og at døden, efter indtag af en dødelig dosis, vil ske som følge af, typisk, indre blødninger. Vitamin K er et vigtigt molekyle for den blodkoagulerende proces. I leveren omdannes vitamin K ved hjælp af enzymet vitamin $K_{2,3}$ epoxid reduktase (VKOR), som gør, at kroppen kan danne prothrombin og dermed opretholde en normal koaguleringsfunktion. Alle antikoagulanter påvirker VKOR og uden omdannelse af vitamin K er kroppen ikke i stand til at opretholde en normal koagulation af blodet. Koagulationsevne påvirkes først efter 3 til 4 dage, da kroppens pulje af vitamin K først skal opbruges. Typisk indtræder døden efter 3 til 6 dage efter indtag af en dødelig dosis.

I 2012 fik vi en revideret bekendtgørelse for rottebekæmpelsen i Danmark. Her blev der skabt et større lovgivningsmæssigt fokus på den forebyggende indsats mod rotter. Med bekendtgørelsen i 2012 blev der for første gang lovgivet om brugen af antikoagulanter i rottebekæmpelsen. Det er nu lovpligtigt at følge den nationale strategi for resistens i brugen af antikoagulanter.

Den nuværende danske resistensstrategi, som evalueres i denne rapport, omhandler, at der altid skal anvendes den mildeste gift muligt i den givne situation. Det betyder, at er der ikke tegn på resistens, skal bekæmpelsesmidler med coumatetralyl anvendes. Med indførelsen af chlorophacinon i 2015, som også er et af de meget milde FGARs, valgte man i Styrelsen for Vand- og Naturforvaltning (tidligere Naturstyrelsen) at sidestille de to FGARs, coumatetralyl og

chlorophacinin, således at rottebekæmperens første valg i en rottebekæmpelse med gift er midler baseret på aktivstofferne coumatetralyl og/eller chlorophacinon. Hvis der opleves problemer med bekæmpelsen, som følge af resistens, så skal midler baseret på den antikoagulant, som er en grad stærkere end coumatetralyl/chlorophacinon, vælges. Efter coumatetralyl og chlorophacinon kommer bromadiolon, en af de milde SGARs. Efter bromadiolon følger difenacoum, og skulle det vise sig, at der er resistens overfor difenacoum, så kan der vælges frit blandt et af de tre meget stærke SGARs (brodifacoum, flocoumafen og difethialon), idet disse betragtes som indbyrdes sideordnede med hensyn til deres styrke og effekt.

I dag ved vi, at antikoagulant resistens, som minimum, skyldes tilstedeværelsen af en lille genetisk ændring i genet, som koder for VKOR. Den lille ændring er en enkelt base ændring (mutation), som resulterer i, at den oprindelige aminosyre ændres. Der er fundet flere af disse mutationer (aminosyre-ændringer) i VKOR genet, men fælles for disse mutationer er, at de alle forekommer i samme underenhed (subunit 1) af VKOR genet. De VKOR mutationerne, som er korreleret til resistens, betegnes alle som *VKORC1*-mutationer. *VKORC1*-mutationerne kan forekomme forskellige steder (positioner) i subunit 1 eller kan forekomme på samme position, men med forskellige baseændringer og dermed forskellige aminosyre ændringer. Den mest almindelige *VKORC1*-mutation er den mutation, som er blevet fundet i de danske resistente rotter. Her er der sket en ændring på aminosyre position 139, hvor den oprindelige (vildtypen) aminosyre tyrosin (Y) er ændret til aminosyren cystein (C), den genetisk betegnelse af denne mutation er Y139C. Y139C er som nævnt den eneste mutation, der er fundet i de danske resistente rotter. Men mutationen er også meget udbredt i Tyskland og forekommer også i mindre grad i f.eks. England, Frankrig og Ungarn. Af andre betydende resistente *VKORC1*-mutationer kan nævnes; 1) Y139F, hvor tyrosin på aminosyre-position 139 er ændret til fenylalanin (F). Denne mutation er meget udbredt i Frankrig, Belgien, Holland og England, 2) L120Q hvor leucin på position 120 er ændret til glutamin, som forekommer i England, Frankrig og Belgien, 3) L128Q hvor leucin er ændret til glutamin, men på position 128, er indtil videre kun fundet i England.

Alle disse resistente *VKORC1* mutationer udviser meget høj grad af resistens overfor alle FGARs. Resistens overfor nogle af SGARs er også udtrykt for nogle af disse mutationer. Forekomst af den danske resistens-mutation Y139C, har vist sig at have en høj grad af resistens overfor bromadiolon, men på et væsentlig lavere niveau end overfor FGARs. Rotter, som har Y139C, kan typisk tåle FGARs i mængde, som ligger fra 40 til flere hundrede gange over den dosis, der skal til for at få en følsom rotte til at reagere. Vi bruger her begrebet Resistens Faktor (RF), som udtrykker det antal gange af ED_{50} (effektiv dosis), som den resistente rotte skal have for at opnå samme respons som den følsomme rotte. En RF på under 1 indikerer, at den undersøgte rotte reagerer mere på giften end den følsomme rotte, som den testes imod. Er RF på 1 så er den undersøgte resistente rotte på samme niveau som den følsomme rottestamme, dvs RF på 1 eller derunder betyder, at den undersøgte rotte er følsom. Er RF på mellem 1 og 2 siges det, at der er indikation for, at der kan forekomme ingen eller mindre bekæmpelsesproblemer med den pågældende antikoagulant – det kalder vi her tekniske resistens. Er RF på over 2 vil der være bekæmpelsesproblemer.

For rotter med Y139C mutationen er der for bromadiolon en resistens faktoren på 17 (hanrotter) og 15 (hunrotter), hvilket betyder, at den Y139C resistente rotte skal have 15 til 17 gange mere gift (ED_{50}) end hvis den ikke var resistent (altså følsom). Overfor difenacoum er resistens faktoren for Y139C 1,6 (hanrotter) og 2,9 (hunrotter).

Der er over de senere år blevet gennemført række feltundersøgelser på landbrugsejendomme i Tyskland, hvor man i forvejen havde identificeret høj grad af resistens (Y139C). Undersøgelserne havde blandt andet til formål at undersøge bekæmpelseseffektiviteten i de resistente rottebestande overfor midlerne bromadiolon, difenacoum og brodifacoum.

I undersøgelsen vedrørende bromadiolon blev der bekæmpet rotter på 4 forskellige landbrugsejendomme. Der blev ædt mellem ca. 10 til 40 kg bromadiolon. Da bekæmpelsen blev

afsluttet efter mellem 35 til 42 dage, blev der på 2 af ejendommen kun bekæmpet henholdsvis 0 og 20 % af den estimerede bestand, som oprindelig var estimeret til mellem ca. 75-100 rotter. På de øvrige to ejendomme blev der bekæmpet ca. 70 % af den oprindelige rottebestand (på mellem 130-225 rotter).

Forsøg med difenacoum på to ejendomme viste, at efter 43-50 dage blev der bekæmpet henholdsvis 60 og 87 % af den oprindelige estimerede rottebestande (på henholdsvis 80 og 340 rotter) ved brug af henholdsvis 8 og 28 kg difenacoum.

Ved forsøg med brodifacoum, hvor der ligeledes blev bekæmpet rotter på to ejendomme og hvor der også var konstateret en udbredt grad af resistens, blev bestandene af rotter 100 % bekæmpet efter 50 til 60 dage (var oprindeligt estimeret til ca. 82 og 150 rotter). På disse ejendomme blev der praktiseret interval udlægning og der blev på de to ejendomme brugt henholdsvis 1,5 og 4 kg brodifacoum.

Det er påvist, at resistente *VKORC1* mutationerne, heriblandt Y139C, ikke er rettet mod en enkelt antikoagulant, men at mutationen derimod dækker resistens over for alle FGARs, bromadiolon og i mindre grad overfor difenacoum.

Er frekvensen af Y139C lav i en rottebestand, når bekæmpelsen påbegyndes, vil man i første omgang opleve en betragtelig nedgang i rottebestanden når FGARs anvendes som første valg af bekæmpelsesmiddel. Men i takt med at bekæmpelsen fortsætter, vil det efterlade de resistente (Y139C) individer (samt individer, som ikke vil spise af giften). Ved fortsat brug af FGARs og/eller bromadiolon og difenacoum vil resistensen ikke udryddes, men derimod blive mere udbredt. Efter noget tid vil man opnå, at rottebestanden har en høj grad af Y139C resistente individer – vi taler her om, at ved fortsat selektion, når FGARs, bromdiolon og/eller difenacoum fortsat anvendes, så vil graden af homozygositet for Y139C være høj, mens graden af homozygositet for den følsomme (uændrede) version af genet vil være meget lav.

Anbefalingerne fra RRAC (Rodenticide Resistance Action Commitee) og fra en rapport bestilt af EU kommissionen er, at når resistente *VKORC1* mutationer, som Y139C optræder i en rottebestand, så kan det ikke anbefales at bekæmpe med alle FGARs, bromadiolon og difenacoum.

Der er endnu ikke konstateret resistens overfor de tre meget stærke SGARs, brodifacoum, flocoumafen og difethialon. Man kunne jo postulere, at det bare er et spørgsmål om tid. Vi har dog haft disse midler siden midt 70'erne og begyndelsen af 80'erne. Dengang var man ligeledes overbevist om, at det var et spørgsmål om tid, baseret på den hurtige resistensudvikling for alle FGARs og de milde SGARs (bromadiolon og difenacoum). Nu mere end 30 år efter og med et relativt stort forbrug af disse midler har vi endnu ikke set resistens mod disse tre midler. Vi betegner derfor disse tre antikoagulanter, som resistens-brydende og disse bør anvendes, når der ikke kan bekæmpes med FGARs som følge af resistens.

Fra et miljømæssigt perspektiv mener rapportens forfattere, at skiftet fra FGARs til et af de tre stærke midler, ved dokumenteret resistens, kan være at foretrække. Ser man på den mængde af bromadiolon og difenacoum, der blev brugt ved bekæmpelse af tyske resistente (Y139C) rottebestande og med en relativ ringe bekæmpelsessucces til følge, i forhold til mængden af brodifacoum, som man anvendte på tilsvarende resistente rottebestande og med en meget høj bekæmpelsessucces, så er der tale om en væsentlig reduktion i den kvantitative mængde gift, når der anvendes brodifacoum i forhold til f.eks. bromadiolon. Med brug af brodifacoum, som må betragtes som resistens-brydende, vil der ikke være overlevende resistente rotter, hvorimod ved bekæmpelse med bromadiolon/difenacoum vil der være u-bekæmpede resistente rotter tilbage, som kan have gift i kroppen. For at minimere risikoen for sekundære forgiftninger, når der anvendes de meget potente SGARs, er det dog væsentligt at pointere, at rotter, som dør som følge af en antikoagulant forgiftning så vidt muligt indsamles, da de ellers vil kunne udgør en risiko for eventuelle ådselsædere.

Der er ingen tvivl om, at der kan ske en kvantitativ reduktion i mængden af forbrugt gift ved at skifte til de potente SGARs, når resistens forekommer, men rapportens forfatter er dog også

opmærksomme på, at selvom der er tale om kvantitativ reduktion, så er der dog et behov for at få belyst den kvalitative betydning, idet disse gifte er så stærke i forhold til bromadiolone og difenacoum. Derfor anbefales det, at der skaffes mere indsigt i hvilken betydning brug af de stærkest SGARs måtte have over for rovfugle og ugler fremfor brug af bromadiolon/difenacoum i bestande af resistente rotter.

Den resistensstrategi, som det anbefales fremover at følge, vil være: at ved al antikoagulant bekæmpelse af rotter skal man som udgangspunkt vælge at benytte FGARs som første valg – kan der dokumenteres resistens, så skal der øjeblikkeligt skiftes over til enten brodifacoum, flocoumafen eller difethialon.

At man i bekæmpelsen vælge enten brodifacoum, flocoumafen og/eller difethialon, ved forekomst af resistens, skyldes 1) at de må betragtes som resistensbrydende og 2) fordi vi pt ikke har andre godkendte og effektive ikke-antikoagulante midler, som ville kunne finde anvendelse overfor resistente rotter. skulle blive tilgængelig for rottebekæmpelsen i Danmark.

1.1 Bekæmpelse med antikoagulanter i forbindelse med forslået strategi

Men resistensstrategien bør og kan ikke stå alene. Der bør tilknyttes krav til en korrekt brug af både FGARs og SGARs, samt at de som udfører rottebekæmpelse har gode muligheder for at vurdere, om resistens er et problem i den enkelte rottebekæmpelsessituation.

Bekæmpelse alene er aldrig løsningen på et rotteproblem. Giften (og eller fælder) kan fjerne problemet midlertidig, men finder man ikke og fjerner man ikke årsagen til rotteforekomsten, ja, så vil det blot være et spørgsmål om tid førend problemet genopstår. Derfor er det vigtigt, at en bekæmpelse altid omfatter den forebyggende indsats, som skal gøre det vanskeligere for rotter at indfinde sig igen. I den danske lovgivning og vejledning til rottebekæmpelse er der allerede taget hånd om det. Nu mangler der blot, at den forebyggende bekæmpelse integreres i endnu højere grad. Hvad der er årsag til, at det ikke sker i højt nok omfang, er ikke belyst i denne rapport, men rapportens forfattere mener, at det måske kan skyldes, at man i kommunerne og i bekæmpelsesfirmaer afsætter for få midler og alt for få ressourcer til særligt den kommunale rottebekæmpelse. Men det skyldes formentlig også, at man i kommunerne ikke er opmærksomme nok på at føre det nødvendige tilsyn med bekæmpelsen og at man ikke håndhæver lovgivningen i det omfang, der er behov for overfor kommunens borgere.

Foruden at have fokus på det forebyggende element så er der nogle forhold, som skal iagttages, når man påbegynder en bekæmpelse. Alle de nedenfor beskrevne forhold er allerede beskrevet i vores nuværende vejledning til bekæmpelse af rotter, men nævnes her ganske kort:

- Forundersøgelsen: her skal bekæmperen foretage en grundig gennemgang af ejendommen. Det er på baggrund af denne forundersøgelse, at den korrekte plan for en bekæmpelse kan lægges.
- Valg af bekæmpelsesmidler: her skal bekæmperen (på baggrund af forundersøgelsen) foretage en vurdering af, om der kan bekæmpes med fælder eller om der skal anvendes gift.
- Forebyggelse og optimering af bekæmpelsesmuligheder – her skal bekæmperen identificere potentielle fødekilder på ejendomme og få dem fjernet eller reduceret. Beskæring af vegetation kan komme på tale. Vegetation er udmærkede steder for rotter at opholde sig og kan i større sammenhænge af vegetation bevæge sig rundt uden at blive set.
- Rottesikring – her tænkes der på de enkelte bygningsdele, hvor bekæmperen skal være opmærksom på alle de forhold, som er uhensigtsmæssigt i forhold til rotter.

Selve bekæmpelsen med brug af gift bør altid ske ud fra en forudgående grundig undersøgelse, således at f.eks. opsætningen af gift bliver tilstrækkelig, både med hensyn til antallet af

giftstationer og med den udlagte giftmængde. Med hensyn til forundersøgelse, planlægning for gift-opsætningen henviser rapportens forfattere til et bekæmpelsesværktøj BayTool, som kan bruges til inspiration til udvikling af et tilsvarende bekæmpelsesværktøj. Forfatterne kender pt ikke andre værktøjer af denne art, men det kan kun opfordres til, at man tænker i udviklingen af lignende programmer.

Det centrale i enhver giftbekæmpelse, når den fornødne giftopsætning er foretaget, er det hyppige tilsyn med giften. Når giften er udlagt bør der kun gå få dage (3-4 dage) inden giftindtaget følges. De efterfølgende tilsyn skal ske med maksimum 7 dages mellemrum i den periode, hvor der er tiltagende og konstant indtag af giften. Der nævnes her 7 dage som maksimum, men er giften ædt i en eller flere giftstationer ved næste tilsyn, skal det næste tilsyn forekomme med færre dages mellemrum. Som tommelfingerregel gælder: 7 dage mellem tilsyn, med mindre flere giftudlægninger er ædt på ejendommen. Tiden til næste tilsyn tilpasses, således at giftstationerne aldrig står tomme or gift imellem tilsynene. Bekæmpelsen fortsætter i op til 35 dage, hvorefter bekæmpelsen burde være afsluttet, forudsat tilsynsfrekvensen har været som foreskrevet.

Der kan være situationer, hvor man kan synes, at have et tilsyn 3 til 4 dage efter en opsætning er meget kort tid. Hvis man står i den situation, så kunne det være værd i stedet at overvej, om bekæmpelsen ligeså godt ville kunne ske med fælder. I rapporten er der givet forslag til (anbefales) at udlægge ugiftig føde eller brug af sporplader forud for en eventuel giftopsætning i de situationer, hvor man mener, der kunne være tale om få rotter, eller at man ikke har den fornødne erfaring eller blot er i tvivl om bestandens størrelse og dermed bekæmpelsens omfang. Den ugiftige føde eller sporplader udlægges ikke i de giftstationer, man senere vil opsætte, men udlægges forskellige steder på ejendommen med udnyttelse af de naturlige skjulesteder, som ejendommen byder på, og som rotter med større sikkerhed vil benytte. På baggrund af indtag eller spor fra de forskellige punkter/plader har man et bedre overblik over bekæmpelsens omfang og dermed behovet for gift og placeringen af giften.

Bekæmperen bør under selve bekæmpelsesforløbet foretage en vurdering af fremgangen i bekæmpelsen og om der på den baggrund er behov for ændringer i den oprindelige plan. Har man f.eks. opsat 10 giftstationer og der efter flere tilsyn stadigvæk er aktivitet på en stor del af disse, så bør bekæmperen overveje, om der er noget han/hun har overset (ved brug af checkliste for bekæmpelse – **Appendix B**). Kan man forholde sig positivt (svare ja til alle punkter), så må man antage, at resistens er sandsynligt, og der bør derfor skiftes til en stærk antikoagulant.

Når de stærke antikoagulanter (brodifacoum, flocoumafen og difethialon) tages i brug, så bør der være et krav om, at der anvendes intervaludlægning. Ved intervaludlægning forstås udlægning af giften i meget små portioner (20-25 g/giftstation) og hvor der følges op på bekæmpelsen og giften med fast 7 dages interval, uagtet at giften er spist op efter ganske få dage.

2. Introduction

Warfarin was the first anticoagulant compound introduced as rodenticide in the 1950s. Resistance to warfarin in Norway rats (*Rattus norvegicus*) was detected for the first time in 1958 in the UK and in Denmark in 1962 (Boyle 1960, Lund 1964). Resistance led to the development of more potent anticoagulant compounds, which were introduced as rodenticides in the following years. Anticoagulants have proven essential for efficient rodent control to protect food stocks, human and animal health. However, the worldwide increasing occurrence of rodent resistance to anticoagulants, resulting in unsuccessful rodent control, poses a serious threat to human and animal health. This also applies to Denmark.

At present anticoagulant compounds are the only rodenticides which are allowed for chemical control of rats throughout the European Union (EU). They are very effective but pose a threat to the environment through primary and secondary exposure risk for non-target species (Christensen *et al.* 2010, Elmeros *et al.* 2015; Geduhn *et al.* 2014, 2015, 2016). The more potent compounds, including the ones which are efficiently in case of resistance, are classified as Persistent, Bio-accumulating and Toxic (PBT compounds). Due to the environmental risks there are restrictions for application of such anticoagulant rodenticides by the EU and by member states of EU (Berny et al. 2014).

One positive effect of the restrictions seems to be that there is an increased interest for developing alternative pest control measures now. However, when dealing with larger rat infestations, non-toxic solutions, which are mainly based on trapping methods, have not proven efficient and cost effective.

Due to spread of resistance and lack of alternative measures for anticoagulant rodenticides the need for a management strategy is apparent.

This report summarizes the development of resistance in general and Denmark, in particular. It presents a recommended future strategy for the use of anticoagulant rodenticides in Denmark, considering best practises, resistance effects and environmental risk.

2.1.1 Rat control in Denmark

In Denmark rat control has been dictated by an Act of law since 1907. Today the outline of rat control is given in the Environmental Act § 17 and § 18 (latest no 1189 of 27th of September 2016). The regulatory details of rat control are furthermore listed in the Statutory Order "Preventative measures and control of rats" (latest version referred to is No. 913 of 27th of June 2016). In the Environmental Act and the Statutory Order of rat control the responsibility for rat control of Norway rats (*Rattus norvegicus*) and Roof rat (*Rattus rattus*) is laid upon the local authority within each of the 98 municipalities. The latter species is only very rarely occurring in Denmark and typical in storage facilities at harbour sites only. Thus all references to rats and rat populations in this report concern the Norway rat.

Rat control in Denmark is meant to be "free of costs". However, in almost all municipalities the costs for carrying out rat control is paid by a small fee on property. All property owners in Denmark pay a small fee to cover all expenses for rat control within the municipality.

Some essential rules of the Danish rat control system are:

- Control of rats is always the responsibility of the local authorities (the municipalities).
- Each rat occurrence has to be reported to the local authorities.

- Every property owner has to ensure that their buildings and sewers are certified as rodent safe and that there are no deposits of waste and food outside the waste containers. If not local authorities can decree legal requirements if necessary.
- The local authorities have to carry out the rat control and can choose to do it themselves or by outsourcing it to a private pest control company.
- Rat control can only be carried out by authorised and trained people.
- Even when controlling mice with anticoagulant compounds authorisation is also required
- All municipalities and each individual pest controller are obliged to follow the strategy for anticoagulant use.
- The authorisation of a person can be withdrawn when the person has violated good control practise.
- Anticoagulants for control of rats and mice must be applied in bait boxes unless when used in sewers, underneath slatted floors and in liquid manure systems in animal stables.
- The anticoagulant rodenticides formulated as contact-foam product can be used outside bait boxes but only for indoor use.

3. The anticoagulant rodenticides

Like in the rest of the EU, Denmark only has anticoagulant rodenticides available for chemical control of rats. The different anticoagulant compounds are described as follows.

The first-generation anticoagulant rodenticides (FGARs) came into use during the early 1950s and revolutionised rodent control, also in Denmark, with outstanding efficacy and safety properties due to a delayed effect reducing bait shyness problems, a high mortality effect of the treatment and the availability of an antidote (Vitamin K) in case of an accident. The second-generation anticoagulant rodenticides (SGARs) were introduced to overcome resistance to the first generation compounds, which was observed for the first time in the late 1950s.

3.1 First generation anticoagulant rodenticides (FGARs)

The FGARs are efficient after multiple doses due to a rather low toxicity. Resistance developed against all FGARs in several regions of the world, including Denmark. There are three compounds in this group:

Warfarin – originally developed as a medicine against blood clotting in humans with cardiovascular problems, this was the first anticoagulant introduced as a rodenticide in 1950. Since 1990 it is no longer allowed in Denmark, due to a case of mistreatment of a pregnant American woman, where the use of warfarin caused damages to the foetus.

Chlorophacinone –was developed in the 1960s and used in several countries but has only since spring 2015 been registered for rodent control in Denmark. Formulated baits have a concentration of chlorophacinone of 50 ppm.

Coumatetralyl –was introduced in Denmark in 1967 as an alternative for the control of warfarin resistant rats. The toxicity of coumatetralyl is slightly higher than chlorophacinone, but the formulated product on the market has a much higher concentration with 375 ppm, compared to the 50 ppm of chlorophacinone baits.

3.2 Second generation anticoagulants (SGARs)

The SGARs are considerably more toxic than the FGARs with a longer half-life in the rodent. They are single-feed substances, meaning that the consumption of a single dose is enough for mortality. The field use of second-generation anticoagulants has resulted in reports of wildlife contamination through non-target poisoning or accumulation through the food chain resulting in secondary poisoning. The SGARs bromadiolone and difenacoum are affected by resistance and are less toxic than the other SGARs brodifacoum, flocoumafen and difethialone which are effective in case of resistance to the former compound. The five SGARs are in detail:

Bromadiolone – was developed in the 1970s and was introduced in Denmark in 1979. In spite of bromadiolone being a SGARs, resistance problems have been encountered. Concentration in baits of 50 ppm.

Difenacoum – was introduced in 1975 in Denmark and proved capable to kill the early strains of resistant rodents found in Denmark, the UK and other parts of Europe. Resistance to difenacoum is found in certain strains of rats and mice. Concentration in baits of 50 ppm.

Brodifacoum – was introduced in Denmark in 1979 and has an important role in controlling rats and mice that have developed resistance to FGARs, bromadiolone and difenacoum. Concentration in baits of 50 ppm.

Flocoumafen –was developed in the early 1980s and introduced in Denmark in 1984. It is similar to brodifacoum in terms of its chemistry, biological activity and potency, persistence, and risk of secondary poisoning. It is effective against rodents that have become resistant to other anticoagulant rodenticides. Concentration in baits of 50 ppm.

Difethialone – is the most recently introduced SGAR, as it was introduced in 1986. The potency of difethialone is very similar to both brodifacoum and flocoumafen. In contrast to the other SGARs, the concentration of difethialone based baits is 25 ppm.

4. Anticoagulant resistance

We use the definition of anticoagulant resistances by Greaves 1994: "*Anticoagulant resistance is a major loss of efficacy in practical conditions where the anticoagulant has been applied correctly, the loss of efficacy being due to the presence of a strain of rodent with a heritable and commensurately reduced sensitivity to the anticoagulant*"

At present, we know that at least one gene*VKORC1*, is involved in the expression of anticoagulant resistance. It codes for the enzyme Vitamin $K_{2\text{-}3}$ epoxide Reductase (VKOR) Complex subunit 1, which is the target for all anticoagulants (Rost *et al.* 2004). The VKOR is involved in the cycling of vitamin K, which is essential for maintaining a normal blood coagulation. When an anticoagulant enters the body, the anticoagulant inhibits the VKOR and the recycling of vitamin K stops. An inactive VKOR leads to impairment of blood coagulation and spontaneous haemorrhages will occur in the animal as soon as the body pool of vitamin K is depleted. Depletion of the internal vitamin K pool is normally depleted after approximately 3 to 4 days, which is why death by anticoagulant poisoning is usually occurring 4 to 6 days after an intake of a lethal dose.

Various changes (single nucleotide mutations) have been identified in *VKORC1* (Pelz *et al.* 2005, Grandemange *et al.* 2009, Rost *et al.* 2009, Baert *et al.* 2012, Buckle 2013, Pelz & Prescott 2015). A majority of these mutations is leading to an amino acid change in VKOR. One of the most widespread mutations found so far is the Tyrosine139Cysteine (Y139C) mutation, where the wildtype[1] amino acid Tyrosine at codon position 139 is changed to amino acid Cysteine leading to resistance. The different single nucleotide mutations found in the mutations in *VKORC1* correspond to the observed susceptible and resistant responses to anticoagulants of rats tested for all FGAR and partly for bromadiolone and difenacoum. The most important resistance mutations are listed in **Table 1**.

One of the most widespread mutations found so far is Y139C (Pelz & Prescott 2015), and is also the mutation found so far in Danish resistant rats. Besides Denmark Y139C has also been found as most common resistance-mutation in Germany and has also been found in France, United Kingdom, Belgium, the Netherlands and Hungary (Rost *et al.* 2009, Grandemange *et al.* 2010, van der Lee *et al.* 2011, Buckle 2012, Baert *et al.* 2012, Baert *et al.* 2016). The resistant mutation Tyrosine139Phenylalanine (Y139F) is prevailing in France and Belgium, but has so far not been found in Denmark. For the distribution of the different mutations see **figure 1**; RRAC 2016.

[1] Rats carrying the wildtype at position 139 will only carry alleles where the amino acid tyrosine has not been changed. A rat being wildtype for Y139C means that this individual is susceptible and thus has no genetic change in the *VKORC1*. Rats not carrying the wildtype allele, will have the modified amino acid cysteine on position 139 in *VKORC1*. These rats will either be heterozygous or homozygous for resistance. Heterozygous rats will have one allele being wildtype and one allele being the resistant mutation. A homozygous resistant rat carries two identical alleles of the Y139C mutation. Resistance level of heterozygous individuals is lower than level of homozygous individuals.

Table 1: Mutations of VKOR in brown rat related to resistance. The table is modified from RRAC 2016.

Base position of the altered amino acid in VKOR	Mutation name	Abbreviated name	Where
120	Leu120Gln	L120Q	the UK, France, Belgium
128	Leu128Gln	L128Q	The UK
139	Tyr139Cys	Y139C	Denmark, Germany, France, the UK, Hungary
139	Tyr139Phe	Y139F	France, Belgium, the Netherlands, the UK
139	Tyr139Ser	Y139S	The UK

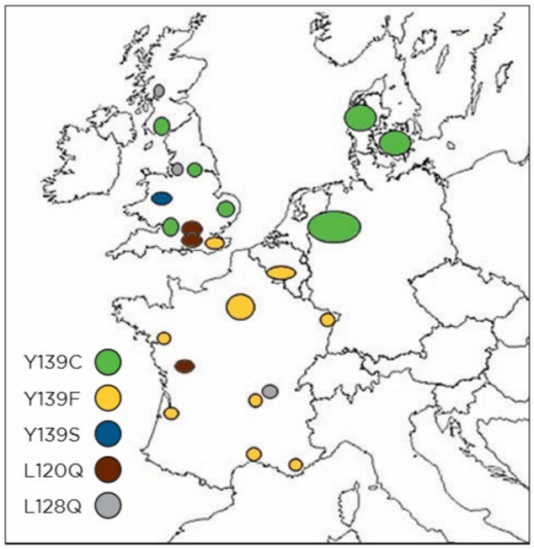


Figure 1: Distribution of anticoagulant-resistant strains of the Norway rat in Europe. The circles show the approximate locations of the different resistance mutations in Europe and not their exact extent. (Figure taken from RRAC 2016).

The Y139C mutation results in strong practical resistance against the FGARs (warfarin and coumatetralyl) (Endepols *et al.* 2007), but rats carrying the Y139C mutation are also less susceptible to both bromadiolone and difenacoum (Endepols *et al.* 2011, Buckle *et al.* 2012, Baert *et al.* 2016). The outcome of practical rat control with bromadiolone is highly correlated with the genetic composition of the rat population: in population of rats where there is a high proportion of Y139C resistant animals, the control will suffer from a severe lack of efficacy using bromadiolone (Endepols *et al.* 2011, Daniels *et al.* 2011). The outcome of rat control with difenacoum in populations were Y139C is present is less conclusive, but it is obvious that a substantial frequency of Y139C within the controlled rat population will lead to a loss of efficacy (Buckle et al 20012, Baert *et al.* 2016). As will be shown later female rats carrying the Y139C mutation are more resistant to difenacoum than Y139C resistant males, whether this may have affected the efficacy of difenacoum control in the study by Buckle *et al.* (2012) can only be hypothesised. However, the animals remaining after a difenacoum treatment will either be rats not feeding on the bait or are resistant. Continuous use of difenacoum will here lead to future exacerbation of the resistance problem (Buckle *et al.* 2012, Berny *et al.* 2014).

Thus, when a rat possesses the Y139C mutation, it is resistant to several anticoagulants of both FGARs and SGARs, and this also is the case for the other types of resistance caused by mutations in *VKORC1* (Pelz & Prescott 2015). So far practical resistance to the most potent anticoagulants (brodifacoum, flocoumafen and difethialone) has not been found (Buckle at al. 2012, Pelz & Prescott 2015).

Due to the speed of development and spreading of resistance to some of the introduced compounds, it was expected that it was only a matter of time, before resistance would develop to one or more of the three very potent anticoagulants (Smith & Greaves 1986). However, these three very potent compounds have been commonly used for the last three decades and there is no indication of practical resistance. One laboratory test has shown that some highly resistant rats had a slightly decreased susceptibility to brodifacoum, but only when using bait as low as 5 ppm (a tenth of the concentration of the commercial bait) (Gill *et al.* 1992).

4.1 Resistance in house mouse (*Mus musculus/domesticus*)

This report is dealing with the use of anticoagulant for rat control, but resistance to anticoagulants in house mouse is also a widespread problem in Europe. Resistance to bromadiolone and difenacoum seems to be common and has also been found in Denmark (Pelz & Prescott 2015). FGARs shouldn't be used for mice management as mice's have low susceptibility to them. Mutations in the *VKORC1* related to anticoagulant resistance in mice were also identified (Pelz *et al.* 2012) and three *VKORC1* sequence variants mediating resistance to anticoagulants seem to be widely distributed all over Europe: Y139C (the same as for Danish and German rats), L128S and the combination Arg12Trp/Ala26Ser/Ala48Thr/Arg61Leu (mutations found in *Mus spretus type*).

House mice carrying the homozygous Y139C sequence variant were found to be highly resistant to FGARs, bromadiolone and partly difenacoum (Pelz *et al.* 2012).

4.2 Resistance factors and practical resistance

As mentioned, resistance towards anticoagulants is not compound specific. When a rat is resistant to more than one compound it is defined as cross-resistance. Typically, the expression of resistance will be higher for the less potent compound compared to the more potent compound. Resistance factor is used to compare the susceptibility of a resistant rat towards compounds. The resistance factor can be expressed as x times the ED_{50} (Effective Dose). The baseline ED_{50} is the dose that is required to get a response in blood coagulation, leading to death in 50% of tested individuals of a susceptible strain of rat. ED_{50} is expressed as mg per kg bodyweight.

All else being equal, the higher resistance factor for a strain of rats the more resistant they are to the anticoagulant. However, it is essential also to consider the baseline ED_{50} of the individual compounds and not only the resistance factor. For bromadiolone the ED_{50} is 0.47 mg/kg bodyweight for male rats and 0.62 mg for female rats (**Table 2**). With a resistance factor of 17 for the Y139C male rats and a resistance factor of 15 for female rats, meaning that for getting an ED_{50} response in Y139C resistant rats 15 to 17 times more bromadiolone is needed, in order to get an ED_{50} response in blood coagulation (RRAC 2016). If we consider an average rat weighs about 250 g the baseline ED_{50} for bromadiolone for a resistant male rat is 0.47 divided by 4. With concentration of a 50 ppm commercial bait of bromadiolone a Y139C male rat will have to eat approximately 40 g to achieve a ED_{50} response and a female rat approximately 46 g (**Table 3**). **Table 2** and **Table 3** shows the different resistance factors and ED_{50} ratios for three *VKORC1* mutations and the amount of formulated bait needed for each of the three *VKORC1* strains.

Table 2: Three of the most important polymorphisms of the *VKORC1* proven to induce resistance to anticoagulants in Norway rats (*Rattus norvegicus*), and resistance factors in male and female resistant rats, based on Blood Clotting Response (BCR) data. Also given are the ED_{50} values for males and females in mg/kg bodyweight of the susceptible baseline strain. (Table from RRAC 2016).

VKOR	Resistance factors in male/female homozygous rats				
	Bromadiolone	Difenacoum	Brodifacoum	Flocoumafen	Difethialon
ED_{50} in susceptible strain (males/females)	0.47 / 0.62	0.65 / 0.79	0.22 / 0.23	0.29 / 0.34	0.43 / 0.49
L120Q	10 / 14	4.8 / 12	2.8 / 6.7	2.5 / 3.2	2.2 / 2.3
Y139C	17 / 15	1.6 / 2.9	1.2 / 1.8	0.8 / 1.0	0.5 / 0.8
Y139F	7 / 9	1.4 / 1.9	1.3 / 1.3	1.0 / 1.0	0.9 / 0.8

Table 3: The amount of formulated bait needed in the three different strains of *VKORC1*-mutations to achieve an ED_{50} response in an average rat of 250 g (*Rattus norvegicus*).

Amount (g) formulated bait per rat (250 g)					
	Bromadiolone	Difenacoum	Brodifacoum	Flocoumafen	Difethialon
Koncentration of bait	*0,005%*	*0,005%*	*0,005%*	*0,005%*	*0,0025*
L120Q	24 / 43	16 / 47	3,1 / 7,7	3,6 / 5,4	10 / 11
Y139C	40 / 47	5 / 12	1,3 / 2,1	1,2 / 1,7	2,2 / 3,9
Y139F	17 / 28	4,6 / 7,5	1,4 / 1,5	1,5 / 1,7	3,9 / 3,9

Resistance factors lower than 1 mean that the tested resistant strain responded a little bit more to the treatment with the anticoagulant than the baseline susceptible strain and a resistance factor of 1 means that there is no difference between the two strains. Resistance factors between 1 and 2 mean that there is only a minor difference which will typically not result in a noticeable loss of efficacy – we could here note it as technical resistance, meaning that the resistant gene is present, but not (yet) playing a role in practical rat control. Resistance factors above 2 are considered indicators of practical control problems (RRAC 2016), but again depending on the ED_{50}.

The higher baseline ED_{50} for difenacoum combined with the resistance factor of 1.6 and 2.9 males and females, respectively, can explain practical control problems of Y139C rats, especially for female rats, as the ED_{50} of 0.79 and a resistance factor of 2.9 means that a Y139C female rat will need to eat approximately 12 g of a commercial difenacoum bait of 50 ppm. In contrast a Y139C male rat will need to only eat 5 g (**Table 3**). As mentioned above control of Y139C resistant rats with difenacoum (Buckle *et al.* 2012) did not lead to full eradication of the controlled rat populations. One of the reasons for a failed control could be due to the fact that female rats carrying the Y139C mutation are more resistant than the Y139C resistant male rats.

For brodifacoum a resistance factor of 1.2 and 1.8 males and females, respectively, for Y139C has no practical relevance because of the low baseline ED_{50} (RRAC 2016). Here male and female rats will need an intake of 1.3 and 2.1 g, respectively, of a commercial brodifacoum bait. In case of L120Q the resistance factors for brodifacoum of 2.8/6.7 for males and females, respectively, but the low ED_{50} means that an average L120Q male rat needs an intake of only 3.1 g of a 50 ppm commercial brodifacoum bait and female rats approximately 8 g in order to have an ED_{50} response.

All ED_{50} and resistance factors are given for homozygous resistant rats, thus for heterozygous rats the ED_{50} and probably also the resistance factors would be considerably lower (not known at present).

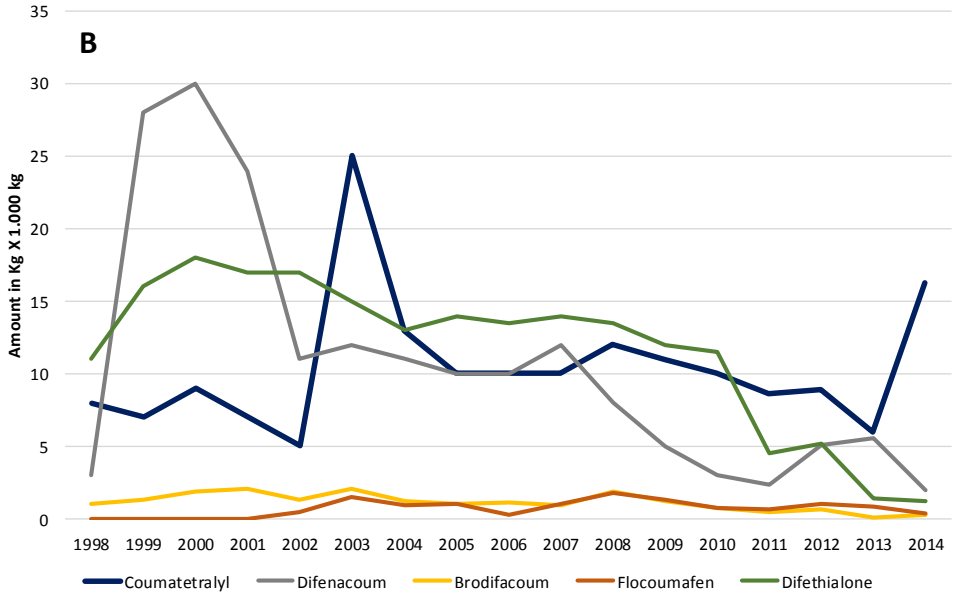
How a population of rats responds to an anticoagulant treatment thus depends on the genetic composition of the *VKORC1* of the population (frequencies of wildtype (susceptible), heterozygous and homozygous resistant rats). If the population is fully susceptible, treatment success will approach 100 % (if all rats eat the bait and the treatment has been carried out correctly). If the opposite is the case, i.e. the majority of rats possess the resistance *VKORC1* allele in the homozygous resistant state, then the anticoagulant treatment with FGARs and bromadiolone will fail and problems with difenacoum are most likely. If resistance occurs, even at very low level, a continued use of that particular anticoagulant, whether it is FGARs, bromadiolone or difenacoum will selectively eradicate susceptible rats and instead the frequency of resistant *VKORC1* allele will rapidly increase in the surviving population and with that the proportion of homozygous resistant rats.

By checking the sequence of nucleotides in the *VKORC1*, we are able to detect resistance and predict the outcome of practical control. Obviously, for the latter to be reliable, enough individuals from a population have to be tested in order to identify the level of resistance within the rat population.

5. Anticoagulant use in Denmark

Each year the Danish municipalities report to the Danish Environmental Protection Agency (DEPA) the amounts of anticoagulants that has been used in the previous year (**figure 2** shows the data for the period 1998 until 2014). From this figure a steady decline in the use of the most commonly used anticoagulant, bromadiolone, is evident. From 1998 to 1999 there was an almost 100 % increase in bromadiolone, correlated to the increase in the number of rat notifications, which in 1999 reached a level of approximately 160,000 notifications throughout Denmark. The number of notifications has varied since 1999, with a few exceptional peak years, like 2008, 2012 and 2014. The amount of bromadiolone has however declined since 1999. The same tendency to decline is also evident for difenacoum and difethialone. In contrast, the use of coumatetralyl has increased in general compared to 1998 and for brodifacoum and flocoumafen the amount used yearly has not changed much with an average yearly use of approximately 0.7 to 1 ton of each. In 2014 the total amounts of anticoagulants used for the rat control carried out by local authorities was approx. 87 tons of formulated anticoagulant bait. It is assumed that approximately the same amount is used in the private professional rodent control every year.

Resistance factors lower than 1 mean that the tested resistant strain responded a little bit more to the treatment with the anticoagulant than the baseline susceptible strain and a resistance factor of 1 means that there is no difference between the two strains. Resistance factors between 1 and 2 mean that there is only a minor difference which will typically not result in a noticeable loss of efficacy – we could here note it as technical resistance, meaning that the resistant gene is present, but not (yet) playing a role in practical rat control. Resistance factors above 2 are considered indicators of practical control problems (RRAC 2016), but again depending on the ED_{50}.



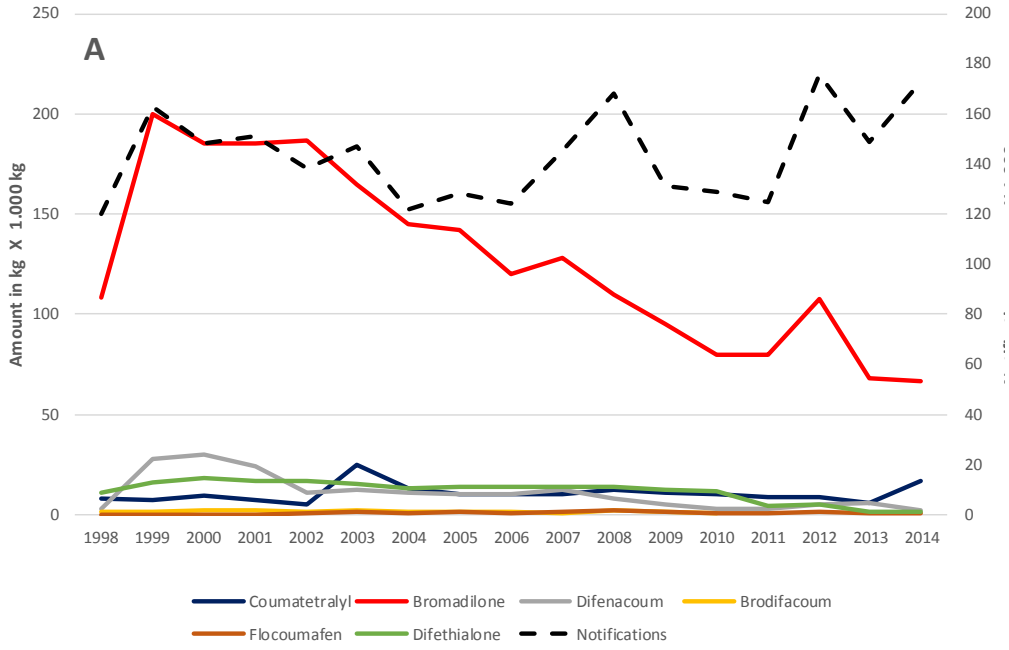


Figure 2: (A) total yearly amount (kg) of anticoagulants used in rat control carried out by the local authorities in Denmark from 1998 till 2014. The broken black line is the total number of notifications. (B) the same numbers but without bromadiolone.

The higher baseline ED_{50} for difenacoum combined with the resistance factor of 1.6 and 2.9 males and females, respectively, can explain practical control problems of Y139C rats, especially for female rats, as the ED_{50} of 0.79 and a resistance factor of 2.9 means that a Y139C female rat will need to eat approximately 12 g of a commercial difenacoum bait of 50 ppm. In contrast a Y139C male rat will need to only eat 5 g (**Table 3**). As mentioned above control of Y139C resistant rats with difenacoum (Buckle *et al.* 2012) did not lead to full eradication of the controlled rat populations. One of the reasons for a failed control could be due to the fact that female rats carrying the Y139C mutation are more resistant than the Y139C resistant male rats.

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VKORC1 allele will rapidly increase in the surviving population and with that the proportion of homozygous resistant rats.

By checking the sequence of nucleotides in the *VKORC1*, we are able to detect resistance and predict the outcome of practical control. Obviously, for the latter to be reliable, enough individuals from a population have to be tested in order to identify the level of resistance within the rat population.

During the last couple of years, the Danish municipalities have digitalised the information on rat control, with information on each individual notification including, for example, the cause of rat problems, actions taken to control the rat infestations, the use of anticoagulants, etc. We were able to obtain data on anticoagulant use for a number of these municipalities (**Figure 3**). The data is assumed to be representative of the anticoagulant usage in the Danish municipalities. The average amount of each of the anticoagulants per municipality resembles the general trends (**Figure 2**) with bromadiolone being the most commonly used anticoagulant in rat control. The large standard deviation indicates the great variation observed in anticoagulant usage in the different municipalities. Some of the observed variation is due to differences in the number of notifications, but also different views of which anticoagulant to use within the municipality and occurrence of resistance may play a significant role. Chlorophacinone was introduced in spring 2015 and does not appear in the data before. The use of FGARs in general seems to be constant, with a decrease in coumatetralyl but a corresponding increase in chlorophacinone. An increase was observed for brodifacoum in 2016. Whether this is due to a continuous high number of rat notifications or pest controllers having increasing problems in controlling rats is not known.

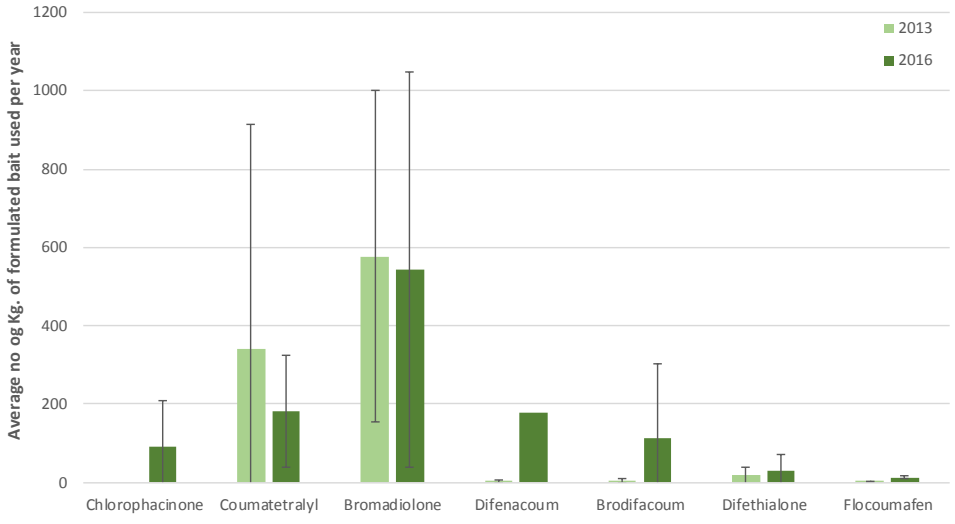


Figure 3: Average amount of kg anticoagulant use in a Danish municipality per year for each of the seven anticoagulant compounds available in Denmark. The average is obtained by using data on registered use of anticoagulant from 5 municipalities (2013) and 8 municipalities (2016). The vertical bars are standard deviation. The data only represent the amount of anticoagulant used in the rat control carried out by the local authorities and do not include the amount used in the private professional control.

6. Anticoagulant resistance in Denmark

Since the first case of resistance in 1962 (Lund 1964) resistance monitoring has been carried out in Denmark. From 1962 until 2001 the resistance testing was based on no-choice feeding tests (basically, feeding the rats a poisonous bait with the investigated anticoagulant for 5 to 6 days and measuring the consumption and time until death). Rats showing resistance to *e.g.* coumatetralyl and surviving was afterwards tested on bromadiolone and so forth until the tested individual died. The most potent anticoagulant, that the rats within a population survived, was registered as the maximum "level of resistance" for the tested rats (**Figure 4A** and **4B**). If more individuals from a population were tested, the "level of resistance" was determined by the rat(s) surviving the most potent anticoagulant.

From 2001 till 2008 a total of 2,334 rats were tested using no-choice feeding tests and Blood Clotting Response (BCR) tests (Lodal 2010) (**Figure 4C**). The BCR test was based on injection of doses of anticoagulant subcutaneously and measurement of the blood clotting time before and after the administration of anticoagulants. The highest "level of resistance" was here determined like it was for the no-choice feeding test, meaning that the last positive anticoagulant test determined the "level of resistance".

Resistance monitoring were for many decades based upon pest controllers trapping rats at locations where they suspected resistance. The trapped rats were send to the testing facilities at the Danish Pest Infestation Laboratory (DPIL) and were here tested for resistance with the no-choice feeding test. Since 2001 a new monitoring programme was established, were each municipality were asked to trap 10 rats for resistance testing. The latter resistance monitoring programme was terminated in 2008 and since then there has been no official monitoring programmes.

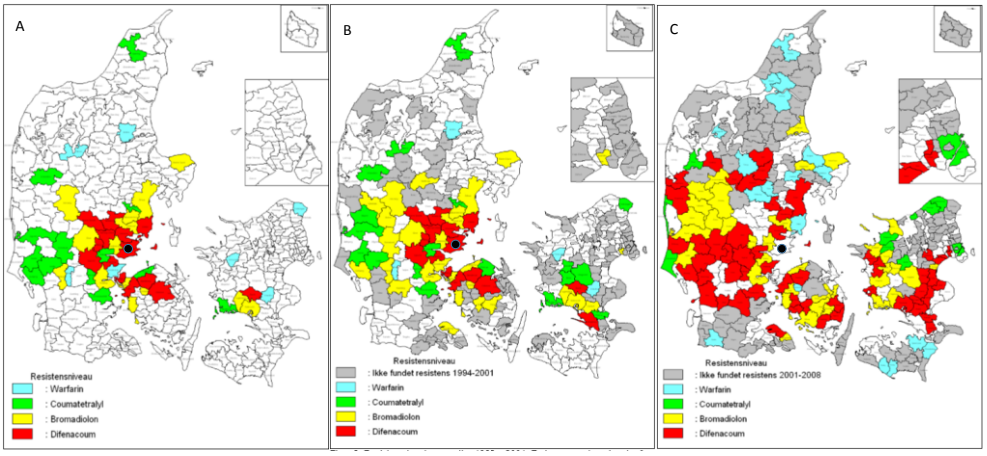


Figure 4. Distribution of anticoagulant resistance in Norway rat from 1962 – 1993 (A), 1994-2001 (B) and 2001-2008 (C). The maps show all municipalities prior to 2007, which is a total of 271 municipalities. The black dot is where resistance to warfarin was first found in 1962. The municipality is coloured according to the knowledge of the highest resistance level within the tested rats. Grey municipalities indicate municipalities where no resistance was documented in the tested rats, for white municipalities no data are available (From Lodal 2010).

The number of municipalities in Denmark has varied in the period of 1970 to 2006. In 2007, after an administrative reorganisation, the number of municipalities was reduced from 271 to 98 municipalities. In the following when referring to anticoagulant resistance in the description of anticoagulant distribution based on the resistance monitoring programmes the number of municipalities refers to the 271 municipalities prior to 2007.

In the first three decades after resistance was first found in 1962 resistance was reported in 48 municipalities (**Figure 4A, Table 4**). In more than half of these 48 municipalities, the highest level of observed resistance was against bromadiolone and difenacoum. In the second period from 1994 till 2001 bromadiolone had become an increasing problem in 27 municipalities compared to the 14 municipalities in the previous period (**Figure 4B, Table 4**). In 2001 till 2008 where a more systematic monitoring program was carried out (municipalities were here requested to provide a minimum of 10 rats for testing) resistance to bromadiolone and difenacoum was evident in 92 municipalities, and now difenacoum was more often the highest level of resistance than bromadiolone (**Figure 4C, Table 4**).

After resistance was found in 1962 near Horsens on Jutland, resistance was gradually found spreading within the municipality and onwards to the neighbouring municipalities. In later years, resistance of a higher level (i.e. to the more potent compounds bromadiolone and difenacoum) seemed to originate from the areas in Jutland where the original resistance to FGARs was first observed. The same pattern was also seen for the first resistance foci on Funen (Middelfart) and Zealand (Vordingborg) (not shown).

As mentioned, there hasn't been any official monitoring of anticoagulant resistance in Denmark since 2008. However, in the last couple of years (2014-2016) the authors of this report have conducted genetic resistance testing on 118 rats sampled from sites with possible control problems, but also from areas were resistance was not expected. In total 57 sampling locations were tested for presence of the Y139C mutation. In 33 of these locations either or both heterozygous and homozygous Y139C resistant rats were identified (**Appendix A**). The resistance monitoring will hopefully continue for at least the next two years in order to obtain knowledge about the distribution of resistance and as a base for the application of appropriate rodenticides to mitigate environmental risks (see below). Ten municipalities will be participating and provide approx. 30 samples per year each (figure 5 shows the location of the 10 participating municipalities).

Table 4: Compiled results from the Danish resistance monitoring program from 1962 until 2008. The number of municipalities where resistance was found is given for each of the three time periods. The definition of municipalities is from before 2007, thus the number of Danish municipalities is 271.

Resistance monitoring in Denmark	Number of municipalities		
	1962-1993	1994 - 2001	2001 - 2008
Warfarin	7	4	17
Coumatetralyl	13	18	10
Bromadiolone	14	27	37
Difenacoum	14	15	55
No resistance	-	80	91

Figure 5: Red dots placed within a municipality area, where sampling and testing for resistance (VKORC1: Y139C mutation) will be carried out in 2017-2018.

7. Resistance management

When controlling rats at sites where resistance occurs, achieving complete eradication of the rats is crucial, therefor using the appropriate anticoagulant and applying the anticoagulant correctly is essential for an effective rat management. A pest controller must, at all times, have an adequate assessment of the remaining population of rats during a control operation, so that the control is not halted when a number of less susceptible rats are still alive, since that would result in a strong selection for resistant individuals.

Choosing the appropriate anticoagulant is important to mitigate the environmental risk during pest control. Using FGARs in case of resistance means unsuccessful pest management connected with accumulation of the toxic compound in the rats and an unnecessary risk for non-target species by primary and secondary exposure.

In Denmark the present resistance strategy is to use less toxic anticoagulant as first choice when there is no prior knowledge of resistance, that means products based on either chlorophacinone or coumatetralyl are chosen as first choice (as internationally accepted, DEPA has categorised chlorophacinone at the same level as coumatetralyl). It has been a general guideline to pest controllers, that when there was a possibility or evidence of resistance, the anticoagulant with next higher potency level should be used.

When looking back only a few years, different concentrations of the commercial product were available like for bromadiolone the two commercial concentrations were 50 and 100 ppm. An alternative when resistance to bromadiolone was detected was to switch from a 50 ppm bait formulation to a 100 ppm bait of the same product.

Since most anticoagulants now only is formulated into baits with concentration of 50 ppm or less with coumatetralyl based bait and contact foam as the only exception (concentration of 375 ppm and 4000 ppm, respectively), the next best choice is the next anticoagulant in line. In this case the requested first choice use would be FGARs followed by bromadiolone (though

slightly more toxic than difenacoum), then difenacoum and then if still experiencing control problems using difenacoum, the pest controller could choose freely amongst the very potent anticoagulants (brodifacoum, flocoumafen and difethialone).

As stated by Greaves (1995) there are only two actions to be taken into consideration if resistance is present; 1) to promote selection in favour of the anticoagulant susceptible rats and 2) to ensure selection against resistant rats. Basically that means stopping the application of the anticoagulant to which resistance is present. Preferentially, the pest controller should switch to a non-anticoagulant rodenticide (but these are at present not available for rats in the EU), or switch to an anticoagulant that does not intensify further selection for resistance. Greaves (1995) proposed to put effort into a progressive selection against the resistant rats like stopping their access to vitamin K_3, of which some anticoagulant resistant rats in UK are known to have a considerably higher vitamin K need than susceptible rats (Hermodson *et al.* 1969, MacNicoll & Gill 1993). However, for Danish resistant rats this may not be the most efficient approach, since Danish heterozygous resistant rats only have a minor increase in vitamin K requirement compared to susceptible rats (Markussen *et al.* 2003).

The Danish resistance strategy was intended to slow down the spread of resistant rat populations and the development of resistance to newer compounds in Denmark (Lodal 2010). The lack of resistance to the more potent SGARs in Denmark has been suggested to be due to the practise of the Danish resistance strategy (Lodal 2010). The fact that practical resistance to none of the more potent SGARs has been found in any rat populations anywhere (Baert *et al.* 2016), shows, however, that the Danish resistance strategy cannot be held responsible for the lack of the mentioned resistance. On the contrary, resistance seems today to be more widespread and controlling rat populations with bromadiolone and difenacoum has become an increasing problem (**figure 4**) (Lodal 2010).

It has been speculated that the Danish resistance strategy using the anticoagulants by a succession of small steps may actually have facilitated the development of resistance in general and especially to bromadiolone and difenacoum (Greaves 1995). In other words, while switching to next level of rodenticide would have meant a selection for a more resistant rats coming from a level that was already somewhat resistant.

Controlling rat populations that were resistant to the weaker rodenticides with one of the most potent SGARs might have been successful in killing off all the resistant rats, in other words "resistance-breaking", after which the low toxicity FGARs could again be used. In the UK the potent SGARs, brodifacoum, flocoumafen and difethialone are, at present, not allowed for controlling rats outdoor. Here the lack of ability to control population of rats highly resistant to bromadiolone and difenacoum has indeed been a challenge and the extensive use of bromadiolone and difenacoum to control already resistant rat populations has instead lead to an extensive spread of resistance and the *VKORC1* mutations (Buckle & Smith 2015).

Our knowledge today on resistance, its mechanisms, genetic characteristic and field trials (*e.g.* Pelz *et al.* 2005, Berny 2011, Berny *et al.* 2014, RRAC 2016, Endepols *et al.* 2007, Endepols *et al.* 2011) shows that FGARs efficacy is severely affected by all resistance mechanisms and especially the *VKORC1* mutations in rats and mice. Bromadiolone is definitely affected and difenacoum is partially affected for rats carrying the Y139C resistant-mutations (Buckle *et al.*, 2012; Buckle *et al.* 2012, Endepols *et al.*, 2012; Endepols *et al.*, 2007). For rats carrying the L120Q mutation only technical resistance to brodifacoum has been demonstrated (Gill *et al.* 1992, RRAC 2016), and as mentioned before no evidence of field (and practical) resistance to brodifacoum, difethialone and flocoumafen has been reported (Baert *et al.* 2016). Thus, the Y139C and the majority of the other *VKORC1* mutations do not exhibit a compound specificity but rather covers resistance to more anticoagulants of both FGARs and SGARs.

It is a fact that resistance to both difenacoum and bromadiolone was found in UK and Denmark only few years after the introduction (Redfern & Gill 1978, Lund 1984). These two compounds were used to control warfarin and coumatetralyl resistant rat populations. Thus when introduc-

ing bromadiolone or difenacoum to these population of rats, resistance to FGARs was already present. The three potent anticoagulants (brodifacoum, flocoumafen and difethialone) were introduced in the late 1970's and mid 1980's and have been used along with the other anticoagulants here in Denmark, but so far no practical resistance towards any of these anticoagulants has been reported, pointing to the use of either of these three anticoagulants as being resistance-breaking (Buckle *et al.* 2012, Pelz & Prescott 2010, RRAC 2016).

It would seem that we, for the years to come, need not worry for having difficulties dealing with rat populations resistant to FGAR and bromadiolone and difenacoum, as control of resistant population of rats are possible, whenever one or more of the resistance-breaking compounds (brodifacoum, flocoumafen and difethialone) are used. The three very potent anticoagulants however pose great risks to the environment and for non-target species and wildlife in general.

7.1 Recommendations for a future resistance strategy/management:

Based on the resistance data available at present it has been recommended in a report commissioned by The European Commission that; "*FGARs, bromadiolone and difenacoum should always be considered first choice products against Norway rats, unless there is local evidence of resistance. If infestation persists after five weeks despite correct application and bait consumption, resistance should be considered and tested for. If resistance is identified and information available on the practical level of resistance, using the most potent anticoagulant should be considered immediately*" (Berny *et al.* 2014).

This statement above addresses factors to be considered when controlling rats using anticoagulants; 1) first choice of anticoagulant, 2) duration of anticoagulant use, 3) correct application of the anticoagulants, 4) testing for resistance and 5) use of resistance breaking compounds. In later sections we will address issues of correct anticoagulant use and duration of an anticoagulant treatment.

The authors of this report strongly support the recommendations made by Berny *et al.* (2014) and RRAC (2016) on first choice of anticoagulant in rat control and choice of anticoagulant compound in the case of resistance. That means, when controlling a population of rats first choice should be either FGARs, bromadiolone or difenacoum. When addressing the issues of resistance and, in particular, the selection for the main resistant traits (the *VKORC1* mutations) the differences between FGARs and bromadiolone are only minor. However, as bromadiolone and difenacoum are classified as PBT compounds, first choice should preferably be FGARs, as they are pose far less risk for the environment.

When resistance to any of these anticoagulant is either suspected or genetically identified, anticoagulants with weak selective properties for the *VKORC1* mutation(s) should be used. As mentioned earlier, we consider the anticoagulants brodifacoum, flocoumafen and difethialone as anticoagulants without selective power against *VKORC1* resistant mutations, and suggest to use either of these when trying to breaking resistance. **Table 5** below shows the recommended use of anticoagulants compounds in case of resistance in Norway rats for various *VKORC1* mutations. When the Y139C mutation is present it means that only the potent SGARs (brodifacoum, flocoumafen and difethialone) should be used as they are the compounds that do not select for resistant rats.

If, however, another non-anticoagulant alternative should become available with an equal potential of control efficiency, safety and palatability as the more potent anticoagulants, we recommend to continue rat control with such an alternative and eventually followed up by using the more potent anticoagulants at the end, if not 100 % control is achieved with the non-anticoagulant choice of control method or to carry out intensive trapping to remove the last remaining resistant rats. It is important to stress, that if a new infestation occurs at a later point

at the same location, first choice should again be either the FGARs, unless resistance seems to be a general problem in the area.

As mentioned before anticoagulants are the only chemicals for controlling rats. But a “Task Force” within the EU is working on bringing back an old non-anticoagulant rodenticide, cholecalciferol, to the European market (Buckle & Smith 2015, RRAC 2016). If cholecalciferol products become available again, hopefully, with a much higher palatability, than in the formulations that used to be on the market, the use of this non-anticoagulant can be recommended to be considered as a resistance-breaking compound.

Table 5: Polymorphisms of the *VKORC1*, and compounds recommended (+) to control these strains of the Norway rat (*Rattus norvegicus*), based on resistance data and field trials. Products containing rodenticides marked with (-) shall not be used to control respective strains. The Y139C mutation is the one present in Danish resistant rats. (modified table from RRAC 2016).

VKOR	Compounds recommended (+) and not recommended (-) for rat control							
Resistant strain	First generation anticoagulants			Second generation anticoagulants				
	Warfarin	Chlorophacinone	Coumatetralyl	Bromadiolone	Difenacoum	Brodifacoum	Flocoumafen	Difethialone
L120Q	-	-	-	-	-	+	+	+
L128Q	-	-	-	-	+	+	+	+
Y139C	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**
Y139F	-	-	-	-	+	+	+	+
Y139S	-	-	-	+	+	+	+	+

7.2 Test for resistance in Danish rats

Whenever a pest controller suspect resistance it would be of great value to establish if the failed rat control is indeed due to resistance or not. Today resistance can easily be confirmed by genetically testing for *VKORC1* mutations. Testing for resistance should be preferred whenever possible in order to assure a correct choice of anticoagulant and especially testing should be preferred whenever the resistance breaking compounds are considered.

Testing for resistance can provide general knowledge on the distribution and dispersal of resistance within the Danish rat population. But also, if more resistance breaking compounds will be used in the future to deal with resistant rats, it will be essential to keep track of the development of resistance as the use of resistance breaking compound along with correct application (see later) should rather decrease and not increase resistance both locally and regional.

7.3 Anticoagulant use in *Mus musculus*

As mentioned, mutation in the *VKORC1* conferring resistance to anticoagulants has also been identified in house mice (*Mus musculus*). Below (**Table 6**) has listed the recommended use of anticoagulant when resistance is occurring. In contrast to rats, difenacoum can still be recommended for controlling Y139C resistant house mice. The high ED_{50} for house mice and difenacoum (ED_{50}: 0,85 mg/kg b.w (male) and 0,56 mg/kg b.w (female) combined with the respective RF of 1,2 and 2,7 indicate that Y139C house mice should only consume 0,4 and 0,6 g of a commercial difenacoum bait to achieve a $ED_{50,}$ and thus it is possible to control resistant mice with difenacoum.

Table 6. Mutation variants of the *VKORC1* proven to induce resistance to warfarin in the house mouse (*Mus musculus*), and compounds recommended to control them (+). Mouse strains resist those compounds that are marked with (-).

VKOR	Compounds recommended (+) and not recommended (-) for control of house mouse							
Resistant strain	First generation anticoagulants			Second generation anticoagulants				
	Warfarin	Chlorophacinone	Coumatetralyl	Bromadiolone	Difenacoum	Brodifacoum	Flocoumafen	Difethialone
L120Q	No data available					+	+	+
L128S	-	-	-	-	+	+	+	+
Y139C	-	-	-	-	+	+	+	+
Y139S	No data available					+	+	+

8. Ecologically-based pest management

We have to keep in mind, that anticoagulant treatments only rarely are the solution to a rat problem. There is always a reason for rats settling. Rat infestation arises because the habitat and the environment (people included) provide the means for the rats to settle and survive. Rat infestations relay on the presence of food, shelter and to a certain degree water (as water is almost always obtainable in our part of the world, it is only very seldom a limiting factor for an infestation of rats). Without shelters the rat cannot breed and its safe dispersal is very limited. Thus rat control should always include the aspects of making the surrounding areas less likely to be invaded by rats again. This holistic way of conducting rat control involving its ecology is termed ecologically-based pest management (EBPM), which has three fundamental goals; 1) minimizing adverse effects on non-target species and the environment, 2) develop an approach that is economic for end-users and 3) the established approached is durable (Singleton *et al.* 1999).

Central when controlling population of rats is that the control is 1) preceded by a proper assessment of the best ways to control the rat population, whether it is chemical or non-chemical, based on thorough investigation of the situation at the premises and surroundings and taking aspects of non-target species into consideration, 2) always follow a best practise for rat control and 3) making sure to identify, remove and/or limit all possible causes for the maintenance of a population of rats in the future.

Thus our recommendation for first choice of anticoagulant in rat control and the use of the more potent anticoagulants in case of resistance does not come without a recommendation for how to conduct rat control in practise.

Already the Danish legislation and guidelines state how rat control should be carried out. In the section “Ecologically-based management” below we describe how and which considerations has to be taken into consideration, when dealing with anticoagulants. This is not new knowledge to pest controllers, pest control companies and local authorities. We, however, recognise that not enough focus has been given to a more thorough overall rat control strategy by all involved parties. The rat control is lacking quality due to underestimation of actual costs invested in rat control in general. We do not suggest a whole new concept; in fact, it is mostly based upon already stated procedures in the Danish guidelines (Naturstyrelsen (now Styrelsen for Vand- og Natur (SVANA)) 2015). Furthermore, our suggestions for anticoagulant applications are based upon a number of guidelines that have appeared in the last couple of years (Links are given below).

- NST – Naturstyrelsen (2015): http://naturstyrelsen.dk/media/133463/vejledning-om-forebyggelse-og-bekaempelse-af-rotter-februar-2015_printversion.pdf
- CRRU – Campaign for Responsible Rodenticide use, UK (2015): http://www.bpca.org.uk/assets/CRRU_COBP.PDF
- CEFIC – European Biocidal Products Forum (2013): http://www.rrac.info/content/uploads/CEFIC-EBPF-RWG-Guideline-Best-Practice-for-Rodenticide-Use-FINAL-S-.pdf

- German Federal Environment Agency (2014): https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/faq_anticoagulant_rodenticides.pdf
- MST – Miljøstyrelsen (2016): http://mst.dk/media/174727/rottevurderingsvejledning2.pdf

8.1 Procedures of rat control

The following is a general description of how to carry out any control of rats with or without anticoagulants. The general outline is based upon the different guidelines to best practice etc. mentioned above. For more details and information, the readers are recommended to become acquainted with the guidelines mentioned previously.

- The first aspect is always to consider, when visiting a location, to characterise the potential rat problem. By investigating the premises (buildings and surroundings). The controller should get an overview of the extent of the rat infestation.

- Next the pest controller should identify possible shelters and available food items. What preliminary actions should be conducted by the property owner and/or the pest controller in order to carry out further investigations? or to be able to begin controlling the rats. What other preventative measures should be conducted along or after the control to ensure that the infestation does not re-establish after control is complete?

- Removal of the food and water source or at least limiting access through exclusion and proofing.

- Identification and removal of the shelters, harbourage and covers used by the rodents is also an integral part of any integrated strategy as this will help in reducing the carrying capacity of the environment. The lower the carrying capacity, the fewer rats there will be. Fewer rats will lead to a minimized level of either chemical and physical control techniques to the benefit of the environment in general. However, it is important to stress, that such actions should not be carried out prior to the control of the rat infestation, but rather going along with the control itself or after the control has finished. Too massive actions prior to controlling can lead to a spreading of the problems, as rats will seek to other places with less disturbances. When recommending needed changes on a premise in order to minimizing the risk of re-infestation, the pest controller, involved in the control authorised by the local authorities, have a valuable tool in using the authorities to demand any changes, proofing etc.

- The cost effective control of rats, either on a small or larger scale, requires a planned strategy. The casual and unplanned implementation of a control program is unlikely to lead to long term effective control. Already today most pest controllers involved in rat control within municipalities are familiar with reporting on the different procedures carried out during controlling of a population of rats using different digitalized platforms. However, what is important here is to emphasize the need of especially the required time for doing a thorough investigation, to come up with an appropriate plan, that has to be described, for the control and preventative measures and to have the necessary time for follow up on the control as often as needed and/or required.

- As mentioned above any EBPM programme comprises a number of practical elements. For eventual success, however, an essential presumption must be that the person applying the EBPM must be suitably trained, which is already the case in Denmark, as only

authorized pest controllers are allowed to carry out rat control and handling anticoagulants.

An effective application of the above mentioned procedures will play a fundamental role in the avoidance of the development and dispersal of resistance to an anticoagulant. The resistance strategy itself has no or only minor influence on; limiting the development and dispersal of resistance and minimizing the environmental impact, if correct application and best practise is not followed.

8.2 Outline of procedures

The following is not a complete protocol for best practise, but it summarises the essential and practical elements of an EBPM programme against rat infestation as described above;

The Survey – is where the pest controller gets a good overview of the extent of the infestation and based on that can make and initiate the most reliable plan of control. When dealing with rat control pest controllers have to take into consideration when planning the control itself, that rats can settle in an area even though distances between shelter/nesting places and food sources are far apart – moving between these two sites can be a few meters up till several 100 meters.

The use of physical control techniques – the pest controller should always consider the possibility of using *e.g*. trapping or other non-toxic alternative for control instead of using anticoagulants.

The use of chemical control techniques – here the pest controller has to take into consideration, which anticoagulant will be appropriate within the “first choice” group? what kind of formulation is the best? and how many bait points are required and how much anticoagulant should be placed in the used bait stations?

Environmental Management – the pest controller identifies and see to that food and other alternative food sources are removed or limited. Inappropriate covers and harbourage are removed or at least trimmed.

Rodent proofing – the pest controller can with the support from the local authorities put legal demands into further rodent proofing in order to protect buildings, homes etc.

Record Keeping and Monitoring – is an essential component of any rodent control programme, as the pest controller, local authorities and the property owner can look into the control operations that have been undertaken and then to utilise these data to monitor progress.

In addition, a good integrated strategy will ensure that essential data are recorded including (modified list made by RRAC 2016):

- Details of who has undertaken the rodenticide application and where
- Environmental Risk Analysis: Possible routes of non-target poisoning, including secondary poisoning, and appropriate risk mitigation measures taken
- Toxicant used
- Where toxicant (in bait boxes) has been placed and how – including mapped distribution of bait placements
- Amount of toxicant used
- Dates of all visits and actions undertaken
- Details of rodent consumption of bait from baiting points
- Records of carcasses recovered
- Records of monitoring and detection (electronic, photographic, tracking plates etc.)
- Details of environmental factors and preventative measures that require attention as a means of reducing carrying capacity in the future
- Baits recovered at the end of the treatment

- Completion and closure dates

Reviews – It is appropriate, in any rodent management programme, that the pest controller has an expectation of the time-scale over which control is to be achieved. As far as the anticoagulants are concerned, an appropriate initial time scale for field control might be set at 14-35 days (see also later). If control is achieved within this time, then targets and expectations have been met, if not the pest controller must review and assess the rat control all over again.

8.3 Use of anticoagulants

High control efficiency can be achieved using FGARs, bromadiolone and/or difenacoum, as long as there is no anticoagulant resistance and that the control is carried out correctly. The most essential part of controlling rats using FGARs, bromadiolone and difenacoum is to make sure that bait is available during the treatment at all times in order to avoid under-baiting, which means avoid feeding the rats sub-lethal doses of anticoagulant. This is especially important, if first choice is one of the FGARs, as rats will need multiple and larger doses in order to obtain a lethal dose.

The most common way to achieve under-baited rat population is when follow-up visits is not done frequently enough. Thus when using anticoagulants follow-up visits should be done with regular intervals. But what are regular intervals?

8.3.1 Frequency of follow up visits

As a rule of thumb: if one or more bait points are empty at the next follow-up visit, then the time between the two visits has been too long. As a general rule;

- First follow-up visit should be within few days (every 2-3 days) (especially when many rats are expected and when FGARs are used) and maximum 7 days for all other infestations.

The following visits should follow with a time interval of maximum 7 days for as long as the bait uptake is increasing or constant. But again if more bait points are empty at the next follow-up visit 7 days are too long a time interval and thus should be shorten.

When the activity on bait is decreasing the interval between visits may be prolonged to maximum of 14 days. But again if bait points are empty, the time before next visit has to be shorten.

It should be noted, that when using anticoagulants, the premise should be investigated minimum twice a week for dead and dying rats. Dead and dying rats shall be removed by the pest controller.

8.3.2 Duration of a treatment

Normally when resistance is not present and the anticoagulant has been applied correctly the treatment should be terminated within 35 days and for most treatment even less. If an infestation cannot be controlled within the 35 days, the controller has to re-evaluate the procedure. In all rat control using anticoagulants the procedure should be evaluated as soon as the controller have visual evidence, that the control carried out is not working. Is it resistance or is it insufficient control techniques?

8.3.3 Number of bait points?

It is essential that enough bait points (mandatory use of bait stations) are laid out. Often the number of bait points used in rat control is too few. The poor number probably often leading back to lack of sufficient survey of the premises. The number of bait points will in most cases be determined by a number of elements and not necessarily only were rodent activities have been notified (Endepols *et al.* 2003), like;

- Buildings
- Animal feed
- Livestock, etc
- Groundcover
- Piles of various materials
- Straw and hay
- Surroundings
- Places with gaming feeding

The pest controller should cover each of these elements with sufficient bait points, whenever they are present on the premises. When setting up bait station be aware of rules dictated by the product label concerning distances between bait points.

We can recommend developing a general tool for planning the individual treatment in order to enable or urge the pest controller to use sufficient bait points and to place them at obvious places. So far we only know of one German digital tool for helping the pest controllers to plan a treatment, for inspiration the programme BayTool and is developed by Bayer (http://agrar.bayer.de/Beratung/Farm%20Protect.aspx) can be used.

8.4 Choosing the right anticoagulant as first choice

The following is a description on the choices and procedures when using anticoagulant for controlling rat. For references see the flow chart in **Figure 6**.

The FGARs, bromadiolone and difenacoum should together be considered possible first choice in all anticoagulant treatment, according to recommendations by Berny *et al.* (2014) and RRAC (2016). However, we recommend that FGARs should always be the first choice, when there is no known history from previous treatment at the site, as the FGARs is not considered PBT compounds as opposed to bromadiolone and difenacoum. Thus the environmental concerns using FGARs should be minimized.

Bromadiolone or difenacoum can be chosen as first choice if none of the FGARs are accepted by the rats or if the different formulations of FGARs should be inappropriate. For example, the quality of bromadiolone products for sewers, slatted floors systems etc. has for long been the most suitable.

Another option to choose bromadiolone or difenacoum as first choice could be when dealing with infestations, where it hasn't been possible to eliminate all or most of the alternative food sources. However, it should be stressed, that the choice of bromadiolone or difenacoum as first choice should only be taken if everything has been done to eliminate the alternative food sources. We know that when using FGARs there is a risk of under-baiting if either we do not provide enough bait, but also if the bait is not attractive enough compared to the alternatives. With the use of bromadiolone or difenacoum as first choice here, we will have a better chance of optimizing the control, under the assumption that resistance is not occurring.

Open Question 1 (see also **figure 6**): As discussed later (Anticoagulants and non-target species) we need further investigation of the risk evaluation for the environment in case of resistance. Here we should consider two alternative scenarios; 1) continue to use low toxicity anticoagulants (which is not resistance breaking) in a resistance situation, will lead to a larger amount of poison being used and getting into the ecosystem, either directly or through secondary poisoning, as more rats with consumed anticoagulants will survive a treatment if resistance is present. 2) Shifting to high toxicity compounds (resistance breaking) may mean a lower amount of poison in the environment, but with a higher toxicity level, which may increase the risk of primary poisoning of non-target species, whereas secondary poisoning (when other predatory species are eating rats containing an anticoagulant) could be reduced, as the number of live rats having consumed the highly potent anticoagulant will be reduced drastically.

8.4.1 Is resistance occurring?

If the treatment is not progressing as expected the whole process has to be evaluated. But what could be the signs of a treatment not going as planned? One good indicator is using the bait take and the number of bait points. Everything being equal, the number of bait point with activity should decrease as the control is progressing. If no decrease or only a small decrease is observed this should lead to concern. The same if there is a decrease in bait points with activity, but the remaining bait points have a continuously high uptake of bait. To evaluate a treatment, we recommend **Appendix B** as a mandatory part of every treatment. By using this checklist, the pest controller is reminded on the different required procedures in rat control and thereby make the needed correction in the application of anticoagulants.

Open question 2: whenever resistance is expected or even prior to a treatment in very large infestations of rats it would be beneficial for the control to obtain knowledge beforehand on resistance (as mentioned earlier). Should there be a demand on resistance testing prior to use of the very potent SGARs? Today laboratory tests are required to genetically verify cases of resistance, but if a field test for resistance was developed, it would be more beneficial for initiating the treatment, as the pest controller, prior to any anticoagulant treatment, could confirm cases of resistance.

8.4.2 Pulsed baiting for brodifacoum, flocoumafen and difethialone

Pulsed baiting is recommended for whenever brodifacoum, flocoumafen and difethialone are used. Compared to a saturated baiting scheme, where anticoagulants are replenished whenever the bait station is empty and typically using 100 to 200 grams of the anticoagulant per bait station, pulsed baiting has the potential of lowering the risk of primary and secondary poisoning. Pulsed baiting is based upon baiting in small quantities (between 20 to 50 grams) at each bait points. The bait points is controlled every 7 days and bait points with activity is replenished till 20 to 50 g. As shown later the amount of bait used to control rats using pulsed baiting leads to a much reduced (ten-folds) amount of bait compared to using bromadiolone and difenacoum (Buckle *et al.* 2012b).

Open question 3: We only have very little knowledge on the environmental risk using these potent SGAR and nothing on the environmental impact when using pulsed baiting as opposed to the saturated baiting using FGARs, bromadiolone and difenacoum. This question is related to open question 1.

8.4.3 Baiting without poison

In the flow chart (**Figure 6**), showing the outline of control with anticoagulants, we have included a mandatory step after the survey (the general observations made prior to setting up the treatment), but before the anticoagulant baiting. We do however recommend this step if the pest controller has no idea of the extent of the infestation. The pest controller should be putting out non-poisonous bait at various places as mentioned under “the number of bait points”. The benefits of this is that the non-poisonous bait can be placed without the use of bait boxes, so we know that the rats will be more likely to accept this bait and thus get a more precise measurement of where activity is and the size of the infestation (minimum number of rats). However, to get a more reliable measurement the baiting could be conducted for three consecutive days. If combined with the use of tracking points the pest controller 1) will be able to observe if consumption of the non-poisonous bait is due to other non-target species and 2) will be able to plan the correct control and placing bait points at the most optimal sites.

If the pre-bait consumption is less than a total of 200 g/24 hours the pest controller will have an opportunity for using traps instead of anticoagulant. If the pre-bait uptake is more than 200 g/24 hours anticoagulants are recommended for control.

8.4.4 How can tracking be used when controlling rats?

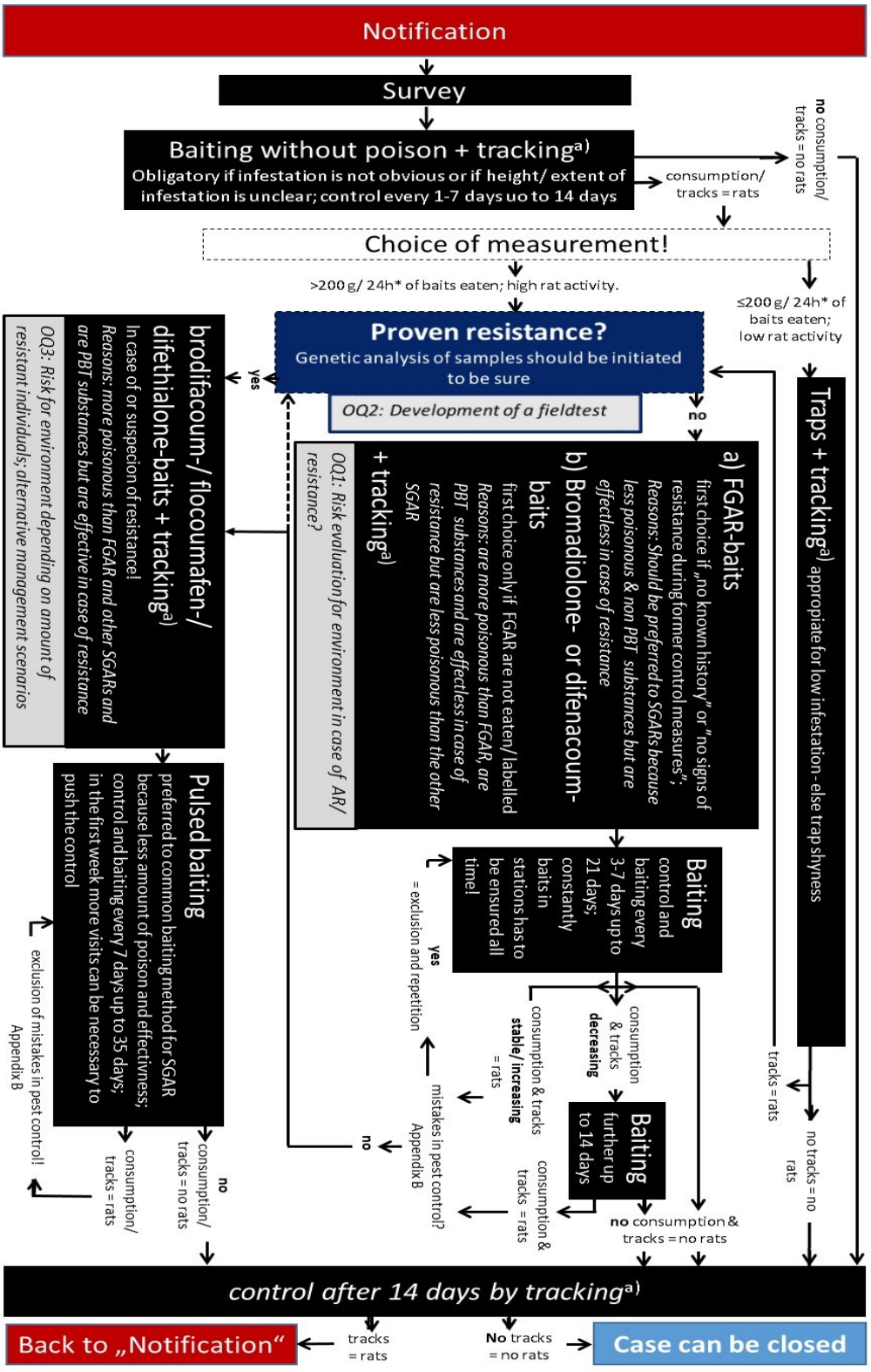
In the following flow chart (**Figure 6**) we have put up the scheme for anticoagulant control. In this we have included an optional action called “tracking”. By tracking we are referring to the use of e.g. cameras, electronic devices, powder or sand (preferably silver sand). By putting out e.g. sand tracks (20 x 20 cm) at various places as mentioned under “the number of bait points”. Ideally to get a good impression or measurement of the population size, three consecutive days are recommended in order to monitore the tracking points. If combined with the non-poisonous pre-baiting the pest controller will have good possibility to plan the correct control and placing bait points at the most optimal sites.

The tracking patches can be scored according to following scale:

- 0 = no signs of rodent tracks
- 1 = < 20% of patch covered with footprints
- 2 = 20–40%
- 3 = 41–60 %
- 4 = 61–80%
- 5 = >80%covered.

If average rat activity is low (0-1) the pest controller will have an opportunity for using traps instead of anticoagulant. But with rat activity of more than 1 anticoagulants should be used.

Figure 6: FGAR (coumatetralyl, chlorophacinon, warfarin), SGAR (bromadiolone, difenacoum, brodifacoum, flocoumafen, difethialone), OQ1-3 open questions1, 2 and 3, PBT potential persistent (P), bioaccumulative (B) und toxic (T); *approximate values; [a)] tracking can be done by cameras or traps (with/ without message systems), powder or sand sheets.



9. Use of bait boxes and the influence on rat control

The advantage of using of bait boxes is that the risk for exposing anticoagulants is limited for other non-target organisms. However, bait boxes do also exhibit a huge challenge especially for rat control because of the neophobic behaviour of the rats. Studies have shown that the bait uptake by rats are severely reduced when using bait boxes. The uptake decreases approximately 8 times compared when using natural coverage etc. when applying the bait (Buckle & Prescott 2010).

In normal situation where anticoagulants are used correctly and no resistance is occurring a full control can be expected within 35 days and often less days (Berny et al. 2014). This however has so far only been obtained in studies, where bait has also been used using natural hiding and coverage, whenever suitable (Buckle *et al.* 2012a+b, Endepols *et al.* 3003 Endepols *et al.* 2011). In a study by Buckle & Prescott (2011) it was shown from trials at three larger farm sites that rats are more prone to eat bait from open bait trays, where the trays were made safe from non-target animals using materials present at the sites like e.g. sheets of corrugated metal, wooden sheetings, slates, bricks, straw or hay bales, pipes etc. Three different kinds of bait boxes were also tested. The uptake of the non-poisonous baits in open trays was approx. 8 times higher compared individually to each of the bait boxes. A study by Quy *et al.* 2003 has demonstrated the potential of various bait boxes, which often are too small and being one of the reasons that rat do not have prolonged feeding time within the boxes. In the same study it was shown, that when baiting in these boxes, the rats were more likely to take/carry the bait with them instead of eating the bait at the bait point, as they did when using open, but covered bait-trays. Whether the use of bait boxes will have an effect on the duration of the control can only be presumed, but it is presumed to be of minor importance.

10. Anticoagulant rodenticides and non-target wildlife

Due to the huge environmental impact of anticoagulants and the risk of secondary poisoning the pest controller most always before carrying out rodent control considering the possibilities of using other alternatives – like use of traps. Traps are always recommended for use when rats occur within the residence itself. However, traps could be applied in order to minimize the environmental impact when dealing with minor rat infestation outdoor.

The Danish reports on the occurrence of anticoagulant residues in Danish birds of prey, owls and the smaller mammalian predators showed that it should be a matter of great concern, as between 84 to 100 % of the investigated non-target species had anticoagulant residues (Christensen et al. 2010).

But let's have a look at a scenario were the more potent anticoagulant are used more often due to suspicion of resistance. Will that lead to an increase threat to the environment? Or could it be that the use of the potent resistance-breaking anticoagulants, like brodifacoum instead of ineffective compounds like bromadiolone and difenacoum, would result in wildlife being exposed to lower absolute quantities of anticoagulant rodenticides at resistance foci?

Studies by Buckle et al. (2012a+b) and Endepols et al. (2011) showed the differences of the amounts of anticoagulants used to control Y139C resistant rats (**Table 7**). They clearly demonstrated the very large differences between using anticoagulants like bromadiolone and difenacoum on populations of rats, where Y139C have been identified compared to the use of a resistance-breaking compound, like brodifacoum. Brodifacoum is far more potent and thus only a very small amount is need for the rats to obtain a lethal dose. In the study, where brodifacoum was used, full control was obtained using a small amount of bait (1.45 and 4 kg, respectively on the two farm sites) (Buckle *et al.* 2012b). Whereas large quantities were used with bromadiolone and difenacoum and a considerably large proportion of the population was not eradicated on the different farm sites (Endepols *et al.* 2011, Buckle *et al.* 2012a). Thus the potential or risk for surviving rats containing anticoagulants after the treatment, when bromadiolone and difenacoum were used, are high and that may pose a threat to the non-target species like birds of prey, owls etc.

Table 7: the quantities of anticoagulant bait used and estimated control efficiency when bromadiolone, difenacoum and brodifacoum were used to control Y139C resistant rats on farms in Westphalia, Germany. (Buckle et al. 2012 a + b, Endepols et al. 2011).

Anticoagulant	site	Total amount of bait taken (kg)	Total no. of bait points	Estimated % efficiency
Bromadiolone	1	9,95	42	71,5
	2	43,4	43	0
	3	25,5	20	20
	4	38,4	43	69
Difenacoum	1	28,2	42	86,8
	2	8,1	37	59,9
Brodifacoum	1	4	89	99,2
	2	1,45	56	100

11. References

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12. Further reading:

CRRU – Campaign for Responsible Rodenticide use, UK (2015):
http://www.bpca.org.uk/assets/CRRU_COBP.PDF

CEFIC – European Biocidal Products Forum (2013):
http://www.rrac.info/content/uploads/CEFIC-EBPF-RWG-Guideline-Best-Practice-for-Rodenticide-Use-FINAL-S-.pdf

German Federal Environment Agency (2014):
https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/faq_anticoagulant_rodenticides.pdf

Bilag

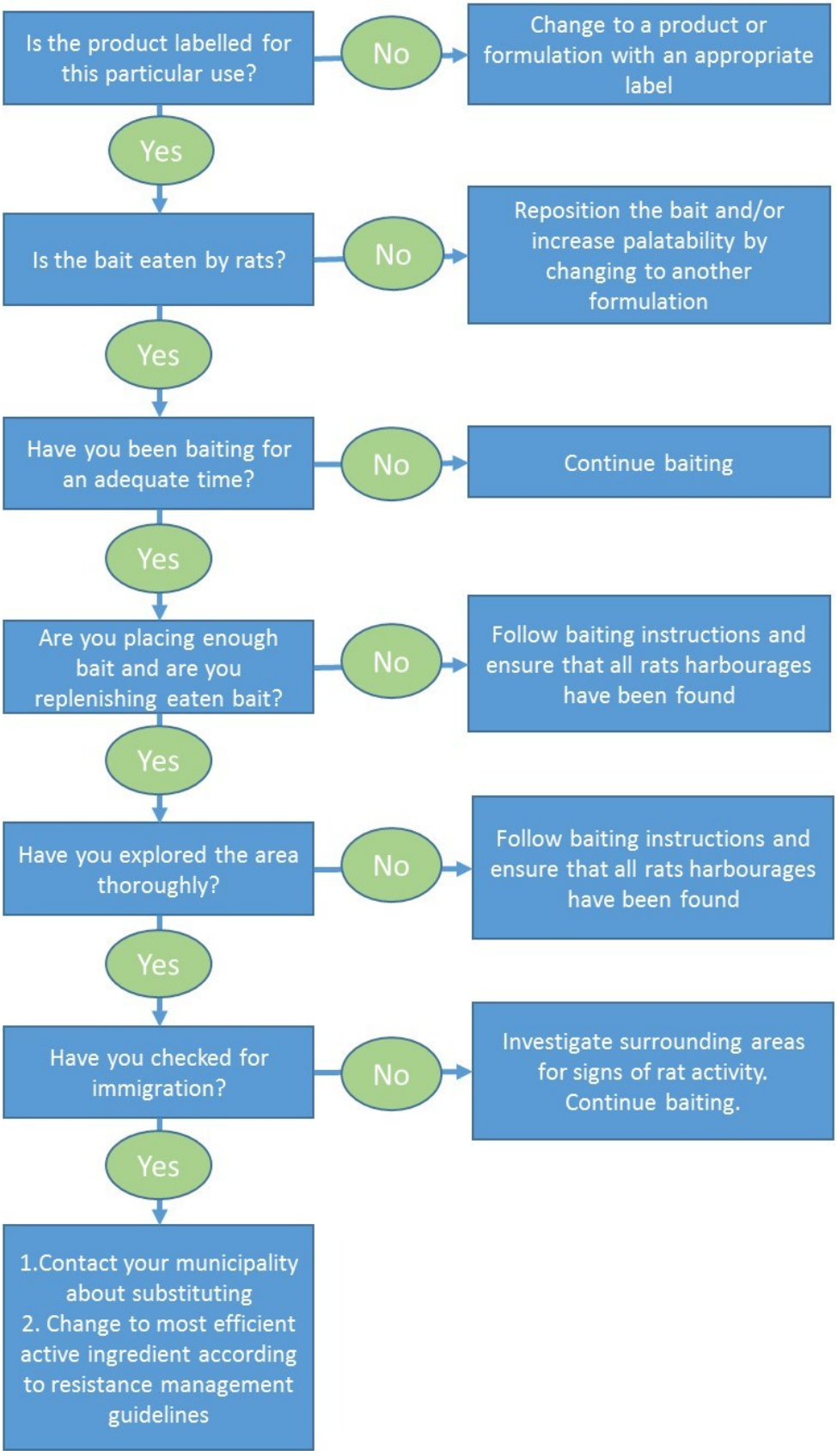
Appendix A

Since 2014 a total of 118 rats has been tested from sites in Denmark and tested for presence of Y139C mutations. A total of 57 sampling sites has been included. Samples originated from location where resistance was considered but also a majority of these trapping sites was chosen randomly and with no suspension of resistance. Of these 33 sites had a least one heterozygous and/or homozygous resistant rat.

sampling location	Genotype for position Y139C			Y139C resistance
	wildtype	heterozygous	Homozygous	
site 1	1	1		Yes
site 2	1			No
site 3			1	Yes
site 4				No
site 5			1	Yes
site 6	2	2		Yes
site 7	1			No
site 8		2		Yes
site 9				No
site 10	1			No
site 11	1		3	Yes
site 12		1		Yes
site 13	1			No
site 14	1			No
site 15		4		Yes
site 16		2	6	Yes
site 17	1			No
site 18	1			No
site 19	1			No
site 20	1	1		Yes
site 21				No
site 22			1	Yes
site 23		1		Yes
site 24				No
site 25	1	2		Yes
site 26	1	1		Yes
site 27	1			No
site 28			3	Yes
site 29				No
site 30	1			No

sampling location	Genotype for position Y139C			Y139C resistance
	wildtype	heterozygous	Homozygous	
site 31		1		Yes
site 32	1			No
site 33				No
site 34			1	Yes
site 35		3		Yes
site 36	1	1		Yes
site 37	1			No
site 38				No
site 39	1	3	2	Yes
site 40	1			No
site 41		2		Yes
site 42		1		Yes
site 43			10	Yes
site 44				No
site 45	3			No
site 46		1	2	Yes
site 47	3			No
site 48	1	2		Yes
site 49		1		Yes
site 50		1	1	Yes
site 51			2	Yes
site 52	1	1		Yes
site 53			1	Yes
site 54	3			No
site 55			1	Yes
site 56		4		Yes
site 57		2		Yes

Appendix B – checklist for rodent control and case of resistance



Evaluation of the Danish resistance strategy
Rapporten anbefaler, at der ved bekæmpelse med antikoagulanter følger en ny resistensstrategi. Ved bekæmpelse af rotter, hvor der benyttes antikoagulanter, skal der som udgangspunkt benyttes en af de svageste antikoagulanter som første valg. Kan der dokumenteres resistens, så skal der øjeblikkeligt skiftes over til en af de stærkeste typer af rottegift.
Samtidig kommer rapporten med anbefalinger til retningslinjer for en mere effektiv rottebekæmpelse herunder bedre anvendelse af antikoagulanter.

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