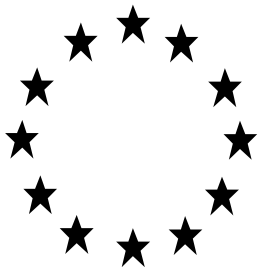
Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products

Evaluation of active substances

Renewal of approval

Assessment Report



Difenacoum

Product-type 14
(Rodenticides)

July 2016

eCA Finland

CONTENTS

1. STATEMENT OF SUBJECT MATTER AND PURPOSE

1.1. Procedure followed

This assessment report has been established as a result of the evaluation of the active substance difenacoum as product-type 14 (rodenticides), carried out in the context of evaluation of applications for renewal provided for in Article 14 of the Biocidal Product Regulation (EU) No 528/2012 (BPR), with a view to the possible renewal of the approval of this substance.

With the intention to streamline the renewal of substance approvals and product authorisations of anticoagulant rodenticides[1] and their comparative assessments, at the 50th CA meeting the document "Substance approval and product authorisation renewals of the anticoagulant rodenticides" (CA-Feb13-Doc.5.2.b – Final) was endorsed. This was confirmed at the 61th CA meeting laid down in the document "Renewal of anticoagulant rodenticides active substances (CA-Sept15-Doc.5.3).

A workshop was held in Brussels on 26 February 2015 regarding the report on *Risk mitigation measures for anticoagulant rodenticides as biocidal products (Final Report October 2014; ISBN 978-92-79-44992-5)* prepared for the European Commission. The revised summary of the workshop was endorsed at the 62nd CA meeting (CA-Nov15-Doc.5.4). The BPC Efficacy Working Group discussed in WGI-2016 some recommendations of the RMM report for anticoagulant rodenticides.

Difenacoum was approved as an existing active substance, in product-type 14 under the Biocidal Products Directive (Commission Directive 2008/81/EC). The renewal of the active substance has been requested by the Difenacoum Renewal Task Force comprised of Activa S.r.l., BASF Agro B.V. Arnhem (NL) Zürich Branch, HENTSCHKE & SAWATZKI KG and PelGar International Limited.

On 20 September 2013, Finland competent authority (eCA) received a dossier Difenacoum Renewal Task Force. The dossier was completed by the applicant by 28 July 2015 as agreed in CA-Feb13-Doc.5.2.b - Final and CA-Sept14-Doc.5.2 – Final.Rev1. The eCA accepted the dossier as complete for the purpose of the evaluation on 28 September 2015. On the basis of the available information the eCA decided that only a limited evaluation in accordance with Article 14(2)(2) of the BPR of the application is necessary.

As all anticoagulant rodenticides meet the exclusion criteria. If approved, stringent risk mitigation measures will need to be applied. Where no new information was available in the application of renewal, the revision of the evaluation applying current guidance is postponed to product authorisation. This decision shall exclusively apply for the renewal of anticoagulant rodenticides. On 24 March 2016, the eCA submitted to the Agency and the applicant the assessment report.

In order to review the assessment report and the comments received on it, consultations of technical experts from all Member States (peer review) were organised by ECHA. Revisions agreed upon were presented at the 16th Biocidal Products Committee and its Environment Working Group meeting (WGI-2016) the assessment report was amended accordingly.

[1] The concerned active substances are: brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difethialone, difenacoum, flocoumafen and warfarin.

1.2. Purpose of the assessment report

The aim of the assessment report is to support the opinion of the Biocidal Products Committee and the decision on the renewal of the approval of difenacoum for product-type 14, and, should it be approved, to facilitate the authorisation of individual biocidal products. In the evaluation of applications for product-authorisation, the provisions of Regulation (EU) No 528/2012 shall be applied, in particular the provisions of Chapter IV, as well as the common principles laid down in Annex VI.

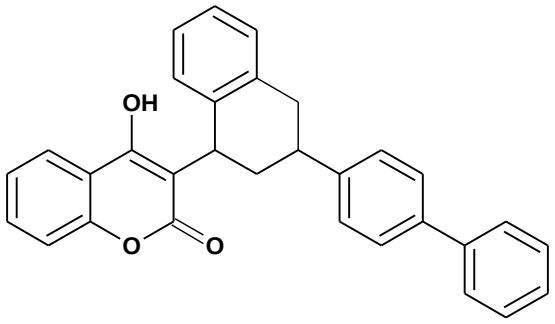
For the implementation of the common principles of Annex VI, the content and conclusions of this assessment report, which is available from the Agency web-site shall be taken into account.

However, where conclusions of this assessment report are based on data protected under the provisions of Regulation (EU) No 528/2012, such conclusions may not be used to the benefit of another applicant, unless access to these data for that purpose has been granted to that applicant.

2. OVERALL SUMMARY AND CONCLUSIONS[2]

2.1. Presentation of the Active Substance

2.1.1. Identity

CAS-No.	56073-07-5
EINECS-No.	259-978-4
Other No. (CIPAC, ELINCS)	67/548/EEC Annex I No.: 607-157-00-X
IUPAC Name	3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin
Common name, synonym	Difenacoum (BSI, ISO), diphenacoum
Molecular formula	$C_{31}H_{24}O_3$
Structural formula	
Molecular weight	444.5 g/mol
Minimum purity	≥960 g/kg

[2] See document CA-Sept15-Doc.5.3 - Renewal anticoagulant rodenticides.doc

Isomers	Isomeric mixture of trans isomer (CAS N. 151986-16-2, CA Index Name: 2H-1-Benzopyran-2-one, 3-(3-[1,1'-biphenyl]-4-yl-1,2,3,4-tetrahydro-1-naphthalenyl)-4-hydroxy-, trans-) and cis isomer (CAS N. 151986-15-1, CA Index Name: 2H-1-Benzopyran-2-one, 3-(3-[1,1'-biphenyl]-4-yl-1,2,3,4-tetrahydro-1-naphthalenyl)-4-hydroxy-, cis-). The range of cis-isomer is 50-80%. Both diastereomers are toxicologically active. More detailed information on isomers is given in Annex Confidential Data and Information (original assessment). More detailed information on isomers is given in Annex Confidential Data and Information of the original CARs and confidential Appendix III.

2.1.2. Intended Uses

Difenacoum is intended to be used as a rodenticide against Norway rats, Roof rats and House mice by different user groups and in different areas of use as described in the table below. The maximum concentration allowed in the ready to use products was 75 mg/kg in the Commission Directive 2008/81/EC.

Species	Area of use	User category	Maximum amount of bait
House mice *Mus musculus/ domesticus*	In and around buildings	General public[1] Professionals Trained professionals	80 g/bait point
Norway rats *Rattus norvegicus*	In and around buildings	General public[1] Professionals Trained professionals	220 g/bait point
	Open area Waste dump Sewer	Professionals Trained professionals	
Roof rats *Rattus rattus*	In and around buildings	General public[1] Professionals Trained professionals	220 g/bait point
	Open area Waste dump	Professionals Trained professionals	

[1] Please refer to the maximum quantity of bait per pack set for the general public in 2.3 B.1.b of the Opinion for difenacoum.

2.2. Summary of the Assessment

2.2.1. Specification of the different sources of the active substances

The 5-batch analyses performed for difenacoum after the original evaluation (Activa s.r.l: Ticco 2011, BASF Agro B.V. Arnhem (NL) and HENTSCHKE & SAWATZKI KG: Walker 2010, PelGar International: Kekulova 2009) confirm the minimum purity of 96.0% (w/w) for each source. The impurity profiles have not remarkable changed but remained within the specifications set during the original evaluation or specifications accepted as results of technical equivalence assessments. None of the applicants have changed the manufacturing method or production site.

However, since the 5-batch studies are more than five years old, quality control data from each source are required in order to confirm that the quality of technical material has remained

unchanged until the present day.

Technical equivalence of difenacoum from Sorex (now BASF) source and PelGar source was shown during the original evaluation. In connection with a product authorisation in 2011, the French CA has assessed and confirmed technical equivalence between the original PelGar's material (5-batch analysis in 2001) and material of more resent production of the same source, by a new 5-batch analysis data (2009). The French CA has also assessed and confirmed (2012) the technical equivalence between PelGar's original source and Activa's source (5-batch analysis in 2011). Independently from French CA's assessments, the Italian CA has performed year 2011 a technical equivalence assessment between PelGar's original source and Activa's source (5-batch analysis in 2011).

2.2.2. Assessment as to whether the conclusion of the initial assessment of approval remain valid

2.2.2.1. **Physico-chemical properties and methods of analysis**

New information has been provided on log Kow since the original approval. At that time value of 7.6 had been estimated by QSAR. These estimations concerned the undissociated species.

The n-octanol/water partition determination for difenacoum has been performed at pHs 3.8, 4.0, 7.0 and 9.0 as these pHs have been assessed as being environmentally relevant, and a partitioning ratio is determined for each ionisation state. The log Kow values obtained were: 7.22 at pH 3.8, 7.16 at pH 4.0, 4.78 at pH 7.0 and 3.35 at pH 9.0.

A new method for water, reaching the agreed (TM IV, 2013) LOQ for drinking water (0.01 µg/L) has been requested from the applicants Activa S.r.l and PelGar Limited. Currently, the LOQ (0.05 µg/L) of their analytical method does not reach this limit.

2.2.2.2. **Classification and Labelling**

Difenacoum was discussed by the Technical Committee on Classification and Labelling of Dangerous Substances (TC C&L) of the European Chemicals Bureau (ECB) together with seven other anticoagulant rodenticides (2006 – 2008) as well as by the Specialised Experts for Reproductive Toxicity (September 2006). No final decision could be made on the human health classification of the substances (classification for reprotoxicity and setting of specific concentration limits for acute and repeated dose toxicity) and, the work was transferred to be coordinated by ECHA. A CLH proposal was prepared by the evaluating Member State (Finland) and submitted to the Committee for Risk Assessment (RAC) of ECHA. The dossiers for the eight rodenticides were handled as a group, but the RAC evaluated the proposals on a substance by substance basis comparing the data available for Warfarin and other AVKs and relying on a weight-of–evidence approach as required by Regulation 1272/2008 (CLP). The RAC-opinion was adopted on 14 March 2014. The proposed classification shall be adopted in the 9th Adaptation of the Regulation of 1272/2008.

Difenacoum has been classified according to the Commission directive 93/72/EEC (19th adaptation of technical process). Main changes from the current classification to the revised classification are the inclusion of all exposure routes (oral, inhalation, dermal) to the classification for acute toxicity 1 and to the repeated dose toxicity and the classification for reproductive toxicity (teratogenicity). In addition, a specific concentration limit (SCL) was added both for reproductive toxicity and to the repeated dose toxicity endpoints as well as the M-factor of 10 for aquatic acute and aquatic chronic toxicity.

Revised Annex VI entry (draft 9th ATP to CLP)	
Hazard Class and Category Codes	Acute Tox. 1 H300 Acute Tox. 1 H310 Acute Tox. 1 H330 STOT RE H372 Repr. 1B H360D Aquatic Acute 1 H400 Aquatic Chronic 1 H410
Labelling	
Pictograms	GHS06 GHS08 GHS09
Signal Word	Danger
Hazard Statement Codes	H300: Fatal if swallowed H310: Fatal in contact with skin H330: Fatal if inhaled H360D: May damage the unborn child H372: Causes damage to the blood through prolonged or repeated exposure H410: Very toxic to aquatic life with long lasting effects
Specific Concentration limits, M-Factors	SCL Repr. 1B: H360D C ≥ 0.003% SCL STOT RE: H372: C ≥ 0.02%; H373: 0.002% ≤ C ≤ 0.02% M=10 Aquatic Acute toxicity M=10 Aquatic Chronic toxicity

2.2.2.3. **Efficacy and resistance**

No new information on efficacy and resistance has been provided since the original approval and hence the conclusions remain the same. A number of scientific articles have been published since the original approval on the resistance of ARs in general. Such studies have not been submitted for the renewal.

According to the conditions for granting an authorisation of a biocidal products in Article 19 (1) (b) ii) of the Biocides Products Regulation (EU) No 528/2012, the products should be *"sufficiently effective and have no unacceptable effect on the target organisms such as resistance, or, in the case of vertebrates, unnecessary suffering and pain".* It is recognised that anticoagulants like difenacoum do cause pain in rodents. However, as long as effective, but comparable less painful alternative biocidal substances or biocidal products or even non-biocidal alternatives are not available anticoagulant rodenticides should be accepted.

2.2.2.4. **Human health assessment**

No new information on the human health assessment has been provided since the original approval and hence the conclusions remain the same. However, in the proposed new classification difenacoum is classified as toxic for reproduction (category 1B). This fulfils the criteria set in the Articles 5(c) and 19(4) of BPR (528/2012), and has to be taken account in the product authorisation stage.

2.2.2.5. **Environmental effects assessment**

An earthworm reproduction study has been provided since the original approval of difenacoum. Difenacoum reduced number of offspring, but did not affect mortality. The study resulted in a NOEC of 62.5 mg/kg dw.

The PNECsoil derived from the test is 0.625 mg/kg dw (assessment factor of 100 agreed at the BPC-16; an acute and a long-term earthworm tests were available, no studies on plants or micro-organisms). The PNEC derived in the original assessment were 0.994 mg/kg dw and 2.3

mg/kg dw (EPM). Thus, the new study resulted in a slightly lower PNEC and subsequently the PEC/PNEC for soil was slightly increased, but remained less than 1.

In the original risk assessment risk was identified in the open area scenario with the EPM PNECsoil. When PNECsoil was derived from the terrestrial acute tests, no risk was identified. No unacceptable risk was identified in any scenarios with the revised PNECsoil based on the earthworm reproduction test.

PEC/PNEC ratios in the original and revised assessment. The PECs and PNECs were given as mg/kg dry weight.

Scenario	PECsoil (worst case+ refined metabolism)	PEC/PNEC Original	PEC/PNEC EPM[1] Original	PEC/PNEC Revised
Sewer sludge appl.	0.00014	0.0001	0.0006	0.0002
In and around building	0.034	0.034	0. 15	0.054
Open areas	0.346	0.348	1.5	0.554
Waste dump	0.0016	0.002	0.007	0.003

[1]PECsoil/PNECsoil was increased by a factor of 10 for compounds with a log Kow > 5 (TGD, Section 3.6.2.1). Multiplication is not any longer relevant due to revised log Kow < 5.

2.2.2.6. **Fate and distribution in the environment**

No new studies were provided on biodegradation or abiotic degradation and conclusions of the original assessment were not changed. New studies on fish and earthworm bioaccumulation were submitted after original evaluation. In addition, an experimentally derived log Kow was provided. In the original assessment no bioaccumulation studies were included and BCFs were calculated from the QSAR log K_{ow} of 7.6. The experimentally derived log Kow of the unionised difenacoum at pH 3.8 is 7.22. At the environmentally more relevant pH of 7 the log D_{ow} is 4.78.

Very low bioaccumulation was observed in the earthworm test. The bioaccumulation factors (BAF) derived from this test were 1.32 kg soil/kg worm (kinetic) and 0.81 kg soil/kg worm (steady state). The low bioaccumulation may be due to strong adsorption to soil. Higher bioaccumulation potential was observed in the fish bioaccumulation study. BPC-2016-I-ENV decided that the growth corrected kinetic BCF of 1100 L/kg shall be used for the risk assessment.

The revised risk assessment of the secondary poisoning via fish in aquatic food chain and via earthworms in terrestrial food chain was done according to Guidance on the Biocidal Products Regulation, Volume IV Environment - Part B Risk Assessment (active substances), 3.8.3.

The experimentally derived BCFs for fish and earthworm were significantly lower compared to calculated QSAR values of 9010 L/kg and 477 729 L/kg used in the original risk assessment. Therefore, the revised risk assessment resulted in an acceptable risk for birds and mammals in the aquatic food chain and for mammals in the terrestrial food chain. An unacceptable risk was still identified for birds in the terrestrial food chain but the PEC/PNEC ratio was lowered from 480 to 6.4.

Original risk assessment for secondary poisoning in aquatic and terrestrial food chains. PECwater (2.23 x 10^{-7} mg/L) was taken from the sewer scenario, PECsoil (0.035 mg/kg dw) and PECporewater (1.1 x 10^{-6} mg/L) from the in and around building scenario of the Doc IIB of Activa/PelGar Task Force.

	Aq. PECoral, predator µg/kg fish	Terr. PECoral predator µg/kg earthworm	PNECoral µg/kg food	PEC/PNEC Aquatic	PEC/PNEC Terrestrial
Scenario	**Sewer**	**In and around building**			

Birds	10	240	0.5	20	480
Mammals	10	240	7	1.43	34.3

Revised risk assessment for secondary poisoning in aquatic food chain (sewer scenario) and terrestrial food chain (in and around building).

	Aq. PECoral, predator µg/kg fish	**Terr. PECoral predator µg/kg earthworm**	**PNECoral µg/kg food**	**PEC/PNEC Aquatic**	**PEC/PNEC Terrestrial**
Scenario	**Sewer**	**In and around building**			
Birds	0.245	3.183	0.5	0.49	6.4
Mammals	0.245	3.183	7	0.035	0.45

It was assumed in the original risk assessment that secondary poisoning via the aquatic food chain would not be significant due to low water solubility and high adsorption tendency of difenacoum. Even though risk is identified in the terrestrial food chain for birds, the risk via poisoned rodents is considered significantly higher compared to risk via earthworms or other invertebrates. Thus, conclusion from the original assessment is not changed.

2.2.2.7. **PBT and POP assessment**

PBT assessment was performed in the original risk assessment, Doc IIC, 2.5. There were three separate Doc IICs for difenacoum (Sorex Limited, HENTSCHKE & SAWATZKI KG and the Activa/PelGar Brodifacoum and Difenacoum Task Force). The PBT assessment was performed according to TGD and at that time no criteria for P in soil existed.

Revised PBT assessment according to Regulation (EU) No 253/2011

P and vP criteria

Difenacoum is not readily or inherently biodegradable and it is hydrolytically stable. Photolytic half-life in water is 3-8 hours in the pH range 5-9. No biodegradation test is available in fresh or marine water or sediment. The half-life of 439 day at 20 °C (833 days at 12 °C) was determined in the aerobic soil degradation test. The half-life in soil exceeds the criteria for P (120 days) and vP (180 days). Difenacoum fulfils the P and vP criteria.

B criterion

The original assessment of the B criterion was based on the calculated log K_{ow} (7.6) and BCF (35 645 L/kg, TGD and 9010 L/kg, EPIWIN). After original assessment log K_{ow} has been experimentally determined for difenacoum. The log K_{ow} was 7.22 for an unionised difenacoum at pH 3.8. For an ionised difenacoum at pHs 4, 7 and 9 the log D_{ow} were 7.16, 4.78 and 3.35, respectively. The log Kow (pH 3.8) and log D_{ow} (pH 7) still exceed the screening criteria of ≥4.5 (Guidance on Information Requirements abd Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. Version 2.0. November 2014).

An aquatic and terrestrial bioaccumulation studies were submitted for the renewal evaluation of difenacoum. The bioaccumulation potential detected in the earthworm *Eisenia fetida* was low, probably due to adsorption of difenacoum to soil. The steady state and kinetic bioaccumulation factors (BAF) for earthworm were 0.81 and 1.32 kg soil/kg worm, respectively. The respective biota-soil accumulation factors (BSAF) were 0.23 and 0.22 kg OC/kg lipid. In the aquatic bioaccumulation study in rainbow trout *Oncorhynchus mykiss* the steady state and kinetic BCFs were 840 L/kg and 920 L/kg, respectively. Growth corrected kinetic BCF was 1100 L/kg and with lipid normalisation 410 L/kg. The uptake rate constant 148 per day was higher than the depuration rate constant 0.161 per day. The growth corrected elimination half-life was 5 days, DT90 was not determined. In the terrestrial bioaccumulation test the DT90 (104 days) was clearly longer than DT50 (7.9 days). It was decided at the BPC-WG-I-2016-ENV that the growth corrected kinetic BCF (1100 L/kg) is to be used without lipid

normalisation.

The BCF 1100 L/kg was below the B criterion of 2000 L/Kg. On the basis of the study difenacoum does not fulfil B. The result was conflicting to the fact that residues of difenacoum are commonly found in non-target species that prey on rodents or feed on carcasses of rodents. The conclusion from the ad hoc follow up after BPC-WG-2016-I-ENV was following:

The commenting WG members agreed that the study was conducted according to the guideline and GLP but due to the high mortality rates in the high concentration, it can be considered as valid only for the lowest tested concentration.

However, for the lowest test concentration the variation between the individual samples was high, especially after 14 days. Regression parameters such as r^2 and 95% confidence intervals were not presented in the study report. Reproducing the regression by one WG members shows that k1=148/d and k2=0.161/d only resulted in a r^2=0.151. The corresponding 95% confidence intervals will be therefore extremely high with an upper limit that may exceed the BCF=2000 L/kg. All tissue concentrations on day 7 and 10 are well above the fitted regression curve, which may indicate that the derived BCF values are too low. In addition, water samples were analysed unpurified, the concentrations in the water phase may therefore be underestimated due to sorption on dissolved organic matter and suspended materials.

In general the relevance of the aquatic BCF study for rodenticides was questioned with regard to the common exposure pathway of anticoagulant rodenticides via terrestrial food chain: Taking into account the high log Kow (at low pH values) of difenacoum a bioaccumulation study with dietary exposure was considered as being more appropriate. Rodenticides do not enter the food chain via passive uptake by partitioning at the lowest level, but via active uptake of feed at higher trophic levels. The findings of difenacoum in terrestrial non-target organisms indicate that the substance is effectively transferred in the food chain; it is taken up from food in an efficient way and is not easily eliminated (e.g. excreted and/or metabolized).

It was further agreed that the aquatic BCF from the study should not be the only aspect considered when discussing the B criterion of difenacoum; the available information on residues of difenacoum in biota in a great variety of non-target species across Europe also needs to be acknowledged. The monitoring data should therefore be applied in addition as part of a weight of evidence approach. The conclusion of the ad hoc follow up was that difenacoum should be considered as bioaccumulative (B.

Difenacoum as well as other anticoagulant rodenticides (AR) have been found in many studies in non-target animals in UK, France, Germany, Spain, the Netherlands, Denmark, Norway, Sweden and Finland (Appendix IV). High prevalence shows high potential for secondary exposure of difenacoum and other ARs in the food chain poisoned rodent → predator or poisoned rodent carcass → scavenger.

High prevalence in the non-target animals is likely due to the delayed mechanism of action of ARs. The rodents feed on the product and may ingest a lethal dose or even more, but they continue to live about week after ingestion of lethal dose. During that time the rodents are available for various predators. It may also happen that at some stage poisoned rodents may even become easier prey for predators than non-poisoned rodents. It has been shown by Elmeros et al. 2015 that small mammals other than target rodents like voles, mice and shrews are contaminated by the ARs within 100 m distance from the baiting point. Many of those species belong commonly to diet of raptorial birds and mammals like e.g. mustelids. In addition, some animals feed on carcasses of poisoned dead rodents.

When concluding on the B criterion available information needs to be considered. The BCF is lower than 2000 L/kg indicating non B, but all other facts indicate B. Difenacoum is a lipophilic substance with a long half-life in the second phase of biphasic elimination (DT50 in rat 118 days). It accumulates specifically in the liver and is commonly found in non-target animals feeding on rodents or their carcasses. It is concluded that difenacoum fulfils the B criterion.

T criterion

NOEC or EC10 for marine or freshwater organisms is not available. Difenacoum is classified as toxic for reproduction, category 1B and STOT RE 1 H372. Difenacoum fulfils the T criterion.

2.2.2.8. **Assessment of endocrine disruptor properties**

No new information on endocrine disruptor properties has been provided since the original approval and hence the conclusions remain the same. Difenacoum does not fulfil the interim criteria for endocrine-disrupting properties set in the Article 5(3) of BPR (528/2012).

2.2.3. Assessment of the recommendations arising from the report[3] on RMM for anticoagulant rodenticides that are relevant for the active substance.

Anticoagulant rodenticides (AR) are divided into First Generation AR (FGAR; warfarin, chlorophacinone, coumatetralyl) and Second Generation ARs (SGARs; bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone). Difethialone, brodifacoum and flocoumafen are often referred to as more potent than bromadiolone and difenacoum.

Anticoagulant rodenticides have been found in many studies in non-target animals. Some new studies were submitted for the renewal of the anticoagulant rodenticides: i) in Denmark coumatetralyl and several SGARs were found in stone martens and polecats; ii) in UK anticoagulant rodenticides are regularly detected in the Predatory Bird Monitoring Scheme and in incidents of suspected poisoning of animals by pesticides investigated under the Wildlife Incident Investigation Scheme; iii) in Germany several FGARs and SGARs were found in the red fox; iv) in Spain SGARs were found in birds of prey and hedgehogs; in France anticoagulant rodenticides have been found in buzzards, red kite and mustelids species; v) in Finland all anticoagulant rodenticides in use (i.e. coumatetralyl and SGARs) were found in predatory and scavenging non-target birds and mammals. More studies are publicly available but these show that there is a concern with respect to secondary exposure of non-target organisms.

Due to the identified risk for environment and human health, anticoagulant rodenticides have to be handled with great caution and all appropriate and available risk mitigation measures (RMMs) have to be applied. As several AR, which are quite similar regarding hazardous properties and associated risks, were assessed for possible renewal at the same time (see also the CA-document "Substance approval and product authorisation renewals of the anticoagulant rodenticides; CA-Feb13-Doc.5.2.b), the Commission initiated a project on possible risk mitigation measures which could be applied for all anticoagulant rodenticides. This resulted in the report "Risk mitigation measures for anticoagulant rodenticides as biocidal products" (Berny, P. et al., October 2014). The report distinguishes between risk mitigation measures at community level through imposing conditions in the approval for the active substance, and measures at national level when products are authorized.

As a follow-up to the report, the Commission organized a workshop on 26 February 2015 with the aim to discuss and agree on RMMs to be recommended for anticoagulant rodenticides. The workshop was attended by representatives of several Member State Competent Authorities, the Commission, the Rodenticide Resistance Action Group (RRAG, UK), CEPA (Confederation of European Pest Management Associations), CEFIC (the European Chemical Industry Council) and members of the Efficacy Working Group. A summary report presenting the results of the workshop was discussed at the CA meetings in March and November 2015 ("Revised version of the summary of the workshop on the RMM report held in Brussels on 26/02/2015"; CA-Nov15-Doc.5.4). The result of an internet survey on the relevant RMMs was included in the report.

[3] Available at https://circabc.europa.eu/w/browse/d66ad096-37a1-4903-a3e0-24607ca3f3ea

A critical review of the RMM was submitted by the applicant of difenacoum when submitting the application for renewal in line with the CA document "Complementary guidance regarding the renewal of anticoagulant rodenticide active substances and biocidal products" (CA-Sept14-Doc.5.2-Final.Rev1).

In this section the risk mitigation measures proposed in the report of Berny et al. (2014) are presented and assessed, distinguishing between the measures at approval and product authorization stage. This assessment includes, if available, the critical review of the applicant and a recommendation or conclusion by the evaluating Competent Authority.

The detailed considerations in this section on the recommendations for renewal of the inclusion in the Union list of approved active substances formed the basis for the renewal conditions and the elements to be taken into account when authorising products as laid down in respectively sections 2.3 and 2.4 of the opinion of the Biocidal Products Committee (BPC).

General recommendations on RMM for anticoagulant rodenticides

RMM to be set at active substance approval

In the survey reported in the summary of the workshop, most member states agreed that the order of use of methods and substances to control rodents, generally should be:
Non chemical methods > FGARs > less potent SGARs > potent SGARs.

For rat control, FGARs and less potent SGARs should always be considered as the first choice. SGARS should only be used against rats, where there is evidence that infestations are resistant.

The applicant commented that ideally products containing the least potent active substance that will effectively complete control should be used first. However, as there currently is no rapid way to determine the resistance status of a rodent infestation prior to treatment, the proposed approach is neither realistic nor practical.

The eCA agrees in the above mentioned order of use of the substances in principle. However, it is unclear how well this order would be followed in practice. It may not be expected that the general public would know the order of potency of different ARs.

For mouse control, SGARs should always be considered as the first choice, as FGARs have low efficacy against House mice. FGARs should only be used against mice where there is evidence that the local strain is susceptible.

At the workshop it was concluded that there is not necessary information or support to restrict FGAR at EU level for use against mice. The authorization of biocidal products should be decided upon the national or regional resistance situation. It was commented that there is a lack of data on resistance in house mice, and that there is a lot of variation throughout Europe. This was further supported in the Efficacy Working Group in January 2016.

The applicant commented that ideally products containing the least potent active substance that will effectively complete control should be used first. However, as there currently is no rapid way to determine the resistance status of a rodent infestation prior to treatment, the proposed approach is neither realistic nor practical.

The eCA agrees that the high potency SGARs should be used in the known resistance cases where other ARs are known to be ineffective. The problem is that the resistance status is usually not known as pointed out by the applicants. Due to lack of information on resistance situation in most MS this restriction is not suggested for difenacoum (or bromadiolone).

Provided the other RMMs are applied (pack size, bait stations see below), there is no reason to restrict the use of SGAR for amateurs, especially in order to control House mice populations, which are the number one problem in the amateur sector.

According to the internet survey referred in the summary of the workshop, the majority of member states authorize both FGARs and SGARs for use by the general public, both for control of mice and rats.

The applicant is of the opinion that use of rodenticides by amateurs is essential for the wider control of rodent infestations in order to protect public health, property and the environment. Furthermore, it is commented that if rodent control were to become completely reliant on professional operators, then this could be the cause of householders ignoring the need for treatment of infestations due to the higher cost and so increase the associated risks to public health. Furthermore, the applicant considers that there are currently insufficient pest control operators to treat the reported number of household infestations. Farmers are considered to be amateurs in some Member States and farmers should not according to the applicant be denied access to rodent control because of the risks that would present to the food chain.

The eCA is of the opinion that SGARs might be authorized for use by the general public against mice as long as only small quantities are allowed and the bait is provided in the non-refillable tamper resistant bait stations.

Pack size should always be limited for amateur use and SGAR should be sold in smaller amounts than FGARs. A precise computation and list of suggestions is provided. Products intended for use by amateurs should be clearly different from products intended for use by professionals and PCOs.

At the workshop it was agreed that products for professionals and the general public should be placed at the market as different products with different pack size and separate labelling. The proposal for maximum pack size in the RMM report was considered as a good starting point and CEFIC proposed a pack size of 1.5 kg.

The applicant agreed in principle with the restriction on pack size, but with a maximum pack size of 1.5 kg. It was argued that the list of pack sizes proposed in the RMM report is simplistic as it does not consider potency and presumes only one bait point.

The eCA agrees that pack size should be limited for the general public.

Amateurs should have the option to use ARs in and around buildings for the control of rat infestations, since there is evidence that rat infestations almost invariably have an outdoor origin (burrows).

At the workshop it was agreed that the control of rats in and around buildings should be allowed for the general public. However, it should be subject to derogations from the mutual recognition at the product authorization stage.

The applicant commented that any restriction of an active substance, or a biocidal product, to use 'indoors only' is a de facto restriction preventing use against most rat infestations.
Virtually all rat infestations are of an outdoor origin as rats will live outdoors and search indoors for food etc.

The eCA agrees that rat control necessities the use of rodenticides in and around buildings. Due to different national situations, the rat control in and around buildings could be subject to derogation from the mutual recognition at the product authorization stage.

Dyes should always be included in the formulations. Using specifically green/blue dyes for ARs which are not absorbed appears as an interesting RMM to monitor both bait uptake (efficacy) and non-target primary exposure.

At the workshop in was unanimously agreed that dyes should be included in bait formulations (including red dyes).

The applicant commented that it is usual practice of industry to include dyes and pigments in rodenticide products to reduce the risk of accidental uptake by humans and birds etc. However, they considered it unnecessary and commercially unwarranted to specify which colors to be used.

The eCA agrees that the addition of a colouring agent to bait should be mandatory. Dyes reduce risk of accidental uptake by humans. Non-metabolized dyes help identifying uptake of target rodents and primary exposure of non-target species like dogs. Dyes seem not to be an effective RMM in preventing primary and secondary poisoning of non-target animals.

Bittering agents should be included in all bait formulations. Denatonium benzoate at 0.01% (10 mg.kg-1)* is currently the most commonly used bittering agent in bait formulations.
*[*Correction by the applicant: The bittering agent is commonly incorporated at 0.001% (10mg/kg)]*

At the workshop it was unanimously agreed that bittering agents should be included in bait formulations.

The applicant commented that Industry introduced the use of denatonium benzoate as a human taste deterrent in the 1980's and will continue to do so. Denatonium benzoate is commonly incorporated at 0.001% (10 mg/kg) not as given in the statement above.

The eCA agrees on the importance to include bittering agents (e.g. denatonium benzoate) in the bait formulations to reduce the likelihood of oral consumption in humans (i.e. to reduce the amount ingested in case of accidental/intentional intake of bait). It should be kept in mind though that the addition of bittering agent would be expected to significantly reduce, but not eliminate, the probability of an accidental ingestion by the youngest children. Bittering agents seem not to be an effective RMM in preventing primary and secondary poisoning of non-target animals.

Baiting area: professionals and trained professionals should conduct surveys prior to application of ARs that consider the extent of the rodent infestation, and the risks posed to humans and non-target species. Information should always be applied on the bait stations but not in the surrounding area.

At the workshop it was agreed that surveys before baiting should be included in code of best practice or be included as a RMM at active substance renewal. As for information in the surrounding area, no position was agreed. Hence, this RMM will be left to the Member States to decide.

The applicant commented that conducting site surveys prior to treatment is considered Best Practice. It is impossible to conduct efficient and effective rodent control with minimal environmental risks without having conducted a survey. Attention should not be drawn to treated areas as this would present evidence of an infestation which could have deleterious effects e.g. on nearby businesses and it would invite the abuse and vandalism of bait points. The text of notices on bait stations should be essential and relevant.

The eCA agrees that a pre-treatment survey of the infested area is necessary to perform by professionals in order to determine the extent of the infestation. The bait stations should be clearly marked to show that they contain rodenticides and that they should not be disturbed. Contact information (e.g. to the Poison Information Centre) and measures to be taken in case of poisonings (most importantly information about antidote) should be included. In addition, contact information to the one responsible for the treatment should be given.

For amateur use, tamper-resistant bait stations should always be mandatory, with baits securely fixed inside the bait stations when possible (wax blocks, paste). Loose baits (such as grain and pellets) cannot be excluded, even for amateur use, because of their higher palatability. Using smaller packs and pre-packed bait stations should reduce the risk of accidental human exposure, and possibly pet exposure.

A large majority of the member states in the survey (reported in the summary of the workshop) agreed that tamper resistant bait stations with securely fixed baits should be mandatory for use by the general public. As for use of loose baits for the general public there were mixed responses.

The applicant commented that the proposal fails as there is no European definition of tamper-resistant. As the use of bait boxes reduces efficacy especially for rat control their use should not be mandatory. Furthermore, there would be situations, e.g. roof voids, locked outbuildings, where bait stations would not be necessary. Loose baits (such as grain and pellets) should in their opinion not be excluded for amateur use, because of their higher palatability.

The eCA considers that non-refillable tamper-resistant bait stations should be mandatory for the general public. The eCA does not consider loose baits such as grains and pellets appropriate for amateur use. Rodents hoard food and will therefore translocate bait from bait stations subsequently making bait available for non-target animals (e.g. birds) and humans. Children and pets are most likely the group most at risk as they may stay inside or around buildings where baits have been placed. As hoarding is more relevant for grains and pellets, they should not be allowed for the general public. The higher palatability of loose baits is not acknowledged in the draft PT 14 efficacy guidance and the eCA has an understanding that bait formulation itself is more important for the palatability than formulation type. Prohibition of loose baits to amateurs should be subject to derogation from the mutual recognition at the product authorization stage.

For PCOs and professionals, bait can either be presented in tamper-resistant bait stations, or in open trays that are protected from non-target species using a combination of natural cover, materials located on site and materials brought onto site specifically for that purpose. **Infestations are likely to be large, and non-target impact will be minimized by optimizing bait presentation to the rodents, and thus minimizing the duration of the treatment. The utility of tamper resistant bait points will vary from site to site and their use should be left to the discretion of the operator, in the light of the risk assessments conducted at the outset of the treatment.**

At the workshop it was agreed that the use of non-conventional bait stations (e.g. open trays or similar) by trained/certified professionals should be possible under certain circumstances. Member states might derogate from mutual recognition at the product authorization stage.

According to the applicant current Best Practice always requires the use of protected bait points. Bait points may be protected by use of bait stations or under covers made from materials found on the site. The use of bait stations is known to limit efficacy as they deter rats from feeding on the bait. The use of materials from the site will result in more efficacious rat control as it will reduce neophobia.

The eCA is of the opinion that covered bait stations can be accepted in specific situations only for trained professionals as long as these bait points provide the same level of protection for non-target species and humans. However, due to the increased risk of poisoning of non-target animals and humans, such covered bait stations should only be accepted for indoor use and be restricted to locations where exposure to children and non-target animals can be excluded.

Pulsed baiting should be used when SGARs are applied to reduce the quantity of bait applied provided data is available to support the efficacy of this practice with particular active substance and biocidal product.

Pulsed baiting is specific for products containing the most potent SGARs. At the workshop it was pinpointed that efficacy needs to be demonstrated. Pulsed baiting, if approved, must be mentioned specifically on the SPC/label of the product.

According to the applicant, pulse baiting is authorized only for products containing brodifacoum and flocoumafen. It is uncertain whether products containing bromadiolone and difenacoum could be used in this manner because of their lower potency. Field trial data would have to be generated to support or dismiss this proposal.

The eCA agrees that the pulse baiting is not appropriate for difenacoum.

Permanent baiting should not be conducted outdoor unless there is a high risk of re-invasion, because it poses a very high risk to non-target species.

At the workshop it was agreed that permanent baiting outdoors should be possible for trained/certified professionals under certain circumstances. This could be defined in a code of Best practice. Member States should be allowed to derogate from mutual recognition (MR) of such use at the product authorization.

The applicant commented that permanent baiting for specific locations could be appropriate as part of an IPM strategy based on site specific risk assessments.

The eCA considers restriction of permanent baiting outdoors critical for limiting exposure of non-target species. Permanent, proactive baiting outdoors is the dominating baiting strategy in professional rodent control in Finland. According to Finnish PCOs and industry that needs rodent control there is a continuous risk of reinvasion of rodents in most places. Permanent baiting with infrequent control visits is a cost-effective way to control rodents. In order to enhance more sustainable use of ARs as well as to prevent development of resistance the minimum frequency of visits should be determined for the permanent baiting.

Permanent baiting may be conducted indoors, particularly where there is a regulatory requirement, or where there is a high risk of re-invasion, because it can be managed to pose a low risk to non-target species.

At the workshop it was agreed that permanent baiting indoors should be possible for trained/certified professionals under certain circumstances. This could be defined in a code of Best practice.

The applicant agrees on the statement.

The eCA considers permanent baiting indoors more acceptable than outdoors, but we do not agree that it poses low risk to non-target species. ARs do not kill rodents instantly and rodents can live several days after ingesting lethal dose. Due to this delayed effect the poisoned rodents are available for predation by non-target species. This happens in particular for rodent species that move outside the buildings where they have eaten the bait. The eCA can still accept that permanent baiting may be necessary in some situations to guarantee hygiene and

quality of food and feed or other products to be protected from contamination of rodents. In our view in such high risk areas the minimum frequency visits should be determined for the permanent baiting.

In the first instance, the duration of outdoor baiting should always be limited to 35 days (5 weeks). Subsequent continued rodent activity could indicate that the rodents are resistant to the rodenticide, or that a significant proportion of the infestation are not being treated, and are continually moving into the treated area.

At the workshop it was agreed that an evaluation should be made after 35 days.

The applicant commented that Best Practice requires that if control has not been achieved within 35 days then the reasons should be investigated and the risk assessment updated accordingly. In some situations, e.g. sensitive areas or areas subject to constant reinvasion, baiting beyond 35 days will be justified.

The eCA agrees that anticoagulant rodenticides shall not be used beyond 35 days without an evaluation of the state of the infestation and of the efficacy of the treatment. This claim is included in the current product labels in Finland but it is frequently misinterpreted as a visiting frequency or an intake of bait after 35 days is considered as a reason to validate the need for permanent baiting. It could be helpful if clear criteria could be created for what are reasons to continue the baiting and what are reasons not to continue the baiting. It should also be clarified whether this evaluation should be done in writing and to whom it should be presented. Or should it only be in possession of the PCO and the client.

Frequency of visits should be left to the discretion of the operator, in the light of the risk assessments conducted at the outset of the treatment. The wide diversity of sites with rodent infestations precludes any strict frequency. However, as a minimum treated sites should be visited once a week.

At the workshop it was agreed that the frequency of visit should be left to the professionals.
A reference to code of best practice should be made by the MS.

The applicant commented that the frequency of visits is dependent on the infestation and site and should be evaluated in the risk assessment. Furthermore, the applicant agrees that treated sites should be visited at least once a week.

The eCA is of opinion that a minimum frequency for visits should be given in the product labels. Minimum frequency of visits would enhance a proper rodent control.

All rodent bodies should be disposed of on each visit by the PCO, and clients should be encouraged to dispose of rodent bodies, taking necessary steps to ensure their safety (providing advice on wearing gloves, minimizing contact, and washing hands after disposal). Specific recommendations for disposal of rodent bodies should be specified (avoid the general sentence "according to local regulations"). For clients and other amateurs, sealing the bodies in two separate plastic bags and safe disposal in the garbage can be considered.

At the workshop the importance to remove and dispose of dead rodent bodies was agreed. However, there were mixed opinions on the method of disposal. Hence, it was proposed to leave the method of disposal and the classification of waste to the Member State.

According to the applicant, disposal of dead and moribund rodents on every site visit is considered to be Best Practice and has been included on product labels for decades.
It was further commented that making specific recommendations for disposal on product labels which are mutually recognized is difficult as different legislation will apply in different Member

States. Thus, the preference is to indicate that the disposal should be done in accordance with local regulations. The pragmatic proposal for disposal by clients and other amateurs is considered to ensure that amateurs will dispose of rodent bodies in a proper manner.

The eCA agrees that dead rodent bodies should be removed and disposed at the end of the treatment. The disposal should be in accordance with local requirements and the method of disposal should be described specifically on the national SPC and on the label of the product. Advice on wearing of gloves when removing dead bodies to minimizing dermal contact with the bodies should be given as well as washing of hands after disposal.

Disposal of dead rodents is not considered as a particularly effective RMM to prevent secondary poisoning. Rodents may continue to live normally a few days after ingestion of lethal dose. Both target rodents and non-target small mammals that feed on the bait around buildings are preyed by predators. Many predators specialized feeding on small mammals take mainly live rodents, not carcasses. In addition, scavengers are assumed to find carcasses faster and more effective than humans do. PCOs have reported that dead rodents are found only seldom.

Uneaten bait should always be removed and disposed of at the end of the treatment. Amateurs may dispose of their remaining uneaten baits by sealing it within two plastic bags and safe disposal in the garbage.

At the workshop the importance to remove and dispose uneaten bait was agreed. However, there were mixed opinions on the method of disposal. Hence, it was proposed to leave the method of disposal and the classification of waste to the Member State.

The applicant commented that removal of uneaten bait at the end of a treatment is Best Practice and has been included on product labels for decades. Furthermore, the pragmatic proposal for disposal by amateurs will ensure that they will dispose of uneaten bait in a proper manner.

The eCA is of the opinion that uneaten bait should be removed after the treatment. The method of disposal should be described specifically on the national SPC and on the label of the product as proposed in the summary of the workshop. Advice on wearing of gloves when removing uneaten bait should be given as well as washing of hands after disposal. In Finland rodenticide baits are considered as dangerous waste.

Resistance in rodent populations should be managed by ensuring that only effective ARs are used to control population rodents. For House mice, first generation anticoagulants should be avoided unless there is good evidence that populations can be controlled with a particular active ingredient, and for House mice and Norway rats, resistance surveys involving the sequencing of the VKORC1 gene should be conducted for any population of rodents where physiological resistance is suspected. Where mutations of the VKORC1 gene are detected, subsequent use of ARs should be restricted to the active ingredients currently believed to be efficacious against that particular mutation. Such information should be made widely available across all MSs in a format similar to that of the Rodenticide Resistance Action Group (see RRAG, 2010), and should be regularly updated in the light of results generated across all member states.

In the long term, mapping of the different VKORC1 mutations across all MSs should also be made available online, to allow predictions to be made for new infestations located within areas that have previously been surveyed.

Monitoring based on sequencing of the VKORC1 gene was generally supported at the workshop. However, the organisation and funding of such a monitoring regime was questioned. The expert team offered to make a proposal in cooperation with CEPA and CAs on the set up of a monitoring system taking into account regional information.

According to the applicant ideally where the resistance status is known prior to treatment, products containing the least potent active substance that will effectively complete control should be used first. FGAR-, bromadiolone- and difenacoum-containing products should not be used where there is evidence of resistance. If there is no evidence of resistance, any authorised product can be used. Evidence includes failing to control an infestation after exclusion of all factors other than resistance. This reflects the position held by Industry as developed by CropLife's Rodenticide Resistance Action Committee, the Rodenticide Resistance Action Group in the UK and similar groups within the EU.

Depending on the feasibility of implementation of a resistance monitoring programme at EU-level, the eCA agrees that information on resistance throughout EU should be made available online.

RMM to be set at the stage of product authorisation

Bait stations should be mandatory for amateur products. Various levels of protection can be obtained with the different bait stations and it is suggested to develop specific requirements for bait stations qualification. Different levels of protection are described in the document and levels 2-3 should be considered for amateurs.

This particular issue was apparently not discussed at the workshop, as not reflected in the summary.

All bait formulations should be available to all user categories, with limited amounts and tamper-resistant bait stations for amateurs.

This particular issue was only partly discussed at the workshop as referred earlier in the text.

A standardized Summary of Product Characteristics (SPC) template should be completed for all products and readily available to all potential users. It should be the basis for label recommendations. It is strongly suggested to have a common and simplified label across MSs.

A work is on-going in EU to harmonise as far as possible the relevant section of the SPCs for anticoagulant rodenticides. A Working Party (WP) was set up in autumn 2015 to discuss the relevant SPC sections, keeping in mind that the risk mitigation measures (RMMs) are also affected by the BPC discussions in the context of the renewal of the active substances.

Product manufacturers should provide a list of the information media available for the various user categories. Information leaflets or labels should be provided at this stage.

Ensuring that appropriate information (label, leaflet) is supplied to the user is essential. In addition easily understandable online information should be available.

2.3. Overall conclusions

The outcome of the assessment for difenacoum in product-type 14 is specified in the BPC opinion following discussions at the 16 meeting of the Biocidal Products Committee (BPC). The BPC opinion is available from the ECHA website.

2.4. Requirement for further information related to the biocidal product

No further information is required. Nevertheless, the authorisation holder shall report any observed resistance incidents to the Competent Authorities of other appointed bodies involved in resistance management.

2.5. List of endpoints

The most important endpoints for the active substance, based on the original evaluation and the revaluation performed for the renewal of approval, are listed in Appendix I.

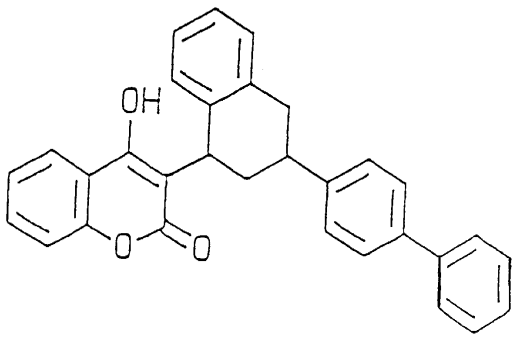
Appendix I: List of endpoints

New data incorporated since first approval are highlighted by yellow.

Chapter 1:Identity, Physical and Chemical Properties, Classification and Labelling

Active substance (ISO Name)	Difenacoum
Product-type	PT 14

Identity

Chemical name (IUPAC)	3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin
Chemical name (CA)	2H-1-Benzo pyran-2-one, 3-(3-[1,1'-biphenyl]-4-yl-1,2,3,4-tetrahydro-1-naphthalenyl)-4-hydroxy-
CAS No	56073-07-5
EC No	259-978-4
Other substance No.	514 (CIPAC No)
Minimum purity of the active substance as manufactured (g/kg or g/l)	960 g/kg
Identity of relevant impurities and additives (substances of concern) in the active substance as manufactured (g/kg)	None
Molecular formula	$C_{31}H_{24}O_3$
Molecular mass	444.5
Structural formula	

Physical and chemical properties

Melting point (state purity)	211 – 215 °C (Purity: 98.7% w/w) (S) An endotherm at 226.3 °C, melting is proposed. (Purity: 99.7% w/w) 216.3 – 226 °C, melting (with signs of degradation) (99.7% w/w) (A/P)

Boiling point (state purity)	No boiling point before start of decomposition. (S) No boiling point detected, In tests up to the temperature of 250 °C. (99.7% w/w) (A/P)
Thermal stability / Temperature of decomposition	>300 °C (96.5%) (S) >250 °C (99.7%) (250 °C was the highest temp. of test) (A/P)
Appearance (state purity)	White fine powder at 20 °C (Purity: 98.7% w/w), off-white for technical grade. (S) Solid off-white powder (99.7%) (A/P) Buff/beige fine powder (technical grade, >90%), (A/P)
Relative density (state purity)	1.27 at 20.5 °C (Purity: 98.7% w/w) (S) 1.1363 at 20 °C (Purity: >99% w/w) (A/P)
Surface tension (state temperature and concentration of the test solution)	Not determined. Not applicable.
Vapour pressure (in Pa, state temperature)	1.9×10^{-11} Pa, with total error of x 352.5, at 25 °C (98.7%), (computer-based estimation). This can be expressed also as a range of 6.7×10^{-9} – 5.4×10^{-14} Pa. The high-end value was used for Henry's law constant. (S) $< 5 \times 10^{-5}$ Pa at 45 °C (99%), an estimation. (A/P)
Henry's law constant (Pa m^3 mol^{-1})	1.75×10^{-6} Pa m^3 mol^{-1} at pH 7 4.9×10^{-8} Pa m^3 mol^{-1} at pH 9 (S) <0.046 Pa m^3 $mole^{-1}$, an estimation (A/P)
Solubility in water (g/l or mg/l, state temperature)	pH 4: <0.05 mg/l at 20 °C (97.8%) (S) pH 5.1: ≤ 0.05 mg/l 20 °C (99.7%) (A/P) pH 7: 1.7 mg/l at 20 °C (S) pH 6.5: 0.43 mg/l at 20 °C (A/P) pH 9: 61 mg/l at 20 °C (S) pH 8.9: 3.72 mg/l at 20 °C (A/P)

Solubility in organic solvents (in g/l or mg/l, state temperature)	Purity: 96.3% w/w Temperature: 20 °C Acetone: 7.6 g/l Propan-2-ol: 1.5 g/l Ethylacetate: 3.7 g/l Toluene: 1.2 g/l Methanol: 1.2 g/l Hexane: 12.1 g/l Dichloromethane: 19.6 g/l (S) Purity: 99.7% w/w Temperature: 20 °C Toluene : 1.49 g/l Ethyl acetate: 3.60 g/l Methanol: 1.00 g/l Acetone: 8.12 g/l Dichloromethane: 17.39 g/l (A/P)
Stability in organic solvents used in biocidal products including relevant breakdown products	Not applicable
Partition coefficient (log P_{OW}) (state temperature)	pH 3.8 at 25 °C: 7.22 pH 4 at 25 °C: 7.16 pH 7 at 25 °C: 4.78 pH 9 at 25 °C: 3.35 The $Log_{10}P_{ow}$ at pH 3.8 is a true $Log_{10}P_{ow}$ as difenacoum is unionised at pH 3.8. At pH 4, 7 and 9 the $Log_{10}P_{ow}$ is a $Log_{10}D_{ow}$ as at those specific pH values difenacoum is ionised.
Dissociation constant	pKa value 4.84 (purity: 96.2%) (S) pKa value 4.5_± 1.00 (a QSAR estimation) (A/P)
UV/VIS absorption (max.) (if absorption > 290 nm state ε at wavelength)	Wavelength of peak (nm) 310.6 and 259.4 $\varepsilon_{310.6}$ = 17 100 $M^{-1}cm^{-1}$ $\varepsilon_{259.4}$ = 46 600 $M^{-1}cm^{-1}$ (98.7%) (S) Wavelength of peak (nm) 308 and 259 ε_{308} = 12926 l/mol.cm^{-1} ε_{259} = 28515 l/mol.cm^{-1} (98.8%) (A/P)
Flammability or flash point	Not highly flammable (96.18%) No self-ignition at temperatures up to melting point (211-215 °C) (S) Not highly flammable (>99%) No self-ignition at temperatures up to 215 °C, high end temperature of the test (99%) (A/P)
Explosive properties	Not explosive (based on expert statement) (S) Not explosive (based on a statement) (A/P)

Oxidising properties	Not oxidizing (96.18%) (S) Not oxidizing (based on studies and a statement) (>99%) (A/P)
Auto-ignition or relative self-ignition temperature	No self-ignition at temperatures up to melting point, 211-215 °C (215 °C is the maximum temperature in the test) (S)

Classification and proposed labelling

with regard to physical hazards	None
with regard to human health hazards	Acute Tox. 1 H300 Acute Tox. 1 H310 Acute Tox. 1 H330 STOT RE H372 Repr. 1B H360D SCL Repr. 1B: H360D C ≥ 0.003% SCL STOT RE: H372: C ≥ 0.02%; H373: 0.002% ≤ C ≤ 0.02%
with regard to environmental hazards	Aquatic Acute 1 H400 Aquatic Chronic 1 H410 M=10 for Aquatic Acute and Chronic

Chapter 2:Methods of Analysis

Analytical methods for the active substance

Technical active substance (principle of method)	Difenacoum quantified in technical grade material by HPLC with U.V. detection at 254 nm using an internal standard. (S, A/P)
Impurities in technical active substance (principle of method)	Impurities in technical grade material quantified by HPLC with U.V. detection using either an internal or external standard. (S, A/P)

Analytical methods for residues

Soil (principle of method and LOQ)	After extraction of the soil samples by acidified dichloromethane: methanol, followed by filtration and evaporation quantification is done by HPLC with MS/MS detector and external standardisation. The method has been acceptably validated for samples of soil containing difenacoum at levels of 0.01-0.1 mg/kg. LOQ is 0.01 mg/kg. (S) After extraction of the soil samples by chloroform:acetone, concentrated extracts are purified with a Florisil-sodium sulphate column. Quantification is done by HPLC-DAD detector. The method has been acceptably validated for samples of soil containing difenacoum at levels of 0.016, 0.063 and 0.158 mg/kg. LOQ is 0.0214 mg/kg. (A/P)

Air (principle of method and LOQ)	Not relevant, due to the low vapour pressure of difenacoum
Water (principle of method and LOQ)	Extraction of difenacoum from surface water involves acidification of the surface water samples, followed by extraction with dichloromethane. Quantification is done by LC-MS/MS in positive chemical ionisation mode. LOQ is 0.01 µg/l. (S & HS) The test method for determination of difenacoum in drinking, ground and surface waters is based on extraction by dichloromethane. Quantification is done by LC-MS/MS (both SIM and SMR mode). LOQ is 0.05 µg/l for drinking water and groundwater and 0.5 µg/l for surface water. The LOQ is not acceptable and a new method for water is required. (A/P)
Sediment (principle of method and LOQ) [Entry copied from the original LOEP]	Extraction of difenacoum from sediment involves a double extraction with acidified dichloromethane:methanol (4:1, v/v), followed by a filtration step. Quantification is done by LC-MS/MS in positive chemical ionisation mode. LOQ is 0.01 mg/kg. (S) Difenacoum is extracted from sediment with acetone/hexane. After centrifugation an aliquot of the extract is purified on SPE cartridges and eluted with ethyl acetate/methanol/formic acid. The samples are dried and re-dissolved in acetonitrile/water. The LOQ is 0.01 mg/kg. Study CEMR 4470 (A/P)
Body fluids and tissues (principle of method and LOQ)	A method is presented for analysis of difenacoum in liver tissue. In this method, difenacoum is extracted from rat liver with acetonitrile and quantified by HPLC with fluorescence detection. The method has been validated for specificity, accuracy, linearity and precision. The LOQ is 0.01 mg/kg (H&S, S) Muscle and liver samples are extracted with acetone/hexane. After centrifugation an aliquot of the extract is purified on SPE cartridges and eluted with ethyl acetate/methanol/formic acid. The samples are dried and re-dissolved in acetonitrile/water. The LOQ is 0.01 mg/kg. Study CEMR-4469 (A/P)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	The DFG method S 19 was validated for the determination of difenacoum residues in food of plant animal origin. In this method difenacoum residues are extracted from cucumber with acetone/water and for liquid /liquid partition, ethyl acetate/ cyclohexane and sodium chloride and the phases are separated. From the citrus samples with acetone/water after neutralizing the acid matrix/water mixture with sodium hydrogen carbonate. The wheat flour samples are thoroughly mixed with water, heated to 40 °C and soaked for 30 min and thereafter extracted with acetone/water. The determinations are performed by LCMS/MS. The LOQ is 0.01 mg/kg for each matrix. (S, HS) Method of residue analysis for cucumber, wheat and lemon has been validated acceptably. The purified extracts are analysed for residues of difenacoum by LC-MS. LOQ is 0.01 mg/kg. (A/P) Oilseed rape seed samples are extracted by liquid/liquid extraction with water/ethyl acetate. After centrifugation the organic layer is purified on SPE cartridges and eluted with ethyl acetate/methanol/formic acid). The samples are re-dissolved in acetonitrile /water. The LOQ is 0.01 mg/kg. Study CEMR-4469 (A/P)
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	An LC MS/MS method for identification and quantification of difenacoum residues in animal origin (meat) has been validated. The method is highly specific, linear, accurate and precise. Samples are mixed with calcium silicate and extracted with acetonitrile/acetone, after that the residue is dissolved in ethyl acetate/ cyclohexane and an aliquot of this solution is cleaned. The LOQ of the method is 0.01 mg/kg. (A/P, H&S, S)

Chapter 3:Impact on Human Health

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of oral absorption:	Peak plasma level reached 4-24 h after dosing. 82% of a low dose and 74% of a high dose absorbed within 168 h (faecal metabolites included). This amount is expected to be the minimum, because the measured metabolite:difenacoum ratio is at 24 h, and it is expected to increase between 24 and 168 h. (S) Bile duct cannulated animals: 68% after a single 0.1 mg/kg dose (bile, urine, liver and carcass included). (A/P)
Rate and extent of dermal absorption*:	The estimated dermal absorption in humans is 3% for the Neosorexa Pellets, based on an *in vitro* study using human skin. (S) 0.047% for the Roban wax block, during 24 h after 8 h exposure in an *in vitro* study with human skin. (A/P)
Distribution:	Widely distributed; highest residues in liver
Potential for accumulation:	Yes, long half-lives for elimination and binding to liver
Rate and extent of excretion:	Slow, biphasic with half-lives of 3 and 118 days. (S). During a five-day sampling, elimination half-lives of 55 and 42 hours in females depending on dose level, and 45 and 31 hours in males, respectively (A/P). Within seven days 37-55% eliminated in faeces and less than 3% in urine
Toxicologically significant metabolite(s)	21-39% of the administered dose is as metabolites in faeces (hydroxylated difenacoum and glucuronide conjugates identified by A/P). 2-5 unidentified metabolites found in liver. Metabolism is assumed to lower the anticoagulant potency significantly

*The dermal absorption value is applicable for the active substance and might not be usable in product authorization as the DA was not evaluated according to the EFSA guidance (2012).

Acute toxicity

Rat LD_{50} oral	1.8 mg/kg bw to the male rat; 2.6 mg/kg bw to the female rat.
Rat LD_{50} dermal	63 mg/kg bw (95% confidence limits 34-85) to the male rat. Two out of five deaths at 20 mg/kg bw (males) 51.54 mg/kg bw (females)

Rat LC_{50} inhalation	3.646 - 5.848 µg/l/4 h, head-only (S) 16.27-20.74 µg/l/4 h, nose only (A/P)
Skin corrosion/irritation	Not irritating
Eye irritation	Not irritating
Respiratory tract irritation	Not irritating
Skin sensitisation (test method used and result)	Negative (Magnusson and Kligman test and Buehler). Overall conclusion: Not a skin sensitizer
Respiratory sensitisation (test method used and result)	-

Repeated dose toxicity

Short term

Species / target / critical effect	Rat, haemorrhage, death
Relevant oral NOAEL / LOAEL	1.8 mg/kg bw to the male rat; 2.6 mg/kg bw to the female rat
Relevant dermal NOAEL / LOAEL	63 mg/kg bw (95% confidence limits 34-85) to the male rat. Two out of five deaths at 20 mg/kg bw (males) 51.54 mg/kg bw (females)
Relevant inhalation NOAEL / LOAEL	3.646 - 5.848 µg/l/4 h, head-only (S) 16.27-20.74 µg/l/4 h, nose only (A/P)

Subchronic

Species/ target / critical effect	Rat (90-day); prothrombin time prolongation, kaolin-cephalin time prolongation, haemorrhage.
Relevant oral NOAEL / LOAEL	0.03 mg/kg bw/day
Relevant dermal NOAEL / LOAEL	-
Relevant inhalation NOAEL / LOAEL	-

Long term

Species/ target / critical effect	-
Relevant oral NOAEL / LOAEL	**-**
Relevant dermal NOAEL / LOAEL	-
Relevant inhalation NOAEL / LOAEL	-

Genotoxicity	*In vitro:* positive result in mammalian gene mutation test and in mammalian chromosome aberration tests. *In-vivo*: Negative results in micronucleus tests and in UDS-tests. Conclusion: No genotoxic effects

Carcinogenicity

Species/type of tumour	Study waived
Relevant NOAEL/LOAEL	-

Reproductive toxicity

Developmental toxicity

Species/ Developmental target / critical effect	Rabbit: increased clotting time and hemorrhage in dams; no clear developmental toxicity in fetuses (some defects or skeletal variations observed without dose-dependence). Rat: Hemorrhages in dams; no effects in fetuses
Relevant maternal NOAEL	Rabbit: 13-day exposure (gestation days 8-20) (S): NOEL/NOAEL: 0.005 mg/kg bw/day 22-day exposure (gestation days 7-28)(A/P): LOAEL: 0.001 mg/kg bw/day Rat: NOEL/NOAEL: 0.03 mg/kg bw/day
Relevant developmental NOAEL	Rabbit: 13-day exposure (gestation days 8-20) (S): NOEL/NOAEL 0.015 mg/kg bw/day 22-day exposure (gestation days 7-28)(A/P): NOEL/NOAEL: 0.01 mg/kg bw/day Rat: NOEL/NOAEL: 0.09 mg/kg bw/day

Fertility

Species/critical effect	Rat: Haemorrhages in parents, no clear effects on fertility, but some indications of possible effects on ovarian function (changes in oestrus cycle and ovarian cysts). (A/P)
Relevant parental NOAEL	No NOEL
Relevant offspring NOAEL	-
Relevant fertility NOAEL	-

Neurotoxicity

Species/ target/critical effect	Not available. No evidence for neurotoxic potential from other studies

Developmental Neurotoxicity

Species/ target/critical effect	No signs of developmental neurotoxicity

Immunotoxicity

Species/ target/critical effect	No signs of immunotoxicity

Developmental Immunotoxicity

Species/ target/critical effect	No signs of developmental immunotoxicity

Other toxicological studies

-

Medical data

Routine monitoring of workers (industrial users) producing the active substance and formulating products has been carried out for the last forty years. Between June 1981 and September 1982, three poisoning incidents occurred with successful recovery. With the exception of these incidents, routine monitoring has shown no clinical effects in any workers. During this time there has been no evidence of allergy, sensitisation or any other abnormal effects induced by repeated and continual exposure to these anticoagulant rodenticides. (S)

Regular health screening of manufacturing workers in one facility producing anticoagulant rodenticides, including difenacoum, since 1970's have not revealed poisoning cases or any other adverse health effect related to difenacoum. (A/P)

Summary

	Value	Study	Safety factor
$AEL_{long\text{-}term}$	0.0000011 mg/kg bw/day	Rabbit teratogenicity study	300 +factor 2 to extrapolate from LOAEL to NOAEL
$AEL_{medium\text{-}term}$	0.0000011 mg/kg bw/day	Rabbit teratogenicity study	300 +factor 2 to extrapolate from LOAEL to NOAEL
$AEL_{short\text{-}term}$	0.0000011 mg/kg bw/day	Rabbit teratogenicity study	300 +factor 2 to extrapolate from LOAEL to NOAEL
ADI[4]	Not applicable		
ARfD	Not applicable		

[4] If residues in food or feed.

MRLs

Relevant commodities	-

Reference value for groundwater

According to BPR Annex VI, point 68	0.01 µg/L

Dermal absorption

Study (*in vitro/vivo*), species tested	In vitro, human skin (S) In vitro, human skin (A/P)
Formulation (formulation type and including concentration(s) tested, vehicle)	Pellet bait, 0.005% (S) Wax block and pasta bait, 0.005% (A/P)
Dermal absorption values used in risk assessment	3% (S) 0.047% (A/P)

Acceptable exposure scenarios (including method of calculation) [5]

Formulation of biocidal product	Formulation of biocidal product was not covered by the BPD and was not calculated.
Intended uses	Rodenticide to be used in and around buildings for *Mus musculus/domesticus, Rattus norvegicus* and *Rattus rattus.* Open area and waste dump: *Rattus norvegicus* and *Rattus rattus.* Sewer: *Rattus rattus.*
Industrial users	Industrial use (manufacture of a.s. and formulation of products) was not covered by the BPD and was not calculated.

[5] New guidance on exposure has been made after the exposure assessment was made (Assessment Report of 2007). More specifically, a harmonised approach for the assessment of anticoagulant rodenticides was made by HEEG in 2010-2012 (HEEG opinion 10 and 12), including agreed numbers of daily manipulations and proposals for harmonised exposure values from the CEFIC Operator exposure studies to be used in the exposure assessment.
The AEL short term (see above) would also influence the outcome of the risk assessment.

Professional users	<u>Application scenario: decanting, placing of pellet or grain bait and clean-up</u> Bait size: 200 g Frequency: 79 exposure situations per day Concentration of a.s.: 0.005% (w/w) Acceptable exposure occurs with gloves (% AOEL91). <u>Application scenario: placing of wax block bait and clean-up</u> Bait size: (200 g) calculations based on number (ten) of baits placed per bait site Frequency: 75 exposure situations per day (60 loadings and 15 clean-ups) Concentration of a.s.: 0.005% /w/w) Acceptable exposure occurs with gloves (%AOEL 11.8). Calculations based on the results of an Operator Exposure study.
Non professional users	<u>Application scenario: placing of pellet or grain bait and clean-up</u> Bait size: 200 g Frequency: 10 exposure situations per day Concentration of a.s.: 0.005% (w/w) Acceptable exposure occurs without gloves (%AOEL 91). <u>Application scenario: placing of wax block bait and clean-up</u> Bait size: (200 g) calculations based on number (ten) of baits per bait site Frequency: 10 exposure situations per day (5 loadings and 5 clean-ups) Concentration of a.s.: 0.005% (w/w) Acceptable exposure occurs when gloves are not worn (% AOEL 10.9). Calculations based on the results of an Operator Exposure study.
General public	Indirect exposure due to transient mouthing by infants is not safe.
Exposure via residue in food	Not applicable

Chapter 4: Fate and Behaviour in the Environment

Route and rate of degradation in water

Hydrolysis of active substance and relevant metabolites (DT_{50}) (state pH and temperature)	
pH 5	At low pH difenacoum insoluble S & HS DT_{50}: > 1year (pH 4 and 25°C) A/P
pH 9	DT_{50}: >1 year (pH 9 and 50°C) S & HS DT_{50}: >1year (pH 9 and 25°C) A/P
Other pH: *7*	DT_{50}: >1 year (pH 7 and 50°C) S & HS DT_{50}: >1year (pH 7 and 25°C) A/P
Photolytic / photo-oxidative degradation of active substance and resulting relevant metabolites	<u>S & HS</u> DT_{50}: 3.26 hours at pH 5 DT_{50}: 8.05 hours at pH 7 DT_{50}: 7.32 hours at pH 9 (Data generated in aqueous solution using local natural midsummer sunlight equivalent exposure periods) No degradation products >10% was found. <u>A/P</u> DT_{50}: 38 minutes (summer) DT_{50}: 227 minutes (winter) DT_{50}: 49 minutes (spring) The half-lives have been recalculated in minutes assuming 12 hour day. Data was generated at a latitude 52° North in the early part of spring. Two degradation products >10% were detected, but not identified.
Readily biodegradable (yes/no)	No (All applicants)
Inherent biodegradable (yes/no)	No (All applicants)
Biodegradation in freshwater	Not available
Biodegradation in seawater	Not available
Non-extractable residues	Not available
Distribution in water / sediment systems (active substance)	Not available (Difenacoum will probably partition into sewage sludge/sediment due to its high log K_{ow} and poor water solubility.)
Distribution in water / sediment systems (metabolites)	Not available

Route and rate of degradation in soil

Mineralization (aerobic)	S Radioactivity extractability decreased with time. After 108 days of incubation, radioactivity extracted from soil had decreased to 78.4% for Speyer 2.2, indicating radioactive binding to soil and/or volatilisation (e.g. to CO_2, but formation of CO_2 was not measured in the study). Thus mineralization is less than 22% after 108 days. A/P The calculated half-life in soil is > 300 days based (TGD, Table 8, Kp 1.34)
Laboratory studies (range or median, with number of measurements, with regression coefficient)	
DT_{50lab} (20°C, aerobic):	439 days (Speyer 2.2 soil) (S)
DT_{90lab} (20°C, aerobic):	Not determined
DT_{50lab} (10°C, aerobic):	Not determined
DT_{50lab} (20°C, anaerobic):	Not determined
degradation in the saturated zone:	Not determined
Field studies (state location, range or median with number of measurements)	
DT_{50f}:	Not determined
DT_{90f}:	Not determined
Anaerobic degradation	Not available
Soil photolysis	Not available
Non-extractable residues	Radioactivity extractability decreased with time. After 108 days of incubation, radioactivity extracted from soil had decreased to 78.4% for Speyer 2.2, indicating radioactive binding to soil and/or volatilisation (e.g. to CO_2; the amount of bound residue was not determined by combustion) (S)
Relevant metabolites - name and/or code, % of applied a.i. (range and maximum)	Non-extractable radioactivity was assumed not to be difenacoum. There were no significant single extractable difenacoum degradates. (S)
Soil accumulation and plateau concentration	Not available

Adsorption/desorption

Ka , Kd Ka_{oc} , Kd_{oc} pH dependence (yes / no) (if yes type of dependence)	S Log Ka_{oc} estimated to be <1.25 (pH 8.46 mobile phase) by HPLC. Log Ka_{oc} estimated to be 2.08 for trans-difenacoum (pH 7.07) by HPLC. Log Ka_{oc} estimated to be 2.32 for cis-difenacoum (pH 7.07) by HPLC. Log Ka_{oc} estimated to be >5.63 (pH 3.29 mobile phase). by HPLC. Log Ka_{oc} estimated to be >5.63 (pH 4.43 mobile phase). by HPLC. A/P Ka_{oc} 67 (pH 7) by HPLC. K_{oc} value of 1 803 018 calculated by the QSAR equation for 'predominantly hydrophobics' according to the TGD part 3, table 4 (log K_{oc}=0.81 log K_{ow} +0.1) (used in PEC and PNEC calculations). (S, HS, A/P)

Fate and behaviour in air

Direct photolysis in air	Not available
Quantum yield of direct photolysis	Not determined
Photo-oxidative degradation in air	S Model calculation (AopWin 1.91): DT_{50} 2.08 h (12 h, c_{OH} = 1.5 × 10^6 molecules/cm³) DT_{50} 6.24 h (24 h, c_{OH} = 0.5 × 10^6 molecules/cm³) A/P Model calculation (EPIWIN v. 3.12): DT_{50} 2.08 h (OH radicals) DT_{50} 2.015 h (ozone)
Volatilization	S Vapour pressure 6.7 x 10^{-9} Pa Henry's law constant 1.75 x 10^{-6} Pa m^3/mol (based on water solubility of 1.7 mg/l) A/P Vapour pressure < 5 x 10^{-5} Pa at 45 °C (99%), an estimation <0.046 Pa m^3 $mole^{-1}$, an estimation Difencoum is not expected to volatilise to air in significant quantities.

Reference value for groundwater

According to BPR Annex VI, point 68	0.1 µg/L

Monitoring data, if available

Soil (indicate location and type of study)	Not available
Surface water (indicate location and type of study)	Not available
Ground water (indicate location and type of study)	Not available
Air (indicate location and type of study)	Not available

Chapter 5: Effects on Non-target Species

Toxicity data for aquatic species (most sensitive species of each group)

Species	Time-scale	Endpoint	Toxicity
Fish			
Rainbow trout (*Oncorhynchus mykiss)*	96 hours	LC_{50}	0.064 mg/l (S) 0.33 mg/l (A/P)
Invertebrates			
Daphnia magna	48 hours	LC_{50}	0.52 mg/l (S) 0.91 (A/P)
Algae			
Green alga (*Selenastrum capricornutum)*	72 hours	E_rC_{50} NOE_rC	0.80 mg/l (S) 0.25 mg/l (S) 0.51 mg/l (A/P) 0.13 mg/l (A/P)
Microorganisms			
Pseudomonas putida	6 hours	EC_{50}	>2.3 mg/l (S) >999.7 mg/l (A/P)

Effects on earthworms or other soil non-target organisms

Acute toxicity to to earthworm (*Eisenia foetida foetida*)……..	>994 mg/kg dry weight (A/P)
Reproductive toxicity to to earthworm (*Eisenia fetida*) ……	NOEC 62.5 mg/kg dw

Effects on soil micro-organisms

Nitrogen mineralization	Not available
Carbon mineralization	Not available

Effects on terrestrial vertebrates

Acute toxicity to mammals	LD_{50} 1.8 mg/kg (male rat) (S) LD_{50} 5-50 mg/kg_{bw} (female rat) (A/P)
Acute toxicity to birds	Bobwhite quail (*Colinus virginianus)* LD_{50}: 56 mg/kg_{bw} (female) (S) Japanese quail (*Coturnix coturnix japonica)* LD_{50}: 133 mg/kg_{bw} (female) (A/P)
Dietary toxicity to birds	Mallard duck (*Anas platyrhynchos)* LC_{50}: 18.9 mg/kg_{food} (S) Japanese quail (*Coturnix coturnix japonica)* LC_{50}: 1.4 mg/kg_{food} (A/P)
Reproductive toxicity to birds	Japanese Quail (*Coturnix coturnix japonica*) NOEC: 0.1 mg/kg_{food} (S, HS) Japanese quail (*Coturnix coturnix japonica*) NOEC: 0.31 mg/$kg_{drinking\ water}$, NOEL 58 µg/kg_{bw} (A/P)

Effects on honeybees

Acute oral toxicity	Not available
Acute contact toxicity	Not available

Effects on other beneficial arthropods

Acute oral toxicity	Not available
Acute contact toxicity	Not available
Acute toxicity to ……..	

Bioconcentration

Bioconcentration factor (BCF), aquatic (*Oncorhynchus mykiss)*	BCF_{Kg} 1100 L/kg, no lipid normalisation; this value shall be used for the risk and PBT assessment BCF_{Kg} 410 L/kg, 5% lipid normalisation
Depuration time (DT_{50})	5.0 days (growth corrected)
Depuration time (DT_{90})	-
Level of metabolites (%) in organisms accounting for > 10 % of residues	-
Bioconcentration factor (BCF), terrestrial (*Eisenia fetida)*	BAFss (steady state) 0.81 BAFk (second-order kinetics) 1.32
	BSAFss 0.23 BSAFk 0.22
Depuration time (DT_{50})	7.91 days
Depuration time (DT_{90})	104.45 days

Chapter 6: Other End Points

Appendix II: List of studies submitted for the renewal of approval process

Data protection is claimed by the applicant in accordance with Article 60 of Regulation (EU) No 528/2012.

Section No / Reference No[6]	Author(s)[7]	Year	Title[8] Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/ No)	Owner
III-A 2	[redacted]	2009	Difenacoum, Five-Batches Analysis and Validation of Methods, Pliva – I.Q.A. a.s, Report number: 284/031/08, March 2010. GLP Unpublished	Yes	PelGar International Ltd
III-A 2	[redacted]	2011	Difenacoum Technical: Complete Analysis of Five Batch Samples, Unpublished report number CH-292, July 2011. GLP Unpublished	Yes	Activa srl
III-A 2	[redacted]	2010	Analytical Profile of Five Batches of Difenacoum Technical, Battelle UK Ltd., Unpublished report No. MX090202, March 2010. GLP Unpublished	Yes	BASF plc Hentschke & Sawatzki KG

[6] ***Section Number/Reference Number*** should refer to the section number in Doc III-A or III-B. If the study is non-key, and hence not summarised in Doc III but mentioned in Doc II, it should be included in the reference list alongside related references and its location in Doc II indicated in brackets. (If there is a need to include a cross-reference to PPP references then an additional column can be inserted).

[7] ***Author's Name*** should include the author's surname before initial (s) to enable the column to be sorted alphabetically. If the Human Rights Charter prevents author's surnames on unpublished references being included in non-confidential documents, then it will be necessary to consider including 'Unpublished [number/year & letter] ' in Doc II, and both ' Unpublished [number/year & letter]' and the 'Authors Name' in the reference list'. This may necessitate the need for an additional column to state whether a reference is unpublished which can then be sorted.

[8] ***Title, Source (where different from company), Company, Report No., GLP (where relevant), (Un)Published*** should contain information relevant to each item (ideally on separate lines within the table cell for clarity). If useful, the name of the electronic file containing the specific study/reference could be added in brackets.

Section No / Reference No[6]	Author(s)[7]	Year	Title[8] Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/ No)	Owner
III-A 3.1.1	[redacted]	2010	Difenacoum (BAS 405 I): Determination of n-octanol/water partition Coefficient. Report no.: BR0101/B. Non-GLP Unpublished	Yes	BASF plc, Hentschke & Sawatzki KG, PelGar International Ltd and Activa srl
III-A 7.4.2	[redacted]	2012	[14C]difenacoum: Determination of the bioconcentration in rainbow trout (Oncorhynchus mykiss), Brixham Environmental Laboratory, AstraZeneca UK Limited, Report No BR0483/B (Study number 11-0087/A). GLP Unpublished	Yes	BASF plc, Hentschke & Sawatzki KG, PelGar International Ltd and Activa srl
III-A 7.5.2.1	[redacted]	2009	Earthworm Reproduction Test – Chronic Effects of Difenacoum on Eisenia fetida, Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Report no.: HEN-001/3-20. GLP Unpublished	Yes	BASF plc, Hentschke & Sawatzki KG, PelGar International Ltd and Activa srl
III-A 7.5.5	[redacted]	2010	Bioaccumulation in Terrestrial Oligochaetes, Uptake and elimination of Difenacoum in Eisenia fetida. Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME), Report no.: HEN-001/3-25. GLP Unpublished	Yes	BASF plc, Hentschke & Sawatzki KG, PelGar International Ltd and Activa srl
AR	[redacted]	1999	Pesticide Poisoning of Animals 1998: Investigations of Suspected Incidents in the United	No	Public

Section No / Reference No[6]	Author(s)[7]	Year	Title[8] Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/ No)	Owner
	[redacted]		Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.		
AR	[redacted]	2000	Pesticide Poisoning of Animals 1999: Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public
AR	[redacted]	2002a	Pesticide Poisoning of Animals 2000: Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public
AR	[redacted]	2002b	Pesticide Poisoning of Animals 2001: Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public
AR	[redacted]	2003	Pesticide Poisoning of Animals 2002: Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public
AR	[redacted]	2003	Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public

Section No / Reference No[6]	Author(s)[7]	Year	Title[8] Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/ No)	Owner
	[redacted]				
AR	[redacted]	2005	Pesticide Poisoning of Animals 2004: Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public
AR	[redacted]	2006	Pesticide Poisoning of Animals 2005: Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public
AR	[redacted]	2007	Pesticide Poisoning of Animals 2006: Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public
AR	[redacted]	2008	Pesticide Poisoning of Animals 2007: Investigations of Suspected Incidents in the United Kingdom. A Report of the Environmental Panel of the Advisory Committee on Pesticides.	No	Public
AR	[redacted]	2007	Pesticides and the intoxication of wild animals. Journal of Veterinary Pharmacology and Therapeutics 30: 93–100.	No	Public
AR	[redacted]	1997	Field evidence of secondary poisoning of foxes (*Vulpes vulpes*) and buzzards	No	Public

Section No / Reference No[6]	Author(s)[7]	Year	Title[8] Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/ No)	Owner
	[redacted]		(*Buteo buteo*) by bromadiolone, a 4-year survey. – Chemosphere 35: 1817–1829.		
AR	[redacted]	2014	Risk mitigation measures for anticoagulant rodenticides as biocidal products. Final Report.	No	Public
AR	[redacted]	2008	Acute poisoning of red kites (*Milvus milvus*) in France: data from the SAGIR network. – Journal of Wildlife Diseases 44: 417–426.	No	Public
AR	[redacted]	2010	Forekomst af antikoagulante rodenticider i danske rovfugle, ugler og små rovpattedyr. Faglig rapport fra DMU nr. 788.	No	Public
AR	[redacted]	2015	62nd meeting of Representatives of Members States Competent Authorities for the implementation of Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products. Revised version of the summary of the workshop on the RMM report held in Brussels on 26/02/2015. CA-Nov15-Doc. 5.4.	No	Public
AR	[redacted]	2004	Field evidence of secondary poisoning of free-ranging riparian mustelids by anticoagulant rodenticides in France: Implications for the conservation of the European mink (Mustela lutreola). – Journal of Wildlife Diseases 40: 688–	No	Public

Section No / Reference No[6]	Author(s)[7]	Year	Title[8] Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/ No)	Owner
	[redacted]		695.		
AR	[redacted]	2015	Relation between intensity of biocide practise and residues of anticoagulant rodenticides in red foxes (*Vulpes vulpes*). PLOS ONE DOI:10.1371/journal.pone. 0139191	No	Public
AR	[redacted]	2016	Prevalence of anticoagulant rodenticides in non-target predators and scavengers in Finland. Report of the Finnish Safety and Chemicals Agency (Tukes).	No	Public
AR	[redacted]	2007	Exposure of raptors and waterbirds to anticoagulant rodenticides (difenacoum, bromadiolone, coumatetralyl, coumafen, brodifacoum): Epidemiological survey in Loire Atlantique (France) – Bulletin of Environmental Contamination and Toxicology 79: 91–94.	No	Public
AR	[redacted]	2015	Interspecific and geographical differences in anticoagulant rodenticide residues of predatory wildlife from the Mediterranean region of Spain. – Science of the Total Environment 511: 259–267.	No	Public
AR	[redacted]	1997	Mortality Causes in British Barn Owls (*Tyto alba*), Based on 1,101 Carcasses Examined during 1963–1996. In Duncan, J. R., Johnson, D. H., Nicholls, T. H. editors. Biology and	No	Public

Section No / Reference No[6]	Author(s)[7]	Year	Title[8] Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/ No)	Owner
			Conservation of Owls in the Northern Hemisphere, Winnipeg, Canada. United States De-partment of Agriculture, p. 299–307.		
AR	[redacted]	2012	Screening of selected alkylphenol compounds, biocides, rodenticides and current use pesticides. Statilig program for forureningsovervåkning Rapportnr. 1116/1012.	No	Public
AR	[redacted]	2009	Subreport 3. Results from the Swedish National Screening Programme 2008. Subreport 3. Biocides: Difenacoum. IVL Swedish Environmental Research Institute.	No	Public
AR	[redacted]	2012	Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. – Science of the Total Environment 420: 280–288.	No	Public
AR	[redacted]	2012	Pesticide Poisoning of Animals in 2012: A Report of Investigations in Scotland. ISBN: 978-1-78412-111-2. www.scotland.gov.uk.	No	Public
AR	[redacted]	2003	Spatial and temporal analysis of second generation anticoagulant rodenticide residues in polecats (*Mustela putorius*) from throughout their range in Britain, 1992–1999. Environmental Pollution 122: 183–193.	No	Public
AR	[redacted]	2013	Anticoagulant rodenticides in predatory birds 2011: a	No	Public

Section No / Reference No[6]	Author(s)[7]	Year	Title[8] Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/ No)	Owner
	[redacted]		Predatory Bird Monitoring Scheme (PBMS) report. Centre for Ecology & Hydrology, Lancaster, UK. 29pp.		
AR	[redacted]	2014	Risico's van anticoagulantia rodenticides voor niet-doelsoorten en predatoren. Een scan van beschikbare kennis in Europa en analyses in roofvogels uit Nederland. Alterra-rapport 2589| ISSN 1566-7197. (In Dutch with English summary)	No	Public

Appendix III: Confidential Annex (separate document)

Appendix IV: Residues of anticoagulant rodenticides in non-target species in some European studies

Please note that this is not an exhaustive review of all studies available on the residues of anticoagulant rodenticides.

UK

WIIS (Wildlife Incident Investigation Scheme) was a program where deaths of wildlife suspected to be caused by pesticides were investigated in the UK in 1998-2006 (Barnett al. 1999-2007). The program seems to have continued in Scotland (SASA 2012). Dead animals were sampled by the public who suspect that pesticides may have been involved in the death of the animals and they delivered the carcasses to authorities who examined carcasses and made chemical analyses. The results have been reported by individual poisoning cases with analyzed pesticides, involved species, and case information. The concentrations of pesticides have not been reported. ARs have been found in many different non-target species. The incidents with ARs belong mostly to a category unspecified use, i.e. the source of found ARs remains unknown. ARs have been found in low concentrations and they were usually not thought to have caused the death of the animals. ARs have been found in many different species including barn owls (*Tyto alba*), tawny owls (*Strix aluco*), eagle owls (*Bubo bubo*), common buzzards (*Buteo buteo*), red kites (*Milvus milvus*), sparrow hawks (*Accipiter nisus*), kestrels (*Falco tinnunculus*), foxes (*Vulpes vulpes*), badgers (*Meles meles*), otters (*Lutra lutra*) and stoats (*Mustela erminea*). The ARs most commonly found were bromadiolone and difenacoum which were assumed to be the most commonly used ARs during 1998-2006. Also brodifacoum was found, but less commonly. Brodifacoum have been earlier restricted to only indoor use in the UK. The exposure to ARs is likely more widespread than the number of incidents suggests.

Newton et al. (1997) found residues of ARs in 24% of 557 barn owls found dead in Britain in 1983-1994. Barn owls carried residues of either difenacoum, brodifacoum, bromadiolone or flocoumafen or more than one of these compounds. The trend of prevalence was increasing over years. Residues of difenacoum and brodifacoum were in the range of 5-106 and 19-515 µg/kg bw, respectively. In most cases the residues were estimated to be sublethal. Eight barn owls were estimated to have died to AR poisoning (or other reason for death was not found). The median concentration in these barn owls was 300 and 245 µg/kg bw for bromadiolone and brodifacoum, respectively. Only one barn owl was estimated to have died to difenacoum (170 µg/kg bw). Shore et al. (2003) found ARs in 36% of 50 studied polecats (*Mustela putorius*). The concentrations of the most commonly found ARs bromadiolone and difenacoum were 34-217 µg/kg and 5-917 µg/kg, respectively.

In Scotland ARs are regularly screened in predatory birds under the Predatory Bird Monitoring Scheme (PBMS, https://wiki.ceh.ac.uk/display/pbms/Home) and incidents of suspected poisoning of animals by pesticides are investigated under the Wildlife Incident Investigation Scheme (WIIS, https://www.sasa.gov.uk/wildlife-environment/wildlife-incident-investigation-scheme-wiis). In WIIS in 2012, residues were detected and identified in the livers of 51 specimens, i.e. in 36% of the total number of samples tested (SASA 2012). Walker et al. (2013) reported that most prevalent AR bromadiolone was found in 69% of barn owls, 83% in kestrels and 100 % in red kites in UK under the predatory Bird Monitoring Scheme (PBMS). Also difenacoum and brodifacoum were found, flocoumafen and difethialone were found only in few samples.

France

Residues of ARs have been studied in the wildlife disease surveillance network (SAGIR) (Berny et al. 1997, Berny 2007, Berny and Gaillet 2008). Bromadiolone was detected in 71% of 31 red foxes, 94% of 16 buzzards and in 100% of 5 red kites. Liver concentrations in foxes were 1500 µg/kg and in buzzards 400 µg/kg. Fournier-Chambrillon et al. (2004) found AR residues in 9% of 122 dead mustelid species: European mink (*Mustela lutreola*), American mink (*M. vision*), polecat and European otter. Liver concentrations ranged from 600 to 900 µg/kg. Lambert et al. (2007) detected ARs in the livers of 22 of 30 raptors. The most contaminated species was buzzard.

Germany

ARs were analysed in 331 liver samples of red fox (Geduhn et al. 2015). Residues were found in 59.8% of foxes. In 20% of foxes residues occurred at levels where biological effects are suspected. The most commonly found AR was brodifacoum followed by bromadiolone, flocoumafen, difenacoum, difethialone, coumatetralyl, warfarin and chorophacinone. Brodifacoum also occurred in the highest concentrations (median) followed by difethialone and bromadiolone. The median concentrations of SGARs ranged from 29 to 91 µg/kg, the median concentration of coumatetralyl was 25 µg/kg. The concentrations of coumatetralyl were well in the range of concentrations of SGARs. Clearly, the prevalence of the second generation ARs was higher than the first generation ARs. No information was given on the use volumes of ARs. However, lesser use of FGARs was assumed to be due to high prevalence of resistance in Germany.

The concentrations (µg/kg) of substances are given below. The limit of detection for all substances was 1 µg/kg for coumatetralyl, 2 µg/kg for warfarin and difenacoum, 3 µg/kg for brodifacoum and bromadiolone, 5 µg/kg difethialone, flocoumafen and chorophacinone.

Substance	N	%	Mean	Median	Min	Max
Brodifacoum	151	45.6	267	91	10	2433
Bromadiolone	125	37.8	185	61	4	1574
Difenacoum	37	11.2	87	29	10	774
Flocoumafen	46	7.9	99	65	17	327
Difethialone	26	13.9	102	48	8	838
Chlorophacinone	1	0.3	13	-	-	-
Coumatetralyl	19	5.7	130	25	1	891
Warfarin	2	0.6	10	10	8	12

Spain

Prevalence of ARs was studied in predatory wildlife in the Mediterranean region of Spain (López-Perea 2015). The studied species included Algerian hedgehog (*Atelerix algirus*), European hedgehog (*Erinaceus europaeus*), scrops owl (*Otus scrops*), barn owl, tawny owl, eagle owl, long-eared owl, little owl and common buzzard. Brodifacoum, bromadiolone, difenacoum, flocoumafen, difethialone and warfarin were found in the liver of 62.8% of 344 individuals. The most commonly detected AR was brodifacoum followed by bromadiolone,

difenacoum, flocoumafen, difethialone and warfarin. No information on use volumes of these substances was given. A single AR was detected in 28.2% of samples, 34.6% had combinations of more than one AR.

The concentrations (µg/kg) of substances are given below. The limit of detection varied between 1-6 µg/kg.

Substance	N	%	Geometric mean	Min	Max
Brodifacoum	138	40.1	81.9	2	2008
Bromadiolone	119	34.6	46.0	0	2548
Difenacoum	90	26.2	12.2	0	1921
Flocoumafen	30	8.7	12.3	0	299
Difethialone	24	7.0	180.5	4	4463
Warfarin	1	0.3	611.8	-	-

The Netherlands

Prevalence of SGARs in rodent-eating avian predators (buzzard, kestrel, barn owl, little owl, eagle owl and long-eared owl) were studied in the Netherlands (van den Brink 2014). 50% of 30 studied individuals carried residues. the most commonly detected SGAR was brodifacoum (detected in 12 birds) while bromadiolone and difenacoum were detected in four birds. Highest concentrations were found in kestrels and eagle owls, generally brodifacoum.

Denmark

Residues of ARs have been studied in Denmark in 2010 and 2015 (Christensen et al. 2010, Elmeros et al. 2015). In first study ARs were detected in 84-100% of studied species. The substances detected in decreasing were difenacoum, bromadiolone, brodifacoum. coumatetralyl and flocoumafen. The most commonly used ARs in Denmark from 2000 to 2008 have been bromadiolone and coumatetralyl, other substances are used considerably less. Majority of animals carried two or more ARs. The average cumulative concentration of ARs in livers of kestrel, tawny owl, barn owl and little owls (*Athene noctua*) ranged from 35 to 58 µg/kg. Higher concentrations of 152 and 162 µg/kg were found in the red kites and eagle owl, respectively. Lower concentrations (9-24 µg/kg) of ARs were found in rough-legged buzzard (*Buteo lagopus*), marsh harrier (*Circus aeruginosus*), long-eared owl (*Asio otus*) and short-eared owl (*A. flammeus*). The highest levels of ARs were found in mustelids where average concentrations were 58 and 63 µg/kg in stoat and weasel (*Mustela nivalis*), respectively. 200 µg/kg was suggested to be a critical contamination level for raptors and owls. Overall, no differences in frequency and level of contamination were detected between sex and age groups, between time of year, or in relation to registered cause of death.

In the second study, Elmeros et al. (2015) showed that small mammals like common shrew, bank vole, field vole, harvest mouse, yellow-necked field mouse and house mouse feed baits containing bromadiolone. The proportion of small mammals with bromadiolone increased with decreased distance to the bait stations. The bromadiolone concentration in the small mammals ranged from 3 to 228 µg/kg. Lowest concentrations were found in the common shrew and highest concentrations in the yellow-necked field mouse. In the same

study AR residues were found in 99% of the stone marten (*Martes foina*) and 94% of the polecats. Bromadiolone was the most commonly detected substance and it is the most commonly used AR in Denmark. 93% of the stone martens and 73% of the polecats had detectable levels of more than one AR. The median and maximum concentrations of bromadiolone in stone marten were 1083 and 2083 µg/kg, respectively. The corresponding concentrations in the polecat were 228 and 1026 µg/kg. The maximum concentrations of brodifacoum, difenacoum, difethialone and flocoumafen ranged from 234 to 505 µg/kg in the stone marten and maximum coumatetralyl concentration in the same species was 47 µg/kg.

Finland

Prevalence of ARs in non-target animals was studied in 2014 (Koivisto et al. 2016). One or more ARs were detected in 87% of 136 studied individuals. ARs were commonly found in eagle owls (100%, sample size 12), tawny owls (85%, sample size 13) , raccoon dogs (*Nyctereutes procyonoides*) (98%, sample size 41), red foxes (100% sample size 12), pine martens (100%, sample size 7), least weasels (100%, sample size 9) and stoats (67%, sample size 12). Residues of ARs were found also in badgers, cats, otters, goshawks, hooded crows and magpies. ARs were found in about 60% of these species (sample size 2-7). ARs were not found in hen harrier, sea eagle or sparrow hawk, but only one individual from each species was studied.

The most prevalent AR was bromadiolone (found in 70% of the samples) which was also the most frequently used AR in Finland in 2014. The second most common AR present in the livers was coumatetralyl (56%) followed by difenacoum (44%), brodifacoum (23%) and flocoumafen (15%). Overall, the prevalence of ARs correlated well with the sales of these substances in the country. The concentrations (µg/kg) of substances are given below. The limit of quantification for all substances was 1 µg/kg.

Substance	Mean	Median	SE	Min	Max	N
Bromadiolone	116	32	21	1.0	920	78
Difenacoum	24	11	4.5	1.2	138	54
Brodifacoum	41	8.3	16	1.5	288	26
Flocoumafen	2.4	1.7	0.9	1.1	7.6	7
Coumatetraly	6.4	4.1	0.9	1.0	20	41

Norway

Residues of brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen were studied in five birds of prey (NIVA 2012). Brodifacoum and bromadiolone were found in 44% of 16 golden eagles (*Aquila chrysaetos*) in concentrations ranging from 11 to 154 µg/kg. Difenacoum and flocoumafen were found in 12% of golden eagles in concentrations 15 to 181 µg/kg. Bromadiolone was detected in 50% of 8 eagle owls in concentrations ranging from 74 to 158 µg/kg. Difethialone was not found in any species. Sales of different ARs studied were not reported. ARs were studied in addition in ospreys, peregrine falcons and gyrfalcons, but no residues were found.

Sweden

Residues of ARs were studied in a routine monitoring program in Sweden (Norström et al. 2009). ARs were not found in surface water, sediment, soil, sludge or fish. ARs were analyzed also in the liver of one eagle owl found dead in a landfill. The eagle owl carried residues of coumatetralyl (125 µg/kg), bromadiolone, difenacoum and brodifacoum (5 µg/kg) in a decreasing order. Coumatetralyl was found most, 125 µg/kg and brodifacoum least, 5 µg/kg. The concentrations of difenacoum and bromadiolone ranged from approximately 15 to 25 µg/kg. Bromadiolone, coumatetralyl and difenacoum were found in muscle tissues of two other eagle owls, but in much lower concentrations. Other animals were not studied.