CONSIDERATION OF THE ENVIRONMENTAL RISK FROM THE USE OF BRODIFACOUM, FLOCOUMAFEN, DIFETHIALONE, DIFENACOUM AND BROMADIOLONE

HSE

Contents

Background

Brodifacoum, flocoumafen, difethialone, difenacoum and bromadiolone are all second-generation anticoagulant rodenticides (SGAR). They have all been assessed by Member States (MS) and are on Annex I of the Biocides Product Directive (BPD).

As a result of the EU review concern was raised regarding the risk to non-target organisms and these are presented in Appendix 1. Of the points noted in Appendix 1, the one key to this paper and the accompanying paper (see HSE, 2012b[1]) on risk mitigation measures is:

> *Primary as well as secondary exposure of humans, non-target animals and the environment are minimised, by considering and applying all appropriate and available risk mitigation measures. These include, amongst others, the restriction to professional use only, setting an upper limit to the package size and laying down obligations to use tamper resistant and secured bait boxes.*

A range of risk mitigation measures are presented in an EU document (Anon 2007[2]) and discussed in HSE (2012b). The EU document states that 'the choice of specific risk mitigations measures should therefore be deferred to product authorisation stage'. It goes on to say that where the 'use of an anticoagulant presents such a risk of primary and secondary poisoning[3] ... the area of use must be confined as much as possible, the authorised use could be limited to use in and around buildings[4] or to indoor use only'.

Outlined below is a consideration of the EU review for each active substance as well as additional data considered by the UK.

(It should be noted that this paper only covers the risk from the individual active substances, it does not cover any potential increase in risk that may be caused due to resistance. This is due to this issue not being considered at the EU level.)

Conclusion of the EU review

[1] Environmental Risk Mitigation Measures for Second Generation Anticoagulant Rodenticides Proposed by the UK (HSE, 2012b).

[2] Anon (2007) Risk mitigation measures for anticoagulants used as rodenticides. ENV B.3/PC d(2007) – 21/03/2007. Available at http://ec.europa.eu/environment/biocides/pdf/anticoagulants.pdf

[3] Primary poisoning in this instance refers to the consumption of the bait itself, whilst secondary poisoning refers to the consumption of treated rats, mice and other organisms by predatory and/or scavenging birds and mammals.

[4] 'In and around buildings' shall be understood as the building itself, and the area around the building that needs to be treated in order to deal with the infestation of the building. This would cover uses in sewer system or ships but not in waste dumps or open areas such as farmlands, parks or golf courses.

Please note that the EU assessment was conducted according to the Emission Scenario Document (ESD)[5] for biocides used for rodenticides or product type 14. There are four main scenarios that are considered by the ESD and these equate to how or where the rodenticide may be used. These scenarios are: exposure scenarios for a sewer system, exposure scenarios in and around buildings, exposure scenarios for open areas, exposure scenario for waste dumps. Risks to the aquatic as well as terrestrial environment are assessed.

Below are the outcomes of the primary and secondary risk to birds and mammals only. The PEC/PNEC[6] presented are these for the various uses assessed, i.e. use in sewers, in and around buildings, open areas and waste dumps. A range is quoted, as the risk assessments considered a number of different exposure situations.

Full details of the EU review for each of the five active substances can be found via the links provided below:

Brodifacoum:

http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/2010_brodifacoum/_EN_1.0_&a=d

Primary poisoning PEC/PNEC ratios for birds ranged from 125000 to 1582031, whilst for mammals they ranged from 181818 to 1269696. Secondary poisoning PEC/PNEC ratios for birds ranged from 18375 to 217188, whilst for mammals they ranged from 15000 to 855855.

The following additional data were presented:

> *A study aimed at estimating the LC50 in captive kestrels upon ingestion of brodifacoum contaminated vole did not meet the goal[7]. The conclusion was that, under field conditions, the degree of exposure to non-target animals would depend on dose and treatment levels, methods of use, local ecological situations and the behaviour of the target and non-target species. Other studies on crows and barn owls did not provide exhaustive conclusions. In the laboratory, dogs and foxes mostly survived periods of 1, 3 or 5 days feeding on brodifacoum contaminated rats only. At worst case, one fox died after eating 5 rats which provided a dose of 4.83 mg a.s./Kg and one dog died upon reaching a dose of 1.85 mg a.s./Kg. Surviving dogs showed severe injuries.*
>
> *The potential for secondary poisoning of brodifacoum was assessed in two laboratory trials where owls were fed contaminated mice....In one study, the*

[5] Supplement to the methodology for risk evaluation of biocides Emission scenario document for biocides used as rodenticides. CA-Jun03-Doc.8.2-PT14.

[6] PEC/PNEC = predicted environmental concentration/predicted no effect concentration. The trigger value is 1, i.e. if the ratio is greater than 1, then there is a perceived risk and no authorisation can be permitted without further consideration of either higher tier data or risk mitigation measures.

[7] The study did not derive an LC50.

consumption of three brodifacoum killed mice (possibly fewer) in a single day caused the death of 4 out of 6 birds. The owl livers contained 0.63-1.25 mg/Kg fresh weight of brodifacoum. In the second study, owls were fed for 15 days poisoned mice containing different concentrations of rodenticide. Liver retained the highest concentration of rodenticide residues. The concentration appears largely independent of dose, providing supporting evidence that the owl liver contains saturable binding sites. All owls that died contained liver residues in excess of Brodifacoum 1.7 mg/kg. One monitoring study was conducted in Britain to investigate the contamination of barn owls with rodenticides. Brodifacoum was found in 4% of dead birds and its concentration in liver was 0.002-0.515 µg/g. No evidence of contribution to the overall mortality of owls was concluded. It can be argued that the mode of action of anticoagulants (death is slow and preceded by lethargy) makes the carcasses of poisoned owls difficult to find.

Bromadiolone:

http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/assessment_16122011pdf/_EN_1.0_&a=d

Primary poisoning PEC/PNEC ratios for birds ranged from 2100 to 22909, whilst for mammals they ranged from 4074 to 26300. Secondary poisoning PEC/PNEC ratios for birds ranged from 705 to 4250, whilst for mammals they ranged from 3242 to 590000.

The following additional data were presented:

*Three studies have been presented ... that were conducted to simulate the secondary poisoning of non-target predatory birds and mammals that may potentially occur following intake of poisoned target rodents containing bromadiolone residues. In the first, rats were first fed with bromadiolone bait pellets for three days, followed by uncontaminated feed for a fourth day, before being euthanised and fed to five great-horned owls (*Bubo virginianus*) at the rate of one carcass per bird per day for seven days. Four of the owls died during the course of the subsequent 30-day observation phase, with inactivity noted in the period immediately prior to death and with widespread and massive haemorrhaging identified at the cause of death post mortem. The sole survivor generally avoided the livers and only partially consumed the intestines of the poisoned rats during the exposure period, but evidence of earlier internal haemorrhaging was also found in this bird following termination at the end of the study. The bromadiolone intake of the owls that died was estimated to between 0.034 and 0.076 mg/kg bw/d with a mean value of 0.056 mg/kg bw/d. This value has been used to assign a $PNEC_{oral}$ for secondary poisoning. An assessment factor of 3000[8] shall be used if the available data is a short term effect value*

[8] This is in line with the Technical Guidance Document on Risk Assessment (part 2). Used in support of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Commission Directive 93/67/EEC on Risk Assessment for new notified substance. EC JRC *20418 EN/2*

(LC_{50}). The suggested assessment factor takes into account interspecies variation, lab to field extrapolation and acute to chronic extrapolation. However, it may be argued that since the tested species is an owl, the interspecies factor can be omitted and the assessment factor can thus be lowered to 300[7]. Further reduction of the assessment factor is not considered possible, due to the uncertainty arising from the fact that the available effect data is LC_{100} and not LC_{50}. The remaining two studies were done on barn owls and stone martens and are described in published scientific literature. In conclusion, the intake of poisoned rats may cause severe effects including death to predatory birds. The effect on wild mammals seems to be less severe, but the submitted study comprised a limited number of animals and the concentration of bromadiolone in the mice fed to the martens was not known. There are several reports on bromadiolone content in, and bromadiolone related effects on non-target species and predators. Studies indicate that bromadiolone is distributed among many species in the environment.

Difenacoum:

http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/final-ar-difenacoumsep09/_EN_1.0_&a=d

PEC/PNEC ratios are not quoted in the above document. On the basis of information in the Competent Authority Report PEC/PNEC ratios are similar to bromadiolone. However the following text is presented:

According to the risk calculations the proposed normal use of difenacoum causes unacceptable risk for primary and secondary poisoning of non-target vertebrates. However, the risk for primary poisoning is assumed to be negligible in the Emission Scenario Document if the rodenticidal baits are used according to the label instructions. In the aquatic food chain (fish-eating birds and mammals) risk for secondary poisoning is considered insignificant. In the terrestrial food chain secondary poisoning is possible via contaminated soil invertebrates and rodents, and the latter animals are the most likely source for difenacoum residues in raptorial birds (i.e. bird of prey) *and mammalian predators. Not only the risk characterisation shows risk for secondary poisoning, but also the published laboratory studies confirm bioaccumulation of difenacoum in the owls. Bioaccumulation of difenacoum in predators has been shown in the measurements of difenacoum residues in the animal carcasses found from the field in the United Kingdom. The target organ for difenacoum is the liver and difenacoum residues reported are generally liver values. In one laboratory study highest residues were measured in the liver, and residues in other tissues including the fat tissue were low. Owls exposed to difenacoum showed variable effects from no foreseeable effects to death. Other observed effects were increased coagulation times and haemorrhages. The effects disappeared gradually after the end of exposure. Population level effects of difenacoum have not been studied.*

In the laboratory studies, the owls fed entirely or mostly on poisoned rodents which is probably more extreme than field conditions. The carcasses found in the

> *field were diagnosed to have died from other causes other than difenacoum but contained difenacoum residues that were assumed to be sub-lethal. It is, however, possible that sublethal difenacoum residues have contributed to the death of predators. Reproductive effects of difenacoum in avian or mammalian predators or scavengers have not been studied in the laboratory or in field experiments. Dose-related effects on the reproduction were observed in Japanese quail in the reproduction study. The NOEC of 0.31 mg/l drinking water and NOEL of 58 µg/kg bw were determined in this study. In another reproduction study no dose-related reproductive effects were observed in Japanese quail resulting in the NOEC of > 0.1 mg/kg diet and NOEL of > 0.01 mg/kg bw/d. Higher concentrations were not tested. The residues in the liver were not measured in either test, and hence comparison to the monitoring data is difficult. The residue levels measured from dead barn owls ranged from 0.05-0.2 mg/kg in liver.*

Difethialone:

http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/difethialone_210607pdf/_EN_1.0_&a=d

Primary poisoning PEC/PNEC ratio for birds ranged from 76000 to 383000, whilst for mammals they ranged from 5700 to 126000. Secondary poisoning PEC/PNEC ratios for birds ranged from 10500 to 33000, whilst for mammals they ranged from 7900 to 68000.

The following additional data were presented:

> *A dietary secondary poisoning study where barn owls were fed with poisoned rats is described in a recent article. The study had some deficiencies; however, it gives valuable insight into the availability of prey ingested difethialone for predators. The study gave a low LD100 in the range of 0.27 to 0.39 mg/kg bw. This indicates that excretion/metabolism during the 56 day period is low in birds and that ingested difethialone in rats is readily available to the owls.*

Flocoumafen:

http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/assessment_cleanpdf/_EN_1.0_&a=d

Primary poisoning PEC/PNEC ratio for birds ranged from 24000 to 98480, whilst for mammals they ranged from 89000 to 297000. Secondary poisoning PEC/PNEC ratios for birds ranged from <3300 to <10440, whilst for mammals they ranged from 12500 to 97000.

The following additional data were presented:

> *The notifier claims that, when the product is applied according to submitted directions for use, i.e., in tamper-resistant bait stations, rat burrow entrances or under equivalent cover, access of non-target organisms to the bait is sufficiently excluded, and therefore estimated daily uptake rates should be negligible for*

non-target species. They refer to field trials, where flocoumafen bait was placed according to the submitted directions for use, or at a higher rate, and conclude that no evidence of primary poisoning hazards to non-target organisms was found. This suggests that when the submitted directions for use are followed, primary poisoning hazards are minimised. From the field tests it can be derived that birds are able to enter bait boxes and that non-target rodents, such as house mouse, wood mouse and vole fed extensively on the bait and the analysed specimens contained flocoumafen residues.

Secondary poisoning

A secondary hazard was identified in field trials in UK at 10 farms which employed an exaggerated baiting scheme (saturation baiting): flocoumafen residues were detected in one barn owl, one cat and one stoat found dead. Also slight primary hazards was found to birds as there were 4 observations of birds entering bait boxes and one observation of a bird pecking at the bait. However, no blue-dyed bird faeces were found. A clear primary hazard was identified in non-target rodents (house mouse, wood mouse and vole) with 60 carcasses containing flocoumafen residues. Trials at 6 other farms in UK using the proposed minimal baiting scheme (3 pulses of 2 blocks per baiting point) however produced no evidence of a secondary hazard. A primary hazard was found for non-target rodents (house mouse, wood mouse and vole) with 12 carcasses. No primary hazard to non-rodents and birds was not identified at any farm.

In the study using saturation baiting, average flocoumafen residues in rat carcasses (0.6 mg/kg bw) were found comparable with the normal case scenario (fraction treated bait in rodent's diet = 20%). For non-target rodents average flocoumafen residues were even higher, comparable with the intermediate case (fraction treated bait in rodent's diet = 50%). In the study using restricted baiting average flocoumafen residues in rat and mouse carcasses (1.1-3.5 mg/kg bw) were ca. a factor 2 higher than the normal case concentrations. It should be noticed that all the flocoumafen residues in both live and dead rodents, exceed the PNECs (>0.0021 and 0.00056 mg/kg diet) for birds and mammals, respectively. It should be noted that flocoumafen may not have appeared significantly in the data because the use of products containing this active substance is not significant compared to other actives.

Therefore, any conclusion made on these data may not be sufficiently robust. The RMS considers that the available field studies can be used as supporting evidence, recognizing that the information on effects to non-target animals is limited.

Additional information

It should be noted that when the UK considered the outdoor use of flocoumafen under the UK Control of Pesticide Regulation (1986), an assessment was carried out (our reference SC9328, 9500 and 9649). In carrying out this assessment all the, then available, data on rodenticides used in the UK were drawn together. This assessment

did not use the Emission Scenario Document but used information on the toxicity of the compounds to birds along with real residues in mice and rats. These residue data were the result of both saturation and pulsed baiting. Data from effects field trials as well as feeding studies were also considered. The results of this comparison were that:

- Brodifacoum was the most toxic rodenticide then in use in the UK and difenacoum the least toxic with flocoumafen intermediate between brodifacoum and bromadiolone.

- Data on single and multiple-dose toxicities indicated that toxicities tended to be slightly lower for multiple-dose than single dose.

- There did not appear to be any order of magnitude differences amongst the active substances in the levels of residues in bodies of exposed animals. However, marginally higher residues were found in animals from brodifacoum trials.

As regards the position of flocoumafen it was considered to be more toxic than either difenacoum or bromadiolone and as persistent as brodifacoum. The Environmental Panel of the Advisory Committee on Pesticides (ACP) considered that the primary risk could be managed appropriately. However, despite the use of various baiting techniques (i.e. pulsed baiting versus saturation baiting), which aimed to reduce the residues in treated rodents, the secondary risk could not be satisfactorily reduced. It was also concluded that:

> *'an effect on predatory animals from the proposed use of flocoumafen has been observed. There is currently no risk management practice available for reducing this potential effect as it is caused by the inherent toxicity and persistent characteristics of the chemical involved. Hence it is considered that flocoumafen poses an unacceptably high risk to non-target animals, therefore it is recommended that approval for the outdoor use is not granted.'*
>
> (SC 9328)

(As a result of the above the use of flocoumafen was restricted to indoor use only[9]. Brodifacoum was also subject to the same restriction.)

Provided below is a brief summary of the field studies used by the Environmental Panel of the Advisory Committee on Pesticides in reaching their decision to recommend restricting the use of brodifacoum and flocoumafen to indoor use only. The studies concern rodenticide use on farms.

[9] Indoors is defined in this context by the registration authorities as:- i) situations where the bait is placed within a building or other enclosed structure and where the target is living or feeding predominantly within that building or structure; and ii) behind closed doors.
If rodents living outside a building can move freely to where the bait is laid within the building, then products containing brodifacoum/flocoumafen should NOT be used. Open barns or buildings and tamper-resistant bait stations placed in open areas are not classified as indoors. However, sewers or closed drains are considered to be 'indoors situations'.

Compound[10]	
Brodifacoum	In 11 trials a total of 41birds mainly small birds were found dead during these trials along with three rabbits and one harvest mouse. Exposure was not confirmed by residue analysis. Most bodies were associated with baiting hedgerows rather than around farm buildings. Treatment was carried out during Jan/Feb. An additional 5 treatments were carried out and one further casualty was found. No details of bait base or whether bait boxes or similar were used was included in the summary.
	A subsequent 9 saturation baiting treatments with brodifacoum around farm buildings led to 32 non-target carcasses being found – 3 cats, 1 fox, 1 rabbit, 2 crow species and 25 passerines. Two other saturation baiting trials involving field use in hedgerows resulted in 25 non-target casualties (1 squirrel, 2 buzzards, 2 tawny owls, 17 crow species and 3 small birds). No details of bait base or whether bait boxes or similar were used was included in the summary.
	Pulsed baiting was carried out on 16 sites with pellet baits. 60 non-target bodies were found – 1 grey squirrel, 4 rabbits, 2 magpies, 2 chickens, 2 pheasants, 49 passerines). Counts of sedentary bird species (e.g. robin, dunnock and chaffinch) showed a decline in their numbers. 14 tawny owl territories were present at the start of the study and this declined to 12 at the end of the study.
	12 non-target deaths were recorded during 10 treatments using pulsed baiting with brodifacoum wax block. These were – 1 cat, 1 stoat, 1 grey squirrel, 2 rabbits, 5 crow species and 2 small birds. Four of the seven casualties involving secondary poisoning were stated to have been the result of a single trial carried out in hedgerows and woodland more than 100 m away from farm buildings.
Flocoumafen	Three trials using wax block saturation baiting resulted in 7 birds, 5 woodmice and 4 vole carcasses being found. Significant flocoumafen residues were obtained from the mice and voles.
	Seven trials using wax block saturation baiting resulted in 18 non-target rodent carcasses – 14 wood mice and 4 voles; 15 non-rodent carcasses – hedgehogs, rabbits, 1 mole, 1 stoat and 1 cat; 67 bird carcasses – 14 blackbirds, 14 house sparrows, 8 woodpigeons, 7 starlings, 5 feral pigeons, 1 barn owl and 1 little owl. Flocoumafen residues were found in 2 of the 25 analysed bird carcasses and in the wood mouse, vole, cat and stoat carcasses. Five mammal carcasses were found on the two control sites – 1 wood mouse, 1 cat, 1 stoat and 2 rabbits; 15 bird carcasses were also found.
	A pulsed baiting regime was used for 6 trials using flocoumafen wax

[10] The data on brodifacoum has been obtained from SC9500 – a review of the toxicity of second-generation anticoagulants. This document was produced when Pesticides Safety Directorate, the Environmental Panel and the ACP were considering the outdoor use of flocoumafen. The aim of the document was to compare the toxicities and potential risks from a range of anticoagulants.

Compound[10]	
	blocks on farms. 12 mammals- 8 mice, 3 voles and 1 cat and one bird (a starling) were found. Flocoumafen residues were found in all the mammal bodies. Another 6 trials of flocoumafen blocks using a pulsed baiting regime were carried out in the same area of Wales. Flocoumafen residues were found in 12 wood mouse carcasses and also in live-caught animals – 5 wood mice, 2 voles and 5 shrews.

In addition to the above field trial data, the UK review of the toxicity of second generation anticoagulants includes reference to the potential risk of secondary poisoning to predatory birds. It is stated that for brodifacoum 'mean levels of residues in the bodies of rats from pulsed baiting with brodifacoum (1.4 mg/kg) are higher than those in mice required to generate substantial mortalities in barn owls (Newton *et al* 1990)[11].' The UK review states that 'the mean levels of flocoumafen residues found in bodies of rats during pulsed baiting (0.79 mg/kg) appear to be sufficient to generate some mortality amongst barn owls (i.e. they are greater than the 0.65 mg/kg in mice used by Newton *et al* 1994[12])'.

In the Environmental Panel assessment of the outdoor use of flocoumafen, three further studies were submitted on the potential secondary poisoning risk. One used the buzzard and indicated that the 'secondary poisoning acute oral dose for the buzzard was 0.76 mg/kg consumed over 5 days.' In a further study, barn owls were fed treated mice containing mean residues 0.65 mg/kg (i.e. in line with mean residues found in the field). One of the five birds died following consumption of 0.93 mg/kg flocoumafen over a six day period; the other owls survived. In the final study the toxicity of brodifacoum, difenacoum and flocoumafen to barn owls was determined. Groups of four barn owls were fed treated mice for a total of 15 consecutive days. Two owls fed flocoumafen died after consuming doses of 2.2 and 2.8 mg/kg, equivalent to 0.15 and 0.19 mg/kg bw/day. This is equivalent to 56 and 85 µg/day over the 15 day period. One owl fed brodifacoum died after consuming a cumulative dose of 5.4 mg/kg over 14 days. This was stated to be equivalent to 133 µg/day or 0.39 mg/kg/day. (In interpreting these data it should be noted that death may have resulted after fewer than 15 days dosing, i.e. mouse consumed on day 1, 2 or 3 may have resulted in death.)

(Please note that predatory bird feeding studies are considered further below.)

The ACP considered the above field trial data along with a range of laboratory data and concluded that due to the potential impact on non-target organisms that the risk from outdoor use of either brodifacoum or flocoumafen was higher and recommended that use should be restricted to indoor use only.

[11] Newton I., Wylie I and Freestone P. (1990) Rodenticides in British barn owls Environmental Pollution 68: 101-117

[12] Newton I., Wylie I., Gray A., and Eadsforth C.V. (1994) The toxicity of the rodenticide flocoumafen to barn owls and its elimination via pellets. Pesticide Science 41: 187-193.

The field studies considered by the ACP in their deliberations in the 1980s and 1990s are not up to modern standards in terms of both field study design or, and perhaps more importantly, pest control practice. The studies were not conducted using *current* best pest control practice techniques and therefore represent a worst case situation in terms of both primary and secondary exposure to targets and non-targets. The studies are also not representative of best practice regarding determining effects on non-targets. However, they do provide limited information on the *possible* impacts of brodifacoum and flocoumafen when used in the same manner as in the field trials.

It should be noted that if the studies were not available the risk assessment would rest on first tier data used in the EU review where PNEC/PEC ratios were much greater than 1.

No regulatory data are available for non-target casualties following use in urban or non-farm areas, which may include a different range of non-target species present.

No regulatory field trial data are available for difenacoum, bromadiolone or difethialone.

Risk from combined exposure

Data from an, as yet, unpublished/un-peer reviewed thesis indicates that when Japanese Quail were dosed twice with a 25 day interval with sub-lethal doses of either difenacoum, brodifacoum or one compound followed by the other, the impact of brodifacoum on clotting times was greater than that of difenacoum. This was somewhat expected based on the known acute toxicity of the compound, however a repeated dose of brodifacoum 25 days later markedly increased the duration and severity of anticoagulant activity compared to a single dose. This was not evident with the repeat doses of difenacoum, i.e. repeat doses of difenacoum 25 days apart lead to a slight increase in anticoagulation time whereas repeat exposure of brodifacoum lead to anticoagulation time over a much longer period and to a much greater extent compared to a single, novel exposure. The degree of anticoagulation would be affected by the time between doses, for example if exposure were closer together, then the effect would be expected to be greater. However, concern was raised regarding the fact that these data are not yet peer reviewed and cannot be cited.

Monitoring of the effectiveness of risk mitigation measures

Shore *et al*[13] considered the importance of appropriate risk mitigation measures. Scavengers, particularly rats, are considered possible vectors of Foot and Mouth

[13] Shore R F, Malcolm H M, McLennan D, Turk A, Walker L A, Wienburg C L and Burn A J (2006) Did Foot-and-Mouth Disease-Control Operations Affect Rodenticide Exposure in Raptors? Journal of Wildlife Management 70(2):588-593.

Disease (FMD).

During the outbreak of FMD in 2001 rodent control was carried out on all premises affected by FMD. Shore *et al* (2006) investigated whether increased rodenticide use during the FMD outbreak in 2001, and the subsequent rodent control, led to an increase in residues in the barn owl and the buzzard. It was estimated that during the FMD outbreak typically no more than 20 kg bait was used on each premises, although more than 100 kg, and occasionally more than 300 kg, was required on a small proportion (<10%) of farms with very high rat populations. These figures compare to a typical usage of 14 kg per year, i.e. 40% increase.

Difenacoum and bromadiolone were most frequently detected in barn owls. Shore *et al* found difenacoum in a significantly greater proportion of owls from non-FMD than from FMD-affected counties, i.e. 45% of carcases from non-FMD counties contained difenacoum, whilst 10% from FMD counties contained difenacoum. Between 1998 and 2000, 42% of the owls found dead in counties subsequently affected by FMD contained difenacoum, a significantly higher proportion than during the FMD epidemic. In contrast, the proportion of barn owls found between 1998 and 2000 in non-FMD counties that had difenacoum residues was 26% which was lower than the equivalent proportion in 2001 (45%). As with barn owls, liver residues were detected in a greater proportion of buzzards from non-FMD areas than from FMD-counties (38% vs. 26%), but this difference was not statistically significant, unlike that for barn owls.

Shore *et al* considered the results and proposed whilst their findings do not prove a direct association between FMD outbreaks and reduced difenacoum exposure, they did propose that if there was any such link, then this may have been largely a result of:

- the careful management of FMD pest control operations, and
- the way normal outdoor use of rodenticides was used in FMD impacted regions. Shore *et al* commented that rodenticide use away from buildings is likely to be a main route of contamination for non-target small mammals and for most predators. Although very large amounts of bait were used the treatment only lasted a short time. There was also a diligent effort to search for and remove the corpses of poisoned rats.
- the heavy disturbance of clean-up operations may also have deterred predators and scavengers from foraging around infected premises.
- baiting was carried out mainly around and in buildings, slaughter areas, and nearby areas of good rat habitat (such as silage clamps, slurry pits, and straw stacks), and it was concentrated around rat burrows and centres of rat activity.
- there was a concomitant reduction in more widespread outdoor baiting. Hedgerows and ditches were not usually baited.
- there was no semi-permanent or permanent baiting away from pest control areas, and other routine pest-control activities were also stopped
- game rearing and shooting was disrupted in FMD-affected regions and may have reduced the use of rodenticides by gamekeepers, many of whom bait game-bird rearing areas.

- as a result of the above, the amount and duration of rodenticide baiting away from buildings may have been substantially reduced in FMD-affected regions, compared to non-FMD regions or standard practice.

Comparison of the risk posed by the five second generation anticoagulant rodenticides to birds and mammals

Outlined below is a consideration of the primary and secondary risk to birds and mammals.

Risk from primary poisoning

Based on the outcome of the EU review, the PEC/PNEC ratios for primary poisoning for birds and mammals are all greater than 1 and hence indicate a high risk. Difenacoum and bromadiolone pose a slightly lower risk (i.e. the PEC/PNEC ratios are slightly smaller than those for difethialone, brodifacoum and flocoumafen).

The formulation type may affect the risk of primary poisoning e.g. some bait formulations (paste baits, wax block baits) might be less attractive to birds than to mammals (including target rodents), whereas grain baits and pellet baits might be equally attractive to birds and mammals. However, it is still likely that resulting PEC/PNEC will be greater than 1. It should also be noted that the bait needs to be attractive to target organisms in order to be efficacious. The risk can, however, be mitigated by ensuring that the product is used in bait boxes or situations where non-target vertebrates cannot get access to the bait or where access is limited. The practicalities and effectiveness of excluding small mammals from baiting points is unknown.

The above approach is in line with Luttik *et al* (1999)[14] where it is stated that rather than trying to refine the risk assessment 'it may be more realistic to develop and validate risk management options such as the use of trained operators, bait boxes and correct baiting techniques'.

Risk from secondary poisoning

Birds

Toxicity

From the EU review it is clear that for all second generation anticoagulant rodenticides all secondary poisoning PEC/PNEC are greater than 1.

For **brodifacoum** and **flocoumafen** additional data, in the form of predatory feeding studies and field studies[15] to some extent confirm the theoretical risk predicted by the

[14] Luttik R., Clook M.A., Taylor M.R., Hart A.D.M., (1999) The regulatory aspects of the ecotoxicological risk assessment of rodenticides. In Advances in Vertebrate Pest Management Pest Management, p 369-385, ed Cowan P.D. and Feare C.J. Filander Verlag, Further Germany.
[15] Field studies were a key part of the original COPR assessment and very limited use was made of field trials in the EU assessments.

Emission Scenario Document. However, it should be noted that as outlined above there is concern regarding the appropriateness of the field trial data. Data are available from predator feeding studies for both brodifacoum and flocoumafen. Four studies using brodifacoum and barn owls were considered by Luttik *et al*. These studies indicate that there were 5/6, 3/4, 1/4 and 4/6 mortalities in exposed barn owls (see below for further details). As regards flocoumafen three studies were considered by Luttik *et al* and these indicated that there were 3/4, 2/4 and 1/5 mortalities in exposed barn owls (see below for further details).

When the use of **difethialone** was considered a comparison with brodifacoum and flocoumafen was carried out (see http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/acp/acp-minutes/minutes-of-acp-346-held-on-16-november-2010.htm). This assessment indicated that the PEC/PNEC are greater than 1 and whilst less than those for brodifacoum were similar to flocoumafen. Additional data were limited to an owl feeding study (see above for details) and this indicated a potential concern. (As a result of the similarity to brodifacoum and flocoumafen, difethialone was also restricted to indoor use only.)

Predatory feeding studies on **difenacoum** indicate that it is possible for barn owls to consume treated rodents and survive (see Mendenhall and Pank (1980)[16] and Newton *et al* (1990)) (see table below for details). Sub-lethal effects (e.g. increase coagulation time) were observed. Mortality, however appears to be dependant upon the feeding regime used as Gray *et al* (1994)[17] (see below) indicated that mortality could occur under their feeding regime with 1/4 owls dying.

For **bromadiolone** a predator feeding study was conducted on great horned owl and resulted in 4/5 owls dying (see EU review). In addition, one study on barn owls resulted in the death of 1/6 owls (Mendenhall and Pank (1980) (see below)) with no symptoms of toxicity observed in the five remaining owls. Additional studies on bromadiolone by Lee (1993) and Newton *et al* (1990 and 1994) resulted in 3/4 and 0/6 dead owls respectively (see table below).

Also included in the tables below are results from other predator feeding studies, including one that used **warfarin**[18].
Some of the above studies were considered as part of the EU review (see above).

As regarding interpreting these studies, Luttik *et al* states that 'little can be deduced from these feeding studies about the relative toxicity of the compounds to barn owls, even when they are included in the same experiment'. They go on to state that the difficulties

[16] Mendenhall V.M., and Pank L.F. (1980) Secondary poisoning of owls by anticoagulants. Wild Soc. Bull 8: 311-315.
[17] Gray A., Eadsforth C.V., Dutton A.J., Vaughan J.A. (1994) toxicity of three second-generation rodenticides to barn owls Pesticide Science 42: 179-184.
[18] Lee C.H. (1993) Secondary toxicity of some rodenticides to barn owls. 4th MAPPS International Conference in the Tropics, Kuala Lumpar.

are due to the lack of consistency in the study designs and the low number of birds tested.

Summary tables from Luttik *et al* are presented below:

Summary of selected predator feeding studies, comparing accumulated doses to lowest available avian LD50 (both in mg/kg bw). The top row shows the period of primary exposure (rat or mouse) and secondary exposure (barn owl) in each study. In the studies by Newton *et al*., data are shown for the third period of exposure (6d) except for brodifacoum where data are shown for the first period (1d).

	Mendenhall and Pank (1980) Rat: 5 d Barn Owl: 1-10 d		Lee (1993) Rat: 4 d Barn Owl: 5-7 d		Gray *et al* (1994) Mice: 1-2 d Barn Owl: 15 d		Newton et al (1990 and 1994) Mice: 1 day Barn Owl: 1+3+6 d		Lowest avian LD50 mg/kg bw
a.s.[1]	Dose-e[2]	Mort[3]	Dose-e	mort	Dose-m[4]	mort	Dose-m	mort	
Brod	1.4-4.9	5/6	8.9-11	3/4	1.9-5.4	1/4	0.12-0.18	4/6	0.95
Brom	2.5-14	1/6	7.6-11	3/4			0.23-0.29	0/6	50
Dif	3.2-12	0/6			1.6-5.5	1/4	0.21-0.27	0/6	>50
Floc			5.3-8.6	3/4	1.8-2.8	2/4	0.78-1.3	1/5	24
Warf			55-94	2/4					500

[1] active substance: brodifacoum, bromadiolone, difenacoum, flocoumafen, warfarin. Bait formulations all at 0.0005% w/w except warfarin (0.025%) and brodifacoum (0.002% in Mendenhall and Pank and Newton *et al* (1994)). Formulation not stated for bromadiolone in Newton *et al* (1994).
[2] dose-e = maximum accumulated dose to owls (mg/kg bw), estimated from total intake of bait by rodents. These values are expected to overestimate actual doses, due to metabolism and excretion.
[3] mort = owl mortality (number died/number survived)
[4] dose-m = accumulated dose to owls (mg/kg bw), estimated from measured residues in rodents.

Summary of selected predator feeding studies, comparing accumulated doses to lowest available avian LC50 (both in mg/kg diet). The top row shows the period of primary exposure (rat or mouse) and secondary exposure (barn owl) in each study. In the studies by Newton et al., data are shown for the third period of exposure (6d) except for brodifacoum where data are shown for the first period (1d). In the studies by Lee (1993) and Gray et al (1994) owls were fed a mixture of

treated and untreated rodents, so the overall concentration of rodenticide in their diets was lower than indicated by the data.

	Mendenhall and Pank (1980) Rat: 5 d Barn Owl: 1-10 d		Lee (1993) Rat: 4 d Barn Owl: 5-7 d		Gray *et al* (1994) Mice: 1-2 d Barn Owl: 15 d		Newton et al (1990 and 1994) Mice: 1 day Barn Owl: 1+3+6 d		Lowest avian LC50 mg/kg bw
a.s.[1]	conc-e[2]	Mort[3]	conc-e	mort	conc-m[4]	mort	conc-m	mort	
Brod	3.9-8.2	5/6	11	3/4	2.1-4.3	1/4	0.44	4/6	1.4
Brom	13-23	1/6	12	3/4			n.d.	0/6	464
Dif	11-24	0/6			1.1-5.1	1/4	0.29	0/6	0.25
Floc			8.0	3/4	1.0-4.3	2/4	0.65	1/5	1.7
Warf			65	2/4					438

[1] see above footnote (1)
[2] conc-e = maximum accumulated concentration in treated rodents offered to owls (mg/kg bw), estimated from intake of bait by rodents. These are expected to over estimate actual concentrations
[3] mort = owl mortality (number died/number survived)
[4] conc-m = measured dose in treated rodents offered to owls (mg/kg bw)

Wildlife Incident Investigation Scheme and Predatory Bird Monitoring Scheme

Information from the Wildlife Incident Investigation Scheme (WIIS) (see Appendix 2) and other monitoring schemes (e.g. Predatory Bird Monitoring Scheme (PBMS)[19]) indicate that:

(i) incidents involving non-target animals and rodenticides do occur and
(ii) residues (sub-lethal and lethal) also occur in a wide range of species.

As regards the PBMS data, the source of the rodenticide is unknown, and there is uncertainty regarding the relevance of the residues. What is clear, however, is that exposure does occur. This contamination may be the result of primary poisoning of small non-target mammals which in turn are consumed by predatory birds and mammals.

As for the WIIS data it is clear that incidents do occur, however it is not always possible, with any degree of certainty, to draw anything conclusive from these data in terms of the conditions under which the incident occurred, i.e. was the incident due to unspecified use, misuse or abuse.

Conclusion of secondary poisoning

[19] See http://pbms.ceh.ac.uk/ for details

As regards relative risks of secondary poisoning, it is possible to conclude the following:

- All secondary poisoning PEC/PNEC ratios are greater than 1.
- The datasets, especially higher tier data, for the five compounds are not equitable and hence direct comparisons are difficult.
- Field data for brodifacoum and flocoumafen provide limited evidence that the predicted risk *may be* realised in the field. However, there are concerns regarding the appropriateness of the pest control practice used in these studies as that they do not reflect *current* best practice. These data were used by the Advisory Committee on Pesticides to conclude that the outdoor use is unacceptable for these products containing these active substances.
- No field trial data are available for difethialone, bromadiolone or difenacoum
- Studies on predatory bird feeding studies indicate that all these active substances are capable of causing mortality as well as sub-lethal effects. The differences are likely to be due to available residues, binding strength, metabolism and excretion as well as feeding strategy of individual birds.
- On the basis of the predatory bird feeding studies 'little can be deduced from these feeding studies about the relative toxicity of the compounds to barn owls, even when they are included in the same experiment' (Luttik *et al* 1999).

(The above has dealt primarily with secondary poisoning of predatory birds; it is also the case that birds will feed on dead rodents. The risk from this route of exposure can be managed via the appropriate label phrases informing users to clear up rodent bodies/carcasses. However, it will be inevitable that some dead rodents (and potentially non-target vertebrates) will be available to be consumed by predatory and/or scavenging birds.)

Mammals

Like the PNEC/PEC ratios for secondary poisoning in birds, the PEC/PNEC ratios are also greater than 1. Little additional information is available. Only one predatory feeding study is available and that is for bromadiolone and is on stone martens. The interpretation of this study is, like the bird studies considered above, difficult and hence cannot be used to refine or further the risk assessment.

Overall conclusion

On the basis of the above the following can be concluded:

- All PEC/PNEC for primary poisoning are greater than one; it is considered that this risk can to a limited extent be mitigated and hence managed via the use of appropriate bait boxes or used in situations where access by non-target vertebrates is limited.

- All PEC/PNEC for secondary poisoning are greater than one; as regards the risk to birds, predator feeding studies have been submitted which indicate that depending on the feeding profile all can cause mortality and sub-lethal effects.

- On the basis of the limited toxicity and exposure data available it is not possible to clearly rank the rodenticides in terms of risk, where risk is an indication of the likelihood, magnitude and frequency of effects.

- Limited evidence from an unpublished PhD thesis indicates that sub-lethal doses of difenacoum (either given as one dose or as two consecutive doses with a 25 day interval) poses a slightly lower risk to birds compared to brodifacoum.

- Residue data from barn owls and buzzards indicate that intensive but carefully managed rodent control can lead to lower occurrence of residues compared to normal practice. This work indicates the importance of duration of the rodent control, risk mitigation measures (e.g. clearing up rodent bodies) and appropriate placement of bait boxes or similar (i.e. avoiding baiting along hedgerows).

Overall, it is not possible to clearly rank the active substances in terms of risk.

Chemicals Regulation Directorate
Health and Safety Executive
August 2012

Appendix 1. Decision regarding Inclusion in Annex I and Elements to be taken into account by Member States when authorising products

The following has been taken from section 3 of the Assessment Report (AR) for difenacoum. It should be noted that similar passages appear in the AR for other rodenticides:

3.2 Decision regarding Inclusion in Annex I

Difenacoum shall be included in Annex I to Directive 98/8/EC as an active substance for use in product-type 14 (rodenticides), subject to the following specific provisions.

In view of the fact that the active substance characteristics render it potentially persistent, liable to bioaccumulate and toxic, the active substance is to be subject to a comparative risk assessment in accordance with the second subparagraph of Article 10(5)(i) of Directive 98/8/EC before its inclusion in this Annex is renewed.

Member States shall ensure that authorisations are subject to the following conditions:

(1) The nominal concentration of the active substance in the products shall not exceed 75 mg/kg and only ready-for-use baits shall be authorised.
(2) Products shall contain an aversive agent and, where appropriate, a dye.
(3) Products shall not be used as tracking powder.
(4) Primary as well as secondary exposure of humans, non-target animals and the environment are minimized, by considering and applying all appropriate and available risk mitigation measures. These include, amongst others, the restriction to professional use only, setting an upper limit to package size and laying down obligations to use tamper resistant and secured bait boxes.

3.3. Elements to be taken into account by Member States when authorising products

The use of appropriate personal protective equipment should be advised in the use instructions.

As professional users are likely to be exposed more often, products containing difenacoum may be used by professional users if data are provided to show that calculated occupational exposure based on the operator exposure study, is acceptable.

The restriction of products to specific areas and manners of use and also restrictions of products to professionals or trained professionals only, should be considered.
The size of the package placed on the market should be proportionate to the duration of the treatment and appropriate to the pattern of use of particular user groups.

Product design and use restrictions should be optimised in order to ensure sufficient efficient rodent control while at the same time minimizing the risk for primary poisoning. This could include the use of tamper resistant bait boxes and the need to secure the baits so that rodents cannot remove the bait from the bait box.

When tamper-resistant bait stations are used, they should be clearly marked to show that they contain rodenticides and that they should not be disturbed.

Difenacoum baits should not be placed where food, feeding stuffs or drinking water could be contaminated.

In case no standard safety phrases are required on the product label, adequate safety instructions should be provided in the use instructions.

In addition to the elements already listed in Article 20(3) of Directive 98/8/EC, all packaging of anticoagulant rodenticides should be marked with the following standard phrases to protect humans, animals or the environment:

Baits must be securely deposited in a way so as to minimize the risk of consumption by other animals or children. Where possible, secure baits so that they cannot be dragged away.

Search for and remove dead rodents at frequent intervals during treatment (unless used in sewers), at least as often as when baits are checked and/or replenished. Dispose of dead rodents in accordance with local requirements.

Unless under the supervision of a pest control operator or other competent person, do not use anticoagulant rodenticides as permanent baits.

Remove all baits after treatment and dispose of them in accordance with local requirements.

Keep out of the reach of children.

This last safety precaution should always be carried on the label of the products, if not already legally required by Directive 1999/45/EC. The others could be stated elsewhere on the packaging or on an accompanying leaflet together with the other directions for use and disposal of the product required by article 20(3) of Directive 98/8/EC.

Member States should encourage the application of Codes of Good Practices in rodent control. These measures could include (but should not be restricted to) the following factors:

The population size of the target rodent should be evaluated before a control campaign. The number of baits and the timing of the control campaign should be in proportion to the size of the infestation.

A complete elimination of rodents in the infested area should be achieved.

The use instruction of products should contain guidance on resistance management for rodenticides.

Resistant management strategies should be developed, and difenacoum should not be used in an area where resistance to this substance is suspected.

The authorisation holder shall report any observed resistance incidents to the Competent Authorities or other appointed bodies involved in resistance management.

When the product is being used in public areas, the areas treated must be marked during the treatment period and a notice explaining the risk of primary or secondary poisoning by the anticoagulant as well as indicating the first measures to be taken in case of poisoning must be made available alongside the baits.

Appendix 2. Wildlife Incident Investigation Scheme data

Outlined below is a summary of Wildlife Incident Investigation Scheme (WIIS) data. These data have been obtained from the Food and Environment Research Agency (Fera).

Summary of Wildlife Incident Investigation Scheme data from 1984 to 2011.

Table 1

1997 to date, 24th June 2011, "group by" category search

	approved use	abuse	misuse	unspecified	Total	%
brodifacoum		5	8	11	24	**9**
bromadiolone	4	12	25	37	78	**30**
difenacoum	1	23	35	28	87	**34**
flocoumafen			2	1	3	**1**
mixture of rodenticides	1	1	32	32	66	**26**
Total	6	41	102	109	258	100
%	**2**	**16**	**40**	**42**		

Table 1 is a summary of all the incidents assigned to the four second generation anticoagulant from 1997 to 2011. This summary may include some "for information only" type incidents where there were no analyses carried out. It will not include any incidents attributed to other categories, where some very low level of anticoagulant residue was found and not considered to be linked to the cause of death. The "mixture of rodenticides" category may include mixtures of first and second generation rodenticides, although it is likely to be mainly second generation.

The categorisation of incidents in to approved, abuse, misuse and unspecified is difficult and there is sometime uncertainty in the classification, especially between misuse and approved use. It is also likely that the unspecified category consists of a mixture of misuse and approved use incidents. Despite the difficult in confidently attributing each incident, it is clear that there have been several incidents involving all rodenticides.

In considering these data the concerns of Luttik *et al*[20] and EFSA (2009)[21] regarding the potential for under reporting should be noted.

Luttik *et al* (1999) compared a subset of these data covering the period 1985-96 with the usage over the same period. They concluded that there had been 8 incidents that were attributable to rodenticide poisoning over that period. These 8 incidents were

[20] Luttik R., Clook M.A., Taylor M.R., Hart A.D.M., (1999) The regulatory aspects of the ecotoxicological risk assessment of rodenticides. In Advances in Vertebrate Pest Management Pest Management, p 369-385, ed Cowan P.D. and Feare C.J. Filander Verlag, Further Germany.
[21] European Food Safety Authority (2009) Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438. Available online: www.efsa.europa.eu

considered to be due to the approved use, however due to the delayed toxicity of SGAR it is difficult to be specific about the source, therefore the 8 incidents considered in detail may have been due to misuse as well as approved use. The analysis by Luttik *et al* indicated that there were 4, 0.2, 0.2 and 0 incidents per 1000 tonne of bait for brodifacoum, bromadiolone, difenacoum and flocoumafen respectively. Caution is needed in interpreting these data as the number of incidents per active substance is small.