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

PRODUCT ASSESSMENT REPORT OF A BIOCIDAL PRODUCT FOR NATIONAL AUTHORISATION APPLICATIONS

Product identifier in R4BP	**Ruby Grain**
Product type:	14 (Rodenticide)
Active ingredient(s):	Difenacoum
Case No. in R4BP	BC-HS001360-44
Asset No. in R4BP	IE-0000671-0000
Evaluating Competent Authority	Ireland – Department of Agriculture, Food & the Marine
Internal registration/file no	**IE/BPA 70529**
Date	30.04.2018 (NA-RNL renewal)

Version 2.0

1 Version History

Date	Version	Reason for revision
2011/06/30	Version 1.0	Initial PAR
2016/05/09	Version 1.1	Revised PAR
2018/04/30	Version 2.0	Updated at 1st Renewal of authorisation RNL

2 Overview of applications

Application type	refMS	Case number in the refMS	Decision date	Assessment carried out (i.e. first authorisation / amendment /renewal)	Page
National Authorisation Dir.98/8/EC	IE	n/a	2011/06/30	1st Authorisation	93
NA-RNL	IE	BC-HS001360-44	2018/04/30	Renewal	33

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1st Renewal PAR – April 2018

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

PRODUCT ASSESSMENT REPORT OF A BIOCIDAL PRODUCT FOR THE RENEWAL OF A NATIONAL AUTHORISATION (NA-RNL)

Product identifier in R4BP	**Ruby Grain**
Product type:	14 (Rodenticide)
Active ingredient(s):	Difenacoum
Case No. in R4BP	BC-HS001360-44
Asset No. in R4BP	IE-0000671-0000
Evaluating Competent Authority	Ireland – Department of Agriculture, Food & the Marine
Internal registration/file no	**IE/BPA 70529**
Date	30.04.2018 (NA-RNL renewal)

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1 Conclusion

The Irish CA for the authorisation of biocidal products has processed an application for renewal for the biocidal product Ruby Grain which contains the active substance Difenacoum (0.005 % w/w).
The assessment presented in the Product Assessment Report for the first authorisation showed acceptable efficacy but unacceptable risks for the environment, if the product is used as a rodenticide (product-type 14) for use in and around buildings, by the general public, professionals and trained professionals, and in open areas and waste dumps by professionals and trained professionals.

The conditions for granting an authorisation according to Article 19 (1) of Regulation (EU) No 528/2012[1] (BPR) are not fulfilled.
In consequence the product can only be authorised in accordance with Article 19 (5) BPR, as this Article provides Member States with the legal basis to authorise products in cases where not authorising the product would result in disproportionate negative impacts for society when compared to the risks to human health arising from the use of the biocidal product.

Detailed information on the uses appropriate at the renewal of authorisation are presented in section 2.4.
General directions for use of the product are summarised in section 2.5.

Prior to renewing the approval of anticoagulant active substances and renewing the authorisations of the respective products discussions took place at EU-level to harmonise use instructions and risk mitigation measures to the greatest possible extend. As an outcome of these discussions a set of three standard SPCs (Summary of Product Characteristics) compiling the relevant sentences for the uses that may be authorised for each of the three user categories (general public, professionals and trained professionals) has been produced (for details please refer to document CA-Nov16-Doc.4.1.b – Final).

The specific conditions from Commission Implementing Regulation (EU) 2017/1379[2] for the active substance Difenacoum were considered for the re-assessment.

The Irish CA concludes that the conditions set out in Article 5(2) b) and c) of the BPR are currently met. Anticoagulant rodenticides are considered essential to ensure appropriate rodent control in Ireland by

[1] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products, last amended by Regulation (EU) No 334/2014 of the European Parliament and of the Council of 11 March 2014.

[2] Commission Implementing Regulation (EU) 2017/1379 of 25 July 2017 renewing the approval of difenacoum as an active substance for use in biocidal products of product-type 14

efficient pest management and as a consequence, to prevent or control any serious danger to human and animal health in which rodents are involved.
Rodent control in Ireland currently relies largely on the use of anticoagulant rodenticides, the non-renewal of which could lead to insufficient rodent control in Ireland. This may not only cause significant negative impacts on human or animal health or the environment, but may also affect the public's perception of its safety with regard to exposure to rodents or the security of a number of economic activities that could be vulnerable to rodents, resulting in economic and social consequences in Ireland.

The product has been classified according to the 9th ATP of Regulation (EC) No 1272/2008[3]. Detailed information on classification and labelling is provided in Section 2.3.
As a consequence of the new harmonised classification, the active substance Difenacoum meets the criteria for exclusion according to Article 5(1) BPR as well as for substitution according to Article 10 BPR Therefore, in line with Article 23 (1) BPR a comparative assessment for the product Ruby Grain has been conducted (for details see Section 3.10).

Comparative assessment
In line with Article 23 (1) BPR a comparative assessment for the product has been conducted (for details see Section 3.10).
In summary it can be concluded that the criteria according Article 23(3) a), b) BPR are not fulfilled.
According to Article 23 (6) BPR the authorisation of the product will be renewed for 5 years.

Approval of the active substance
The active substance Difenacoum is included in the Union list of approved active substances and the specific provisions laid down there are fulfilled:

The authorisations of biocidal products containing Difenacoum are subject to the conditions listed in the Annex to Commission Implementing Regulation (EU) 2017/1379:

Composition and formulation
The ready-to-use product is a grain bait and contains the active substance Difenacoum.
No substance of concern has been identified.
Please refer to section 5.1 for detailed information.

Physical, chemical and technical properties
No new data was provided nor had new guidance to be taken into account for the renewal evaluation.

[3] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Accordingly, the conclusion from the former assessment regarding physical, chemical and technical properties remains valid.

Physical hazards and respective characteristics

No new data was provided, nor had new guidance to be taken into account for the renewal evaluation. Accordingly, the conclusion from the former assessment regarding physical hazards and respective characteristics remains valid.

Methods for detection and identification

No new data was provided, nor had new guidance to be taken into account for the renewal evaluation. Accordingly, the conclusion from the former assessment regarding methods for detection and identification remains valid.

Efficacy

The IE CA considers that the efficacy data has confirmed that Ruby Grain is effective in the proposed areas for use, at the recommended dose rate when used as per label recommendations. Apart from two studies using 3-year aged bait no new data was provided nor had new guidance to be taken into account for re-assessment.

An evaluation of the studies provided demonstrated that the ready-to-use block formulation proved to be both palatable to and effective against infestations of rats (*Rattus norvegicus* and *Rattus rattus*) and house mice (*Mus musculus / domesticus*).

Consequently, the conclusion from the former assessment regarding the product's efficacy against target organisms remains valid.

The conclusion of the evaluation is that the product may be authorised.

Risk assessment for human health

The human health risk assessment for this product is based on the active substance.

According to the BPC Opinion the EFSA-Guidance on dermal absorption had been taken into account when reviewing the dermal absorption of the product.

Based on the risk assessment of the active substance, a risk for professional users resulting from the intended use is unlikely.

For risk mitigation measures please refer to section 2.

Due to the new classification (Repr.1B) it is not allowed to grant authorisation for the use by general public (Article 19 (4) and (5) BPR). Therefore the product will not be authorised for the non-professional user.

Based on the risk assessment it is unlikely that the intended use(s) cause any unacceptable acute or chronic risk to professional users, bystanders and residents. Regarding the trained professional users health protection, there are no objections against the intended uses if the directions for use are followed (For details see section 2).

Risk assessment for the environment

No new data was provided. The only area where new guidance was relevant was with respect to the groundwater assessment. Following discussion at the CG-18 meeting and subsequent agreement, Tier II PEC groundwater was calculated using the FOCUS models PEARL or PELMO in the instances where Tier I indicated an exceedance of the relevant trigger value.

According to the risk assessment, the risk for poisoning of non-target predator birds and mammals during primary (acute and long-term exposure) and secondary poisoning is high as the trigger value is exceeded in all cases.

No safe use was established for the Difenacoum product at a concentration of 50 ppm in the ecotoxicology risk assessment.

In consequence the product can only be authorised in accordance with Article 19 (5) BPR.

Overall conclusion

The assessment of the biocidal product Ruby Grain remains valid. However, the authorisation has to be adapted where necessary taking into account the points mentioned above.

The biocidal product will be authorised according to Article 19 (5) BPR in conjunction with Article 23 (6) BPR.

According to Article 23 (6) BPR the authorisation of the product will be renewed for 5 years.

2 Summary of the product assessment

2.1 Administrative information

2.1.1 Identifier in R4BP

Ruby Grain
Additional trade name(s): Roded Grain Bait

2.1.2 Authorisation holder

Name and address of the authorisation holder	**Name**	LODI S.A.S.
	Address	Parc d'Activités des Quatre Routes 35390 Grand Fougeray France
Authorisation number	IE/BPA 70529	
Date of the authorisation	30.04.18	
Expiry date of the authorisation	30.04.23	

2.1.3 Manufacturer(s) of the product

Name of manufacturer	LODI S.A.S.
Address of manufacturer	Parc d'Activités des Quatre Routes 35390 Grand Fougeray France
Location of manufacturing sites	Parc d'Activités des Quatre Routes 35390 Grand Fougeray France

2.1.4 Manufacturer(s) of the active substance(s)

Active substance	**Difenacoum**
Name of manufacturer	PelGar International Limited

Address of manufacturer	Unit 13, Newman Lane Alton Hampshire GU34 2QR UK
Location of manufacturing sites	Prazska 54, 280 02 Kolin, Czech Republic

2.2 Product composition and formulation

2.2.1 Qualitative and quantitative information on the composition

Table 1

Common name	IUPAC name	Function	CAS number	EC number	Content (%)
Difenacoum	3-(3biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphtyl)-4-hydroxycoumarin	Active Substance	56073-07-5	259-978-4	0.005

- The product contains a bittering agent and a dye.
  - ➢ Information on the full composition is provided in the confidential[4] annex (see chapter 4).
- According to the information provided the product contains no nanomaterials as defined in Article 3 paragraph 1 (z) of Regulation No. 528/2012:

2.2.2 Information on the substance(s) of concern

There are no substances of concern.

2.2.3 Candidate(s) for substitution

The following substance was identified as a candidate for substitution:

- **Difenacoum**

Difenacoum meets the following exclusion criteria according to Article 5(1) BPR:

- toxic for reproduction category 1B
- persistent and very persistent, bioaccumulative and toxic

[4] Access level: "Restricted" to applicant and authority

Therefore Difenacoum meets the conditions laid down in Article 10 BPR, and is consequently a candidate for substitution.

2.2.4 Type of formulation

Ready-to-use bait: grain

2.3 Classification and Labelling according to the Regulation (EC) No 1272/2008[5]

Table 2

Classification **Hazard classes, Hazard categories**	**Hazard statements**
STOT RE 2	H373: May cause damage to organs (blood) through prolonged or repeated exposure
Repr. 1B	H360D: May damage the unborn child.

Table 3

Labelling	**Code**	**Pictogram / Wording**
	GHS08	
Signal word		Danger
Hazard statements	STOT RE 2	H373: May cause damage to organs (Blood) through prolonged or repeated exposure
	Repr. 1B	H360D: May damage the unborn child.
Supplemental label elements		
Precautionary statements:	P201	Obtain special instructions before use

5 Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

	P202	Do not handle until all safety precautions have been read and understood.
	P260	Do not breathe dust.
	P280	Wear protective gloves.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P314	Get Medical advice/attention if you feel unwell.
	P405	Store locked up.
	P501	Dispose of contents in accordance with local/regional/national /international regulations
Note		

2.4 Uses appropriate for further authorisation[6]

Table 4: Summary Table of Uses

No.	Use
1	House mice – professionals – indoor
2	Rats – professionals – indoor
3	House mice and/or rats – professionals – outdoor around buildings
4	House mice and/or rats – trained professionals – indoor
5	House mice and/or rats – trained professionals – outdoor around buildings
6	Rats – trained professionals – Outdoor open areas & waste dumps

2.4.1 Use 1 appropriate after renewal of the authorisation – House mice – professionals – indoor

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	House mouse (*Mus musculus / domesticus*) – adults and juveniles
Field(s) of use	Indoors
Application method(s)	Ready-to-use bait to be used in tamper-resistant bait stations

6 Member States might refuse to grant an authorisation or adjust the terms and conditions of the authorisation to be granted according to Article 37 BPR.

Application rate(s) and frequency	20-30 g of bait per bait station. 20-30 g of bait spaced 3 meters apart in case of high infestation and 5 meters apart in case of low infestation
Category(ies) of users	Professionals
Pack sizes and packaging material	Min pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachet:** 25g **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. Packaging material: **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) Loose bait: 1 kg, 1,5 kg, 2 kg, 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE / paper craft bag with inner liner in PE (loose bait)** : 5 kg, 10 kg and 20 kg **Cardboard box of wrapped sachets of 25g (PP/PE):** 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25)

2.4.1.1 Use-specific instructions for use

- The bait stations should be visited at least every 2 to 3 days at the beginning of the treatment and at least weekly afterwards, in order to check whether the bait is accepted, the bait stations are intact and to remove rodent bodies. Re-fill bait when necessary.
- [When available] Follow any additional instructions provided by the relevant code of best practice.

2.4.1.2 Use-specific risk mitigation measures

None

2.4.1.3 Where specific to the use, the particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment

When placing bait stations close to water drainage systems, ensure that bait contact with water is avoided.

2.4.1.4 Where specific to the use, the instructions for safe disposal of the product and its packaging

None

2.4.1.5 Where specific to the use, the conditions of storage and shelf-life of the product under normal conditions of storage

None

2.4.2 Use 2 appropriate after renewal of the authorisation – Rats – professionals – indoor

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Indoors
Application method(s)	Ready-to-use bait to be used in tamper-resistant bait stations
Application rate(s) and frequency	90-100 g of bait per bait station. If more than one bait station is needed, the minimum distance between bait stations should be of 5 meters (high infestation). If there is a low infestation the distance between bait stations should be 10 meters.
Category(ies) of users	Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachet: 25, 50 or 100 or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg.

	Packaging material: **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) 50g: 2.5 kg (50*50), 3 kg (60*50), 3.5 kg (70*50), 4 kg (80*50), 4.5 kg (90*50), 5 kg (100*50), 5.5 kg (110*50), 6 kg (120*50), 6.5 kg (130*50), 7 kg (140*25), 7.5 kg (150*50), 8 kg (160*50), 8.5 kg (170*50), 9 kg (180*50), 9.5 kg (190*50), 10 kg (200*50) 100g: 2.5 kg (25*100), 3 kg (30*100), 3.5 kg (35*100), 4 kg (40*100), 4.5 kg (45*100), 5 kg (50*100), 5.5 kg (55*100), 6 kg (60*100), 6.5 kg (65*100), 7 kg (70*100), 7.5 kg (75*100), 8 kg (80*100), 8.5 kg (85*100), 9 kg (90*100), 9.5 kg (95*100), 10 kg (100*100) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE / paper craft bag with inner liner in PE (loose bait) :** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets** of 25, 50 or 100 g: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) 50g: 2.5 kg (50*50), 3 kg (60*50), 3.5 kg (70*50), 4 kg (80*50), 4.5 kg (90*50), 5 kg (100*50), 5.5 kg (110*50), 6 kg (120*50), 6.5 kg (130*50), 7 kg (140*25), 7.5 kg (150*50), 8 kg (160*50), 8.5 kg (170*50), 9 kg (180*50), 9.5 kg (190*50), 10 kg (200*50) 100g: 2.5 kg (25*100), 3 kg (30*100), 3.5 kg (35*100), 4 kg (40*100), 4.5 kg (45*100), 5 kg (50*100), 5.5 kg (55*100), 6 kg (60*100), 6.5 kg (65*100), 7 kg (70*100), 7.5 kg (75*100), 8 kg (80*100), 8.5 kg (85*100), 9 kg (90*100), 9.5 kg (95*100), 10 kg (100*100)

2.4.2.1 Use-specific instructions for use

- The bait stations should be visited only 5 to 7 days after the beginning of the treatment and at least weekly afterwards, in order to check whether the bait is accepted, the bait stations are intact and to remove rodent bodies. Re-fill bait when necessary.
- [When available] Follow any additional instructions provided by the relevant code of best practice.

2.4.2.2 Use-specific risk mitigation measures

None

2.4.2.3 Where specific to the use, the particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment

When placing bait stations close to water drainage systems, ensure that bait contact with water is avoided.

2.4.2.4 Where specific to the use, the instructions for safe disposal of the product and its packaging

None

2.4.2.5 Where specific to the use, the conditions of storage and shelf-life of the product under normal conditions of storage

None

2.4.3 Use 3 appropriate after renewal of the authorisation – House mice and/or rats – professionals – outdoor around buildings

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	House mouse (*Mus musculus / domesticus*) – adults and juveniles Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Outdoors around buildings
Application method(s)	Ready-to-use bait to be used in tamper-resistant bait stations
Application rate(s) and frequency	Mice : 20-30 g / Rats 90-100 g of bait **Mice** Low infestation – 20-30g bait in bait points every 5 metres High infestation – 20-30g bait in bait points every 3 metres **Rats** Low infestation – 90 - 100g bait in bait points every 10 metres High infestation – 90 - 100g bait in bait points every 5 metres
Category(ies) of users	Professionals
Pack sizes and packaging	Minimum pack size 2.5kg

material	**Grams of bait wrapped individually sachets (PE/PP):** 25g **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE/ paper craft bag with inner liner in PE (loose bait):** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets** of 25g: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25)

2.4.3.1 Use-specific instructions for use

- Protect bait from the atmospheric conditions (e.g. rain, snow, etc.). Place the bait stations in areas not liable to flooding.
- The bait stations should be visited [for mice - at least every 2 to 3 days at] [for rats - only 5 to 7 days after] the beginning of the treatment and at least weekly afterwards, in order to check whether the bait is accepted, the bait stations are intact and to remove rodent bodies. Re-fill bait when necessary.
- Replace any bait in a bait station in which bait has been damaged by water or contaminated by dirt.
- [*When available]* Follow any additional instructions provided by the relevant code of best practice.

2.4.3.2 Use-specific risk mitigation measures

- Do not apply this product directly in the burrows.

2.4.3.3 Where specific to the use, the particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment

When placing bait stations close to water drainage systems, ensure that bait contact with water is avoided.

2.4.3.4 Where specific to the use, the instructions for safe disposal of the product and its packaging

None

2.4.3.5 Where specific to the use, the conditions of storage and shelf-life of the product under normal conditions of storage

None

2.4.4 Use 4 appropriate after renewal of the authorisation – House mice and/or rats – trained professionals – indoor

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	House mouse (*Mus musculus / domesticus*) – adults and juveniles Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Indoors
Application method(s)	Ready-to-use bait to be used in covered bait points or in tamper-resistant bait stations
Application rate(s) and frequency	For rats 90-100g, for mice 20-30g. **Mice** Low infestation – 20-30g bait in bait points every 5 metres High infestation – 20-30g bait in bait points every 3 metres **Rats** Low infestation – 90 - 100g bait in bait points every 10 metres High infestation – 90 - 100g bait in bait points every 5 metres - Permanent baiting – **Mice** - High infestation: (20-30) g of bait per baiting point every 3 meters

	- Low infestation: (20-30) g of bait per baiting point every 5 meters **Rats** - High infestation: (90-100) g of bait per baiting point every 5 meters - Low infestation: (90-100) g of bait per baiting point every 10 meters
Category(ies) of users	Trained Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachets:** 25g **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE/ paper craft bag with inner liner in PE (loose bait):** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets** of 25 g: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25)

2.4.4.1 Use-specific instructions for use

- Remove the remaining product at the end of treatment period.
- *[When available]* Follow any additional instructions provided by the relevant code of best practice.
- For permanent baiting - Where possible, it is recommended that the treated area is revisited every 4 weeks at the latest in order to avoid any selection of a resistant population.
  [When available] Follow any additional instructions provided by the relevant code of best practice.

2.4.4.2 Use-specific risk mitigation measures

- Where possible, prior to the treatment inform any possible bystanders (e.g. users of the treated area and their surroundings) about the rodent control campaign *[in accordance with the applicable code of good practice, if any]*.

- Consider preventive control measures (e.g. plug holes, remove potential food and drinking as far as possible) to improve product intake and reduce the likelihood of reinvasion.
- To reduce risk of secondary poisoning, search for and remove dead rodents during treatment at frequent intervals, in line with the recommendations provided by the relevant code of best practice
- Permanent baiting is strictly limited to sites with a high potential for reinvasion when other methods of control have proven insufficient.
  The permanent baiting strategy shall be periodically reviewed in the context of integrated pest management (IPM) and the assessment of the risk for re-infestation.
- Do not use this product in pulsed baiting treatments.

2.4.4.3 Where specific to the use, the particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment

When placing bait stations close to water drainage systems, ensure that bait contact with water is avoided.

2.4.4.4 Where specific to the use, the instructions for safe disposal of the product and its packaging

None

2.4.4.5 Where specific to the use, the conditions of storage and shelf-life of the product under normal conditions of storage

None

2.4.5 Use 5 appropriate after renewal of the authorisation – House mice and/or rats – trained professionals – outdoor around buildings

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide

Target organism(s) (including development stage)	House mouse (*Mus musculus / domesticus*) – adults and juveniles Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Outdoors around buildings
Application method(s)	Ready-to-use bait to be used in covered bait points or in tamper-resistant bait stations, or in direct application of ready-to-use bait into the burrow.
Application rate(s) and frequency	Rats: 90-100g. Mice: 20-30g **Mice** Low infestation – 20-30 g bait in bait points every 5 metres High infestation – 20-30 g bait in bait points every 3 metres **Rats** Low infestation – 90 - 100g bait in bait points every 10 metres High infestation – 90 - 100g bait in bait points every 5 metres - In burrows: 90-100g of bait per burrow. - Permanent baiting – **Mice** - High infestation: (20-30) g of bait per baiting point every 3 meters - Low infestation: (20-30) g of bait per baiting point every 5 meters **Rats** - High infestation: (90-100) g of bait per baiting point every 5 meters - Low infestation: (90-100) g of bait per baiting point every 10 meters
Category(ies) of users	Trained Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachets**: 25g **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE/ paper craft bag with inner liner in PE (loose bait):** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets of 25 g**: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*

2.4.5.1 Use-specific instructions for use

• Protect bait from the atmospheric conditions (e.g. rain, snow, etc.). Place the bait stations in

areas not liable to flooding.

- Replace any bait in baiting points in which bait has been damaged by water or contaminated by dirt.
- Remove the remaining product at the end of treatment period.
- For permanent baiting - Where possible, it is recommended that the treated area is revisited every 4 weeks at the latest in order to avoid any selection of a resistant population.
- [When available] Follow any additional instructions provided by the relevant code of best practice.
- *[For outdoor use, baiting points must be covered and placed in strategic sites to minimise the exposure to non-target species]. [When available]* Follow any additional instructions provided by the relevant code of best practice.
- When used in burrows: Baits must be placed to minimise the exposure to non-target species and children. Cover or block the entrances of baited burrows to reduce the risks of bait being rejected and spilled. *[When available]* Follow any additional instructions provided by the relevant code of best practice.

2.4.5.2 Use-specific risk mitigation measures

- Where possible, prior to the treatment inform any possible bystanders (e.g. users of the treated area and their surroundings) about the rodent control campaign *[in accordance with the applicable code of good practice, if any]*.
- Consider preventive control measures (e.g. plug holes, remove potential food and drink as far as possible) to improve product intake and reduce the likelihood of reinvasion.
- To reduce risk of secondary poisoning, search for and remove dead rodents during treatment at frequent intervals, in line with the recommendations provided by the relevant code of best practice
- Permanent baiting is strictly limited to sites with a high potential for reinvasion when other methods of control have proven insufficient.
  The permanent baiting strategy shall be periodically reviewed in the context of integrated pest management (IPM) and the assessment of the risk for re-infestation.
- Do not use this product in pulsed baiting treatments.

2.4.5.3 Where specific to the use, the particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment

When placing bait points close to surface waters (e.g. rivers, ponds, water channels, dykes, irrigation ditches) or water drainage systems, ensure that bait contact with water is avoided.

2.4.5.4 Where specific to the use, the instructions for safe disposal of the product and its packaging

None

2.4.5.5 Where specific to the use, the conditions of storage and shelf-life of the product under normal conditions of storage

None

2.4.6 Use 6 appropriate after renewal of the authorisation – Rats – trained professionals – Outdoor open areas & waste dumps

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Outdoor open areas & waste dumps
Application method(s)	Ready-to-use bait to be used in covered bait points or in tamper-resistant bait stations, or in direct application of ready-to-use bait into the burrow.
Application rate(s) and frequency	90-100g spaced 10m apart (5m apart in areas of high infestation) **Rats** Low infestation – 90 - 100g bait in bait points every 10 metres High infestation – 90 - 100g bait in bait points every 5 metres - In burrows: 90-100g of bait per burrow. - Permanent baiting – **Mice** - High infestation: (20-30) g of bait per baiting point every 3 meters - Low infestation: (20-30) g of bait per baiting point every 5 meters

	Rats - High infestation: (90-100) g of bait per baiting point every 5 meters - Low infestation: (90-100) g of bait per baiting point every 10 meters
Category(ies) of users	Trained Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachet:** 25, 50 or 100 **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) 50g: 2.5 kg (50*50), 3 kg (60*50), 3.5 kg (70*50), 4 kg (80*50), 4.5 kg (90*50), 5 kg (100*50), 5.5 kg (110*50), 6 kg (120*50), 6.5 kg (130*50), 7 kg (140*25), 7.5 kg (150*50), 8 kg (160*50), 8.5 kg (170*50), 9 kg (180*50), 9.5 kg (190*50), 10 kg (200*50) 100g: 2.5 kg (25*100), 3 kg (30*100), 3.5 kg (35*100), 4 kg (40*100), 4.5 kg (45*100), 5 kg (50*100), 5.5 kg (55*100), 6 kg (60*100), 6.5 kg (65*100), 7 kg (70*100), 7.5 kg (75*100), 8 kg (80*100), 8.5 kg (85*100), 9 kg (90*100), 9.5 kg (95*100), 10 kg (100*100) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE / paper craft bag with inner liner in PE (loose bait) :** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets** of 25, 50 or 100 g: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) 50g: 2.5 kg (50*50), 3 kg (60*50), 3.5 kg (70*50), 4 kg (80*50), 4.5 kg (90*50), 5 kg (100*50), 5.5 kg (110*50), 6 kg (120*50), 6.5 kg (130*50), 7 kg (140*25), 7.5 kg (150*50), 8 kg (160*50), 8.5 kg (170*50), 9 kg (180*50), 9.5 kg (190*50), 10 kg (200*50) 100g: 2.5 kg (25*100), 3 kg (30*100), 3.5 kg (35*100), 4 kg (40*100), 4.5 kg (45*100), 5 kg (50*100), 5.5 kg (55*100), 6 kg (60*100), 6.5 kg (65*100), 7 kg (70*100), 7.5 kg (75*100), 8 kg (80*100), 8.5 kg (85*100), 9 kg (90*100), 9.5 kg (95*100), 10 kg (100*100)

2.4.6.1 Use-specific instructions for use

- Protect bait from the atmospheric conditions (e.g. rain, snow, etc.). Place the bait stations in areas not liable to flooding.
- Replace any bait in baiting points in which bait has been damaged by water or contaminated by dirt.

- Remove the remaining product at the end of treatment period.
- *[When available]* Follow any additional instructions provided by the relevant code of best practice.
- For permanent baiting - Where possible, it is recommended that the treated area is revisited every 4 weeks at the latest in order to avoid any selection of a resistant population.
  [When available] Follow any additional instructions provided by the relevant code of best practice.
- *[For outdoor use, baiting points must be covered and placed in strategic sites to minimise the exposure to non-target species]. [When available]* Follow any additional instructions provided by the relevant code of best practice.
- When used in burrows: Baits must be placed to minimise the exposure to non-target species and children. Cover or block the entrances of baited burrows to reduce the risks of bait being rejected and spilled. *[When available]* Follow any additional instructions provided by the relevant code of best practice.

2.4.6.2 Use-specific risk mitigation measures

- Where possible, prior to the treatment inform any possible bystanders (e.g. users of the treated area and their surroundings) about the rodent control campaign *[in accordance with the applicable code of good practice, if any]*.
- To reduce risk of secondary poisoning, search for and remove dead rodents during treatment at frequent intervals, in line with the recommendations provided by the relevant code of best practice.
- Permanent baiting is strictly limited to sites with a high potential for reinvasion when other methods of control have proven insufficient.
- The permanent baiting strategy shall be periodically reviewed in the context of integrated pest management (IPM) and the assessment of the risk for re-infestation.
- Do not use this product for pulsed baiting.

2.4.6.3 Where specific to the use, the particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment

When placing bait points close to surface waters (e.g. rivers, ponds, water channels, dykes, irrigation ditches) or water drainage systems, ensure that bait contact with water is avoided.

2.4.6.4 Where specific to the use, the instructions for safe disposal of the product and its packaging

None

2.4.6.5 Where specific to the use, the conditions of storage and shelf-life of the product under normal conditions of storage

None

2.5 General directions for use

2.5.1 Instructions for use

2.5.1.1 Instructions for Use - Professionals

- Read and follow the product information as well as any information accompanying the product or provided at the point of sale before using it.
- Carry out a pre-baiting survey of the infested area and an on-site assessment in order to identify the rodent species, their places of activity and determine the likely cause and the extent of the infestation.
- Remove food which is readily attainable for rodents (e.g. spilled grain or food waste). Apart from this, do not clean up the infested area just before the treatment, as this only disturbs the rodent population and makes bait acceptance more difficult to achieve.
- The product should only be used as part of an integrated pest management (IPM) system, including, amongst others, hygiene measures and, where possible, physical methods of control.
- Consider preventive control measures (e.g. plug holes, remove potential food and drink as far as possible) to improve product intake and reduce the likelihood of reinvasion.
- Bait stations/ points should be placed in the immediate vicinity of places where rodent activity has been previously observed (e.g. travel paths, nesting sites, feedlots, holes, burrows etc.).
- Where possible, bait stations must be fixed to the ground or other structures.

- Bait stations must be clearly labelled to show they contain rodenticides and that they must not be moved or opened (see section 2.5.3 for the information to be shown on the label).
- [If national policy or legislation require it] When the product is being used in public areas, the areas treated should be marked during the treatment period and a notice explaining the risk of primary or secondary poisoning by the anticoagulant as well as indicating the first measures to be taken in case of poisoning must be made available alongside the baits.
- Bait should be secured so that it cannot be dragged away from the bait station.
- Place the product out of the reach of children, birds, pets, farm animals and other non-target animals.
- Place the product away from food, drink and animal feeding stuffs, as well as from utensils or surfaces that have contact with these.
- Wear protective chemical resistant gloves during product handling phase (glove material to be specified by the authorisation holder within the product information).
- When using the product do not eat, drink or smoke. Wash hands and directly exposed skin after using the product.
- If bait uptake is low relative to the apparent size of the infestation, consider the replacement of bait stations to further places and the possibility to change to another bait formulation.
- If after a treatment period of 35 days baits are continued to be consumed and no decline in rodent activity can be observed, the likely cause has to be determined. Where other elements have been excluded, it is likely that there are resistant rodents so consider the use of a non-anticoagulant rodenticide, where available, or a more potent anticoagulant rodenticide. Also consider the use of traps as an alternative control measure.
- Remove the remaining bait or the bait stations at the end of the treatment period.
- Bait in sachets: Do not open the sachets containing the bait.
- Loose pellets-granules, grains: Place the bait in the bait station by using a dosage devise. Specify the methods to minimise dust (e.g. wet wiping).

2.5.1.2 Instructions for Use – Trained Professionals

- Read and follow the product information as well as any information accompanying the product or provided at the point of sale before using it.

- Carry out a pre-baiting survey of the infested area and an on-site assessment in order to identify the rodent species, their places of activity and determine the likely cause and the extent of the infestation.

- Remove food which is readily attainable for rodents (e.g. spilled grain or food waste). Apart from this, do not clean up the infested area just before the treatment, as this only disturbs the rodent

population and makes bait acceptance more difficult to achieve.

- The product should only be used as part of an integrated pest management (IPM) system, including, amongst others, hygiene measures and, where possible, physical methods of control.

- The product should be placed in the immediate vicinity of places where rodent activity has been previously explored (e.g. travel paths, nesting sites, feedlots, holes, burrows etc.).

- Where possible, bait stations must be fixed to the ground or other structures.

- Bait stations must be clearly labelled to show they contain rodenticides and that they must not be moved or opened *(see section 2.5.3 for the information to be shown on the label)*.

- *[If national policy or legislation requires it]* When the product is being used in public areas, the areas treated should be marked during the treatment period and a notice explaining the risk of primary or secondary poisoning by the anticoagulant as well as indicating the first measures to be taken in case of poisoning must be made available alongside the baits.

- Bait should be secured so that it cannot be dragged away from the bait station.

- Place the product out of the reach of children, birds, pets and farm animals and other non-target animals.

- Place the product away from food, drink and animal feeding stuffs, as well as from utensils or surfaces that have contact with these.

- Wear protective chemical resistant gloves during product handling phase (glove material to be specified by the authorisation holder within the product information).

- When using the product do not eat, drink or smoke. Wash hands and directly exposed skin after using the product.

- The frequency of visits to the treated area should be at the discretion of the operator, in the light of the survey conducted at the outset of the treatment. That frequency should be consistent with the recommendations provided by the relevant code of best practice.

- If bait uptake is low relative to the apparent size of the infestation, consider the replacement of bait points to further places and the possibility to change to another bait formulation.

- If after a treatment period of 35 days baits are continued to be consumed and no decline in rodent activity can be observed, the likely cause has to be determined. Where other elements have been excluded, it is likely that there are resistant rodent so consider the use of a non-anticoagulant rodenticide, where available, or a more potent anticoagulant rodenticide. Also consider the use of traps as an alternative control measure.

Bait in sachets: [For non-emptiable sachets - Do not open the sachets containing the bait].

Loose pellets-granules, grains: Place the bait in the baiting point by using a dosage devise. Specify

the methods to minimise dust (e.g. wet wiping).

IE Only: The resistance status of the target population should be taken into account when considering the choice of rodenticide to be used. In those areas where evidence of resistance to specific active ingredients is suspected, avoid their use. To control the spreading of resistance, it is advisable to alternate baits containing different anticoagulant active ingredients.

2.5.2 Risk mitigation measures

2.5.2.1 Risk mitigation measures - Professionals

- Where possible, prior to the treatment inform any possible bystanders (e.g. users of the treated area and their surroundings) about the rodent control campaign [*in accordance with the applicable code of good practice, if any*]".
- To reduce risk of secondary poisoning, search for and remove dead rodents at frequent intervals during treatment (e.g. at least twice a week). *[Where relevant, specify if more frequent or daily inspection is required*].
- Products shall not be used beyond 35 days without an evaluation of the state of the infestation and of the efficacy of the treatment.
- Do not use baits containing anticoagulant active substances as permanent baits for the prevention of rodent infestation or monitoring of rodent activities.
- The product information (i.e. label and/or leaflet) shall clearly show that:
- -the product shall not be supplied to the general public (e.g. "for professionals only").
- - the product shall be used in adequate tamper resistant bait stations (e.g. "use in tamper resistant bait stations only").
- -users shall properly label bait stations with the information referred to in section 5.3 of the SPC (e.g. label bait stations according to the product recommendations").
- Using this product should eliminate rodents within 35 days. The product information (i.e. label and/or leaflet) shall clearly recommend that in case of suspected lack of efficacy by the end of the treatment (i.e. rodent activity is still observed), the user should seek advice from the product supplier or call a pest control service.
- Do not wash the bait stations with water between applications.
- Dispose dead rodents in accordance with local requirements *[The method of disposal shall be described specifically in the national SPC and be reflected on the product label].*

2.5.2.2 Risk mitigation measures – Trained Professionals

- Where possible, prior to the treatment inform any possible bystanders about the rodent control campaign *[in accordance with the applicable code of good practice, if any]*".

- The product information (i.e. label and/or leaflet) shall clearly show that the product shall only be supplied to trained professional users holding certification demonstrating compliance with the applicable training requirements (e.g. "for trained professionals only".

- Do not use in areas where resistance to the active substance can be suspected.

- Products shall not be used beyond 35 days without an evaluation of the state of the infestation and of the efficacy of the treatment

- Do not rotate the use of different anticoagulants with comparable or weaker potency for resistance management purposes. For rotational use, consider using a non-anticoagulant rodenticide, if available, or a more potent anticoagulant.

- Do not wash the bait stations or utensils used in covered and protected bait points with water between applications.

- Dispose of dead rodents in accordance with local requirements *[The method of disposal shall be described specifically in the national SPC and be reflected on the product label]*.

2.5.3 Particulars of likely direct or indirect effects, first aid instructions and emergency measures to protect the environment

This product contains an anticoagulant substance. If ingested, symptoms, which may be delayed, may include nosebleed and bleeding gums. In severe cases, there may be bruising and blood present in the faeces or urine.
Antidote: Vitamin K1 administered by medical/veterinary personnel only.

In case of: Dermal exposure, wash skin with water and then with water and soap.
Eye exposure, rinse eyes with eyes-rinse liquid or water, keep eyes lids open at least 10 minutes.
Oral exposure, rinse mouth carefully with water. Never give anything by mouth to unconscious person. Do not provoke vomiting. If swallowed, seek medical advice immediately and show the product's container or label.
Contact a veterinary surgeon in case of ingestion by a pet.

Bait stations must be labelled with the following information: "do not move or open"; "contains a rodenticide"; "product name or authorisation number"; "active substance(s)" and "in case of incident, call a poison centre [insert national phone number]".

Hazardous to wildlife.

2.5.4 Instructions for safe disposal of the product and its packaging

At the end of the treatment, dispose of uneaten bait and the packaging in accordance with local requirements. Use of gloves is recommended.

2.5.5 Conditions of storage and shelf-life of the product under normal conditions of storage

Shelf-life: 24 months

Store in a dry, cool and well ventilated place. Keep the container closed and away from direct sunlight.

Store in places prevented from the access of children, birds, pets and farm animals.

Keep only in original container.

2.5.6 Other information

Because of their delayed mode of action, anticoagulant rodenticides may take from 4 to 10 days to be effective after consumption of the bait.

Rodents can be disease carriers. Do not touch dead rodents with bare hands, use gloves or use tools such as tongs when disposing them.

This product contains a bittering agent and a dye.

2.5.7 Documentation

2.5.7.1 Data submitted in relation to product application

Please see General Annexes section 4.1

2.5.7.2 Access to documentation

The applicant supported the evaluation of the active substance at EU level and has full access to the documents submitted by the taskforce for the EU review programme.

3 Assessment of the product

3.1 Proposed Uses

3.1.1 Use 1 – House mice – professionals – indoor

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	House mouse (*Mus musculus / domesticus*) – adults and juveniles
Field(s) of use	Indoors
Application method(s)	Ready-to-use bait to be used in tamper-resistant bait stations
Application rate(s) and frequency	20-30 g of bait per bait station. 20-30 g of bait spaced 3 meters apart in case of high infestation and 5 meters apart in case of low infestation
Category(ies) of users	Professionals
Pack sizes and packaging material	Min pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachet:** 25g **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. Packaging material: **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) Loose bait: 1 kg, 1,5 kg, 2 kg, 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE / paper craft bag with inner liner in PE (loose bait)** : 5 kg, 10 kg and 20 kg **Cardboard box of wrapped sachets of 25g (PP/PE):** 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25)

3.1.2 Use 2 – Rats – professionals – indoor

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide

Target organism(s) (including development stage)	Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Indoors
Application method(s)	Ready-to-use bait to be used in tamper-resistant bait stations
Application rate(s) and frequency	90-100 g of bait per bait station. If more than one bait station is needed, the minimum distance between bait stations should be of 5 meters (high infestation). If there is a low infestation the distance between bait stations should be 10 meters.
Category(ies) of users	Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachet: 25, 50 or 100 or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) 50g: 2.5 kg (50*50), 3 kg (60*50), 3.5 kg (70*50), 4 kg (80*50), 4.5 kg (90*50), 5 kg (100*50), 5.5 kg (110*50), 6 kg (120*50), 6.5 kg (130*50), 7 kg (140*25), 7.5 kg (150*50), 8 kg (160*50), 8.5 kg (170*50), 9 kg (180*50), 9.5 kg (190*50), 10 kg (200*50) 100g: 2.5 kg (25*100), 3 kg (30*100), 3.5 kg (35*100), 4 kg (40*100), 4.5 kg (45*100), 5 kg (50*100), 5.5 kg (55*100), 6 kg (60*100), 6.5 kg (65*100), 7 kg (70*100), 7.5 kg (75*100), 8 kg (80*100), 8.5 kg (85*100), 9 kg (90*100), 9.5 kg (95*100), 10 kg (100*100) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE / paper craft bag with inner liner in PE (loose bait) :** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets** of 25, 50 or 100 g: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) 50g: 2.5 kg (50*50), 3 kg (60*50), 3.5 kg (70*50), 4 kg (80*50), 4.5 kg (90*50), 5 kg (100*50), 5.5 kg (110*50), 6 kg (120*50), 6.5 kg (130*50), 7 kg (140*25), 7.5 kg (150*50), 8 kg (160*50), 8.5 kg (170*50), 9 kg (180*50), 9.5 kg (190*50), 10 kg (200*50) 100g: 2.5 kg (25*100), 3 kg (30*100), 3.5 kg (35*100), 4 kg (40*100), 4.5 kg (45*100), 5 kg (50*100), 5.5 kg (55*100), 6 kg (60*100), 6.5 kg (65*100), 7 kg (70*100), 7.5 kg (75*100), 8 kg (80*100), 8.5 kg (85*100), 9 kg (90*100), 9.5 kg (95*100), 10 kg (100*100)

3.1.3 Use 3 - House mice and/or rats – professionals – outdoor around buildings

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	House mouse (*Mus musculus / domesticus*) – adults and juveniles Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Outdoors around buildings
Application method(s)	Ready-to-use bait to be used in tamper-resistant bait stations
Application rate(s) and frequency	Mice : 20-30 g / Rats 90-100 g of bait **Mice** Low infestation – 20-30g bait in bait points every 5 metres High infestation – 20-30g bait in bait points every 3 metres **Rats** Low infestation – 90 - 100g bait in bait points every 10 metres High infestation – 90 - 100g bait in bait points every 5 metres
Category(ies) of users	Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually sachets (PE/PP):** 25g **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE/ paper craft bag with inner liner in PE (loose bait):** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets** of 25g: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25)

3.1.4 Use 4 - House mice and/or rats – trained professionals – indoor

Product Type(s)	14

Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	House mouse (*Mus musculus / domesticus*) – adults and juveniles Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Indoors
Application method(s)	Ready-to-use bait to be used in covered bait points or in tamper-resistant bait stations
Application rate(s) and frequency	For rats 90-100g, for mice 20-30g. **Mice** Low infestation – 20-30g bait in bait points every 5 metres High infestation – 20-30g bait in bait points every 3 metres **Rats** Low infestation – 90 - 100g bait in bait points every 10 metres High infestation – 90 - 100g bait in bait points every 5 metres
Category(ies) of users	Trained Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachets:** 25g **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE/ paper craft bag with inner liner in PE (loose bait):** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets** of 25 g: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25)

3.1.5 Use 5 - House mice and/or rats – trained professionals – outdoor around buildings

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including	House mouse (*Mus musculus / domesticus*) – adults and juveniles

development stage)	Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Outdoors around buildings
Application method(s)	Ready-to-use bait to be used in covered bait points or in tamper-resistant bait stations
Application rate(s) and frequency	Rats: 90-100g. Mice: 20-30g **Mice** Low infestation – 20-30 g bait in bait points every 5 metres High infestation – 20-30 g bait in bait points every 3 metres **Rats** Low infestation – 90 - 100g bait in bait points every 10 metres High infestation – 90 - 100g bait in bait points every 5 metres
Category(ies) of users	Trained Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachets**: 25g **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE/ paper craft bag with inner liner in PE (loose bait):** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets of 25 g**: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*

3.1.6 Use 6 - Rats – trained professionals – Outdoor open areas & waste dumps

Product Type(s)	14
Where relevant, an exact description of the use	Rodenticide
Target organism(s) (including development stage)	Brown rats (*Rattus norvegicus*) – adults and juveniles Roof rats (*Rattus rattus*) – adults and juveniles
Field(s) of use	Outdoor open areas & waste dumps
Application method(s)	Ready-to-use bait to be used in covered bait points or in tamper-resistant bait stations

Application rate(s) and frequency	90-100g spaced 10m apart (5m apart in areas of high infestation) **Rats** Low infestation – 90 - 100g bait in bait points every 10 metres High infestation – 90 - 100g bait in bait points every 5 metres
Category(ies) of users	Trained Professionals
Pack sizes and packaging material	Minimum pack size 2.5kg **Grams of bait wrapped individually in PE/PP sachet:** 25, 50 or 100 **or loose bait** Package is restricted to separately packed bags with a maximum bag size of 10 kg. **Packaging material:** **Bucket (PP,PE):** 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) 50g: 2.5 kg (50*50), 3 kg (60*50), 3.5 kg (70*50), 4 kg (80*50), 4.5 kg (90*50), 5 kg (100*50), 5.5 kg (110*50), 6 kg (120*50), 6.5 kg (130*50), 7 kg (140*25), 7.5 kg (150*50), 8 kg (160*50), 8.5 kg (170*50), 9 kg (180*50), 9.5 kg (190*50), 10 kg (200*50) 100g: 2.5 kg (25*100), 3 kg (30*100), 3.5 kg (35*100), 4 kg (40*100), 4.5 kg (45*100), 5 kg (50*100), 5.5 kg (55*100), 6 kg (60*100), 6.5 kg (65*100), 7 kg (70*100), 7.5 kg (75*100), 8 kg (80*100), 8.5 kg (85*100), 9 kg (90*100), 9.5 kg (95*100), 10 kg (100*100) Loose bait: 2.5 kg, 3 kg, 3.5 kg, 4 kg, 4.5 kg, 5 kg, 5.5 kg, 6 kg, 6.5 kg, 7 kg, 7.5 kg, 8 kg, 8.5 kg, 9 kg, 9.5 kg and 10 kg **Cardboard box with inner liner in PE / paper craft bag with inner liner in PE (loose bait) :** 5 kg, 10 kg and 20 kg **Cardboard box with wrapped PE/PP sachets** of 25, 50 or 100 g: 25g: 2.5 kg (100*25), 3 kg (120*25), 3.5 kg (140*25), 4 kg (160*25), 4.5 kg (180*25), 5 kg (200*25), 5.5 kg (220*25), 6 kg (240*25), 6.5 kg (260*25), 7 kg (280*25), 7.5 kg (300*25), 8 kg (320*25), 8.5 kg (340*25), 9 kg (360*25), 9.5 kg (380*25), 10 kg (400*25) 50g: 2.5 kg (50*50), 3 kg (60*50), 3.5 kg (70*50), 4 kg (80*50), 4.5 kg (90*50), 5 kg (100*50), 5.5 kg (110*50), 6 kg (120*50), 6.5 kg (130*50), 7 kg (140*25), 7.5 kg (150*50), 8 kg (160*50), 8.5 kg (170*50), 9 kg (180*50), 9.5 kg (190*50), 10 kg (200*50) 100g: 2.5 kg (25*100), 3 kg (30*100), 3.5 kg (35*100), 4 kg (40*100), 4.5 kg (45*100), 5 kg (50*100), 5.5 kg (55*100), 6 kg (60*100), 6.5 kg (65*100), 7 kg (70*100), 7.5 kg (75*100), 8 kg (80*100), 8.5 kg (85*100), 9 kg (90*100), 9.5 kg (95*100), 10 kg (100*100)

3.2 Physical, chemical and technical properties

Four new studies were provided and are evaluated below. All other conclusions from the former assessments (Original PAR and the Addendum to the Product Assessment Report, April 2012) regarding physical, chemical and technical properties remain valid. No new guidance had to be taken into account for the renewal evaluation.

<table>
<tr><th>Property</th><th>Guideline and Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>Storage stability test – accelerated storage

(8 weeks storage at 40°C ±2°C.)</td><td>CIPAC MT 46</td><td>
<table>
<tr><th>Time</th><th>Mean Conc. (ppm)</th><th>Deviation with declared value (%)</th><th>Deviation between t_0 and t_x (%)</th></tr>
<tr><td>T_0</td><td>54.52</td><td>+9.04</td><td>--</td></tr>
<tr><td>T_{8weeks}</td><td>52.34</td><td>+4.68</td><td>4.00</td></tr>
</table>
Note: The declared value was 50ppm.
</td><td>“Chemical Stability after accelerated storage of Difenacoum whole grain baits 0.005%”

Study No. LODI 16/2009
Magnier, 2010
Version Date: 2010-02-02</td></tr>
<tr><td>Storage stability test – long term storage at ambient temperature

(2 years storage at 20°C ±2°C.)</td><td>GLP</td><td>
<table>
<tr><th>Time</th><th>Mean Conc. (ppm)</th><th>Deviation with declared value (%)</th><th>Deviation between t_0 and t_x (%)</th></tr>
<tr><td>T_0</td><td>51.1</td><td>+2.2</td><td>--</td></tr>
<tr><td>T_{2yr}</td><td>47.6</td><td>-4.8</td><td>-6.8%</td></tr>
</table>
Note: The declared value was 50ppm.
</td><td>“Chemical stability and physical state of Difenacoum broken grain bait after 2 years of storage at 20 °C ± 2°C”

Study No. LODI 67/2011
Richerioux, 2011
Version Date: 2012-01-12</td></tr>
</table>

<table>
<tr><th>Property</th><th>Guideline and Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>Storage stability test – further long term storage at ambient temperature

(4 years storage at 20°C)</td><td></td><td>This test examines the chemical stability after storage at of Difenacoum red whole grain baits at 6 months, 1 year, 2 years, 3 years and 4 years.
<table>
<tr><th>Time</th><th>Conc (ppm)</th><th>Deviation between t_0 and t_x (%)</th></tr>
<tr><td>T_0</td><td>54.52</td><td>--</td></tr>
<tr><td>$T_{6\ months}$</td><td>43.96</td><td>-19.37</td></tr>
<tr><td>$T_{1\ yr}$</td><td>43.2</td><td>-20.76</td></tr>
<tr><td>$T_{14\ months}$</td><td>43.14</td><td>-20.87</td></tr>
<tr><td>$T_{2\ yr}$</td><td>42.4</td><td>-22.23</td></tr>
<tr><td>$T_{3\ yr}$</td><td>45.6</td><td>-16.36</td></tr>
<tr><td>$T_{4\ yr}$</td><td>42.3</td><td>-22.39</td></tr>
<tr><td></td><td></td><td></td></tr>
</table></td><td>“Chemical stability after storage at 20 °C after 6 months, 1 year and 3 years of difenacoum whole grain baits 0.005%”

Study No. LODI 19/2009
Richerioux, 2013
Date: 2013-12-18</td></tr>
<tr><td>Flowability/Pourability/ Dustability</td><td>CIPAC MT 172</td><td>The mean percentage of test item retained on the 5mm sieve after 5 liftings was 0.5% w/w.
No test item remained on the 5mm sieve after 20 liftings.</td><td>“Flowability of granules test on RUBIS GRAIN”

Report No. 12-912011-003
Grevin, 2012
Date: 17 October 2012</td></tr>
</table>

Conclusion on the physical, chemical and technical properties of the product

Storage stability test – accelerated storage

This study was carried out to GLP. The study was carried out at 40°C ± 2°C for 8 weeks. The deviation of difenacoum content between time zero and after 8 weeks storage at 40°C ±2°C was -4.00%, this is within 10% deviation. The study is acceptable.

Storage stability test- long term storage at ambient temperature (2 years)

This study was carried out to GLP. Relative deviation of difenacoum content in broken grain bait between analysis at initial time and after 2 years at 20°C is -6.8%, this is within 10% deviation. The test item was stable for two years at ambient temperatures. The study is acceptable.

Storage stability test – further long term storage at ambient temperature

Carried out to GLP**.** Relative deviation of difenacoum content in whole grain bait between analysis at initial time and over all time points to $T_{4\ years}$ at 20°C is -22.39% Full argumentation can be found for this large variation which was investigated previously in the Addendum to the Product Assessment Report, April 2012. This cites that Difenacoum does not degrade over time but becomes bound to the matrix and therefore becomes harder to extract. The results of the study investigating the degradation products of Difenacoum under heat and acid degradation show that Difenacoum does not degrade during storage for two years at ambient temperatures and that the efficacy of the product was not affected.

Proposed Shelf Life

A shelf life of 2 years is proposed.

Flowability

This study was carried out to GLP. Results are acceptable.

3.3 Physical hazards and respective characteristics

No new data was provided, nor had new guidance to be taken into account for the renewal evaluation. Accordingly, the conclusion from the former assessment regarding physical hazards and respective characteristics remains valid.

3.4 Methods for detection and identification

No new data was provided, nor had new guidance to be taken into account for the renewal evaluation. Accordingly, the conclusion from the former assessment regarding methods for detection and identification remains valid.

3.5 Efficacy against target organisms

The results from laboratory palatability and efficacy studies and field trials previously evaluated demonstrate that the product is both palatable to, and effective in controlling target populations of rats (*Rattus norvegicus* and *Rattus rattus*) and house mice (*Mus musculus / domesticus*) when applied according to the label advice. The grain bait formulation proved to be both attractive to and effective against infestations of brown rats and house mice in the trials and provided excellent control of the infestations treated based upon census baiting and tracking data. Thus, the previously evaluated laboratory palatability and field studies remain valid.
The results of two new laboratory trials demonstrated that the product is both palatable to, and effective in controlling target populations of brown rats and house mice after storage at ambient temperature for 3 years (36 months).
3-year palatability and efficacy data is required on the roof rat (*Rattus rattus*) to extend the proposed 36 months storage stability claim to all target organisms. In light of the demonstrated palatability and efficacy against brown rats and house mice this can be added as a post-authorisation data requirement.

Resistance to the first generation anticoagulants has been widely reported in both *Rattus norvegicus* and *Mus domesticus* since the late 1950's. The incidence of resistance to first generation anticoagulants in areas in which it is established is commonly 25-85%.
The enzyme vitamin K 2, 3 epoxide reductase (VKOR) is the target for anticoagulants. Modifications in the protein structure due to polymorphisms on the gene coding the VKOR may induce anticoagulant resistance. Most resistant strains are characterised by one single nucleotide polymorphism (SNP).

These SNPs cause the exchange of one amino acid in the VKOR enzyme. The biochemical mechanism of anticoagulant resistance has been studied in several geographic strains/VKORC1-variants of the Norway rat. Amino acid substitutions in the VKOR seem to alter its structure and function, resulting in decreased sensitivity to anticoagulant inhibition, depending on strain characteristics.

For house mice, a dominant autosomal warfarin-resistance gene was determined on chromosome 7 in house mice. Three VKORC1 sequence variants mediating resistance to anticoagulants seem to be widely distributed. House Mice carrying the homozygous of one of these variants (Y139C) were found highly resistant to warfarin and bromadiolone.

For roof rats, experiments on warfarin resistant rats indicated considerable instability in the resistance and suggested a multifactorial basis for resistance.

Some degree of resistance to difenacoum has been reported in the UK, Denmark, France and Germany but this is usually found in certain populations of rodents highly resistant to first generation anti-coagulants (Greaves et al., 1982[7]; Lund, 1984[8]; Pelz et al. 1995[9]). The resistance factor tells how much the anticoagulant dose has to be multiplied to kill resistant individuals compared to sensitive ones. The resistant factors for difenacoum in the brown rats ranged from 1.1 to 8.6 (Greaves and Cullen-Ayres 1988[10]). The study included rats resistant to warfarin and difenacoum. Resistance factors for warfarin ranged from approx. 50 to 2300. Greaves et al. (1982) reported a fivefold difenacoum dose needed to kill difenacoum resistant rats. Considerable doubt exists as to the significance of reports in UK of resistance to second-generation anticoagulants and in the UK control failures with the second-generation products are increasingly being attributed to baiting problems rather than physiological resistance (Greaves and Cullen Ayres, 1988; Quy et al. 1992a,b[11]).

Studies carried out in different European countries, in the UK more particularly (Kerins et al, 2001; see annex 1) revealed the occasional occurrence of cross-resistances to second-generation anticoagulants, such as difenacoum and bromadiolone on resistant brown rats populations to coumafene. Moreover, a publication (Baer et al., 2012) has demonstrated that the majority (91%) of warfarin resistant rat trapped in East and West parts of Belgium were also resistant to bromadiolone. The rats trapped in the region of Flanders (Northern Belgium) carried mutation Y139F. This mutation is found extensively in France where it also confers resistance to bromadiolone (Grandemange et al., 2009). The same mutation was

7 Greaves J. H.; Shepherd D. S.; Gill, J. E. (1982): An investigation of difenacoum resistance in Norway rat populations in Hampshire. *Annals of Applied Biology* 100, 581–587.

8 LUND, M. (1984): Resistance to the second generation anticoagulant rodenticides. *In Proceedings of 11th vertebrate pest conference*, Sacramento, Ca. March 6-8, 1984: 89-94.

9 Pelz H-J, Ha¨nisch D, Lauenstein G (1995) Resistance to anticoagulant rodenticides in Germany and future strategies to control *Rattus norvegicus. Pestic Sci* 43, 61–67

10 Greaves J. H.; Cullen-Ayres P. B. (1988): Genetics of difenacoum resistance in the rat. In: J. W. Suttie (Ed.), Current advances in vitamin K research, Elsevier, N.Y., 381–388.

11 Quy R.J., Shepherd D.S., Inglis I.R. (1992): Bait avoidance and effectiveness of anticoagulant rodenticides against warfarin- and difenacoum-resistant populations of Norway rats (Rattus norvegicus). *Crop Protection*, Volume 11, Issue 1, February 1992, Pages 14-20

also found in UK (Prescott et al., 2011) where applications of bromadiolone had been unsuccessful. Difenacoum is also thought to be partially resisted by rats which carry Y139F.

House mice carrying the homozygous Y139C sequence variant were found to be highly resistant to warfarin and bromadiolone. It is important to understand that all known resistance mutations, in both rats and mice, are capable of effective control with applications of the most potent second-generation anticoagulants (brodifacoum, difethialone and flocoumafen) and that no practical resistance to any of these active substances is presently known.

So, resistance to second generation anticoagulant rodenticides should not be underestimated.

An exhaustive study carried out at the French and European levels could enable to point-out resistant areas with first generation anticoagulants and potential cross-resistances to second-generation anticoagulants. It is one of the actions undertaken since 2010 in France by a group of scientists (Rodent program “impacts of anticoagulants rodenticides on ecosystems-adaptations of target rodents and effects on their predators”).

The document CropLife International (RRAC 2015) provides guidance to advisors, national authorities, professionals, practitioners and others on the nature of anticoagulant resistance in rodents, the identification of anticoagulant resistance, strategies for rodenticide application that will avoid the development of resistance and the management of resistance where it occurs.

The following are the essential elements of an effective program: survey, use of physical and chemical control techniques, environmental management, record keeping, monitoring and review.

The authorization holder should report any observed resistance incidents to the Competent Authorities or other appointed bodies involved in resistance management at the renewal of the product.

To ensure a satisfactory level of efficacy and avoid the development of resistance, the recommendations proposed in the SPC have to be implemented.

3.6 Risk assessment for human health

A dermal absorption value of 4% was used for difenacoum for the grain product..

3.6.1 Assessment of effects of the active substance on human health

See section 3.6.3.

3.6.2 Assessment of effects of the product on human health

See section 3.6.3.

3.6.3 Exposure assessment

A dermal absorption value of 4% was used for difenacoum.

The chronic AEL (**1.1*10^{-6} mg/kg bw/day) was used for the risk assessment for trained and non-trained professional users. The risk assessment utilised the recommendation of HEEG 9, 10 and 12.**

For the 'transient mouthing of poison bait' scenario, 10 mg (TNsG, with bittering agent/repellent) of the product is assumed to be swallowed by an infant per poisoning event as stated in: The Human Exposure to Biocidal Products (Technical Notes for Guidance – June 2002). The weight of the infant is assumed to be 10 Kg. The acute AEL (1.1 x 10-6 mg/kg bw/day) was used for the toddler risk assessment. An oral absorption of 100% was assumed for the mounting scenarios.

Biocidal Exposure Risk assessment for Ruby Grain difenacoum rodenticide (50 ppm) .

Professional user	
	Grain
Without PPE	573.2% of AEL (0.00000631 mg/kg bw/day)
With PPE	28.7% of AEL (0.000000315 mg/kg bw/day)
Non-trained professional user (farmer)	
	Grain
Without PPE	88.3% of AEL (0.000000972 mg/kg bw/day)
With PPE	4.4% of AEL (0.0000000486 mg/kg bw/day)
Exposure to children (Toddlers)	

	Grain
Oral exposure -treated with repellent	4545.45% AEL (0.00005 mg/kg bw/day)
Oral exposure - without repellent	2272727.27% AEL (0.025 mg/kg bw/day)

Derived values indicated a no safe usage scenarios for professional users handling the difenacoum grain product without PPE, though when PPE are utilised a safe usage scenario was obtained. Derived values for professional users handling the grain product without PPE were 0.00000631 mg/kg bw/day (573.2% AEL). Derived values for professional users handling the grain product with PPE were 0.000000315 mg/kg bw/day (28.7% AEL).

Derived values indicated safe usage for non-trained professional users handling the grain product with PPE and no safe usage without PPE. Derived values for non-trained professional users handling the grain product without PPE were 0.000000972 mg/kg bw/day (88.3% AEL). Derived values indicated safe usage for non-trained professional users handling the grain product with PPE. Derived values for non-trained professional users handling the grain product with PPE were 0.0000000486 mg/kg bw/day (4.4% AEL).

Derived values indicated no safe exposure scenarios for toddlers through oral exposure/transient mouthing of the grain product. Derived values for oral exposures in the toddler found transient mounting of a grain not containing a repellent to result in a dose of 0.025 mg (2272727.27% AEL). Derived values for oral exposures in the toddler found transient mounting of a grain containing a repellent to result in a dose of 0.00005 mg (4545.45% AEL). However, the design of the rat bait boxes will incorporate a tamper-proof seal system to prevent easy access to internal compartments. As a result of incorporating a tamper proof seal system toddlers are not expected to be able to gain access to the rodenticides and subsequent mouthing scenarios are deemed unlikely.

3.6.4 Risk characterisation for human health

3.6.4.1 Risk for professional users

As shown in section 3.6.2.

3.6.4.2 Risk for the general public

Not relevant.

3.6.4.3 Risk for consumers via residues in food

No new data was provided nor had new guidance to be taken into account for the renewal evaluation. Accordingly, the conclusion from the former assessment regarding risks for consumers via residues in food remain valid.

3.6.4.4 Risk characterisation from combined exposure to several active substances or substances of concern within a biocidal product[12]

The biocidal product does not contain other substances in quantities that would be of toxicological concern in the production formulation.

3.6.4.5 Summary of risk characterisation

Derived values indicated a no safe usage scenarios for professional users handling the difenacoum grain product without PPE, though when PPE are utilised a safe usage scenario was obtained. Derived values for professional users handling the grain product without PPE were 0.00000631 mg/kg bw/day (573.2% AEL). Derived values for professional users handling the grain product with PPE were 0.000000315 mg/kg bw/day (28.7% AEL).

Derived values indicated safe usage for non-trained professional users handling the grain product with PPE and no safe usage without PPE. Derived values for non-trained professional users handling the grain product without PPE were 0.000000972 mg/kg bw/day (88.3% AEL). Derived values indicated safe usage for non-trained professional users handling the grain product with PPE. Derived values for non-trained professional users handling the grain product with PPE were 0.0000000486 mg/kg bw/day (4.4% AEL).

Derived values indicated no safe exposure scenarios for toddlers through oral exposure/transient mouthing of the grain product. Derived values for oral exposures in the toddler found transient mounting of a grain not containing a repellent to result in a dose of 0.025 mg (2272727.27% AEL). Derived values for oral exposures in the toddler found transient mounting of a grain containing a repellent to result in a dose of 0.00005 mg (4545.45% AEL). However, the design of the rat bait boxes will incorporate a tamper-proof seal system to prevent easy access to internal compartments. As a result of incorporating a tamper proof seal system toddlers are not expected to be able to gain access to the rodenticides and subsequent mouthing scenarios are deemed unlikely.

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3.7 Risk assessment for animal health

No new data was provided, nor had new guidance to be taken into account for the renewal evaluation. Accordingly, the conclusion from the former assessment regarding animal health remains valid.

3.8 Risk assessment for the environment

The exposure assessment carried out for this product in 2013 is still valid. Regarding groundwater, the recent CG decision requires this now be assessed:

Groundwater assessment for rodenticides
As required by Article 31(3) of the BPR and Article 2(1)(f) of Regulation 492/2014, when carrying out their assessment of whether the conclusions of the first authorisation regarding Article 19(1)(iv) remain valid, applicants will have to address the groundwater assessment. Since no new guidance was agreed in the past that could become applicable at the time of the completion of the applications for renewal by 28/02/2017, the guidance of reference are the existing methods that are applied since years as standard tools for the assessment of active substances:
- Tier I according to Vol. IV Part B (the former TGD), as provided in chapter 2.3.8.6 of this guidance document.
- Tier II using the FOCUS models PEARL or PELMO for refinements in case Tier I would lead to an exceedance of the relevant trigger values.

The previous exposure assessment contained a Tier 1 assessment of groundwater PECs. The following is an extract from the report:

Exposure of groundwater may occur as a result of soil exposure which occurs via residues present in sewage sludge after using the bait in sewers and via direct (spillages) and disperse release (urine and faeces) after the use of the product in the scenarios in and around buildings, open areas and waste dumps. As an indication for potential groundwater levels, the concentration in porewater of agricultural soil was taken. It should be noted that this is a worst-case assumption, neglecting transformation and dilution in deeper soil layers. A summary of the PECs obtained are presented in ***Table 3.3.6.4-1****. All concentrations are less than the EU trigger value of 0.1 µg/L.*

Table 3.3.6.4-1. Predicted Environmental Concentration (µg/L) of difenacoum in groundwater

Compartment/Scenario	***ESD realistic***	***ESD realistic worst***	***ESD normal use***

	worst case scenario	***case scenario with modified input parameters***	***scenario with modified input parameters***
Sewer scenario			
Groundwater/porewater	*9.94 x 10^{-5}*	*7.29 x 10^{-5}*	
In and around buildings scenario			
Groundwater/porewater	*1.5 x 10^{-3}*	*1.1 x 10^{-3}*	*3.2 x 10^{-4}*
Open areas			
Groundwater/porewater	*5.23 x 10^{-3}*	*1.05 x 10^{-2}*	*---*
Waste dump			
Groundwater/porewater	*2.24 x 10^{-4}*	*2.5 x 10^{-4}**	*---*

*******For high infestations of rats the blocks are spaced 5 m apart. According to calculations provided by the Reviewer this could potentially result in a maximum of ~441 (21, 100 m lines of 21 blocks, 5 m apart) blocks in a 1 ha area during high infestations. This corresponds to ~44.1 kg of product, which is greater than the quantity considered under realistic worst-case conditions in the ESD. Consequently the notifiers exposure calculation is not sufficient to support this use. The Reviewer generated new exposure calculations for this use*

However, during the 2016 renewal of the active substance difenacoum, the reference value for groundwater according to BPR Annex VI, point 68, was lowered to 0.01 μg/L. As the value for the open areas scenario exceeds the trigger (0.0105μg/L) the eCA has performed a Tier II assessment using FOCUS PEARL v4.4.4. The open areas scenario exceeds the trigger (0.196μg/L) the eCA has performed a Tier II assessment using FOCUS PEARL v4.4.4. The open areas scenario outlined in the PT14 ESD describes placement of the grain bait at the bottom of a cylindrical hole of radius 4cm and depth 30cm. A larger soil cylinder of radius 28cm is assumed to be exposed to the bait. From the soil exposure performed in the 2013 evaluation, 0.0025g of active substance is deposited each campaign (Elocalsoil). The base of the cylinder has an area of 0.062m^2 (π x 0.14^2). 0.0025g spread over an area of 0.062m^2 gives an application rate of 0.0406gm^{-2} or 0.406$kgha^{-1}$. This application rate assumes the bait is placed uniformly across the field or park. In reality bait is placed in specific burrows at distances of 5m or greater where rodents are active. Therefore the actual use rate will be considerably lower than 0.406kg/ha. The ESD proposes a 6 day campaign during which the rodenticide is applied. This allows for a possibility of approximately 50 campaign per year. Again this is likely to be significantly greater than the actual number of campaigns per year so our assessment is expected to be highly conservative in nature. The input parameters are summarised below:

Input parameter	**Unit**	**Difenacoum**
Physicochemical parameters		
Molecular weight	g mol^{-1}	444.5

Water solubility	mg L^{-1}	0.43 (20°C)
Molar enthalpy of dissolution	kJ mol^{-1}	27 (default)
Saturated vapor pressure	Pa	5.4E-14 (25°C)
Molar enthalpy of vaporisation	kJ mol^{-1}	95 (default)
Diffusion coefficient in water	m^2 d^{-1}	4.3E-05 (default)
Diffusion coefficient in air	m^2 d^{-1}	0.43 (default)
Degradation parameters		
Half-life at reference condition	d	439 (20°C)
Molar activation energy	kJ mol^{-1}	65.4 (default)
Exponent for the effect of liquid	-	0.7 (default)
Sorption parameters		
Kom value (=Koc/1.724)	L kg^{-1}	1.1E06 (QSAR value)
Freundlich exponent 1/n	-	1.0 (worst case assumption)
Method of subroutine	-	pH independent
Crop related parameters		
FOCUS crop	-	Grassland
Crop uptake factor	-	0
Application parameters		
Number of applications per annum	-	50
Application rate	kg ha^{-1}	0.406
Application type	-	Injection at 30 cm
Number of applications per annum	-	50

The 80th percentile PEC_{GW} values are shown below. Based on this assessment it can be concluded that there is no risk to groundwater from use of the product.

PEARL SCENARIO	**$PEC_{groundwater}$ (µg/L)**
Châteaudun	<0.001
Hamburg	<0.001
Jokioinen	<0.001
Kremsmünster	<0.001
Okehampton	<0.001
Piacenza	<0.001
Porto	<0.001

Seville	<0.001
Thiva	<0.001
• Levels above 0.1 µg/L exceed the drinking water limit for pesticides	

Primary and Secondary Poisoning

Primary Poisoning

The Tier 1 assessment assumes that there is no bait avoidance by the non-target animals, and that they obtain 100% of their diet in the treated area and have access to the difenacoum product. The worst case Tier 1 PEC_{oral} is 50 mg/kg and is used in quantitative risk assessment for the long-term situation. The LD_{50} values are 56 mg/kg bw for birds (AF 3000) and 1.8 mg/kg bw for mammals (AF 90) (List of Endpoints in the Assessment Report (17-09-2009). The Tier 1 Primary poisoning PEC/PNEC ratios are provided below:

Tier 1 Primary poisoning PEC/PNEC ratios

Exposed Organism	PNEC µg/kg food	PNEC[1] µg/kg bw/d	PEC	PEC/PNEC
Birds	0.5	0.1	50 mg/kg food	500000
Mammals	7	0. 3	50 mg/kg food	166667

[1] Appendix V- Assessment Report (17-09-2009)

Acute risk assessment for primary poisoning of a non-target organism:

Tier 2:

In the refined risk assessment the daily uptake (ETE) is compared to the PNEC for birds and mammals. The PNEC values for each representative animal are compared with the ETE values to provide an indication of the risk to non-target animals ingesting a daily dose of the product.

Tier 2 acute risk assessment: $PEC_{oral}/PNEC_{oral}$ for non-target animals accidentally exposed to bait containing Difenacoum after one meal

Non-target animals	ETE, concentration of Difenacoum after one meal (one day) (mg/kg b.w.)		$PNEC_{oral}$ (dose, mg/kg b.w./d)	PEC/PNEC	
	Step 1	Step 2		Step 1	Step 2
Tree sparrow	17.3	12.44	0.0001	173000	124400
Chaffinch	15.00	10.8	0.0001	150000	108000
Wood pigeon	5.42	3.9	0.0001	54200	39000
Pheasant	5.39	3.9	0.0001	53900	39000
Dog	3.0	2.16	0.0003	10000	7200
Pig	0.375	0.27	0.0003	1250	900
Pig, young	1.2	0.864	0.0003	4000	2880

The ratios PEC/PNEC are above 1 indicating a potential risk even after refinement.

Long-risk assessment for primary poisoning of a non-target organism:

Tier 2:

In the long-term risk assessment, the EC (expected concentration of active substance in the animal) after metabolism and other elimination is calculated and used to calculate the $EC_{oral/}PNEC_{ratio}$after 1-day and 5-day elimination of Difenacoum. The $EC_{oral/}PNEC_{ratio}$ are above 1 after 1-day elimination of Difenacoum indicating a potential risk (data not shown). The $EC_{oral/}PNEC_{ratio}$ for the 5-day elimination of Difenacoum are shown below.

Tier 2 long-term risk assessment: $EC_{oral}/PNEC_{oral}$ ratio after 5-day elimination

Species	EC_{oral} after 5 days (mg/kg b.w./d) with excretion factor = .4, AV = 1, PT = 1 (mg/kg bw)[a]	EC_{oral} after 5 days (mg/kg b.w./d) with excretion factor = 0.4, AV = 0.9, PT = 0.8 (mg/kg bw)[a]	$PNEC_{oral}$ (mg/kg b.w./d)	Ratio $EC_{oral}/PNEC_{oral}$
Tree sparrow	23.03	13.8	0.0001	138191
Chaffinch	19.97	11.98	0.0001	119836
Wood pigeon	7.21	4.32	0.0001	43297
Pheasant	7.18	6.30	0.0001	43086
Dog	3.99	2.39	0.0003	7989
Pig	0.499	0.299	0.0003	998
Pig, young	1.59	1.34	0.0003	4491

[a] calculation according to equation 21 in the ESD

The ratios PEC/PNEC are above 1 indicating a potential risk even after refinement.

Conclusion:
Overall, all acute and long-term $PEC_{oral}/PNEC_{oral}$ ratios are still above the trigger value of 1 indicating acute and long-term unacceptable risks.

Secondary Poisoning

A Tier 1 risk assessment was carried out to assess the risk for poisoning of non-target predator birds and mammals during acute and long-term exposure via rodents poisoned. The $PEC_{oral}/PNEC_{oral}$ values exceeded the trigger value of 1 (data not shown). Therefore, a refined tier 2 assessment was carried out, based on representative species. The refined tier 2 risk assessment considers exposure of relevant species of predators, based on their bodyweights and food intakes. The Difenacoum concentrations in non-target mammals and birds consuming contaminated rodents is calculated ($ETE_{oral\ predators}$) and compared to the $PNEC_{oral}$.

Tier 2 risk assessment of secondary poisoning (non-resistant and resistant rodents)

Species	Exposure	$ETE_{oral\ predators}$ (mg a.s./kg/d)	$PNEC_{oral}$ (mg a.s./kg/d)	Ratio $ETE_{oral\ predators}$ / $PNEC_{oral}$
Barn owl	Day 5 before the last meal	0.80	0.0001	8058
	Day 5 after the last meal	1.42		14257
	Day 14 after the last meal	1.54		15497
Kestrel	Day 5 before the last meal	1.22	0.0001	12238
	Day 5 after the last meal	2.16		21651
	Day 14 after the last meal	2.35		23534
Little owl	Day 5 before the last meal	0.91	0.0001	9195
	Day 5 after the last meal	1.62		16268
	Day 14 after the last meal	1.76		17682
Tawny owl	Day 5 before the last meal	0.74	0.0001	7407
	Day 5 after the last meal	1.31		13106
	Day 14 after the last meal	1.42		14245
Fox	Day 5 before the last meal	0.29	0.0003	988
	Day 5 after the last meal	0.52		1749
	Day 14 after the last meal	0.57		1901
Polecat	Day 5 before the last meal	0.61	0.0003	2058

Species	Exposure	ETE $_{oral\ predators}$ (mg a.s./kg/d)	PNEC$_{oral}$ (mg a.s./kg/d)	Ratio ETE $_{oral\ predators}$ / PNEC$_{oral}$
	Day 5 after the last meal	1.09		3641
	Day 14 after the last meal	1.18		3958
Stoat	Day 5 before the last meal	0.88	0.0003	2943
	Day 5 after the last meal	1.56		5207
	Day 14 after the last meal	1.69		5660
Weasel	Day 5 before the last meal	1.27	0.0003	4247
	Day 5 after the last meal	2.25		7514
	Day 14 after the last meal	2.45		8167

All ratios ETE$_{oral\ predators}$ / PNEC$_{oral}$ are above the trigger value of 1 indicating an unacceptable risk of secondary poisoning.

Overall conclusion

According to this risk assessment the risk for poisoning of non-target predator birds and mammals during primary (acute and long-term exposure) and secondary poisoning is high as the trigger value is exceeded in all cases.

No safe use was established for the Difenacoum product at a concentration of 50 ppm in the ecotoxicology risk assessment.

3.9 Assessment of a combination of biocidal products

A use with other biocidal products is not intended.

3.10 Comparative assessment

The Irish CA for biocides has processed an application for renewal for this biocidal product which contains the active substance Difenacoum. The active substance Difenacoum meets the criteria for exclusion according to Article 5(1) BPR as well as for substitution according to Article 10 BPR (for details see chapter 2.2.3).

Therefore, in line with Article 23 (1) BPR, a comparative assessment for this product has to be conducted.

At the 60th meeting of representatives of Members States Competent Authorities for the implementation of the BPR held on 20 and 21 May 2015, all Member States submitted to the Commission a number of questions to be addressed at Union level in the context of the comparative assessment to be carried out

at the renewal of anticoagulant rodenticide biocidal products ('anticoagulant rodenticides'). The questions submitted were the following:

(a) Is the chemical diversity of the active substances in authorised rodenticides in the Union adequate to minimise the occurrence of resistance in the target harmful organisms?;
(b) For the different uses specified in the applications for renewal, are alternative authorised biocidal products or non-chemical means of control and prevention methods available?;
(c) Do these alternatives present a significantly lower overall risk for human health, animal health and the environment?;
(d) Are these alternatives sufficiently effective?;
(e) Do these alternatives present no other significant economic or practical disadvantages?

The information addressing these questions is provided in the Annex of the Commission Implementing Decision (EU) 2017/1532[13]. In accordance with Article 1 of Commission Implementing Decision (EU) 2017/1532, the Irish CA considered the information in the Annex during the comparative assessment of anticoagulant rodenticide biocidal products.

Conclusion

Based on the information provided in the Annex of the Commission Implementing Decision (EU) 2017/1532 the Irish CA came to the conclusion that in the absence of anticoagulant rodenticides, the use of rodenticides containing other active substances would lead to an inadequate chemical diversity to minimize the occurrence of resistance in the target harmful organisms. These products also showed some significant practical or economical disadvantages for the relevant uses.

The Irish CA also considered a number of non-chemical control or prevention methods ("non-chemical alternatives"), which in our view do not provide sufficient alternatives to anticoagulant rodenticides.

In summary it can be concluded that the criteria according Article 23(3) a), b) BPR are not fulfilled. Therefore, the authorisation of this product will be renewed for 5 years.

[13] Commission Implementing Decision (EU) 2017/532 of 7 September 2017 addressing questions regarding the comparative assessment of anticoagulant rodenticides in accordance with Article 23(5) of Regulation (EU) No 528/2012 of the European Parliament and of the Council.

4 General Annexes

4.1 List of studies for the biocidal product (family)

Author	Year	Title	Publication	Report no.	Legal entity owner	Report date	GLP/ GEP	Data Protection Claimed

4.2 Output tables from exposure assessment tools

None

4.3 New information on the active substance

Under the 9th Adaptation to Technical Progress of the Classification and Labelling regulation (Commission Regulation (EU) 2016/1179), anticoagulant rodenticides were classified as Toxic to Reproduction Category 1A or 1B with a specific concentration limit of 0.003%. Under Article 19 of the Biocidal Products Regulation, biocidal products with such classifications (including anticoagulant rodenticides at this and higher concentrations) shall not be authorised for use by the general public.

4.4 Residue behaviour

No assessment necessary.

4.5 Summaries of the efficacy studies (B.5.10.1-xx)[14]

Function and field of use envisaged	Test substance	Test organism(s)	Test method, test system/concentrations applied/ exposure time	Test results; effects	Reference
PT14 RODENTICIDE	BELGASOURIS, containing 0.005% difenacoum	Grey mice (*Mus musculus*)	Lab conditions, rodents were housed in individual cages. Test was performed on fresh product. Mice were captured in field then housed in individual cages and offered a choice of safe crushed wheat and poisoned crushed bait.	All tested animals died, n=10. The efficacy of the product is very good because all the test animals offered the rodenticide, BELGASOURIS, died within an average of 9 days, which is a normal timing for an anticoagulant product. The efficacy is 100%.	Latteur G., 1996, CRA, Efficacy test on rodenticide product, BELGASOURIS: whole and crushed wheat, containing 0.005% of Difenacoum, against grey mice (*Mus musculus*), report 947, Unpublished
PT14 RODENTICIDE	DISOURICIDE PESCE, containing 0.005ppm difenacoum	Wild grey mice (*Mus musculus*)	Field study: experiment conducted in 3 aviaries. Test was performed on: Fresh product (T0) The method used has been inspired by the French method called “method no. 002 from Biological Trials Commission (C.E.B) ”, Method for practical efficacy trials of raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. Revised by OEPP in 1980.	*Grain bait/ Field efficacy/ Mice /Product at T0* The wheat consumption has decreased from around 100g to around 5g, namely a 95% efficacy. Disouricide pesce is effective at 95% against grey mice.	Pest Control Assistance (PCA), Appetition and efficacy trial of « DISOURICIDE PESCE » on grey mice (*Mus musculus*), For LODI, Le Cosquer (56), 2002 Unpublished
PT14 RODENTICIDE	RACO GRAIN BAITS, containing 0.005% difenacoum	Black rats/ Roof rats (*Rattus rattus*)	Field: study conducted in pig stables. Estimated population: 20 rats. The experiment was conducted on fresh product. The method used has been inspired by the French method called “method no. 002 from Biological Trials Commission (C.E.B) ”, Method for practical efficacy trials of raticides:	*Grain bait/ Field efficacy/ Roof rat /Product at T0* DIFENACOUM is said to kill rodents in 5 to 21 days. In the test the first signs of illness started after 8 days; dead rats were found after 12 days. After sixteen days there was still some activity, which ended later (unrecorded). 2142g of bait consumed during the trial. These results are consistent with the results expected with difenacoum baits.	Feys J-L., Field trial with RACO GRAIN BAITS against ROOF RATS 11 November 2009_08 03 December 2009, batch PB 091109 Belgagri. Unpublished

14 If an IUCLID file is not available, please indicate here the summaries of the efficacy studies.

			• Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. Revised by OEPP in 1980.	One can conclude that RACO Grain Baits is very well suited for the control of *Rattus rattus.*	
PT14 RODENTICIDE	DIRATICIDE, containing 0.005% difenacoum	Wild brown Rats *(Rattus norvegicus)*	Field study: experiment conducted in silos for cereal storage Test was performed on: • Fresh product (T0) The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. Revised by OEPP in 1980.	*Grain bait/ Field efficacy/ Rats /Product at T0* The wheat consumption decreased from around 2.1Kg to around 140g, namely a decrease of 93%. The efficacy of the product is 93%. Diraticide is efficacious against Brown rats.	Pest Control Assistance (PCA), Appetition and efficacy trial of « DIRATICIDE » on brown rats (*Rattus norvegicus*), For LODI, U.K.L (56), 2002 Unpublished
PT14 RODENTICIDE	BELGASOURIS, containing 0.005% difenacoum	Albino mice (*Mus musculus*)	Lab conditions, rodents were housed in individual cages Test was performed on different stages product: - Fresh product (T0) - 6 months (T6) Test was carried out in accordance with Guideline for the Rodenticide assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides).*	*Grain bait/ Lab/Choice test/ Mice (albino)/ Product at T6 months* All tested animal died excepted one female, at T0. (n=20 animals at each time period). Fresh BELGASOURIS is efficient at 95%. The appetizing rate for BELGASOURIS does not decrease trough the time period of 6 months.	Ryckel (de) B., Meeus P., 1997, CRA, Appetizing test through different period of time, performed on BELGASOURIS, rodenticide containing 0.005% of Difenacoum, against grey mice (*Mus musculus*), report 972 (complement to rapport 947), Unpublished.
PT14 RODENTICIDE	BELGASOURIS, containing 0.005% difenacoum	Albino mice (*Mus musculus*)	Lab conditions, rodents were housed in individual cages Test was performed on different stages product: - Fresh product (T0) - 12 months (T12) Test was carried out in accordance with Guideline for the Rodenticide assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides).*	*Grain bait/ Lab/Choice test/ Mice (albino)/ Product at T12 months* All tested animal died excepted:: - 2 animals at T0 (n=20animals). - 1 animal at T12 (n=20animals). Palatability of BELGASOURIS did not decrease after 12 months of storage at ambient temperature (20°C). The efficacy at T0 is 90% and 95% at T12 months.	De Proft M., Meeus P., 2001, CRA, Appetizing behaviour with BELGASOURIS at different period of time, bait ready to use, containing 0.005% of Difenacoum, used in a bino mice in order to be applied against grey mice (*Mus musculus*), complement report

					10.312. Unpublished
PT14 RODENTICIDE	1. Difenacoum grain bait whole grain 2. Difenacoum grain bait broken grain Containing 0.005% difenacoum	Albino Rats *(Rattus norvegicus)* Albino Mice *(Mus musculus)*	Laboratory Test was performed on: • Fresh product (T0) Product stored for 12 months (T12) Test was carried out in accordance with the Guideline for the Rodenticide assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides).*	*Grain bait/ Laboratory efficacy/ Mice and Rats/ Product at T0 and T12* *Between fresh product and the 12 months aged product, acceptance loss/palatability is not significant.* Both tests used 11 males and 11 females with one male and one female acting as controls. 1. DIFENACOUM GRAIN BAIT WHOLE GRAIN • T0: 15 dead rats at the end of the trial • T12: 18 dead rats at the end of trial. 2. DIFENACOUM GRAIN BAIT BROKEN GRAIN For both fresh and 12 months aged product (100% dead mice for both trials), no problem of acceptance loss has observed.	De Proft M., CRA Gembloux, Study of ageing behavior of ready-to-use baits containing 0.005% of Difenacoum (effect on palatability), PART 2: Grain Bait, report number ROD 2008 11 BIO 6-Part 2: Grains baits Unpublished
PT14 RODENTICIDE	DISOURICIDE PESCE, containing 0.005ppm difenacoum	Wild grey mice *(Mus musculus)*	Field study: experiment conducted in domestic house. Test was performed on: Fresh product (T0) The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. Revised by OEPP in 1980.	*Grain bait/ Field efficacy/ Mice /Product at T 2 years* The wheat consumption before treatment was around 300g, and after treatment the wheat consumption was around 5g. Therefore, Disouricide pesce is effective at 98% against grey mice, even 2 years after manufacture.	Pest Control Assistance (PCA), Appetition and efficacy trial of « DISOURICIDE PESCE » on grey mice (*Mus musculus*), For LODI, Mme Rigal, 56150 Baud, 2002 Unpublished
PT14 RODENTICIDE	DIRATICIDE, containing 0.005ppm difenacoum	Wild brown Rats *(Rattus norvegicus)*	Field study: experiment conducted in 4 aviaries for wildfowl breeding Test was performed on: Product stored during 2 years The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. • Revised by OEPP in 1980.	*Grain bait/ Field efficacy/ Rats /Product at T 2 years* The wheat consumption has decreased from around 1,900g to around 40g, namely a decrease of 98%. Consequently, DIRATICIDE is effective at 98% on Brown Rats, even 2 years after manufacturing.	Pest Control Assistance (PCA), Appetition and efficacy trial of « DIRATICIDE» on brown rat (*Rattus norvegicus*), For LODI, Mr LAMOURIC Maurice, Tréviol, 56480 CLEGUEREC Baud, 2002 Unpublished
PT14	Difenacoum Grain	Albino house	Difenacoum block bait (batch No.	During the 9-day testing period, the percentage	Bureau, M, Choice

RODENTICIDE	Bait 0.005% difenacoum	mice (*Mus musculus*)	09415) (aged; 3 years at room temperature) was provided by the Sponsor and stored at Biotrial Pharmacology at room temperature. The test was performed on 3-years aged product in comparison with challenged diet (non-poisoned source).	intake of challenged diet was 51.2±6.4% for female mice and 33.1±9.5% for male mice. The percentage intake of difenacoum block bait was 48.8±6.4% for female mice and 66.9±9.5% for male mice. Globally, mortality occurred in 100% of male and female mice with a mean day to death of 5.7±1.9 days (range 3 to 9 days). Furthermore acceptance of difenacoum block bait on D7, D8, D9, D10, D11 and D12 was 58% (n=10), 63% (n=10), 66% (n=10), 51% (n=9), 43% (n=6) and 15% (n=5), respectively, for male and female mice.	feeding trials for difenacoum block bait (aged product) against house mice, 0LODI15. Unpublished
PT14 RODENTICIDE	Difenacoum Block Bait 0.005% difenacoum	Albino brown rats (*Rattus norvegicus*)	Difenacoum block bait (batch No. 09415) (aged; 3 years at room temperature) was provided by the Sponsor and stored at Biotrial Pharmacology at room temperature. The test was performed on 3-years aged product in comparison with challenged diet (non-poisoned source).	During the 11-day testing period, the percentage intake of challenged diet was 61.9±15.9% for female rats and 72.6±13.0% for male rats. The percentage intake of difenacoum grain bait was 38.1±15.9% for female rats and 27.4±13.0% for male rats. Globally, mortality occurred in 90% of male and female rats with a mean day to death of 6.6±1.7 days (range 4 to 9 days) with a surviving male mouse at the end of the experiment (D20).	Bureau, M, Choice feeding trials for difenacoum block bait (aged product) against rats, 0LODI18. Unpublished

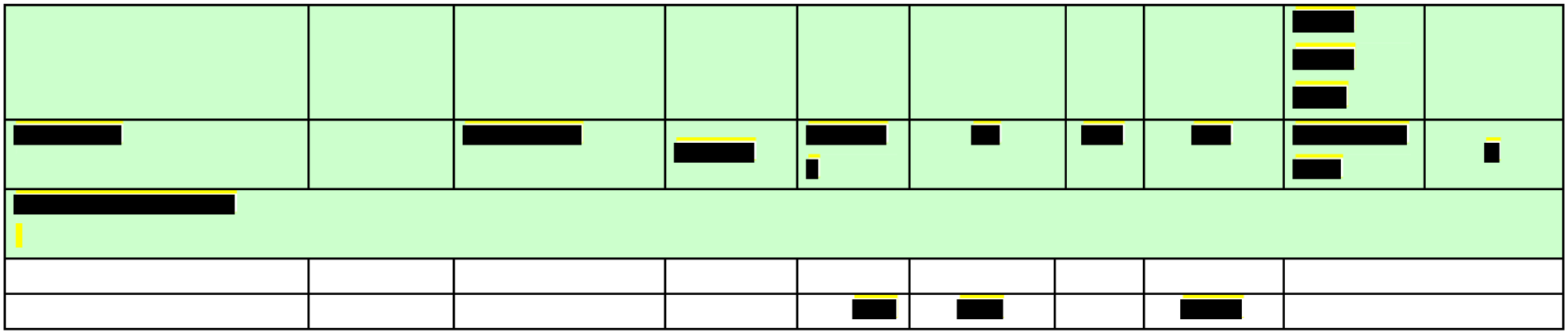
4.6 Other

None.

Active substance(s)				Contents				
Common name	IUPAC name	CAS No.	EC No.	Concentration	Unit 15	w/w (%)	Minimum purity (% w/w)	Same source as for Annex I inclusion (Y/N)
Difenacoum	3-(3biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphtyl)-4-hydroxycoumarin	56073-07-5	259-978-4	0.05	g/kg	[redacted]	[redacted]	[redacted]

Co-formulants					Contents				
Common name	IUPAC name	Function	CAS No.	EC No.	Concentration	Unit (g/kg)	w/w (%)	Classification	Substance of concern (Y/N)
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[15] g/l, g/kg, other. For biological products, the concentration should state the number of activity units/units of potency (as appropriate) per defined unit of formulation (e.g. per gram or per litre).



5 Confidential annex (Access level: “Restricted” to applicant and authority)

5.1 Full composition of the product

Annex 1 - Initial PAR – June 2011

PAR Lodi - Ruby Grain 2011-06-30.pdf

ANNEXES to Initial PAR – June 2011

ANNEXES

Annex:

1. Confidential Information and Data
2. Summary of the Product Characteristics (SPC)
3. Study Summaries of Studies Reviewed
4. List of Studies Reviewed
5. Toxicology Calculations
6. Environmental Calculations
7. Residue Calculations

ANNEX I: Confidential Information and Data

Manufacturing site(s) of the active substance(s)[16]

Manufacturing site of the active substance(s):	
Company Name:	Pelgar International Ltd.
Address:	Prazska 54, 280 02 Kolin, Czech Republic c/o Pelgar International Ltd. Unit 13, Newman Lane, Alton, Hants. GU34 2QR, UK
Tel:	[redacted]
E-mail:	[redacted]
Contact:	[redacted]

Manufacturing site(s) of the biocidal product[3]

Manufacturing site of the biocidal product:	
Company Name:	LODI S.A.
Address:	Parc d'activities des quatre routes Grand Fougeray 35390 France
Tel:	[redacted]
E-mail:	[redacted]
Contact:	[redacted]

[16] All sites involved in the manufacturing process of each active substance and of the product must be listed.

Study summaries of new data[17] submitted in support of the evaluation of the active substance (IIIA)

A new 5-batch analysis for Difenacoum was submitted. This information was assessed by France and was found to be acceptable. Ireland accepts France's assessment.

[redacted]

[17] Data which have not been already submitted for the purpose of the Annex I inclusion.

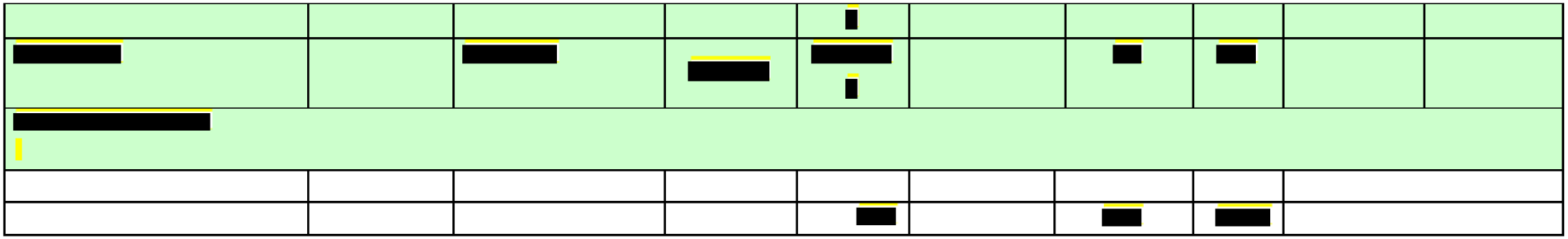
Product trade name: Ruby Grain

Qualitative and quantitative information on the composition/specification of the biocidal product

Active substance(s)				Contents				
Common name	IUPAC name	CAS No.	EC No.	Concentration	Unit[18]	w/w (%)	Minimum purity (% w/w)	Same source as for Annex I inclusion (Y/N)
Difenacoum	3-(3biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphtyl)-4-hydroxycoumarin	56073-07-5	259-978-4		0.05 g/kg	[redacted]	[redacted]	[redacted]

Co-formulants					Contents				
Common name	IUPAC name	Function	CAS No.	EC No.	Concentration	Unit (g/kg)	w/w (%)	Classification	Substance of concern (Y/N)
[redacted]		[redacted]	[redacted]	[redacted]		[redacted]	[redacted]		[redacted]
[redacted]		[redacted]	[redacted]	[redacted]		[redacted]	[redacted]		
[redacted]		[redacted]	[redacted]	[redacted]		[redacted]	[redacted]		
[redacted]		[redacted]	[redacted]	[redacted]		[redacted]	[redacted]		

[18] g/l, g/kg, other. For biological products, the concentration should state the number of activity units/units of potency (as appropriate) per defined unit of formulation (e.g. per gram or per litre).



Annex II: Summary of the Products Characteristics (SPC)

Annex III: Study Summaries of Studies Reviewed

Study summaries of new data[19] submitted in support of the evaluation of the active substance (IIIA)

Physical Chemical Characteristics

New data was submitted in support of PelGar's Difenacoum source of active substance. This included a study report to demonstrate the appearance of the technical substance. This information was assessed by France and was found to be acceptable. Ireland accepts France's assessment.

Methods of Analysis

New data was submitted in support of PelGar's Difenacoum source of active substance. This included a validated method of analysis for difenacoum in animal and human tissues, validation data for the analytical method for the determination of residues of difenacoum in meat and oil-seed rape (food/feeding stuffs) and validation data for the analytical method for determination of difenacoum in sediment (based on the analysis method for difenacoum in soil). This information was assessed by France and was found to be acceptable. Ireland accepts France's assessment.

Efficacy

Not applicable.

Toxicology

Not applicable

Environment (including Eco-Toxicology)

Not applicable

Confidential Section:

See confidential section (Annex I).

19 Data which have not been already submitted for the purpose of the Annex I inclusion.

Study summaries of new data submitted in support of the evaluation of the biocidal product (IIIB)

Physical Chemical Characteristics For Ruby Grain

Subsection (Annex Point/TNsG)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official Use only
3.1 Appearance (IIB3.1/Pt. I-B3.1)	Red grains							
3.1.1 Physical state and nature	Solid							
3.1.2 Colour	Red							
3.1.3 Odour	/							
3.2 Explosive properties (IIB3.2/Pt. I-B3.2)				The absence of certain reactive groups in the structural formula of the a.s., difenacoum (CAS 56073-07-5) *{Ref: Brethrick, Handbook of Reactive Chemical Hazards, Butterworths, London 1979}*, and it oxygen balance, establish beyond reasonable doubt that difenacoum is incapable of decomposing, forming gases, or realising heat very rapidly.				

Subsection (Annex Point/TNsG)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official Use only
				There are no other components in the formulation which present any explosive properties.				
3.3 Oxidising properties (IIB3.3/Pt. I-B3.3)				Neither the a.s. or the solvent present oxidising properties Examination of the chemical structural establish beyond reasonable doubt that the a.s., difenacoum (CAS 56073-07-5) is incapable of reacting exothermically with a combustible material *(refer to Explosive Properties)*. There are no other components in the formulation which present any oxidising properties.				
3.4 Flash-point and other indications of flammability or spontaneous ignition (IIB3.4/Pt. I-B3.4)	EPA 830.6315	-	**flammability** : None observed when heated to 100°C	There are no other components present in the formulation which present flammability properties.				

Subsection (Annex Point/TNsG)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official Use only
Flammable properties				There are no other components present in the formulation which present flammability properties.				
Autoflammability				There are no other components present in the formulation which present flammability properties.				
Other indications of flammability				Not applicable				
3.5 Acidity/Alkalinity (IIB3.5/Pt. I-B3.5)				Not applicable, the product is a ready to use bait which is a solid block at ambient temperature.				
3.6 Relative density/bulk density (IIB3.6/Pt. I-B3.6)	CIPAC MT 159	Grain baits contained 0.005% Difenacoum	D_P = 0.735 ±0.003 g/ml (Pour density) D_T= 0.773 ±0.001 g/ml (Tap density)	The test was performed in duplicate resulting in values of 0.738 and 0.732 g/ml for Pour Density and 0.772 and 0.773g/ml for Tap density. The mean values being respectively 0.735 g/ml and 0.773g/ml.	Y	1	Ferron N, Defitraces,2009 Draft Report N° 09-902018-001	
3.7 Storage stability - stability and shelf life (IIB3.7/Pt. I-B3.7)								

Subsection (Annex Point/TNsG)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official Use only
Effects of temperature (IV.B3.7.1) (IV.B3.7.2)	- GIFAP Monography n°17, CIPAC MT 46.3 - HPLC(UV) and Azur after 6 months and 2 years storage at ambient T°.	Grain baits contained 0.005% Difenacoum Grain baits contained 0.005% Difenacoum	Degradation: > 25% after 2 weeks at 54°C =25% after 16 weeks at 40°C = 22% after 2 years at T°N.	Sample was stable during 16 weeks at 40°C meaning it is considered to be stable after 4 years at T°N. No significant change was observed in the characteristics of the items, neither in the Difenacoum content after the accelerated storage procedures. The test items were considered to be stable.	Y Y	1 1	Biannic ML., LODI-Group, 2008-01-07 Biannic ML, LODI-Group, 2009-11-12	
Effects of light				None, see packaging				
Reactivity towards container material				[redacted] Needs comment on data request. These references need to be taken out				
Other	give in months if							

Subsection (Annex Point/TNsG)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official Use only
	shelf life is < 2 years							
3.8 Technical characteristics (IIB3.8/Pt. I-B3.8)								
Wettability/ Suspensibility	Only solid preparations			Not applicable, the product is a ready-to-use grain bait.				
Wet sieve analysis	for WPs, SCs, granules, tablets			Not applicable, the product is a grain.				
Emulsifiability	only forECs and ready for use emulsions			Not applicable, the product is a grain.				
Disintegration time				Not applicable, the product is a ready-to-use grain bait				
Attrition/friability of granules; integrity of tablets				Not applicable, the product is a ready-to-use grain bait				
Persistence of foaming				Not applicable, the product is a grain.				
Flowability/Pourability				Not applicable, the product is a				

Subsection (Annex Point/TNsG)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official Use only
				ready-to-use grain bait.				
Dustability				Not applicable, the product is a ready-to-use grain bait.				
3.9 Compatibility with other products (IIB3.9/Pt. I-B3.9)				Not applicable, the product is a ready-to-use grain bait and is not intended to be added or mixed with any other product.				
3.10 Surface tension (Pt. I-B3.10)				Not applicable, the product is a grain bait.				
3.11 Viscosity (Pt. I-B3.10)				Not applicable, the product is a grain bait.				
3.12 Particle size distribution (Pt. I-B3.11)	Only for powders and granules			Not applicable, the product is a ready-to-use grain bait				

Conclusion:

The biocidal product Ruby Grain is not explosive, oxidising or flammable and does not classify from a phys.chem. point of view. The test item is stable after storage for two years at ambient temperatures. The test item is a ready-to-use grain bait and is not intended to be added or mixed with any other product.

Data requirements:

Information on the reactivity of the grain bait towards the container material is outstanding.

Methods of Analysis

Doc IIIB Section 4.1 **BPD Data Set IIB/** **Annex Point III.4.**		**Analytical Method for Detection and Identification** Analytical method validation for the determination of difenacoum in grain bait	
		1 Reference: IIIB4.1a	**Official use only**
1.1	***Reference***	Ricau H, Analytical method validation for the determination of Difenacoum in Difenacoum Grain Bait, Anadiag group-Defitraces, Study Report n°09-902018-003, 19 pages, Bio6. Unpublished	
1.2	***Data protection***	Yes	
1.2.1	**Data owner**	Bio6 s.a.	
1.2.2	**Companies with letter of Access**	PelGar International Ltd	
1.2.3	**Criteria for data protection**	Data on existing [a.s. / b.p.] submitted under national legislation for Post Inclusion of a.s. authorisation Data on existing [a.s./b.p.] submitted for the first time for Post Inclusion of a.s.	
		2 Guidelines and Quality Assurance	
2.1	***Guideline study***	CIPAC/3807R	
2.2	***GLP***	Yes	
2.3	***Deviations***	One deviation was recorded. Due to a presence of an interferent in the test item a second reverse phase column C8 was used. This deviation has not affected the quality or the interpretation of the results obtained.	
		3 Materials and Methods	
3.1	***Preliminary treatment***		
3.1.1	**Enrichment**	Difenacoum was extracted from the grain bait using Methanol and heated under reflux for about 90 minutes at 80°C.	
3.1.2	**Cleanup**	Extract was filtered through a Whatman filter N°1 and diluted in Methanol and Acetonitrile before injection.	
3.2	***Detection***		

3.2.1	**Separation method**	HPLC using a Phenomenex Hyperclone Mos C8 + Luna 5µC8 ((10+25)*(4.6+4.0)ID) column with a flow rate of 0.8 ml/min and a mobile phase of Methanol.	
3.2.2	**Detector**	UV detection at 310 nm	
3.2.3	**Standard (s)**	Difenacoum standard (Cluzeau Info Labo) for reference item solution preparation	
3.2.4	**Interfering substance(s)**	No peak was observed in the blank solvent, in the blank formulation and in the reference item.	
3.3	***Linearity***	(Ref IVB.4.1b-R05-912011-001)	
3.3.1	**Calibration range**	The response of difenacoum is linear within the range of 0.0008mg/ml to 0.0012 mg/ml.	
3.3.2	**Number of measurements**	6	
3.3.3	**Linearity**	Correlation coefficient = 1.000	
3.4	***Specificity:*** Interfering substances	The specificity of the method was evaluated by the absence of interfering peaks in the area of interest (3.24minutes). When injecting blank samples, no interfering peak shows up at the retention time where the analyte signal was expected. No other peak was found in the reference item and in the test item. The specificity was therefore defined.	
3.5	***Recovery rates at different levels***	The method has been validated at 0.92mg/ml (100%level) and at 0.46mg/ml (50%level). Recovery found respectively, 111 and 103%	
3.5.1	**Recovery results**	Between 103% and 111% in conformity with the CIPAC Guideline requirements which recommend recovery results in the range 80%-120%	
3.6	***Limit of determination***		
3.7	***Precision***		
3.7.1	**Repeatability**	The concentration of difenacoum in the test item is equal to 0.005% (m/m) or 0.50g/kg. In the case of difenacoum, the precision is acceptable as the RSD is lower than the result of the modified Horwitz equation: 3.40 < 5.95 (C=0.0001%). (Ref IVB.4.1b-R05-912011-001).	

3.7.2	**Independent laboratory validation**	Not available	
		4 Applicant's summary and conclusion	
4.1	***Materials and methods***	After a methanol dilution and heated under reflux during 90 minutes, extract was filtered and diluted again in methanol and acetonitrile. Determination of difenacoum was made by liquid chromatography on a reversed phase analytical column using UV detection at 310nm.	
4.2	***Conclusion***	The analytical method showed a good specificity for difenacoum analysis. The accuracy results of difenacoum were in conformity with the CIPAC Guidelines requirements for formulations containing less than 0.1% of an active substance. Indeed, the recovery results should be in the range 80-120% and they were experimentally between 103 and 111%.	
4.2.1	**Reliability**	1	
4.2.2	**Deficiencies**	No	

	Evaluation by Competent Authorities	

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Reference Member State	
Date	7.1.2011	
Materials and Methods	The method of analysis presented above was only validated in terms of its accuracy and specificity. The outstanding validated data (linearity & precision) was presented in report number R05-912011-001.	
Results and discussion	Accept the results of the Notifier.	
Conclusion	Accept the conclusion of the Notifier.	
Reliability	1	
Acceptability	Acceptable. Note that the outstanding validation data was presented in report no: R05-912011-001.	
Remarks	None.	

Doc IIIB Section 4.1 BPD Data Set IIB/ Annex Point III.4.		Analytical Method for Detection and Identification Analytical method validation for the determination of difenacoum in grain bait	
		1 Reference: IIIB4.1B	**Official use only**
1.1	***Reference***	Ricau H, Quantification of Difenacoum 0.005% m/m in a rat poison bait., Defitraces, Study Report n°05-912011-001, 22 pages, LODI sa. Unpublished	
1.2	***Data protection***	Yes	
1.2.1	**Data owner**	LODI s.a.	
1.2.2	**Companies with letter of Access**	PelGar International Ltd	
1.2.3	**Criteria for data protection**	Data on existing [a.s. / b.p.] submitted under national legislation for Post Inclusion of a.s. authorisation Data on existing [a.s./b.p.] submitted for the first time for Post Inclusion of a.s.	
		2 Guidelines and Quality Assurance	
2.1	***Guideline study***	Method was developed in compliance with the Standard Operating Procedures in uses at DEFITRACES.	
2.2	***GLP***	Yes	
2.3	***Deviations***	One deviation was recorded. Issue of the draft report in March 2005 instead of February 2005 as described in the study plan. This deviation has no adverse effect on the study.	
		3 Materials and Methods	
3.1	***Preliminary treatment***		
3.1.1	**Enrichment**	Difenacoum was extracted from the grain bait using Methanol and heated under reflux for about 90 minutes at 80°C.	X
3.1.2	**Cleanup**	Extract was filtered through a Whatman filter N°40 and diluted in Methanol and Acetonitrile before injection.	
3.2	***Detection***		
3.2.1	**Separation method**	HPLC using a Supelcosil LC-8 (25*4.0 ID) column with a flow rate	

		of 0.3 ml/min and a mobile phase of Methanol.	
3.2.2	**Detector**	UV detection at 310 nm	
3.2.3	**Standard (s)**	Difenacoum standard (Cluzeau Info Labo) for reference item solution preparation	
3.2.4	**Interfering substance(s)**	No peak was observed in the blank solvent, in the blank formulation and in the reference item.	
3.3	***Linearity***		X
3.3.1	**Calibration range**	The response of difenacoum is linear within the range of 0.0008mg/ml to 0.0012 mg/ml.	
3.3.2	**Number of measurements**	6	
3.3.3	**Linearity**	Correlation coefficient = 1.000	
3.4	***Specificity:*** Interfering substances	A shift of difenacoum retention time was always observed in the test item presumably due to the presence of waxy co-extracts. By comparison of the UV spectra at the level of the reference item peak and the test item peak, it was shown that the peak at around 4.60 represents difenacoum. The retention time of difenacoum in the test item changes from about 4.60 to 4.80. It was concluded that the analytical method showed a good specificity.	
3.5	***Recovery rates at different levels***	The method has been validated at 0.005 % (m/m).	
3.5.1	**Recovery results**	Between 102% and 105% in conformity with the CIPAC Guideline requirements which recommend recovery results in the range 102%-105% for formulations containing less than 1% of an active substance.	
3.6	***Limit of determination***		
3.7	***Precision***		
3.7.1	**Repeatability**	The concentration of difenacoum in the test item is equal to 0.005%, m/m or 0.50g/kg. In the case of difenacoum, the precision is acceptable as the RSD is lower than the result of the modified Horwitz equation: 3.40 < 5.95 (C=0.0001%).	X
3.7.2	**Independent laboratory validation**	Not available	

		4 Applicant's summary and conclusion	
4.1	***Materials and methods***	After a methanol dilution and heated under reflux during 90 minutes, extract was filtered and diluted again in methanol and acetonitrile. Determination of difenacoum was made by liquid chromatography on a reversed phase analytical column using UV detection at 310nm.	
4.2	***Conclusion***	The analytical method showed a good specificity for difenacoum analysis. The response of difenacoum was linear within the range of 0.0008 mg/ml to 0.0012 mg/ml. The precision was acceptable as the RSD was lower than the modified Horwitz equation. The accuracy results of difenacoum were in conformity with the CIPAC Guidelines requirements for formulations containing less than 1% of an active substance. Indeed, the recovery results should be in the range 95-105% and they were experimentally between 102 and 105%.	
4.2.1	**Reliability**	1	
4.2.2	**Deficiencies**	No	

	Evaluation by Competent Authorities	

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Reference Member State	
Date	7.1..2011	
Materials and Methods	The method of analysis presented above was not validated for the grain bait only the block bait and therefore is unacceptable. However, the information on the linearity and precision can be used to cover the lack of linearity and precision data in study 09-90218-003.	
Results and discussion	X Enrichment It states that "Difenacoum was extracted from the grain bait ....". However the study was carried out on awax block bait. X Linearity The linearity data presented in this study was carried out using standard solutions and the same analytical method as in 09-902018-003 therefore it covers the data requirement for linearity for that method. X Repeatability. A correction should be made, the concentration of Difenacoum in the test item is equal to 0.005%, m/m or 0.05 g/kg not 0.50 g/kg as stated in the above text.	
Conclusion	The information on linearity and precision provided in this study is acceptable and covers the data requirements from study 09-902018-003.	
Reliability	2	
Acceptability	Acceptable in terms of the linearity and precision data.	

Remarks	The method of analysis presented above was not validated for the grain bait only the block bait and therefore it cannot be used to cover the grain bait. However, the information on the linearity and precision can be used to cover the lack of linearity or precision data in study 09-90218-003.

Doc IIIB Section 4.2	**Analytical Method for Detection and Identification**	
BPD Data Set IIB/ Annex Point III.4.	Analytical method validation for the determination of difenacoum in grain bait	
	1 Reference: IIIB4.2	**Official use only**
1.1 Reference	Bucciarelli B., Determination of concentration of difenacoum in anticoagulant rodenticide cereal based, Laboratorio Bucciarelli, Study Report n°24/2008, 20 pages, LODI sa. Unpublished	
1.2 Data protection	Yes	
1.2.1 Data owner	LODI s.a.	
1.2.2 Companies with letter of Access	PelGar International Ltd	
1.2.3 Criteria for data protection	Data on existing [a.s. / b.p.] submitted under national legislation for Post Inclusion of a.s. authorisation Data on existing [a.s./b.p.] submitted for the first time for Post Inclusion of a.s.	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	The study was done according to the described working procedures and according to the Study plan as agreed with the Sponsor.	
2.2 GLP	Yes	
2.3 Deviations	No deviation	
	3 MATERIALS AND MethodS	
3.1 Preliminary treatment		
3.1.1 Enrichment	Difenacoum was extracted using a Hexane, Dichloromethane, Methanol and acetic acid extraction solution. After ultrasonication for 15 minutes, a 4 hour decantation step was required.	
3.1.2 Cleanup	Extract was filtered through a Whatman filter N°41. After addition of internal standard (triphenylbenzene), solution was diluted in extraction solution before injection.	
3.2 Detection		
3.2.1 Separation method	HPLC Dionex instrument using a Partisil ODS-3 (4.6*100mm, 5µm, 85A) column with a flow rate of 1ml/min and methanol/water/acetic	

		acid solution as mobile phase. Chromtaographic separation was coupled with a UV detector	
3.2.2	**Detector**	UV detection at 310 nm	
3.2.3	**Standard (s)**	Difenacoum standard solution 2.5% Tec (Pelgar International) for reference item solution preparation and 1.3.5 triphenylbenzene (Sigma-aldrich) as internal standard	
3.2.4	**Interfering substance(s)**	No peak was observed in the blank solvent, in the blank formulation and in the reference item.	
3.3	***Linearity***		
3.3.1	**Calibration range**	The response of difenacoum is linear within the range of 1 to 4 mg/l	
3.3.2	**Number of measurements**	5	
3.3.3	**Linearity**	Correlation coefficient > 0.995	
3.4	***Specificity:*** Interfering substances	No peak interfered in the analysis. The analytical method showed a good specificity.	
3.5	***Recovery rates at different levels***	The method has been validated at several levels: from 0.0025 to 0.01% of active ingredient.	
3.5.1	**Recovery results**	Between 95.00% and 105% for 0.0025-0.01% of difenacoum.	
3.6	***Limit of determination***	Limit of detection = 1 mg/l	
3.7	***Precision***		
3.7.1	**Repeatability**	RSD < 3%	
3.7.2	**Independent laboratory validation**	Not available	
		4 Applicant's summary and conclusion	
4.1	***Materials and methods***	After mixing with extraction solution, difenacoum extract was filtered before injection. Internal standard was used for determination and quantification. HPLC-UV system allowed determination of difenacoum.	
4.2	***Conclusion***	The analytical method showed a good specificity for difenacoum. Over the range of active ingredient from 1 to 4 mg/l, the correlation R^2 observed was	

	not less than 0.995. The precision was acceptable with a RSD < 3% for 0.0025- 0.01% of difenacoum And recovery results were between 95% and 105% for 0.0025-0.01% of difenacoum.	
4.2.1 Reliability	1	
4.2.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Reference Member State	
Date	7.1.2011	
Materials and Methods	The method of analysis presented above is acceptable.	
Results and discussion	The results are acceptable.	
Conclusion	The method of analysis is acceptable.	
Reliability	1	
Acceptability	Acceptable.	
Remarks	None.	

Doc IIIB Section 4 litt-01 BPD Data Set IIB/ Annex Point III.4.	**Analytical Method for Detection and Identification** Analytical method validation for the determination of difenacoum in grain bait	
	1 Reference: IIIB4.litt-01	**Official use only**
1.1 Reference	Magnier C., Analytical method validation for determination of Difenacoum in Difenacoum Bait (pasta, grain and block), LodiGroup, Study Report n°LODI17/2009, 21 pages, LODI sa. Unpublished	
1.2 Data protection	Yes	
1.2.1 Data owner	LODI s.a.	

1.2.2	**Companies with letter of Access**	PelGar International Ltd	
1.2.3	**Criteria for data protection**	Data on existing [a.s. / b.p.] submitted under national legislation for Post Inclusion of a.s. authorisation Data on existing [a.s./b.p.] submitted for the first time for Post Inclusion of a.s.	
		2 Guidelines and Quality Assurance	
2.1	***Guideline study***	CITAC/EURACHEM	
2.2	***GLP***	Yes	
2.3	***Deviations***	No deviation	
		3 Materials And Methods	
3.1	***Preliminary treatment***		
3.1.1	**Enrichment**	Not available	
3.1.2	**Cleanup**	Not available	
3.2	***Detection***		
3.2.1	**Separation method**	HPLC using a reverse phase column and an UV detector	
3.2.2	**Detector**	Not available	
3.2.3	**Standard (s)**	Not available	
3.2.4	**Interfering substance(s)**	Not available	
3.3	***Linearity***		
3.3.1	**Calibration range**	The response of difenacoum is linear within the range of 80% to 120% of the item concentration.	
3.3.2	**Number of measurements**	5*3 Is this 5 sets of 3?? Or 15 in total	
3.3.3	**Linearity**	Correlation coefficient > 0.99	
3.4	***Specificity:*** Interfering substances	No peak was observed in the extraction solution, in the placebo whole and broken grain and in the stressed whole and broken grain. The analytical method showed a good specificity.	
3.5	***Recovery rates at different levels***	The method has been validated at several levels: 50 – 100 and	X

		150% doped placebo.	
3.5.1	**Recovery results**	Between 97.00% and 103.59% for whole grain. And between 101.50% and 103.15% for broken grain. Those values are in conformity with the requirements which recommend recovery results in the range 95%-105%.	X
3.6	***Limit of determination***	Limit of detection = 0.05ppm Limit of quantification = 0.25ppm	X
3.7	***Precision***		
3.7.1	**Repeatability**	RSD <1.168	
3.7.2	**Independent laboratory validation**	Not available	
		4 Applicant's summary and conclusion	
4.1	***Materials and methods***	Test item was quantified by liquid chromatography on a reversed phase analytical column using an UV detector. Quality criteria applied on the method allowed to validate this analytical method for determination of difenacoum in baits.	
4.2	***Conclusion***	The analytical method showed a good specificity for difenacoum analysis. The response of difenacoum was linear within the range of 80 to 120% of the concentration in the test item. The precision was acceptable as the RSD was lower than the modified Horwitz equation. The accuracy results of difenacoum translate the narrowness between the found value and the value of reference. ??The recovery results were between 95% and 105%	
4.2.1	**Reliability**	2	
4.2.2	**Deficiencies**	No	
		Evaluation by Competent Authorities	

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Reference Member State	
Date	10.1.2011	
Materials and Methods	X The Notifer gave no information on the principle of the method only that HPLC was used with UV detection. The company clarified (1.3.2011) that the method is similar to the principle of the method used in reports 09-902018-005 and 05-912011-001. X Three injections were carried out at each of the different levels (50, 100 and 150% doped placebo) for the recovery experiment. The mean recovery at each of the fortification levels was 100.43%, 97.22% and 98.99% respectively. The overall mean was 98.88%. X LOD: the operator injected a solution containing 10 ppm of test item to calculate the S/N ratio. The operator divided by 10, then by 2, the concentration of test item until obtaining a ratio lower than 3 (S/N $\geq$ 3). LOQ: The operator injected a solution containing 50 ppm of test item to calculate the S/N ratio. The operator divided by 10, and then by 2, the concentration of test item until obtaining a ratio lower than 10 (S/N $\geq$ 10).	
Results and discussion	The results are acceptable.	
Conclusion	The information provided in this study is considered extra information only, with the exception of the LOD and LOQ information.	
Reliability	2	
Acceptability	Acceptable.	
Remarks	The company clarified that the method is similar to the principle of the method used in reports 09-902018-005 and 05-912011-001. The company also clarified that the units for the concentrations of the solutions used in the precision experiment were mg/l.	

Efficacy

Section B5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Product type(s) and field(s) of use envisaged (IIB5.1)**		
5.1.1	**Product type(s)**		
	MG03: Pest control Further specification	Product types PT14 - Rodenticides Grain bait	
5.1.2	**Overall use pattern**	Rodenticidal bait, containing 0.005% difenacoum as the active substance, may be used: • indoors, • around buildings, • away from building; • around waste sites and sewers. The product is used in the manner in all of these situations, the bait is placed in discrete locations within the infested area, and it is not disperses or broadcast within the environment. The products are primarily used to treat existing infestations. ***or rat:*** lace 100g, in appropriate dry location, at protected bait points, every 5 - 10 metres apart, until no bait is consumed during three days. The distance has to be adapted to the infestation. ***For mice*** Place baits in the points visited by mice. As mice are sporadic feeders, use many lure points, about 3 m apart, wherever droppings, damage or other signs of activity are seen. osage: 25 g every 3 to 5 m. The distance has to be adapted to the infestation. The distance has to be adapted to the	

infestation.

An adequate of baits points are placed in dry locations, protected from the weather and in an appropriate positions to help prevent access by non-target animals.

The number of bait point employed and the amount of the product used is dependent on:

- The treatment site
- The size and the severity of the infestations
- The users, and
- The user's requirement and needs.

A large number of bait points would be used on a site where immigrations pressure is high, the existing infestations is heavy, the users is professionally competent and requires maximum control. Conversely, a low number of bait points would be used in domestic premises where the householder had sightings of a rodent pest and considered it necessary to take some action.

The common strategy for best rat control, given that rats generally live outdoors, is to place protected baits between where rats live and feed so that they encounter the bait before encountering alternative foods. Bait points are thus best placed around burrows and living area, along runs where rats habitually travels, at entry points into buildings and around area where rats are known to feed.

As mice are sporadic feeders and more confidents than rats, and they generally live indoors within inaccessible spaces and voids, the strategy for best mouse control is to place many bait points throughout the area where mice are known to feed.

Bait points are inspected frequently and the bait point is filled in when a decrease in bait is observed. When the amount bait is stabilised for more than three days it is considered that control has been achieved and bait points are removed from the site. It

is normally expected that a typical baiting treatment of an infestation will not exceed 35 day duration.

At the conclusions of a rodent control treatment all remains of bait and bait containers are removed from the site and disposal safety, in accordance with the local/national safety regulations into force.

Some Members States have specifics disposal requirement; for example, in the UK non professional users can dispose of their waste direct to landfill sites (via domestic refuse but professional users have to dispose of waste as controlled wastes under EU waste legislation. Rodents bodies must be disposed of using the same way.

5.2 Method of application including description of system used (IIB5.2)

a) Include code(s) and term(s)
b) Give name of substances used for dilution including their concentration in the biocidal product. State any other substance(s) added including purpose and concentration in the product. Describe the application technique(s). Particularly if more than one product type or application method is applicable, you may summarize these data in tabular form (see example Table A5-1 below).

The codes and terms for the Product Type 14 - Rodenticides is:

Product	Codes*	Terms*	GIFAP codes
Cereals	VIII.3.1	Granular bait	AB,

Application codes for encoding Rodenticides (PT14), edited the 16 January 2009 on website Ex-ECB.* ***In point IVB5-0_01 of the dossier**)*

The product is ready to use and contains 50 ppm difenacoum,

as the active substance. Other components are added at the production phase of the product, but the product is not intended to be diluted with any other substance or preparation prior to use.

The product is applied but manually placing measured amounts of baits points, at discrete locations throughout a rodent infested area.

5.3 Application rate and if appropriate, the final concentration of the biocidal product and active substance in the system in which the preparation is to be used, e.g. cooling water, surface water, water used for heating purposes (IIB5.3)

For each product type and application technique give the recommended dose of the biocidal product and the active substance per object (e.g. per surface area of the material to be protected or as a concentration in a water system)

Product Type 14 - This product is ready to use and contains 50 ppm difenacoum, as the active substance.

or rat:

lace 100g, in appropriate dry location, at protected bait points, every 5 - 10 metres apart, until no bait is consumed during three days. The distance has to be adapted to the infestation.

For mice

Place baits in the points visited by mice. As mice are sporadic feeders, use many lure points, about 3 m apart, wherever droppings, damage or other signs of activity are seen.

osage: 25 g every 3 to 5 m. The distance has to be adapted to the infestation. The distance has to be adapted to the infestation.

Rodenticidal bait can be used indoors, around buildings, away from building, around waste sites and sewers. The amount of product laid is influenced by different factors, including the treatment site, the size and severity of infestation, the user and their requirement and needs.

5.4 Number and timing of applications, and where relevant, any particular information relating to geographical variations, climatic variations, or necessary waiting periods to protect man and animals (IIB5.4)

Indicate the recommended number and timing, i.e. duration of application and possible reapplications as well as waiting periods considered necessary. Where relevant, describe how the application should be varied in different parts of the Community. Particularly if more than one product type or application method is applicable, you may summarize these data in tabular form (see example Table A5-2 below).

Rodent control is undertaken by users in response to a rodent infestation. Rodenticidal products are used in the same manner whatever the geographical are or the climate, as the intended purpose for using the product is the same, *i.e.* to control rodent infestations. Therefore, the number and timings of applications is dependent on the presence of a rodent infestation.

An average rodent treatment should not continue beyond 35 days. (*British Pest control Association, 2001, Guidelines for the use of anticoagulant rodenticide by professional users, PT-958-1225*, ***in point IVB5-0_02 of the dossier****)*

5.5 Function (IIB5.5)

Include code(s) and term(s) for fungicide, rodenticide, insecticide, bactericide or other

The codes and terms for the Product Type 14 - Rodenticides is:

Product	Codes*	Terms*	GIFAP codes
Cereals	VIII.3.1	Granular bait	AB,

Application codes for encoding Rodenticides (PT14), edited the 16 January 2009 on website Ex-ECB, **in point IVB5-0_01 of the dossier)*

5.6 Pest organism(s) to be controlled and products, organisms or objects to be protected
(IIB5.6)

5.6.1 Pest organism(s) to be controlled

Include code(s) and term(s) and state common name, scientific name, sex, strain and stadia if relevant

Rodents (I.1), Murids (I.1.1):

Codes*	Specific names*	Common English Terms*
I.1.1.1	*Rattus Norvegicus*	Brown rats
I.1.1.2	*Rattus rattus*	Roof rat, House rat
I.1.1.3	*Mus musculus*	House mouse

Application codes for encoding Rodenticides (PT14), edited the 16 January 2009 on website Ex-ECB.* ***In point IVB5-0_01 of the dossier**)*

5.6.2 Products, organisms or objects to be protected

Include code(s) and term(s) for products, organisms or objects to be protected and the application aim

For the purpose of the protection of public health, including:

- Prevention of transmission disease;
- Prevention of the contamination of food and feeding stuffs and other materials, with urine, faeces and rodent hairs, at all stages of their production, storage and use;
- Protection of buildings and structures including pipes, cables and overall integrity;
- Protection of livestock, wild and domestic;
- Social abhorrence and stigma
- Legal requirement, for example, UK Prevention of Damage by Pest Act 1954.

Please find codes and term(s) for products, organisms or objects to be protected and the application aim in the following

table:

Codes *	Terms*
VII.1	Stored product protection/food protection
VII.2	Health protection
VII.3	Material protection (i.e. historical buildings, technical objects)

Application codes for encoding Rodenticides (PT14), edited the 16 January 2009 on website Ex-ECB.* ***In point IVB5-0_01 of the dossier**)*

5.7 Effects on target organisms (IIB5.7)

Describe the effects on the target organisms required for the claimed efficacy and specify these for each product type and method of application if appropriate.

Anticoagulant rodenticides disrupt the normal blood-clotting, mechanisms, resulting in increased bleeding tendency and eventually, and profuse haemorrhage.

Signs of anticoagulant poisoning in rats and mice included lethargy, hunched posture and vain clearing in the ears. Blood around the eyes, mouth and anus, indicating internal haemorrhaging, appears prior to death*. (Extract from WHO, 1995. Environmental Health Criteria 175 – Anticoagulant Rodenticides, International Programme on Chemical Safety, pages 22 and 55,* ***in point IVB5-0_03 of the dossier****)*

5.8 Mode of action (including time delay) in so far as not covered by section A5.4 (IIB5.8)

Refer to data given for the active substance or describe here. If appropriate, refer to experimental studies summarized in section 5.10 or any other studies.

Difenacoum is a second generation anticoagulant which prevents blood clotting in the target organisms by inhibiting regeneration of the active form of vitamin K1.

(*Extract Assessment Report – Difenacoum, Product-type 14 (Rodenticides), Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC, 17 September 2009, Annex I – Finland, p9,* ***in point IVB5-0_04 of the dossier***).

Anticoagulant rodenticides are vitamin K antagonists. The main site of their action is the liver, where several of the blood coagulation precursors undergo vitamin-K-dependent post-translation processing before they are converted into the respective procoagulant zymogens. The point of action appears to be the inhibition of K1 epoxide reductase.

Anticoagulant rodenticides are easily absorbed from the gastrointestinal tract, and may also be absorbed through the skin and respiratory system. After oral administration, the major route of elimination in various species is through the faeces.

The metabolic degradation of warfarin and indandiones in rats mainly involves hydroxylation. However, the second-generation anticoagulants are mainly eliminated as unchanged compounds. The low urinary excretion precludes isolation of metabolites from the urine.

(Extract from WHO, 1995. Environmental Health Criteria 175 – Anticoagulant Rodenticides, International Programme on Chemical Safety, pages 20, ***in point IVB5-0_03 of the dossier****).*

The liver is the main organ for accumulation and storage of rodenticide anticoagulants. Difenacoum has been found in the liver as both the parent compound and metabolites. The metabolism and elimination of the *trans*-isomer was more rapid than those of the *cis*-isomer.

The elimination from the liver and kidney is biphasic with an initial rapid phase of three days and a slower phase with a half-life of 118-120 days. In the pancreas, the concentration declined more slowly (a half-life of 182 days). No data are available for the kinetics and metabolism of difenacoum in humans.

(*Extract from IPCS International Programme On Chemical Safety, Health and Safety Guide No. 95, Difenacoum Health And Safety Guide, United Nations Environment Programme, International Labour Organisation, World Health Organization, World Health Organization, Geneva 1995,* ***in point IVB5-0_05 of the dossier***)

Accumulation also occurs in the fat.

Clinical signs are progressive and occur within 18 hours after ingestion of a toxic dose, ultimately leading to death from 3 to 10 days later. Effects are reversible by administration of the antidote vitamin K1 which stimulates the regeneration of the clotting factors.

(*Extract Assessment Report – Difenacoum, Product-type 14 (Rodenticides), Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC, 17 September 2009, Annex I – Finland, p9,* ***in point IVB5-0_03 of the dossier****).*

5.9 User: industrial, *Include code(s) and term(s) and briefly describe the use*

professsional, general public (non-professional) (IIB5.9)

conditions

Please find codes and term(s) for products, organisms or objects to be protected and the application aim in the following table:

Codes *	Terms*
V.1	Non professional/general public
V.2	Professional
V.3	Specialised professional

Application codes for encoding Rodenticides (PT14), edited the 16 January 2009 on website Ex-ECB., **in point IVB5-0_01 of the dossier).*

1. Industrial

[The inclusion of further exposure information is possible, see e.g. EASE (LEV, Full containment etc.)]

ormulation of the product requires a number of stages:

he batch process is performed at least once per week, as and when orders and stock level require it. Preparation, i.e. charging the mixer with the formulation components, takes 30minutes with a mixing time of 5 minutes.

ppropriate RPE/PPE is used at each stage. This prevents exposure by inhalation and dermal routes. Routine worker monitoring confirms no exposure.

Please refer to Manufacturing Process description in Doc IVB 1 (Confidential)

Please refer also to DOC I_Appendix 2_ description of packaging

2. Professional

his user group is not exposed to the active substance, except when formulated in a rodenticidal product at the concentration of 50 ppm.

he following tasks are undertaken when using rodenticidal baits.

- Decanting of bait from bulk container may occur;

- Loading of bait point with bait;
- Topping-up bait points when bait has been consumed, and
- Clean-up and disposal of spent baits at the end of the treatment.

oading the bait point with bait and topping up bait points when bait has been consumed are essentially identical tasks.

lthough gloves are not necessary when handling the product they are recommended for protection against exposure to rodent-borne diseases.

is expected that a professional user would undertake a risk assessment to the standard required by chemical Agents Directive 98/24/EC o, order to determine if any exposure controls are required for any specific tasks on specific treatment sites.

Refer to DOC I_Appendix 2_ description of packaging

3. General public

his user group is not exposed to the active substance, except when formulated in a rodenticidal product at the concentration of 50 ppm.

he following tasks are undertaken when using rodenticidal baits.

- Decanting of bait from bulk container may occur;
- Loading of bait point with bait;
- Topping-up bait points when bait has been consumed, and
- Clean-up and disposal of spent baits at the end of the treatment

oading the bait point with bait and topping up bait points when bait has been consumed are essentially identical tasks.

lthough gloves are not necessary when handling the product they are recommended for protection against exposure to rodent-borne diseases.

xposure is indirectly limited by controls on pack sizes available to this user group.

Please refer to DOC I_Appendix 2_ description of packaging

5.10 Efficacy data: The proposed label claims for the product and efficacy data to support these claims, including any available standard protocols used, laboratory tests, or field trials, where appropriate (IIB5.10)		
5.10.1 Proposed label claims for the product	or the control of rats and mice by professional and non – professional users. ***or rat:*** lace 100g, in appropriate dry location, at protected bait points, every 5 - 10 metres apart, until no bait is consumed during three days. The distance has to be adapted to the infestation. ***For mice*** Place baits in the points visited by mice. As mice are sporadic feeders, use many lure points, about 3 m apart, wherever droppings, damage or other signs of activity are seen. osage: 25 g every 3 to 5 m. The distance has to be adapted to the infestation. The distance has to be adapted to the infestation. general rodenticide treatment with anticoagulant rodenticides would be expected to achieve control within 35 days. ***Refer to DOC I_Appendix 1_ proposed draft label text for this representative product.***	
5.10.2 Efficacy data	*Include efficacy data; use standard format B5_10 to summarize any efficacy tests* ***All efficacy studies have been summarised using the standard format B5_10.***	
5.11 Any other known limitations on	*Give information on the occurrence of resistance or possible occurrence of the development of resistance and appropriate*	

efficacy including resistance (IIB5.10)	*management strategies. If appropriate, refer to test results described in section 5.10.2.* Difenacoum resistant brown rats are found in limited areas of Denmark, Germany and Great Britain. Monitoring of resistance occurs only in these countries and lack of information does not necessarily mean lack of resistance in the other countries. The incidence of resistance ranges from 2 to 84%. About 5-9-fold doses are needed to kill difenacoum resistant rats. No reports have been submitted to the Rapporteur Member State about the distribution and incidence of resistance in the house mouse or black rat in Europe. (*Extract Assessment Report – Difenacoum, Product-type 14 (Rodenticides), Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC, 17 September 2009, Annex I – Finland, p9 and 21,* ***in point IVB5-0_03 of the dossier****).* ***Please also refer to efficacy studies summarised in B5_10 of the dossier.***	
5.11.1 Use-related restrictions	*Describe possible restrictions or recommendations concerning the use of the product in specific environmental or other conditions.* It is widely accepted as good general practice of rodent control that removal of alternative food and feedstuffs, clearing up any spillages of possible food sources and containment of stocks of feedstuffs will promote the take of the bait. Also, following a successful rodenticide treatment the removal of vegetation, rubbish and any other potential burrows will help maintain a rodent free site. This information is communicated to the user via industry and through product-related literature, in the form of leaflets or web pages.	

(*Extract Assessment Report – Difenacoum, Product-type 14 (Rodenticides), Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC, 17 September 2009, Annex I – Finland, p9 and 21,* ***in point IVB5-0_03 of the dossier****).*

5.11.2 Prevention of the development of resistance

Describe and give reasons for possible recommendations concerning the avoidance of the continuous use of the product in order to prevent the development of resistant strains.

Application of area or block rodent control to eliminate resistance:

- Where individual infestations are found to be resistant or contain resistant individuals it is possible that the resistance extends further to neighbouring properties.
- Where there are indications that resistance may be more extensive than a single infestation, apply area or block control rodent programmes.
- The area under such management should extend at least to the boundaries of the area of known resistance and ideally beyond.
- These programmes must be effectively coordinated and should encompass the procedures identified above.

(Extract Anticoagulant resistance management strategy for pest management professionals, central and local government and other competent users of rodenticides. Crop Life International RRAC (Rodenticide Resistance Action Committee) Technical Monograph, Brussels, p. 18 and www.croplife.org, 2003, p11, ***in point IVB5-0_06 of the dossier***)

Resistance Management Strategies:

The important issues here are firstly to identify strategies for avoiding the development of resistance in susceptible rodent populations and secondly to identify strategies for managing resistance to the anticoagulants when it is suspected or identified.

Remember that the normal strategy used for managing resistance in populations of insects, weeds or other pests is to rotate the control between different groups of pesticide,

targeting as they do, different control mechanisms.

Unfortunately, the anticoagulant rodenticides all work in much the same way and the nature of the resistance to the different anticoagulants is so similar that simply rotating between the anticoagulants is not a reliable means of managing anticoagulant resistance. However, using anticoagulants of higher toxicity plays a major part in resistance management. In case of confirmed practical resistance, an anticoagulant rodenticide of higher toxicity compared to that, which is hit by resistance, should be used to eradicate the infestation. In some cases, especially with mice, alternations with non-anticoagulants can be part of the strategy.

(Extract Anticoagulant resistance management strategy for pest management professionals, central and local government and other competent users of rodenticides. CropLife International RRAC (Rodenticide Resistance Action Committee) Technical Monograph, Brussels, p. 18 and www.croplife.org, 2003, p8, ***in point IVB5-0_06 of the dossier***)

5.11.3 Concomittant use with other (biocidal) products

State if the product cannot be mixed with other substances, particularly other biocidal products, or if the use of the product with other biocidal products is recommended.

The product is ready to use and is not intended to be mixed with any other substance or preparation

Section B5.10_01

Official use only

5 Reference

5.1 Reference

Latteur G., CRA Gembloux, Efficacy test on rodenticide product, BELGASOURIS: whole and crushed wheat, containing 0.005% of Difenacoum, against grey mice (*Mus musculus*), report 947, November 1996.

CRA (Agronomic Research Center), Phytopharmacological department, Rue du Bordia, 11, 5030 Gembloux Belgium. Unpublished

5.2 Data protection

Yes

5.2.1 Data owner

BELGAGRI

Industrial Zone of Noville-les-Bois

14, rue du Grand Champ

5380 FERNELMONT, Belgium

5.2.2 Criteria for data protection

Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post inclusion

5.3 Guideline study	Guideline for the Rodenticide assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides)*
5.4 Deviations	No

6 Method

Test Substance (Biocidal Product)	as given in section 2 deviating from specification given in section 2 *(Fill in the fields 3.1.2 and 3.1.3)*
Trade name/ proposed trade name	BELGASOURIS
Composition of Product tested	0.005 % of Difenacoum
Physical state and nature	Bait ready to use: whole and crushed grains
Monitoring of active substance concentration	Yes, Chemical analyse of the BLEGASOURIS was determined before the efficacy trials at 0.0047% m/m.
Method of analysis	HPLC
Reference substance	No.
Method of analysis for reference substance	Not applicable
Testing procedure	

Test population / inoculum / test organism	10 grey mice (*Mus musculus*).	X
Test system	Mice are housed in individual cage.	
Application of TS	Mice received a portion of 10 g of crushed wheat in their mangers. Every day, mangers were weighed in order to estimate the consumption. Safe and poisoning wheat are placed in two different mangers. At each weight measures, mangers of safe and poisoning wheat are alternated.	
Test conditions	Minimum three weeks were observed between the first and the last captured mice, in order to suppress pregnant female. Before starting of experiment, mice were acclimatized in cages were they received crushed wheat and water *ad libitum*.	
Duration of the test / Exposure time	Please find the duration by phase: • Pre-baiting with safe crushed wheat: 5 days • Poisoning bait Vs safe crushed bait: 21 days	
Number of replicates performed	No replicates	
Controls	No	

Examination

Effect investigated	Efficacy of product BELGASOURIS used against mice population (*Mus musculus*). Appetizing behaviour of mice with safe and poisoning product.	
Method for recording	The method is to estimate the food consumption, by weighing every	

/ scoring of the effect	day the mangers and compares values obtains with the pre-baiting with safe crushed wheat and the competitive condition with safe crushed wheat and poisoning wheat.
Intervals of examination	Daily
Statistics	Total and average amount consumed by mice population based on the number dead animals.
Post monitoring of the test organism	Yes. The poisoning and the post monitoring phases are grouped in the same period, until the death of animal is observed.

7 Results

Efficacy

All tested animal died.

The efficacy is 100%.

Dose/Efficacy curve

Based on the food preference ratio between safe crushed wheat and poisoning wheat, we can observe the poisoning bait is consumed at half of the safe wheat.

Dead mice	Total (g) of consumed wheat during the poisoning phase		Ratio (PCW/SCW)
	Poisoning crushed wheat (PCW)	Safe crushed wheat (SCW)	
10	66.3	139.8	**0.47**

Begin and duration of effects

The results show that mice died between day 6 and 17 after the first ingestion of rodenticide bait, either nearly 9 days after the first ingestion.

Observed effects in the post monitoring phase

- Death of mice.
- The amount of poisoning bait is eaten sufficiently to cause death among the tested animal
- Comparison between pre-baiting and poisoning phase shows a decrease in the daily consumed food, whatever the sex is, mice eat in average 3.52 in pre-baiting versus an average of 2.42grams for the poisoning phase.
- Few days before days, mice eat less and less their daily portion of wheat.

Effects against organisms or objects to be protected

Not applicable

Other effects

none

Efficacy of the reference substance

Not applicable

Tabular and/or graphical presentation of the summarised results

Poisoning and Post monitoring phase results				
Dead mice	Total (g) of consumed wheat during the poisoning phase		Ratio (PCW/SCW)	Living day (average)
	Poisoning crushed wheat (PCW)	Safe crushed wheat (SCW)		
10	66.3	139.8	**0.47**	**9**

Table 1: Poisoning and Post monitoring phase results

Average (g) consumption by mice per days.(n=10)	
Pre-baiting phase	**3.52**
Poisoning and post monitoring phase	**2.42**

Table 2: comparison of daily consumption at different phase of the test.

The daily consumption during the poisoning phase corresponds to the sum of the safe and the poisoning wheat consumption. The living days of each animal are also take in account in the calculus.

Efficacy limiting factors

Occurrences of resistances: Not applicable

Other limiting factors: Not applicable

8 Relevance of the results compared to field conditions

Reasons for laboratory testing

The laboratory conditions shows the :

- Daily amount of food consumed by mice
- Timing needed for the product efficacy after ingestion
- Mice's behaviour in competitive food condition.
- Mice's behaviour with an older product stored in realistic conditions.

All these parameters are important when the scaling will be settled down.

Intended actual scale of biocide application

Not applicable

Relevance compared to field conditions

The parameters explained in 4.1 are estimated, the individual specification of mice can varied in an open space. Moreover, in nature rodent have access to other kind of food.

Application method: In this laboratory experiment, mice have accessed to one kind of food crushed wheat.

In nature condition, mice have access to other kind of food, which can

run in competition with the poisoned wheat.

It is very interesting to observe and compare their behaviour in the field condition.

Moreover, nature trials are closer to real condition of use than a laboratory process.

Test organism	YES	X
Observed effect	YES	X

Relevance for read-across

Yes,
This experiment demonstrated that stored and fresh products are both accepted by mice, despite the difference in time and their chemical variation in active ingredient.
We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier.

9 Applicant's Summary and conclusion

Materials and methods

The aim of the experiment is to compare appetizing behaviour of mice with safe and poisoning product.

BELGASOURIS, the tested product is a bait ready to use presented under whole and crushed grains, containing 0.005 % of Difenacoum.

Mice (*Mus musculus)* were acclimated during at least 8 days in their housed cages before starting the experiment.

During the test, mice received a portion of crushed wheat in their mangers, each kind of food were weighed in order to estimate the consumption.

The process is established by this following steps:

- Pre-baiting with crushed wheat: 5 days
- Poisoning and monitoring phase: safe crushed wheat and poisoning wheat: until death or 21 days after the first bait exposure.

The concentration in active ingredient was also determined before the experiment.

Reliability

1, Study conducted in compliance with agreed protocols.

The experiment was conducted on product and the results are based on the number of dead mice.

Assessment of efficacy, data analysis and interpretation

The experiment was conducted on rodenticide product with wild grey mice.

All mice died, nine animals on ten ate less bait than their usual food. The preference food ratio is nearly for poison is nearly the half of the usual wheat.

Conclusion

The efficacy of the product is very good because all the tested animals in presence of the rodenticide, BELGASOURIS, died an average of 9 days, which is a normal timing for an anticoagulant product.

The efficacy is 100%.

Proposed efficacy specification

BELGASOURIS is appropriate to fight against *Mus musculus.*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	10 Evaluation by Rapporteur Member State
Date	April 2011.
Comments	**1.3** The guidelines followed were not attached to the test report. **2.3.1** TNsG on product evaluation recommends that twenty mice should be used (10 male and 10 female). **4.3.2** Test organism – wild grey mice (*Mus musculus*). **4.3.3** Observed effect – 100% mortality. **5.2** Reliability of 2 is more appropriate.
Summary and conclusion	Although the number of animals tested was below those recommended in the TNsG, the palatability and efficacy of the fresh BELGASOURIS gain bait proved excellent. Although ingestion levels were slightly below those for un baited feed the dose consumed was sufficient to achieve total control of the mice tested.
	11 Comments from ... *(specify)*
Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	BELGASOURIS: rodenticide cereals bait. Containing 0.005 % of Difenacoum
Origin	Record number R290.896 Date of production: August 1996 Date of arrival in lab: 29 August 1996
Initial biomass	Not applicable
Reference of methods	Not mentioned
Collection / storage of samples	By comparative measure between results obtained at with safe food and poisoning food.
Preparation of inoculum for exposure	The measures on fresh product started on 15/09/1996.
Pretreatment	Not applicable
~~**Initial density of test population in the test system/**~~ **Active substance determined in the product**	Chemical analyse in Difenacoum on fresh product of 0.0047% m/m Difenacoum. (analyse Bulletin N° 8513/ch.1135/1996/107)

1.2 Test organism (*if applicable*)

Criteria	Details
Species	Grey mice *(Mus musculus)*
Strain	Wild
Source	Not mentioned
Laboratory culture	No applicable
Stage of life cycle and stage of stadia	Not applicable due to the test conditions
Mixed age population	Not mentioned
Other specification	Not applicable due to the test conditions
Number of organisms tested	10 mice captured in the field area of Gembloux and housed in individual cages.
Method of cultivation	Mangers were weighted daily.
Pretreatment of test organisms before exposure	Before the experiment, mice are acclimatizing at least 8 days, in their individual cage, where they received water and crushed wheat *ad libitum*.
Initial density/number of test organisms in the test system	10 mice by experiment.

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions
Number of vessels / concentration	Not applicable due to the test conditions
Test culture media and/or carrier material	Not applicable due to the test conditions
Nutrient supply	Not applicable due to the test conditions
Measuring equipment	Not applicable due to the test conditions

1.4 Application of test substance

Criteria	Details
Application procedure	During pre-baiting, mice received crushed wheat. During the poison bait, mice received 10g of safe crushed wheat and 10g of poison crushed bait, in two different mangers. Each manger is weighed every day.
Delivery method	In mangers
Dosage rate	Pre-baiting: 7g of safe crushed wheat
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Not applicable due to the test conditions

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	Not applicable due to the test conditions
Moisture	-
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	-

Section B5.10_02

Reference

Official use only

Reference

Ryckel (de) B., Meeus P., CRA Gembloux, Choice test through different period of time, performed on BELGASOURIS, rodenticide containing 0.005% of Difenacoum, against grey mice (*Mus musculus*), report 972 (complement to rapport 947), July 1997.

CRA(Agronomic Research Center), Phytopharmacological department, Rue du Bordia, 11, 5030 Gembloux Belgium

Data protection

Yes

Data owner

BELGAGRI

Industrial Zone of Noville-les-Bois

14, rue du Grand Champ

5380 FERNELMONT, Belgium

Criteria for data protection	Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post inclusion
Guideline study	Guideline for the Rodenticide assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides)*
Deviations	No

12 Method

Test Substance (Biocidal Product)	as given in section 2 deviating from specification given in section 2 *(Fill in the fields 3.1.2 and 3.1.3)*
Trade name/ proposed trade name	BELGASOURIS
Composition of Product tested	0.005 % of Difenacoum
Physical state and nature	Bait ready to use: whole and crushed grains
Monitoring of active substance concentration	Yes, Chemical analyse of the BLEGASOURIS was determined before the appetizing trials.
Method of analysis	HPLC
Reference substance	No.
Method of analysis	Not applicable

for reference substance		
Testing procedure		
Test population / inoculum / test organism	20 albinos mice (*Mus musculus*) by experiment: - 10 males - 10 females	X
Test system	Mice are housed in individual cage.	
Application of TS	Mice received a portion of 5 g of crushed wheat in their mangers. Every day, mangers were weighed in order to estimate the consumption. Safe and poisoning wheat are placed in two different mangers. At each weight measures, mangers of safe and poisoning wheat are alternated.	
Test conditions	Minimum three weeks were observed between the first and the last captured mice, in order to suppress pregnant female. Before starting of experiment, mice were acclimatized during 8 days in cages where they received crushed wheat and water *ad libitum*.	
Duration of the test / Exposure time	Please find the duration by phase: • Pre-baiting with safe crushed wheat: 5 days • Poisoning bait Vs safe crushed bait: 21 days	
Number of replicates performed	No replicates	
Controls	Yes, two controls by experiment: One male and one female were fed with crushed wheat, like the pre bating phase of the experiment	
Examination		

Effect investigated	Assessment of mice appetizing toward product BELGASOURIS at different period of time: T0 and T6 months Efficacy of stored product BELGASOURIS used against mice population (*Mus musculus*).
Method for recording / scoring of the effect	The method is to estimate the food consumption, by weighing every day the mangers and compares values obtained from safe crushed wheat and poisoning wheat.
Intervals of examination	Daily
Statistics	Total and average amount ate by mice population based on the number dead animals. Decision critters: Trough the time, bait can lose its efficacy due to a decrease in active substance or due to the appetizing or maybe both. The decrease in active ingredient can be measured by a chemical analyse performed on the fresh product and then on the stored/old product. The appetizing loss can be measured, theoretically, by comparison between the total consumed at T0 and the total consumed at T6 months tested on albinos mice, from same strain, same age and same food history. However, an appetizing loss can also cause an efficacy loss, so the decision is based on the mortality results.
Post monitoring of the test organism	Yes. The poisoning and the post monitoring phases are grouped in the same period, until the death of animal is observed.

13 Results

Efficacy	All tested animal died excepted one female, at T0.

The efficacy at T0 is 95% and 100% at T6 months.

Dose/Efficacy curve

Based on the preference ratio between safe crushed wheat and poisoning wheat, in the following table, we can observe:

- Ratios at T6 are higher than T0, so the stored bait did not lose appetizing properties.

Timing	Sex	n of dead	Total (g) of consumed wheat during the poisoning phase		Ratio (PCW/SCW)	Mortality
			Poisoning crushed wheat (PCW)	Safe crushed wheat (SCW)		
T0	Male	10	53.8	165.1	**0.33**	**95%**
	Female	9	27.3	125.2	**0.22**	
T6	Male	10	80.9	161.4	**0.50**	**100%**
	Female	10	68.2	119.4	**0.57**	

Begin and duration of effects

After the first application of the tested product until the animal's death, the number of living days is:

- At T0, an average of 7 days for males and 6 for females.
- At T6, an average of 6 days for males and 8 for females.
    - The living day decrease for males at T6 is explained by the higher amount of ingested bait.

Observed effects in the post monitoring phase

Death of mice

Comparison between pre-baiting and poisoning phase shows a decrease in the daily consumed food.

Few days before days, mice eat less and less their daily portion of wheat.

Effects against organisms

Not applicable

or objects to be protected

Other effects

At T0, one female (number 6) seems less sensitize than other mice to the rodenticide bait.

.

Efficacy of the reference substance

Not applicable

Tabular and/or graphical presentation of the summarised results

Poisoning and Post monitoring phase results						
Timing	Sex	n of dead	Total (g) of consumed wheat		Ratio (PCW/ SCW)	Living day (average)
			(PCW)	(SCW)		
T 0	Male	10	53.8	165.1	**0.33**	**7**
	Female	9	27.3	125.2	**0.22**	**6**
T 6	Male	10	80.9	161.4	**0.50**	**6**
	Female	10	68.2	119.4	**0.57**	**8**

Chemical analyse of Difenacoum in tested product at:

- T0 (fresh product) is 47.2 mg/kg
- T6 (stored product) is 40.9 mg/kg.

After 6 months, the rodenticide product lost 12% of Difenacoum

Efficacy limiting factors

Occurrences of resistances

Not applicable

Other limiting factors

Not applicable

14 Relevance of the results compared to field conditions

Reasons for laboratory testing	The laboratory conditions shows the : • Daily amount of food consumed by mice • Timing needed for the product efficacy after ingestion • Mice's behaviour in competitive food condition. • Mice's behaviour with an older product stored in realistic conditions. All these parameters are important when the scaling will be settled down.	
Intended actual scale of biocide application	Not applicable	
Relevance compared to field conditions	The parameters explained in 4.1 are estimated, the individual specification of mice can varied in an open space. Moreover, in nature rodent have access to other kind of food.	
Application method	In this laboratory experiment, mice have accessed to one kind of food crushed wheat. In nature condition, mice have access to other kind of food, which can run in competition with the poisoned wheat. It is very interesting to observe and compare their behaviour in the field condition. Moreover, nature trials are closer to real condition of use than a laboratory process.	
Test organism	YES	X
Observed effect	YES	X
Relevance for read-across	Yes, This experiment demonstrated that stored and fresh products are both accepted by mice, despite the difference in time and their chemical	

variation in active ingredient.
We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier.

15 Applicant's Summary and conclusion

Materials and methods

The aim of the experiment is to compare appetizing behaviour of mice with fresh bait product and a stored bait product. The protocol can also defined the preference food ratio between safe and poisoning product.

BELGASOURIS, the tested product is a bait ready to use presented under whole and crushed grains, containing 0.005 % of Difenacoum.

Mice (*Mus musculus)* were acclimated during at least 8 days in their housed cages before starting the experiment.

During the test, mice received a portion of crushed wheat in their mangers.

Poison bait and safe wheat are in two different mangers, each kind of food is weighed to determine the daily consumption of each kind. During the poisoning phase, after each measure, mangers are alternated.

The process is established by this following steps:

- Pre-baiting with crushed wheat: 5 days
- Poisoning and monitoring phase: safe crushed wheat and poisoning wheat: until death or 21 days after the first bait exposure.

The concentration in active ingredient was also determined before the experiment:

Reliability

1, Study conducted in compliance with agreed protocols.

The experiment was conducted on product and the results are based

	on the number of dead mice.
Assessment of efficacy, data analysis and interpretation	The experiment was conducted on rodenticide product with albinos mice. One on 40 tested animal survived to the experiment: female number 6 at T0, so neither the decrease in active substance nor the appetizing loss is not involved in this result
Conclusion	Fresh BELGASOURIS is efficient at 95%. The appetizing rate for BELGASOURIS does not decrease trough the time period of 6 months.
Proposed efficacy specification	BELGASOURIS is appropriate to fight against *Mus musculus.*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	16 Evaluation by Rapporteur Member State
Date	April 2011.
Comments	**2.3.1** In total 40 mice were used (20 for the fresh bait and 20 for the aged bait experiments). **4.3.2** Test organism – albino mice (*Mus musculus*). **4.3.3** Observed effect – 100% mortality.
Summary and conclusion	The fresh BELGASOURIS bait achieved 95% mortality in mice (1 female survived) whilst the 6 month aged bait achieved 100% control of mice.
	17 Comments from ... *(specify)*
Date	*Give date of comments submitted*

Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	BELGASOURIS: rodenticide cereals bait. Containing 0.005 % of Difenacoum
Origin	Record number R290.896 Date of production: August 1996 Date of arrival in lab: 29 August 1996 Efficacy test on wild grey mice: rapport 947.
Initial biomass	Not applicable
Reference of methods	Not mentioned
Collection / storage of samples	By comparative measure between results obtained at with safe food and poisoning food.
Preparation of inoculum for exposure	The measures began the: - 6th November 1996 on the fresh product. - 30th May 1997 on the stored product.
Pretreatment	At its arrival, product with its originally packaging is placed in dark condition at 5°C. The day before the appetizing trials, the amount required for the three trials at different time of storage, is stored at 20°C, always in the initial packaging.
~~Initial density of test population in the test system/~~ Active substance determined	Chemical analyse in Difenacoum at: - T0 (fresh product) is 47.2 mg/kg (analyse Bulletin N° 8513/ch.1135/1996/107) - T6 (stored product) is 40.9 mg/kg. (analyse Bulletin N° 8686-Ch.1267-1997/47) After 6 months, the rodenticide product lost 12% of

in the product	Difenacoum.

1.2 Test organism (*if applicable*)

Criteria	Details
Species	mice *Mus musculus*
Strain	Albinos
Source	Not mentioned
Laboratory culture	No applicable
Stage of life cycle and stage of stadia	Not applicable due to the test conditions
Mixed age population	Between 10 and 20 weeks old.
Other specification	Not applicable due to the test conditions
Number of organisms tested	22 mice per experiment: T0 and T6.
Method of cultivation	Mangers were weighted daily.
Pretreatment of test organisms before exposure	Before the experiment, mice are acclimatizing at least 8 days, in their individual cage, where they received water and crushed wheat *ad libitum*.
Initial density/number of test organisms in the test system	20 tested mice by experiment and 2 controls.

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions
Number of vessels / concentration	Not applicable due to the test conditions
Test culture media and/or carrier material	Not applicable due to the test conditions
Nutrient supply	Not applicable due to the test conditions
Measuring equipment	Not applicable due to the test conditions

1.4 Application of test substance

Criteria	Details
Application procedure	During pre-baiting, mice received crushed wheat. During the poison bait, mice received 5g of safe crushed wheat and 5g of poison crushed bait, in two different mangers. Safe and poisoning bait are in two different mangers, every day, after the weight measures and the refill in, mangers are alternated.
Delivery method	In mangers
Dosage rate	Pre-baiting: 5g of safe crushed wheat
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Decision critters: Trough the time, bait can lose its efficacy due to a decrease in active substance or due to the appetizing or maybe both. The decrease in active ingredient can be measured by a chemical analyse performed on the fresh product and then on the stored/old product. The appetizing loss can be measured, theoretically, by comparison between the total consumed at T0 and the total consumed at T6 months tested on albinos mice, from same strain, same age and same food history. However, an appetizing loss can also cause an efficacy loss, so the decision is based on the mortality results.

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	
Moisture	
Aeration	
Method of exposure	
Aging of samples	
Other conditions	

Section B5.10_03

Reference

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Reference

De Proft M., Meeus P., CRA Gembloux, Choice test with BELGASOURIS at different period of time, bait ready to use, containing 0.005% of Difenacoum, used in albinos mice in order to be applied against grey mice (*Mus musculus*), complement report 10.312, February 2001.

CRA(Agronomic Research Center), Phytopharmacological department, Rue du Bordia, 11, 5030 Gembloux Belgium

Data protection

Yes

Data owner	BELGAGRI Industrial Zone of Noville-les-Bois 14, rue du Grand Champ 5380 FERNELMONT, Belgium	
Criteria for data protection	Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post inclusion	
Guideline study	Guideline for the Rodenticide assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides)*	X
Deviations	No	

18 Method

Test Substance (Biocidal Product)	as given in section 2 deviating from specification given in section 2 *(Fill in the fields 3.1.2 and 3.1.3)*	
Trade name/ proposed trade name	BELGASOURIS	
Composition of Product tested	0.005 % of Difenacoum	
Physical state and nature	Bait ready to use being presented under whole grains, crushed barley grains, corn cracks and cornflakes, coloured in red.	
Monitoring of active substance	Yes, Chemical analyse of the BLEGASOURIS was used to determine the	

concentration	concentration on fresh product and product stored during 12 months.
Method of analysis	HPLC

Reference substance

	No.
Method of analysis for reference substance	Not applicable

Testing procedure

Test population / inoculum / test organism	22 albinos mice (*Mus musculus*) by test. • 11 males • 11 females
Test system	Mice are housed in individual cage.
Application of TS	Mice received a portion of 7.0 g of crushed wheat in their mangers. Every day, mangers were weighed in order to estimate the consumption.
Test conditions	Mice are between 10 and 20 weeks old. After the pre-baiting phase, mice received in addition of their crushed wheat, portion of poisoned crushed wheat, BELGASOURIS.
Duration of the test / Exposure time	Please find the duration by phase: • Pre-baiting with crushed wheat: 5 days • Poisoning bait Vs safe crushed bait: 21 days
Number of replicates performed	No replicates.

Controls	Yes. One male and one female were fed with crushed wheat, like the pre bating phase of the experiment.

Examination

Effect investigated	Assessment of mice appetizing toward product BELGASOURIS at different period of time: T0 and T12 months Efficacy of fresh and stored product BELGASOURIS used against mice population (*Mus musculus*).
Method for recording / scoring of the effect	The method is to estimate the food consumption, by weighing every day the mangers and compares values obtained from safe crushed wheat and poisoning wheat.
Intervals of examination	Daily
Statistics	Total and average amount ate by mice population based on the number dead animals.
Post monitoring of the test organism	Yes. The poisoning and the post monitoring phases are grouped in the same period, until the death of animal is observed.

19 Results

Efficacy

All tested animal died excepted three females:

- Numbers 2 and 6 at T0.
- Number 6 at T12.

The efficacy at T0 is 90% and 95% at T12 months.

Dose/Efficacy curve	Based on the food preference ratio between safe crushed wheat and poisoning wheat, in the following table, we can observe:

- Ratios at T12 are higher than T0, so the stored bait did not lose appetizing properties.

Timing	Sex	n of dead	Total (g) of consumed wheat during the poisoning phase		Ratio (PCW/SCW)	Mortality
			Poisoning crushed wheat (PCW)	Safe crushed wheat (SCW)		
T0	Male	10	12.3	263.9	**0.047**	90 %
	Female	8	14.3	237.5	**0.060**	
T12	Male	10	71.5	200.2	**0.36**	95 %
	Female	9	39.5	179	**0.22**	

Table 1: Food preference ratio through storage time

Begin and duration of effects

After the first application of the tested product until the animal's death, the number of living days is:

- At T0, an average of 7.5 days for males and 12 for females.
- At T12, an average of 6 days for males and 7 for females.

Observed effects in the post monitoring phase

Death of mice is observed despite the very low preference food ratio observed at T0.

Comparison between pre-baiting and poisoning phase shows a decrease in the daily consumed food.

Few days before days, mice eat less and less their daily portion of wheat.

Effects against organisms or objects to be protected

Not applicable

Other effects

Some animals seems less sensitized than other mice to the rodenticide bait.

Efficacy of the reference substance

After 12 months of storage, we observed an efficiency as good as the efficiency obtained at T0 in report 972, with similar preference food ratio. The timing required to obtain same results is longer after 12 months, it can be easily explain by the loss in active substance through the time.

Tabular and/or graphical presentation of the summarised results

Poisoning and Post monitoring phase results						
Timing	Sex	n of dead	Total (g) of consumed wheat		Ratio (PCW/ SCW)	Living day (average)
			(PCW)	(SCW)		
T 0	Male	10	12.3	263.9	**0.047**	**7.5**
	Female	8	14.3	237.5	**0.060**	**6**
T 6	Male	10	71.5	200.2	**0.36**	**12**
	Female	9	39.5	179	**0.22**	**7**

Efficacy limiting factors

Occurrences of resistances: Not applicable

Other limiting factors: Not applicable

20 Relevance of the results compared to field conditions

Reasons for laboratory testing

The laboratory conditions shows the :

- Daily amount of food consumed by mice

	• Timing needed for the product efficacy after ingestion • Mice's behaviour in competitive food condition. • Mice's behaviour with an older product stored in realistic conditions. All these parameters are important when the scaling will be settled down.	
Intended actual scale of biocide application	Not applicable	
Relevance compared to field conditions	The parameters explained in 4.1 are estimated, the individual specification of mice can varied in an open space. Moreover, in nature rodent have access to other kind of food.	
Application method	In this laboratory experiment, mice have accessed to one kind of food crushed wheat. In nature condition, mice have access to other kind of food, which can run in competition with the poisoned wheat. It is very interesting to observe and compare their behaviour in the field condition. Moreover, nature trials are closer to real condition of use than a laboratory process.	
Test organism	YES	X
Observed effect	YES	X
Relevance for read-across	Yes, This experiment demonstrated that stored and fresh products are both accepted by mice. We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier.	

21 Applicant's Summary and conclusion

Materials and methods

The aim of the experiment is to compare appetizing behaviour of mice with fresh and stored product.

BELGASOURIS, the tested product is a bait ready to use presented under whole grains, crushed barley grains, corn cracks and cornflakes, coloured in red, containing 0.005 % of Difenacoum.

Mice (*Mus musculus)* were acclimated during at least 8 days in their housed cages before starting the experiment.

During the test, mice received a portion of crushed wheat in their mangers, each kind of food were weighed in order to estimate the consumption.

The process is established by this following steps:

- Pre-baiting with crushed wheat: 5 days
- Poisoning and monitoring phase: safe crushed wheat and poisoning wheat: until death or 21 days after the first bait exposure.

The concentration in active ingredient was also determined before the experiment.

Reliability

1, Study conducted in compliance with agreed protocols.

The experiment was conducted on product and the results are based on the number of dead mice.

Moreover a timing scale of active substance and BELGASOURIS efficacy has been made.

Assessment of efficacy,

The experiment was conducted on rodenticide product with albinos mice. The condition on fresh and stored product were identical

data analysis and interpretation	Three on 40 tested animal survived to the experiment: female number 2 and 6 at T0, female number 6 at T6, so neither the decrease in active substance nor the appetizing loss is not involved in this result
Conclusion	Mice appetizing for BELGASOURIS have not decreased during the last 12 months of storage at ambient temperature (20°C), despite a reduction of 24% in active substance. The efficacy at T0 is 90% and 95% at T12 months.
Proposed efficacy specification	BELGASOURIS is appropriate to fight against *Mus musculus.* Based on these results, The conformity time of the product can be fixed at least to 12 months. For the 24 months conformity please refer to study in B5_10_06.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	22 Evaluation by Rapporteur Member State
Date	April 2011.
Comments	**1.3** The guidelines followed were not attached to the test report. **4.3.2** Test organism – albino mice (*Mus musculus*). **4.3.3** Observed effect – 100% mortality. **5.2** Reliability of 2 is appropriate for this test.
Summary and conclusion	Levels of mortality observed for the T0 and T12 aged baits were 90% and 95% respectively. This was a good level of kill despite the fact that the amount of active substance had dropped by almost a quarter in the stored product in relation to the fresh. This can be explained another way however whereby the fresh product had 72.6 mg/kg when analysed and the stored product contained 53.6 mg/kg difenacoum (i.e. both in excess of the rate proposed). In this test the grain bait formulation appeared to be far less palatable when compared to the block and pasta bait formulations.

23 Comments from ... *(specify)*

Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	BELGASOURIS: rodenticide grains bait. Containing 0.005 % of Difenacoum
Origin	Record number R160201
Initial biomass	Not applicable
Reference of methods	Not mentioned
Collection / storage of samples	By comparative measure between results obtained at T0 and T12 months stored product. From the same origin.
Preparation of inoculum for exposure	The measures on fresh product started on 07/03/2001 and on stored product on 27/02/2002.
Pretreatment	The product when it arrived at lab was considered as fresh, three samples were prepared as follows: • 200g stored at -18°C for the chemical analyse on fresh product. • The half part is stored in room at 20°C, in order to test the chemical analysis and the appetizing after 12 months. • The other half part is stored at 4°C for the appetizing test on fresh product. Products were always stored in dark conditions
~~**Initial density of test population in the test system/**~~ **Active substance determined**	Chemical analyse in Difenacoum at: - T0 (fresh product) is 72.6 mg/kg - T12 (stored product) is 53.6 mg/kg. After 12 months, the rodenticide product lost 26% of

in the product	Difenacoum

1.2 Test organism (*if applicable*)

Criteria	Details
Species	mice *Mus musculus*
Strain	Albinos
Source	Not mentioned
Laboratory culture	No applicable
Stage of life cycle and stage of stadia	Not applicable due to the test conditions
Mixed age population	Between 10 and 20 weeks
Other specification	Not applicable due to the test conditions
Number of organisms tested	20 mice (10 males and 10 females) and 2 controls, by experiment (T0 and T12 months)
Method of cultivation	Mangers were weighted daily.
Pretreatment of test organisms before exposure	Before the experiment, mice are acclimatizing at least 8 days, in their individual cage, where they received water and fresh crushed wheat *ad libitum*.
Initial density/number of test organisms in the test system	22 mice by experiment (T0 and T12 months)

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions
Number of vessels / concentration	Not applicable due to the test conditions
Test culture media and/or carrier material	Not applicable due to the test conditions
Nutrient supply	Not applicable due to the test conditions
Measuring equipment	Not applicable due to the test conditions

1.4 Application of test substance

Criteria	Details
Application procedure	During pre-baiting, mice received crushed wheat. During the poison bait, mice received safe crushed wheat and poison crushed bait.
Delivery method	In mangers
Dosage rate	Pre-baiting: 7g of safe crushed wheat
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Not applicable due to the test conditions

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	Not applicable due to the test conditions
Moisture	-
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	-

Section B5.10_04

Reference

Official use only

Reference

-, Pest Control Assistance (PCA), Appetition and efficacy trial of « DISOURICIDE PESCE » on grey mice (*Mus musculus*), For LODI, Le Cosquer (56), 2002

PCA, 3 rue Constantin Le Priol 56150 BAUD (France),

Unpublished

Data protection

Yes

Data owner

LODI S.A.,

Parc d'activité des Quatre Routes,

35390 Grand Fougeray, France

Criteria for data protection

Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post

inclusion

Guideline study

Yes,

The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides:

- Adopted on 1960, derived from the work of Chitty and Dotty in the 1940.
- Revised by OEPP in 1980.

Deviations

No

24 Method

Test Substance (Biocidal Product)

as given in section 2

deviating from specification given in section 2

(Fill in the fields 3.1.2 and 3.1.3)

Trade name/ proposed trade name

DISOURICIDE PESCE

Composition of Product tested

0.005 % of Difenacoum

Physical state and nature

Wheat rodenticide bait

Monitoring of active substance concentration

No

Method of analysis

Testing method of practical efficacy of raticides of the CEB, revised by OEPP:

This method has been adapted to anticoagulants. This is a relative method, which consists in the comparison of two stages of consumption of a placebo: one before, on after bait.

	It is nearly impossible to know the number mice, it can only be estimated. The method consisting offering the placebo of the bait, in bait station that permit to carry out consumption measurements, and to daily statements with the placing of new baits, until we obtain a global consuming stabilised over 3 consecutive days. Then an estimation of the whole population can be made on basis of the food consumed. After obtaining this stage the placebo is replaced by toxic bait a period between 7 to 10 days. Regarding the slow mode of action of anticoagulant, one week is needed without toxic bait or placebo, so that death rate we can hope over, and then we go post baiting with the placebo, to establish the second consumption stage. To obtain the first stage, 2 to 3 weeks are necessary depending on the importance of the mice population. For the post-baiting, it does not exceed 5 days in general, in order to avoid eventual recontamination by mice coming from the surroundings of the site, which would lead to a wrong estimation of consumption.	
Reference substance	No	
Method of analysis for reference substance	-	
Testing procedure		
Test population / inoculum / test organism	Not mentioned please find details of estimation in table 1.2.	X
Test system	The experimental site is 3 aviaries for exotic birds. Address Collin J-L, Le Cosquer 56300 Malguenac, France. (75002 Paris). According to the data shown in field, the infestation seems to be solely localized around the aviaries (bird food available in mangers in the aviaries).	

Application of TS

Nine bait boxes "PS LODI PVC SOUPLE" with 100g of wheat in each are places in the site. Every day each spots were weighed until graph reaches a plateau in the food consumption.

Test conditions

According to the data shown in field, the infestation seems to be solely localized around the aviaries (bird food available in mangers in the aviaries).

Duration of the test / Exposure time

The experiment was settled down all along the month of march.

- Step 0: Inspection of the trial place and setting up of the baiting boxes
- Pre-baiting: Determination of initial consumption with wheat= 23 DAYS, initial amount placed 100g of safe wheat.
- Poisoning bait : Treatment with 100g of rodenticide bait for each bait point= 19 DAYS
- Post-baiting: Determination of final consumption= 14 DAYS

Any rest period was observed.

Number of replicates performed

No replicates

Controls

No control.

Examination

Effect investigated

Reduction of mice population by poisoning with rodenticide wheat bait produced in the year.

Method for recording / scoring of the effect

The method is to estimate by indirect observation, the bait consumption, a decrease of population before and after poisoning bait.

Intervals of examination

Daily

Statistics

[Average Pre-btg (grams) – Average Post-btg (grams)] x100/ Average Pre-btg (grams) = Efficacy

Btg= baiting

Post monitoring of the test organism

Yes,
After the poisoning phase, safe wheat replaced poisoning wheat at same spot. It is called, the post-baiting phase, where the reduction in population is estimated.

25 Results

Efficacy

The wheat consumption has decreased from around 100g to around 5g, namely a 95% efficacy.

Dose/Efficacy curve

The consumption of wheat has not begun at the beginning of the trial, probably due to the food competition and because mice are neophob animals

An important decrease in wheat consumption was observed the 23 February. Either, 13 days after the first poisoning bait.

The changing in food, wheat to poisoned wheat has seemed create a phenomena of mistrust among mice, which was observed by a low consumption the first days.

Begin and duration of effects

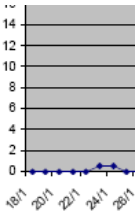
The consumption of poisoned bait felt on the 13th day of the treatment phase.

Observed effects in the post monitoring phase

1. The post baiting happened normally. The flow of consumption remains as low during the post period than the end of the treatment period.
2. By indirect observation, we suppose the targeted animals are died from the ingestion of poisoning bait.

Effects against organisms or objects

Not applicable

to be protected	
Other effects	-
Efficacy of the reference substance	Not applicable
Tabular and/or graphical presentation of the summarised results	Please find in the following graph, the daily consumption in the bait boxes: 
Efficacy limiting factors	
Occurrences of resistances	Not applicable
Other limiting factors	Not applicable

26 Relevance of the results compared to field conditions

Reasons for laboratory testing	This experiment is a scaling-up. This experiment is closer to reality than laboratory process. Moreover, aviaries are exposed to mice invasions. Please note that both conditions are tested in the dossier.
Intended actual scale of biocide application	Not applicable

Relevance compared to field conditions	Not applicable	X
Application method	Not applicable, this study is closer to field condition than laboratory process.	X
Test organism	YES	X
Observed effect	Not applicable	X
Relevance for read-across	Yes, This experiment shows results in a specific area with real conditions and constraints related to architecture and uses of the building in process of treatment. Moreover, rodents are very attracted by any food storages, which offer them a huge supply of their needs. We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier.	

27 Applicant's Summary and conclusion

Materials and methods

The experimental site has been chosen to their natural condition opportunities: aviaries with food supply for birds.

The aviaries, “Le Cosquer”, are located in MALGUENAC 56300. Baits were placed where evident traces of mice were observed and in their possible access used by them.

This method used has been adapted to anticoagulants. This is a relative method, which consists in the comparison of two stages of consumption of a placebo: one before and one after the poisoning bait.

Pre-baiting phase:

It is nearly impossible to know the number of mice, it can only be estimated. The method consisting offering the placebo of the bait, in bait station that permit to carry out consumption measurements, and to daily statements with the placing of new baits, until we obtain a global consuming stabilised what this translated by a plateau on the graph. Then an estimation of the whole population can be made on basis of the food consumed.

Poisoning phase:

After obtaining the estimated population, the placebo is replaced by toxic bait for 19 days.

The changing of food, the passage of whole wheat towards poisoning bait can cause mistrust in mice behaviour. This phenomenon is translated to the field by a low consumption. Generally, this phenomenon is passed within 2 days.

Post-baiting:

Placebo was put in place during 14 days but the average consumption. This time corresponds to the surviving mice brings back to the bait stations

Reliability

1, Study conducted in compliance with agreed protocols.

The consumption rate established during the poisoning phase corresponds to the expectations, but a comparison with the post baiting values is needed to relatives the all experiment.

Assessment of efficacy, data analysis and interpretati

The consumption of wheat has not begun at the beginning of the trial, probably due to the food competition and because mice are neophob animals.

The wheat has been replaced by Disouricide pesce when the wheat

on	consumption was constant. The daily consumption has decreased when wheat has been replaced by Disouricide pesce, probably because of the neophobia once again, then the mice have consumed more Disouricide pesce than wheat.
Conclusion	The wheat consumption has decreased from around 100g to around 5g, namely a 95% efficacy. Consequently, Disouricide pesce is effective at 95% on grey mice.
Proposed efficacy specification	According to the point, we can declare the product as very efficiency with the rate of 95% find in this experiment, which is compliance with the rodenticide guidelines.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	28 Evaluation by Rapporteur Member State
Date	April 2011.
Comments	**2.3.1** Estimated population was 100 wild mice (*Mus musculus*). **4.3** Study was conducted under field conditions. **4.3.1** Application method – oral. Grain bait placed in a purpose made bait box. **4.3.2** Target organism – wild mice (*Mus musculus*). **4.3.3** Observed effect – reduction in consumption of 95% indicating efficacy of treatment.
Summary and conclusion	DISCOURICIDE PESCE appeared to be both palatable and effective in this field study given that the mice had free access to feed on site. The estimated population was 100 mice based on census baiting but their bait consumption levels dropped by 95% from the peak consumption plateau indicating excellent control.
	29 Comments from ... *(specify)*
Date	*Give date of comments submitted*

Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	DISOURICIDE PESCE: grain rodenticide bait. Containing 0.005 % of Difenacoum
Origin	LODI Manufacturing date: December 2001
Initial biomass	Not applicable
Reference of methods	Testing method of practical efficacy of raticides of the CEB, revised by OEPP: First step: Pre-baiting: wheat without toxic substance. New baits are put in place daily until the consumption is stabilised over 3 consecutive days. Second step with the toxic substance Last step: Post-baiting with safe wheat.
Collection / storage of samples	By comparative measure between before and after baiting with placebo (wheat)
Preparation of inoculum for exposure	The measures for the pre-baiting started the 18th February 2002, at the rate of 100g of wheat by bait boxes. Nine bait boxes were dispatched around the aviaries. Twenty-three days were necessary to obtain a stabilised consumption of wheat. The 9/02/02, the wheat is replaced by Disouricide pesce, 100g in each bait box. Bait boxes emptied every day and refill with 100g of Disouricide pesce. The consumption in all the bait boxes is recorded every day. The rodenticide baits were placed during 19days. From 01/03/02 to 14/03/02: Bait boxes emptied every day and refill with 100g of wheat. The consumption in all the bait boxes is recorded every day. The 14/03/02, all the bait boxed are removed with in the same time as wheat.

Pretreatment	Any
~~Initial density of test population in the test system/~~ Active substance determined in the product	Containing 0.005 % of Difenacoum

1.2 Test organism (*if applicable*)

Criteria	Details
Species	Mouse *(Mus musculus)*
Strain	Wild
Source	From the surrounding areas of the 3 aviaries for exotic birds
Laboratory culture	No, the aim of the study is to be as much as close of the reality.
Stage of life cycle and stage of stadia	Not applicable due to the test conditions
Mixed age population	Not applicable due to the test conditions
Other specification	Not applicable due to the test conditions
Number of organisms tested	The average consumption of last 3 days of pre-baiting shows: (105+100+95)/3= 300 grams / day. Based on the average and if we allocate an effective consumption of 3 g per mice, we could estimate the test population to nearly 100 mice.
Method of cultivation	Bait stations were weighted daily.
Pretreatment of test organisms before exposure	Preliminary step was put in place to bring as many mice as possible.

Initial density/number of test organisms in the test system	Based on the pre-baiting step and an average of 3g per mouse, the population is estimated to 100 mice.

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions
Number of vessels / concentration	Not applicable due to the test conditions
Test culture media and/or carrier material	Not applicable due to the test conditions
Nutrient supply	Not applicable due to the test conditions
Measuring equipment	Not applicable due to the test conditions

1.4 Application of test substance

Criteria	Details
Application procedure	Whole wheat during the pre-baiting and post baiting phase and Grains bait during the poisoning phase
Delivery method	In bait boxes “PAS PVS SOUPLE LODI”
Dosage rate	Weighted the daily consumption and refill with 100g of wheat, safe or rodenticide.
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Not applicable due to the test conditions

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	Not applicable due to the test conditions
Moisture	-
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	-

Section B5.10_05

Reference

Official use only

Reference

-, Pest Control Assistance (PCA), Appetition and efficacy trial of « DISOURICIDE PESCE » on grey mice (*Mus musculus*), For LODI, Mme Rigal, 56150 Baud, 2002

PCA, 3 rue Constantin Le Priol 56150 BAUD (France),

Unpublished

Data protection

Yes

Data owner

LODI S.A.,

Parc d'activité des Quatre Routes,

35390 Grand Fougeray, France

Criteria for data protection

Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post inclustion

Guideline study Yes,

The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides:

- Adopted on 1960, derived from the work of Chitty and Dotty in the 1940.
- Revised by OEPP in 1980.

Deviations No

30 Method

Test Substance (Biocidal Product) as given in section 2

deviating from specification given in section 2

(Fill in the fields 3.1.2 and 3.1.3)

Trade name/ proposed trade name DISOURICIDE PESCE

Composition of Product tested 0.005 % of Difenacoum

Physical state and nature Wheat rodenticide bait

Monitoring of active substance concentration No

Method of analysis Testing method of practical efficacy of raticides of the CEB, revised by OEPP:

This method has been adapted to anticoagulants. This is a relative method, which consists in the comparison of two stages of consumption of a placebo: one before, on after bait.

	It is nearly impossible to know the number mice, it can only be estimated. The method consisting offering the placebo of the bait, in bait station that permit to carry out consumption measurements, and to daily statements with the placing of new baits, until we obtain a global consuming stabilised over 3 consecutive days. Then an estimation of the whole population can be made on basis of the food consumed. After obtaining this stage the placebo is replaced by toxic bait a period between 7 to 10 days. Regarding the slow mode of action of anticoagulant, one week is needed without toxic bait or placebo, so that death rate we can hope over, and then we go post baiting with the placebo, to establish the second consumption stage. To obtain the first stage, 2 to 3 weeks are necessary depending on the importance of the mice population. For the post-baiting, it does not exceed 5 days in general, in order to avoid eventual recontamination by mice coming from the surroundings of the site, which would lead to a wrong estimation of consumption.	
Reference substance	No	
Method of analysis for reference substance	-	
Testing procedure		
Test population / inoculum / test organism	Not mentioned please find details of estimation in table 1.2.	X

Test system

The experimental site is a house with kitchen, dining room, cellar, basement and garage. Address: Mme Rigal, Rue de la Libération, 56150 BAUD, France.

Application of TS

Bait boxes have been placed in strategic places where mice (and tracks) have been seen a lot of time.

- 3 Bait boxes in the kitchen
- 2Bait boxes in the dining room
- 2 Bait boxes in the garage
- 3 Bait boxes in the cellar
- 2 Bait boxes in the basement

100 grams of wheat were placed in each bait box, either 1200g of wheat available every day.

Advice to the proprietary: tidy all the food that was available to mice, close the dustbins

Test conditions

Bait boxes have been placed in strategic places where mice (and tracks) have been seen a lot of time.

Duration of the test / Exposure time

The experiment was settled down all along the month of march.

- Step 0: Inspection of the trial place and setting up of the baiting boxes
- Pre-baiting: Determination of initial consumption with wheat= 8 DAYS, initial amount placed 100g of safe wheat.
- Poisoning bait : Treatment with 100g of rodenticide baitt for each bait point= 7 DAYS
- Post-baiting: Determination of final consumption= 5 DAYS

Any rest period was observed.

A check visit is observed on the 12th March, either one week after the remove of all boxes.

X

Number of replicates performed

No replicates

Controls

No control.

Examination

Effect investigated	Reduction of mice population by poisoning with ageing rodenticide of two years.
Method for recording / scoring of the effect	The method is to estimate by indirect observation, the bait consumption, a decrease of population before and after poisoning bait.
Intervals of examination	Daily
Statistics	[Average Pre-btg (grams) – Average Post-btg (grams)] x100/ Average Pre-btg (grams) = Efficacy Btg= baiting
Post monitoring of the test organism	Yes, After the poisoning phase, safe wheat replaced poisoning wheat at same spot. It is called, the post-baiting phase, where the reduction in population is estimated.

31 Results

Efficacy	The wheat consumption before treatment was around 300g, and after treatment the wheat consumption was around 5g. Consequently Disouricide pesce is effective at 98% on grey mice, even 2 years after the manufacturing
Dose/Efficacy curve	The wheat consumption has quickly reached a maximal level. The first day of rodenticide bait, the consumption of wheat has decreased, but the consumption was restored the next day. This phenomena is probably due to neophob mice.
Begin and duration	The consumption of poisoned bait felt on the 17th February (6th day after the first rodenticide bait)

of effects

Observed effects in the post monitoring phase

3. The post baiting happened normally. The flow of consumption remains as low during the post period than the end of the treatment period.
4. By indirect observation, we suppose the targeted animals are died from the ingestion of poisoning bait.
5. One week after the test, a check visit was made: No mice, no droppings.

Effects against organisms or objects to be protected

Not applicable

Other effects

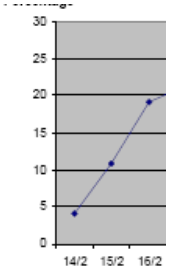
-

Efficacy of the reference substance

Not applicable

Tabular and/or graphical presentation of the summarised results

Please find in the following graph, the daily consumption in the bait boxes:



Efficacy limiting factors

Occurrences of resistances

Not applicable

Other limiting factors

Not applicable

32 Relevance of the results compared

to field conditions

Reasons for laboratory testing	This experiment is a scaling-up. This experiment is closer to reality than laboratory process. Moreover, private houses are exposed to mice invasions. Please note that both conditions are tested in the dossier.	
Intended actual scale of biocide application	Not applicable	
Relevance compared to field conditions	Not applicable	X
Application method	Not applicable, this study is closer to field condition than laboratory process.	X
Test organism	YES	X
Observed effect	Not applicable	X
Relevance for read-across	Yes, This experiment shows results in a specific area with real conditions and constraints related to architecture and uses of the building in process of treatment. Moreover, rodents are very attracted by any food storages, which offer them a huge supply of their needs. We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier.	

33 Applicant's Summary and conclusion

Materials and	The experimental site has been chosen to their natural condition	

methods

opportunities: domestic/private house with kitchen, dining room, cellar, basement and garage. Address: Mme Rigal, Rue de la Libération, 56150 BAUD, France.

This method used has been adapted to anticoagulants. This is a relative method, which consists in the comparison of two stages of consumption of a placebo: one before and one after the poisoning bait.

Pre-baiting phase:

It is nearly impossible to know the number of mice, it can only be estimated. The method consisting offering the placebo of the bait, in bait station that permit to carry out consumption measurements, and to daily statements with the placing of new baits, until we obtain a global consuming stabilised what this translated by a plateau on the graph. Then an estimation of the whole population can be made on basis of the food consumed.

Poisoning phase:

After obtaining the estimated population, the placebo is replaced by toxic bait for 7 days.

The changing of food, the passage of whole wheat towards poisoning bait can cause mistrust in mice behaviour. This phenomenon is translated to the field by a low consumption. Generally, this phenomenon is passed within 2 days.

Post-baiting:

Placebo was put in place during 5 days but the average consumption. This time corresponds to the surviving mice brings back to the bait stations

One week after the test, a check was made.

Reliability	1, Study conducted in compliance with agreed protocols. The consumption rate established during the poisoning phase corresponds to the expectations, but a comparison with the post baiting values is needed to relatives the all experiment.
Assessment of efficacy, data analysis and interpretation	The consumption of wheat has not begun at the beginning of the trial, probably due to the food competition and because mice are neophob animals. The wheat has been replaced by Disouricide pesce when the wheat consumption was constant. The daily consumption has decreased when wheat has been replaced by Disouricide pesce, probably because of the neophobia once again, then the mice have consumed more Disouricide pesce than wheat.
Conclusion	The wheat consumption has quickly reached a maximal level The wheat consumption has decreased from around 25% to around 0%, the efficacy is almost totale. The wheat consumption before treatment was around 300g, and after treatment the wheat consumption was around 5g. Consequently Disouricide pesce is effective at 98% on grey mice, even 2 years after the manufacturing.
Proposed efficacy specification	According to the point, we can declare the product as very efficiency with the rate of 98% find in this experiment, which is compliance with the rodenticide guidelines.

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
34 Evaluation by Rapporteur Member State

Date	April 2011.
Comments	**2.3.1** Test organism was a wild strain of mouse (*Mus musculus*). **2.3.5** Both the poisoning period and the post-baiting period could have easily been extended and may have resulted in 100% mortality of the test population. **2.4.1** Rodenticide bait was aged for 2 years prior to commencement of study. **4.3** Relevant for field conditions as test was conducted in the field. **4.3.1** Application method is oral, with baits placed in a tamper proof bait station. **4.3.2** Test organism was wild strain of mouse (*Mus musculus*). **4.3.3** Observed effect was drop in consumption indication death of the target pests.
Summary and conclusion	12 strategically placed baiting stations were used in the study, conducted in a private dwelling house. Following an initial pre-baiting phase the population was estimated at ~100 mice. The bait used had been aged for 2 years. Based on calculation from consumption levels observed during the pre-baiting, baiting phase and post-baiting consumption indicated 98% control of the target organisms (drop in daily consumption from 300g to 5g).

35 Comments from ... *(specify)*

Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	DISOURICIDE PESCE: grain rodenticide bait. Containing 0.005 % of Difenacoum
Origin	LODI Manufacturing date: April 2000
Initial biomass	Not applicable
Reference of methods	Testing method of practical efficacy of raticides of the CEB, revised by OEPP: First step: Pre-baiting: wheat without toxic substance. New baits are put in place daily until the consumption is stabilised over 3 consecutive days. Second step with the toxic substance Last step: Post-baiting with safe wheat.
Collection / storage of samples	By comparative measure between before and after baiting with placebo (wheat)
Preparation of inoculum for exposure	**13/02/02:** Bait boxes have been placed in strategic places where mice (and tracks) have been seen a lot of time **From 14/02/02 to 21/02/02:** Bait boxes emptied every day and refill with 100g of wheat. The consumption in all the bait boxes is recorded every day. The 21/02/02, the wheat is replaced by Disouricide pesce (Same quantity = 100g per box) **From 22/02/02 to 28/02/02:** Bait boxes emptied every day and refill with 100g of Disouricide pesce. The consumption in all the bait boxes is recorded every day. The 28/02/02, Disouricide pesce is replaced by wheat (Same quantity = 100g per box)

	From 01/03/02 au 05/03/02: Bait boxes emptied every day and refill with 100g of wheat. The consumption in all the bait boxes is recorded every day. The 05/03/02, all the bait boxed are removed with in the same time as wheat.
Pretreatment	Any
~~**Initial density of test population in the test system/**~~ **Active substance determined in the product**	Containing 0.005 % of Difenacoum

1.2 Test organism (*if applicable*)

Criteria	Details
Species	Mouse *(Mus musculus)*
Strain	Wild
Source	From the surrounding areas of the house
Laboratory culture	No, the aim of the study is to be as much as close of the reality.
Stage of life cycle and stage of stadia	Not applicable due to the test conditions
Mixed age population	Not applicable due to the test conditions
Other specification	Not applicable due to the test conditions
Number of organisms tested	The average consumption of last 3 days of pre-baiting shows: (300+305+310)/3= 305 grams / day. Based on the average and if we allocate an effective consumption of 3 g per mice, we could estimate the test population to nearly 100 mice.
Method of cultivation	Bait stations were weighted daily.
Pretreatment of test organisms before exposure	Preliminary step was put in place to bring as many mice as possible.

Initial density/number of test organisms in the test system	Based on the pre-baiting step and an average of 3g per mouse, the population is estimated to 100 mice.

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions
Number of vessels / concentration	Not applicable due to the test conditions
Test culture media and/or carrier material	Not applicable due to the test conditions
Nutrient supply	Not applicable due to the test conditions
Measuring equipment	Not applicable due to the test conditions

1.4 Application of test substance

Criteria	Details
Application procedure	Whole wheat during the pre-baiting and post baiting phase and Grains bait during the poisoning phase
Delivery method	In bait boxes “PAS PVS SOUPLE LODI”
Dosage rate	Weighted the daily consumption and refill with 100g of wheat, safe or rodenticide.
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Not applicable due to the test conditions

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	Not applicable due to the test conditions
Moisture	-
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	-

Section B5.10_06

Reference

Official use only

Reference

De Proft M., CRA Gembloux, Study of ageing behavior of ready-to-use baits containing 0.005% of Difenacoum (effect on palatability), PART 2: Grain Bait, report number ROD 2008 11 BIO 6-Part 2: Grains baits

CRA (Agronomic Research Center), Phytopharmacological department, Rue du Bordia, 11, 5030 Gembloux Belgium

Data protection

Yes

Data owner

BIO 6

Industrial Zone of Noville-les-Bois

14, rue du Grand Champ

5380 FERNELMONT, Belgium

Criteria for data protection

Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post

inclusion

Guideline study

Yes,

The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides:

- Adopted on 1960, derived from the work of Chitty and Dotty in the 1940.
- Revised by OEPP in 1981, J. Giban.

Deviations

No

36 Method

Test Substance (Biocidal Product)

as given in section 2

deviating from specification given in section 2

(Fill in the fields 3.1.2 and 3.1.3)

Trade name/ proposed trade name

1. DIFENACOUM GRAIN BAIT WHOLE GRAIN

2. DIFENACOUM GRAIN BAIT BROKEN GRAIN

Composition of Product tested

0.005 % of Difenacoum

Physical state and nature

1. DIFENACOUM GRAIN BAIT WHOLE GRAIN
Whole wheat grains
2. DIFENACOUM GRAIN BAIT BROKEN GRAIN
Broken wheat grains

Monitoring of active substance concentration

No X

Method of analysis

The study protocol included the following :

- An acceptance test of the fresh product with albinos' rodents and in individuals cages fresh product.

- An acceptance test of the product aged for 12 months with albinos' rodents in individuals
- An acceptance of the product aged for 24 months with albinos' rodents in individuals

Acceptance loss is measured by comparing results of several acceptance trials. Each of these trails uses 22 albino rats (11 males and 11 females) 10 to 20 weeks old, from the same origin, the same strain, and the same alimentation story at the trial start.

The first trial uses fresh product and the following aged product (respectively 12 and 24 month). Each trials begins when rats have been acclimatized at least 8 days in individual cages in the lab, where they receive as much water and crushed wheat as they want.

During the first 5 days, all the rodents received crushed wheat in a feeding dish:

- 30g for rats
- 7g for mice

Daily consumption of each rodent was measure by calculating the difference between weight of the full feeding dish and this one of this dish after 24 hours. In a second time, another dish containing the study bait was added, except for 2 control rodents (one male and one female) which continued to be fed only with crushed wheat. Trials last 20 days.

Reference substance

No

Method of analysis for reference substance

-

Testing procedure

Test population / inoculum / test organism

Rats *(Rattus norvegicus)*

Mice (*Mus musculus*)

Test system	Rodents were housed individually in cages.
Application of TS	Daily, the bait stations were measured.
Test conditions	Each trials begins when rodents have been acclimatized at least 8 days in individual cages in the lab, where they receive as much water and crushed wheat as they want.
Duration of the test / Exposure time	Trials last 20 days for each experiment.
Number of replicates performed	No replicates
Controls	Yes: one male and one female. They only received crushed wheat.

Examination

Effect investigated	Determination of bait acceptance by rodents.
Method for recording / scoring of the effect	Daily consumption of each rodent was measure by calculating the difference between weight of the full feeding dish and this one of this dish after 24 hours.
Intervals of examination	Daily
Statistics	Calculating the difference between weight of the full feeding dish and this one of this dish after 24 hours.
Post monitoring of the test organism	Yes, The post-baiting is required to estimate the reduction in rats' population.

37 Results

Efficacy

1. DIFENACOUM GRAIN BAIT WHOLE GRAIN

- T0: 15 dead rats at the end of the trial
- T12: 18 dead rats at the end of trial.

No problem of acceptance loss has been observed

2. DIFENACOUM GRAIN BAIT BROKEN GRAIN

Between fresh product and the 12 months aged product (100% dead mice for both trials), no problem of acceptance loss has observed.

Dose/Efficacy curve

Between fresh product and the 12 months aged product, acceptance loss is not significant.

Begin and duration of effects

Not applicable

Observed effects in the post monitoring phase

Not applicable

Effects against organisms or objects to be protected

Not applicable

Other effects

-

Efficacy of the reference substance

Not applicable

Tabular and/or graphical presentation of the summarised results

Between fresh product and the 12 months aged product, acceptance loss is not significant.

Efficacy limiting factors Not applicable

Occurrences of resistances Not applicable

Other limiting factors Not applicable

38 Relevance of the results compared to field conditions

Reasons for laboratory testing

The laboratory conditions shows the :

- Daily amount of food consumed by rodents
- Timing needed for the product efficacy after ingestion
- Rodent's behaviour in competitive food condition (appetizing behaviour of mice in presence of product)

All these parameters are important when the scaling will be settled down.

Intended actual scale of biocide application Not applicable

Relevance compared to field conditions

The parameters explained in 4.1 are estimated, the individual specification of mice can varied in an open space. Moreover, in nature rodent have access to other kind of food.

Application method

In this laboratory experiment, rodents have accessed to two types of food.

In nature condition, rodents have access to other kind of food, which

	can run in competition with the poisoned bait. Moreover the change in food can cause mistruth and modify the alimentary behaviour in mice. It is very interesting to observe and compare their behaviour in the field condition. Moreover, nature trials are closer to real condition of use than a laboratory process.	
Test organism	YES	X
Observed effect	YES	X
Relevance for read-across	Yes, We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier.	

39 Applicant's Summary and conclusion

Materials and methods

The study protocol included the following :

- An acceptance test of the fresh product with albinos' rodents and in individuals cages fresh product.
- An acceptance test of the product aged for 12 months with albinos' rodents in individuals
- An acceptance of the product aged for 24 months with albinos' rodents in individuals

Acceptance loss is measured by comparing results of several acceptance trials. Each of these trails uses 22 albino rats (11 males and 11 females) 10 to 20 weeks old, from the same origin, the same strain, and the same alimentation story at the trial start.

The first trial uses fresh product and the following aged product (respectively 12 and 24 month). Each trials begins when rats have been acclimatized at least 8 days in individual cages in the lab, where they receive as much water and crushed wheat as they want.

During the first 5 days, all the rodents received crushed wheat in a feeding dish:

- 30g for rats

- 7g for mice

Daily consumption of each rodent was measure by calculating the difference between weight of the full feeding dish and this one of this dish after 24 hours. In a second time, another dish containing the study bait was added, except for 2 control rodents (one male and one female) which continued to be fed only with crushed wheat. Trials last 20 days.

Reliability

1, Study conducted in compliance with agreed protocols.

Assessment of efficacy, data analysis and interpretation

1. DIFENACOUM GRAIN BAIT WHOLE GRAIN

- T0: 15 dead rats at the end of the trial
- T12: 18 dead rats at the end of trial.

No problem of acceptance loss has been observed

2. DIFENACOUM GRAIN BAIT BROKEN GRAIN

Between fresh product and the 12 months aged product (100% dead mice for both trials), no problem of acceptance loss has observed.

Conclusion

Between fresh product and the 12 months aged product, acceptance loss is not significant.

Proposed efficacy specification

Between fresh product and the 12 months aged product, acceptance loss is not significant.

The last trials have to be conducted on 24months –aged products, at the end of 2010.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	40 Evaluation by Rapporteur Member State
Date	April 2011.
Comments	**2.1.4** The active substance concentration was monitored and the results presented in Doc IVB. **4.3.2** Test organism – Rats (*Rattus norvegicus*). Mice (*Mus musculus*). Details of the strains used were not provided. **4.3.3** Observed effect – mortality.
Summary and conclusion	The whole grain bait achieved 75% and 90% efficacy against rats with the fresh and 12-month aged bait respectively. No comment was provided on the surviving animals. The broken gain bait achieved 100% control of mice for both the fresh and aged (12-month) baits.
	41 Comments from ... *(specify)*
Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	DIFENACOUM GRAIN BAIT : Containing 0.005 % of Difenacoum
Origin	Batch BS291008: broken wheat Batch BR291008: whole wheat
Initial biomass	Not applicable
Reference of methods	The study protocol included the following : 1) An acceptance of the fresh product with albinos' rats and in individuals cages fresh product. 2) An acceptance of the product aged for 12 months with albinos' rats in individuals 3) An acceptance of the product aged for 24 months with albinos' rats in individuals
Collection / storage of samples	By comparative measure between before and after baiting with placebo (wheat).
Preparation of inoculum for exposure	First Pre-baiting: GRAINS baiting: Post bait phase:

<table>
<tr><td>Pretreatment</td><td>Not applicable</td></tr>
<tr><td><s>Initial density of test population in the test system/</s> Active substance determined in the product</td><td>The product DIFENACOUM GRAIN BAIT was tested at different time in the same lab conditions. The product was stored at room temperature.
<table>
<tr><th></th><th>whole</th><th>broken</th></tr>
<tr><td>Production date</td><td>0.00466
-6.8%**
(2008/11/04)</td><td>0.00472
-5.6%**
(2008/11/05)</td></tr>
<tr><td>T0 (start of trial,</td><td>0.00453
-9.4%**
(2009/01/20)</td><td>0.00459
-8.2%**
(2009/04/17)</td></tr>
<tr><td>T12</td><td>0.00419
-16.2%**
(2009/10/16)</td><td>0.00424
-15.2%**
(2009/10/16)</td></tr>
</table>
**deviation for the measured content from the declared value</td></tr>
</table>

1.2 Test organism (if applicable)

Criteria	Details
Species	Rats*(Rattus norvegicus)* Mice *(Mus musculus)*
Strain	Albinos
Source	From the same origin, the same strain, and the same alimentation story at the trial start.
Laboratory culture	No, the aim of the study is to be as much as close of the reality.
Stage of life cycle and stage of stadia	10 to 20 weeks old,
Mixed age population	
Other specification	Not applicable
Number of organisms tested	20 tested animal, 10 of each sex.
Method of cultivation	Measurement in bait station every day.
Pretreatment of test organisms before exposure	Not applicable
Initial density/number of test organisms in the test system	Not applicable

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions.
Number of vessels / concentration	
Test culture media and/or carrier material	
Nutrient supply	
Measuring equipment	

1.4 Application of test substance

Criteria	Details
Application procedure	Wheat during the pre-baiting and post baiting phase and paste during the poisoning phase.
Delivery method	manger
Dosage rate	Measurement of consumption was measured every day.
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Not applicable due to the test conditions

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	Not applicable due to the test conditions
Moisture	-
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	-

Section B5.10_07

Reference

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Reference

-, Pest Control Assistance (PCA), Appetition and efficacy trial of « DIRATICIDE » on brown rats (*Rattus norvegicus*), For LODI, U.K.L (56), 2002

PCA, 3 rue Constantin Le Priol 56150 BAUD (France),

Unpublished

Data protection	Yes
Data owner	LODI S.A., Parc d'activité des Quatre Routes, 35390 Grand Fougeray, France
Criteria for data protection	Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post inclusion
Guideline study	Yes, The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. • Revised by OEPP in 1980.
Deviations	No

42 Method

Test Substance (Biocidal Product)	as given in section 2 deviating from specification given in section 2 *(Fill in the fields 3.1.2 and 3.1.3)*
Trade name/ proposed trade name	DIRATICIDE
Composition of Product tested	0.005 % of Difenacoum
Physical state and	Wheat rodenticide bait

nature	
Monitoring of active substance concentration	No
Method of analysis	Testing method of practical efficacy of raticides of the CEB, revised by OEPP: This method has been adapted to anticoagulants. This is a relative method, which consists in the comparison of two stages of consumption of a placebo: one before, on after bait. It is nearly impossible to know the number rats, it can only be estimated. The method consisting offering the placebo of the bait, in bait station that permit to carry out consumption measurements, and to daily statements with the placing of new baits, until we obtain a global consuming stabilised over 3 consecutive days. Then an estimation of the whole population can be made on basis of the food consumed. After obtaining this stage the placebo is replaced by toxic bait a period between 7 to 10 days. Regarding the slow mode of action of anticoagulant, one week is needed without toxic bait or placebo, so that death rate we can hope over, and then we go post baiting with the placebo, to establish the second consumption stage. To obtain the first stage, 2 to 3 weeks are necessary depending on the importance of the rodent population. For the post-baiting, it does not exceed 5 days in general, in order to avoid eventual recontamination by rats coming from the surroundings of the site, which would lead to a wrong estimation of consumption.
Reference substance	No
Method of analysis for reference substance	-

Testing procedure

Test population / inoculum / test organism

Not mentioned please find details of estimation in table 1.2.

Test system

The experimental site is silos for cereals' storage. Address U.K.L., Zone artisanale Lanveur, 56440 LANGUIDIC, France.

Classic storage of cereals with the particularities as follows: the silos are totally emptied and all the premises are cleaned in order to remove dust, and a cleaning with water is just about to be performed.

The site in infested with the species: *Rattus norvegicus*

It is decided to wait for the end of the cleaning before proceeding to the treatment.

Application of TS

The rodenticide treatment begins at the end of the cleaning

07/02/02: 14 bait boxes are placed, with 500g of wheat in each:

Elevator pit No 1:

- 3 bait boxes in the redler

Big silo:

- 1 bait box where there are cables
- 1 bait box under the pit

Elevator pit No 2:

- 1 bait box under the pit
- 1 bait box in the redler
- 1 bait box where there are cables
- 1 bait box in the tunnel

Ventilation area:

- 2 bait boxes

Outdoor shelter for the engines:

- 1 bait box

« Pont-bascule » (area to weigh the trucks):

- 2 bait boxes

500 grams of wheat were placed in each bait box, either 7000g of wheat available every day.

Test conditions

Classic storage of cereals with the particularities as follows: the silos are totally emptied and all the premises are cleaned in order to remove dust, and a cleaning with water is just about to be performed.

The site in infested with the species: *Rattus norvegicus*

It is decided to wait for the end of the cleaning before proceeding to the treatment.

Duration of the test / Exposure time

The experiment was settled down all along the month of march.

- Step 0: Inspection of the trial place and setting up of the baiting boxes
- Pre-baiting: Determination of initial consumption with wheat= 13 DAYS, initial amount placed 500g of safe wheat.
- Poisoning bait : Treatment with 500g of rodenticide bait for each bait point= 8 DAYS
- Post-baiting: Determination of final consumption= 4 DAYS

Any rest period was observed.

A check visit is observed on the 11th March, either one week after the remove of all boxes.

Number of replicates performed

No replicates

Controls

No control.

Examination

Effect investigated

Reduction of rat population by poisoning with rodenticide wheat bait produced in the year.

Method for recording / scoring of the effect

The method is to estimate by indirect observation, the bait consumption, a decrease of population before and after poisoning bait.

Intervals of examination

Daily

Statistics

[Average Pre-btg (grams) – Average Post-btg (grams)] x100/ Average Pre-btg (grams) = Efficacy

Btg= baiting

Post monitoring of the test

Yes,

organism

After the poisoning phase, safe wheat replaced poisoning wheat at same spot. It is called, the post-baiting phase, where the reduction in population is estimated.

43 Results

Efficacy

The wheat consumption has decreased from around 2.1Kg to around 140g, namely a decrease of 93%.

The efficacy of the product is 93%.

Dose/Efficacy curve

The first day of rodenticide bait, the consumption of wheat has decreased, but the consumption was restored the next day. This phenomena is probably due to the mistruth of rat which is has a neophobia behaviour with food.

Begin and duration of effects

The consumption of poisoned bait felt on the 26th February (6th day after the first rodenticide bait) .

Observed effects in the post monitoring phase

6. The post baiting happened normally. The flow of consumption remains as low during the post period than the end of the treatment period.
7. By indirect observation, we suppose the targeted animals are died from the ingestion of poisoning bait.
8. One week after the test, a check visit was made: No rat was seen.

Effects against organisms or objects to be protected

Not applicable

Other effects

-

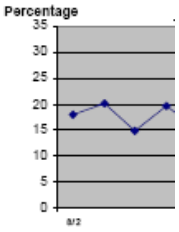
Efficacy of the reference

Not applicable

substance

Tabular and/or graphical presentation of the summarised results

Please find in the following graph, the daily consumption in the bait boxes:



Efficacy limiting factors

Occurrences of resistances

Not applicable

Other limiting factors

Not applicable

44 Relevance of the results compared to field conditions

Reasons for laboratory testing	This experiment is a scaling-up. This experiment is closer to reality than laboratory process. Moreover, aviaries are exposed to rodent invasions. Please note that both conditions are tested in the dossier.	
Intended actual scale of biocide application	Not applicable	
Relevance compared to field conditions	Not applicable	
Application method	Not applicable, this study is closer to field condition than laboratory process.	X
Test organism	YES	X
Observed effect	Not applicable	X
Relevance for read-across	Yes, This experiment shows results in a specific area with real conditions and constraints related to architecture and uses of the building in process of treatment. Moreover, rodents are very attracted by any food storages, which offer them a huge supply of their needs. We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier.	

45 Applicant's Summary and conclusion

Materials and methods

The experimental site has been chosen to their natural condition opportunities: The experimental site is silos for cereals' storage. Address U.K.L., Zone artisanale Lanveur, 56440 LANGUIDIC, France.

Classic storage of cereals with the particularities as follows: the silos are totally emptied and all the premises are cleaned in order to remove dust, and a cleaning with water is just about to be performed.
The site in infested with the species: *Rattus norvegicus*

After the first visit, end of January, it is decided to wait for the end of the cleaning before proceeding to the treatment.

This method used has been adapted to anticoagulants. This is a relative method, which consists in the comparison of two stages of consumption of a placebo: one before and one after the poisoning bait.

Pre-baiting phase:

It is nearly impossible to know the number of rodent, it can only be estimated. The method consisting offering the placebo of the bait, in bait station that permit to carry out consumption measurements, and to daily statements with the placing of new baits, until we obtain a global consuming stabilised what this translated by a plateau on the graph. Then an estimation of the whole population can be made on basis of the food consumed.

Poisoning phase:

After obtaining the estimated population, the placebo is replaced by toxic bait for 19 days.

The changing of food, the passage of whole wheat towards poisoning bait can cause mistrust in rodent behaviour. This phenomenon is translated to the field by a low consumption. Generally, this phenomenon is passed within 2 days.

Post-baiting:

Placebo was put in place during 14 days but the average

consumption. This time corresponds to the surviving rodent brings back to the bait stations

Reliability

1, Study conducted in compliance with agreed protocols.

The consumption rate established during the poisoning phase corresponds to the expectations, but a comparison with the post baiting values is needed to relatives the all experiment.

Assessment of efficacy, data analysis and interpretation

The consumption of wheat has not begun at the beginning of the trial, probably due to the food competition and because rats are neophob animals.

The wheat has been replaced by DIRATICIDE when the wheat consumption was constant.

The daily consumption has decreased when wheat has been replaced by DIRATICIDE, probably because of the neophobia.

Conclusion

The wheat consumption has decreased from around 2.1Kg to around 140g, namely a decrease of 93%.
The efficacy of the product is 93%.

Diraticide is efficient to fight against Brown rats.

Proposed efficacy specification

According to the point, we can declare the product as efficiency with the rate of nearly 95% find in this experiment, which is compliance with the rodenticide guidelines.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	46 Evaluation by Rapporteur Member State
Date	April 2011.
Comments	**4.3.1** Application method – baits placed in tamper proof baiting boxes, route oral. **4.3.2** Test organism – wild strain of brown rats (*Rattus norvegicus*). **4.3.3** Reduction in bait consumption indicating effectiveness of the poisoning bait.
Summary and conclusion	A reduction in the post-baiting consumption of 93% was observed clearly demonstrating the palatability and effectiveness of DIRATICIDE grain bait. This equates to a reduction from an estimated population of 144 rats to 10 in the experimental phase considered. A longer baiting phase may have resulted in higher efficacy being achieved. This field study is acceptable for product authorisation.
	47 Comments from ... *(specify)*
Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	DIRATICIDE: grain rodenticide bait. Containing 0.005 % of Difenacoum
Origin	LODI Manufacturing date: December 2001
Initial biomass	Not applicable
Reference of methods	Testing method of practical efficacy of raticides of the CEB, revised by OEPP: First step: Pre-baiting: wheat without toxic substance. New baits are put in place daily until the consumption is stabilised over 3 consecutive days. Second step with the toxic substance Last step: Post-baiting with safe wheat.
Collection / storage of samples	By comparative measure between before and after baiting with placebo (wheat)
Preparation of inoculum for exposure	**31/01/02:** Visit of the site **07/02/02:** 14 bait boxes are placed, with 500g of wheat in each **From 08/02/02 to 20/02/02** Bait boxes emptied every day and refill with 500g of wheat. The consumption in all the bait boxes is weighed recorded every day. The wheat has been replaced by the poison Diraticide when the consumption was regular during at least 3 days (20/02/02) **From 21/02/02 to 28/02/02:** Bait boxes emptied every day and refill with 500g of Diraticide. The consumption in all the bait boxes is weighed and recorded every day. 28/02/02: Diraticide is replaced by wheat

	From 01/03/02 to 04/04/02: Bait boxes emptied every day and refill with 500g of wheat. The consumption in all the bait boxes is weighed recorded every day. The 04/04/02: All the bait boxes are collected in the same time as wheat.
Pretreatment	Any
~~**Initial density of test population in the test system/**~~ **Active substance determined in the product**	Containing 0.005 % of Difenacoum

1.2 Test organism (*if applicable*)

Criteria	Details
Species	Brown rats (*Rattus norvegicus*)
Strain	Wild
Source	From the surrounding areas of silos for cereals' storage.
Laboratory culture	No, the aim of the study is to be as much as close of the reality.
Stage of life cycle and stage of stadia	Not applicable due to the test conditions
Mixed age population	Not applicable due to the test conditions
Other specification	Not applicable due to the test conditions
Number of organisms tested	The average consumption of last 3 days of pre-baiting shows: (2110+2280+2070)/3= 2154 grams / day. Based on the average and if we allocate an effective consumption of 15 g per rats, we could estimate the test population to nearly 144 rats.
Method of cultivation	Bait stations were weighted daily.
Pretreatment of test organisms before exposure	Preliminary step was put in place to bring as many rats as possible.

Initial density/number of test organisms in the test system	Based on the pre-baiting step and an average of 15g per rat, the population is estimated to 144 animals.

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions
Number of vessels / concentration	Not applicable due to the test conditions
Test culture media and/or carrier material	Not applicable due to the test conditions
Nutrient supply	Not applicable due to the test conditions
Measuring equipment	Not applicable due to the test conditions

1.4 Application of test substance

Criteria	Details
Application procedure	Whole wheat during the pre-baiting and post baiting phase and Grains bait during the poisoning phase
Delivery method	In bait boxes.
Dosage rate	Weighted the daily consumption and refill with 500g of wheat, safe or rodenticide.
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Not applicable due to the test conditions

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	Not applicable due to the test conditions
Moisture	-
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	-

Section B5.10_08

Reference

Official use only

Reference

-, Pest Control Assistance (PCA), Appetition and efficacy trial of « DIRATICIDE» on brown rat (*Rattus norvegicus*), For LODI, Mr LAMOURIC Maurice, Tréviol, 56480 CLEGUEREC Baud, 2002

PCA, 3 rue Constantin Le Priol 56150 BAUD (France),

Unpublished

Data protection

Yes

Data owner

LODI S.A.,

Parc d'activité des Quatre Routes,

35390 Grand Fougeray, France

Criteria for data protection

Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post inclusion

Guideline study

Yes,

The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides:

- Adopted on 1960, derived from the work of Chitty and Dotty in the 1940.
- Revised by OEPP in 1980.

Deviations

No

48 Method

Test Substance (Biocidal Product)

as given in section 2

deviating from specification given in section 2

(Fill in the fields 3.1.2 and 3.1.3)

Trade name/ proposed trade name

DIRATICIDE

Composition of Product tested

0.005 % of Difenacoum

Physical state and nature

Wheat rodenticide bait

Monitoring of active substance concentration

No

Method of analysis

Testing method of practical efficacy of raticides of the CEB, revised by OEPP:

This method has been adapted to anticoagulants. This is a relative method, which consists in the comparison of two stages of consumption of a placebo: one before, on after bait.

It is nearly impossible to know the number of rats, it can only be estimated. The method consisting offering the placebo of the bait, in bait station that permit to carry out consumption measurements, and to daily statements with the placing of new baits, until we obtain a global consuming stabilised over 3 consecutive days. Then an estimation of the whole population can be made on basis of the food consumed. After obtaining this stage the placebo is replaced by toxic bait a period between 7 to 10 days.

Regarding the slow mode of action of anticoagulant, one week is needed without toxic bait or placebo, so that death rate we can hope over, and then we go post baiting with the placebo, to establish the second consumption stage.

To obtain the first stage, 2 to 3 weeks are necessary depending on the importance of the rat population. For the post-baiting, it does not exceed 5 days in general, in order to avoid eventual recontamination by rats coming from the surroundings of the site, which would lead to a wrong estimation of consumption.

Reference substance

No

Method of analysis for reference substance

-

Testing procedure

Test population / inoculum / test organism

Not mentioned please find details of estimation in table 1.2.

Test system	The experimental site is 4 aviaries for wildfowl breeding (young partridge, pheasants). Address: Mr LAMOURIC Maurice, Tréviol, 56480 CLEGUEREC Baud, 2002
Application of TS	Four bait boxes have been placed around each aviary, out of them, and have been filled in with 500g of wheat
Test conditions	Around each aviary
Duration of the test / Exposure time	The experiment was settled down all along the month of march. • Step 0: Inspection of the trial place and setting up of the baiting boxes • Pre-baiting: Determination of initial consumption with wheat= 20 DAYS, initial amount placed 500g of safe wheat. • Poisoning bait : Treatment with 500g of rodenticide bait for each bait point= 13 DAYS • Post-baiting: Determination of final consumption= 5 DAYS Any rest period was observed.
Number of replicates performed	No replicates
Controls	No control.

Examination

Effect investigated	Reduction of rat population by poisoning with ageing rodenticide of two years.
Method for recording / scoring of the effect	The method is to estimate by indirect observation, the bait consumption, a decrease of population before and after poisoning bait.
Intervals of examination	Daily

Statistics

[Average Pre-btg (grams) – Average Post-btg (grams)] x100/ Average Pre-btg (grams) = Efficacy

Btg= baiting

Post monitoring of the test organism

Yes,
After the poisoning phase, safe wheat replaced poisoning wheat at same spot. It is called, the post-baiting phase, where the reduction in population is estimated.

49 Results

Efficacy

The wheat consumption has decreased from around 1900g to around 40g, namely a decrease of 98%.

Consequently, DIRATICIDE is effective at 98% on Brown Rats, even 2 years after the manufacturing.

Dose/Efficacy curve

The first day of rodenticide bait, the consumption of wheat has decreased, but the consumption was restored the next day. This phenomenon is probably due to neophobia behaviour of rats.

Begin and duration of effects

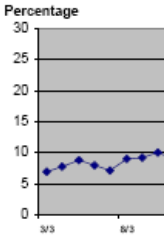
The consumption of poisoned bait felt on the 28th March (6th day after the first rodenticide bait) and the consumption continued to decrease to 30g per day to the 2d April, where a stabilisation occurred around the

Observed effects in the post monitoring phase

9. The post baiting happened normally. The flow of consumption remains as low during the post period than the end of the treatment period.
10. By indirect observation, we suppose the targeted animals are died from the ingestion of poisoning bait.
11. The observations have been pursued until 19/04/02 – as a security – because there was residual wheat consumption

Effects against organisms or objects to be

Not applicable

protected	
Other effects	-
Efficacy of the reference substance	Not applicable
Tabular and/or graphical presentation of the summarised results	Please find in the following graph, the daily consumption in the bait boxes: 
Efficacy limiting factors	
Occurrences of resistances	Not applicable
Other limiting factors	Not applicable

50 Relevance of the results compared to field conditions

Reasons for laboratory testing	This experiment is a scaling-up. This experiment is closer to reality than laboratory process. Moreover, aviaries are exposed to rat due to food for birds and birds. Please note that both conditions are tested in the dossier.
Intended actual scale of biocide	Not applicable

application		
Relevance compared to field conditions	Not applicable	
Application method	Not applicable, this study is closer to field condition than laboratory process.	X
Test organism	YES	X
Observed effect	Not applicable	X
Relevance for read-across	Yes, This experiment shows results in a specific area with real conditions and constraints related to architecture and uses of the building in process of treatment. Moreover, rodents are very attracted by any food storages, which offer them a huge supply of their needs. We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier.	

51 Applicant's Summary and conclusion

Materials and methods

The experimental site has been chosen to their natural condition opportunities: aviaries with food supply for birds and young birds.

The 4 aviaries for wildfowl breeding (young partridge, pheasants) belong to Mr LAMOURIC Maurice, Tréviol, 56480 CLEGUEREC Baud, 2002

This method used has been adapted to anticoagulants. This is a relative method, which consists in the comparison of two stages of consumption of a placebo: one before and one after the poisoning bait.

Pre-baiting phase:

It is nearly impossible to know the number of rats, it can only be estimated. The method consisting offering the placebo of the bait, in bait station that permit to carry out consumption measurements, and to daily statements with the placing of new baits, until we obtain a global consuming stabilised what this translated by a plateau on the graph. Then an estimation of the whole population can be made on basis of the food consumed.

Poisoning phase:

After obtaining the estimated population, the placebo is replaced by toxic bait for 7 days.

The changing of food, the passage of whole wheat towards poisoning bait can cause mistrust in rat’s behaviour. This phenomenon is translated to the field by a low consumption. Generally, this phenomenon is passed within 2 days.

Post-baiting:

Placebo was put in place during 5 days but the average consumption.

This time corresponds to the surviving rats brings back to the bait stations

Reliability

1, Study conducted in compliance with agreed protocols.

The consumption rate established during the poisoning phase corresponds to the expectations, but a comparison with the post baiting values is needed to relatives the all experiment.

Assessment of efficacy, data analysis and interpretation

The consumption of wheat has not begun at the beginning of the trial, probably due to the food competition and because rats are neophob animals.

The wheat has been replaced by DIRATICIDE when the wheat consumption was constant.

The daily consumption has decreased when wheat has been replaced by DIRATICIDE, probably because of the neophobia once again, then the rats have consumed more DIRATICIDE than wheat.

Conclusion

The wheat consumption has decreased from around 1900g to around 40g, namely a decrease of 98%

Consequently, DIRATICIDE is effective at 98% on Brown Rats, even 2 years after the manufacturing.

Proposed efficacy specification

According to the point, we can declare the product as very efficiency with the rate of 98% find in this experiment, which is compliance with the rodenticide guidelines.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	52 Evaluation by Rapporteur Member State

Date	April 2011.
Comments	**4.3.1** Application method – baits placed in tamper proof baiting boxes, route oral. **4.3.2** Test organism – wild strain of brown rats (*Rattus norvegicus*). **4.3.3** Reduction in bait consumption indicating effectiveness of the poisoning bait.
Summary and conclusion	Excellent reductions in consumption (98%) demonstrated the palatability and effectiveness of the 2-year aged grain bait despite the fact that food was present in abundance on site. The efficacy equates to a *pro-rata* reduction in the population from an estimated 124 rats to just 2.

53 Comments from ... *(specify)*

Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	DIRATICIDE: grain rodenticide bait. Containing 0.005 % of Difenacoum
Origin	LODI Manufacturing date: January 2000
Initial biomass	Not applicable
Reference of methods	Testing method of practical efficacy of raticides of the CEB, revised by OEPP: First step: Pre-baiting: wheat without toxic substance. New baits are put in place daily until the consumption is stabilised over 3 consecutive days. Second step with the toxic substance Last step: Post-baiting with safe wheat.
Collection / storage of samples	By comparative measure between before and after baiting with placebo (wheat)
Preparation of inoculum for exposure	**02/03/02:** 4 bait boxes have been placed around each aviary, out of them, and have been filled in with 500g of wheat. **From 03/03/02 to 22/03/02:** Bait boxes emptied every day and refill with 500g of wheat. The consumption in all the bait boxes is weighed recorded every day. The 22/03/02, the wheat is collected a last time then replaced by the poisoned product Diraticide (total wheat consumption was stabilized) **From 23/03/02 to 06/04/02:** Bait boxes emptied every day and refill with 500g of Diraticide. The consumption in all the bait boxes is weighed and recorded every day. The 06/04/02, Diraticide is collected for the last time

	then replaced by wheat (500g in each box) **From 07/04/02 to 19/04/02:** Bait boxes emptied every day and refill with 500g of wheat. The consumption in all the bait boxes is weighed recorded every day. The observations have been pursued until 19/04/02 – as a security – because there was residual wheat consumption.
Pretreatment	Any
~~**Initial density of test population in the test system/**~~ **Active substance determined in the product**	Containing 0.005 % of Difenacoum

1.2 Test organism (*if applicable*)

Criteria	Details
Species	Brown rat *(Rattus norvegicus)*
Strain	Wild
Source	From the surrounding areas of the house
Laboratory culture	No, the aim of the study is to be as much as close of the reality.
Stage of life cycle and stage of stadia	Not applicable due to the test conditions
Mixed age population	Not applicable due to the test conditions
Other specification	Not applicable due to the test conditions
Number of organisms tested	The average consumption of last 3 days of pre-baiting shows: (1840+1910+1850)/3= 1867 grams / day. Based on the average and if we allocate an effective consumption of 15 g per rat, we could estimate the test population to nearly 124 rats.
Method of cultivation	Bait stations were weighted daily.
Pretreatment of test organisms before exposure	Preliminary step was put in place to bring as many rats as possible.

Initial density/number of test organisms in the test system	Based on the pre-baiting step and an average of 15g per rat, the population is estimated to 124 rats.

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions
Number of vessels / concentration	Not applicable due to the test conditions
Test culture media and/or carrier material	Not applicable due to the test conditions
Nutrient supply	Not applicable due to the test conditions
Measuring equipment	Not applicable due to the test conditions

1.4 Application of test substance

Criteria	Details
Application procedure	Whole wheat during the pre-baiting and post baiting phase and Grains bait during the poisoning phase
Delivery method	In bait boxes .
Dosage rate	Weighted the daily consumption and refill with 100g of wheat, safe or rodenticide.
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Not applicable due to the test conditions

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	Not applicable due to the test conditions
Moisture	-
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	-

Section B5.10_09

Reference

Official use only

Reference

Feys J-L., Field trial with RACO GRAIN BAITS against ROOF RATS 11 November 2009_08 03 December 2009, batch PB 091109 Belgagri.

Unpublished

Data protection

Yes

Data owner

BELGAGRI

Industrial Zone of Noville-les-Bois

14, rue du Grand Champ

5380 FERNELMONT, Belgium

Criteria for data protection

Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation] / Post inclusion

Guideline study

Yes,

The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides:

- Adopted on 1960, derived from the work of Chitty and Dotty in the 1940.
- Revised by OEPP in 1981, J. Giban.

Deviations No

54 Method

Test Substance (Biocidal Product) as given in section 2
deviating from specification given in section 2
(Fill in the fields 3.1.2 and 3.1.3)

Trade name/ proposed trade name RACO GRAIN BAITS

Composition of Product tested 0.005 % of Difenacoum

Physical state and nature cereal bait, mixture of cereals

Monitoring of active substance concentration No

Method of analysis Field test to control the attractivity, the uptake and the efficacy of RACO Grain Baits on roof rats (*Rattus rattus*).

Reference substance No

Method of analysis for reference substance -

Testing procedure

Test population / inoculum / test organism	*Rattus rattus (*Roof rats; Black rats*)* Population estimation: 20 rats.
Test system	The field test was performed in one of the pig stables of the company NAVAR ADDRESS: 10, Hanestraat in B- 9810 NAZARETH, Belgium
Application of TS	Daily, the bait stations were measured.
Test conditions	The field test was performed in one of the pig stables of the company NAVAR ADDRESS: 10, Hanestraat in B- 9810 NAZARETH, Belgium The stable where the test was performed is the biggest of 5 stables, see photo above. This stable is separated from the others, although a very small stable is very near and another big stable is quite near also. This stable was chosen because the Pest Controller, Mr. demyttenaere, had a clear view in this stable about the degree of infestation and the resulting damage to the roof, which were not too severe. If the damage were too severe it would have been difficult to affix the bait points. (Small baskets affixed in the holes, see below). The stable is 50 metres long and 18 m large. There is a central alley and left and right of this alley there are 26 pig boxes at each side, 52 in total. These boxes measure 1.90 x 8 m and contained an average of 15 pigs. The stable is completely automized; the pigs receive their food and water automatically. Every two boxes there are concrete beams supporting the roof and, in the middle of the box, beams down from the roof to the floor. The roof of the stable is insulated with polystyrene boards and the rats had made holes in these boards, mainly there where the beams go down. From there they had very easy access to the pig food.
Duration of the test / Exposure	Prebating: 8 days Poisoning bait: 14 days

time	
Number of replicates performed	No replicates
Controls	No control

Examination

Effect investigated	Killing the rat population with a fresh poisoning bait Field test to control the attractivity, the uptake and the efficacy of RACO Grain Baits on roof rats (*Rattus rattus*)
Method for recording / scoring of the effect	The method is to estimate by indirect observation, the bait consumption and a decrease of population before and after poisoning bait.
Intervals of examination	Daily
Statistics	Observation of the consumed baits and traces of rats in their usual environment.
Post monitoring of the test organism	Yes, The post-baiting is required to estimate the reduction in rats' population.

55 Results

Efficacy	The prebaiting showed a small but active group of Rattus rattus, estimated around 20 pieces. The tested product RACO was taken by the roof rats almost as well as the placebo bait.
Dose/Efficacy curve	The uptake of RACO dropped slowly from the eighth day of the test. Probably, the rats showed first signs of sickness after 8 days.

	The twelfth day, 2 dead rats were found. The following days the uptake diminished to a very low level, showing most of the rats were eliminated. Pest Controllers use as a standard rule 10 % of the dead rats are found, so 2 rats brings us back to a population of around 20 rats. The total amount of bait used was 3 x 1.8 kg = 5.4 kg • This can seem to be a big amount for an estimated population of 20 rats, but The stable is quite big, 18 baiting points of 100 g seemed the minimum. • The actual measured uptake was only 2142 g • The last refreshment of 1800 g was not really necessary, but was done to have a good view of the uptake as the old bait was dirty. The sixteenth day, the test was stopped; there was still some small activity, which came to an end later on (follow-up by Pest Controller, without uptake control or any other measurements) Although known as difficult to bait, the roof rats did take well RACO and this in the presence of pig feed..
Begin and duration of effects	Not applicable
Observed effects in the post monitoring phase	Not applicable
Effects against organisms or objects to be protected	Not applicable
Other effects	-
Efficacy of the reference substance	Not applicable
Tabular and/or graphical presentation of the	Not supplied

summarised results		
Efficacy limiting factors	Not applicable	
Occurrences of resistances	Not applicable	
Other limiting factors	Not applicable	

56 Relevance of the results compared to field conditions

Reasons for laboratory testing	This experiment is a scaling-up. Moreover this experiment is closer to reality than laboratory process.	
Intended actual scale of biocide application	Not applicable	
Relevance compared to field conditions	Not applicable	X
Application method	Not applicable. This study is closer to field condition than laboratory process, rodent have access to plenty alternative food which is in competition with the poison bait.	X
Test organism	YES.	X
Observed effect	Not applicable. The conclusions have been made from indirect observations:	X

decreased of food consumption)

Relevance for read-across

Yes,
This experiment shows results in a specific area with real conditions and constraints related to architecture and uses of the building in process of treatment.
Moreover, rodents are very attracted by any food storages, which offer them a huge supply of their needs.
We can refer to the study, which regrouped all excellent parameters, as a relevant example of efficacy test for the dossier

57 Applicant's Summary and conclusion

Materials and methods

Before the test started the Pest Control operator, with years of experience in the destruction of *Rattus rattus* in stables estimated the population in the stable to somewhat 20 to 25 rats.

To control the extension of the population, the uptake of 18 x 100 g non treated bait was monitored.
The test was started and the non treated bait was placed November 11th 2009.

After a week, November 18th, the uptake by the rats was steady; around 270 grams per day, and the baskets were almost empty.

As the rats had also access to the pig food, the uptake of 270 grams /day can be the result of 15 to 25 rats. It certainly showed that 18 baskets of 100 g was enough to control the population, assumed the test product was equally taken as the placebo bait.
November 18th, the placebo bait was replaced by the test product RACO.

The uptake of the RACO was measured daily (not the first day after), the bait replaced twice. The bait was replaced a first time when the baskets were almost empty, a second time when the
uptake became scarcer and less uniform.

From November 22nd to 24th, the fifth to the seventh day, the uptake of RACO was almost as high as the placebo bait, around 260 grams a day.
November 25th, the uptake was measured and then the bait refreshed as it was almost completely gone in most baskets.
From November 25th on, the eighth day of the test, the uptake dropped, first slowly, then quicker.

November 29th, the twelfth day of the test, two dead rats were found .The uptake had dropped to a total of 124 gram/day.
The bait was refreshed as it contained a lot of droppings and was dirty.
From there the uptake dropped dramatically and only on a few bait points there was some uptake, showing most of the rats must have died.
November 30th the total uptake dropped to 59 grams from 4 bait points; the 14 other bait points remained untouched.

December 2nd the total uptake dropped to 30 grams from 2 bait points; the 16 other bait points remained untouched.

December 3rd, after sixteen days of baiting with RACO, the total uptake dropped to 25 grams from 2 bait points. The test was stopped.

The Pest Controller took over and finished the extermination. The following month the insulation was completely restored and until now not any new damages had been recorded.

Reliability

2, Study conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological

deficiencies, which do not affect the quality of relevant results

Assessment of efficacy, data analysis and interpretation

The extermination of *Rattus rattus* in pig stables is known to be very difficult; *Rattus rattus* are very smart and very shy, difficult to bait, living above the insulation of the stables and coming out at night, descending right to the pig feed , avoiding baits.

This test was brought to a good end thanks to the skills and advice of the AHBO company.

The prebaiting showed a small but active group of *Rattus rattus*, estimated around 20 pieces.
The tested product RACO was taken by the roof rats almost as well as the placebo bait.

The uptake of RACO dropped slowly from the eighth day of the test.
Probably, the rats showed first signs of sickness after 8 days.
The twelfth day, 2 dead rats were found. The following days the uptake diminished to a very low level, showing most of the rats were eliminated.
Pest Controllers use as a standard rule 10 % of the dead rats are found, so 2 rats brings us back to a population of around 20 rats.
The total amount of bait used was 3 x 1.8 kg = 5.4 kg
This can seem to be a big amount for an estimated population of 20 rats, but The stable is quite big, 18 baiting points of 100 g seemed the minimum The actual measured uptake was only 2142 g.

The last refreshment of 1800 g was not really necessary, but was done to have a good view of the uptake as the old bait was dirty.
The sixteenth day, the test was stopped; there was still some small activity, which came to an end later on (follow-up by Pest Controller, without uptake control or any other measurements) Although known as difficult to bait, the roof rats did take well RACO and this in the presence of pig feed

Conclusion

DIFENACOUM is said to kill rodents in 5 to 21 days.
In these test the first signs of illness started after 8 days; dead rats

were found after 12 days.
After sixteen days there was still some activity, which ended later (unrecorded).

These results are consistent with the results expected with difenacoum baits.
One can conclude that RACO Grain Baits is very well suited for the extermination of *Rattus rattus* in stables.

Proposed efficacy specification

RACO GRAIN Baits is very well suited for the extermination of Rattus rattus in stables

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

58 Evaluation by Rapporteur Member State

Date

April 2011.

Comments

4.3 Relevant for field conditions as test was conducted in the field.

4.3.1 Application method is oral, with baits placed in a tamper proof bait station (e.g. baskets affixed to the roof where rats were entering the building).

4.3.2 Test organism was wild strain of Roof rats; Black rats *(Rattus rattus)*

4.3.3 Observed effect was drop in consumption indication death of the target pests, 2 dead rats were seen.

Summary and conclusion

The reduction in the bait consumption from 270g per day in the presence of ad-lib pig feed to 25g after 16 days of baiting suggests a significant reduction in the population of the rat population in the pig shed. Neophobia, especially in roof rats, is a likely result of the reason why such a long pre-baiting period was required. The pest control operator claims that feeding and any signs of activity came to a stop soon after the 16 day recording period.

The test is deemed valid for product authorisation as the RACO GRAIN proved both palatable and effective.

59 Comments from ... *(specify)*

Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Nature</td><td>RACO GRAIN BAITS: Containing 0.005 % of Difenacoum</td></tr>
<tr><td>Origin</td><td>Batch N°: PB 091109
Product manufactured: November 3rd 2009</td></tr>
<tr><td>Initial biomass</td><td>Not applicable</td></tr>
<tr><td>Reference of methods</td><td>Not applicable</td></tr>
<tr><td>Collection / storage of samples</td><td>By comparative measure between before and after baiting with placebo (wheat).</td></tr>
<tr><td>Preparation of inoculum for exposure</td><td>First Pre-baiting:
RACO GRAIN BAITS baiting:</td></tr>
<tr><td>Pretreatment</td><td>Not applicable</td></tr>
<tr><td><s>Initial density of test population in the test system/</s> Active substance determined in the product</td><td>The product RACO GRAIN BAITS was tested at lab conditions, the 10/11/2009.
<table>
<tr><th></th><th>Specification</th><th>Results</th><th>Decision</th></tr>
<tr><td>Aspect</td><td>Red wheat grain</td><td>Red wheat grain</td><td>OK</td></tr>
<tr><td>Composition</td><td>Difenacoum 50ppm±12.5 ppm</td><td>47.75</td><td>OK</td></tr>
</table></td></tr>
</table>

1.2 Test organism (if applicable)

Criteria	Details
Species	Roof rats; Black rats *(Rattus rattus)*
Strain	Wild
Source	From the surrounding tested area
Laboratory culture	No, the aim of the study is to be as much as close of the reality.
Stage of life cycle and stage of stadia	Not mentioned
Mixed age population	
Other specification	Not applicable
Number of organisms tested	Not mentioned, only estimation could be performed based on the prebaiting. (Population estimation: 20 rats.)
Method of cultivation	Measurement in bait station every day.
Pretreatment of test organisms before exposure	Not applicable
Initial density/number of test organisms in the test system	Not applicable

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	Not applicable due to the test conditions.
Number of vessels / concentration	
Test culture media and/or carrier material	
Nutrient supply	
Measuring equipment	

1.4 Application of test substance

Criteria	Details
Application procedure	Placebo grain bait during the pre-baiting phase (100g by station) and RACO GRAIN during the poisoning phase.
Delivery method	-
Dosage rate	Measurement of consumption was measured every day.
Carrier	Not applicable due to the test conditions
Concentration of liquid carrier	Not applicable due to the test conditions
Liquid carrier control	Not applicable due to the test conditions
Other procedures	Not applicable due to the test conditions

1.5 Test conditions

Criteria	Details
Substrate	Not applicable due to the test conditions
Incubation temperature	Not applicable due to the test conditions
Moisture	-
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	-

Toxicology

Doc IIIB Section 6.1.1 BPD Data Set IIB Annex Point VI.6.1.1	**Acute Oral Toxicity**	
	Reference	**Official use only**
Reference	[redacted]Difenacoum grain bait - Acute Oral Toxicity in the rat - Acute toxic class method, [redacted] study number TAO423-PH-09/0087, 8 December 2009, 40 pages, Bio6. Unpublished	
Data protection	YES	
Data owner	Bio6 S.A,	
Companies with letter of	Letter of authorisation from PelGar International (UK) to Bio6 S.A.	

Access	(Belgium)	
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
	Guidelines and Quality Assurance	
Guideline study	OECD n° 423 (24 April 2002) Test method B.1ter Council regulation No 440/2008	
GLP	YES	
Deviations	Any	
	MATERIALS AND MethodS	
Test material	**Difenacoum grain bait** It was identified under the code number in the laboratory as **PH-09/0087.**	
Lot/Batch number	600300	
Specification	CAS No: 56073-07-5	
Description	Granular and red	
Purity	Difenacoum 0.005 % m/m (nominal value). Please see the analysis certificate.	
Stability	2 years	
Test Animals		
Species	Rat	
Strain	Sprague-Dawley, SPF Caw	
Source	[redacted]	
Sex	Female	
Age/weight at study initiation	Females weighed between 191 g and 217 g and were 8 or 9 weeks old	
Number of animals per group	Two groups of three females	
Control animals	No	
Administration/ Exposure	**Oral**	
Post exposure period	14 days	
Type	Administered by gavage	
Concentration	2000 mg/kg	
Vehicle	A suitable syringe graduated fitted with an oesophageal metal canula.	
Concentration in vehicle	2000 mg/kg (2 g of the test item were weighed in a 10 ml volumetric flask completed with distilled water)	X
Total volume applied	10 mL/kg body weight	
Controls	No	
Examinations	Clinical signs (every day), body weights (D0, D2, D7 and D14), and necropsy findings (D14)	

Method of determination of LD_{50}	No mortality occurred during the study. The LD50 of the test item Difenacoum grain bait is higher than 2000 mg/kg body weight by oral route in the rat. In accordance with the OECD guideline n°423, the LD50 cut-off of the test item may be considered higher than 5000 mg/kg body weight by oral route in the rat.	
Further remarks	-	
	Results and Discussion	
Clinical signs	Daily examinations were carried out to identify any behavioural or toxic effects on the major physiological functions 14 days after administration of the test item. This examination focuses particularly on a list of symptoms, recorded as "present" or "absent" on the observation sheet. These observations were compared to historical control data. Observations and a mortality report were then carried out every day for 14 days. Bodyweight were recorded at the day 0, 2, 7 and 14 (death day). The animal appeared normal for the duration of the study.	
Pathology	This was not investigated during study.	
Other	On D14, the animals were anaesthetised with sodium pentobarbital and administration continued to fatal levels. Macroscopic observations were entered on individual autopsy sheets. Only those organs likely to be modified in cases of acute toxicity were examined. Those presenting macroscopic anomalies can be removed and preserved in view to microscopic examinations.	
LD_{50}	No mortality occurred during the study at 2000mg/kg. The estimated acute LD50, as indicated by the data, was determined to be greater than 5000mg/kg	

	Applicant's Summary and conclusion	
Materials and methods	Six healthy female rats (Sprague Dawley, SPF Caw) originated from Elevage JANVIER were used after an acclimatization period of at least five days. Rats were housed by group of three in solid-bottomed clear polycarbonate cages with a stainless steel mesh lid. Drinking water (tap-water from public distribution system) and foodstuff were supplied freely. Food was removed at D-1 and then redistributed 4 hours after the test item administration. The animals of the treated group, received an effective dose of 2000 mg/kg body weight of the test item Difenacoum grain bait, prepared extemporaneously in distilled water and administered by gavage under a volume of 10 mL/kg body weight using a suitable syringe graduated fitted with an oesophageal metal canula. The test item was first reduced in fine powder using a coffee mill. Then, 2 g of the test item were weighed in a 10 mL volumetric flask completed with distilled water. The formulation obtained was placed under magnetic stirring up to obtain a homogeneous suspension. Then, the suspension was filtered using a sieve and a pestle. Systematic examinations were carried out to identify any behavioural or toxic effects on the major physiological functions 14 days after administration of the test item. This examination focuses particularly on a list of symptoms, recorded as "present" or "absent" on the observation sheet. These observations were compared to historical control data. Observations and a mortality report were then carried out every day for 14 days. On D14, the animals were anaesthetised with sodium pentobarbital and administration continued to fatal levels.	
Results and discussion	No mortality occurred during the study. No clinical signs related to the administration of the test item were observed. The body weight evolution of the animals remained normal throughout the study. The macroscopical examination of the animals at the end of the study revealed a thickening of the corpus with presence of red spots (3/6 animals).	

Conclusion	The LD50 of the test item Difenacoum grain bait is higher than 2000 mg/kg body weight by oral route in the rat. In accordance with the OECD guideline n°423, the LD50 cut-off of the test item may be considered higher than 5000 mg/kg body weight by oral route in the rat. According to the criteria for classification, packaging and labelling of dangerous substances and preparations in accordance with the E.E.C. Directives 67/548, 2001/59 and 99/45, the test item Difenacoum grain bait must not be classified. No symbol and risk phrase are required. In accordance with the Globally Harmonized System (Regulation (EC) No 1272/2008), the test item must not be classified in category 4. No signal word and hazard statement are required.	
Reliability	1	
Deficiencies	No	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Rapporteur Member State	
Date	*30 May 2011*	
Materials and Methods	*Adopt applicant's version.*	
Results and discussion	*Adopt applicant's version.*	
Conclusion	Other conclusions: *LD50 > 2000mg/kg bw*	
Reliability	*2*	
Acceptability	acceptable Difenacoum is lipid soluble. An aqueous extract will not recover all of the active substance from the sample. An emulsion will form and the majority of the difenacoum will partition into the oil phase. Cannot be certain of actual dose.	
Remarks		
	Comments from ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIB Section 6.1.2 BPD Data Set IIB Annex Point VI.6.1.2	**Acute Dermal Toxicity**	
	Reference	**Official use only**
Reference	[redacted] Difenacoum grain bait - Acute Dermal Toxicity in the rat - Acute toxic class method, [redacted] study number TAD-PH-09/0087, December 2009, 39 pages, Bio6. Unpublished	
Data protection	YES	
Data owner	Bio6 S.A,	
Companies with letter of Access	Letter of authorisation from PelGar International (UK) to Bio6 S.A. (Belgium)	
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
	Guidelines and Quality Assurance	
Guideline study	OECD n° 402 (24 February 1987) Test method B.3 Council regulation No 440/2008	
GLP	YES	
Deviations	Any	
	MATERIALS AND MethodS	
Test material	**Difenacoum grain bait** It was identified under the code number in the laboratory as **PH-09/0087.**	
Lot/Batch number	PB090209	
Specification	CAS No: 56073-07-5	
Description	Granular and red	
Purity	Difenacoum 0.005 % m/m (nominal value). Please see the analysis certificate.	
Stability	2 years	

Test Animals		
Species	Rat	
Strain	Sprague-Dawley, SPF Caw	
Source	[redacted]	
Sex	Males and females	
Age/weight at study initiation	Males weighed between 214 g and 238 g and were 7 weeks old Females weighed between 209 g and 220 g and were 8 weeks old	
Number of animals per group	One group of 5 males and the other of 5 females.	
Control animals	No	
Administration/ Exposure	**Dermal**	
Post exposure period	14 days	
Area covered	10% of the total surface area (from the dorsal area of the trunk of the test animals)	
Occlusion	Occlusive	
Vehicle	None.	
Concentration in vehicle	2000mg/kg	
Total volume applied	10ml/kg	
Duration of exposure	24h	
Removal of test substance	The gauze dressings were removed and the treated site was rinsed with distilled water.	
Controls	None.	
Examinations	Clinical signs, body weights, and necropsy findings.	
Method of determination of LD_{50}	There was no mortality during the study. The LD50 of the test item Difenacoum grain bait is higher than 2000 mg/kg body weight by dermal route in the rat	
Further remarks	-	

	Results and Discussion	
Clinical signs	Daily examinations were carried out to identify any behavioural or toxic effects on the major physiological functions 14 days after administration of the test item. This examination focuses particularly on a list of symptoms, recorded as "present" or "absent" on the observation sheet. These observations were compared to historical control data. Observations and a mortality report were then carried out every day for 14 days. Bodyweight were recorded at the day 0, 2, 7 and 14 (death day). The animal appeared normal for the duration of the study.	
Pathology	It was not investigated during study.	
Other	On D14, the animals were anaesthetised with sodium pentobarbital and administration continued to fatal levels. Macroscopic observations were entered on individual autopsy sheets. Only those organs likely to be modified in cases of acute toxicity were examined. Those presenting macroscopic anomalies can be removed and preserved in view to microscopic examinations.	
LD_{50}	There was no mortality during the study. The estimated acute LD_{50}, as indicated by the data, was determined to be greater than 2000mg/kg body weight.	

	Applicant's Summary and conclusion	

Materials and methods	During the treatment, the animals were kept in individual cage. On D3, the animals were put into their cage by 2 or 3. The rats were kept in solid-bottomed clear polycarbonate cages with a stainless steel mesh lid. Each cage contains sawdust bedding which was changed at least 2 times a week. Each cage was installed in conventional air conditioned animal husbandry. Drinking water (tap-water from public distribution system) and foodstuff were supplied freely. Approximately 24 hours before the treatment, fur was removed from the dorsal area of the trunk of the test animals by clipping. At least 10 per cent of the body surface area was clear for the application of the test item. The test item was first reduced in fine powder using a coffee mill. Then, 2 g of the test item were weighed in a 10 mL volumetric flask completed with distilled water. The formulation obtained was placed under magnetic stirring up to obtain a homogeneous suspension. Then, the suspension was filtered using a sieve and a pestle. Animals from treated group received by topical application, under porous gauze dressing, an effective dose of 2000 mg/kg body weight of Difenacoum grain bait, administered under a volume of 10 mL/kg body weight, during 24 hours. After 24-hour exposure period, the gauze dressings were removed and the treatment site was rinsed with distilled water. Systematic examinations were carried out to identify any behavioural or toxic effects on the major physiological functions 14 days after administration of the test item. This examination focuses particularly on a list of symptoms, recorded as "present" or "absent" on the observation sheet. These observations were compared to historical control data. Observations and a mortality report were then carried out every day for 14 days On D14, the animals were anaesthetised with sodium pentobarbital and administration continued to fatal levels.	

Results and discussion	No mortality occurred during the study. Neither cutaneous reactions nor systemic clinical signs related to the administration of the test item were observed. It was only noted a depilation and a pink coloration, which did not prevent the observations, after rinsing of the remaining test item. The body weight evolution of the animals remained normal throughout the study. The macroscopical examination of the animals at the end of the study did not reveal treatment-relatedchanges. It was only noted a pink coloration of the treated site	
Conclusion	The LD50 of the test item Difenacoum grain bait is higher than 2000 mg/kg body weight by dermal route in the rat. According to the criteria for classification, packaging and labelling of dangerous substances and preparations in accordance with the E.E.C. Directives 67/548, 2001/59 and 99/45, the test item Difenacoum grain bait must not be classified. No symbol and risk phrase are required. In accordance with the Globally Harmonized System (Regulation (EC) No 1272/2008), the test item must not be classified in category 4. No signal word and hazard statement are required.	
Reliability	1	
Deficiencies	No	
	Evaluation by Competent Authorities	

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Rapporteur Member State	
Date	*30 May 2011*	
Materials and Methods	*Adopt applicant's version*	
Results and discussion	*Adopt applicant's version*	
Conclusion	Other conclusions: *Adopt applicant's version*	
Reliability	*1*	
Acceptability	acceptable	
Remarks		
	Comments from ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

III B Section 6.1.3 BPD Data Set IIB Annex Point VI.6.1.3	**Inhalation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. *The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [x]**	
Detailed justification:	The active substance and the other co-formulant have low vapor pressures and are present only at low concentration in the product (with the obvious exception of the bait base). For example, difenacoum is present at 0.005% w/W and has a vapor pressure of 6.7×10^{-9} – 5.4×10^{-14} Pa.	
	According exposure assessment performed on measurements of a surrogate in simulated use conditions and on daily exposure frequencies according to a questionnaire answered by selected pest control companies in several EU countries. In primary exposure, the skin is the main exposure route, and only a small proportion of inhalation exposure to dust from decanting of pellets or grain baits is included in the total exposure. Inhalation exposure is not included for wax block formulation. Oral exposure is not considered relevant in primary exposure. Dermal absorption of 0.047% and body weight of 60 kg for an adult is used for the calculations	
	Source: *Assessment Report – Difenacoum, Product-type 14 (Rodenticides), Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC, 17 September 2009, Annex I – Finland, p14.*	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*30 May 2011*	

Evaluation of applicant's justification	*Accept applicant's justification*
Conclusion	*Accept applicant's justification*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

III B Section 6.1.4 BPD Data Set IIB Annex Point VI.6.1.4	**Information on Mixture of Biocidal Product**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. *The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Not applicable since following the proposed uses of **BLOCK BAIT** and the label claims, the rodenticide **BLOCK BAIT** is not intended to be used in mix with other Biocidal products.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*30 May 2011*	
Evaluation of applicant's justification	*Accept applicant's justification*	
Conclusion	*Accept applicant's justification*	

Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

IIIB Section 6.2._01 BPD Data Set IIB/ Annex Point VI.6.2	**Acute Dermal Irritation**	
	Reference	**Official use only**
Reference	[redacted] Difenacoum block bait – Skin Irritation test in the rabbit, [redacted] study number IC-OCDE-PH-09/0087, 8 December 2009, 36 pages, Bio6. Unpublished	
Data protection	YES	
Data owner	Bio6 S.A,	
Companies with letter of Access	Letter of authorisation from PelGar International (UK) to Bio6 S.A. (Belgium)	
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
	Guidelines and Quality Assurance	
Guideline study	OECD n° 404 (24 April 2002) Test method B.4 Council regulation No 440/2008	
GLP	YES	
Deviations	Any	

	MATERIALS AND MethodS	
Test material	**Difenacoum block bait** It was identified under the code number in the laboratory as **PH-09/0085.**	
Lot/Batch number	PB090209	
Specification	CAS No: 56073-07-5	
Description	Granular and red	
Purity	Difenacoum 0.005 % m/m (nominal value). Please see the analysis certificate.	
Stability	2 years	
Test Animals		
Species	Albino rabbit	
Strain	New Zealand	
Source	[redacted]	
Sex	Female ??	X
Age/weight at study initiation	The animals weighed between 2.44 kg and 3.28 kg. At the beginning of the test, the animals were 13 weeks old.	
Number of animals per group	One group of 3 males ??	X
Control animals	No, but there was for each animal two kind of area, one for the test site and on other for control site.	
Administration/ Exposure	Dermal	
Application		
Preparation of test substance	The test item was previously reduced in fine powder with a coffee mill. As no tissue destruction was noted after a treatment during 3 minutes and 1 hour, the test item was applied, as supplied.	
Test site and Preparation of Test Site	The test site was the undamaged skin area of one flank of each animal	
Occlusion	Semi-occlusive dressing, the patch was secured in position with a strip of surgical adhesive tape	
Vehicle	None, application directly on the skin.	
Concentration in vehicle	A dose of 0.5 g	
Total volume applied	0.5g	
Removal of test substance	Distilled water	X
Duration of exposure	4h	

<table>
<tr><td>Postexposure period</td><td>If no reaction is observed 72 hours after the treatment, the study is terminated.

In case of persistent reactions, additional observations can be carried out from D4 to D14 in order to determine the reversible character of the lesions observed.</td><td></td></tr>
<tr><td>Controls</td><td>No specified by the laboratory</td><td></td></tr>
<tr><td>Examinations</td><td></td><td></td></tr>
<tr><td>Clinical signs</td><td>No</td><td></td></tr>
<tr><td>Dermal examination</td><td>Yes</td><td></td></tr>
<tr><td>Scoring system</td><td>The state scoring system is explained to the fallowing table:

<table>
<tr><td>Score</td><td colspan="2">Evaluation of skins reactions</td></tr>
<tr><td></td><td>Erythema Formation</td><td>Oedema formation</td></tr>
<tr><td>0 (min)</td><td>No erythema</td><td>No oedema</td></tr>
<tr><td>1</td><td>Very slight (Barely perceptible)</td><td>Very slight (Barely perceptible)</td></tr>
<tr><td>2</td><td>Well-defined</td><td>Slight (contour clearly defined)</td></tr>
<tr><td>3</td><td>Moderate to severe</td><td>Moderate (Raised approximately 1mm)</td></tr>
<tr><td>4 (max)</td><td>Severe (beet redness) with eschars formation preventing gradin of erythema</td><td>Severe (raised than 1mm and extending beyond the area of exposure</td></tr>
</table></td><td></td></tr>
<tr><td>Examination time points</td><td>The animals were examined at 1, 24, 48 and 72 hours.</td><td></td></tr>
<tr><td>Other examinations</td><td>No other signs of dermal irritation.
A pink or red coloration was noted on the treated area but did not prevent from quotation</td><td></td></tr>
<tr><td>Further remarks</td><td>Initially, a single animal was treated. After consideration of the cutaneous responses produced in the first treated animal, two additional animals were treated during 4 hours.</td><td></td></tr>
</table>

	Results and Discussion	

<table>
<tr><td>Average score</td><td></td><td></td></tr>
<tr><td>Erythema</td><td>The average score for all animals is given at the following table:
<table>
<tr><td rowspan="2">Animal number</td><td colspan="4">Hours of examination</td></tr>
<tr><td>1</td><td>24</td><td>48</td><td>72</td></tr>
<tr><td>A9659 (12 May 09)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>A9660 (19 May 09)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>A9662 (19 May 09)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
0= Non irritating</td><td></td></tr>
<tr><td>Edema</td><td>The average score for all animals is given at the following table:
<table>
<tr><td rowspan="2">Animal number</td><td colspan="4">Hours of examination</td></tr>
<tr><td>1</td><td>24</td><td>48</td><td>72</td></tr>
<tr><td>A9659 (12 May 09)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>A9660 (19 May 09)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>A9662 (19 May 09)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
0= Non irritating</td><td></td></tr>
<tr><td>Reversibility</td><td>Yes</td><td></td></tr>
<tr><td>Other examinations</td><td>No other signs of dermal irritation</td><td></td></tr>
<tr><td>Overall result</td><td>No cutaneous reactions (erythema and oedema) were observed, on the treated area, whatever the examination times (ie 1, 24, 48 and 72 hours).</td><td></td></tr>
</table>

	Applicant's Summary and conclusion	

Materials and methods	Three **female** albino New Zealand rabbits were used for this experiment. They were kept during minimal 5-day acclimatization. Each animal was kept in an individual box installed in conventional air conditioned animal husbanding. Drinking water (tap-water from public distribution system) and foodstuffs (SDS – C15) were supplied freely. Approximately 24 hours before the test, the rabbit's back and flanks were shorn using electric clippers equipped with a fine comb, so as to expose an area of skin about 6 cm^2. The test item was previously reduced in fine powder with a coffee mill. As no tissue destruction was noted after a treatment during 3 minutes and 1 hour, the test item was applied, as supplied, at a dose of 0.5 g, on an undamaged skin area of one flank of each animal, during 4 hours. The patch was secured in position with a strip of surgical adhesive tape under semi-occlusive dressing. After the removal of the patch, the treated area was rinsed with distilled water. On the opposite flank an untreated area was served as the control. Initially, a single animal was treated. After consideration of the cutaneous responses produced in the first treated animal, two additional animals were treated during 4 hours. The irritation scoring was observed at 1, 24, 48 and 72 hours after the substance exposure.	
Results and discussion	No cutaneous reactions (erythema and oedema) were observed, on the treated area, whatever the examination times (ie 1, 24, 48 and 72 hours).	
Conclusion	The results obtained, under these experimental conditions, enable to conclude that the test item Difenacoum block bait, according to the scales of interpretation retained: - is non irritant to skin (PSi = 0.00) according to the classification established in the Journal Officiel de la République Française dated February 21st, 1982, - and, must not be classified, according to the criteria for classification, packaging and labelling of dangerous substances and preparations in compliance with the E.E.C. Directives 67/548, 2001/59 and 99/45. No symbol and risk phrase are required. In accordance with the Globally Harmonized System (Regulation (EC) No 1272/2008), the test item must not be classified in category 2. No signal word and hazard statement are required.	

Reliability	1	
Deficiencies	No	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Rapporteur Member State	
Date	*30 May 2011*	
Materials and Methods	*Adopt applicant's version.*	
Results and discussion	*Adopt applicant's version*	
Conclusion	Other conclusions: *Adopt applicant's version*	
Reliability	*1*	
Acceptability	acceptable *Difenacoum is water insoluble. Cleaning of the site with an aqueous medium is not suitable to ensure complete removal of product.*	
Remarks		
	Comments from ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

IIIB Section 6.2_02 BPD Data Set IIB/ Annex Point VI.6.2	**Acute Eye Irritation**	
	Reference	**Official use only**
Reference	[redacted] Difenacoum block bait – Skin Irritation test in the rabbit, [redacted] study number IC-OCDE-PH-09/0087, 8 December 2009, 39 pages, Bio6. Unpublished	
Data protection	YES	
Data owner	Bio6 S.A,	
Companies with letter of Access	Letter of authorisation from PelGar International (UK) to Bio6 S.A. (Belgium)	
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
	Guidelines and Quality Assurance	
Guideline study	OECD n° 405 (24 April 2002) Test method B.5 Council regulation No 440/2008	
GLP	YES	
Deviations	Any	

	MATERIALS AND MethodS	
Test material	**Difenacoum block bait** It was identified under the code number in the laboratory as **PH-09/0087.**	
Lot/Batch number	52-600300	
Specification	CAS No: 56073-07-5	
Description	Granular and red	
Purity	Difenacoum 0.005 % m/m (nominal value). Please see the analysis certificate.	
Stability	2 years	
Test Animals		
Species	Albino rabbit	
Strain	New Zealand	
Source	[redacted]	
Sex	Male	
Age/weight at study initiation	The animals weighed between 2.29 kg and 2.64 kg. At the beginning of the test, the animals were 11 weeks old.	
Number of animals per group	One group of 3 males	
Control animals	No, but one yes received the test item, the second is used as control.	

Administration/ Exposure		
Preparation of test substance	The test item was previously reduced in fine powder with a coffee-mill.	
Amount of active substance instilled	0.1 g of the test item	
Exposure period	24h	
Postexposure period	If no reaction is observed 72 hours after instillation, the study is terminated. In case of persistent reactions, additional observations can be carried out from D4 to D21 in order to determine the reversible character of the lesions observed	
Examinations		
Ophthalmoscopic examination	Yes	

Scoring system

Chemosis (A)	
No swelling	0
Slight swelling, including the nictitating membrane	1
Swelling with eversion of the eyelid	2
Swelling with eyelid half-closed	3
Swelling with eyelid more than half-closed	4
Discharge (B)	
No discharge	0
Slight discharge (normal slight secretions in the inner corner not to be taken into account	1
Discharge with moistening of the eyelids and neighbouring hairs	2
Discharge with moistening of the eyelids and large areas around the eye	3
Redness (C)	
Blood vessels normal	0
Vessels significantly more prominent than normal	1
Vessels individually distinguishable with difficulty	-
• Generalised red coloration	2
• Generalised deep red coloration	3
Iris (D)	
Normal	0
Iris significantly more wrinkled than normal, congestion, swelling of the iris which continues to react to light, even slowly	1
No reaction to light, haemorrhage, significant damage (any or all of these characteristics	2
Cornea: Degree of opacity (E)	
No modification visible either directly or after instillation of fluorescein (no loss of glint or polish)	0
Translucent areas (diffuse or disseminated), iris details clearly visible	1
Easily identifiable translucent area, iris details slightly obscured	2
Opalescent area, no iris details visible, pupil outline scarcely distinguishable	3
Total corneal opacity, completely obscuring the iris and pupil	4
Cornea: Extent of opacity (F)	
Opaque area present but covering one quarter or less	1
Between one quarter and half	2
Between half and three quarters	3
Between three quarters and the entire surface	4

<table>
<tr><td></td><td>The calculs for the total maximum score for:
<table>
<tr><th></th><th></th><th>Maximum score</th></tr>
<tr><td>CONJUNCTIVAE</td><td>(A+B+C)x2 = X</td><td>20</td></tr>
<tr><td>IRIS</td><td>Dx5 =Y</td><td>10</td></tr>
<tr><td>CORNEA</td><td>ExFx5= Z</td><td>80</td></tr>
<tr><td colspan="2">TOTAL</td><td>110</td></tr>
</table></td><td></td></tr>
<tr><td>Examination time points</td><td>60min, 24h, 48h, 72h</td><td></td></tr>
<tr><td>Other investigations</td><td>None</td><td></td></tr>
<tr><td>Further remarks</td><td>Initially, a single animal was treated. After consideration of the ocular responses produced in the first treated animal at D1, two additional animals were treated.

At the reading time 1 hour, for the animals A9666 and A9667, residual test item was still noted. Therefore, the treated eye was rinse with a physiological saline solution</td><td></td></tr>
</table>

<table>
<tr><td></td><td>Results and Discussion</td><td></td></tr>
<tr><td>Clinical signs</td><td>No effects</td><td></td></tr>
<tr><td>Average score</td><td></td><td></td></tr>
<tr><td>Cornea</td><td>The average score for the cornea is given at the following table:
<table>
<tr><td>Animal number</td><td colspan="3">A9663</td><td colspan="3">A9666</td><td colspan="3">A9667</td></tr>
<tr><td>Hours of examination</td><td>24</td><td>48</td><td>72</td><td>24</td><td>48</td><td>72</td><td>24</td><td>48</td><td>72</td></tr>
<tr><td>Opacity (E)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>TOTAL</td><td colspan="3">0</td><td colspan="3">0</td><td colspan="3">0</td></tr>
<tr><td>MEAN</td><td colspan="3">0.0</td><td colspan="3">0.0</td><td colspan="3">0.0</td></tr>
</table></td><td></td></tr>
<tr><td>Iris</td><td>The average score for the iris is given at the following table:
<table>
<tr><td>Animal number</td><td colspan="3">A9663</td><td colspan="3">A9666</td><td colspan="3">A9667</td></tr>
<tr><td>Hours of examination</td><td>24</td><td>48</td><td>72</td><td>24</td><td>48</td><td>72</td><td>24</td><td>48</td><td>72</td></tr>
<tr><td>Opacity (E)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>TOTAL</td><td colspan="3">0</td><td colspan="3">0</td><td colspan="3">0</td></tr>
<tr><td>MEAN</td><td colspan="3">0.0</td><td colspan="3">0.0</td><td colspan="3">0.0</td></tr>
</table></td><td></td></tr>
<tr><td>Conjunctiva</td><td></td><td></td></tr>
<tr><td>Redness</td><td>The average score for the redness is given at the following table:
<table>
<tr><td>Animal number</td><td colspan="3">A9663</td><td colspan="3">A9666</td><td colspan="3">A9667</td></tr>
<tr><td>Hours of examination</td><td>24</td><td>48</td><td>72</td><td>24</td><td>48</td><td>72</td><td>24</td><td>48</td><td>72</td></tr>
<tr><td>Opacity (E)</td><td>2</td><td>1</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>1</td><td>2</td></tr>
<tr><td>TOTAL</td><td colspan="3">4</td><td colspan="3">6</td><td colspan="3">3</td></tr>
<tr><td>MEAN</td><td colspan="3">1.3</td><td colspan="3">2.0</td><td colspan="3">1.0</td></tr>
</table></td><td></td></tr>
</table>

<table>
<tr><td>Chemosis</td><td>The average score for the chemosis is given at the following table:
<table>
<tr><td>Animal number</td><td colspan="3">A9663</td><td colspan="3">A9666</td><td colspan="3">A9667</td></tr>
<tr><td>Hours of examination</td><td>24</td><td>48</td><td>72</td><td>24</td><td>48</td><td>72</td><td>24</td><td>48</td><td>72</td></tr>
<tr><td>Chemosis (A)</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td><td>0</td></tr>
<tr><td>TOTAL</td><td colspan="3">3</td><td colspan="3">3</td><td colspan="3">2</td></tr>
<tr><td>MEAN</td><td colspan="3">1.0</td><td colspan="3">1.0</td><td colspan="3">0.7</td></tr>
</table></td><td></td></tr>
<tr><td>Reversibility</td><td>Yes, the redness and the chemosis disappeared after 72 hours.</td><td></td></tr>
<tr><td>Other</td><td>None</td><td></td></tr>
<tr><td>Overall result</td><td>According to the calculated means and the European regulation, the calculated means, the item must not be classified.

According to the calculated means and the GHS regulation, the item must not be classified</td><td></td></tr>
</table>

	Applicant's Summary and conclusion	
Materials and methods	Three male albino New Zealand rabbits were used for this experiment. They were kept during minimal 5-day acclimatization. Each animal was kept in an individual box installed in conventional air conditioned animal husbanding. Drinking water (tap-water from public distribution system) and foodstuffs (SDS – C15) were supplied freely. The test item was previously reduced in fine powder with a coffee-mill. 0.1 g of the test item was instilled into the conjunctival sac of one eye; the other eye remained untreated serving as control. Initially, a single animal was treated. After consideration of the ocular responses produced in the first treated animal at D1, two additional animals were treated. Ocular examinations were performed on both right and left eyes 1 hour, 24, 48 and 72 hours following treatment,	
Results and discussion	The ocular conjunctivae reactions observed during the study have been slight to moderate and totally reversible in the three animals; a slight to moderate redness, noted 1 hour after the test item instillation and totally reversible between day 3 and day 4, associated with a slight to moderate chemosis, noted 1 hour after the test item instillation and totally reversible between day 2 and day 3.	
Conclusion	The results obtained, under these experimental conditions, enable to conclude that the test item Difenacoum block bait: - is slightly irritant for the eye (Max. O.I = 12.7) according to the classification established in the *Journal Officiel de la République Française* dated July 10th, 1992. - and, must not be classified according to the criteria for the classification, packaging and labelling of dangerous substances and preparations in compliance with the E.E.C. Directives n° 67/548,n°2001/59 and n°99/45. No symbol and risk phrase are required. In accordance with the Globally Harmonized System (Regulation (EC) No 1272/2008), the test item must not be classified in category 2. No signal word and hazard statement are required.	
Reliability	1	
Deficiencies	No	
	Evaluation by Competent Authorities	

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Rapporteur Member State	
Date	*30 May 2011*	
Materials and Methods	*Adopt applicant's version.*	
Results and discussion	*Adopt applicant's version.*	
Conclusion	*Adopt applicant's version*	
Reliability	*1*	
Acceptability	acceptable	
Remarks		
	Comments from ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

IIIB Section 6.3 BPD Data Set IIB/ Annex Point VI.6.3	**Skin Sensitisation**	
	Reference	**Official use only**
Reference	[redacted] block bait – Skin sensitisation in the guinea pig - Magnusson and Kligman maximisation method [redacted] study number SMK-PH-09/0087, 8 December 2009, 42 pages, Bio6. Unpublished	
Data protection	YES	
Data owner	Bio6 S.A,	
Companies with letter of Access	Letter of authorisation from PelGar International (UK) to Bio6 S.A. (Belgium)	
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
	Guidelines and Quality Assurance	
Guideline study	OECD n° 406 (17 July 1992) Test method B.6 Council regulation No.440/2008	X
GLP	YES	
Deviations	Any	X
	MATERIALS AND MethodS	
Test material	**Difenacoum block bait** It was identified under the code number in the laboratory as **PH-09/0085.**	
Lot/Batch number	600300	
Specification	CAS No: 56073-07-5	
Description	Granular and red	
Purity	Difenacoum 0.005 % m/m (nominal value). Please see the analysis certificate.	
Stability	2 years	

Preparation of test substance for application	The following table shows the dose for the induction and for the challenge for the test substance and for the positive control substance: Preparation of the test substance	

	<table><tr><td></td><td></td><td>Difenacoum block bait</td></tr><tr><td rowspan="3">Concentration administrated</td><td>Induction</td><td>50% in distilled water</td></tr><tr><td rowspan="2">Challenge</td><td>50% in distilled water</td></tr><tr><td>25% in distilled water</td></tr></table>	
Pretest performed on irritant effects	Yes, preliminary tests were performed in order to determine, by topical application the Pre-Maximal Non Irritant Concentration (Pre-MNIC), which allowed to evaluate the irritant potential of the test item, defined whether an application of sodium lauryl sulphate would be needed during topical induction phase The MNIC test was carried out for the purpose of determining the of the test item without risk of an irritant effect during the challenge phase	
Test Animals		
Species	Guinea pigs	
Strain	Dunkin-Hartley strain	
Source	[redacted]	
Sex	Female	
Age/weight at study initiation	The animals weighed between 274 g and 300 g at the beginning of the test and were 4 weeks old.	
Number of animals per group	<table><tr><td></td><td>GROUP 1 negative control</td><td>GROUP 2 treated</td></tr><tr><td>Female/group</td><td>5 n° C1898 to C1902</td><td>11 n° C1903 to C1913</td></tr></table>	
Control animals	Negative control (5 for the group)	

<table>
<tr><td>Administration/ Exposure</td><td>The aim of the study was to evaluate the possible allergenic activity of the test item after topical administration in guinea pigs.</td><td></td></tr>
<tr><td>Induction schedule</td><td>Day 1 – Day 6 – Day 7</td><td></td></tr>
<tr><td>Way of Induction</td><td>Topical</td><td rowspan="2"></td></tr>
<tr><td></td><td>Occlusive</td></tr>
<tr><td>Concentrations used for induction</td><td>The concentration used for the induction was 50% of the test item in distilled water.
<table><tr><td colspan="2"></td><td>Preparation of the test substance</td></tr><tr><td colspan="2"></td><td>Difenacoum block bait</td></tr><tr><td rowspan="3">Concentration administrated</td><td>Induction</td><td>60% in distilled water</td></tr><tr><td rowspan="2">Challenge</td><td>60% in distilled water</td></tr><tr><td>30% in distilled water</td></tr></table></td><td>X</td></tr>
<tr><td>Concentration Freunds Complete Adjuvant (FCA)</td><td>50 % FCA in isotonic sodium chloride</td><td></td></tr>
<tr><td>Challenge schedule</td><td>Day 20</td><td></td></tr>
<tr><td>Concentrations used for challenge</td><td>The concentrations used for challenge were 50% (MNIC) and 25% (1/2 MNIC) of the test item in distilled water.</td><td>X</td></tr>
<tr><td>Rechallenge</td><td>No</td><td></td></tr>
<tr><td>Scoring schedule</td><td>24h, 48h after challenge</td><td></td></tr>
<tr><td>Removal of the test substance</td><td>Not specified.</td><td></td></tr>
<tr><td>Positive control substance</td><td>α-Hexylcinnamaldehyde</td><td></td></tr>
<tr><td>Examinations</td><td></td><td></td></tr>
<tr><td>Pilot study</td><td>Yes</td><td></td></tr>
<tr><td>Further remarks</td><td>-</td><td></td></tr>
</table>

	Results and Discussion	
Results of pilot studies	- Pre MNIC determination: 24 hours after the removal of the occlusive dressings, no cutaneous reaction was recorded whatever the tested concentration (60%, 30%, 15% and 7.5% diluted in distilled water, after being reduced to fine powder with a coffee mill). In view of these results, the concentration selected was 60% for the 2nd induction of the Group 2 and the MNIC determination began at this concentration of 60%. - MNIC determination: 24 hours after removal of the occlusive dressings, no cutaneous reaction was recorded whatever the tested concentration (table 2, page 12). In view of this result, the concentrations selected were 60% (MNIC) and 30% (1/2 MNIC) for the challenge phase.	
Results of test		
24h after challenge	No macroscopic cutaneous reactions was recorded during the examination following the removal of the occlusive dressing (challenge phase) from the animals of the treated group with the test item at 60% and 30%. It was only noted a depilation at the reading time 24 hours on the treated area at 60% in one animal (1/11) and on the treated area at 30 % in one animals (1/11). A slight pink coloration, not preventing from scoring, was also noted on the treated areas.	
48h after challenge	No macroscopic cutaneous reactions was recorded during the examination following the removal of the occlusive dressing (challenge phase) from the animals of the treated group with the test item at 60% and 30%.	

Other findings

No cutaneous intolerance reaction was recorded in animals from the negative control group after the challenge phase, on the treated area with the test item at 60% and 30%. It was only noted a depilation at the reading time 24 hours on the treated area at 60% in two animals (2/5) and on the treated area at 30% in all animals (5/5). A slight pink coloration, not preventing from scoring, was also noted on the treated areas.

Overall result

The following tables show the macroscopic evaluation at 24 and 48 hours after the challenge with the test substance:

Groups	Reading time	Conc	Quotations				% of positive responses ≥1	% of animal sensitized
Negative control group	24	60%	0	1	2	3or>		
	48	30%	0	0	0	0	0%	
	24	60%	0	0	0	0	0%	
	48	30%	0	0	0	0	0%	
Treated Group	24	60%	0	0	0	0	0%	0%
	48	30%	0	0	0	0	0%	0%
	24	60%	0	0	0	0	0%	0%
	48	30%	0	0	0	0	0%	0%

0: No reaction.

	Applicant's Summary and conclusion	

Materials and methods

Sixteen female albino pigs of Dunkin-Hartley strain, supplied by Charles River (F-69592 L'ARBRESLE) were exposed to the test item after an acclimatisation period of at least five days. For the main study, the animals weighed between 274 g and 300 g at the beginning of the test and were 4 weeks old.

Prior to the test, the animals were kept for a minimum acclimatization period of 5 days, under stabling and nutritional conditions identical to those of the test.

Before the experimentation process, they were identified individually by marking with picric acid and a tattoo placed on their ear. The animals were carefully shorn before each test item application:
- On the inter-scapular zone for the induction phase,
- On the dorso-lumbar zone for the challenge phase.

At least 3 hours before the first reading (challenge phase) they were shorn a second time in this dorsolumbar zone. The animals were weighed at the beginning and at the end of the study.

Preliminary tests were performed to determine the dose in the main study:

- As the test item was not administrable by the intradermal route, the induction in the main study was performed by topical route and no MNNC (Maximal Non Necrotizing Concentration) determination was performed.

- The Maximal Non Irritant Concentration test, was determine with several concentration (60%, 30%, 15% and 7.5% in distilled water, after being reduced in fine powder with a coffee mill) applied on the dorso-lumbar zone of two guinea pigs shorn beforehand, with occlusive dressing for 24 hours.

Animals were split in two groups for the main study:

	GROUP 1	GROUP 2
	negative control	treated
Female/group	5 n° C1898 to C1902	11 n° C1903 to C1913

Calendar of the main study	
Day 0	**Intradermal induction** After shearing the scapular zone, two (2) pairs of intradermal injections (ID) of 0.1 ml of Freund's Complete Adjuvant diluted at 50 % in isotonic sodium chloride were performed on the scarified scapular zone in such a way as an injection on each pair is placed to either side of the spine. A topical application under occlusive dressing for 48 hours was performed on the injection sites of each animal.
Day 6	**Topical induction** The scapular zone of all the animals in each group, shorn beforehand, was brushed with a solution of sodium lauryl sulfate at 10% in thick vaseline, in order to create a local irritation
Day 7	**Topical induction** A topical application under occlusive dressing for 48 hours was performed on the injection sites of each animal. GROUP 1 (Negative control): 0.5 ml of distilled water GROUP 2 (treated): 0.5 ml of the test item at 60%
Rest period	
Day 20	**Challenge phase** The experimental procedure of this phase was identical for both groups GROUP 1 (Negative control) and GROUP 2 (Treated) submitted to this experimentation: on the previously shorn dorso-lumbar zone, an application on either side of the spine, under occlusive dressing, was performed during 24 hours: - 1 sample cup containing the test item at 60% (MNIC) and at 30% (1/2 MNIC).

Results and discussion	No macroscopic cutaneous reactions was recorded during the examination following the removal of the occlusive dressing (challenge phase) from the animals of the treated group with the test item at 60% and 30%. It was only noted a depilation at the reading time 24 hours on the treated area at 60% in one animal (1/11) and on the treated area at 30 % in one animal (1/11). A slight pink coloration, not preventing from scoring, was also noted on the treated areas. No cutaneous intolerance reaction was recorded in animals from the negative control group after the challenge phase, on the treated area with the test item at 60% and 30%. It was only noted a depilation at the reading time 24 hours on the treated area at 60% in two animals (2/5) and on the treated area at 30 % in all animals (5/5). A slight pink coloration, not preventing from scoring, was also noted on the treated areas	
Conclusion	In view of these results, under these experimental conditions, the test item Difenacoum block bait must not be classified as a skin sensitiser, in accordance with the criteria for classification, packaging and labelling of dangerous substances and preparations of the E.E.C. Directives 67/548, 2001/59 and 99/45. No symbol and risk phrase are required. In accordance with the Globally Harmonized System (Regulation (EC) No 1272/2008), the test item must not be classified in category 1. No signal word and hazard statement are required.	
Reliability	1	
Deficiencies	No	
	Evaluation by Competent Authorities	

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30 May 2011	
Materials and Methods	Applicant's version is not acceptable.	
Results and discussion	Applicant's version is not acceptable.	
Conclusion	Other conclusions:	
Reliability	4	
Acceptability	not acceptable - The test substance is finely ground and then diluted with distilled water. However, the test material contains an active substance that is not water soluble that is applied to the surface of a kernel (grain) matrix that is also not water soluble. At best a fine suspension is created that is unsuitable for intradermal injection. - This procedure cannot be identified as a Guinea Pig Maximisation Test, no intradermal induction can occur as outlined in the materials and methods. - Changes were made to the procedure so that it no longer conforms to the OECD 406 guidelines. - At best this might be described as a modified type of Buehler test, primary induction is by way of topical application (over FCA injection sites). - too few animals to consider results in a meaningful way. - no requirement to repeat this study, the results of a GPMT and Buehler study carried out on the active substance difenacoum and submitted in support of the CAR provide no evidence of sensitising potential.	
Remarks		
	COMMENTS FROM ...	
Date	Give date of comments submitted	
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state	
Results and discussion	Discuss if deviating from view of rapporteur member state	
Conclusion	Discuss if deviating from view of rapporteur member state	
Reliability	Discuss if deviating from view of rapporteur member state	
Acceptability	Discuss if deviating from view of rapporteur member state	
Remarks		

III B Section 6.4 BPD Data Set IIB Annex Point VI.6.4	**INFORMATION ON DERMAL ABSORPTION**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. *The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [x]**	
Detailed justification:	More details are explained in the Risk Assessment for the human and environmental exposure, where each step of the process was evaluated.	
	According exposure assessment performed on measurements of a surrogate in simulated use conditions and on daily exposure frequencies according to a questionnaire answered by selected pest control companies in several EU countries. In primary exposure, the skin is the main exposure route, and only a small proportion of inhalation exposure to dust from decanting of pellets or grain baits is included in the total exposure. Inhalation exposure is not included for wax block formulation. Oral exposure is not considered relevant in primary exposure. Dermal absorption of 3% (pellets and grain baits) or 0.047% (wax block bait) and body weight of 60 kg for an adult is used for the calculations. The dermal absorption value of 3 % used in the CAR may overestimate the exposure taking into account that the dermal absorption value was much lower (0.047%) for the wax block formulation containing 50 mg/kg difenacoum. Calculations using a product specific dermal absorption value are expected to indicate acceptable risks.	
	Source: *Assessment Report – Difenacoum, Product-type 14 (Rodenticides), Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC, 17 September 2009, Annex I – Finland, p14.*	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*30 May 2011*	
Evaluation of applicant's justification	*Applicant's justification is acceptable*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

III B Section 6.5 BPD Data Set IIB Annex Point VI. 6.5	**AVAILABLE TOXICOLOGICAL DATA RELATING TO TOXICOLOGICALLY RELEVANT NON-ACTIVE SUBSTANCES (I.E. SUBSTANCES OF CONCERN)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. *The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [x]**	

Detailed justification:	In the formulated product, **GRANULAR BAIT**, containing 0.005% difenacoum, there is no presence of co-formulant of toxicological concern. The only substance of concern could be Sorbic acid (CAS 110-44-1), used as fungistat has the following classification: - Xi: irritant - R 36/37/38: Irritating to eyes, respiratory system and skin. Due to this low level, 0.02%, we can consider the substance has no influenced on the formulated product. No other studies have been deemed necessary	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*30 May 2011*	
Evaluation of applicant's justification	*Applicant's justification is acceptable.*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

III B Section 6.6 BPD Data Set IIB Annex Point VI.6.6	**INFORMATION RELATED TO THE EXPOSURE OF THE BIOCIDAL PRODUCT**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. *The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [x]**	

Detailed justification:	In competent authority reports, exposure and risk from the use of the representative products are calculated based on the dossiers submitted by the relevant applicants. Due to different data base (different repeated dose toxicity NOAEL/LOAEL-values and different bioavailability), different AOEL-values were set in competent authority reports. In this assessment report, the exposure to the products is compared to the lowest relevant repeated dose NOAEL/LOAEL- and AOEL-values identified in competent authority reports. This leads to higher risks for the products which were evaluated using a higher repeated dose NOAEL- and AOEL-values in competent authority reports. In most cases, gloves must be used to reduce the exposure below the AOEL-value for trained professionals. For non-trained professionals and amateurs, the use is generally acceptable also without gloves. Exposure from use of pellets or grain baits to a trained professional, covering daily application and post-application tasks (79 daily exposures), results in $1.0x10^{-6}$ mg/kg bw/day systemic dose with protective gloves. The exposure is approx. 91% of the AOEL (0.0000011 mg/kg bw/day). Because non-trained-professionals (e.g. farmers) and amateurs are expected to handle much smaller amounts of baits daily, the exposure is at lower level than for the pest control operators. The calculated systemic dose (for 10 daily exposure) is $1.0x10^{-6}$ without protective gloves which is below the AOEL-value (91% of the AOEL). Thus, it is concluded that non-trained professional/amateur use of pellet or grain baits does not result in unacceptable health risk. Exposure for a trained professional covering daily application and post-application tasks (75 daily exposures, 60 loadings and 15 clean-ups) from use of wax block bait, results in 1.3x10-7 mg/kg bw/day systemic dose with protective gloves. If protective gloves are worn, the risk is at acceptable level for wax block, bait (12% of the AOEL-value of 0.0000011 mg/kg bw/day). Non-trained-professionals (e.g. farmers) and amateurs are expected to handle much smaller amounts of baits daily, and the exposure is at lower level than for the pest control operators. The calculated systemic dose for wax blocks and 10 daily exposure is 1.2x10-7 without protective gloves which is below the AOEL-value (11% of the AOEL). It is concluded that non-trained professional/amateur use of wax block baits does not result in unacceptable health risk.	

	Placing of pellet or grain bait and clean-up, non-trained professional Placing of pellet or grain bait and clean-up, amateur Information related to the toxicity of the BPD to human is presented in documents IIB and IIC of the present application. A description and an assessment of the intended use for Professional, non trained professionals and amateurs were carried out in doc IIB. Calculations were then compared against the relevant end points in doc IIC. Results of the risk characterization show that worker wearing appropriate PPE, as recommended on the label, are not at potential risk. Source: *Assessment Report – Difenacoum, Product-type 14 (Rodenticides), Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC, 17 September 2009, Annex I – Finland, p14-15 and 40.* *Documents IIB and IIC of the present application.*	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*30 May 2011*	
Evaluation of applicant's justification	*Applicant's justification is acceptable.*	

Conclusion	*Applicant's justification is acceptable.*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Environment (including Eco-Toxicology)

III B Section 7.1 **BPD Data Set IIB** **Annex Point VII.7.1**	**Foreseeable routes of entry into the environment on the basis of the use envisaged**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements.* *The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [x]**	

Detailed justification:

Route of entry in the environment have been assessed in documents IIB and IIC. Following the results of the risk assessment carried out, and the nature of the molecule, physico-chemical properties and the relation structure/function, there is no foreseen route of entry in the environment that are of concern.

Following results on the a.s., nature of the molecule, physico-chemical properties and the relation structure/function, there is no foreseen route of entry in the environment that are of concern

Water justifications:

Difenacoum is only slightly soluble in water in neutral conditions, and it is hydrolytically stable. Difenacoum undergoes rapid phototransformation in water (half-life about 8 hours or less). Two applicants did not identify transformation products, because individual transformation products were formed less than 10% of the active substance added.

In the photolysis study of Activa/Pelgar Brodifacoum and Difenacoum Task Force two breakdown products above 10% were detected, but not chemically identified. Because the photodegradation is regarded as a minor removal process for difenacoum and the exposure to water is low no further characterization of metabolites was deemed necessary.

PEC surface water were calculated and compared against the relevant end points in Doc IIC. PEC surface water were calculated for the representative uses, i.e. sewer systems, in and around buildings, open areas and landfills/dump. No concern has been raised

Air justifications:

Difenacoum has a low vapour pressure (< 5 x 10-5 Pa) and

Henry's Law constant (0.046 - 0.0129 x 10-2 Pa.m3mol-1). Release to air via water is expected to be negligible. This is also supported by calculations using the TGD on risk assessment for percent release to air from a sewage treatment plant (section 3.3.2) where no release to air is predicted. Releases to air from use of wax blocks within bait boxes are considered to be negligible. The manufacture of the active substance is in a closed system. There are no releases to air of difenacoum from manufacturing, formulating, use or disposal phases

Soil justifications:

Difenacoum is not readily or inherently biodegradable. Difenacoum degrades slowly under aerobic conditions in soil, with a measured DT50 of 439 days. Photolysis may contribute to the degradation in soil, but in the lack of experimental evidence, soil photolysis cannot be taken into account.

PEC soil were calculated and compared against the relevant end points in Doc IIC. PEC soil were calculated for the representative uses, i.e. sewer systems, in and around buildings, open areas and landfills/dump. No concern has been raised.

Groundwater justifications:

The QSAR Koc value of 1.8×10^6 is used in the risk assessment instead of the experimentally derived Koc values, because they were regarded unreliable. The Koc values were determined with the HPLC method and although the studies *per se* were regarded valid, the test method appeared to be unsuitable for difenacoum.

The HPLC method (OECD 121) is not an actual study with measurements in real soil, but only an estimation based on the comparison of test substance to reference substances under artificial system, and hence there may be more uncertainties than in the adsorption/desorption batch-test (OECD 106).

The experimentally derived Koc values were inversely related to pH, so that high values were obtained in acidic conditions (Koc of

		426 579 at pH 3-4) and low values in neutral or alkaline conditions (17-165 at pH 7-8.5). The experimentally derived Koc values are not supported by the physical and chemical properties of difenacoum. Difenacoum is a large aromatic molecule with two polar groups which can potentially ionize at environmental relevant pH. Difenacoum has also low water solubility and a high log Kow. The HLPC-method gives quite low Koc value suggesting that ionized form of difenacoum will not have great affinity to organic matter. Although difenacoum is a weak acid with probably two dissociable sites, it might not be in ionized form with low adsorption in natural environment, or ionizable form might behave like a neutral form if the charge is shielded by the large molecule size. Also comparison to similar anticoagulant molecules supports the expert view that due to the intrinsic properties of these molecules the adsorption to particles is probable. One applicant has also experimental data which show that difenacoum is not mobile in soil, as concentrations in leachate from column leaching studies conducted with both the active substance and the product were non-determinable. Difenacoum is therefore not expected to contaminate groundwater. Calculated PECgw leads to concentration far below the EU trigger value for drinking water of 0.1 µg/l <u>Source:</u> *Assessment Report – Difenacoum, Product-type 14 (Rodenticides), Directive 98/8/EC concerning the placing of biocidal products on the market. Inclusion of active substances in Annex I or IA to Directive 98/8/EC, 17 September 2009, Annex I – Finland, p15-16.* *Documents IIB and IIC of the present application.*	
Undertaking of intended data submission	**[ ]**	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data*	

submission.)	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02-02-11
Evaluation of applicant's justification	The Reviewer is only considering the first part of the applicant's justification. Foreseeable routes of entry into the environment on the basis of the use envisaged are assessed in the environmental exposure and risk assessment (please see the PAR for further details). The rest of the justification is largely taken from the difenacoum assessment report (17-09-2009) section 2.2.2.1 except where reference is made to PEC calculations.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

III B Section 7.2 **BPD Data Set IIB** **Annex Point VII.7.2**	**Information on the ecotoxicology of the active substance in the product, where this cannot be extrapolated from the information on the active substance itself**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data*	Official use only

	requirements. *The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [x]**	
Detailed justification:	Information on the a.s., regarding ecotoxicology, could easily be extrapolated from active substance difenacoum. Indeed, co-formulants used in the final product do not have an impact on the toxicology, ecotoxicology or e-fate. No other studies have been deemed necessary	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	26/01/11	

Evaluation of applicant's justification	According to the Final AR (Sept 2009) on Difenacoum, Difenacoum classifies as R50/53 under Directive 67/548/EEC. However, it is stated that no classification of products containing 50 mg/kg or 75 mg/kg would be necessary according to Directive 1999/45/EC and GHS Regulation (EC) No 1272/2008. Similarly, according to Directive 67/548/EEC, the co-formulant, Denatonium Benzoate, which is a bittering agent added as a safety measure to protect non-target organisms classifies as R52/53 (MSDS PelGar). However, according to Directive 1999/45/EC and GHS Regulation (EC) No 1272/2008, since the concentration of this co-formulant in the product is only 0.195% w/w, it does not classify. Therefore Applicant's justification is acceptable assuming the test material is used according to the supported GAP.
Conclusion	C.A. considers applicant's justification to be acceptable.
Remarks	No further remarks.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

III B Section 7.3 **BPD Data Set IIB Annex Point VII.7.3**	**Available ecotoxicological information relating to exotoxicological relevant non-active substances (i.e substances of concern), such as information from safety data sheet.**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements.* *The justifications are to be included in the respective location*	Official use only

	(section) of the dossier. *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [x]**	
Detailed justification:	Information on the a.s., regarding toxicology, could easily be extrapolated from active substance difenacoum. Indeed, co-formulants used in the final product do not have an impact on the toxicology, ecotoxicology or e-fate. No other studies have been deemed necessary	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*26/01/11*	

Evaluation of applicant's justification	According to the Final AR (Sept 2009) on Difenacoum, Difenacoum classifies as R50/53 under Directive 67/548/EEC. However, it is stated that no classification of products containing 50 mg/kg or 75 mg/kg would be necessary according to Directive 1999/45/EC and GHS Regulation (EC) No 1272/2008. Similarly, according to Directive 67/548/EEC, the co-formulant, Denatonium Benzoate, which is a bittering agent added as a safety measure to protect non-target organisms classifies as R52/53 (MSDS PelGar). However, according to Directive 1999/45/EC and GHS Regulation (EC) No 1272/2008, since the concentration of this co-formulant in the product is only 0.195% w/w, it does not classify. Therefore Applicant's justification is acceptable assuming the test material is used according to the supported GAP.
Conclusion	C.A. considers applicant's justification to be acceptable.
Remarks	No further remarks.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Annex IV: List of studies reviewed

List of new data[20] submitted in support of the evaluation of the active substance (IIIA)

Not Applicable

List of new data submitted in support of the evaluation of the biocidal product (IIIB)

Identity:

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	Data owner	LoA# (Y/N)	DPC* (Y/N)
B1	-	-	Statement confidential data Manufacturing process.	Bio6		Y
B2.1_0	-	-	Difenacoum Grain: composition	Bio6		Y
B2.1_1	Porte P., Denny O.	2009	Analytical Certificate Product name: Difenacoum grain bait Batch number: 60030, date of analysis: 5 May 2009. Defitraces, 69126 Brindas, France, 19th October 2009. GLP. Unpublished.	Bio6		Y
B2.1_2	Angelis R., Bucciarelli B.	2008	Analisi Chimiche-Microbiologiche Servizi. Diraticide pesce-anticoagulant rodenticide cereal based, batch number 260608 Laboratori Bucciarelli, Rapporto di Proca N 15192/08., 11/09/08.	LODI		Y
B2.2_01	Anonym	2010	Saftey Data Sheet_Component 1: Difenacoum concentrate 2.5% (Red) Denatonium Benzoate. PELGAR International, UK. Not GLP, Published	Pelgar		Y
B2.2_02	Anonym	2010	[redacted]	Colorey SAS		Y
B2.2_03	Anonym	2006	[redacted]	Quaron		Y
B2.2_04	Anonym	2010	[redacted]	Quaron		Y
B2.2_05	Anonym	-	[redacted]	Bio6		Y

20 Data which have not been already submitted for the purpose of the Annex I inclusion.

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Physical/Chemical Properties:

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	Data owner	LoA# (Y/N)	DPC* (Y/N)
B3.6_1	Demangel B.	2008	Pour and tap density of granular materials on difenacoum grain bait, In compliance with CIPAC MT 159 (CIPAC Handbook F – 1994). Andiag Group – Defitraces, Z.A. des ”Andrés” 150, rue Pré-Magne, 69126 Brindas, France, Report no. 09-902018-001. GLP Unpublished.	Bio6		Y
B.3.7_1	Biannic M-L., Magnier C.	2008	Study report – Stability of Difenacoum baits after accelerated storage procedure. Test item: Baits containing 0.005% of Difenacoum: pasta, block and cereals. LODI Group, Parc d’activité des Quartre Routes, 35390 Grand Fougeray, FRANCE. Version date: 2008-01-07 Unpublished	LODI		Y
B.3.7_2	Biannic M-L., Magnier C.	2009	Study Report –stability of Difenacoum baits after storage at ambient temperature. Test item: Baits containing 0.005% of Difenacoum: baits, block and cereals. LODI Group, Parc d’activité des Quartre Routes, 35390 Grand Fougeray, FRANCE. Version date: 2009-11-12 Unpublished	LODI		Y
B.3.7_3	Brekelmans, Ir. M.J.C.	2010	Study Report –Determination of physic-chemical properties of difenacoum grain baits. NOTOX B.V., Hambakenwetering 7, 5231 DD ’s-Hertogenbosch, The Netherlands. Version date: 17th September 2010 Project no: 490523. Unpublished	Bio6		Y

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Methods of Analysis:

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	Data owner	LoA# (Y/N)	DPC* (Y/N)
B4_1a	Ricau, H.	2009	Analytical method validation for the determination of difenacoum in Difenacoum grain bait, in compliance with CIPAC/3807R. Anadiag Group - Defitraces, 69126 Brindas, France. Report No. 09-902018-003, of 19 October 2009. GLP. Unpublished	Bio6		Y

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	Data owner	LoA# (Y/N)	DPC* (Y/N)
B4_1b	Ricau, H.	2009	Quantification of difenacoum 0.005% m/m in a rat poison bait. Anadiag Group - Defitraces, 69126 Brindas, France. Report No. 05-912011-001, 16 June 2005, 22 pages, LODI sa. GLP. Unpublished	LODI		Y
B4_1c	Porte P., Denny O.	2009	Analytical Certificate – Product name: Difenacoum grain bait, batch number: 600300, date of analysis: 5th May 2009. Anadiag Group - Defitraces, 69126 Brindas, France, belong to study 09-902018-003. GLP. Unpublished.	Bio6		Y
B4_2	Bucciarelli B.	2008	Analytical certificate – Determination of concentration of difenacoum in anti-coagulant rodenticide cereal based. Laboratorio Bucciarelli, Analisi Chimiche-Microbiologiche Servizi, via del commerci, 112 Zona industriale Basso Marino, 63100 Asoli Piceno, Italia, Study number 24/2008, 18/09/2008. GLP Unpublished	Bio6		Y
B4_Litt-01	Magnier C., Biannic ML.	2009	Analytical method validation for the determination of difenacoum in Difenacoum bait (pasta, grain and block). LODI Group, Parc d'activité des Quartre Routes, 35390 Grand Fougeray, FRANCE. Study No. LODI 17/2009_Version date 2009-11-04. Unpublished	LODI		Y

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Efficacy

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	DPC* (Y/N)	Data owner
B5.0_01	Anonym	2004	Application Codes fo Encoding Rodenticides (PT14) No GLP, Published	N	E.U
B5.0_02	Anonym	2001	Guidelines fort he safe use of Anticogulant Rodenticodes by professional BPCA: Bristih Pest Control Association	N	BPCA

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	DPC* (Y/N)	Data owner
			No GLP, Published		
B5.0_03	Anonym	1995	Anticoagulant rodentices (EHC 175, 1995) International Programme on Chemical Safety No GLP, Published	N	INCHEM
B5.0_04	Anonym	2009	Assessment report Difenacoum Product type 14 17th September 2009 No GLP, Published	N	Finland RMS
B5.0_05	Anonym	1995	IPCS INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY: Health and Safety Guide No. 95 DIFENACOUM - HEALTH AND SAFETY GUIDE No GLP, Published	N	IPCS
B5.0_06	Anonym	2003	Technical Monograph 2003 Anticoagulant Resistance Management Strategy For Pest Management Professionals, Central And Local Government and Other Competent Users Of Rodenticides, No GLP, Published	N	CropLife International
B5.10.01a	Latteur G.,	1996	Efficacy test on rodenticide product, BELGASOURIS: whole and crushed wheat, containing 0.005% of Difenacoum, against grey mice (*Mus musculus*). - *Efficacité du rodenticide Belgasouris, mélange de grains de cereals entiers ou aplatis à base de 0.005% Difénacoum contre la souris grise (Mus musculus).* Grain bait/ Semi field efficacy/ Mice / Fresh Product (T0) CRA (Agronomic Research Center), Phytopharmacological department, Rue du	Y	Belgagri

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	DPC* (Y/N)	Data owner
			Bordia, 11, 5030 Gembloux Belgium report 947, November 1996. GLP, Unpublished		
B5.10.01b	-	-	Calculus addenda report 947	Y	Belgagri
B5.10.02a	Ryckel (de) B., Meeus P.,	1997	Appetizing test through different period of time, performed on BELGASOURIS, rodenticide containing 0.005% of Difenacoum, against grey mice (*Mus musculus). Evaluation de la perte d'efficacité au cours du vieillissement du "Belgasouris", rodenticide à base de 0,00% de difénacoum pour lutter contre la souris grise (Mus musculus).* *Grain bait/ Lab/Choice test/ Mice (albinos)/ Product at T6 months* CRA (Agronomic Research Center), Phytopharmacological department, Rue du Bordia, 11, 5030 Gembloux Belgium report 947, November 1996*.),* report 972 (complement to report 947), July 1997. GLP, Unpublished	Y	Belgagri
B5.10.02b	Ryckel (de) B., Meeus P.,	1997	Analyse certificate N°8686-Ch1267-1997-47, Personnalité Juridique De La Station De Phytopharmacie, Rue du Bordia, ll B - 5030 - GEMBLOUX – Belgique, N°8882Ch.1440/1997 GLP, Unpublished	Y	Belgagri
B5.10.02c	-	-	Calculus addenda report 972	Y	Belgagri
B5.10.03a, b	De Proft M., Meeus P	2001	Appetizing behaviour with BELGASOURIS at different period of time, bait ready to use, containing 0.005% of Difenacoum, used in albinos mice in order to be applied against grey mice (*Mus musculus*). *Etude du comportement du BELGASOURIS, appât prêt à l'emploi, contenant 0.005% Difenacoum,*	Y	Belgagri

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	DPC* (Y/N)	Data owner
			destine à lutter contre la souris grise (Mus musculus) au cour s du vieillissement. *Grain bait/ Lab/Choice test/ Mice (albinos)/ Product at T12 months* CRA(Agronomic Research Center), Phytopharmacological department, Rue du Bordia, 11, 5030 Gembloux Belgium ,complement report 10.312, February 2001 GLP, Unpublished		
B5.10.03c	Ryckel (de) B., Meeus P.,	2001	Analyse certificate N°329-Ch2329-2001-37, Personnalité Juridique De La Station De Phytopharmacie, Rue du Bordia, ll B - 5030 - GEMBLOUX – Belgique, 329-Ch2329-2001-37, 14/02/2001 GLP, Unpublished	Y	Belgagri
B5.10.03d	-	-	Calculus addenda report 10.312	Y	Belgagri
B5.10.04	-	2002	-, Pest Control Assistance (PCA), Appetition and efficacy trial of « DISOURICIDE PESCE » on grey mice (Mus musculus), For LODI, Le Cosquer (56), 2002 Grain bait/ Field efficacy/ Mice /Product at T0 PCA, 3 rue Constantin Le Priol 56150 BAUD (France), Unpublished	Y	LODI
B5.10.05	-	2002	Appetition and efficacy trial of « DISOURICIDE PESCE » on grey mice (Mus musculus), For LODI, Mme Rigal, 56150 Baud, 2002. Grain bait/ Field efficacy/ Mice /Product at T 2 years Pest Control Assistance (PCA), 3 rue Constantin Le Priol 56150 BAUD (France), Unpublished	Y	LODI
B5.10.06b	Biannic M-L	2008	Intermediate report – Quantification of Difenacoum in Grain Bait (Whole grain), version date: November 5th, 2008. Test item at production date, batch BR291008 LODI S.A, FRANCE No GLP, Unpublished	Y	LODI
B5.10.06c	Biannic	2009	Intermediate report – Quantification of Difenacoum in Grain Bait (Whole grain), version	Y	LODI

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	DPC* (Y/N)	Data owner
	M-L		date: January 20th, 2009. Test item at start of test, batch BR291008 LODI S.A, FRANCE No GLP, Unpublished		
B5.10.06d	Biannic M-L	2009	Intermediate report – Quantification of Difenacoum in Grain Bait (Whole grain), version date: October 16th, 2008. Test item after 11 months, batch BR291008 LODI S.A, FRANCE No GLP, Unpublished	Y	LODI
B5.10.06e	Biannic M-L	2008	Intermediate report – Quantification of Difenacoum in Grain Bait (Broken grain), version date: November 5th, 2008. Test item at production date, batch BS291008 LODI S.A, FRANCE No GLP, Unpublished	Y	LODI
B5.10.06f	Biannic M-L	2009	Intermediate report – Quantification of Difenacoum in Grain Bait (Broken grain), version date: April 17th, 2009. Test item at start of test, batch BS291008 LODI S.A, FRANCE No GLP, Unpublished	Y	LODI
B5.10.06g	Biannic M-L	2009	Intermediate report – Quantification of Difenacoum in Grain Bait (Broken grain), version date: October 16th, 2009. Test item after 11 months, batch BS291008 LODI S.A, FRANCE No GLP, Unpublished	Y	LODI
B5.10.07	-	2002	Appetition and efficacy trial of « DIRATICIDE » on brown rats (Rattus norvegicus), For LODI, U.K.L (56), 2002 Grain bait/ Field efficacy/ Rats /Product at T0 Pest Control Assistance (PCA), 3 rue Constantin Le Priol 56150 BAUD (France), Unpublished	Y	LODI
B5.10.08	-	2002	Appetition and efficacy trial of « DIRATICIDE» on brown rat (Rattus norvegicus), For LODI, Mr LAMOURIC Maurice, Tréviol, 56480 CLEGUEREC Baud, 2002. Grain bait/ Field efficacy/ Rats /Product at T 2 years Pest Control Assistance (PCA), 3 rue Constantin Le Priol 56150 BAUD (France), Unpublished	Y	LODI
B5.10.09a	Feys J-L.	2009	Field trial with RACO GRAIN BAITS against ROOF RATS 11 November 2009_03 December 2009, batch PB 091109	Y	Belgagri

Ref No	Author	Year	Title Source Company, Report No. GLP (where relevant)/ (Un)Published	DPC* (Y/N)	Data owner
			Grain bait/ Field efficacy/ Rats /Product at T0 Belgagri. Unpublished		
B5.10.09b	Feys J-L.	2009	Field trial *Rattus rattus* 11 November 2009_03 December 2009: scheme	Y	Belgagri
B5.10.09c	Feys J-L.	2009	Field trial RACO ROOF Rats (11/11/2009) test results	Y	Belgagri
B5.10.09d	Feys J-L.	2009	Field trial with RACO GRAIN BAITS against ROOF RATS 11 November 2009_03 December 2009_Summary	Y	Belgagri
B5.10.09e	Magnier C	2009	Analyse certificate, batch PB 091109	Y	LODI

* Data Protection Claimed

Toxicology

Ref No	Author	Year	Title	Data owner	LoA# (Y/N)	DPC* (Y/N)
B6.1.1	[redacted]	2009	Difenacoum grain bait - Acute Oral Toxicity in the rat - Acute toxic class method	Bio6 S.A.	Y	Y
B6.1.2	[redacted]	2009	Difenacoum grain bait - Acute Dermal Toxicity in the rat - Acute toxic class method	Bio6 S.A.	Y	Y
B6.2	[redacted]	2009	Difenacoum block bait – Skin Irritation test in the rabbit	Bio6 S.A.	Y	Y
B6.2	[redacted]	2009	Difenacoum block bait – Eye Irritation test in the rabbit	Bio6 S.A.	Y	Y
B6.3	[redacted]	2009	Difenacoum block bait – Skin sensitisation in the guinea pig - Magnusson and Kligman maximisation method	Bio6 S.A.	Y	Y

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ANNEX V: Toxicology Calculations

Insert relevant exposure/effect calculations undertaken, if applicable.

ANNEX VI: Environmental Calculations

The Notifier submitted the same assessment that was used to support Annex I inclusion.

A summary of the Environmental exposure assessment

PEC in surface water, sewage treatment plant, ground water and sediment

Using the scenarios outlined in the ESD for rodenticides and the TGD on risk assessment, and the calculations and assumptions presented in the previous sections above, the following PEC locals presented below have been derived for the aquatic compartment. No risk to ground water ($PEC_{groundwater}$ < 0.1 µg/L) was identified when the product is used in accordance with the assumptions made in the exposure assessment. The maximum permissible concentration by directive 80/778/EEC (amended by 98/83/EC) of 0.1 µg/L is not exceeded in surface waters.

PEC in surface water, sewage treatment plant, groundwater and sediment

Compartment/Scenario	ESD realistic worst case scenario	ESD realistic worst case scenario with modified input parameters	ESD normal use scenario with modified input parameters
Sewer scenario (30 kg of product used in control operation)			
PEC for microorganism in the STP	8.06×10^{-6} mg/L	5.91×10^{-6} mg/L	---
Local PEC in surface water during emission an episode (dissolved)	2.11×10^{-7} mg/L	1.55×10^{-7} mg/L	---
Local PEC in freshwater sediment during an emission episode	8.61×10^{-3} mg/kg wwt	6.32×10^{-3} mg/kg wwt	---
Groundwater/porewater	9.94×10^{-5} µg/L	7.29×10^{-5} µg/L	
In and around buildings scenario			
Groundwater/porewater	1.5×10^{-3} µg/L	1.1×10^{-3} µg/L	3.2×10^{-4} µg/L
Open areas			
Groundwater/porewater	0.00523 µg/L	0.0105 µg/L	---
Waste dump			
Groundwater/porewater	0.000224 µg/L	~0.00025 µg/L*	

*****For high infestations of rats the blocks are spaced 5 m apart. According to calculations provided by the Reviewer this could potentially result in a maximum of ~441 (21, 100 m lines of 21 blocks, 5 m apart) blocks in a 1 ha area during high infestations. This corresponds to ~44.1 kg of product, which is greater than the quantity considered under realistic worst-case conditions in the ESD. Consequently the notifiers exposure calculation is not sufficient to support this use. The Reviewer generated new exposure calculations for this use

PEC in air

Difenacoum is not expected to partition to the atmosphere to any significant extent due to low vapour pressure and Henry's Law constant. Difenacoum has athe potential for rapid photo-oxidative degradation in the air (half-life about two hours). Difenacoum is not expected to have a the potential for long-range atmospheric transport or contribute to global warming, ozone depletion or acidification on the basis of its physical and chemical properties.

PEC in soil

A summary of the soil exposure assessment is presented below:

PEC in soil

Compartment/Scenario	ESD realistic worst case scenario	ESD realistic worst case scenario with modified input parameters	ESD normal use scenario with modified input parameters
Sewer scenario (sludge application)			
Local PEC in agric. Soil (total) average over 30 d	3.29 x 10^{-3} mg/kg wwt	2.41 x10^{-3} mg/kg wwt	---
Local PEC in agric. Soil (total) average over 180 d	3.29 x 10^{-3} mg/kg wwt	2.41 x 10^{-3} mg/kg wwt	---
Local PEC in grassland. Soil (total) average over 180 d	1.31 x 10^{-3} mg/kg wwt	9.64 x 10^{-4} mg/kg wwt	---
In and around buildings scenario			
Total concentration in soil	0.047 mg/kg wwt	0.0348 mg/kg wwt	0.01 mg/kg wwt
Open areas			
Local concentration in soil after a Campaign	0.173 mg/kg wwt	0.346 mg/kg wwt	---
Waste dump			
Local concentration in soil after a Campaign	0.0074 mg/kg wwt	0.0082 mg/kg wwt*	---

*****For high infestations of rats the blocks are spaced 5 m apart. According to calculations provided by the Reviewer this could potentially result in a maximum of ~441 (21, 100 m lines of 21 blocks, 5 m apart) blocks in a 1 ha area during high infestations. This corresponds to ~44.1 kg of product, which is greater than the quantity considered under realistic worst-case conditions in the ESD. Consequently the notifiers exposure calculation is not sufficient to support this use. The Reviewer generated new exposure calculations for this use

Environmental Risk Assessment

Risk Characterisation for surface water, groundwater and sediment after elimination processes in STP

Difenacoum is very toxic to fish, aquatic invertebrates and algae. Toxicity to fish, the most sensitive species, is based on the inhibition of blood clotting. The mode of action in aquatic invertebrates and algae is unknown. The PNEC value was calculated according to ESD guidelines (Larsen, 2003), applying an Assessment Factor of 1000 to the lowest endpoint from studies on three trophic levels. According to the Assessment Report (17-09-2009), the limit of solubility was the PNEC for STP (480 µg/l). The risk characterisation for the STP and aquatic compartment including sediment is presented below:

Aquatic PEC/PNEC ratios using realistic worst case scenario with normal use after elimination processes in STP

Exposed Compartment	Endpoint	PNEC	PEC	PEC/PNEC
Surface water	LC_{50} 0.064 mg/l	0.06 µg/l	2.11 x 10^{-4} µg/l	3.5 x 10^{-3}

Sediment	-[1]	2.51[1] mg/kg ww	8.61×10^{-3} mg /kg ww	3.4×10^{-3}
STP	Solubility limit	480 µg/l	8.06×10^{-3} µg/l	1.6×10^{-5}

[1]In the absence of any ecotoxicological data for sediment-dwelling organisms and as PECsediment is calculated using EUSES 2.0.3, an aquatic PEC/PNEC ratio is used for sediment risk characterisation increasing it according to TGD (Part II, Section 3.5.2) with a factor of 10 as difenacoum has a log Kow > 5. PNEC reported as 2.51mg/kg ww in the Assessment Report (17-09-2009)

The PEC/PNEC ratios were less than 1 in all compartments indicating that difenacoum, following recommended use of Ruby Block, does not cause unacceptable risk to aquatic organisms, sediment-dwelling organisms or biological processes at the sewage treatment plant. As difenacoum is not readily biodegradable, the degradation of difenacoum in sediment is also anticipated to be low. However, according to the PEC calculations, concentrations in sediment would be low (8.61×10^{-3} mg /kg ww) and below the level that causes unacceptable risk, thus risk for unacceptable accumulation in sediment can be regarded as low. No risk is identified to either groundwater/porewater or surface water used as drinking as in both cases the maximum permissible concentration by directive 80/778/EEC (amended by 98/83/EC) of 0.1 µg/l is not exceeded in the ESD realistic worst case scenarios for uses in sewer, in and around buildings, open areas and waste dumps.

Risk Characterisation for Terrestrial Compartments

The PNEC applied in the risk characterisation for soil is one derived from the endpoint of an acute toxicity study on earthworms with an Assessment Factor of 1000. The risk characterisation for the terrestrial compartment including is presented below:

Terrestrial PEC/PNEC ratios using realistic worst case scenario with normal use

Exposed Compartment		PNEC	PEC	PEC/PNEC
Sewer-application of sewage sludge	Local PEC in agric. soil (total) average over 30 d	0.877 mg/kg ww	3.29×10^{-3} mg/kg ww	3.38×10^{-3}
	Local PEC in agric. soil (total) average over 180 d	0.877 mg/kg ww	3.29×10^{-3} mg/kg ww	3.38×10^{-3}
	Local PEC in grassland. soil (total) average over 180 d	0.877 mg/kg ww	1.31×10^{-3} mg/kg ww	1.5×10^{-3}
In and around buildings	Direct	0.877 mg/kg ww	4.1×10^{-2} mg/kg ww	4.7×10^{-2}
	Indirect	0.877 mg/kg ww	6.0×10^{-3} mg/kg ww	6.8×10^{-3}
	Total	0.877 mg/kg ww	4.7×10^{-2} mg/kg ww	5.4×10^{-2}
Open areas		0.877 mg/kg ww	1.73×10^{-1} mg/kg ww	0.197
Waste dump		0.877 mg/kg ww	8.2×10^{-3} mg/kg ww*	9.4×10^{-3}

* Value calculated by Environmental Fate and Behaviour Reviewer for High infestations of rats.

The PEC/PNEC ratios were less than 1 in all compartments indicating that difenacoum, following recommended use of Ruby Block, does not cause unacceptable risk to organisms in any of the terrestrial compartments assessed.

Primary poisoning

The Tier 1 assessment assumes that there is no bait avoidance by the non-target animals, and that they obtain 100% of their diet in the treated area and have access to the difenacoum product. The worst case Tier 1 PEC_{oral} is 50 mg/kg (difenacoum present at 0.005% w/w in Ruby Block) and is used in quantitative risk assessment for the long-term situation. The LD_{50} values are 56 mg/kg bw for birds (AF 3000) and 1.8 mg/kg bw for mammals (AF 90) (List of Endpoints in the Assessment Report (17-09-2009). The Tier 1 Primary poisoning PEC/PNEC ratios are provided below:

Tier 1 Primary poisoning PEC/PNEC ratios

Exposed Organism	PNEC µg/kg food	PNEC[1] µg/kg bw/d	PEC	PEC/PNEC
Birds	0.5	0.1	50 mg/kg food	500000
Mammals	7	0. 3	50 mg/kg food	166667

[1] Appendix V- Assessment Report (17-09-2009)

According to ESD (Larsen, 2003) a Tier 2 evaluation assessment can be done estimating daily uptake of a compound (ETE) by non-target animals according to the equation 19 of ESD (ETE = (FIR/BW) * C * AV * PT * PD (mg/kg bw/day);

FIR: food intake rate of the indicator species,
BW: indicator species body weight,
C: concentration of the active substance in fresh diet,
AV: avoidance factor,
PT: fraction of diet obtained in treated area and
PD: the fraction of the food type in the diet.

In Tier 2 Step 1 (worst case) AV, PT and PD are all set at 1, in Step 2 (realistic worst case) these AV and PT are refined to 0.9 and 0.8, respectively.

When elimination of active substance is taken into account the expected concentration of active substance (EC) in animals is calculated with equation 20 (ESD), EC = ETE x (1-El), where El is fraction of daily uptake eliminated (number between 0 and 1, default 0.3). According to the toxicokinetic study[9], average level of radioactivity in excreta of rats was 23% of total administered radioactivity during the first day after single dose and daily average 25% during 7 consecutive daily dosing. Difenacoum is also eliminated in the rat body through metabolism, average proportion of difenacoum in extract of liver was 30% on day 168 (and thus metabolites can be assumed to account for 70%). 24.3% of total administered radioactivity was found in liver, so 17% of total administered dose is (liver) metabolites (metabolites in other tissues were not studied and thus not taken into account). Thus the total daily elimination in rats taking into account excretion through faeces and metabolism of difenacoum in rat liver, is approximately 40% (**elimination factor 0.4**), which is also used in calculations for non-target animals as there are no other data available.

For the acute exposure situation, no $PNEC_{oral}$ is determined and no quantitative risk characterisation is performed. Instead a qualitative assessment is done by comparing LD_{50} values to the expected contents of the active substances in birds and mammals. According to the guidance agreed at 23rd CA, these values are used for qualitative risk assessment of **acute primary poisoning**. The values obtained are provided below:

Tier 2 Expected concentrations of difenacoum in non-target animals in the worst case (Step 1) and realistic worst case (Step 2) for acute situations with and without elimination

Species		Body weigh t (g)	Daily mean food intake (dw) (g)	Rodentic ide consum ption (g)	Estimated daily uptake of difenacoum (ETE) after single meal (mg/kg bw)	Expected concentration (EC) of a.i. in the animal after one day elimination (mg/kg bw)

					Step 1 [1]	Step [2]	Step 1[1]	Step 2[2]
Dog	*Canis familiaris*	10000	456	600	2.28	1.64	1.37	0.98
Pig	*Sus scrofa*	80000	25203 (600)[4]	600	0.4	0.27	0.23	0.16
Pig, young	*Sus scrofa*	25000	969[3] (600)[4]	600	1.2	0.86	0.72	0.52
Fox Representing General non-target mammal	*Vulpes vulpes*	5700 5700	520 [5] 287[3]	520 287	4.56 2.5	3.28 1.8	2.74 1.5	1.97 1.08
Tree sparrow	*Passer montanus*	22	7.6	7.6	17.3	12.44	10.36	7.46
Chaffinch	*Fringilla coelebs*	21.4	6.42	6.42	15.0	10.8	9.0	6.48
Wood pigeon	*Columba palumbus*	490	53.1	53.1	5.4	3.9	3.25	2.34
Pheasant	*Phasianus colchicus*	953	102.7	102.7	5.4	3.9	3.23	2.33

[1] avoidance (AV), Fraction of diet from treated area (PT) and Fraction of food type in diet (PD) are set at 1.

[2] according to ESD AV to 0.9 and PT 0.8.

[3] according to ESD 3.2.1. logFIR = 0.822 logBW – 0.629.

[4] according to ESD 600g is maximum for rodenticide consumption in one daily meal.

[5] ESD table 3.5.

The qualitative assessment of acute primary poisoning is presented below:

Qualitative assessment of acute primary poisoning. The expected concentrations (EC) in the non-target animals after one day exposure with and without elimination. The EC have been calculated with the Step 2 assumptions, i.e, PT=0.8 and AV=0.9

Species		**EC after one day exposure without elimination mg/kg bw**	**EC after one day exposure and elimination mg/kg bw**	**LD_{50}**
Dog	*Canis familiaris*	1.64	0.98	1.8

Pig	*Sus scrofa*	0.27	0.16	1.8
Pig, young	*Sus scrofa*	0.86	0.52	1.8
Fox	*Vulpes vulpes*	3.28	1.97	1.8
Fox, representing general non-target mammal		1.8	1.08	1.8
Tree sparrow	*Passer montanus*	12.44	7.46	56
Chaffinch	*Fringilla coelebs*	10.8	6.48	56
Wood pigeon	*Columba palumbus*	3.9	2.34	56
Pheasant	*Phasianus colchicus*	3.9	2.33	56

According to the ESD the comparison of concentration in the non-target animals and the $PNEC_{oral}$ describes the **long-term risk for primary poisoning**. Calculations of the expected concentrations (EC) for 5 days exposure considering elimination are calculated according to ESD equation 21[1]. The Tier 1 calculations represent the a worst case i.e. AV, PT and PD are set to 1. In the Tier 2 calculations, the PT and AV have been modified according to the ESD to the realistic worst case values of 0.8 and 0.9 respectively According to the guidance agreed at 23rd CA meeting, EC_5 values are used for quantitative risk assessment of primary poisoning in the long-term situation. EC_5 values represent the expected concentration of the difenacoum after 5 days of exposure with elimination over the five day period (including the fifth day after exposure). The values obtained are provided below:

Expected concentrations of difenacoum (EC_5) in non-target animals for the long-term situations

Species		**Body weight(g)**	**Daily mean food intake (dw) (g)**	**Rodenticide consumption (g)**	**Expected concentration (EC_5) of a.i. in the animal after 5 days exposure, elimination taken into account (mg/kg bw)**	
					Tier 1	Tier 2
Dog	*Canis familiaris*	10000	456[2]	456	3.15	2.27
Pig	*Sus scrofa*	80000	2520[2] (600)[3]	600	0.52	0.37

Pig, young	*Sus scrofa*	25000	969[2] (600)[3]	600	1.66	1.19
Fox Representing General non-target mammal	*Vulpes vulpes*	5700 5700	520[4] 287[2]	520 287	6.31 3.48	4.54 2.51
Tree sparrow	*Passer montanus*	22	7.6	7.6	23.89	17.2
Chaffinch	*Fringilla coelebs*	21.4	6.42	6.42	20.75	14.94
Wood pigeon	*Columba palumbus*	490	53.1	53.1	7.49	5.39
Pheasant	*Phasianus colchicus*	953	102.7	102.7	7.45	5.37

[1]$ECn = \sum_{n=1}^{n-1} ETE * (1\ EL)^n$.

[2] according to ESD3.2.1. logFIR = 0.822 logBW – 0.629.
[3] according to ESD 600g is maximum for rodenticide consumption in one daily meal.

[4] ESD table 3.5.

The results of the risk assessment for long-term primary poisoning are provided below:

Tier 2 risk characterisation of primary poisoning. The expected concentrations (EC) in the non-target animals after five days exposure have been calculated with the Step 2 assumptions, i.e, PT=0.8 and AV=0.9. The $PNEC_{oral}$ is expressed as the daily dose

Species		PEC EC_5 µg/kg bw	$PNEC_{oral}$ µg/kg bw/d	PEC/PNEC
Dog	*Canis familiaris*	2270	0.3	7567
Pig	*Sus scrofa*	370	0.3	1233
Pig, young	*Sus scrofa*	1190	0.3	3967
Fox	*Vulpes vulpes*	4540	0.3	15133
Fox, representing general non-target mammal		2510	0.3	11 100
Tree sparrow	*Passer montanus*	17200	0.1	172000
Chaffinch	*Fringilla coelebs*	14940	0.1	149400
Wood pigeon	*Columba palumbus*	5390	0.1	53900
Pheasant	*Phasianus colchicus*	5370	0.1	53700

Secondary poisoning

Calculations of the PEC_{oral} predator for the possible exposure routes are shown below with the relevant re-calculated values from the Environmental Fate and Behaviour section. The waiving of fish bioconcentration test was accepted, because the test was judged not possible to perform technically, and because an estimated BCF value could be used in the risk assessment. The calculated BCFs range from 9010 (aquatic) to 477 729 (terrestrial).These are based on the estimated log P_{ow} of 7.6 (EPIWIN v. 3.1.2) in the absence of valid measured log P_{ow}.

Fish-eating birds and mammals

$PEC_{oral,\ predator}$ = PEC_{water} * BCF_{fish} * BMF (eq 76, TGD,2003):
= 2.11 x 10^{-7} mg/l * 9010 $l/kg_{wetfish}$ * 10 = 0.02 $mg/kg_{wet\ fish}$ (concentration in fish)

The PEC_{water} applied here is the ESD realistic worst case scenario. According to TGD (p. 127) the most appropriate scenario is that 50% of the diet comes from the local area and 50% comes from the regional area, thus when the PEC_{local} water is used in calculation, the PEC_{oral},predator to be used in risk assessment is 0.02 $mg/kg_{wet\ fish}$ *0.5 = **0.01 $mg/kg_{wet\ fish}$.**

Earthworm-eating birds and mammals

The Reviewer has recalculated the PEC_{oral} values by applying the revised exposure estimates provided by Environmental Fate and Behaviour.

PEC $oral,_{predator}$ = $C_{earthworm}$ (eq 80, TGD, 2003)

$C_{earthworm}$ = ($BCF_{earthworm}$*$C_{porewater}$+ C_{soil}*F_{gut}*$CONV_{soil}$)/ (1+$Fg_{ut\ kgdwt/kgwwt}$*$CONV_{soil\ kgwwt/kgdwt}$) (eq 82c, TGD 2003).

No measured BCF for earthworm is available and the calculated BCF of 4.80 x 10^5 $l/kg_{wetearthworm}$ (see Assessment Report, 2009) is used in calculations. The $C_{earthworm}$ is different for each compartment and the equations are given below for ESD realistic worst case scenarios.
According to the TGD (p. 131) the most appropriate scenario is that 50% of the diet comes from a local area and 50% comes from the regional area, thus when the PEClocal,soil is used in calculation, the PECoral, Predator to be used in risk assessment is 50% of the calculated $C_{earthworm}$.

Sewer Scenario

$C_{earthworm}$ = (4.80 x 10^5 $l/kg_{wetearthworm}$ x 9.94 x 10^{-8} mg/l (max $C_{porewater}$) + 3.29 x 10^{-3} mg/kg (max C_{soil}) x $0.1_{kgdwt/kgwwt}$ x 1.13 $_{kgwwt/kgdwt}$)/(1+0.1 *1.13) = 0.043 $mg/kg_{wetearthworm}$ x 0.5 = **0.022 $mg/kg_{wetearthworm}$.**

In and around buildings scenario

$C_{earthworm}$ = (4.80 x 10^5 $l/kg_{wetearthworm}$ x 1.5 x 10^{-6} mg/l (max $C_{porewater}$) + 0.047 mg/kg (max C_{soil}) x $0.1_{kgdwt/kgwwt}$ x 1.13 $_{kgwwt/kgdwt}$)/(1+0.1 *1.13) = 0.652 $mg/kg_{wetearthworm}$ x 0.5 = **0.326 $mg/kg_{wetearthworm}$.**

Open areas

$C_{earthworm}$ = (4.80 x 10^5 $l/kg_{wetearthworm}$ x 5.23 x 10^{-6} mg/l (max $C_{porewater}$) + 0.173 mg/kg (max C_{soil}) x $0.1_{kgdwt/kgwwt}$ x 1.13 $_{kgwwt/kgdwt}$)/(1+0.1 *1.13) = 2.273 $mg/kg_{wetearthworm}$ x 0.5 = **1.137 $mg/kg_{wetearthworm}$.**

Waste dump

$C_{earthworm}$ = (4.80 x 10^5 l/$kg_{wetearthworm}$ x 2.25 x 10^{-7} mg/l (max $C_{porewater}$) + 0.0082mg/kg (max C_{soil}) x $0.1_{kgdwt/kgwwt}$ x 1.13 $_{kgwwt/kgdwt}$)/(1+0.1 *1.13) = 0.098 mg/$kg_{wetearthworm}$ x 0.5 = **0.049 mg/$kg_{wetearthworm}$.**

The results of the quantitative assessment of acute secondary poisoning for birds and mammals via the aquatic food chain are provided below. The Reviewer has revised the $PNEC_{oral}$ to the daily dose as recommended by SANCO/4145/2000 (Sept 2002).

Secondary poisoning via aquatic food chain

	Aquatic $PEC_{oral,\ predator}$, µg/kg wet fish	**$PNEC_{oral}$ µg/kg bw/day**	**Aquatic PEC/PNEC**
Birds	10	0.1	100
Mammals	10	0.3	33

The results of the quantitative assessment of acute secondary poisoning for birds and mammals via the terrestrial food chain are provided below. The Reviewer has revised the $PNEC_{oral}$ to the daily dose as recommended by SANCO/4145/2000 (Sept 2002).

Table 6.5.3.2-2. Secondary poisoning via terrestrial food chain

	Terrestrial compartment	**Terrestrial $PEC_{oral,\ predator}$, µg/kg wet earthworm**	**$PNEC_{oral}$ µg/kg bw/day**	**Terrestrial PEC/PNEC**
Birds	Sewer	22	0.1	220
	In and around buildings scenario	326	0.1	3260
	Open areas	1137	0.1	11370
	Waste dump	49	0.1	490
Mammals	Sewer	22	0.3	73
	In and around buildings scenario	326	0.3	1087
	Open areas	1137	0.3	3790
	Waste dump	49	0.3	490

Rodent-eating birds and mammals

For estimation of secondary poisoning risk through poisoned rats, the amount of difenacoum in rats is estimated according to equations 19 and 21 in ESD (ETE = (FIR/BW) * C * AV * PT * PD (mg/kg bw/day), $EC_n = \sum^{n-1}_{n=1}$ ETE * $(1 - EL)^n$. In calculations AV and PT for rodent are set to 1 and PD values to 1 and 0.5 and 0.2. The daily elimination is assumed to be 40% (see Section 6.5.2). Tier 1

PEC_{oral} for short term situation is calculated according to the equation 22 in ESD (Larsen, 2003); $PEC_{oral, predator} = (ECn + ETE) \times F_{rodent}$) using value 1 for F_{rodent} (non-target animal consume 100% of their daily intake on poisoned rodents).

F_{rodent}; fraction of poisoned rodents in predator's diet
EC_n : expected concentration of a.s. in the rodent on day 'n' before the last meal
n; the number of days the rodent is eating rodenticide until caught, default 5.

Results are provided below. These values are used for qualitative risk assessment of **secondary poisoning in acute situation.**

Estimated concentration (EC) of difenacoum in target rodents (rats) in mg a.s./kg bw at different times during a control operation

	Residues of rodenticide in target rodent, mg/kg		
	Worst case 100% bait consumption by rodent (PD 1)	**Normal case 50% bait consumption by rodent (PD 0.5)**	**ESD minimum 20% bait consumption by rodent (PD 0.2)**
normal non-resistant target rodent which stops eating on day 5			
Day 1 after 1st meal	5.0	2.5	1.0
Day 2 before new meal	3.0	1.5	0.6
Day 5 before meal	6.53	3.26	1.31
Day 5 after last meal	11.53	5.76	2.31
Day 6*	6.92	3.46	1.38
Day 7 (mean time to death)*	4.15	2.08	0.83
Extreme case – rodent continues eating due to resistance			
Day 14 after the meal	12.49	6.25	2.5

* - The feeding period has been set to a default value of 5 days until the onset of symptoms after which it eats nothing until its death.

A qualitative assessment of the acute secondary poisoning is made by comparing the concentration in the rodents to LD_{50} values from acute oral studies. Rodents are assumed to feed entirely on bait containing difenacoum and the non-target animals are assumed to consume only poisoned rodents. The results of the qualitative assessment are provided below**.**

Qualitative assessment of acute secondary poisoning for rodent-eating birds and mammals

	EC in rat on day 5 after last meal mg/kg	Birds LD_{50} mg/kg bw	Mammals LD50 mg/kg bw
PD=1	11.53	56	1.8
PD=0.5	5.76	56	1.8
PD=0.2	2.31	56	1.8

Tier 1 quantitative assessment of secondary poisoning

The Tier 1 assessment of secondary poisoning for the long term situation is calculated in the way outlined for acute situations but is based on the concentration in the predator's or scavenger's food, i.e. poisoned rodents. The rodents are assumed to consume only bait (PD = 1), while half of the predator's or scavenger's daily food intake is poisoned rodents (F_{rodent} = 0.5). The rodents are assumed to eat the bait over five or fourteen successive days, whereas the predator or the scavenger is assumed to eat the poisoned rodents during one day. The predator is assumed to have caught the rodent after the last meal on day 5 or day 14. Only resistant rodents are assumed to eat bait over 14 days. The results are provided below:

Estimated concentration (EC) of difenacoum in target rodents (rats) in mg a.s./kg bw for acute and long term situations

$PEC_{oral,predato}$,mg/kg			
	Worst case 100% bait consumption by rodent (PD 1)	Normal case 50% bait consumption by rodent (PD 0.5)	ESD minimum 20% bait consumption by rodent (PD 0.2)
Normal non-resistant target rodent which stops eating on day 5			
PEC_{oral} on day 5 for 'acute situation'	11.53	5.76	2.31
PEC_{oral} on day 5 for 'long term situation'	5.76	2.88	1.15
Extreme case – rodent continues eating due to resistance			
$PEC_{oral,predator}$ on day 14 'acute'[1]	12.49	6.25	2.5
$PEC_{oral,predator}$ on day 14	6.25	3.13	1.25

'chronic'			

[1] Day 14 after the meal, from Table 6.5.3.2-3. This is different to the figure presented in the CAR.

The results of the Tier 1 assessment of secondary poisoning are provided below.

Tier 1 risk characterisation of secondary poisoning. Expected concentration in target rodents is compared to the $PNEC_{oral}$ expressed as concentration in food. Rodents are assumed to consume only bait (PD=1). Half of the predator's diet is poisoned rodents (F_{rodent}=0.5 equivalent to PD=0.5)

	PEC EC in rodent µg/kg	**$PNEC_{oral}$ µg/kg bw/day**	**PEC/PNEC**
Rodents caught on day 5 after meal			
Birds	5760	0.1	57600
Mammals	5760	0.3	19200
Rodents caught on day 14 after meal			
Birds	6250	0.1	62500
Mammals	6250	0.3	20833

Tier 2 assessment of secondary poisoning

Tier 2 for long-term exposure:

According to guidance agreed by the CA the PEC_{oral} is the concentration in non-target animals after a single day of exposure (mg/kg bw) using values PD of 1 (100% bait consumption by rodent) and F_{rodent} of 0.5. PEC_{oral} values are presented in below are used for Tier 2 quantitative risk assessment of secondary poisoning in the long-term situation (supporting information from Table 3.5 ESD).

Expected concentrations of difenacoum in non-target animals due to secondary poisoning after a single day exposure (concentration of difenacoum in rodenticide bait 0.005 %); rodents caught by predators on day 5 and 14 (after feeding), PD 1, F_{rodent} 0.5

Species	**Body wt [g]**	**Daily FIR [g]**	**Rodent caught on day 5 after feeding mg ai/kg predator**	**Rodent caught on day 14 after feeding mg ai/kg predator**

Barn owl	*Tyto alba*	294	72.9	1.43	1.55
Kestrel	*Falco tinnunculus*	209	78.7	2.17	2.35
Little owl	*Athene noctua*	164	46.4	1.63	1.77
Tawny owl	*Strix aluco*	426	97.1	1.31	1.42
Fox	*Vulpes vulpes*	5700	520.2	0.53	0.57
Polecat	*Mustela putorius*	689	130.9	1.10	1.19
Stoat	*Mustela erminea*	205	55.7	1.57	1.70
Weasel	*Mustela nivalis*	63	24.7	2.26	2.45

In applying the predicted difenacoum concentrations in predatory birds and mammals, the Tier 2 risk characterisation was conducted and the results of which are provided below.

Tier 2 risk characterisation of secondary poisoning. The expected concentrations in predatory birds and mammals are compared to the $PNEC_{oral}$ expressed as daily dose

Species		**PEC EC in predator µg/kg bw Rodent caught on day 5**	**PEC EC in predator µg/kg bw Rodent caught on day 14**	**$PNEC_{oral}$ µg/kg bw/d**	**PEC/PNEC Rodent caught on day 5**	**PEC/PNEC Rodent caught on day 14**
Barn owl	*Tyto alba*	1430	1550	0.1	14 300	15 500
Kestrel	*Falco tinnunculus*	2170	2350	0.1	21 700	23 500
Little owl	*Athene noctua*	1603	1770	0.1	16 030	17 700
Tawny owl	*Strix aluco*	1310	1420	0.1	13 100	14 200
Fox	*Vulpes vulpes*	530	570	0.3	1 767	1 900
Polecat	*Mustela putorius*	1100	1190	0.3	3 667	3 967
Stoat	*Mustela erminea*	1570	1700	0.3	5 233	5 667
Weasel	*Mustela nivalis*	2260	2450	0.3	7 533	8 167

In conclusion, the PEC/PNEC ratios based from the Annex I inclusion CAR on the measured concentration in rats and mice were lower than the respective figures calculated according to the ESD, but still considerably higher than 1 indicating risk for secondary poisoning. Risk mitigation measures need to be applied.

ANNEX VII: Residue Calculations

No residue calculations are required as Ruby Grain is a ready to use bait, which is used to kill rats and mice. Ruby Grain will not come into contact with the human food chain. The bait may be used indoors, around buildings, away from buildings and around waste sites and sewers. The bait will be placed at protected bait points in dry locations, protected from the weather to help prevent access by non target animals.

Annex 2 – Revised PAR – May 2016

Product Assessment Report

Ruby Grain

Active substance:	**Difenacoum**
Product-type:	**PT 14: Rodenticide**
Type of application:	**Authorisation**
Authorisation No:	**IE/BPA 70003 (Non-professional product)** **IE/BPA 70027 (Professional product)**
Date:	**09 May 2016**

Biocidal Product Assessment Report (PAR) related to Product Authorisation under Directive 98/8/EC.

Department of Agriculture, Fisheries and Food
An Roinn Talmhaíochta, Iascaigh agus Bia

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1. General information about the product application

An application for authorisation was made to the Pesticide Registration and Control Division of the Department of Agriculture Fisheries and Food by Lodi S.A.S for the biocidal product Ruby Grain on 1st April 2010 in accordance with the provisions set out by Commission Directive 2008/81/EC.

This Product Assessment Report is for:

Trade name:	Ruby Grain
Authorisation No.:	IE/BPA 70003 (Non-professional) IE/BPA 70027 (Professional and Trained Professional)

The following authorisations in Ireland are linked to the above product authorisation:

Trade name	Authorisation No.	Marketing/Distribution Co.	Authorisation Type
Roded Grain Bait	PCS 70028	Hygeia Chemicals Ltd.	Supplemental Authorisation (Back-2-Back Authorisation)

59.1 Applicant/Authorization Holder

Company Name:	LODI S.A.
Address:	Parc d'activities des quatre routes Grand Fougeray 35390 France
Tel:	[redacted]
E-mail:	[redacted]

[redacted]

Company Name:	[redacted]
Address:	[redacted]
Tel:	[redacted]

59.3 Marketing/Distributing Company (where applicable)

Company Name:	LODI UK
Address:	Pensnett Trading Estate Building 69 3rd Avenue Kingswinford West Midlands, DY6 7FD UK

Tel:	[redacted]

59.4 General Information on the Biocidal Product

Trade name:	Ruby Grain
Manufacturer's development code number(s):	N/A
Active substance content (% w/w):	0.005% w/w difenacoum
Main group:	MG3 – Pest control
Product type:	PT14 - Rodenticides
Product Specification:	See Confidential Annex
Site of product formulation:	See Confidential Annex
Formulation type:	Ready-to-use (RB) Grain bait (AB)
Ready to use product (yes/no):	Yes (Only RTU products to be authorised)
Chemical/micro-organism:	Chemical substance
Contain or consist of GMOs[21] (yes/no):	N/A
Is the product already notified /authorised (yes/no); If yes: product name:	Yes (Notified under transitional arrangements with the PRCD) Ruby Grain, PCS 94705
Is the biocidal product equivalent to the product assessed for the purpose of Annex I inclusion to 98/8/EC (yes/no):	No.

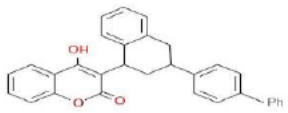
Manufacturer of Formulated Product:	LODI S.A.
Address:	Parc d'activities des quatre routes Grand Fougeray 35390 France
Tel:	[redacted]
[redacted]	[redacted]

59.5 Information on active substance(s)[22]

Active substance chemical name:	Difenacoum
IUPAC name:	3-(3biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphtyl)-4-hydroxycoumarin
CAS No:	56073-07-5
EC No:	259-978-4
Purity (minimum, g/kg or g/l):	>960 g/kg (96.0% w/w)

21 A copy of any written consent(s) of the competent authorities to the deliberate release into the environment of the GMOs for research and development purposes where provided for by Part B of the above-mentioned Directive was provided.

22 Please insert additional columns as necessary

Structural formula:	
Manufacturing site:	See Confidential Annex
Specification of pure active substance:	See Confidential Annex
Is a new active substance data package (source) supplied (yes/no):	No
If yes, Is the active substance equivalent to the active substance listed in Annex I to 98/8/EC (yes/no):	N/A
If no, does the applicant have a LoA to the active substance data packaged used to support Annex I inclusion (yes/no):	Yes (Pelgar International Ltd.)

Manufacturer of active substance(s):	Pelgar International Ltd.
Address:	Unit 13 Newman Lane Alton Hants. GU34 2QR UK
Tel:	[redacted]
E-mail:	[redacted]

59.6 Information on the intended use(s) of the biocidal product

Main Group:	MG03 (Pest control)
Product-type:	PT14 (Rodenticide)
Intended use:	Difenacoum grain bait to control rodents indoors and outdoors for the protection of public health, stored products and materials.
Target organisms:	(I.1) Rodents (I.1.1) Murids (I.1.1.1) Brown rats (*Rattus Norvegicus*) (I.1.1.2) House rat (*Rattus rattus*) (I.1.1.3) House mouse (*Mus musculus*)
Development stage:	(II.1) Juveniles (II.2) Adults
Function:	Rodenticide
Mode of action:	Anticoagulant III.2 long-term action III.2.1 anticoagulant III.2.1.1 ingestion toxin III.2.1.1.1 ingestion by eating
Application aim:	Protection of: Public health/hygiene, materials and Stored products
Category of users:	Trained professionals, professionals and non-professional

	(general public/amateur)
Area of use (indoors/outdoors):	Indoors (warehouses, outbuildings) Outdoors (in and around buildings, waste dumps, open areas)
Directions for use including minimum and maximum application rates, typical size of application area:	Rats: 90-100 g of grain bait per bait point spaced at 10m (spaced at 5m in high infestation areas). Typical treatment time 6 weeks. Mice: 20-30 g of bait per bait point spaced at 5m (spaced at 3m in high infestation areas). Typical treatment time 6 weeks.
Application method:	Grain bait contained in secured bait stations
Interval between applications:	When required. Regularly check bait consumption and replace consumed or spoilt bait until consumption has stopped. Repeat treatment in case of new infestation, new tracks or fresh droppings.
Typical treatment time:	6 weeks for rats and mice
Potential for release into the environment (yes/no):	Yes
Potential for contamination of food/feedingstuff (yes/no):	No

59.7 Documentation

59.7.1 Data submitted in relation to product application

A full new product dossier was submitted by Lodi S.A. in support of the product Ruby Grain containing difenacoum.

Please see the attached reference list in Annex IV.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2. *Classification, labelling and packaging*

Under this heading the assessment of the classification, labelling and packaging should be summarised. Further, any result of the assessments made under the following headings that require recommendations or restrictions appearing on the label should be summarised here.

2.1. Harmonised classification of the active substance

The current classification of the active substance based on the proposals resulting from the review programme for difenacoum, according to Directive 67/548/EEC, is provided in the table below. Additionally, the extrapolation of these proposals using the BG RCI converter tool (http://www.gischem.de/ghs/konverter) is also provided in the table below in accordance with Regulation (EC) 1272/2008.

Classification of the active substance, difenacoum, according to Directive 67/548/EEC and CLP Regulation (EC) 1272/2008:

Symbol(s):	 	**Pictogram(s):**	
Indication(s) of danger:	Very Toxic Dangerous for the Environment	**Signal word(s):**	Danger
Risk phrases:	R26/27/28: Very Toxic by inhalation, in contact with skin and if swallowed. R48/23/24/25: Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed. R61: May cause harm to the unborn child. R50/53: Very Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.	**Hazard statements:**	H300: Fatal if swallowed. H310: Fatal in contact with skin. H330: Fatal if inhaled. H360D: Suspected of damaging the unborn child. H372: Causes damage to organs through prolonged or repeated exposure through inhalation . H410: Very toxic to aquatic life with long lasting effects.
Safety phrases:	S45: In case of accident or if you feel unwell, seek medical advice immediately (show label where possible). S53: Avoid exposure - obtain special instruction before use. S60: This material and/or its container must be disposed of as hazardous waste. S61: Avoid release to the environment. Refer to special instructions/safety data sheet.	**Precautionary statements:**	P201: Obtain special instructions before use. P273: Avoid release to the environment. P308 + P313: IF exposed or concerned: Get medical advice/attention. P314: Get medical advice/attention if you feel unwell. P501: Dispose of contents/container to hazardous waste facilities in accordance with national regulations.

2.2. Harmonised classification and labelling of the biocidal product

The current classification and labelling according to Directive 99/45/EC and Regulation (EC) 1272/2008, Annex VI, Part 3 are provided in the tables below.

Classification and Labelling of the biocidal product, Ruby Grain, according to Directive 99/45/EC:

Symbol(s):	None
Indication(s) of danger:	None
Risk phrases:	None
Safety phrases:	S1+S2: Keep locked up and out of reach of children S13: Keep away from food, drink and animal feedingstuffs S37: Wear suitable gloves S46: If swallowed, seek medical advice immediately and show this container or label S57: Use appropriate containment to avoid environmental contamination. S35: This material and its container must be disposed of in a safe way.

Classification and Labelling of the biocidal product, Ruby Grain, according to the CLP Regulation (EC) 1272/2008:

Pictogram(s):	None
Signal word(s):	None
Hazard statements:	None
Precautionary statements	P102: Keep out of reach of children. P103: Read label before use. P220: Keep/Store away from food, drink and animal feedingstuffs. P270: Do not eat, drink or smoke when using this product. P273: Avoid release to the environment. P280: Wear protective gloves P301+310: IF SWALLOWED: Immediately call a poison centre or doctor/physician. P404+405: Store locked up in a closed container. P501: Dispose of contents/container in accordance with national regulations.

Further, the content of the label should be updated to comply with the labelling requirements established (for biocidal products) where the labelling requirements in Article 20(3) of Directive 98/8/EC has been implemented. The safety data sheet should comply with the requirements in Regulation (EC) 1907/2006.

Additional Labelling Requirements:

Addition safety Information:	To avoid risks to human health and the environment, comply with the instructions for use. Use bait containers clearly marked “poison” at all surface baiting points. Remove all remains of bait, dead rodents during and after treatment and dispose of safely. Apply only in positions inaccessible to children and pets.
Special labelling provisions for Ireland:	Use Biocides Safely and Sustainably (IE/BPA 70027) Not For Amateur Sale It is illegal to use this product for uses or in a manner other than that prescribed on this label.
If a separate leaflet is attached to or supplied with the product, add the following information to the front label:	Read attached instructions before use

2.3. Packaging

The packaging details for the biocidal product, Ruby Grain, are outlined below for amateur and professional users.

Nomenclature: PP = polypropylene, PS = polystyrene, PE = polyethylene, HDPE = high-density polyethylene, PVC = polyvinylchloride

Amateur product packaging:

Container description:	Sachets and sachets (with standing pouch)		
Pack size(s):	100g	250g	500g
Baits/sachets per pack:	Loose bait	Loose bait	Loose bait
Pack dimensions (LxWxH):	150x60x190	150x60x190	180x100x260
Packaging materials:	PE (with PE + Aluminium standing pouch)		
Ready-to-use (yes/no)	Yes		
Shelf-life:	4 years		
Conditions of storage:	Store in dry, cool area. Store in tightly closed packings. Keep in original containers. Store away from damp or wet conditions. Keep away from children.		

Container description:	Can container		Pot container	Bucket container	
Pack size(s):	400g	500g	~~1kg~~	~~1kg~~	~~2.5kg~~
Baits/sachets per pack:	Loose bait	Loose bait	~~Loose bait~~	~~Loose bait~~ ~~10x100g~~ ~~20x50g~~ ~~40x25g~~	~~Loose bait~~ ~~25x100g~~ ~~50x50g~~ ~~100x25g~~
Pack dimensions (LxWxH):	120x70x140	120x70x140	~~110x110x200~~ ~~180x70x260~~	~~240x170x130~~	~~290x200x210~~
Packaging materials:	PE		PET or HDPE	PP or PE	
Ready-to-use (yes/no)	Yes				
Shelf-life:	4 years				
Conditions of storage:	Store in dry, cool area. Store in tightly closed packings. Keep in original containers. Store away from damp or wet conditions. Keep away from children.				

Container description:	Box container			
Pack size(s):	100g	150g	200g	500g
Baits/sachets per pack:	Loose bait 4x25g	Loose bait 3x50g 6x25g 15x10g	Loose bait 2x100g 4x50g 8x25g 16x10g	Loose bait 5x100g 10x50g 20x25g
Pack dimensions (LxWxH):	100x45x70	100x45x160 140x70x190	100x45x160 140x70x190	100x70x190 140x70x210
Packaging materials:	Cardboard			
Ready-to-use (yes/no)	Yes			
Shelf-life:	4 years			
Conditions of storage:	Store in dry, cool area. Store in tightly closed packings. Keep in original containers. Store away from damp or wet conditions. Keep away from children.			

Professional product packaging:

Container description:	Bucket container

Pack size(s):	1kg	2.5kg	5kg	10kg
Baits/sachets per pack:	Loose bait 10x100g 20x50g 40x25g	Loose bait 25x100g 50x50g 100x25g	Loose bait 50x100g 100x50g 200x25g	Loose bait 100x100g 200x50g 400x25g
Pack dimensions (LxWxH):	240x170x130	290x200x210	290x200x270	380x290x450
Packaging materials:	PE or PP			
Ready-to-use (yes/no)	Yes			
Shelf-life:	4 years			
Conditions of storage:	Store in dry, cool area. Store in tightly closed packings. Keep in original containers. Store away from damp or wet conditions. Keep away from children.			

Container description:	Box container	Bag container (with inner lining)
Pack size(s):	10kg	20kg
Baits/sachets per pack:	Loose bait 100x100g 200x50g 400x25g	Loose bait
Pack dimensions (LxWxH):	350x230x240	71x50x10
Packaging materials:	Cardboard	Paper or PE or PP (with PE inner lining)
Ready-to-use (yes/no)	Yes	
Shelf-life:	4 years	
Conditions of storage:	Store in dry, cool area. Store in tightly closed packings. Keep in original containers. Store away from damp or wet conditions. Keep away from children.	

On the basis of the packaging details presented, it is considered appropriate to limit aspects of the packaging for amateur users as a risk mitigation measure. Packaging restrictions are to be limited to pre-baited bait stations and refill packs with a maximum pack-size of 500g. Additionally, the grain bait should be supplied to the amateur market in sachets/wrapped in order to reduce exposure risks to amateur operators during application to bait stations.

Packaging details:

Pack size: IE/BPA 70003 – Maximum pack size of 500g

	Packs: 100g, 150g, 200g, 250g, 400g and 500g (in all cases the bait must be supplied in inner packs or units, each containing enough bait for one point)
	IE/BPA 70027
	Packs: 1kg, 2.5kg, 5kg, 10kg and 20kg (in all cases the bait should be supplied in inner packs or units, each containing enough bait for one point)
Container materials[23]:	Box (cardboard with PE inner lining)
	Bucket (PP or PE)
	Bag (paper with PE inner lining)
Safety features:	Covered bait stations (tamper resistant)
	Wrapped bait (sachets)

[23] PP = polypropylene, PS = polystyrene, PE = polyethylene, HDPE = high-density polyethylene, PVC = polyvinylchloride

3. Summary of the product assessment

3.1. Physical/chemical properties and analytical methods

Active substance (taken from the CAR):
Difenacoum does not exhibit hazardous physical-chemical properties. Difenacoum is a white to off-white powder (off-white to beige, technical grade). It has low vapour pressure; Henry's Law constant (1.75×10^{-6} Pa m^3 mol^{-1} or <0.046 Pa m^3 mol^{-1}) was calculated based on an estimated value of 6.7×10^{-9} Pa at 25°C or on an estimated vapour pressure of less than 5×10^{-5} Pa at 45°C. Difenacoum is a weak acid with a pKa value of 4.84 or with an estimated pKa value of 4.5+1. The water solubility is pH dependent and it increases with increasing pH. At neutral conditions the water solubility of Difenacoum is low, 1.7 mg/l (at pH 7 at 20°C), or in 0.48 mg/l (at 20°C at pH 6.5). Solubility in organic solvents tested ranged from 1 to 20 g/l. The estimated log K_{ow} value is 7.6. The experimental information available on Difenacoum suggests that it may be beyond the performance ranges of the experimental tests for log K_{ow}. The substance is thermally stable up to about 300°C or up to 250°C. No boiling point was detected before start of decomposition. Difenacoum is not highly flammable and shows no self-ignition at temperatures up to melting point, 211-215°C or 215°C, the maximum temperature in the test. Corrosiveness to containers has not been observed. Comment that we are awaiting the reactivity test to containers. Difenacoum does not show oxidising or explosive properties.

Biocidal product:
The biocidal product Ruby Grain is not explosive, oxidising or flammable and does not classify from a phys.chem. point of view. The test item is stable after storage for two years at ambient temperatures. The test item is a ready-to-use grain bait and is not intended to be added or mixed with any other product.

3.1.1. Identity related issues

The source of active substance used in the biocidal product Ruby Grain is the same source of active substance that is listed in Annex I of 98/8/EC (Pelgar International Ltd.).

Table 3.1.1: Composition of the biocidal product Ruby Grain

Component	% w/w	g/kg	Chemical name	CAS no	Function
Concentrate containing - Difenacoum 2.5% (Purity 96%, Technical 0.005 %) + other components which are identified in the confidential section	0.20 (0.005 % technical active substance)	2.00 (0.05 g/kg technical active substance)	3-(3biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphtyl)-4-hydroxycoumarin	56073-07-5	Active substance
Co-formulants	See Confidential Data and Information (Annex I)				

Note: The biocidal product Ruby Grain is not the same as the representative biocidal product accompanying the Annex I inclusion. See confidential information and data for details of composition.

3.1.2. Physical/chemical properties

The source of active substance used in the biocidal product Ruby Grain is the same source of active substance that is listed in Annex I of 98/8/EC (Pelgar International Ltd.). Pelgar International Ltd. provided a letter of access for LODI S.A for their source of active substance.

3.1.3. Physical, Chemical and Technical Properties of the Biocidal Product

Summary of the Physical and Chemical Properties of the Biocidal Product Ruby Grain

Section	Study	Method	Results	Comment	Reference
1.1	Appearance	Observation.	Appearance: Red grains. Physical state: solid		
1.1	Appearance	OPPTS 830.6302 OPPTS 830.6303 OPPTS 830.6304	Colour (Munsell code): Red (7.5 R 4/14) Physical state: grain Odour: characteristic	Carried out to GLP. Study is acceptable.	NOTOX Project 490523. "Determination of physic-chemical properties of difenacoum grain baits". Brekelmans, Ir. M.J.C. 17th September 2010.
1.2.1	Explosive properties		The absence of certain reactive groups in the structural formula of the a.s., difenacoum (CAS 56073-07-5) *{Ref: Brethrick, Handbook of Reactive Chemical Hazards, Butterworths, London 1979}*, and its oxygen balance, establish beyond reasonable doubt that difenacoum is incapable of decomposing, forming gases, or realising heat very rapidly. There are no other components in the formulation, which present any explosive properties.	The IE-CA accepts that Difenacoum was determined not to be explosive as part of the Annex I inclusion process (expert statement). IE-CA accepts the justification provided by the notifier that Ruby Grain is not explosive.	
1.2.1	Explosive properties		A reasoned statement was provided by the Notifier. Difenacoum grain bait is not explosive.	The Ref MS accepts the Notifiers justification. Difenacoum grain bait is not explosive.	NOTOX Project 490523. "Determination of physic-chemical properties of difenacoum grain baits". Brekelmans, Ir. M.J.C. 17th September 2010.

Section	Study	Method	Results	Comment	Reference
1.2.2	Oxidising properties		Neither the active substance nor the solvent present oxidising properties. Examination of the structure establishes beyond reasonable doubt that the a.s., difenacoum (CAS 56073-07-5) is incapable of reacting exothermically with a combustible material *(refer to Explosive Properties)*. There are no other components in the formulation which present any oxidising properties.	The Ref MS accepts that Difenacoum was determined not to be oxidising as part of the Annex I inclusion process. IE-CA accepts the justification provided by the notifier that Ruby Grain is not oxidising.	
1.2.2	Oxidising properties		A reasoned statement was provided by the Notifier. Difenacoum grain bait is not oxidising.	The IE-CA accepts the Notifiers justification. Difenacoum grain bait is not oxidising.	NOTOX Project 490523. "Determination of physic-chemical properties of difenacoum grain baits". Brekelmans, Ir. M.J.C. 17th September 2010.
1.3.1	Flash point		No flash point data is required for solids. See 1.3.2, Flammability below.		
1.3.2	Flammability	EEC A.10 (flammability (solids)).	Flammability: Not highly flammable. The flame of the gas burner did ignite the test substance . The test substance glowed and burned with a yellow flame and turned into a charred residue. After removal of the ignition source, the flame extinguished after 4 seconds and no propagation of combustion was observed. Performance of the main test was not	Carried out to GLP. The test substance is considered "not highly flammable". The study is acceptable.	NOTOX Project 490523. "Determination of physic-chemical properties of difenacoum grain baits". Brekelmans, Ir. M.J.C. 17th September 2010.

Section	Study	Method	Results	Comment	Reference
			required.		
1.3.3	Auto-flammability	EEC A.16 (relative self-ignition temperature for solids)	The test item is considered not self-ignitable.	Carried out to GLP. The test item is considered not self-ignitable. The study is acceptable.	NOTOX Project 490523. "Determination of physic-chemical properties of difenacoum grain baits". Brekelmans, Ir. M.J.C. 17th September 2010.
1.4.1	Free acidity/ Alkalinity			Not applicable. The product is a ready to use bait which is a solid Grain at ambient temperature.	
1.4.1	Free acidity/ Alkalinity		The determination of acidity or alkalinity is required if the pH of the 1% (w/v) aqueous test substance dispersion is <4 or >10. The pH of a 1% (w/v) aqueous test substance solution was determined during NOTOX project 490525 to be 6.7. Therefore since this pH was within the pH range 4-10 the acidity/alkalinity test was not required and thus not performed.	IE-CA agrees that the acidity/alkalinity test is not required.	NOTOX Project 490523. "Determination of physic-chemical properties of difenacoum grain baits". Brekelmans, Ir. M.J.C. 17th September 2010.
1.4.2	pH (1 %)			See 1.7.1a below.	
1.5.1	Viscosity			Not applicable. The product is a grain bait.	
1.5.2	Surface tension			Not applicable. The product is a grain bait.	
1.6	Density	CIPAC MT 159 (CIPAC	Pour density: 0.735 ± 0.003 g/ml Tap density: 0.773 ± 0.001 g/ml	Carried out to GLP. The study is acceptable.	Report No.: 09-902018-001. Ferron, Nadège.

Section	Study	Method	Results	Comment	Reference
		Handbook F – 1994)			26th March 2010.
1.6	Density	CIPAC MT 186 (bulk density)	Pour density: 0.7 g/ml Tap density: 0.76 g/ml	Carried out to GLP. The study is acceptable.	NOTOX Project 490523. "Determination of physic-chemical properties of difenacoum grain baits". Brekelmans, Ir. M.J.C. 17th September 2010.
1.7.1a	Storage stability (accelerated storage 2 weeks at 54°C)	CIPAC MT 46.3 FAO SANCO/3030/99 (a.i content) OPPTS 830.6302 (colour, Munsell code) OPPTS 830.6303 (physical state) OPPTS 830.6304 (odour) CIPAC MT 75.3 (pH (1%)) CIPAC MT 178 (Attrition resistance)	**Difenacoum content (g/kg):** Before storage: 0.0411 After storage: 0.0413 **Appearance:** Before: Red (7.5R 4/14), grain, characteristic odour After: Red (7.5R 4/14), grain, characteristic odour **pH (1% in water):** Before: 6.7 After: 6.1 **Attrition resistance:** Before: 100% After: 100%	Carried out to GLP. The results are acceptable. The test substance is dust free before and after storage. Difenacoum Grain Bait is stable after 14 days storage at 54°C, which is equivalent to 2 years at ambient temperatures. Storage of the container and the reactivity with the product needs to be mentioned?	NOTOX Project 490525. "Determination of the accelerated storage stability of difenacoum grain baits by heating". Brekelmans, Ir. M.J.C. 17th September 2010.

<table>
<tr><th>Section</th><th>Study</th><th>Method</th><th>Results</th><th>Comment</th><th>Reference</th></tr>
<tr><td>1.7.1b</td><td>Storage stability
(accelerated storage
5 weeks at 54^{o}C and 20 weeks at 40^{o}C)</td><td>CIPAC MT 46.3
GIFAP Monograph no. 17.</td><td>The study examined the Difenacoum content before and after accelerated storage for three different products (paste, Grain and cereals). Only the Difenacoum cereals (0.005%) results are given below:

<table><tr><th>Weeks at 54^{o}C</th><th>0</th><th>2</th><th>3</th><th>4</th><th>5</th></tr><tr><td>Agent conc. in ppm</td><td>48.8</td><td>35.7</td><td>27.6</td><td>14.6</td><td>13.3</td></tr><tr><td>Deviation from the declared value</td><td>- 2.4%</td><td>- 28.6%</td><td>- 44.8%</td><td>- 70.8%</td><td>- 73.4%</td></tr><tr><td>Min. Tolerance in ppm</td><td>37.5</td><td>37.5</td><td>37.5</td><td>37.5</td><td>37.5</td></tr></table>
The loss of agent was greater than 25% during the 5 weeks storage at 54^{o}C, therefore it was decided to age the sample more slowly i.e. 20 weeks at 40^{o}C (see below).</td><td>The rat poison is considered stable when less than 25% agent or AS?? breakdown is observed.

The product is unstable when stored for 5 weeks at 54^{o}C as indicated by loss of agent of ≥ 25% over the duration of the test. It was decided to age the sample more slowly i.e. 20 weeks at 40^{o}C.

The sample was stable during 16 weeks at 40^{o}C. The results show that the test item would be stable for 2 years at ambient temperatures. The study is acceptable.</td><td>Biannic, Marie-Laure. LODI-Group. 7th January 2008.</td></tr>
<tr><td>1.7.1</td><td>Storage stability</td><td></td><td></td><td></td><td></td></tr>
</table>

<table>
<tr><th>Section</th><th>Study</th><th>Method</th><th>Results</th><th>Comment</th><th>Reference</th></tr>
<tr><td></td><td>(accelerated storage
5 weeks at 54^{o}C and 20 weeks at 40^{o}C)
continued</td><td></td><td>
<table>
<tr><th>Weeks at 40^{o}C</th><th>0</th><th>8</th><th>12</th><th>16</th><th>20</th></tr>
<tr><td>Agent AS conc. in ppm</td><td>50</td><td>40.2</td><td>40.2</td><td>37.5</td><td>33.6</td></tr>
<tr><td>Deviation from the declared value</td><td>0%</td><td>-19.6%</td><td>-19.6%</td><td>-25%</td><td>-32.8%</td></tr>
<tr><td>Min. Tolerance in ppm</td><td>37.5</td><td>37.5</td><td>37.5</td><td>37.5</td><td>37.5</td></tr>
</table>

The sample was stable during 16 weeks at 40^{o}C, which would indicate that the test item would be stable for 2 years at ambient temperatures.</td><td></td><td></td></tr>
<tr><td>1.7.2</td><td>Shelf life (storage for two years at ambient temperatures)</td><td></td><td>The study examined the stability of Difenacoum in the test item for three different products (paste, block and cereals). Only the Difenacoum cereals (0.005%) results are given below:</td><td>The rat poison is considered stable when less than 25% agent breakdown is observed. The test item was stable for two years at ambient temperatures.
The study is acceptable.</td><td>Biannic, Marie-Laure. LODI-Group. 12th November 2009.</td></tr>
</table>

<table>
<tr><th>Section</th><th>Study</th><th>Method</th><th>Results</th><th>Comment</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><th>Time</th><th>0</th><th>6 months</th><th>2 years</th></tr>
<tr><td>Agent AS conc. In ppm</td><td>48.8</td><td>47.3</td><td>38.2</td></tr>
<tr><td>Deviation from the declared value</td><td>-2.40%</td><td>-3.07%</td><td>-21.72%</td></tr>
<tr><td>Min. Tolerance in ppm.</td><td>37.5</td><td>37.5</td><td>37.5</td></tr>
</table>

The test item was stable for two years at ambient temperatures.</td><td></td><td></td></tr>
<tr><td>1.7.3</td><td>Packaging stability (20^{o}C)</td><td></td><td>
PP Bucket (individual PP sachet):
<table>
<tr><th rowspan="2"></th><th colspan="3">Weight</th></tr>
<tr><th>Bucket (g)</th><th>Test item (g)</th><th>Total (g)</th></tr>
<tr><td>T_0</td><td>53.100</td><td>131.66</td><td>184.76</td></tr>
<tr><td>$T_{6months}$</td><td>53.111</td><td>130.51</td><td>183.62</td></tr>
<tr><td>Deviation</td><td>+0.02%</td><td>-0.87%</td><td>-0.62%</td></tr>
<tr><td>T_{1year}</td><td>53.125</td><td>129.76</td><td>182.89</td></tr>
<tr><td>Deviation</td><td>+0.05%</td><td>-1.44%</td><td>-1.01%</td></tr>
<tr><td>$T_{18\ months}$</td><td>53.118</td><td>129.72</td><td>182.85</td></tr>
<tr><td>Deviation</td><td>+0.03%</td><td>-1.47%</td><td>-1.03%</td></tr>
</table>
</td><td>Carried out to GLP.
The deviation weights (packaging weights and test item weights) after 2 years at 20 ± 2^{o}C are lower than 5% for all the packaging. Moreover, no significant changes were observed on the packaging and on the test item.
The packaging is stable for 2 years at ambient temperature.
The results are acceptable.</td><td>Richerioux, Sandra. LODI-Group. 15th April 2016. “Compatibility between difenacoum grain bait and packagings after 3 years of storage at 20^{o}C”</td></tr>
</table>

Section	Study	Method	Results	Comment	Reference
				Note: The results for the 3-year time point have not been submitted as the study is still on-going.	

T_{2years}	53.121	129.58	182.70
Deviation	+0.04%	-1.58%	-1.11%

T_0 = White opaque bucket with smooth, dry and clean internal wall. Red wheat slightly dusty in individual PP sachet sealed on both side and on the centre.

$T_{6months}$ = White opaque bucket with smooth and clean internal wall. Red wheat slightly dusty. Individual properly sealed PP sachet. No hole.

T_{1year} = White opaque bucket with smooth and clean internal wall. Red wheat slightly dusty. Individual properly sealed PP sachet. No hole.

$T_{18\ months}$ = White opaque bucket with smooth and clean internal wall. Red wheat slightly dusty. Individual properly sealed PP sachet. No hole.

$T_{2\ years}$ = White opaque bucket with smooth and clean internal wall. Red wheat slightly dusty. Individual properly sealed PP sachet. No hole.

PP Bucket (individual PE sachet):

	Weight		
	Bucket (g)	**Test item (g)**	**Total (g)**
T_0	53.393	128.68	182.07
$T_{6months}$	53.410	128.37	181.78
Deviation	+0.03%	-0.24%	-0.16%
T_{1year}	53.438	128.16	181.59
Deviation	+0.08%	-0.40%	-0.26%
$T_{18\ months}$	53.412	128.01	181.42
Deviation	+0.04%	-0.52%	-0.36%
T_{2years}	53.413	127.74	181.15
Deviation	+0.04%	-0.73%	-0.51%

Section	Study	Method	Results	Comment	Reference
			T_0 = White opaque bucket with smooth, dry and clean internal wall. Red wheat slightly dusty in individual PE sachet sealed on both side and on the centre. Presence of dust in weld. $T_{6months}$ = White opaque bucket with smooth, dry and clean internal wall. Red wheat slightly dusty in individual PE sachet. No hole. Welds intact. Presence of dust in weld. T_{1year} = White opaque bucket with smooth, dry and clean internal wall. Red wheat slightly dusty in individual PE sachet. No hole. Welds intact. Presence of dust in weld. $T_{18\ months}$ = White opaque bucket with smooth and clean internal wall. Red wheat slightly dusty in individual PE sachet. Properly sealed sachet, no hole. Presence of dust in weld. $T_{2\ years}$ = White opaque bucket with smooth and clean internal wall. Red wheat slightly dusty in individual PE sachet. Properly sealed sachet, no hole. Presence of dust in weld. **Cardboard box (individual PP sachet):** **Weight** **Cardboard box (g)** / **Test item (g)** / **Total (g)** T_0: 49.621 / 101.97 / 151.59 $T_{6months}$: 50.379 / 101.05 / 151.43 Deviation: +1.53% / -0.90% / -0.11% T_{1year}: 49.462 / 99.591 / 149.05 Deviation: -0.32% / -2.33% / -1.68% $T_{18\ months}$: 50.143 / 99.682 / 149.83 Deviation: +1.05% / -2.24% / -1.16% T_{2years}: 49.412 / 98.942 / 148.36 Deviation: -0.42% / -2.97% / -2.13%		

<table>
<tr><th>Section</th><th>Study</th><th>Method</th><th>Results</th><th>Comment</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
T_0 = Grey, clean and dry internal wall. Red wheat slightly dusty in 100g individual PP sachet, sealed on both side and on the centre.

$T_{6months}$ = Grey, clean and dry internal wall. Red wheat slightly dusty in sealed individual sachet. No hole in the sachet or welds.

T_{1year} = Grey, clean and dry internal wall. Red wheat slightly dusty in sealed individual sachet. No hole in the sachet or welds.

$T_{18\ months}$ = Grey, clean and dry internal wall. Red wheat slightly dusty in individual PP sachet. Properly sealed sachet. No hole in the sachet or welds.

$T_{2\ years}$ = Grey, clean and dry internal wall. Red wheat slightly dusty in individual PP sachet. Properly sealed sachet. No hole in the sachet or welds.

Cardboard box (individual PE sachet):
<table>
<tr><th rowspan="2"></th><th colspan="3">Weight</th></tr>
<tr><th>Cardboard box (g)</th><th>Test item (g)</th><th>Total (g)</th></tr>
<tr><td>T_0</td><td>42.890</td><td>103.30</td><td>146.20</td></tr>
<tr><td>$T_{6months}$</td><td>43.391</td><td>102.43</td><td>145.82</td></tr>
<tr><td>Deviation</td><td>+1.17%</td><td>-0.84%</td><td>-0.26%</td></tr>
<tr><td>T_{1year}</td><td>42.610</td><td>100.98</td><td>143.58</td></tr>
<tr><td>Deviation</td><td>-0.65%</td><td>-2.25%</td><td>-1.79%</td></tr>
<tr><td>$T_{18\ months}$</td><td>43.188</td><td>101.16</td><td>144.35</td></tr>
<tr><td>Deviation</td><td>+0.69%</td><td>-2.07%</td><td>-1.27%</td></tr>
<tr><td>T_{2years}</td><td>42.555</td><td>100.47</td><td>143.02</td></tr>
<tr><td>Deviation</td><td>-0.78%</td><td>-2.74%</td><td>-2.18%</td></tr>
</table>
T_0 = Grey, clean and dry internal wall. Red wheat slightly dusty in 100g individual PE sachet, sealed on both side and on the centre.
</td><td></td><td></td></tr>
</table>

<table>
<tr><th>Section</th><th>Study</th><th>Method</th><th>Results</th><th>Comment</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
$T_{6months}$ = Grey, clean and dry internal wall. Red wheat slightly dusty in sealed individual sachet. No hole in the sachet or welds.

T_{1year} = Grey, clean and dry internal wall. Red wheat slightly dusty in individual PE sachet. No hole in the sachet or welds.

$T_{18\ months}$ = Grey, clean and dry internal wall. Red wheat slightly dusty. Properly sealed PE sachet. No hole in the sachet or welds.

$T_{2\ years}$ = Grey, clean and dry internal wall. Red wheat slightly dusty. Properly sealed PE sachet. No hole in the sachet or welds.

HDPE drum:
<table>
<tr><th></th><th colspan="3">Weight</th></tr>
<tr><th></th><th>Drum (g)</th><th>Test item (g)</th><th>Total (g)</th></tr>
<tr><td>T_0</td><td>54.612*</td><td>346.03</td><td>400.64</td></tr>
<tr><td>$T_{6months}$</td><td>54.649*</td><td>345.85</td><td>400.50</td></tr>
<tr><td>Deviation</td><td>+0.07%</td><td>-0.05%</td><td>-0.03%</td></tr>
<tr><td>T_{1year}</td><td>54.663*</td><td>345.56</td><td>400.25</td></tr>
<tr><td>Deviation</td><td>+0.09%</td><td>-0.14%</td><td>-0.10%</td></tr>
<tr><td>$T_{18\ months}$</td><td>54.651*</td><td>345.33</td><td>400.01</td></tr>
<tr><td>Deviation</td><td>+0.07%</td><td>-0.20%</td><td>-0.16%</td></tr>
<tr><td>T_{2years}</td><td>54.657</td><td>345.11</td><td>399.74</td></tr>
<tr><td>Deviation</td><td>+0.08%</td><td>-0.27%</td><td>-0.22%</td></tr>
<tr><td colspan="4">* With stopper</td></tr>
</table>
T_0 = White and opaque drum. Interior clean and dry. Red wheat slightly dusty.

$T_{6months}$ = White and opaque drum. Presence of dust on internal wall of the drum. Red wheat slightly dusty.

T_{1year} = White and opaque drum. Presence of dust on
</td><td></td><td></td></tr>
</table>

<table>
<tr><th>Section</th><th>Study</th><th>Method</th><th>Results</th><th>Comment</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
internal wall of the drum. Red wheat slightly dusty.
$T_{18\ months}$ = White and opaque drum. Presence of dust on internal wall of the drum. Red wheat slightly dusty.
$T_{2\ years}$ = White and opaque drum. Presence of dust on internal wall of the drum. Red wheat slightly dusty.

PP bucket:
<table>
<tr><th rowspan="2"></th><th colspan="3">Weight</th></tr>
<tr><th>Bucket (g)</th><th>Test item (g)</th><th>Total (g)</th></tr>
<tr><td>T_0</td><td>53.055*</td><td>250.82</td><td>303.89</td></tr>
<tr><td>$T_{6months}$</td><td>53.106*</td><td>250.62</td><td>303.75</td></tr>
<tr><td>Deviation</td><td>+0.10%</td><td>-0.08%</td><td>-0.05%</td></tr>
<tr><td>T_{1year}</td><td>53.130*</td><td>249.90</td><td>303.19</td></tr>
<tr><td>Deviation</td><td>+0.14%</td><td>-0.37%</td><td>-0.23%</td></tr>
<tr><td>$T_{18\ months}$</td><td>53.099*</td><td>249.32</td><td>302.43</td></tr>
<tr><td>Deviation</td><td>+0.08%</td><td>-0.60%</td><td>-0.48%</td></tr>
<tr><td>T_{2years}</td><td>53.085</td><td>248.57</td><td>301.66</td></tr>
<tr><td>Deviation</td><td>+0.06%</td><td>-0.90%</td><td>-0.73%</td></tr>
<tr><td colspan="4">* With stopper</td></tr>
</table>
T_0 = White and opaque bucket. Smooth, clean and dry internal wall. Red wheat slightly dusty.
$T_{6months}$ = White and opaque bucket. Presence of dust on internal wall of bucket. Red wheat slightly dusty.
T_{1year} = White and opaque bucket. Presence of dust on internal wall of bucket. Red wheat slightly dusty.
$T_{18\ months}$ = White and opaque bucket. Presence of dust on internal wall of bucket. Red wheat slightly dusty.
$T_{2\ years}$ = White and opaque bucket. Presence of dust on internal wall of bucket. Red wheat slightly dusty.
</td><td></td><td></td></tr>
</table>

Section	Study	Method	Results	Comment	Reference
1.8.1	Wettability			Not applicable. The product is a ready to use grain bait. This is only required for solid preparations.	
1.8.2	Persistent foaming			Not applicable. The product is a grain.	
1.8.3.1	Suspensibility			Not applicable. The product is a ready to use grain bait. This is only required for solid preparations.	
1.8.3.2	Dispersibility			Not applicable. The product is a ready to use grain bait.	
1.8.4	Wet/dry sieving test			Not applicable. The product is a ready to use grain bait. This is only required for WPs, SCs, granules and tablets.	
1.8.5	Particle size distribution			Not applicable. The product is a ready to use grain bait. This is only required for powders and granules.	
1.8.6	Water content			Not applicable. The product is a ready to use grain bait.	
1.8.7	Emulsion stability			Not applicable, the product is a	

Section	Study	Method	Results	Comment	Reference
				grain. This is only required for ECs and ready to use emulsions.	
1.8.8	Flowability, pourability and dustability ??			Not applicable, the product is a ready to use grain bait.	
1.9	Physical compatibility			Not applicable. The product is a ready to use grain bait and is not intended to be added or mixed with any other product.	

Conclusion:

The biocidal product Ruby Grain is not explosive, oxidising or flammable and does not classify from a phys/chem. aspect. The test item is stable after storage for two years at ambient temperatures. The test item is ready-to-use grain bait and is not intended to be added or mixed with any other product.

Compatibility with packaging material:

The test item is compatible with the following packaging for two years at ambient temperatures (20^{o}C):

PP bucket (individual PP sachet)

PP bucket (individual PE sachet)

Cardboard box (individual PP sachet)

Cardboard box (individual PE sachet)

HDPE drum

PP bucket

Data requirements:

None.

3.1.4. Analytical methods

Ruby Grain was not assessed as part of the Annex I inclusion process therefore the Notifer has submitted the following methods of analysis to cover the outstanding data gaps.

Table 3.1.4.1:

<table>
<tr><td>Report:</td><td>09-902018-003</td></tr>
<tr><td>Title:</td><td>“Analytical method validation for the determination of difenacoum in Difenacoum Grain Bait”</td></tr>
<tr><td>Author(s):</td><td>Ricau, Hélène</td></tr>
<tr><td>Date:</td><td>19th October 2009</td></tr>
<tr><td>GLP: Yes/No</td><td>Yes.</td></tr>
<tr><td>Principle of the Method:</td><td>CIPAC/3807R.
After a methanol dilution and heating under reflux for 90 minutes, the extract was filtered and diluted again in methanol and acetonitrile. Difenacoum was quantified by liquid chromatography using a reverse phase column and a UV detector (310 nm).</td></tr>
<tr><td>Linearity:</td><td>Refer to the linearity data presented in Table 3.1.4.2.</td></tr>
<tr><td>Precision/repeatability:</td><td>Refer to the precision/repeatability data presented in Table 3.1.4.2.</td></tr>
<tr><td>Accuracy:</td><td>Accuracy determination at the 100% level:
<table>
<tr><th>Item solutions</th><th>Reconstituted (mg/l)</th><th>Conc. found (mg/l)</th><th>Recovery (%)</th></tr>
<tr><td>Exact 1 100%</td><td>0.92</td><td>1.02</td><td>111</td></tr>
<tr><td>Exact 1 100%</td><td>0.92</td><td>1.01</td><td></td></tr>
<tr><td>Exact 2 100%</td><td>0.92</td><td>1.02</td><td>111</td></tr>
<tr><td>Exact 2 100%</td><td>0.92</td><td>1.02</td><td></td></tr>
</table>
Accuracy determination at the 50% level:
<table>
<tr><th>Item solutions</th><th>Reconstituted (mg/l)</th><th>Conc. Found (mg/l)</th><th>Recovery (%)</th></tr>
<tr><td>Exact 1 50%</td><td>0.46</td><td>0.47</td><td>103</td></tr>
<tr><td>Exact 1 50%</td><td>0.46</td><td>0.47</td><td></td></tr>
<tr><td>Exact 2 50%</td><td>0.46</td><td>0.49</td><td>106</td></tr>
<tr><td>Exact 2 50%</td><td>0.46</td><td>0.48</td><td></td></tr>
</table></td></tr>
<tr><td>Specificity/Interferences</td><td>To define the specificity of the analytical method, blank solvent, blank formulation, reference item and the test item were analysed. No peak was observed in the blank solvent or in the blank formulation. The peak at a retention time around 3.24min represented difenacoum in both the reference item and test item. No other peak was found in either the reference item or test item, therefore the method is specific.</td></tr>
<tr><td>Limit of quantification:</td><td>-</td></tr>
</table>

Conclusion:

No information on the linearity or precision of the method was given in this study. However, the linearity data presented in method 05-912011-001 (see Table 3.1.4.2) was carried out with standard solutions using the same analytical method and therefore covers the data gap for this study. The precision data in method 05-912011-001 (see Table 3.1.4.2) covers the data gap for precision in this study. The analytical method showed good specificity for the analysis of difenacoum. The accuracy results were within the range 80-120% and are acceptable.

Data requirements:

None.

Table 3.1.4.2:

Report No:	05-912011-001
Title:	"Quantification of Difenacoum 0.005% m/m in a rat poison bait"
Author(s):	Ricau, Hélène
Date:	16th June 2005
GLP: Yes/No	Yes
Guideline study:	-
Principle of the Method:	After a methanol dilution and heating under reflux for 90minutes the extract was filtered and diluted again in methanol and acetonitrile. Difenacoum was quantified by liquid chromatography using a reverse phase column and a UV detector at 310 nm. The purity of the reference standard for Difenacoum was 975 g/kg. Note: The method is the same as the method outlined in Table 3.1.4.1 above with the exception of a Whatman filter no.40 being used instead of filter no.1.
Linearity:	The response of difenacoum is linear within the range of 0.0008 mg/ml to 0.0012 mg/ml (3 concentrations analysed twice). Correlation coefficient r^2 = 1.000. A calibration plot was included and was acceptable.
Precision/repeatability:	The precision was determined by analysing six samples (in duplicate) for the content of difenacoum. The concentration of difenacoum in the test item equalled 0.005% w/w or 0.05 g/kg. The % RSD = 3.40, which is within the acceptable criteria (<20%).
Accuracy:	The accuracy was determined by analysing two samples in duplicate for the content of difenacoum. The accuracy results are between 102-105%, which is in line with current guidelines.

Sample	Content (% w/w)	Average (% w/w)	Recovery (%)
DEF05-0062B	0.0049	0.0049	102
DEF05-0062B	0.0049		
DEF05-0062C	0.0050	0.0050	105
DEF05-0062C	0.0051		

Specificity	The specificity was determined by injecting the blank solvent, the reference item and the test item. A shift of difenacoum retention time was observed in the test item due to the presence of waxy co-extracts. By comparison of the UV spectra at the level of the reference item peak (at 4.20 min) and the test item peak, it was shown that the peak at around 4.60 represents difenacoum. The retention time of difenacoum in the test item changes from about 4.60 to 4.80. No peak was observed in the blank solvent.
Active substance concentration	Two independent analysis of the test item were made.

	<table><tr><th></th><th>Difenacoum concentration (% w/w)</th><th>Average Difenacoum concentration (% w/w)</th></tr><tr><td>DEF05-0062</td><td>0.005</td><td rowspan="2">0.005</td></tr><tr><td>DEF05-0062</td><td>0.005</td></tr><tr><td>DEF05-0062A</td><td>0.005</td><td rowspan="2">0.005</td></tr><tr><td>DEF05-0062A</td><td>0.005</td></tr></table>
Limit of quantification:	-

Conclusion:

The analytical method described above (05-912011-001) was not validated for the grain bait only the block bait. However the linearity and precision information covers the data gaps in study no. 09-902018-003 (see Table 3.1.4.1 above).

Data requirements:

None.

Table 3.1.4.3:

Report:	24/2008.
Title:	"Determination of concentration of difenacoum in anticoagulant rodenticide cereal based"
Author(s):	Bucciarelli, Bruno.
Date:	18th September 2008
GLP: Yes/No	Yes.
Principle of the Method:	After mixing with extraction solution, the difenacoum extract was filtered before injection. An internal standard was used for determination and quantification. The content of difenacoum was determined by reversed-phase HPLC-UV and triphenylbenzene as internal standard (λ = 310 nm).
Linearity:	The response of Difenacoum was linear within the range of 1 mg/l to 4 mg/l (3 concentrations analysed twice). Correlation coefficient r^2 > 0.995. A calibration plot was included and was acceptable.
Precision/repeatability:	Five replicates were analysed. The RSD was <3%for 0.0025-0.01% of active ingredient.
Accuracy:	The recovery of four laboratory synthetic samples was in the range: 95% to 105% for 0.0025-0.01% of active ingredient.
Specificity/Interferences	Comparison of the chromatograms for samples 1 and 2 with the chromatograms for the calibration solutions 1 and 2 show no interfering peaks at the retention time for Difenacoum.
Limit of quantification:	-

Conclusion:

The analytical method is acceptable for the determination of difenacoum in cereal based bait.

Data requirements:

None.

Table 3.1.4.4:

Report:	Study No. LODI.17/2009
Title:	"Analytical method validation for determination of difenacoum in difenacoum bait (pasta grain and block)."
Author(s):	Magnier, Claire.
Date:	4th November 2009.
GLP: Yes/No	Yes.
Guideline:	CITAC/EURACHEM
Principle of the Method:	The test item was quantified by liquid chromatography using a reverse phase

<table>
<tr><td></td><td>column and a UV detector.
Note that no exact information on the principle of the method was provided. The company clarified that the method is similar to the principle of the method used in reports 09-902018-003 and 05-912011-001.</td></tr>
<tr><td>Linearity:</td><td>The response of difenacoum was linear over the range 80% - 120% of the test item concentration. Five measurements were made in triplicate. The correlation coefficient $r^2 > 0.99$. Calibration curves were provided and were acceptable.</td></tr>
<tr><td>Precision/repeatability:</td><td>Three solutions were prepared of a concentration C (~ 2.367 mg/l) of the product. Three injections of each solution were carried out and the RSD was calculated.
RSD <1.168</td></tr>
<tr><td>Accuracy:</td><td>The method was validated at 50%, 100% and 150% doped placebo. Three injections were carried out per solution and the average recoveries are reported below.
<table>
<tr><th></th><th>50% doped placebo</th><th>100% doped placebo</th><th>150% doped placebo</th><th>Average recovery</th></tr>
<tr><td>Whole grain</td><td>103.59%</td><td>101.30%</td><td>97.00%</td><td>100.63%</td></tr>
<tr><td>Broken grain</td><td>101.76%</td><td>103.15%</td><td>101.50%</td><td>102.14%</td></tr>
</table></td></tr>
<tr><td>Specificity:</td><td>There was no peak observed in either the whole grain or broken grain placebo or extraction solution chromatograms. An adjacent peak appeared in the stressed whole grain (R = 2.33) and broken grain (R = 2.17) but with the resolution being higher than 2, the quantification was considered acceptable.</td></tr>
<tr><td>Limit of quantification:</td><td>0.25 mg/kg (ppm)</td></tr>
<tr><td>Limit of detection:</td><td>0.05 mg/kg (ppm)</td></tr>
</table>

Conclusion:

The method is acceptable. The information provided in this study is considered extra information only, with the exception of the LOD and LOQ information.

Data requirements:

None.

3.1.5 Analytical method for the relevant impurities and isomers in the biocidal product

There are no relevant impurities or isomers in the biocidal product therefore no analytical method is required.

3.2. Efficacy of the Biocidal Product

Ruby grain is a ready-to-use rodenticide wheat grain bait containing 0.005% (w/w) difenacoum or 50 ppm difenacoum. The efficacy of the product was assessed against the proposed label claims. Both amateur and professional uses are proposed in and around buildings.

The applicant submitted new data in the form of 9 trial reports where both fresh and aged grain baits were used in both laboratory and field situations to assess the palatability and effectiveness of the product. Studies were conducted according to a variety of standards and protocols.

In the first laboratory choice test 100% control was achieved using the fresh grain bait on average 9 days after treatments were first offered (range 6-17 days). The second laboratory trial used 20 albino mice with fresh and aged bait (6 months). All mice died with the 6 month aged bait whilst 95% efficacy was achieved with the fresh grain bait. The surviving mouse appeared less sensitised to the bait. The third study used 22 albino mice in a laboratory choice test. Fresh and aged (12 month old) bait was offered. 90% and 95% efficacy was recorded for the T0 and T12 treatment respectively. A commercial aviary for exotic birds was chosen for the next study. The wild mouse population was estimated at ~100 individuals based on pre-baiting census. A 95% reduction in the population based on consumption levels was achieved after a 19-day baiting phase. A private dwelling house with a mouse infestation estimated at approximately 100 individuals was used for the next study. A 2-year old bait was used which achieved 98% efficacy after what could be considered a relatively short baiting and post-baiting monitoring period. A laboratory choice study using fresh and year old bait on mice and rats was considered in the next study conducted in Belgium. The whole grain bait achieved 75% and 90% efficacy against rats with the fresh and 12-month aged bait respectively. No comment was provided on the surviving animals. The broken gain bait achieved 100% control of mice for both the fresh and aged (12-month) baits. A commercial grain silo premises was chosen for the next study where a population of rats was estimated at ~144 individuals. After an 8 day baiting phase using fresh bait, a reduction of 93% based on pre and post-baiting census tracking was observed. An aviary for wildfowl breeding was chosen for a study on the control of brown rats with aged bait (2 years). The report confirmed that the farm contained a plentiful supply food and water with nearby harbourage for the rats. Population tracking estimated that there were ~124 rats onsite. A 98% reduction in consumption levels/efficacy was achieved after a 13 day baiting phase. A pig production building with a black rat problem (20 rats by estimate) was chosen as the final site. The reduction in the bait consumption from 270g per day in the presence of ad-lib pig feed to 25g after 16 days of baiting suggests a significant reduction in the population of the rat population in the pig shed. Neophobia, especially in roof rats, is a likely result of the reason why such a long pre-baiting period was required. The pest control operator claims that feeding and any signs of activity came to a stop soon after the 16 day recording period.

The grain bait formulation proved to be sufficiently palatable and effective against both rats and mice in the tests. Both fresh and aged baits (12 and 24 months after manufacture) also provided excellent control of the test animals with the ageing process not adversely affecting the active substance content, palatability or the effectiveness of the product. The product is concluded to be effective against brown rats, black rats and mice.

The grain bait formulation is not suitable for baiting in damp or wet conditions (i.e. sewers).

3.2.1. Function/Field of use

Main Group (MG):	3 – Pest control
Product-type (PT):	14
Function:	Rodenticide

Difenacoum is intended to be used to control rodent pests, both indoors and outdoors, in and around buildings, sewers, open areas and waste sites. The target species are brown rat (*Rattus norvegicus),* black rat (*Rattus rattus)* and house mouse (*Mus musculus/domesticus)*. Comprehensive laboratory and field data submitted for Annex I inclusion and evaluated in the CAR confirmed that difenacoum is an effective rodenticide for the control of mice and rats. In addition new data on the grain

formulation was provided in the form of laboratory and field studies to verify the proposed label claims.

Product	Codes*	Terms*	GIFAP codes
Cereals	VIII.3.1	Granular bait	AB

3.2.2. Dose/Mode of action

Ruby Grain should be placed in discrete locations within the infested area and placed in appropriate, secure, (preferably dry) tamper-proof baiting stations, bait boxes or pipe sections.

For mice: place 25g every 3 to 5 metres.
For rats: place 100g every 5 to 10 metres.
The distance has to be adapted to the infestation level.

Difenacoum is a second generation anticoagulant which prevents blood clotting in the target organisms by inhibiting regeneration of the active form of vitamin K1. Clinical signs are progressive and occur within 2-3 days after ingestion of a toxic dose, ultimately leading to death from 4-5 days later. Effects are reversible by administration of the antidote vitamin K1 which stimulates the regeneration of the clotting factors.

Anticoagulant rodenticides are vitamin K antagonists. The main site of their action is the liver, where several of the blood coagulation precursors undergo vitamin K dependent post translation processing before they are converted into the respective procoagulant zymogens. The specific point of action is thought to be the inhibition of K1 epoxide reductase. The anticoagulants accumulate and are stored in the liver until broken down. The plasma prothrombin (pro-coagulant factor II) concentration provides a suitable guide to the severity of acute intoxication and to the effectiveness and required duration of the antidoting therapy (vitamin K1).

Signs of poisoning in rodents and other mammals are those associated with an increased tendency to bleed leading ultimately to profuse haemorrhage. After feeding on bait containing the active ingredient for 2 – 3 days the animal becomes lethargic and slow moving. Signs of bleeding are often noticeable and blood may be seen around the nose and anus. As symptoms develop the animal will lose its appetite and will remain in its burrow or nest for increasingly long periods of time. Death will usually occur within 4-5 days of ingesting a lethal dose and animals often die out of sight in their nest or burrow.

The standard concentration at which difenacoum is typically used in ready for use baits is 0.005% w/w. This concentration has been standardised over the last 25 years as the optimal concentration to deliver the benefits of the active substance. Difenacoum is inherently not very palatable and at concentrations above 50 ppm there is a risk that it can be detected by the target species. Difenacoum, even at 50 ppm, is a multi-feed product and if this concentration was lower then the time to control the target population would be extended to several weeks or even months, which is unlikely to be acceptable where there is a rodent population that needs to be controlled for public health reasons. A further disadvantage of reducing the concentration is that it takes longer to accumulate a lethal dose in the target species such that moribund rodents containing residues of the anticoagulants will be active above ground over a longer period. Because of the poisoning effects of general lethargy these are likely to be the individuals targeted by predators. Maintaining and perhaps limiting the use rate at 50 ppm ensures a lethal dose is quickly ingested and death also follows quickly.

The assessment of the biocidal activity of difenacoum demonstrates that it has a sufficient level of efficacy against the target organisms in concentration of 50 mg/kg and the evaluation of the summary data provided in support of the efficacy of the accompanying product, establishes that the product may be expected to be efficacious. Difenacoum content in the representative product is 50 mg/kg.

3.2.3. Organisms to be controlled

Pest organisms to be controlled by the formulated product are animals belonging to:

- Order: Rodents (I.1).
- Family: Murids (I.1.1).

Please find the specific species in the following table:

Codes*	Specific names*	Common English Terms*
I.1.1.1	*Rattus norvegicus*	Brown rats
I.1.1.2	*Rattus rattus*	Roof rat, House rat
I.1.1.3	*Mus musculus*	House mouse

Developmental stages of target organisms to be controlled

II.1	Juveniles
II.2	Adults

*Application codes for encoding Rodenticides (PT14), edited the 16 January 2009 on website Ex-ECB, in point IVB5-0_01 of the dossier).

3.2.4. Effects on the target organisms (efficacy)

Anticoagulant rodenticides disrupt the normal blood-clotting, mechanisms, resulting in increased bleeding tendency and eventually, and profuse haemorrhage.
Signs of anticoagulant poisoning in rats and mice included lethargy, hunched posture and vain clearing in the ears. Blood around the eyes, mouth and anus, indicating internal haemorrhaging, appears prior to death.

Data requirements: None.

3.2.5. Known limitations (e.g. resistance)

Difenacoum resistant brown rats are found in limited areas of Denmark, Germany and Great Britain. Monitoring of resistance occurs only in these countries and lack of information does not necessarily mean lack of resistance in the other countries. The incidence of resistance ranges from 2 to 84%. About 5-9-fold doses are needed to kill difenacoum resistant rats. No reports have been submitted to the Rapporteur Member State about the distribution and incidence of resistance in the house mouse or black rat in Europe.

Resistance management strategies

The immediate aim of resistance management is to prevent or retard the development of resistance to a given anticoagulant while, as far as is not counterproductive, permitting its continued use. The ultimate aim is to reduce or eliminate the adverse consequences of resistance.

CropLife International has published a strategy for resistant management of rodenticides (RRAC 2003). The habitat management is addressed in the strategy in addition to chemical control. The access of rodents should be restricted by physical barriers and no food should be available for rodents. Rotation between different anticoagulants is not a reliable means of managing the anticoagulant resistance, as all anticoagulants have the same mode of action and the nature of resistance is also similar. The resistant individuals can be identified by conducting a blood clotting response (BCR) test (Gill et al. 1993, RRAC 2003). The problem with the BCR test is that it has proven difficult to standardise and it produces both false positives and negatives (Pelz et al. 2005). In order to follow the occurrence and spread of difenacoum resistance, wild rats should be continuously monitored for resistance in the rodent controlled area. The recommendations of CropLife International are quoted below.

To avoid the development of resistance in susceptible rodent populations:

- When anticoagulant rodenticide is used, ensure that all baiting points are inspected weekly and old bait replaced where necessary.
- Undertake treatment according to the label until the infestation is completely cleared.
- On completion of the treatment remove all unused baits.
- Do not use anticoagulant rodenticides as permanent baits routinely. Use permanent baits only where there is a clear and identified risk of immigration or introduction or where protection is afforded to high-risk areas.
- Monitoring of rodent activity should be undertaken using visual survey, through the use of non-toxic placebo monitors or by other effective means.
- Record details of treatment.
- Where rodent activity persists due to problems other than resistance, use alternative baits or baiting strategies, extend the baiting programme or apply alternative control techniques to eliminate the residual infestation (acute or sub-acute rodenticides, gassing or trapping).
- Ensure that complete elimination of the infestation is achieved.
- As appropriate during the rodenticide treatment, apply effective Integrated Pest Management measures (remove alternative food sources, remove water sources, remove harbourage and proof susceptible areas against rodent access).

Treatment of rodent infestations containing resistant individuals:

- Where rodent infestations containing resistant individuals are identified, immediately use an alternative anticoagulant of higher potency. If in doubt, seek expert advice on the local circumstances.
- Alternatively use an acute or sub-acute but non-anticoagulant rodenticide.
- In both cases it is essential that complete elimination of the rodent population is achieved. Where residual activity is identified apply intensive trapping to eliminate remaining rodents. Gassing or fumigation may be useful in specific situations.
- Apply thorough Integrated Pest Management procedures (environmental hygiene, proofing and exclusion).
- Do not use anticoagulant rodenticides as permanent baits as routine. Use permanent baits only where there is a clear and identified risk of immigration or introduction or where protection is afforded to high risk areas.

- Record details of treatment.

Application of area or block rodent control to eliminate resistance:

- Where individual infestations are found to be resistant or contain resistant individuals it is possible that the resistance extends further to neighbouring properties.
- Where there are indications that resistance may be more extensive than a single infestation, apply area or block control rodent programmes.
- The area under such management should extend at least to the boundaries of the area known resistance and ideally beyond.
- These programmes must be effectively coordinated and should encompass the procedures identified above.

3.2.6. Humaneness

The use of difenacoum as a rodenticide could cause suffering of vertebrate target organisms. The use of anti-coagulant rodenticides is necessary as there are at present no other viable measures available to control the rodent population in the European Union. Rodent control is needed to prevent disease transmission, contamination of food and feeding stuffs and structural damage. It is recognised that such substances do cause pain in rodents but it is considered that this is not in conflict with the requirements of Article 5.1 of Directive 98/8/EC 'to avoid unnecessary pain and suffering of vertebrates', as long as effective, but comparable less painful alternative biocidal substances or biocidal products or even non-biocidal alternatives are not available.

Experimental data on the effectiveness of the biocidal product Ruby Grain against the intended target organisms

Test substance	Test organism (s)	Test system	Test conditions	Test results, mode of action, resistance	References
BELGASOURIS, containing 0.005ppm difenacoum	Grey mice (*Mus musculus*)	Lab conditions, rodents were housed in individual cages. Test was performed on fresh product. Mice were captured in field then housed in individual cages.	Test was carried out in accordance with Guideline for the Rodenticide assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides).*	*Grain bait/ Semi field efficacy/ Mice / Fresh Product (T0)* All tested animal died, n=10. The efficacy of the product is very good because all the test animals offered the rodenticide, BELGASOURIS, died within an average of 9 days, which is a normal timing for an anticoagulant product. The efficacy is 100%.	***IIIB5-10_01*** Latteur G., 1996, CRA, Efficacy test on rodenticide product, BELGASOURIS: whole and crushed wheat, containing 0.005% of Difenacoum, against grey mice (*Mus musculus*), report 947, Unpublished
BELGASOURIS, containing 0.005ppm difenacoum	Albinos mice (*Mus musculus*)	Lab conditions, rodents were housed in individual cages	Test was carried out in accordance with Guideline for the Rodenticide	*Grain bait/ Lab/Choice test/ Mice (albinos)/ Product at T6 months* All tested animal died excepted one	***IIIB5-10_02*** Ryckel (de) B., Meeus P., 1997, CRA, Appetizing test

Test substance	Test organism (s)	Test system	Test conditions	Test results, mode of action, resistance	References
		Test was performed on different stages product: - Fresh product (T0) - 6 months (T6)	assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides).*	female, at T0. (n=20 animals at each time period). Fresh BELGASOURIS is efficient at 95%. The appetizing rate for BELGASOURIS does not decrease trough the time period of 6 months.	through different period of time, performed on BELGASOURIS, rodenticide containing 0.005% of Difenacoum, against grey mice (*Mus musculus*), report 972 (complement to rapport 947), Unpublished.
BELGASOURIS, containing 0.005ppm difenacoum	Albinos mice (*Mus musculus*)	Lab conditions, rodents were housed in individual cages Test was performed on different stages product: - Fresh product (T0) - 12 months (T12)	Test was carried out in accordance with Guideline for the Rodenticide assessment edited by Ministry for the Middle-	*Grain bait/ Lab/Choice test/ Mice (albinos)/ Product at T12 months* All tested animal died excepted:: - 2 animals at T0 (n=20animals). - 1 animal at T12 (n=20animals). Palatability of BELGASOURIS did not	***IIIB5-10_03*** De Proft M., Meeus P., 2001, CRA, Appetizing behaviour with BELGASOURIS at

Test substance	Test organism (s)	Test system	Test conditions	Test results, mode of action, resistance	References
			classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides).*	decrease after 12 months of storage at ambient temperature (20°C). The efficacy at T0 is 90% and 95% at T12 months.	different period of time, bait ready to use, containing 0.005% of Difenacoum, used in albinos mice in order to be applied against grey mice (*Mus musculus*), complement report 10.312. Unpublished
DISOURICIDE PESCE, containing 0.005ppm difenacoum	Wild grey mice *(Mus musculus)*	Field study: experiment conducted in 3 aviaries. Test was performed on: - Fresh product (T0)	The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides:	*Grain bait/ Field efficacy/ Mice /Product at T0* The wheat consumption has decreased from around 100g to around 5g, namely a 95% efficacy. Disouricide pesce is effective at 95%	***IIIB5-10_04*** -, Pest Control Assistance (PCA), Appetition and efficacy trial of « DISOURICIDE PESCE » on grey mice (*Mus*

Test substance	Test organism (s)	Test system	Test conditions	Test results, mode of action, resistance	References
			• Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. • Revised by OEPP in 1980.	against grey mice.	*musculus*), For LODI, Le Cosquer (56), 2002 Unpublished
DISOURICIDE PESCE, containing 0.005ppm difenacoum	Wild grey mice *(Mus musculus)*	Field study: experiment conducted in domestic house. Test was performed on: Fresh product (T0)	The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. • Revised by OEPP in 1980.	*Grain bait/ Field efficacy/ Mice /Product at T 2 years* The wheat consumption before treatment was around 300g, and after treatment the wheat consumption was around 5g. Therefore, Disouricide pesce is effective at 98% against grey mice, even 2 years after manufacture.	***IIIB5-10_05*** -, Pest Control Assistance (PCA), Appetition and efficacy trial of « DISOURICIDE PESCE » on grey mice (*Mus musculus*), For LODI, Mme Rigal, 56150 Baud, 2002 Unpublished
1. Difenacoum grain bait whole grain	Albinos Rats	Laboratory	Test was carried out in	*Grain bait/ Laboratory efficacy/ Mice and Rats/ Product at T0 and T12*	***IIIB5-10_06***

Test substance	Test organism (s)	Test system	Test conditions	Test results, mode of action, resistance	References
2. Difenacoum grain bait broken grain Containing 0.005ppm difenacoum	*(Rattus norvegicus)* Albinos Mice *(Mus musculus)*	Test was performed on: • Fresh product (T0) • Product stored for 12 months (T12)	accordance with the Guideline for the Rodenticide assessment edited by Ministry for the Middle-classes and Agriculture (*Lignes Directrices du Ministère des Classes Moyennes et de l'Agriculture pour l'évaluation des Rodenticides).*	Between fresh product and the 12 months aged product, acceptance loss/palatability is not significant.	De Proft M., CRA Gembloux, Study of ageing behavior of ready-to-use baits containing 0.005% of Difenacoum (effect on palatability), PART 2: Grain Bait, report number ROD 2008 11 BIO 6-Part 2: Grains baits Unpublished
DIRATICIDE, containing 0.005ppm difenacoum	Wild brown Rats *(Rattus norvegicus)*	Field study: experiment conducted in silos for cereal storage Test was performed on: • Fresh product (T0)	The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of	*Grain bait/ Field efficacy/ Rats /Product at T0* The wheat consumption decreased from around 2.1Kg to around 140g, namely a decrease of 93%. The efficacy of the product is 93%. Diraticide is efficacious against Brown rats.	***IIIB5-10_07*** -, Pest Control Assistance (PCA), Appetition and efficacy trial of « DIRATICIDE » on brown rats (*Rattus*

Test substance	Test organism (s)	Test system	Test conditions	Test results, mode of action, resistance	References
			raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. • Revised by OEPP in 1980.		*norvegicus*), For LODI, U.K.L (56), 2002 Unpublished
DIRATICIDE, containing 0.005ppm difenacoum	Wild brown Rats *(Rattus norvegicus)*	Field study: experiment conducted in 4 aviaries for wildfowl breeding Test was performed on: • Product stored during 2 years	The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. • Revised by OEPP in 1980.	*Grain bait/ Field efficacy/ Rats /Product at T 2 years* The wheat consumption has decreased from around 1,900g to around 40g, namely a decrease of 98%. Consequently, DIRATICIDE is effective at 98% on Brown Rats, even 2 years after manufacturing.	***IIIB5-10_8*** -, Pest Control Assistance (PCA), Appetition and efficacy trial of « DIRATICIDE» on brown rat (*Rattus norvegicus*), For LODI, Mr LAMOURIC Maurice, Tréviol, 56480 CLEGUEREC Baud, 2002 Unpublished

Test substance	Test organism (s)	Test system	Test conditions	Test results, mode of action, resistance	References
RACO GRAIN BAITS, containing 0.005ppm difenacoum	Black rats/ Roof rats (*Rattus rattus*)	Field: study conducted in pig stables. The experiment was conducted on fresh product.	The method used has been inspired by the French method called "method no. 002 from Biological Trials Commission (C.E.B) ", Method for practical efficacy trials of raticides: • Adopted on 1960, derived from the work of Chitty and Dotty in the 1940. • Revised by OEPP in 1980.	*Grain bait/ Field efficacy/ Roof rat /Product at T0* DIFENACOUM is said to kill rodents in 5 to 21 days. In the test the first signs of illness started after 8 days; dead rats were found after 12 days. After sixteen days there was still some activity, which ended later (unrecorded). These results are consistent with the results expected with difenacoum baits. One can conclude that RACO Grain Baits is very well suited for the control of *Rattus rattus*.	***IIIB5-10_9*** Feys J-L., Field trial with RACO GRAIN BAITS against ROOF RATS 11 November 2009_08 03 December 2009, batch PB 091109 Belgagri. Unpublished

3.3. Biocidal Product Risk Assessment (Human Health and the Environment)

3.3.1. Description of the intended use(s)

Ruby Grain is a rodenticide grain bait for the effective control of rodent species, both indoors and outdoors, in and around a variety of places including but not limited to buildings, sewers, open areas and waste dumps. Ruby Grain takes the form of ready to use grain bait, packaged in ready to use bags of 25 and 100g, containing 0.005% w/w (50 ppm) difenacoum, a second generation 4-hydroxy coumarin or superwafarin anticoagulant, which causes death due to massive internal haemorrhages after several days of ingestion as a consequence of an accumulated lethal dose. The target species are brown rat (*Rattus norvegicus*), black rat (*Rattus rattus*) and house mouse (*Musmusculus / domesticus*). Other than the active ingredient, the product is composed of food-grade materials forming a grain bait base. The grain is dyed red to make it unattractive to wildlife, birds in particular.

3.3.2. Hazard Assessment for Human Health

No new exposure studies have been submitted for evaluation. Signs of poisoning in rodents and other mammals are those associated with an increased tendency to bleed, leading ultimately to profuse haemorrhage. Non-target organisms are most at risk from secondary poisoning, i.e. consumption of rodent carcasses by predators such as raptors. Difenacoum is highly lipid soluble and persists with a long half life once ingested. This is in contrast to warfarin and is a characteristic of some of the second generation 4-hydroxy coumarin derivatives that makes them particularly hazardous with repeated exposure because of their ability to bioaccumulate and display very prolonged anticoagulant activity in exposed mammals including humans.

3.3.2.1. Toxicology of the active substance

The toxicology of the active substance was examined extensively according to standard requirements. The results of this toxicological assessment can be found in the CAR for difenacoum prepared by the Rapporteur Member State Finland. The threshold limits and labelling regarding human health risks listed in Annex 4 "Toxicology and metabolism" must be taken into consideration. There are no new studies post annex I, that impact on the original toxicological assessment carried out by the RMS.

Summary of acute toxicity data for the active substance Difenacoum

Parameter	Test material	Species	Result	Classification	Ref.
Acute Oral Toxicity	Difenacoum technical, 99.7 % w/w purity	Rat CRL:(WI)BR (Wistar), Female: 3/dose,(two low dose groups)	5 < LD_{50}< 50 mg/kg bw	T+; R28 / Acute Tox. 2; H300	[redacted] Study Code: 04/904-001P
	Acceptability (Y/N): Y		**Method**: OECD Guidelines 423 (2001)		**GLP (Y/N): Y**
	Comments: No deviations. The method used was not intended to allow the calculation of a precise LD50 value.				
Acute Dermal Toxicity	Difenacoum technical, 99.7 % w/w purity	Rat CRL:(WI)BR (Wistar), female / male: 5/sex/group	LD_{50} = 51.5 mg/kg bw (females)	T+; R27 / Acute Tox. 1; H310	[redacted] Study Code: 04/904-002P
	Acceptability (Y/N): Yes		**Method:** OECD Guidelines 402		**GLP (Y/N): Yes**
	Comments: Males and females in low dose group (20 mg/kg bw) only. Only females in the other 2 dosing groups (55 & 155 mg/kg bw). 2 out of 5 males died in the low dose group, compared with 3 out of 5 for the mid and 5 out of 5 for the top dose groups. The LD_{50} value				

Parameter	Test material	Species	Result	Classification	Ref.
	was calculated for female rats only (51.5 mg/kg bw) even though males were apparently more sensitive. Due to the overall mortality (both sexes) the risk phrase R27; Very toxic in contact with skin, was warranted by the RMS.				
Acute Inhalation Toxicity	Difenacoum technical, 97.7 % w/w purity	Rat CRL:(WI)BR (Wistar), female / male	Males: LC_{50} = 20.74µg/L/4h Females: LC_{50} = 16.27µg/L/4h	T+; R26 / Acute Tox. 2; H330	[redacted] Report no. MLS/9825
	Acceptability (Y/N): Yes		**Method:** Complies with OECD 403		**GLP (Y/N): Yes**
	Comments: Groups of 5 male and 5 female rats were exposed, nose only for a single four hour period to aerosols of difenacoum technical material. The aerosols had concentrations of 3.28, 7.52 and 20.33µg/L. Two males and four females were killed in extremis following exposure to 20.33µg/l. Clinical signs, delayed deaths and post mortem findings were consistent with anti-coagulent poisoning. Only slight signs of toxicity were seen in animals exposed to the lower concentrations. The LC_{50} value is 20.74µg/L/4h (95% confidence limits 12.03-39.76) for males and 16.27 µg/L/4h (95% confidence limits 10.03-26.24) for females.				
Acute Dermal Irritation	Difenacoum technical, 99.7 % w/w purity. Batch 03652.	Rabbit, male, NZW, 3 in total	No irritation.	none	[redacted] Study code: 04/904-006N
	Acceptability (Y/N): Yes		**Method:** Complies with OECD 404		**GLP (Y/N): Yes**
	Comments: Pure difenacoum technical was applied in a single dose of 0.5 g to the shaven skin of all experimental animals. After 4 hours test article was removed and animals were examined 1, 24, 48 and 72 hours after patch removal. No irritation symptoms (erythema and oedema) or other signs were recorded (Draize scores of 0, all time points). Difenacoum is not a skin irritant.				
Acute Eye Irritation	Difenacoum technical, 99.7 % w/w purity. Batch 03652.	Rabbit, male, NZW, 3 in total	No irritation.	none	[redacted] Study code: 04/904-005N
	Acceptability (Y/N): Yes		**Method:** OECD 405 (2002)		**GLP (Y/N): Yes**
	Comments: 0.1 g of difenacoum technical was applied to the left eye of each animal. The untreated right eye served as control. The treated eyes of the test animals were not washed out following the instillation of 0.1g of test item. The eyes were examined at 1, 24, 48, and 72 hours after application. There was no evidence of irritation by the active substance (Draize scores of 0 for 24, 48, & 72 hour time points). Difenacoum is not an eye irritant.				
Skin Sensitisation (M & K study)	Difenacoum, as a technical concentrate of the a.s. (2.6% w/v) in solvent. Batch SC7396.	Guinea Pig, (Dunkin-Hartley), male & female. Control group: 5 male, 5 female. Test group: 10 male & 10 female.	No sensitisation.	none	[redacted] Report number CIT/14302
	Acceptability (Y/N): Yes		**Method:** OECD 406		**GLP (Y/N): Yes**
	Comments: Preparation for induction; intradermal injections at day 0, a 1% (w/w) preparation of the technical concentrate in isotonic saline solution and Freund's complete adjuvant. On day 7, sodium lauryl sulphate in vaseline (10%w/w) was applied on the test site to induce local irritation. On day 8, this same test site was treated by topical application of the test substance (technical concentrate with 2.6% difenacoum w/v) or the vehicle (control group) and was covered by an occlusive dressing for 48 hours. Challenge was performed on day 22 with undiluted test substance (technical concentrate with 2.6% difenacoum w/v). Test substance and vehicle were maintained under an occlusive dressing for 24 hours. Skin reactions were evaluated at 24 and 48 hours. There were no clinical signs or mortalities during the study. No cutaneous reactions were recorded after the challenge application. Positive controls were acceptable. Dilution of a liquid sample of very low water solubility with isotonic saline solution is highly questionable.				
Skin Sensitisation (Buehler study)	Difenacoum, asa technical concentrate of the a.s. (2.6% w/v) in solvent.	Guinea Pig, (Dunkin-Hartley), male & female. Control group: 5 male, 5	No sensitisation.	none	[redacted] Report No. MLS/10009

Parameter	Test material	Species	Result	Classification	Ref.
	Batch TCP 0047/94.	female. Test group: 10 male & 10 female.			
	Acceptability (Y/N): Yes		**Method:** OECD 406		**GLP (Y/N): Yes**
	Comments: On day 1 the test site was treated by topical application of the test substance (10 % w/v preparation of the formulation in deionised water) or the vehicle (control group) and was covered by an occlusive dressing for 6 hours. This was repeated at 7 day intervals to give a total of three 6 hour exposures over 14 days. The animals were left untreated for 14 days prior to challenge. Challenge consisted of topical application of test substance (10 % and 3% w/v preparation of the formulation in deionised water) and vehicle were maintained under an occlusive dressing for 6 hours. Skin reactions were evaluated at 24 and 48 hours. There were no clinical signs or mortalities during the study. No cutaneous reactions were recorded after the challenge application. Dilution of a liquid sample of very low water solubility with deionised water is highly questionable.				

Difenacoum is acutely very toxic by the oral and inhalation routes. Difenacoum may also be considered very toxic by the dermal route. It is not a skin or eye irritant. Difenacoum is not a skin sensitiser.

Summary of difenacoum subchronic, chronic, mutagenic and reproductive toxicity.

Repeated oral administration of difenacoum to rats in diet at doses up to 0.06 mg/kg bw/day for 90 days gave rise to increased kaolin-cephalin times and histological findings indicative of toxic effects related to anticoagulation only at the highest dose level. No other adverse effects were observed. A suggestive NOAEL value can be established at 0.03 mg/kg bw/day.

Repeated oral exposure to difenacoum results in toxic effects related to anticoagulation giving cause to concern for serious damage to health by prolonged exposure. Furthermore, based on the results of the acute dermal and inhalation toxicity studies and route-to-route extrapolation, it is justified to assume a similar concern for serious damage to health by prolonged exposure through dermal and inhalation routes also. Difenacoum classifies for repeated dose toxicity; T; R48/23/24/25, Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed.

Difenacoum was not mutagenic in bacterial cells, but the mutation frequency and chromosome aberrations were increased in mammalian cells *in vitro*. All *in vivo* genotoxicity tests were negative. It can be concluded that difenacoum does not classify as mutagenic.

Teratogenicity tests have been performed in two species. In the rabbit, the LOAEL value for maternal toxicity is 0.001 mg/kg bw/day. A higher incidence of foetal effects (skeletal variations) was observed at two dose levels compared to controls, but the incidence was not dose dependent. The NOEL/NOAEL value for developmental toxicity is 0.01 mg/kg bw/day. The NOEL/NOAEL for maternal toxicity in rats is 0.03 mg/kg bw/day. There was no evidence of embryotoxic or teratogenic potential following oral exposure of pregnant rats at 0.09 mg/kg bw/day (=NOEL/NOAEL for developmental toxicity).

Clear developmental toxicity was not observed in rabbits or rats. However, difenacoum should be considered teratogenic to humans because it contains the same chemical moiety responsible for the teratogenicity of warfarin, a known human teratogenic agent, and it has the same mode of action that is a known mechanism of teratogenicity in humans. The possible teratogenic effects of coumarin-related compounds cannot be detected using the standard OECD 414 study design, because the exposure period has to be adjusted to correspond to the critical periods in rat for the observed effects in humans. Furthermore, maternal bleeding has to be prevented, e.g. by vitamin K supplementation, to achieve a biochemical blockade of net extrahepatic vitamin K – dependent processes. Based on read across from warfarin, difenacoum is classified for reproductive toxicity, Repr. Cat. 1; R61, "May cause harm to the

unborn child". In addition, specific concentration limits have been set by the RMS due to the very high acute toxicity associated with difenacoum.

Effects on fertility have been studied in a rat multi-generation study. In this study, dose levels had to be lowered twice during the course of the study due to extensive mortality. Regardless of the very low doses, it can be concluded that difenacoum does not have clear effects on fertility. However, there were indications of disturbed oestrous cycling perhaps due to ovarian hormonal disturbances. Because the main findings related to fertility (irregular oestrous cycles in treated animals in both generations and ovarian cysts at a maternally toxic dose of 0.06 mg/kg bw/day in F0 females) did not affect the fertility index, no severe increase in post-implantation loss (increased spontaneous abortions have been associated with warfarin treatment in humans) were observed, and warfarin is not classified for fertility, it is considered that classification for fertility effects is not necessary for difenacoum. In the literature, there are no indications of adverse fertility effects associated with warfarin or vitamin K recycling blockade. It is considered that the possible effects on ovarian function are adequately covered by the risk phrase R48/23/24/25.

There are no studies on neurotoxicity. Other studies with difenacoum did not reveal any neurotoxic potential and there are no structural alerts evident for this endpoint.

Data requirements: (List if applicable)
None.

3.3.2.2. Toxicology of the biocidal product

The toxicology of the biocidal product was examined appropriately according to standard requirements. The product was not a dummy product in the EU- review program for inclusion of the active substance in Annex I of Directive 98/8/EC.

Summary of acute toxicity data for the biocidal product: Ruby Grain Bait

Parameter	Test material	Species	Result	Classification	Ref.
Acute Oral Toxicity	Difenacoum Grain bait Batch: 600300	Rat, female, Sprague-Dawley, SPF Caw, 6 in total.	LD_{50}> 2000 mg/kg bw	none.	[redacted] (2009). study [redacted]mber: TAO-423-PH-09/0087
	Acceptable (Y/N): Yes		**Method:** OECD 423 (24 April 2002)		**GLP (Y/N): Yes**
	Comments: No mortality occurred during the study at 2000mg/kg. There were no clinical signs observed. Macroscopical examination of the animals at the end of the study revealed a thickening of the corpus (5/6 animals) with presence of red spots (3/6 animals).				
Acute Dermal Toxicity	Difenacoum Grain bait. Batch: PB090209	Rat, Sprague-Dawley, SPF Caw, 5/sex.	LD_{50}> 2000 mg/kg bw	none.	[redacted] (2009). study number: TAD-PH-09/0087
	Acceptable (Y/N): Yes		**Method:** OECD 402 (24 Feb1987)		**GLP (Y/N): Yes**
	Comments: No mortality occurred during the study at 2000mg/kg.No cutaneous reactions or systemic clinical signs related to the administration of the test item were observed. Some slight pink colouration of the test site was observed.				
Acute Inhalation Toxicity	none	none	none	none	none
	Acceptable (Y/N):		**Method:**		**GLP (Y/N):Yes**
	Comments: Inhalation exposure is not appropriate for a wrapped paste formulation. Active substance has very low volatility and is only present at 0.005% (w/w) in the product. Company justification accepted.				
Information on mixture of	none	none	none	none	none
	Acceptable (Y/N): Yes		**Method:**		**GLP (Y/N):Yes**

<table>
<tr><th>Parameter</th><th>Test material</th><th>Species</th><th>Result</th><th>Classification</th><th>Ref.</th></tr>
<tr><td>biocidal products</td><td colspan="5">Not applicable since following the proposed uses of the product and the label claims, the rodenticide is not intended to be used in a mix with other biocidal products. Company justification accepted.</td></tr>
<tr><td rowspan="3">Acute Skin Irritation</td><td>Difenacoum Grain bait

Batch: LAB090209</td><td>3 female NZW rabbits.</td><td>No irritation</td><td>none</td><td>[redacted] (2009). study number:

IC-OCDE-PH-09/0087</td></tr>
<tr><td colspan="2">Acceptable (Y/N): Yes</td><td colspan="2">Method: OECD 404 (24 April 2002)</td><td>GLP (Y/N): Yes</td></tr>
<tr><td colspan="5">Comments: The test item was ground to a fine powder and applied at a dose of 0.5 g, on an undamaged skin area of one flank of each animal for 4 hours. No cutaneous reactions (erythema and oedema) were observed on the treated areas. Company report accepted. Results do not warrant classification under the conditions of the study.</td></tr>
<tr><td rowspan="3">Acute Eye Irritation</td><td>Difenacoum paste bait

Batch: 52-600300</td><td>3 male NZW rabbits.</td><td>Slight irritation</td><td>none</td><td>[redacted] (2009). study number:

IO-OCDE-PH-09/0087</td></tr>
<tr><td colspan="2">Acceptable (Y/N): Yes</td><td colspan="2">Method: OECD 405 (24 April 2002)</td><td>GLP (Y/N): Yes</td></tr>
<tr><td colspan="5">Comments: The test item was applied at a dose of 0.1 g instilled into the conjunctival sac of one eye in each animal. Ocular conjunctivae reactions observed during the study were slight to moderate and totally reversible by day 6. Company report accepted. Results do not warrant classification under the conditions of the study.
<table>
<tr><th>Animal number</th><th>A9661</th><th>A9678</th><th>A9679</th></tr>
<tr><td></td><td colspan="3">24, 48, 72 hour mean</td></tr>
<tr><td>Corneal Opacity</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Iritis</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Conjunctivae: - Redness</td><td>1.3</td><td>2.0</td><td>1.0</td></tr>
<tr><td>Chemosis</td><td>1.0</td><td>1.0</td><td>0.7</td></tr>
<tr><td></td><td colspan="3">Day 6</td></tr>
<tr><td>Conjunctivae</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td></td><td></td><td></td><td></td></tr>
</table></td></tr>
<tr><td rowspan="3">Skin Sensitisation (M&K)</td><td>Difenacoum Grain bait

Batch: 600300</td><td>Guinea Pig, female, Dunkin-Hartley strain, 5 in negative control, 11 in treated groups.</td><td>negative</td><td>none</td><td>[redacted] (2009). study number:

SMK-PH-09/0087</td></tr>
<tr><td colspan="2">Acceptable (Y/N):Yes</td><td colspan="2">Method: OECD 406 (17 July 1992)</td><td>GLP (Y/N): Yes</td></tr>
<tr><td colspan="5">Comments: The study format was a Guinea Pig maximisation method skin sensitization test. The test item was reduced to a fine powder with a coffee mill but then assessed as unsuitable for intradermal injection. . Changes made to the protocol of the GPMT included induction by topical application only. This test should have being revised and concluded as a Buehler test instead of an M&K test in order to carefully ascertain the results. In its present form it is similar to a Buehler but with too few animals in the study. Potentiation by injection of test material with Freund's Complete Adjuvant has not been performed, taking all these things into consideration the company report is rejected. Suitable positive controls were reported. In the original CAR, the applicant submitted two sensitisation studies with a 2.5% liquid concentrate of difenacoum, one Magnusson & Kligman test and one Buehler test (see Doc IIIA, CAR). The RMS concluded that the available studies (both negative) provided sufficient evidence for no sensitisation potential by the active substance. It is therefore unlikely that the product ruby wax is a skin sensitiser on the basis of its difenacoum content.</td></tr>
</table>

Conclusion:

According to the results of the toxicological studies, Ruby Grain (containing 50mg/kg difenacoum) does not classify with respect to Directive 1999/45/EC or Regulation (EC) No 1272/2008. However, safety phrases and precautionary statements are proposed by the Rapporteur. One issue that does not seem to be addressed by the acute studies above is the solubility of difenacoum in aqueous media. According to the physical / chemistry properties of the active substance, difenacoum has incredibly low water solubility (4.83 x 10^{-4} g/l at pH 6.5 or < 0.5mg per litre, 3.72 x 10^{-3} g/l at pH 8.9). This affects the amount of active substance in a dose such that between 5 – 40% of the expected amount might be present in the acute oral study for example.

Data requirements: (List if applicable)
None.

3.3.2.3. Toxicology of the co-formulants (substances of concern)

The biocidal product contains no other substances in quantities that would be of toxicological concern. The majority of these components are food grade materials and are not classified.

Summary of toxicological properties of the co-formulants in Ruby Grain Bait

3.3.3. Exposure Assessment for Human Health

The most relevant route of exposure to the active substance is the dermal route. Ruby Grain takes the form of ready to use grain bait, packaged in ready to use bags of 25 – 100g & containing 0.005% w/w (50 ppm) difenacoum.

The active substance has a low vapour pressure, therefore the potential for evaporation is low, and hence the potential for inhalation exposure is low. Inhalation exposure is generally only of concern during the formulation process where the active substance has a potential for becoming airborne when mixed with dry bait ingredients. The treated grain bait is typically of low dustiness and inhalation exposure is only likely during the decanting process for professional users. Inhalation exposure is negligible for all other activities (loading/application and cleaning) and non-professional users. The only relevant inhalation exposure is assumed to be that from the decanting of loose grain, pellets and granules due to the potential release of airborne dusts.

Any potential oral exposure will be indirect exposure via possible release to the environment. Other possible exposure scenarios include dermal contact with dead animals and accidental ingestion of poison baits by children.

In general there is very little data available for use in modelling human exposure to rodenticides. Any calculations must be viewed in the context of the use of many assumptions and extrapolations from only a few studies. The values presented for exposure assessment and risk characterisation must be viewed at best as being crude estimates.

Key Endpoints for Exposure Assessment

The key endpoints for exposure assessment are the No Observed Adverse Effect Level (NOAEL) for Margin of Exposure (MOE) estimates and the Acceptable Exposure Level (AEL). The lowest Low Observed Adverse Effect Level (LOAEL) in a repeated dose study, (developmental toxicity study in rabbits, LOAEL value for maternal toxicity is 0.001 mg/kg bw/day, Difenacoum CAR, 2009), was chosen as the basis to establish the AEL and calculate an NOAEL for MOE. Risk characterisation in the original CAR for difenacoum and in documents supplied by the notifier in support of Ruby Grain state the bioavailability of difenacoum as 68% following oral absorption of a single low dose in bile duct cannulated rats (Swan, 2006, Difenacoum – Metabolism in Rats. Report no. PLG 0005). However, a true measure of bioavailability must also consider enterohepatic circulation because it is important to consider the reabsorption of lipophilic compounds with long half-lives from the gastrointestinal tract such as difenacoum. Bioavailability may be under-estimated in this case but it is taken as 68% for the purpose of exposure assessment in this document. Details for the derivation of each endpoint are described below.

NOAEL for MOE:

LOAEL value for rabbit maternal toxicity is 0.001 mg/kg bw/day. To extrapolate from LOAEL to NOAEL an assessment factor of 2 is considered justified due to the steep dose response to acute effects such as lethality. Correction for bioavailability of 68% is applied.

$(0.001 \div 2) \times (68/100) =$ **3.4×10^{-4} mg/kg bw/day**

AEL:

LOAEL value for rabbit maternal toxicity is 0.001 mg/kg bw/day. Default assessment factors of 10 for inter-species variability and 10 for inter-individual variability are applied. Furthermore, due to the toxicological significance and uncertainty in the database, an additional safety factor of 3 for teratogenicity is used for all anticoagulant rodenticides. An additional assessment factor of 2 is supported due to concern over the higher potency of the second generation anticoagulants compared to warfarin and the much higher vulnerability of human foetuses to disturbances in vitamin K recycling and availability compared to rodents. Correction for bioavailability of 68% is applied.

$((0.001 \div (10 \times 10 \times 3)) / 2 = 1.67\times10^{-6}$ mg/kg bw/day

taking into account 68% bioavailability...

$(1.67\times10^{-6}) \times (68/100) =$ **1.13×10^{-6} mg/kg bw/day**

PPE (coverall, boots and gloves) is required as standard when the bait is used in sewage systems.

3.3.3.1. Exposure to professional users

Grain and pelleted bait is used by amateurs and professionals in and around buildings. Professionals can also use the product in sewers once it is held above the water line. For professional use, the operator is trained in the correct use of the bait, i.e. placement, number of bait points required based on the infestation rate area, the number of bait blocks per bait point and safe handling procedures. The use of PPE i.e., disposable gloves and a dust mask may be employed when decanting bait and disposable gloves may be employed when loading bait boxes and disposing of remaining bait and carcasses. However, when pelleted bait is contained within a bait trap there will be no exposure of the

operator to the product. Full PPE (coverall, boots and gloves) is required as standard when the blocks are used in sewage systems.

For rats, each bait point or box will contain up to 200g bait. A mouse bait point or box will only contain up to 40 g bait. Bait points for mice should be placed 5m apart, although this can be reduced to 2m in areas of high infestation and for rats, bait points should be 10m apart reduced to 5m apart in high infestation areas. Bait points should be checked frequently, at least every 2 to 3 days during the first 10-14 days of treatment and any carcasses removed. Operators should search for all rodent bodies in and around the baited area for disposal. Bait points should be removed, in a typical campaign, 6 weeks after initial placement. Sites should not be re-baited until a new infestation is observed.

During use, professional pest control operators will be exposed to rodenticide product during (1) decanting the product, or the mixing and loading phase, (2) loading of bait boxes/bait points, (3) post application activities including the disposal of old bait and carcasses. For the decanting process, exposure will be via the dermal and inhalation routes. For the loading, application and clean-up processes, exposure will be via the dermal route with the theoretical inhalation exposure being negligible.

Exposure calculations – professionals

The CEFIC/EBPF Rodenticides Data Development Group conducted an operator exposure study using flocoumafen (which may be considered a suitable surrogate for all other second generation anti-coagulants) to determine exposure during simulated use of rodenticide baits (*Chambers* 2004, unpublished, confidential). This study examined exposure to wax blocks and grain bait. Guidance is also taken from a confidential paper entitled "Harmonised Approach for Rodenticides" by the German Competent Authority, Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA).

The daily exposure frequency and its division between different tasks are based on a survey organised by CEFIC (and based on a questionnaire answered by selected pest control companies in several EU countries), and on an agreement between Member States on the common approach for exposure assessment and ECB guidelines (see CAR September 2009). A dermal absorption of 3% is used for all pellets and grains in all exposure calculations based on the final Assessment Report by Finland dated 17 September 2009.

Dermal Exposure during the decanting phase:

The Chambers study determined exposure from the decanting phase from the following scenario: 3kg grain bait is decanted from 25kg drums into a 10L plastic bucket (termed 1 manipulation). Decanting of 3kg portions are performed 1, 5, and 10 times. The results show an increase in exposure with increasing manipulations. The determined value is lower than that used by Finland in their exposure estimates in the CAR. The proposed value of **52.34mg (of grain bait) per decanting of 3kg grain bait** is determined to represent the dermal exposure for this manipulation. The following assessment considers both the total used amount of grain in the decanting process and the number of bait station manipulations per day.

For professional operators the potential total daily dermal exposure (assuming the previously agreed number of 63bait station loadings from TM III/10 is applied and a total of 200g bait is applied per bait station, thus requiring 12.6kg grain bait in total) from the decanting-phase is **220mg** grain product per day (i.e. 52.3mg × 12.6kg / 3kg).

Dermal Exposure during the loading and placement of bait stations:

The Chambers study determined exposure from the application phase from the following scenario: 5 operators transferred 200g of loose grain bait from a 10L bucket using a plastic scoop into a bait station, this was repeated to give a total of 1, 5 and 10 manipulations. The proposed value of **2.04mg (of grain bait) per bait station application** is determined to represent the dermal exposure for this manipulation. If we consider the total daily number of applications to 63 bait stations then this represents a total calculated daily dermal exposure of **128mg** grain product per day (i.e. 2.04mg × 63). No linear relationship was found between exposure and the handled amount of grain per bait station, therefore the value of 2.04mg per bait station application is assumed regardless of the total amount of grain bait loaded into each bait station.

Dermal Exposure during the cleaning of bait stations:

The Chambers study determined exposure from the cleaning phase from the following scenario: 5 operators emptied a loaded bait station containing 200g of grain bait, into a 10L bucket. This was repeated to give a total of 1, 5 and 10 such manipulations. The proposed value of **3.79mg (of grain bait) per bait station manipulation** is determined to represent the potential dermal exposure for this activity. If we consider the total daily number of cleaning manipulations to be done on 16 bait stations then this represents a total calculated daily dermal exposure of **60.6mg** grain product per day (i.e. 3.79mg × 16). No linear relationship was found between exposure and the handled amount of grain per bait station, therefore the value of 3.79mg per bait station cleanup is assumed regardless of the total amount of grain bait emptied from each bait station.

Inhalation Exposure:

A pilot study (*Snowdon*2003, unpublished, confidential) done previously determined the only relevant inhalation exposure occurred during the decanting of loose treated grain. Inhalation exposure measurements from the handling of grain bait during loading and cleaning phases was negligible (similar results obtained for wax blocks). Inhalation exposure is only assessed for the decanting phase.

Inhalation Exposure during the decanting of grain bait:

The Chambers study determined exposure from the decanting phase from the following scenario: 3kg grain bait is decanted from 25kg drums into a 10L plastic bucket (termed 1 manipulation). Decanting of 3kg portions are performed 1, 5, and 10 times. A statistical comparison of the inhalation data for 5 and 10 manipulations of these 3kg grain portions indicates no difference between the datasets. This implies that the inhalation exposure is similar whether 3kg, 15kg or 30kg of grain is decanted in total. The proposed 75th percentile air concentration value of **9.62mg/m^3 (of grain bait) per decanting event of grain bait** is determined to represent the inhalation exposure for this manipulation. If we consider the total daily number of 63 bait stations for loading with 200g in each, then a total of 12.6kg of treated grain is required. The results of the Chambers Study indicate that the total inhalation exposure to grain dusts will be **9.62mg/m3** air and that the time required for 5 and 10 × 3kg manipulations varied from 1 – 4 minutes. For the purposes of exposure assessment the following values are taken as defaults: total time for decanting = 5 minutes; inhalation rate = 1.25m^3/hr; inhalation absorption = 100%; operator body weight = 60kg.

The calculation of PCO (pest control operator) and amateur dermal exposure in decanting, placing and clean-up of rodenticidal grain bait stations, taking into account measured values (75th percentiles), defaults according to ECB guidelines and the common agreement on daily exposure frequencies (TM III/10, BAuA) is presented in the following table.

Pest Control Operator, No PPE:

Inhalation Exposure:	
Air concentration of dusts from the decanting phase	**9.62mg/m^3**
Exposure to dusts inhaled while decanting: (respiration 1.25m^3/hr, 5min decanting time)	9.62 mg/m^3 × (1.25m^3/hr × 5/60) = 1.002 mg
Systemic dose from inhaled dusts: (inhalation absorption 100%, bw 60kg)	(1.002 mg / 60kg) × (0.005 / 100) = **8.35×10^{-7} mg/kg**
Dermal Exposure:	
Amount of exposure to product (75th percentile) following decanting of 12.6kg treated grain.	**220 mg**
Amount of difenacoum on fingers/hands (0.005% in grain)	220 mg × (0.005 / 100) = 1.1×10^{-2} mg
Amount of exposure to product (75th percentile) during loading and placement of 63 bait stations in one day.	(2.04 mg per bait station) **128mg**
Amount of difenacoum on fingers/hands (0.005% in grain)	128 mg × (0.005 / 100) = 6.4×10^{-3} mg
Amount of exposure to product (75th percentile) during clean-up and disposal of 16 bait stations	(3.79 mg per bait station) **60.6mg**
Amount of difenacoum on fingers/hands (0.005% in grain)	60.6 mg × (0.005 / 100) = 3.0×10^{-3} mg
Total Dermal dose of product dusts per day:	(1.1×10^{-2} mg + 6.4×10^{-3} mg + 3.0×10^{-3} mg) = 2.04×10^{-2} mg
Total Dermal Systemic dose per day (difenacoum concentration 0.005%, dermal absorption 3%, bw 60 kg).	(2.04×10^{-2} mg × (3 / 100)) / 60kg = 1.02×10^{-5} mg/kg
Total Systemic Dose per day: (Inhaled dose + dermal dose)	(1.02×10^{-5}+ 8.35×10^{-7}) mg/kg = **1.105×10^{-5} mg/kgbw/day**
Expressed as a % of the AEL: AEL = 1.13×10^{-6} mg/kg bw/day	**977%**

Pest Control Operator,With PPE (gloves)

Default 10-fold reduction of exposure.	**1.105×10^{-6} mg/kg/day**
Expressed as a % of the AEL: AEL = 1.13×10^{-6} mg/kg bw/day	**98%**

Non-Trained Professional (e.g. farmer), No PPE:

Amount of exposure to product (75th percentile) during loading and placement a single bait station.	2.04 mg
Amount of difenacoum on fingers/hands (0.005% in grain)	2.04 mg ×(0.005 / 100) = 1.02× 10^{-4} mg
Systemic dose after a single manipulation: (assuming 3% dermal absorption, bw 60kg)	(1.02 × 10^{-4} mg × (3 / 100)) / 60kg = 5.1× 10^{-8} mg/kg
Amount of exposure to product (75th percentile) during clean-up of a single bait station.	3.79mg
Amount of difenacoum on fingers/hands after 1 manipulation (0.005% in grain)	3.79 mg ×(0.005 / 100) = 1.875× 10^{-4} mg
Systemic dose after a single manipulation: (assuming 3% dermal absorption, bw 60kg)	(1.875 × 10^{-4} mg × (3 / 100)) / 60kg = 9.4× 10^{-8} mg/kg
Systemic dose resulting from application of grain product to 10 bait sites plus 10 bait sites cleaned per day, no PPE (difenacoum concentration 0.005%, dermal absorption 3%, bw 60 kg). For non-trained professionals and amateurs, following the approach taken by the Finnish RMS, 10 manipulations per day are assumed in this risk assessment because non-trained-professionals (e.g. farmers) and amateurs are expected to handle much smaller amounts of baits daily, thus the exposure is at a lower level than for the pest control operators. Decanting is not taken into account for these users.	((5.1×10^{-8} mg/kg x 10) + (9.4×10^{-8} mg/kg x 10)) = **1.45×10^{-6} mg/kg/day**
<u>Expressed as a % of the AOEL:</u> AOEL = 1.13 × 10^{-6} mg/kg bw/day	**128%**

Non-Trained Professional (e.g. farmer), With PPE (gloves):

Default 10-fold reduction of exposure.	**1.45×10^{-7} mg/kg/day**
<u>Expressed as a % of the AOEL:</u> AOEL = 1.13 × 10^{-6} mg/kg bw/day	**12.8%**

3.3.3.2. Exposure to non-professional users

Description of tasks and amateur exposure to Difenacoum

Bait boxes for use by the general public may be supplied as sealed units ("so called "tea bags" of 10 g presented in bag of 250 g, and 1 kg bag) or as lockable, tamper-proof units that may be refilled by the user. Bait may be used in covered/protected bait points, rather than bait boxes, where appropriate.

Calculations for non-professional exposure are presented below, the first scenario with no exposure during application phase and the second scenario assuming that the bait boxes would have to be loaded by the user. As for the non-trained professionals, it is assumed that a non-professional user performs 10 manipulations per day. Decanting is not taken into account for these users as non-trained professionals and amateurs are assumed to use smaller packs than trained professionals, excluding the need for decanting.

Product type	Exposure scenario	PPE	Inhalation uptake	Dermal uptake
14	Non-professional (amateur)	None	Not relevant	9.4×10^{-7} mg/kg/day[1)]
14	Non- professional (amateur)	None	Not relevant	1.45×10^{-6}mg/kg/day[2)]

1) scenario 1; 2) scenario 2.

Scenario 1: No dermal contact during placing of baits due to sealed bait boxes and / or sealed bait. Potential exposure is only during clean-up. Default exposure value for cleanup is 3.79mg product per bait site, difenacoum present at a concentration of 0.005% (w/w), 60kg body mass, 3% dermal absorption value. The total value is calculated from the cleanup exposure per bait station of ((9.4×10^{-8} mg/kg) × 10). There is no safe use with Scenario 2 without gloves, consider only scenario 1 which is taken forward to risk assessment.

Scenario 2: Assuming that conventional bait boxes are loaded then the exposure is equal to that of the non-trained professional (e.g. farmer) with no PPE. This scenario does not present a safe use without gloves.

3.3.3.3. Exposure to children/workers/general public

Bait points should be covered or protected in such a way to prevent access to the bait. However, the ingestion of grain bait by infants has been assessed as a potential secondary exposure route associated with the use of difenacoum in rodenticide products. Secondary exposure is anticipated to be acute in nature. Two different scenarios of secondary exposure are available, the 'handling of dead rodents' scenario and the 'transient mouthing of poison bait' scenario. The former is excluded from the risk assessment due to unrealistic assumptions. The estimated exposure for the 'transient mouthing of poison bait' scenario is either, 2.5×10^{-2} mg/kg or 5.0×10^{-5} mg/kg, depending on the default assumptions. This results in Margin of Exposure (MOE) values of 0.01 or 6.8, respectively. It shows that infants are at significant risk for secondary exposure, i.e. there is no safe use for children.

For the 'transient mouthing of poison bait' scenario, either 5g (User Guidance) or 10 mg (TNsG, with bittering agent) of the product is assumed to be swallowed by an infant per poisoning event.

TNsG Assumptions: Transient mouthing of poison bait (10mg) containing bittering agent:
(10mg × 0.00005) / 10kg bw
=
5.0×10^{-5} mg/kg bw.

Calculated Exposure Value Relative to the calculated NOAEL for MOE:
3.4×10^{-4} / 5.0×10^{-5} = **6.8**

User Guidance Assumptions: Transient mouthing of poison bait (5000mg) treated with repellent;
(5000mg × 0.00005) / 10kg bw
=
2.5×10^{-2} mg/kg bw.

Calculated Exposure Value Relative to the calculated NOAEL for MOE:
3.4×10^{-4} / 2.5×10^{-2} = **0.01**

The RMS considered that in connection with transient mouthing of poison baits, infants are also exposed via the dermal route while handling the bait. This however is assumed to play a minor role relative to the amount that could be ingested. It is therefore not included in the overall exposure scenario.

3.3.3.4. Exposure to consumers from residues in food

Not applicable.

3.3.3.5. Overall Summary

The exposure data based on measurements in simulated use conditions are used in the following risk assessment. The measurements reveal that inhalation exposure is of minor importance compared with dermal exposure. The calculations have been made with the assumptions of rat control, and there are no separate calculations to assess exposure in mice control in which smaller bait sizes are used.

3.3.4. Risk Characterisation for Human Health

3.3.4.1. Professional users

The exposure assessment for professional pest control operators (PCOs) under reasonable worst case assumptions (12.6kg decanting, 63 loadings and 16 clean-ups/day), as presented in section 3.3.3.1, yielded a potential dermal exposure leading to a systemic dose of 1.105×10^{-5} mg/kg/day for an unprotected operator during bait handling operations. Comparison to calculated NOAEL for MOE shows that the use of rodenticide baits containing 0.005% difenacoum results in a margin of exposure of only 31.

Since pest control operators wear protective gloves by default during pest control operations, a refined assessment is conducted. The resulting margin of exposure (MOE = 308) indicates that

the use of rodenticide baits containing 0.005% difenacoum does not cause a risk for PCOs if gloves are worn.

Likewise, the exposure assessment for non-trained professionals (e.g., farmers) under reasonable worst case assumptions (10 loadings and 10 clean-ups/day), yielded a potential dermal exposure leading to a systemic dose of 1.45×10^{-6}mg/kg/day for an unprotected person. Even without PPE, the resulting margin of exposure (MOE = 234) indicates that use of rodenticide baits containing 0.005% difenacoum is not a risk at the stated exposure frequency. A refined assessment was, nevertheless, conducted since wearing of protective gloves is recommended in the instructions for use. The resulting margin of exposure (MOE = 2345) indicates a sufficient protection level for non-trained professional users when gloves are worn.

The result of the risk assessment concerning use of difenacoum in grain bait indicates that the acceptable exposure level is exceeded for trained professionals (PCOs) without PPE (gloves, face mask, coveralls) and non-trained professionals also using the product without PPE (gloves). The risk is acceptable only with the use of appropriate PPE. Exposure during manufacture of the active substance and formulation of products is beyond the scope of BPD and therefore has not been addressed.

3.3.4.2. Non-professional users

Grains are supplied either in pre-sealed bags or for professionals as loose, treated grain for use in covered/protected bait points or refillable bait boxes. An exposure assessment has been performed taking into account potential exposure both from application and post-application tasks as a worst-case scenario. In the calculations, amateurs were assumed to load 10 bait points and clean 10 bait points per day in the absence of PPE. The estimated daily systemic dose, 1.45×10^{-6} mg/kg/day, results in an MOE value of 234 showing that there is little risk to amateurs.

3.3.4.3. Children/Workers/general public

As a potential secondary exposure route, associated with the use of difenacoum in rodenticide products, ingestion of bait by infants has been assessed. Secondary exposure is anticipated to be acute in nature. The estimated exposure for the scenario, 2.5×10^{-2} mg/kg/day or 5.0×10^{-5} mg/kg/day, depending on the default assumptions, results in MOE values of 0.01 or 6.8, respectively indicating that infants are at risk of poisoning. This should be addressed by ensuring all difenacoum products targeted for amateur use are provided in sealed packs and tamper resistant bait boxes with a bittering agent. The potential exposure due to dermal contact with poisoned rodents is not included in the risk assessment because the available scenarios are unrealistic.

3.3.4.4. Consumers from residues in food

Not applicable, product is not used to treat food stuffs.

3.3.4.5. Overall Summary

The calculations presented have been made with the assumptions of rat control, and there are no separate calculations to assess exposure for mice control in which smaller bait sizes are used. Thus this is worst case.

Using both the MOE and AEL approaches for risk assessment indicates that there is a satisfactory margin between the predicted exposure and the NOAEL (LOAEL) as well as being below the threshold value for the AEL for all intended uses by trained professionals and untrained professionals wearing PPE and amateurs without PPE. The product is deemed suitable for authorisation and appropriate personal protective equipment is advised.

Secondary exposure from transient mouthing of the product exceeds the AEL reference value (1.13×10^{-6} mg/kg bw/day), both with the assumption of 0.01 g and 5 g of product ingested by infants. This is of concern. There is no margin of safety using the existing data and models. There is no safe scenario for indirect exposure if estimated according to TNsG and User Guidance. Mitigation and protection measures such as the inclusion of bittering agents and the enclosure of product in sealed bags and tamper resistant bait boxes are essential to reducing the risk of secondary exposure. Baits should not be placed where food, feeding stuffs or drinking water could be contaminated.

Workplace operation	PPE	Exposure path	Dose (mg/kg bw/day)	MOE	%AEL
Trained Professional: Decanting, placing of grain baits and clean-up	None	Dermal, hands Inhalation	1.1×10^{-5}	31	977
Trained Professional: Decanting, placing of grain baits and clean-up	Protective gloves, face mask and coveralls.	Dermal, hands Inhalation	1.1×10^{-6}	308	98
Non-Trained Professional: Placing of grain baits and clean-up	None	Dermal, hands	1.45×10^{-6}	234	128
Non-Trained Professional: Placing of grain baits and clean-up	Protective gloves	Dermal, hands	1.45×10^{-7}	2345	12.8
Amateur: Placing of grain baits and clean-up	None	Dermal, hands	9.4×10^{-7}	362	83
Secondary Exposure Transient Mouthing of bait by infants	--	Oral	5.0×10^{-5} (TNsG)	7	--
			2.5×10^{-2} (User Guidance)	0.01	--

3.3.5. Hazard Assessment for the Environment

The Finnish Competent Authority evaluated the active substance difenacoum in 2009. No further fate and behaviour studies were identified as necessary to support the authorisation of the active substance. An overview of the EU fate and behaviour and the ecotoxicology of difenacoum in the environment is presented hereunder:

Environmental fate and behaviour

Difenacoum has two stereogenic centres and thus consists of four diastereoisomers (two enantiomer pairs). The methods of analysis used in the available environmental fate and behaviour studies did not resolve the enantiomers, therefore no information is available on the rate of breakdown or transformation of the different individual enantiomers.

Difenacoum is hydrolytically stable at pH 4, 7 and 9 at 25°C (DT_{50} >1 yr). Under aqueous photolysis degradation is rapid (half-life about 8 hours or less). In the photolysis study of Activa/Pelgar two breakdown products above 10% were detected, and a proposal for the identification of structures was made. In the natural aquatic environment photodegradation is regarded to be of minor significance since surface water is normally deeper and muddier compared to conditions in laboratory studies. Therefore the aqueous photolysis metabolites were not considered in the exposure assessment.

Difenacoum has an estimated half-life of approximately 2 hours in air. Consequently, it is predicted to have a negligible effect on stratospheric ozone. Difenacoum shows no absorption in the so-called atmospheric window (800-1,200 nm) and therefore, according to the TGD on risk assessment (Part II, Section 3.7.2) is not a potential greenhouse gas.

Difenacoum is not readily or inherently biodegradable. Difenacoum degrades slowly under aerobic conditions in soil, with a measured DT_{50} of 439 days (20°C). Photolysis may contribute to the degradation in soil. No information is provided on soil metabolites in the CAR. The CA for difenacoum (FI) stated *"due to the low direct exposure and difenacoum being not ready biodegradable and probably absorbed to soil, the ecotoxicological significance of soil metabolites is regarded low".*[24]

Difenacoum has a measured pKa of 4.84 (20°C) and a water solubility that is pH dependent (range < 0.05 mg/L at pH 4 to 61 mg/L at pH 9, pH 7 value 1.7 mg/L all at 20°C). Therefore, in the environmentally relevant pH range of soils, adsorption of difenacoum would be expected to be pH dependent, with adsorption being lower in alkaline soils. No batch soil adsorption experiments were provided for difenacoum. The experimentally derived Koc (HPLC method) was considered as unreliable during the Annex I evaluation for difenacoum. A QSAR (Koc value of 1.8×10^6 (EUSES- Predominantly hydrophobic) was used in the EU exposure assessment instead of the experimentally derived value. The IE-CA notes this value is only relevant for the undissociated form of difenacoum, which will not reflect the dissociation state of difenacoum in the normal pH range of most agricultural soils. The IE-CA also notes the value of the Koc strongly influences the distribution of the active substance to water/sediment, water/sludge and water/soil. The CA for difenacoum stated they do *"..not require more data on Koc, because the significance of Koc is low when uses in sewer and in and around buildings are considered. The choice of Koc does not change the conclusions of the risk assessment. See rationale below:-The surface water PEC calculated using measured (OECD 121) Koc of 67 is appr. 10^{-5} mg/l, with PNECwater of 0.06 µg/l the risk ratio will be 0.00016*[25]*. Low Koc will give lower PECs for soil through sewage sludge and thus high Koc is the worst case. In*

24 Response to Comments from Member States and Participant on the Draft Competent Authority Report on Difenacoum of the Activa/Pelgar Brodifacoum and Difenacoum Task Force (3.7.08) 34/46

25 The Reviewer notes this is two orders of magnitude higher than the PEC specifed in the CAR (PEClocal water 2.35×10^{-7} mg/L) which was calaucated with the QSAR Koc.

direct soil exposure from bait boxes (1%) only initial PECs without degradation or further distribution have been calculated and thus the choice of Koc value does not have any impact on the soil risk from direct exposure. The same applies for indirect exposure via faeces and urine. The secondary poisoning risk through earthworm would be higher with low Koc, because of higher porewater concentrations, but there is a secondary poisoning risk also with the high Koc. The applicant does not have access to data in other dossiers."[24]

In a rat metabolism study 41-71% of the dose administered was excreted according to analysis of rat faeces and urine (7 days after single dosing, low and high dose). Four major metabolites >10 %AR were identified:

Isomers of hydroxylated difenacoum
F7 (11.3 %)
F8 (7.3 %)

Isomers of difenacoum-based structure, which formed glucuronide conjugates
F5 (12.2 %)
F6 (8.0%)

No data on the toxicity of the four major metabolites are available. The 4-hydroxy coumarin moiety is still present and thus the metabolites could be potent as anticoagulants. For the EU risk assessment the metabolites were treated collectively as one and were assumed to have the same toxicity as the parent. The IE-CA notes no PECs for metabolites are provided in the difenacoum CAR. This is presumably because it is covered by the risk assessment for difenacoum based on the assumptions stated in the CAR. To refine the EU exposure assessment for the active substance it was assumed 40% of the excreted amount in urine and faeces is metabolised and that 40 % of the administered total amount is unchanged difenacoum in faeces.[26] The IE-CA notes unchanged difenacoum was present at maximum at 2.9 %applied in faeces. Consequently, assuming that ~40% of the excreted amount in urine and faeces is metabolised is conservative.

Ecotoxicology

No further ecotoxicological studies were identified as necessary to support the authorisation of the active substance and no studies were submitted to support the authorisation of the product. Based on the environmental fate and behaviour of difenacoum, as outlined above, the environmental exposure assessment was conducted.

Difenacoum is very toxic to fish, aquatic invertebrates and algae. Toxicity to fish, the most sensitive species, is based on the inhibition of blood clotting. The mode of action in aquatic invertebrates and algae is unknown. The $PNEC_{water}$ is 0.06 µg/l based on the LC_{50} for Rainbow Trout. Difenacoum did not inhibit growth or respiration of aquatic microbes. The PNEC for sewage treatment plant (STP) micro-organisms is 480µg/l (the limit of solubility). In the absence of any ecotoxicological data for sediment-dwelling organisms, the $PNEC_{sediment}$ was calculated using the equilibrium partitioning method resulting in a value of 2.51 mg/kg (wet weight).

Exposure of soil organisms to difenacoum by direct contamination of soil may occur following use in and around buildings and waste dumps. It is also possible that soil may become exposed

26 "*40% is from the total administered radioactivity, part of the radioactivity remains in the rat (30-60%). Non-identified radioactivity in urine and faeces is minor part and individual unidentified metabolites each account for <4%"* Source: Response to Comments from Member States and Participant on the Draft Competent Authority Report on Difenacoum of the Activa/Pelgar Brodifacoum and Difenacoum Task Force (3.7.08)

following the spreading of sewage sludge from a sewage treatment plant that has been exposed to difenacoum used in sewers. Difenacoum caused no toxic effects in the acute earthworm test and a $PNEC_{soil}$ of 0.877 mg/kg wet weight was determined.

No tests on the soil micro-organisms or plants are required, because difenacoum is not expected to be particularly toxic to them on the basis of the mode of action and available data (Activated sludge, respiration inhibition test).

Difenacoum is very toxic to birds the $PNEC_{oral}$ of birds was determined to be 0.5 µg/kg food or 0.1 µg/kg bw/d. Difenacoum is also very toxic to mammals The $PNEC_{oral}$ for mammals is 7 µg/kg in food or 0.3 µg/kg bw/d. These $PNEC_{oral}$ values were used in risk characterisation of primary and secondary poisoning.

Difenacoum has a considerable bioaccumulation potential in aquatic and terrestrial organisms. One applicant submitted a fish bioconcentration test, but it was not considered as acceptable by the RMS. The waiving of fish bioconcentration test was accepted, because the test was judged not possible to perform technically, and because an estimated BCF value could be used in the risk assessment. The calculated BCFs range from 9010 (aquatic), to 477 729 (terrestrial). As outlined in the Assessment Report for Difenacoum (17-09-2009) the calculated BCFs estimate bioconcentration in the whole animal and not in the fat tissue, so BCF for difenacoum in fat tissue of the non-target vertebrates is unknown. The risk assessment indicates that accumulation of difenacoum in predators results in unacceptable effects when compared with the environmental acceptance criteria given in the Directive and TNsG on Annex I Inclusion. However, as outlined below, the proposed use of Ruby Grain, according to instructions, by professional users, should minimise the impact of such high calculated BCF values.

3.3.6. Exposure Assessment for the Environment

An overview of the environmental exposure assessment for Ruby Grain is presented in this section. Detailed calculations are provided in the Annexes accompanying this Report. Ruby Grain contains 50 mg difenacoum per kg of product and is used to control rats and mice. The proposed use of the product is indoors in warehouses and outbuildings and outdoors in and around buildings, waste dumps and open areas. The product is applied as loose grain/sachets in secured bait stations. The directions for use including minimum and maximum application rates are:

Rats: 100 g of grain bait/bait point spaced 10 m apart (5 m apart in high infestation areas). Typical treatment time 6 weeks.

Mice: 20-25 g of grain bait/bait point spaced 5 m apart (3 m apart in high infestation areas). Typical treatment time 6 weeks.

3.3.6-1. Aquatic compartment

Ruby Grain, whilst not being supported for use in sewers, was assessed in sewer systems to control rats as a worst-case situation for the STP and aquatic compartment. Consequently, exposure to the aquatic compartment occurs when sewage treatment plants make releases to water bodies. Based on worst case assumptions[27] taking the metabolism of difenacoum into account the maximum predicted environmental concentration (PEC) of the active substance for microorganisms in the STP is 5.91 x 10^{-6} mg/L. The corresponding amount in surface water is 1.55 x 10^{-7} mg/L. The maximum permissible concentration by directive 80/778/EEC (amended by 98/83/EC) of 0.1 µg/L is not exceeded in surface waters. 6.32 x 10^{-3} mg/kg wwt is predicted to occur in sediment during an emission episode. Full details of the calculations are contained in the Annexes.

Exposure of surface water to the active substance following its use in the scenario "in and around buildings" is considered negligible according to the ESD. This argumentation was also accepted for the Annex I inclusion of difenacoum.

3.3.6-2. Atmosphere

The use pattern and means by which difenacoum is deployed together with its low volatility, ensure that exposure of the atmosphere is highly unlikely. Difenacoum has an estimated half-life of approximately 2 hours in air. Consequently, it is predicted to have a negligible effect on stratospheric ozone. Difenacoum shows no absorption in the so-called atmospheric window (800-1,200 nm) and therefore, according to the TGD on risk assessment (Part II, Section 3.7.2) is not a potential greenhouse gas.

3.3.6-3. Terrestrial compartment

Exposure of soil to the active substance occurs via residues present in sewage sludge after using the product in sewers and via direct (spillages) and disperse release (deposition by urine and faeces) after the use of the product in and around buildings, open areas and waste dumps.

[27] Realistic worst-case: 21 days campaign

Day 0: 300 wax blocks, Day 7: 100 wax blocks replen. Day 14: 50 wax blocks replen. Day 21: 0 wax blocks replen

Maximum emission during 1st week: 100 blocks

Amount of product used in control operation: 30 kg

Fraction of a.i. (substance) released: 0.66. Difenacoum metabolism data taken into account.

Standard STP scenario (TGD) 200 L/day, 10,000 inhabitants

To refine the EU exposure assessment for the active substance it was assumed 40% of the excreted amount in urine and faeces is metabolised and that 40 % of administered total amount is unchanged difenacoum in faeces. This was also used in the current exposure assessment.

The Reviewer notes the ESD states *"Instead of wax blocks, a container with impregnated grains or pellets may be used. The container is like the wax block left hanging in a wire just above the bottom of the cesspools."* Consequently, the ESD sewer scenario is deemed suitable to evaluate the proposed use of Ruby Grain in sewers.

Based on worst-case assumptions of these typical usage patterns and release mechanisms, the maximum concentration in agricultural soil (averaged over 30 d) after 10 years of sludge application from STP is 2.41 x 10^{-3} mg/kg wwt. The highest concentration of difenacoum in soil from in and around buildings[28] is 0.0348 mg/kg wwt under realistic worst case conditions (200 g of product/bait point, each bait point is 5 m apart). The corresponding amount in open areas is 0.346 mg/kg wwt (200 g of product/bait point).

Based on worst case assumptions, usage patterns and release mechanisms[29], the maximum concentration in soil from applications in waste dumps is predicted to be 0.0082 mg/kg wwt.

According to the Assessment Report (17-09-2009), difenacoum is not readily or inherently biodegradable. Difenacoum degrades slowly under aerobic conditions in soil, with a measured DT_{50} of 439 days. This suggests difenacoum has the potential to accumulate in soil if applications were made in consecutive years to the same area. However, even in the unlikely event of such use soil accumulation would not be expected to pose a problem given the large margins of safety observed for the terrestrial compartment.

3.3.6-4. Groundwater

Exposure of groundwater may occur as a result of soil exposure which occurs via residues present in sewage sludge after using the bait in sewers and via direct (spillages) and disperse release (urine and faeces) after the use of the product in the scenarios in and around buildings, open areas and waste dumps. As an indication for potential groundwater levels, the concentration in porewater of agricultural soil was taken. It should be noted that this is a worst-case assumption, neglecting transformation and

28 In and around buildings (bait boxes)

Amount of product used in control operation for each bait box: 0.25 kg (ESD) and 0.2 kg, which is double the proposed amount.

Realistic worst-case: 21 day campaign

Bait stations: 10 No. of replenishments: 5 Bait stations are 5 m apart.

Fraction released due to spillage: 0.01 Fraction ingested: 0.99

Fraction released of ingested: 0.4 (Difenacoum metabolism data taken into account)

Spillage area: 0.09 m^2 (0.1 m around station) Frequented area: 550 m^2 (10 m around building)

Open areas (grain scenario)

Amount of product used at each refilling in the control operation: 200 g

Realistic worst-case: 6 day campaign Bait points: 1 No. of replenishments: 2

Fraction of product released to soil during application 0.05 Fraction of product released to soil during use 0.2

29 Waste dumps

Amount of product used in control operation per application: 44.1 kg of product No. of replenishments: 7

Fraction of active ingredient released to soil through excreta and dead bodies 0.9. Area of waste dump: 1 ha

dilution in deeper soil layers. A summary of the PECs obtained are presented in **Table 3.3.6.4-1**. All concentrations are less than the EU trigger value of 0.1 μg/L.

Table 3.3.6.4-1. Predicted Environmental Concentration (μg/L) of difenacoum in groundwater

Compartment/Scenario	ESD realistic worst case scenario	ESD realistic worst case scenario with modified input parameters	ESD normal use scenario with modified input parameters
Sewer scenario			
Groundwater/porewater	9.94×10^{-5}	7.29×10^{-5}	
In and around buildings scenario			
Groundwater/porewater	1.5×10^{-3}	1.1×10^{-3}	3.2×10^{-4}
Open areas			
Groundwater/porewater	5.23×10^{-3}	1.05×10^{-2}	---
Waste dump			
Groundwater/porewater	2.24×10^{-4}	2.5×10^{-4}*	---

*****For high infestations of rats the bait points are spaced 5 m apart. According to calculations provided by the IE-CA this could potentially result in a maximum of ~441 bait points (21, 100 m lines of 21 bait points, spaced 5 m apart) in a 1 ha area during high infestations. This corresponds to ~44.1 kg of product, which is greater than the quantity considered under realistic worst-case conditions in the ESD. Consequently the notifiers exposure calculation is not sufficient to support this use. The IE-CA generated new exposure calculations for this use

3.3.6-5 Primary and Secondary poisoning

A clear risk exists for primary and secondary poisoning in both the aquatic and terrestrial compartments for birds and mammals. The empirical risk assumes direct or indirect consumption of the deployed baits. For primary poisoning the initial PEC_{oral} values as outlined above (Section 3.3.5) assume that there is no bait avoidance by the non-target animals and that they obtain 100% of their diet in the treated area and have access to Ruby Grain. Even when avoidance and elimination are taken into account the empirical exposure levels result in unacceptable risks to birds and mammals (see ANNEX VI).
The PEC_{oral} values determined for characterising the risk of secondary poisoning to fish, earthworm and rodent eating birds and mammals is unacceptable. The values assume accumulation based on the PEC values determined for each relevant compartment. Even when avoidance and elimination are taken into account the empirical exposure levels to difenacoum from Ruby Grain result in unacceptable risks to birds and mammals (see ANNEX VI).

3.3.7. Risk Characterisation for the Environment

Ruby Grain is used in and around buildings, open areas and waste to control rats and mice. Ruby Grain, whilst not being supported for use in sewers, was assessed in sewer systems to control rats as a worst-case situation for the STP and aquatic compartment. Consequently, exposure to the aquatic compartment occurs through the STP route. Exposure of soil to the active substance occurs via residues present in sewage sludge after using grain bait in sewers and via direct (spillages) and disperse release

(deposition only by urine and faeces) after the use of the product in the scenarios in and around buildings, open areas and waste dumps. No new data related to the environment fate and behaviour or the ecotoxicology of the active substance has been submitted by the applicant. PECs were calculated in accordance with the ESD for PT14. These calculations are outlined in the previous section.

3.3.7-1 Aquatic compartment

The use of Ruby Grain containing difenacoum may lead to contamination of surface waters and sediment through sewage water and STP. Exposure of surface water to the active substance following its use in the scenario *"in and around buildings"* is considered negligible according to the ESD. The derivation of the PEC and PNEC values is outlined in ANNEX VI. The PEC values, as determined by fate and behaviour, reflect the predicted concentrations of difenacoum in water following the use of Ruby Grain in the relevant scenarios. Aquatic organisms are therefore assessed for effects of difenacoum in their environment for the relevant use scenarios. The PEC/PNEC ratios, for the realistic worst case scenarios with normal use, were less than 1 in all compartments indicating that difenacoum does not cause unacceptable risk to aquatic organisms, sediment-dwelling organisms or biological processes at the sewage treatment plant. As difenacoum is not readily biodegradable, the degradation of difenacoum in sediment is also anticipated to be low. However, according to the PEC calculations, concentrations in sediment would be low (6.32×10^{-3} mg/kg wwt), and below the level that causes unacceptable risk, thus risk for unacceptable accumulation in sediment can be regarded low.

No risk is identified to either groundwater/porewater or surface water used as drinking as in both cases the maximum permissible concentration by directive 80/778/EEC (amended by 98/83/EC) of 0.1 μg/l is not exceeded in the ESD realistic worst case scenarios for uses in sewer, in and around buildings, open areas and waste dumps.

3.3.7-2 Atmospheric compartment

The use pattern and means by which difenacoum is deployed together with its low volatility, ensure that exposure of the atmosphere is highly unlikely. Difenacoum has an estimated half-life of approximately 2 hours in air. Consequently, it is predicted to have a negligible effect on stratospheric ozone. Difenacoum shows no absorption in the so-called atmospheric window (800-1,200 nm) and therefore, according to the TGD on risk assessment (Part II, Section 3.7.2) is not a potential greenhouse gas.

3.3.7-3 Terrestrial compartment

Exposure of soil to the active substance can occur via residues present in sewage sludge and via direct (spillages) and disperse release (deposition by urine and faeces) after the use of the product in and around buildings, open areas and waste dumps. The derivation of the PEC and PNEC values is outlined in ANNEX VI. The PEC values, as determined by fate and behaviour, reflect the predicted concentration of difenacoum in soil following the use of Ruby Grain in the relevant scenarios. Terrestrial organisms are therefore assessed for effects of difenacoum in their environment for the relevant use scenarios. The PEC/PNEC ratios, for the realistic worst case scenarios with normal use, were less than 1 for all the compartments assessed: sewer, in and around buildings, open areas and waste dumps. Therefore, normal use of Ruby Grain does not cause unacceptable risk to terrestrial organisms.

3.3.7-4 Primary poisoning

Acute risk

For the acute exposure situation, no $PNEC_{oral}$ is determined and no quantitative risk characterisation is performed. Instead a qualitative assessment is done by comparing LD_{50} values to the expected concentration of the active substance in birds and mammals following their direct ingestion of Ruby Grain bait.. One day consumption of difenacoum containing baits is not assumed to kill birds and mammals with the exception of foxes. The other animals would suffer from sublethal effects, although mortality cannot be excluded. The assumption is based on the comparison of expected concentration in animals after one day exposure without elimination. The species specific sensitivity differences are not taken into account in this assumption (i.e. no assessment factor is applied to the LD_{50} values), and hence this description must not be considered as a risk characterisation.

Long-term risk

According to the ESD the comparison of concentration in the non-target animals and the $PNEC_{oral}$ describes the long-term risk for primary poisoning. The PEC values generated for the long-term risk assessment were calculated assuming direct ingestion of Ruby Grain by non-target birds and mammals. The expected concentration in the non-target animals are calculated after five days intake and elimination. The elimination is assumed to be 40%. The Step 2 assumptions are used for the calculation of the expected concentrations (see Annex VI for the calculations). The calculations show that mammals and birds would suffer long-term effects of difenacoum if they ingested Ruby Grain. Due to high food intake in relation to the body weight the birds are at considerably higher risk than mammals.

Primary poisoning incidents can be minimised by preventing the access of non-target animals, including companion animals, to the baits. Ruby Grain contains the bittering agent, denatonium benzoate, as a deterrent (0.195 % w/w) which may further reduce the risk of primary poisoning of non-target birds and mammals. It is assumed in the ESD that if the rodenticide baits are used according to the label instructions, the risk for primary poisoning is negligible. However, it may not be possible to exclude exposure of all non-target animals, as the baits have to be accessible to target rodents, they may as well be accessible to non-target mammals and birds of equal or smaller size than the target rodents.

3.3.7-5 Secondary poisoning

In the terrestrial and aquatic environments birds and mammals may be at risk of secondary poisoning if they feed on contaminated organisms following the use of Ruby Grain. The derivation of $PNEC_{oral}$ for birds and mammals is outlined in Annex VI. The derivation of PEC values for fish eating and earthworm eating birds and mammals is outlined in ANNEX VI. These values assume direct ingestion of Ruby Grain by the prey and rely on PEC values generated by environmental fate and behaviour for the relevant compartments. The risk assessment for rodent eating birds and mammals applies an estimated concentration in rodent prey based on the assumption of direct ingestion of Ruby Grain by rodents (see ANNEX VI).

Aquatic

For the aquatic food chain, the PEC/PNEC ratios exceed 1 for both fish eating birds and mammals. Despite this calculation, the risk of secondary poisoning via the aquatic food chain is considered insignificant due to low water solubility and high adsorption tendency of difenacoum. It is also assumed that mechanical screening of sewage water reduces the concentration in the recipient water, although this reduction cannot be quantified. The negligible risk of secondary poisoning of fish-eating birds is supported by the monitoring data in the UK where the fish-eating birds, cormorants, herons, goosanders and red-breasted mergansers have not been involved in any of the reported incidents.

Terrestrial

For the terrestrial environment, following the use of Ruby Grain, the PEC/PNEC ratios exceed 1 for earthworm and rodent eating birds and mammals indicating unacceptable risk. Contaminated rodents are the most likely source for difenacoum residues in raptorial birds and mammalian predators.

Acute risk-Rodent eating birds and mammals

A qualitative assessment of the acute secondary poisoning is made by comparing the concentration in the rodents to LD_{50} values from acute oral studies. Rodents are assumed to eat entirely on bait containing difenacoum and the non-target animals are assumed to consume entirely poisoned rodents. The calculations of PEC_{oral} values are outlined in Annex VI. The results indicate that birds are likely to survive and mammals are likely to die if they eat poisoned rats. The species specific sensitivity differences or other aspects normally covered by the assessment factors are not taken into account in the qualitative assessment.

Long-term risk-Rodent eating birds and mammals

The quantitative risk assessment for long-term exposure to Ruby Grain, based on ESD guidance parameters, for susceptible and resistant rodents indicate that difenacoum causes unacceptable risk for non-target vertebrates. In laboratory studies on Barn Owls, fed on contaminated rodents, accumulation of difenacoum was noted. The target organ for difenacoum is liver and difenacoum residues in the carcasses have been measured from the liver. In one laboratory study highest residues were measured in the liver, and residues in other tissues including the fat tissue were low. Owls exposed to difenacoum showed variable effects, from no foreseeable effects, to death. Other observed effects were increased coagulation times and haemorrhages. The effects disappeared gradually after the end of exposure.

Bioaccumulation of difenacoum in predators has been shown in the measurements of difenacoum residues in the animal carcasses found from the field in the United Kingdom during monitoring campaigns (for details see Annex VI). While the PEC/PNEC ratios based on measured concentration in rats and mice were lower than the respective figures calculated according to the ESD, they were still considerably higher than 1 indicating risk of secondary poisoning of Barn Owls. Population level effects of difenacoum have not been studied and while all available information indicates risk, it does not tell the frequency of secondary poisoning incidents among wildlife. The conclusion, however, is that difenacoum causes a high risk for secondary poisoning.

The risk for secondary poisoning is more difficult to control than that for primary poisoning, as poisoned rodents may be available for predators for several days after intake of difenacoum. The use of difenacoum inside the buildings may reduce the secondary poisoning risk, but does not exclude it as the exposed rodents may move out from the building. The secondary poisoning can be excluded only in fully enclosed spaces where rodents cannot move to outdoor areas or to areas where predators may have access. When using difenacoum as a rodenticide all possible measures have to be taken in order to minimize secondary poisoning of the non-target animals. The measures include use of tamper resistant bait boxes, collection of unconsumed baits after termination of the control campaign and collection of dead rodents during and after the control campaign.

3.4. Measures to protect man, animals and the environment

The information submitted covering the requirements as described in the TNsG on Data Requirements, common core data for the product, section 8, points 8.1 to 8.8 is provided below.

3.4.1. Methods and precautions concerning handling, use, storage, transport or fire

Methods and precautions concerning handling and use:

- Always read the label before use and follow the instructions provided.
- Do not decant product into unlabelled containers.
- Avoid all unnecessary exposure, in particular avoid ingestion.
- Keep away from food, drink and animal feeding stuffs.
- Do not smoke eat or drink while handling this product.
- Baits must be secured in tamper resistant bait boxes to minimise the risk of consumption and poisoning to children, companion animals and other non-target animals.
- Bait boxes must be placed in areas inaccessible to children, companion animals and non-target animals.
- Bait boxes must always be clearly labelled “Do Not Touch” and warn of the contents.
- In public areas (such as business premises, schools, hospitals etc) it must be clearly signed that rodenticide control is in operation. Signage must provide information on the risks of interfering with the product and dead rodents.
- Dead rodent bodies must be collected during all control operations to minimise the risk of consumption and poisoning to children, companion animals and other non-target animals.
- It is illegal to use this product for the intentional poisoning of non-target, beneficial and protected animals.
- Wash hands and face after application and use of the product, and before eating, drinking or smoking.

Methods and precautions concerning storage:

- Store in a cool, dry, well-ventilated place
- Store locked up in the original container
- Store original container tightly closed
- Keep/store out of reach of children and companion animals
- Keep/store away from food, drink and animal feedstuffs.

Methods and precautions concerning transport:

Not classified as dangerous for transport.

Methods and precautions concerning fire:

Suitable Extinguishing Media:

Keep fire exposed containers cool by spraying with water if exposed to fire. Carbon dioxide (CO2), alcohol-resistant foam, dry powder, water spray, mist or foam.

Extinguishing media which must not be used for safety reasons:

Avoid the use of water jets to prevent dispersion.

Specific hazards:

Not applicable

Special protective equipment for fire-fighters:

In the event of fire, wear self contained breathing apparatus, suitable gloves and boots

Residues:

Dispose of residues to certified waste disposal operator for incineration and licensed waste disposal site.

3.4.2. Specific precautions and treatment in case of an accident

Personal precautions

Wear suitable protective clothing, gloves and eye/face protection, if applicable and where appropriate.

- Respiratory Protection: No special respiratory protection equipment is recommended under normal conditions of use with adequate ventilation. However, for professionals it is advised to wear a face-mask when applying the product indoors.
- Hand protection: Wear gloves.
- Skin protection: No special clothing/skin protection equipment is recommended under normal conditions of use.
- Eye protection: Not required.

- Ingestion: When using this product, do not eat, drink or smoke

Personal treatment

- General advice: In the case of accident or if you feel unwell, seek medical advice immediately (show the label where possible and report the authorisation number).
- Skin contact: May cause skin irritation. Remove contaminated clothing Wash off immediately with soap and plenty of water. If irritation persists obtain medical attention Contaminated clothing should be washed and dried before re-use.
- Eye contact: May cause eye irritation. Rinse immediately with plenty of water and seek medical advice.
- Inhalation: Unlikely to present an inhalation hazard unless excessive dust is present. Move to fresh air. Obtain medical advice immediately.
- Ingestion: If swallowed, seek medical advice immediately.

ADVICE FOR DOCTORS:

Difenacoum is an indirect anti-coagulant. Phytomenadione, Vitamin K1, is antidotal. Determine prothrombin times not less than 18 hours after consumption. If elevated, administer Vitamin K1 until prothrombin time normalises. Continue determination of prothrombin time for two weeks after withdrawal of antidote and resume treatment if elevation occurs in that time.

Report all incidents of poisonings to the relevant national poisons centre; include information on the product authorisation number, product trade name and active substance. In Ireland, this is the National Poisons Information Centre, Beaumont Hospital, Dublin (01-8092166)

Environmental precautions

- Prevent accidental exposure of the product to the environment.
- Keep un-used bait locked-up and in secure storage containers
- Bait must be secured in tamper resistant bait boxes in areas away from drains, water courses and non-target organisms.

Environmental treatment

- Clean up accidental spillages promptly by sweeping or vacuum.
- If the product gets into water or soil, it should be removed mechanically.
- Transfer to a suitably labelled container and dispose of to a certified waste disposal operator for incineration and licensed waste disposal site.
- Subsequently, wash the contaminated area with water, taking care to prevent the washings entering sewers or drains.
- For further instructions, see section 3.4.6 below.

3.4.3. Procedures for cleaning application equipment

No application equipment is required; therefore, no specific cleaning for equipment is required.

If necessary, following use, bait boxes should be washed with detergent and water. The bait box should be washed out 3 times (triple rinsed).

3.4.4. Identity of relevant combustion products in cases of fire

Not applicable.

3.4.5. Procedures for waste management of the biocidal product and its packaging

Dispose of packaging, remains of unused product and dead rodents to a certified waste disposal operator for incineration and licensed waste disposal site.

3.4.6. Possibility of destruction or decontamination following accidental release

Air:
Difenacoum has a very low vapour pressure, and decomposes at around 220°C and therefore does not boil. The formulated product is a grain bait. The risk of release of the active ingredient or the product to the atmosphere is negligible.

Water (including drinking water):
The octanol-water partition coefficient of difenacoum is high, and hence the active ingredient will remain in the product. The product is know not to inhibit activate sludge respiration, and the rapid partitioning to the solid phase and very low water solubility, would suggest that product exposure by use in sewer systems, would not result in contamination of water, but would contaminate the sludge.

Directions for use of the product require users **not** to place bait points where water could become contaminated (excepting sewers), so there will be no direct exposure to surface or drinking water.

Indirect exposure by leaching is very unlikely, as the very low water solubility of the active ingredient, and its affinity for soil means that any release into an environmental aquatic compartment will result in rapid partitioning to the solid phase, usually soil.

Soil:
Sources for release to the soil compartment include: sludge spreading, transport of bait by rodents, degradation of dead rodent remains hidden in burrows and excretion of the active ingredient by poisoned rodents. Bioremediation will probably prove the most effective method of decontamination, as 30% biodegradation in a 28 day ready biodegradation study suggests.

In the event of spillage of an appreciable amount of product, this material should be collected for incineration.

3.4.7. Undesirable or unintended side-effects

Toxic to mammalian and avian species, including domesticated animals, wildlife and humans. Therefore the risk to these non-target species must be considered and avoided when using bait.

3.4.8. Poison control measures

The grain bait are dyed (e.g. red or blue) to make them unattractive to wildlife, and birds in particular. In addition, in case of accidental ingestion, the presence of a dye may help to confirm that there has been ingestion and thus facilitate antidote treatment.

The product contains a human taste deterrent (adversive agent – Bitrex).

To report human poisoning incidents call the relevant national poison information centre. Include information on the product authorisation number, product trade name and active substance. Where possible provide a copy of the label or safety data sheet (SDS).

In Ireland to report a poisoning incident, call: 01 (8092566 / 8379964) The Poisons Information Centre of Ireland, Beaumont Hospital, Beaumont Road, Dublin 9.

ADVICE FOR DOCTORS:

Difenacoum is an indirect anti-coagulant. Phytomenadione, Vitamin K1, is antidotal. Determine prothrombin times not less than 18 hours after consumption. If elevated, administer Vitamin K1 until prothrombin time normalises. Continue determination of prothrombin time for two weeks after withdrawal of antidote and resume treatment if elevation occurs in that time.

Report all incidents of poisonings to the relevant national poisons centre (include information on the product authorisation number, product trade name and active substance)

4. Proposal for Decision

The assessment presented in this report has shown that the ready-to-use product, Ruby Grain, formulated by Lodi S.A. with the active substance difenacoum, at a level of 0.005% w/w, may be authorised for use as a rodenticide (product-type 14) for the control of rodents (rats and mice).

This authorisation of the product Ruby Grain has duly taken in to consideration the conclusions and recommendations of both the Finnish Assessment Report for the active substance, difenacoum and Commission Directive 2008/81/EC including difenacoum in Annex I of Directive 98/8/EC.

The product has been shown not to present a physical-chemical hazard to end users and does not classify as flammable, oxidising or explosive.

The product was shown to be efficacious against the intended target organisms, in the proposed areas for use at the proposed dose rate. However, cereal grain bait is not suitable for damp or wet conditions, such as in sewers. Therefore, this use area is not supported by this authorisation.

Acute toxicology studies presented for the product indicated that Ruby Grain (containing 0.005% w/w difenacoum) does not classify with respect to Directive 1999/45/EC or Regulation (EC) No 1272/2008. However, safety phrases and precautionary statements are proposed by the Rapporteur.

A human health exposure and effects assessment for the product was carried out for professionals and amateurs on the product Ruby Grain, based on the larger baiting quantities for rats. Using both the MOE and AEL approaches for risk assessment indicates that there is a satisfactory margin between the predicted exposure and the NOAEL (LOAEL) as well as being below the threshold value for the AEL for all intended uses by trained professionals and untrained professionals wearing PPE and amateurs without PPE. The product is deemed suitable for authorisation and appropriate personal protective equipment is advised.

Secondary exposure from transient mouthing of the product exceeds the AEL reference value (1.13×10^{-6} mg/kg bw/day), both with the assumption of 0.01 g and 5 g of product ingested by infants. This is of concern. There is no margin of safety using the existing data and models. There is no safe scenario for indirect exposure if estimated according to TNsG and User Guidance. Mitigation and protection measures such as the inclusion of bittering agents and the enclosure of product in sealed bags and tamper resistant bait boxes are essential to reducing the risk of secondary exposure. Baits should not be placed where food, feeding stuffs or drinking water could be contaminated.

An environmental exposure and effects assessment for the product indicated that difenacoum in Ruby Grain does not pose a threat to groundwater ($PEC_{GW} < 0.1$ µg/L) and does not infinitely accumulate in soil when used according to label instructions. Difenacoum has an estimated half-life of approximately 2 hours in air. Consequently, it is predicted to have a negligible effect on stratospheric ozone. Difenacoum shows no absorption in the so-called atmospheric window (800-1,200 nm) and therefore, according to the TGD on risk assessment (Part II, Section 3.7.2) is not a potential greenhouse gas.

Difenacoum in Ruby Grain does not adversely impact non-target organisms in the aquatic or terrestrial compartments when used according to label instructions. There is a high potential risk for primary and secondary poisoning for non-target vertebrates. Additionally, difenacoum is a potential PBT substance

(see Difenacoum Assessment Report (17-09-2009)) . These identified risks are minimized by applying all apropriate and available risk mitigation measures.

During the active substance review of difenacoum by Finland, primary and secondary poisoning risks were identified for non-target organisms and for potential accidental incidents involving children. The assessment of those EU identified risks during the product authorisation evaluation of Ruby Grain have also indicated a potential risk of primary and secondary poisoning to no-target animals and the potential for the accidental primary poisoning of children. As such risk mitigation measures are applied to product authorisation.

Additionally, as the target rodents are vermin and are both direct transmitters of disease (such as through biting or contamination of food/feed by urine or faeces) or indirect carriers of disease (such as disease vectors, where fleas move from rat to humans) to humans and other animals. Transmitted diseases can include leptospirosis (or Weil's disease), trichinosis and salmonella. Authorisation of this product is considered necessary on the basis of public health grounds, since rodent populations are considered to constitute a danger to public health through the transmission of disease.

Conditions of authorisation

Two authorisations should be issued. The first authorisation covers professional and trained professional use product. The second authorisation covers amateur use product.

This authorisation of Ruby Grain is for a period of 5-years with an annual renewal.

The concentration of the active substance, difenacoum, in Ruby Grain shall **not** exceed 0.05 g/kg (0.005% w/w).

Only ready-to-use Ruby Grain product is authorised.

As a poison control measure, the authorisation requires that the product shall contain an aversive, bittering agent.

The authorisation requires that the product be dyed with a colour to make them unattractive to wildlife, and birds in particular.

This product shall **not** be used as a tracking poison.

The product is authorised only for use against rodents (for example brown rats, house rats and house mice). Authorisation of this product does **not** allow use against non-target organisms.

The authorisation of this product for professionals and trained professionals allows for use indoors and outdoors in the following areas: Indoors, including areas such as houses, warehouses, outbuildings and commercial premises. Outdoors uses include areas such as in-and-around buildings, waste dumps and open areas. Difenacoum baits must not be placed where food, feeding stuffs or drinking water can become contaminated.

The authorisation of this product for amateurs allows for use of this product indoors and outdoors in the following areas: Indoors, including only privates houses and outbuildings. Outdoors uses, including only in-and-around private building premises and private gardens. Difenacoum baits should not be placed where food, feeding stuffs or drinking water can become contaminated.

The product should only be used for rodent control in tamper resistant, secured bait stations or other secure coverings.

Bait stations should be clearly marked to show that they contain rodenticides and that they should not be disturbed.

Grain bait sachets shall be secured to the bait station(s) so that rodents can not remove bait from the bait box.

For amateur use products placed on the market in Ireland packaging restrictions are to be limited to pre-baited bait stations and refill packs with a maximum pack-size of 500g. Additionally, the grain bait shall be supplied to the amateur market in sachets and where relevant to professionals in order to reduce exposure risks to amateur operators during application to bait stations.

All product placed on the Irish market after the date of authorisation must be in compliance with the conditions of this authorisation and shall carry the approved label with the IE/BPA authorisation number and be packaged in the approved packaging.

Prior to any amendment relating to this authorised product, such as specification, use, labelling or administrative changes, application must be made to this Authority to do so

Upon annual renewal of the product Ruby Grain, the authorisation holder shall provide statistics to PRCD on the import and export from Ireland and also manufacture statistics where appropriate for Ruby Grain for the given full annual period or part thereof.

Authorisation of the biocidal product may be subject to review, following a detailed assessment of the risks involved, in accordance with the European Communities (Authorisation, Placing on the Market, Use and Control of Biocidal Products) Regulations, 2001, as amended. This review may lead to changes in or revocation of this authorisation.