Product Assessment Report

Biocidal product assessment report related to product authorisation under Directive 98/8/EC

NYNA D+ PATE

TRIPLAN SA

December 2011

Internal registration/file no:	PB-10-00096
Authorisation/Registration no:	FR-2012-0003 (professional) / FR-2012-0051 (non-professional)
Granting date/entry into force of authorisation/ registration:	23 February 2012
Expiry date of authorisation/ registration:	31/03/2015 except where a decision of the European Commission extends the registration of the active substance
Active ingredient:	DIFENACOUM (CAS 56073-07-5)
Product type:	14 - Rodenticide

Competent Authority in charge of delivering the product authorisation:
French Ministry of Ecology
Department for Nuisance Prevention and Quality of the Environment
Chemical Substances and Preparation Unit
Grande Arche, Paroi Nord
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Authority in charge of the efficacy and risk assessment:
Anses – French agency for food, environmental and occupational health and safety
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Contents

1 General information about the product application

1.1 Applicant

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E-mail address:	triplan@andorra.ad

1.1.1 Person authorised for communication on behalf of the applicant

Name:	Fredy LACROUX
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1.2 Current authorisation holder[1]

Company Name:	TRIPLAN SA
Address:	BP258 La Poste Française
City:	Andorre la Vieille
Postal Code:	AD500
Country:	Principauté d'Andorre
Telephone:	+376 741 445
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E-mail address:	triplan@andorra.ad
Letter of	No

[1] Applies only to existing authorisations

appointment for the applicant to represent the authorisation holder provided (yes/no):	

1.3 Proposed authorisation holder

Company Name:	TRIPLAN SA
Address:	BP258 La Poste Française
City:	Andorre la Vieille
Postal Code:	AD500
Country:	Principauté d'Andorre
Telephone:	+376 741 445
Fax:	+376 741 450
E-mail address:	triplan@andorra.ad
Letter of appointment for the applicant to represent the authorisation holder provided (yes/no):	No

1.4 Information about the product application

Application received:	31/03/2010
Application reported complete:	30/08/2010
Authorisation granted:	23 february 2012
Type of application:	Product authorisation
Further information:	-

1.5 Information about the biocidal product

1.5.1 General information

Trade name:	NYNA D+PATE
Manufacturer's development code number(s), if appropriate:	Not reported
Product type:	PT14 - Rodenticide
Composition of the product (identity and content of active substance(s) and substances of concern; full composition see confidential annex):	Active substance's identity and content: Difenacoum 0.005% w/w No substance of concern
Formulation type:	Paste
Ready to use product (yes/no):	Yes
Is the product the very same (identity and content) to another product already authorised under the regime of directive 98/8/EC (yes/no); **If yes: authorisation/registration no. and product name:** **or** **Has the product the same identity and composition like the product evaluated in connection with the approval for listing of active substance(s) on to Annex I to directive 98/8/EC (yes/no):**	No No

1.5.2 Information on the intended use(s)

Overall use pattern (manner and area of use):	NYNA D+ PATE is intended to be used for control of mice, brown rats and black rats inside buildings (private, public including farm buildings).
Target organisms:	I.1.1.1 Brown rat: *Rattus norvegicus* I.1.1.2 Roof rat, House rat: *Rattus rattus* I.1.1.3 House mouse: *Mus musculus*
Category of users:	V.1 Non Professional/general public V.2 Professional
Directions for use including minimum and maximum application rates, application rates per time unit (e.g. number of treatments per day), typical size of application area:	VI.2 Covered application VI.2.1 Covered application in bait stations. The product is a ready to use paste bait and contains 0.005% of difenacoum <u>Professionals / Non-professionals</u> Rat: 180 g paste/secured bait point separated by 5-10 m. Mice: 30g paste/secured bait point separated by 1-2 m. For professionals and non-professionals, the product is wrapped individually in heat-sealed paper sachet of 10 g (3 sachets for mice and 18 sachets for rat). Secondary packaging proposed for only professional are following: - Cardboard boxes : 100 g, 200g, 500g, 1000 g - Boxes with a plastic cap: 200g, 500g, 600 g - Plastic buckets from 2 to 18 kg.
Potential for release into the environment (yes/no):	Yes
Potential for contamination of food/feedingstuff (yes/no)	No
Proposed Label:	Control of rats (*Rattus norvegicus* and *Rattus rattus*) and mice (*Mus musculus*) inside buildings. Professional et non professional (sachets 10 g): Rat: 18 sachets /secured bait point separated by 5-6 m. Mice: 3 sachets /secured bait point separated by 1-2 m. Over a period of 28 days for application, cleaning, refilling and collect of dead rodents
Use Restrictions:	Use only inside buildings in secured bait stations out of reach of children and domestic animals.

1.5.3 Information on active substance(s)

Active substance chemical name:	Difenacoum
CAS No:	56073-07-5
EC No:	259-978-4
Purity (minimum, g/kg or g/l):	960 g/kg
Inclusion directive:	2008/81/EC
Date of inclusion:	01/04/2010
Is the active substance equivalent to the active substance listed in Annex I to 98/8/EC (yes/no):	Yes
Manufacturer* of active substance(s) used in the biocidal product:	
Company Name:	PM TEZZA SRL
Address:	Via Tre Ponti 22
City:	S. Maria di Zevio (VR)
Postal Code:	37050
Country:	Italy
Telephone:	Not reported
Fax:	Not reported
E-mail address:	Not reported

*Activa is the applicant of the active substance but not the manufacturer. Tezza SRL is the manufacturer of the active substance as mentioned in the Final CAR of difenacoum of the Activa / PelGar Brodifacoum and Difenacoum Task Force.

1.5.4 Information on the substance(s) of concern

NYNA D+ PATE does not contain any substance of concern according to the Technical Notes for Guidance on data requirements[2].

1.6 Documentation

1.6.1 Data submitted in relation to product application

Identity, physicochemical and analytical method data

Physico-chemical properties studies were provided by Triplan. Some data have been provided using product with old composition and some others with the new composition:

- Explosive properties, oxidising properties performed on NYNA D+ old formulation. The results were extrapolated for the current formulation NYNA D+ PATE.

[2] Technical guidance document in support of the directive 98/8/ec concerning the placing of biocidal products on the market - Guidance on data requirements for active substances and biocidal products, October 2000.

- The other required physico-chemical properties performed on NYNA D+ PATE, current formulation.

An analytical method to determine the active substance in the formulation NYNA D+ PATE (current formulation) was provided by Triplan.

Data on the active substance required at the product authorization stage as stated in the Assessment Report of the active substance and provided by Activa:

- Analytical data to prove the isomeric composition and impurity profile of the active substance,
- Appearance of the active substance,
- A validated method for the analysis of difenacoum in animal and human tissues,
- Validation data for the determination of residues of difenacoum in meat and oil-seed rape (food/feeding stuffs),
- Validation data for the determination of difenacoum in sediment.

Efficacy data

The following efficacy studies were submitted:

- Efficacy laboratory study of NYNA D+ paste rodenticide containing 0.005% difenacoum with albino house mice (*Mus musculus*).
- Efficacy field study for NYNA D+, paste rodenticide, containing 0.005% difenacoum with black rats (*Rattus rattus*).

Studies were performed with the old formulation NYNA D+ (see detailed composition in confidential document). This formulation is different from the NYNA D+ PATE because of the type of two preservatives not listed in PT6, the pigment and it also contains fewer appetent agents. But it is a paste formulation containing 0.005% p/p of difenacoum and it is the same rate of bittering agent then results can be taken into account in order to support the product authorization of NYNA D+ PATE. Moreover, in order to support the resistance information, new data carried out with literature references were submitted during the evaluation.

Toxicology data

The applicant did not submit new toxicological data on active substance. The following toxicological studies performed with NYNA D+ BLOC SP were submitted:

- Acute dermal and oral studies,
- Skin and eye irritation studies,
- Sensibilisation study.

The extrapolation of the results to NYNA D+ PATE has been accepted because it is not expected that the difference between the two formulations impacts the toxicity.

Ecotoxicology data

The applicant has not provided ecotoxicological study with the biocidal product. The environmental risk assessment for NYNA D+ PATE has been done by the authority in charge of the risk assessment, using the Competent Authority Report on the active substance supported by the Task Force Activa/Pelgar.

1.6.2 Access to documentation

In the frame of the authorization of NYNA D+ PATE supported by TRIPLAN SA, the applicant Activa Srl has submitted a letter of access to all data on difenacoum submitted by the Activa/Pelgar Brodifacoum and Difenacoum Task Force under directive 98/8/EC for the purpose of Annex I listing.

2 Summary of the product assessment

2.1 Identity related issues

Data were required at the product authorization stage as stated in the AR about the active substance and were provided by Activa:
- Analytical data to prove the isomeric composition and impurity profile of the active substance.

The assessment of the technical equivalence of the source of difenacoum from Activa versus the reference source of Pelgar used for annex I inclusion has been performed. The conclusion is that the source of Activa used in NYNA D+ PATE is technically equivalent to the source of Pelgar assessed for annex I inclusion. The confidential document is attached to this PAR as the addendum to the CAR of difenacoum is not available yet. See the confidential appendix “Technical equivalence Difenacoum Activa” for detailed information.

The composition of the product is confidential and is presented in a confidential annex.
There is no substance of concern.

2.2 Classification, labelling and packaging

2.2.1 Harmonised classification of the biocidal product

No classification is required for NYNA D+ PATE.

2.2.2 Labelling of the biocidal product

No labelling is required for NYNA D+ PATE.

2.2.3 Packaging of the biocidal product

Primary packaging:
For professionals and non-professionals, the product is wrapped individually in heat-sealed paper sachet of 10 g.

Secondary packaging:
The 10 g wrapped pieces of paste are put in plastic heat-sealed bags (100 g, 200 g, 500 g, and 1000 g).

Tertiary packaging:
The bags are put in cardboard boxes (quality: chromoduplex GD2) (100 g, 200, 500, 1000 g) or in varnished iron circular boxes with a plastic cap (200, 500, 600 g) or in waterproof plastic buckets (polyethylene) (from 2 to 18 kg) with a waterproof cape

2.3 Physico/chemical properties and analytical methods

Data on the active substance difenacoum required at the product authorization stage as stated in the Assessment Report (AR) of the active substance and provided by Activa:

- Appearance of the active substance.

Results of the assessment: for appearance, the data provided are acceptable. The results are reported in 2.3.1.

2.3.1 Physico-chemical properties

Table 1: Physico-chemical properties of the active substance:

	Method/ Guideline	**Purity/Specification**	**Result**	Reference
Physical state	Visual examination	99.5% w/w difenacoum Batch number 03090205	Solid powder at ca. 22℃	CH-082/2010
Colour	Visual examination	99.5% w/w difenacoum Batch number 03090205	Faint beige (Sigma-aldrich Color Chart)	
Odour	Olfactory test	99.5% w/w difenacoum Batch number 03090205	Characteristic	

Other physico-chemical properties are presented in the CAR of difenacoum of the Activa / Pelgar Brodifacoum and Difenacoum Task Force. Triplan has a letter of access to these data.

Table 2: Physico-chemical properties of the biocidal product:

For the studies performed on NYNA D+ old formulation, results from these studies could be extrapolated to the current formulation of NYNA D+ PATE. The differences in composition between the two formulations were evaluated and considered as acceptable for each property under consideration.

	Method	Purity/Specification	Results	Reference
Physical state and nature	Visual inspection at room temperature	0.048 g/kg difenacoum	Homogenous paste Bait ready for use (RB)	10-920010-009
Colour			Turquoise blue	
Odour			Not determined	
Explosive properties	Differential Scanning Calorimetric method (DSC)	0.045 g/kg difenacoum tested on NYNA D+ (old formulation)	Not explosive	09-920010-001
Oxidizing properties	Literature survey	0.045 g/kg difenacoum tested on NYNA D+ (old formulation)	No oxidizing properties	09-920010-001
Flash point	Not applicable			
Autoflammability	EC A16	0.048 g/kg difenacoum	No self ignition up to 400℃	10-920010-009
Other indications of flammability	EC A10	0.048 g/kg difenacoum	Not highly flammable	10-920010-009
Acidity / Alkalinity	CIPAC MT 75.3	0.048 g/kg difenacoum	1% m/v in standard water D 6.64 at 20.3℃ after 1 min. 6.32 at 20.6℃ after 10 min. The measured pH value is higher than 4 and lower than 10, therefore no further testing is required	10-920010-010
Relative density / bulk density	EC A3	0.048 g/kg difenacoum	D (19.7℃/4.0℃) = 1.335 ± 0.003 g/cm^3	10-920010-009
Storage stability – stability and shelf life	2-years storage stability		See conclusion below the table	
Effects of temperature	CIPAC MT 46.3	0.048 g/kg difenacoum	The aspect of the test item was considered to be stable after an accelerated storage procedure for 14 days at 54℃. Difference of content of the active substance:	10-920010-010

	Method	Purity/Specification	Results	Reference
			- 4.2% deviation from T=0 value after the accelerated storage procedure for 14 days at 54℃ See comment and conclusion below the table	
Effects of light			Not required since the product will be stored protected from light.	
Reactivity towards container material	Not submitted		See conclusion below the table	
Technical characteristics in dependence of the formulation type	Not applicable			
Compatibility with other products			The product is never used with other products including biocidal products	
Surface tension	Not applicable			
Viscosity	Not applicable			
Particle size distribution	Not applicable			

Storage stability:
The pH was measured after 14 days at 54℃ and no si gnificant changes were observed.

Conclusion:

The shelf life study (2 years at room temperature) is missing and is required in post registration. The study should be performed with test items in quantity sufficient to overcome the heterogeneity problem. Intermediate results at one year should be provided.

The reactivity toward heat-sealed paper sachet of 10g is missing and is required in post registration. The tested material should be clearly identified in the study.

2.3.2 Analytical methods

Data on the active substance difenacoum were required at the product authorization stage as stated in the AR of the active substance and were provided by Activa:
- Analytical data to prove the isomeric composition and impurity profile of the active substance,
- A validated method for the analysis of difenacoum in animal and human tissues,
- Validation data for the determination of residues of difenacoum in meat and oil-seed rape (food/feeding stuffs),
- Validation data for the determination of difenacoum in sediment.

Results of the assessment of the analytical methods provided by Activa on the active substance as required in the CAR:

- Analytical data to prove the isomeric composition and impurity profile of the active substance

Results of the assessment:

→ The method provided doesn't allow to identify and quantify separately the two diastereoisomers. Nevertheless FR CA considers that the provided data allow the determination of the isomeric composition.

→ The submitted data allow to determine the impurity profile.

See table below and the confidential appendix "Technical equivalence Difenacoum Activa" for detailed information.

- A validated method for the analysis of difenacoum in animal and human tissues

Results of the assessment: The method is validated and is acceptable.

- Validation data for the analytical method for determination of residues of difenacoum in meat and oil-seed rape (food/feeding stuffs)

Results of the assessment: The data provided were not validation data based on the analysis method already provided in the dossier, as requested. The submitted study report provided a new method with validation data. This new method is validated and is acceptable.

- Validation data for analytical method for determination of difenacoum in sediment (based on the analysis method for difenacoum in soil)

Results of the assessment: The data provided were not validation data based on the analysis method for difenacoum in soil, as requested. The submitted study report provided a new method with validation data. This new method is validated and is acceptable.

	Principle of method
Technical active substance as manufactured:	HPLC-UV
Impurities in technical active substance:	-
Active substance in the formulation:	HPLC-UV

Technical active substance as manufactured:

The determination of the active substance was performed by HPLC using an internal standard and UV detector at 275nm. The quantification of difenacoum is achieved by comparing the ratio of the analytical standard peak area versus 1,3,5-triphenylbenzene internal standard (IS) peak area and the same ratio determined for a sample containing a known amount of internal standard (I.S). The analytical method is considered to be acceptable.

Impurities in technical active substance:

No methods required since there are no impurities higher than 0.1% w/w.

Active substance in the formulation:

Difenacoum is analyzed after extraction from the formulation and quantified by liquid chromatography using a reverse phase column and an UV detector. Two validated analytical methods have been provided. An analytical method validation was performed on

another difenacoum-based formulation, NYNA D+ BLOC SP, by definition of the specificity, the linearity, the precision and the accuracy of the method. This is acceptable for NYNA D+ PATE. A complementary analytical method for the determination of difenacoum in NYNA D+ PATE was performed by definition of the specificity and the accuracy of the method.

2.4 Risk assessment for Physico-chemical properties

NYNA D+ PATE is a ready-to-use rodenticide. It is a homogenous paste, not highly flammable, not auto-flammable (up to 400°C), not explosive and does not have oxidizing properties.
The accelerated storage (14 days at 54°C) shows that NYNA D+ PATE is stable. Other data are missing (shelf life and reactivity toward container material) and are required in post registration.

2.5 Effectiveness against target organisms

2.5.1 Function

MG 03: Pest Control
Product Type 14: Rodenticide

2.5.2 Organism(s) to be controlled and products, organisms or objects to be protected.

According to the uses claimed by Triplan, NYNA D+ PATE is intended to be used to control rodents inside buildings (private, public including farm buildings).. The target organisms to be controlled are brown rat *(Rattus norvegicus),* roof rat or house rat (*Rattus rattus)* and wild and house mouse *(Mus musculus).*

The products, organisms or objects to be protected are public health, domestic animal heath, and material protection (historical building, technical objects).

2.5.3 Effects on Target organisms

Anticoagulants rodenticides disrupt the blood-cutting mechanisms. Signs of poisoning in rodents are those associated with an increased tendency to bleed, leading ultimately to profuse haemorrhage. After feeding on bait containing the active substance 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. As the active substance has a long acting action, death will usually occur within 4 -10 days of ingesting a lethal dose and animals often die out of sight in their nest or burrow.

<u>The application rates recommended by the applicant are the following:</u>

<u>Rats</u>: (*Rattus norvegicus and Rattus rattus)*

180 g paste/secured bait point separated by 5-10 m.

Mice: (*Mus musculus*)

30 g paste/secured bait point separated by 1-2 m

The product is intended to apply in secured bait stations by professional and non-professional users on infested areas with obvious tracking of feces, and smears next to holes and harbourages. Distances between each bait station, so as the number and timings of application and the amount of product, depend on several factors: the treatment site, the size and severity of the infestation.

The applicant submitted following studies:

Laboratory study with albino house mice *(Mus musculus)*:

In this study, the test system was well conduced with the old formulation NYNA D+ (free and no choice food, and control lot). Within 3 days baiting, the results pointed out that, the consumption of the bait is lower than the control animals' usual food (average of 55%), but sufficient to obtain a total efficacy (100% of mortality) at day 10. Average mortality time has been 6.7 days for the free choice lot and 7.4 days for the efficacy.

Despite a low acceptance of bait, this laboratory trial has proved a high level of efficacy (100% of mortality).

Field trial on Black rat wild strain (*Rattus rattus*):

A field study with black rats was conducted using the old formulation NYNA D+, with a pig farm (black rats). The application rate applied in secured bait was 100 g of bait. Within 8 days baiting, the field trial showed a large range of acceptability. However, the pre-baiting stage was very long (67 days) due to the high competition of food in pig farm and the unusual food behavior of rats.

In spite of these factors, the consumption of bait was sufficient to obtain 94 % of mortality. This mortality rate is in adequacy with the results assessed in the laboratory with albino house mice and confirms the efficacy of the product NYNA D+ PATE.

All efficacy studies results are presented in annex 3.

2.5.4 Occurrence of resistance

The use of massive anticoagulants in the management of rodents since the 1970's has been at the origin of the first batches of resistance (genetic and not behavioral) to the first generation of anticoagulants (coumafene in particular).

Recent 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 (*Rattus norvegicus*) populations to coumafene.

Only an exhaustive study carried out at the French and European levels could enable pointed-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*").

Indeed, we cannot sustain that resistance to difenacoum in all geographical areas where it could be used cannot occur and the occurrence of resistance has an impact on the dosages and efficacy of rodenticides used in a more consequent way. Thus, it compels users to take into account the following precautions to reduce the possibility of rodents developing a resistance to difenacoum:

- Products have always to be used in accordance with the label.
- Efficacy level has to be monitored (periodic check) and the case of reduced efficacy has to be investigated for possible evidence of resistance.
- Treatment has to be alternated with active substances having different mode of action.
- Integrated pest management (combination of chemical control, physical and hygienic measures) has to be taken into account.
- Difenacoum must not be used in an area where resistance to this active substance is suspected or established.
- If signs of resistance begin to appear, then, every effort has to be made to eradicate the population. The measures necessary for eradication will vary in different situations; they may involve a number of procedures using both chemical and non-chemical ways.

The authorization holder should report any observed resistance incidents to the Competent Authorities (CA) or other appointed bodies involved in resistance management every two years.

2.5.5 Evaluation of the Label Claims

The authority in charge of the risk assessment assessed that the product NYNA D+ PATE has shown a sufficient efficacy for the control of mice and rats for an indoor use in domestic, public and private including farm buildings.

<u>The application rates validated are the following:</u>

Rats: (*Rattus norvegicus and Rattus rattus)*

- 180g paste/secured bait point separated by 5-10 m (instead of 5-6 m presented in the label). These intervals between bait points have to be corrected in the product label in accordance with those validated.

Mice: (*Mus musculus*)

- 30 g paste/secured bait point separated by 1-2 m.

According to Triplan, users have to apply 18 sachets/bait point for rats and 3 sachets/bait point for mice. The applicant has to adapt the amount per sachet and bait boxes to the efficient doses. The amount of bait per bait station must not exceed the validated application rates.

The label claim reflects the efficacy data of the product. Nevertheless because of cross-resistances occurrence to second-generation anticoagulants, the product label has to contain information on resistance management for rodenticides:

- Products have always to be used in accordance with the label.
- Efficacy level has to be monitored (periodic check) and the case of reduced efficacy has to be investigated for possible evidence of resistance.
- Treatment has to be alternated with active substances having different mode of action.
- Integrated pest management (combination of chemical control, physical and hygienic measures) has to be taken into account.
- Difenacoum must not be used in an area where resistance to this substance is suspected or established.
- Users should report straightforward to the registration holder any alarming signals which could be assumed to be resistance development.

2.6 Exposure assessment

2.6.1 Description of the intended use(s)

The doses and uses validated are the following:

Product	Field of use envisaged	User	Likely concentration at which active substance will be used
Main group 03; PT 14 NYNA D+ PATE Paste containing 0.005% p/p of difenacoum	In buildings for control of rats (brown and black rats)	Professionals	180 g paste/secured bait point separated by 5-10 m.
	In buildings for control of mice.	Professionals	30 g paste/secured bait point separated by 1-2 m.
	In buildings for control of rats (brown and black rats).	Non professionals	180 g paste/secured bait point separated by 5-10 m.
	In buildings for control of mice.	Non professionals	30 g paste/secured bait point separated by 1-2 m.

According to Triplan, NYNA D+ PATE is intended to be used inside buildings (public, private and farms buildings) for control of house mice (*Mus musculus*), brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*).
The control of mice and rats is based on the principle of applying baits on infested areas with obvious tracking of feces, and smears next to holes and harbourages.

The product is ready-to-use (paste) and it is manually applied by trained professional users and by non-professional users in secured bait boxes or bait stations.

Over a period of 28 days for application, cleaning, refilling (4 times over 28 days period) and collect of dead rodents.

Professionals:

According to Triplan, a professional applies 180 g baits per secured point for the control of rats (18 sachets of 10 g pieces of paste) and 30 g baits per secured points for the control of mice (3 sachets of 10 g pieces of paste).

According to Triplan, the worst case is 30 bait points treated per day plus remains of 30 bait points collected. However, in the *HEEG opinion on harmonizing the number of manipulations in the assessment of rodenticides (anticoagulants)* agreed at the European Technical Meeting TM III 2010, 60 loadings and 15 cleanings bait stations per day are considered for professional using wax block/paste bait in sachets.

Non-professionals:

According to Triplan, a non professional applies 180 g baits per secured point for the control of rats (18 sachets of 10 g pieces of paste) and 30 g baits per secured points for the control of mice (3 sachets of 10 g pieces of paste).

According to Triplan, the worst case is 4 bait points treated per day plus remains of 4 bait points collected. However, in the *HEEG opinion on harmonizing the number of manipulations in the assessment of rodenticides (anticoagulants)* agreed at TM III 2010, 5 loadings and 5 cleanings bait stations per day are considered for non-professional using wax block/paste bait in sachets.

The professional or non-professional users are exposed to ready-to-use paste containing 0.005% (w/w) difenacoum.

2.6.2 Assessment of exposure to humans and the environment

Assessment of human exposure

No new human exposure studies have been submitted. In the dossier, Triplan assessed the human exposure based on the TNsG on human exposure, section 7.2 of part 3 – June 2002. This document only contains a series of examples for human exposure assessment and should not be considered as reference data. Therefore, since Triplan provided a letter of access for the unpublished CEFIC study "*Snowdon P.J. Study to determine potential exposure to operators during simulated use of anticoagulant rodenticide baits*", the FR CA decided to base the human exposure assessment for professionals on this study as done by the RMS (Finland) of the active substance in the Assessment report on difenacoum. This study examined exposure to 20 g wax block baits containing 0.004 % flocoumafen (five blocks/bait box) using 10 replicates for each measurement. This study is considered as representative of the human exposure of wax block rodenticide baits. Considering that similar dermal absorptions were obtained for wax blocks and pastes in the Assessment report on difenacoum and that a similar application/manipulation is expected with these

products, the FR CA decides to use the exposure estimations issued from the CEFIC study for the assessment of NYNA D+ PATE. Furthermore, this study could be considered as very worst case concerning the application since NYNA D+ PATE is wrapped individually in heat-sealed paper sachet.

For non professional users, the same CEFIC study and assumptions were used for the estimation of human exposure since the values available in the TNsG and User Guidance (Human exposure to biocidal products – TNsG June 2002 – version 1) are considered as unrealistic (see argumentation in the Assessment report on difenacoum).

Additionally, the Human Exposure Expert Group (HEEG) opinion on harmonising the number of manipulations in the assessment of rodenticides (anticoagulant), agreed at TMII2010 and the HEEG opinion on an harmonised approach for the assessment of rodenticides (anticoagulants) agreed at TMII2011 were taken into account for the estimation of exposure for professionals and non professionals.

Assessment of environmental exposure

It is important to notice that the applicant did not provide ecotoxicological data about the biocidal product NYNA D+ PATE. So all the environment risk assessment is based on data extrapolated from the active substance, difenacoum.

Bronopol is used in the biocidal product as preservative. This substance is classified as ”Very toxic for aquatic organisms” according to the Directive 67/548/CEE. Moreover, the substance is also notified in the frame of biocidal directive as product type 6 : in-can preservatives and evaluated by Spanish Competent Authority to be included in the Annex I of the Directive 98/8/CE. Therefore, as no data with the biocidal product is available, the FR CA considered that the environmental risk assessment should take into account bronopol. The environmental risk assessment is summarized in section 2.8 of this document.

2.7 Risk assessment for human health

2.7.1 Hazard potential

2.7.1.1 Toxicology of the active substance

The toxicology of the active substance was examined extensively according to standard requirements of Directive 98/8/EC. The results of this toxicological assessment can be found in the CAR. The threshold limits and labelling regarding human health risks listed in Annex 4 of this report “Toxicology and metabolism” must be taken into consideration.

The following corresponds to the summary of the derivation of the AELs from the final Assessment report of difenacoum:
“The lowest LOAEL in a repeated dose study, i.e. the teratogenicity study in rabbits, is chosen as the basis to establish the AOEL (there was no NOAEL). In this study, the maternal LOAEL was 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 according to the agreement during peer-review discussion. A further supportive argument for an additional assessment factor comes from the higher potency of the second generation anticoagulants compared to warfarin, and from the much higher vulnerability of human foetuses to vitamin K deficiency

compared to rodents. To extrapolate from LOAEL to NOAEL an assessment factor of 2 is considered justified due to the deep slope of the dose response curve. After correction for bioavailability of 68%, a NOAEL for MOE (0.00034 mg/kg bw/day) and an AOEL of 0.0000011 mg/kg bw/day are used for risk characterisation. These values are applied both to acute and repeated exposure scenarios."

2.7.1.2 Toxicology of the substance(s) of concern

Considering the following definition of a substance of concern set in the TNsG on data requirement chapter 4 (2000), "*the substance is regarded as a substance of concern if [...] it is classified as dangerous **and** its concentration in the product exceeds the classification limit set in the Council Directive 88/379/EEC, as amended by Directive 1999/45/EC, for a particular dangerous property **or** the other classification limit indicated for the substance in a preparation set in Annex I of Council Directive 67/548/EEC **or** causes that the overall sum of the concentrations of dangerous substances in the product exceeds the limit for classification of the preparation set in Council Directive 88/379/EEC, as amended by Directive 1999/45/EC, for a particular dangerous property*", NYNA D+ PATE does not contain any substance of concern.

2.7.1.3 Toxicology of the biocidal product

The toxicology of the biocidal product was examined according to standard requirements of Directive 98/8/EC. 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. The basis for the health assessment of the biocidal product is laid out in Annex 5 of this report "Toxicology – biocidal product".

<u>New data:</u>
Acute oral and dermal toxicity, skin and eye irritation and skin sensitisation studies have been submitted on the product NYNA D+ BLOC SP. Since it is not expected that the differences of composition between NYNA D+ BLOC SP and NYNA D+ PATE impact the toxicity, the extrapolation of study results from NYNA D+ BLOC SP was accepted.

- Acute oral and dermal toxicity

No mortality, systemic or local effects were observed in these studies. The only reported effect was a slight blue coloration (probably due to the colorant present in the product), in the acute dermal study, noted at 24 hours post-dose on the treated area of all animals and totally reversible on day 2.
Based on the results, no classification is required for these endpoints.

- Irritation and corrosivity

Based on the results of the irritation assays on rabbit's skin and eye, no classification is required.

- Sensitisation

A Magnusson and Kligman sensitisation test was submitted. Due to deviations (such as first induction phase performed by topical route) from the OECD guideline 406, the FR CA does not accept this study. However, based on the composition of NYNA D+ PATE, no ingredients were listed as skin sensitisers.
Therefore, it is expected that NYNA D+ PATE is not a skin sensitiser.

<u>Justification for non submission:</u>

- Dermal absorption:

According to the Competent Autority Report on difenacoum (Doc IIIB6.4), a dermal absorption of 0.047% for wax blocks and pastes containing 0.005 % of difenacoum was considered, based on an *in vitro* study with human skin (8 hours of exposure). Read-across from this study is considered acceptable since NYNA D+ PATE is also a paste bait containing 0.005 % of difenacoum.

- Acute inhalation toxicity:

As the product is a solid bait, the generation of inhalable particle is not expected. Additionally, the vapor pressure of difenacoum is very low (< $5x10^{-5}$ Pa at 45°C based on an Activa/Pelgar estimation). Therefore, an acute toxicity test by inhalation is not required.

The current harmonised classification of the active substance is the following:

Classification under directive 67/548/EEC	Classification under regulation (EC) 1272/2008
T+ R28 T R48/25 N, R50/53 No specific concentration limit	Acute Tox. 2 H300 STOT Rep. 1 H372 Aquatic. Acute 1 H400 Aquatic Chronic 1 H410 No specific concentration limit

Based on the results of the studies, the concentration of the active substance and of other components contained in the product and according to the above classification, NYNA D+ PATE is not classified.

- Other studies

The product is not used with other biocidal products. Therefore, no additional study was conducted.

The product is a solid bait only used indoors in secured bait points. Collecting unconsumed baits and dead rodents must be done every week during the treatment so in these recommended conditions, no contamination is expected for feeding stuffs. Finally, according to the Assessment report on difenacoum, "*difenacoum baits should not be placed where food, feedingstuffs or drinking water could be contaminated*". Therefore, no data on residue was submitted.

2.7.2 Exposure

NYNA D+ PATE (PT14) is a ready-to-use paste rodenticide containing 0.005% of difenacoum (pure: 960 g/kg) intended to be applied by professionals and non-professionals. The baits are placed in bait stations (bait boxes or secured bait stations) out of reach of children and domestic animals. Baits are packaged wrapped individually in heat-sealed plastic film sachet for professional and non professional uses.

2.7.2.1 Exposure of professional users

Primary exposure

During professional use, the major route of primary exposure is dermal. The inhalation exposure could be considered as negligible considering the low vapour pressure of difenacoum (< $5x10^{-5}$ Pa at 45°C based on an Activa/Pelgar estimation), the physical state of the product and because the baits are individually wrapped in sachet.

Based on all the measured exposure data (75th percentile) in the CEFIC study, the amount of exposure to product **during loading** of 5 wax blocks of 20 g per one manipulation was 27.79 mg (value adopted by the HEEG[3]). The following parameters were taken into account:

- Active substance in product: 0.005%
- Number of pastes (considered as similar to wax blocks) per bait site[4]: 18 for control of rats and 3 for control of mice
- Dermal absorption: 0.047%
- Body weight: 60 kg

Thus, the systemic dose of difenacoum per placing of one bait site is 3.92 $x10^{-8}$ mg/kg bw/event for control of rats and 6.53 $x10^{-9}$ mg/kg bw/event for control of mice.

Based on all the measured exposure data (75th percentile) in the CEFIC study, the amount of exposure to product is 5.70 mg **during the cleaning** of one bait site (value adopted by the HEEG[5]). Considering a content of 0.005% of difenacoum in the product, a dermal absorption of 0.047% and a body weight of 60 kg, the systemic dose of difenacoum per cleaning of one bait site is $2.23x10^{-9}$ mg/kg bw/event (for rats and mice because the amount of disposed bait is not taken into account).

Although Triplan considers that 30 bait points are treated and 30 bait points are collected per day, FR CA has used the harmonized number of manipulations for rodenticides anticoagulant set in the HEEG opinion agreed at TM III 2010. Considering that 60 loadings and 15 cleaning are done per day for paste bait in sachets, the overall systemic dose via skin (loading + cleaning) is $2.38x10^{-6}$ mg a.s/kg bw/day without gloves and $2.38x10^{-7}$ mg a.s/kg bw/day (penetration factor of 10)[5] for control of rats. For control of mice, the systemic dose via skin is $4.25x10^{-7}$ mg a.s/kg bw/day without gloves.

This scenario considering exposure during loading and cleaning represents a very worst case since NYNA D+ PATE is only supplied in sachets. As a reasonable case, no exposure is expected during loading as the sachet prevents dermal contacts and the exposure can be reduced to $3.35x10^{-8}$ mg/kg bw/day (without gloves) for both rats and mice because the amount of disposed bait is not taken into account during cleaning.

Secondary exposure

Secondary exposure of users could result in the handling of dead rodents. However, this scenario is excluded due to unrealistic assumptions (very low amount of difenacoum is expected on the fur because NYNA D+ PATE is an oral bait and toxicokinetics data showed that urine is a minor route of excretion for difenacoum).

In Annex 6 “Safety for professional operators”, results of the exposure calculations for the active substance for the professional user are laid out.

[3] HEEG opinion on an harmonised approach for the assessment of rodenticides (anticoagulants) agreed at TMII2011.
[4] Although the bait weights 10 g and not 20 g as in the CEFIC study, it was considered that the important parameter is the number of pastes loaded rather than the weight.
[5] HEEG opinion Default protection factors for protective clothing and gloves, agreed at TMI2010

2.7.2.2 Exposure of non-professional users and the general public

Primary exposure

During non-professional use, the major route of primary exposure is dermal. The inhalation exposure could be considered as negligible considering the low vapour pressure of difenacoum (< $5x10^{-5}$ Pa at 45℃ based on an Activa/Pelgar estimation), the physical state of the product and because the baits are individually wrapped in sachet.

As a worst case, the same assumptions as for professional exposure was considered except for the number of manipulations set at 5 loadings and 5 cleaning per day for non-professional according to the HEEG opinion document[6] and in the absence of PPE. The overall systemic exposure via skin (loading + cleaning) is therefore at $2.08x10^{-7}$ mg a.s/kg bw/day for control of rats and $4.38x10^{-8}$ mg a.s/kg bw/day for control of mice.

As a reasonable case, since NYNA D+ PATE is only supplied in sachets, no exposure is expected during loading as the sachet prevents dermal contacts. Therefore, the exposure can be reduced to $1.1x10^{-8}$ mg/kg bw/day for both rats and mice because the amount of disposed bait is not taken into account during cleaning.

Secondary exposure

Exposure of non users, especially infants, could result from the handling of dead rodents or ingesting poison baits. The "*handling of dead rodents*" scenario is excluded due to unrealistic assumptions (very low amount of difenacoum is expected on the fur because NYNA D+ PATE is an oral bait and toxicokinetics data showed that urine is a minor route of excretion for difenacoum).

For the scenario "*oral exposure by ingesting bait*", a reverse scenario was calculated. Based on the AEL of $1.1x10^{-6}$ mg a.s/kg bw/day, a body weight of 10 kg and an oral absorption of 68% (as stated in the Assesment report of difenacoum [Activa/Pelgar Study]), ingestion of more than 0.3 mg of product per day (corresponding to about 0.003 % of a 10g paste of NYNA D+ PATE) is needed to exceed the AEL.

In Annex 7 "Safety for non-professional operators and the general public", the results of the exposure calculations for the active substance for the non-professional user and the general public are laid out.

2.7.2.3 Exposure to residues in food

Based on the intended uses, no residue assessment was performed (Annex 8 "Residue behaviour").

2.7.3 Risk characterisation

2.7.3.1 Risk for professional users

The estimated exposures for the professional users are compared to the systemic AEL of difenacoum set in the Assessment report ($1.1x10^{-6}$ mg/kg bw/day for short, medium and long-term exposures).

[6] HEEG opinion on harmonising the number of manipulations in the assessment of rodenticides (anticoagulant), agreed at TMII2010

Primary exposure

Based on the risk assessment of the active substance, the risk for professional users resulting from the intended uses is acceptable. The %AEL is set at 3% (without gloves) when exposure is only considered during cleaning since NYNA D+ PATE is supplied in sachet (see Annex 6 for detailed calculations). However, gloves are recommended to help prevent rodent-borne disease. Moreover, the mention “do not open the sachet” has to be added in the label of the product.

Secondary exposure

No relevant secondary exposure is expected for professional users, thus no unacceptable risk has been identified.

2.7.3.2 Risk for non-professional users and the general public

The estimated exposures for the non-professional users are compared to the systemic AEL of difenacoum set in the Assessment report ($1.1x10^{-6}$ mg/kg bw/day for short, medium and long-term exposures).

Primary exposure

Based on the risk assessment of the active substance, the risk for non-professional users resulting from the intended uses is acceptable. The %AEL is set at 1% when exposure is only considered during cleaning since NYNA D+ PATE is only supplied in sachet (see Annex 7 for detailed calculations).

Secondary exposure

Based on a reverse scenario, more than 0.3 mg of product per day (corresponding to 0.003% of a 10 g paste NYNA D+ PATE) should be ingested by infant to exceed the AEL. This indicates that infants are at significant risk of poisoning. Therefore, even if NYNA D+ PATE contains a bittering agent which reduces the likelihood of ingestion, the baits should be placed in bait boxes which do not allow access to children in secured areas. Product label (“do not open the sachet”) and good practice advise users to prevent access to bait by children and infants.

2.7.3.3 Risk for consumers via residues

Based on the intended uses, no food risk assessment was performed.

Table 2.7.3-1: Summary of risk characterisation for professional and non-professional users

Scenario	AEL (mg/kg bw/d)	Exposure (mg/kg bw/d)	%AEL	Risk
Exposure only during cleaning (NYNA D+ PATE supplied and applied in sachet)				

Professional (without gloves)	$1,1x10^{-6}$	$3,4x10^{-8}$	3	**Acceptable**
Non-professional (without gloves)	$1,1x10^{-6}$	$1,1x10^{-8}$	1	**Acceptable**

2.8 Risk assessment for the environment

2.8.1 Fate and distribution of the active substance, difenacoum, in the environment

The summary of information about the active substance is carried out with the data from the CAR of difenacoum owned by the Activa/Pelgar Difenacoum & Brodifacoum Task Force. No new ecotoxicological information on the active substance difenacoum has been submitted in the product dossier.

2.8.1.1 Biodegradation of difenacoum

According to the OECD tests 301B and 302D, difenacoum is not readily or inherently biodegradable. No studies on degradation in soil is available, but using the calculated value of Kp of 1.34 and considering the absence of biodegradation of difenacoum, it can be assumed that half life in soil is over 300 days. It was assumed during technical meeting (TMII-04) that no further degradation studies are needed for intended uses in building.

So the risk assessment is based on the assumption that difenacoum is not readily biodegradable and a half life in soil is over 300 days.

2.8.1.2 Hydrolysis as a function of pH

According to the test OECD 111, the half-life (DT_{50}) of difenacoum is over 1 year at pH 4, 7 and 9 at 25℃. The active substance is hydrolytically stable.

2.8.1.3 Photolysis in water

The active substance undergoes rapid photodegradation. Half-life varied from 0.6 hours to 3.8 hours. Greater than 80% photolysis was noted to have occurred by around five hours. Two breakdown products above 10% of the initial difenacoum concentration were detected and the proposal for the identification of structures was made. The phodegradation is regarded as a minor removal process for difenacoum and the exposure to water is low, therefore it was stated that no further characterisation of metabolites was requested.

2.8.1.4 Photodegradation in air

Photodegradation characteristics of the active substance have been estimated using the EPIWIN v. 3.12 programme in the CAR of the Task Force Difenacoum dossier. Difenacoum has an estimated half-life of approximately 2 hours, therefore it is predicted to have a negligible effect on stratospheric ozone. It is predicted not to be a potential greenhouse

gas. Finally, difenacoum has a low volatility (Henry's law constant< 0.046 $Pa.m^3.mol^{-1}$) and emissions to the air compartment are expected to be low.

2.8.1.5 Distribution

2.8.1.5.1 Adsorption/desorption

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 ionise at environmental relevant pH. Difenacoum has also a low water solubility and a high log Kow.
According to the Technical Guidance Document (TGD) Part 3, Table 4, the QSAR equation used to calculate log Koc from log Kow (7.62, a QSAR estimation) is:

log Koc = 0.81 log Kow + 0.1 (chemical class: Predominantly hydrophobics)

The properties of difenacoum may hamper the estimation of log Kow that is why it should be considered with some caution. The calculated log Koc is 6.27 and Koc = 1 871 544.

In the difenacoum dossier it has been stated that, according to its behaviour, the active substance would not be mobile and would be expected to absorb irreversibly to soil particles. Significant leaching could be expected to occur only in recently contaminated soil under alkaline conditions. Under other conditions, binding to the inorganic component of soil would be largely irreversible. The rate of binding is likely to be limited by steric hindrance of reaction in forming the cation bridge from the organic material.

2.8.1.5.2 Accumulation

The aquatic BCF has been estimated with calculation method because the fish bioconcentration test was invalid. In the absence of valid measured log Kow, the estimated value of log Kow used is 7.6. This value allows to calculate an estimated BCF for fish : 9010 (according to EPIWIN v 3.12) and 35 645 (Equation 75, TGD).
This log Kow is also entered the equation 82d of the TGD to get a $BCF_{earthworm}$ equal to 477 729.

The calculations show that difenacoum has a considerable bioaccumulation potential in aquatic and terrestrial organisms.

2.8.2 Effects of the active substance on environmental organisms

2.8.2.1 Aquatic compartment (including water, sediment and STP)

Difenacoum is very toxic to aquatic organisms. Difenacoum was equally toxic to fish (LC_{50}= 0.33 mg a.s/L, OECD 203), daphnia (EC_{50}= 0.91 mg a.s/L, OECD 202) and algae (E_bC_{50} =0.14 mg a.s/L, OECD 201). Nevertheless, a lower fish test result (LC_{50}=0.064 mg/L) is available in the difenacoum dossier of Sorex Limited. Therefore, it is used for the derivation of $PNEC_{water}$ in the difenacoum Task force dossier as recommended in the CAR of the difenacoum dossier.

In the absence of any ecotoxicological data for sediment-dwelling organisms, the $PNEC_{sediment}$ was calculated using the equilibrium partitioning method.

Difenacoum has shown to degrade photolytically in water in laboratory conditions and it may form degradation products exceeding 10% of the parent compound. The metabolites are not considered to have ecotoxicological significance, because photolysis is considered to be a minor transformation path for difenacoum and the exposure to water via the STP is expected to be low.

Difenacoum did not cause any effects on the activated sludge respiration inhibition up to the nominal concentration of 999.7 mg/L (OECD 209). Because all test concentrations exceeded the water solubility of difenacoum, the water solubility of 0.48 mg/L will be used as $PNEC_{STP}$.

2.8.2.2 Atmosphere

No data are available on the biotic effects in the atmosphere. Difenacoum is not expected to contribute to global warming, ozone depletion in the stratosphere, or acidification on the basis of its physical or chemical properties.

2.8.2.3 Terrestrial compartment

Difenacoum caused no toxic effects on earthworms up to the nominal concentration of 994 mg/kg dry weight (OECD 207). Difenacoum may not be bioavailable to earthworms in soil which would explain the low toxicity. No studies on soil microorganisms or plants were submitted.

The photolysis degradation products are not considered ecotoxicologically relevant because the direct exposure of difenacoum to soil is expected to be low.

Toxicity of difenacoum in birds increased with exposure time. Difenacoum was considered as moderately toxic in acute oral exposure (LD_{50}= 153 mg/kg bw), toxic in 5-day dietary test (LC_{50}=1.4 mg/kg feed) and very toxic in the reproduction test (NOEC= 0.31 mg/kg water, exposure via drinking water). Several dose related effects were detected in the reproduction test: increased adult mortality, increased mortality of 14-day old hatchlings, increased liver and spleen weights in adult females, a declining trend in number of eggs laid/hen/day, declining trend in viability of eggs. Due to methodological deficiencies the reproduction test is not considered to represent the worst case, and therefore the $PNEC_{oral}$ of birds was derived from the dietary test. Difenacoum is very toxic to mammals, and rats seem to be particularly susceptible. The $PNEC_{oral}$ for birds and mammals has been used for the risk characterization of primary and secondary poisoning.

2.8.2.4 PBT assessment

Due to the properties of persistence, accumulation and toxicity of difenacoum, this substance fulfills the PBT criteria.

2.8.2.5 Non compartment specific effects relevant to the food chain

As already stated in the previous sections, difenacoum is concern for bioaccumulation with a calculated log Kow of 7.62, a high predicted aquatic BCF of 9 010 (US EPA EPIWIN) or 35 645 (TGD) and a high predicted terrestrial BCF of 477 729 (TGD). The active substance is not readily biodegradable and is of low solubility (0.5 mg/L pH7). Therefore, difenacoum has a considerable bioaccumulation potential in aquatic and terrestrial organisms.

The primary concern is from predators eating the rodent carcasses and earthworms which have ingested the active substance absorbed to soil. In guidance document for PT14, the active substance is considered to be placed in protected bait point. Therefore, a risk should be taken into account for primary poisoning mainly for birds and mammals of equal or smaller size than the target rodents. Also when target animals carry bait away from e.g. bait stations, non-target animals may be exposed. For the risk characterization of primary poisoning, the $PNEC_{oral}$ described in section 2.8.2.7 will be used.

Also requiring consideration are predators eating fish or earthworms which have accumulated difenacoum from water and soil. The secondary exposure should be taken in consideration. In the CAR of difenacoum, one acceptable study was reported where effects of difenacoum are studied in Barn Owls which have been exposed to poisoned mice. However, the $PNEC_{oral}$ for birds and mammals are derived from a bird 5-day dietary test and a 90-day subchronic test in rat provided in the Activa/Pelgar Difenacoum Task Force dossier as described below (section 2.8.2.6)

2.8.2.6 Effects assessment of metabolites formed in target organisms

A metabolism study presented in the Activa/Pelgar Difenacoum Task Force Annex I inclusion dossier (doc IIIA-6.4 of the CAR of difenacoum) shows that total excreted radioactivity in rat faeces and urine (7 days after single dosing, low and high dose) was 41-71% of the dose administered. Two major faecal metabolites F7 and F8 (max 11.3% and 7.3%, respectively) were identified as isomers of hydroxylated difenacoum. Two other major metabolites, F5 and F6 (max 12.2% and 8.0 %, respectively) were characterised as isomers of difenacoum-based structure which formed glucuronide conjugates. Unchanged difenacoum was present at maximum at 2.9 %. The excretion and retention of radioactivity was also investigated after the final dose following administration of seven consecutive daily oral doses, no substantial differences in excretion patterns between single and repeated level oral doses was observed.

No information on toxicity of these four major metabolites is available. Considering that the metabolites could be potent as anticoagulants, the sum of these four metabolites and unchanged difenacoum in faeces will be taken into account in PEC calculation with assumption that the toxicity of metabolites is comparable to parent (data from the validated CAR of the Activa/Pelgar Difenacoum Task Force Annex I inclusion dossier). Therefore in the environmental exposure calculations, it is assumed that 40% of excreted amount in urine and faeces is metabolised and that 40 % of administered total amount is unchanged difenacoum in faeces (data from the validated CAR of the Activa/Pelgar Difenacoum Task

Force Annex I inclusion dossier). These assumptions represent a worst case for release.

2.8.2.7 Summary of PNEC

2.8.2.7.1 PNEC for aquatic organisms:

The $PNEC_{water}$ is derived from the lowest available LC_{50} value 0.064 mg/L (fish test) with an assessment factor of 1000 as only data on acute toxicity is available. Therefore,

PNECwater = 0.06 µg/L

2.8.2.7.2 PNEC for sediment-dwelling organisms:

In the absence of data on sediment-dwelling organisms, the $PNEC_{sediment}$ is derived from the equilibrium partitioning method.

$PNEC_{sediment}$ = 2.51 mg/kg wet weight.

2.8.2.7.3 PNEC for STP micro-organisms:

As described in section 2.8.2.1, the water solubility of 0.48 mg/L is used as the $PNEC_{STP}$.

$PNEC_{STP}$ = 0.48 mg/L

2.8.2.7.4 PNEC for terrestrial organisms:

The $PNEC_{soil}$ is derived from the experimental data. An assessment factor of 1000 was applied to the LC_{50} > 994 mg/kg issued from an earthworms study to derive the $PNEC_{soil}$.
$PNEC_{soil}$ = 0.994 mg/kg dry weight (0.877 mg/kg wet weight)

Nevertheless, as only one experimental test result is available, the $PNEC_{soil}$ derived with the equilibrium partitioning method (EPM) from the aquatic PNEC has also be taken into account :
$PNEC_{soil}$ = 2.04 mg/kg wet weight

Because the $PNEC_{soil}$ derived from the earthworms test is the lowest, it will be used for the risk characterization. So,

PNECsoil = 0.994 mg/kg dry weight (0.877 mg/kg wet weight)

2.8.2.7.5 PNEC for birds and mammals

$PNEC_{oral}$ for birds is derived from the LC_{50} of 1.4 mg/kg food origin from the 5-day dietary test. The appropriate assessment factor according to the TGD is 3000. In order to

transform the LC_{50} to LD_{50}, LC_{50} is multiplied with average food consumption (13.5 g) and divided by average body weight 71.3 g. The food consumption and body weight are averaged for all treatment groups and over the 5-day exposure period. The resulting LD_{50} is 0.3 mg/kg bw/d. The $PNEC_{oral}$ value kept for the risk assessment is:

$PNEC_{oral}$ for birds = 0.5 µg/kg food equivalent to
$PNEC_{oral}$ for birds = 0.1 µg/kg bw/d

$PNEC_{oral}$ for mammals is derived from the NOAEL of 0.03 mg/kg bw/d origin from the 90-day subchronic test in rat (Doc IIIA6.4.1 of the CAR of difenacoum). The NOAEL is transformed to NOEC (concentration in food) by multiplying with the conversion factor of 20 (TGD, Table 22). The appropriate assessment factor according to the TGD is 90. The $PNEC_{oral}$ value kept for the risk assessment is:

$PNEC_{oral}$ for mammals = 7 µg/kg food equivalent to
$PNEC_{oral}$ for mammals = 0.3 µg/kg bw/d

The $PNEC_{oral}$ for birds and mammals have been used for the risk characterization of primary and secondary poisoning.

Table 2.8.2-1: summary of the difenacoum PNECs

Compartment		**Test Value**	**AF**	**PNEC Unit**
Aquatic	$PNEC_{water}$	LC_{50} =0.064 mg/L	1000	0.064 µg/L
	$PNEC_{sediment}$	PNECwater in eq. 70 (TGD)		2.51 mg/kg wet weight
	$PNEC_{STP}$	Water solubility= 0.48 mg/L		0.48 mg/L
Terrestre	$PNEC_{soil}$	LC50 >994 mg/kg	1000	0.994 mg/kg dry weight 0.877 mg/kg wet weight
	$PNEC_{oral\ for\ birds}$	LC_{50} =1.4 mg/kg food LD_{50}= 0.3 mg/kg bw/d	3000	0.5 µg/kg food eq. to 0.1 µg/kg bw/d
	$PNEC_{oral\ for\ mammals}$	NOEC= 0.6 mg/kg food NOAEL=0.03 mg/kg bw/d	90	7 µg/kg food eq. to 0.3 µg/kg bw/d

2.8.3 Effects on environmental organisms for biocidal product NYNA D+ PATE

It is important to notice that the applicant did not provide ecotoxicological data about the biocidal product NYNA D+ PATE. So all the environment risk assessment of NYNA D+ PATE is based on data obtained from the active substance, difenacoum.

Bronopol is used in the biocidal product as preservative. This substance is classified as ”Very toxic for aquatic organisms” according to the Directive 67/548/CEE. Moreover, the substance is also notified in the frame of biocidal directive and evaluated by Spanish Competent Authority to be included in the Annex I of the Directive 98/8/CE.

Therefore, as no data with the biocidal product is available, FR CA considered that the environmental risk assessment should take into account bronopol.

Data of the acute toxicity of bronopol on aquatic organisms are available in the material safety data sheet:

EC_{50} (72 h) = 0.4 mg/L (*Algae*)
EC_{50} (48 h) = 1.4 mg/L (*Daphnia magna*)
LC_{50} (96 h) = 41.2 mg/L (*Oncorhynchus mykiss*)
EC_{50} > 50 mg/L (Bacteria)

According to the Directive 67/548/CEE, bronopol is classified R50: Very toxic to aquatic organisms with a specific concentration limit: C>2.5%.

Based on CLP regulation 1272/2008, the classification of bronopol is Aquatic Acute 1 – H400: Very toxic to aquatic life.

Nevertheless, in the concentration used in NYNA D+ PATE, the substance does not contribute to the classification of the biocidal product.

As no enough data is available to carry out a complete risk assessment with the substance bronopol, this assessment should be reviewed after the inclusion of bronopol in the Annex I of the Directive 98/8/CE.

2.8.4 Environmental exposure assessment

Exposure scenarios are defined as a set of conditions about sources, pathways and use patterns that quantify the release of the substance from processing, use and disposal into soil, water, air and waste. To describe the possible release of rodenticides from its use and disposal, the exposure scenarios for PT14 introduced in EUBEES ESD (2003), with an addendum endorsed at the 23rd CA meeting Nov. 2006 are used.

In accordance with EUBEES ESD (2003) and TGD for Risk Assessment (2003), a quantitative approach is used in the risk assessment for NYNA D+ PATE biocidal product. Quantitative PEC estimations are performed for the relevant environmental compartments for difenacoum. The different PEC values are derived from model calculations, but available measured data (e.g. difenacoum metabolism in rat) are also taken into consideration.

The product NYNA D+ PATE is a ready-to-use impregnated paste product with 0.005% of difenacoum, the active substance, and bronopol as preservative. The product is used as 10 g paste portions packed in paper sachet. The packed impregnated paste is placed in secured bait stations. According to the applicant, the product is intended to be used in bait boxes inside industrial, commercial and residential buildings. Bait points are inspected and replenished once a week when bait taken is observed.

The available data about the treatment campaign are extracted from the applicant's dossier:

- Duration of a treatment campaign: 28 d,

- Rat application rates: up to 180 g of product / bait point separated by 5-10 meters,
- Mouse application rates: up to 30 g of product / bait point separated by 1-2 meters
- The NYNA D+ PATE baits are placed only in bait stations,
- The product is used inside buildings only,
- Number of bait stations: 20 inside, 5 meters apart for rats, 1 meter for mice,
- Day 1: Treatment with 18 x 10 g product per box for rat, 3 x 10 g per box for mouse,
- Day 7, 14 and 21: bait refilling.

As the product is applied indoor only, no environmental compartment is exposed to NYNA D+ PATE. Nevertheless primary and secondary poisoning cannot be excluded. Indeed, pets living in treated buildings could be exposed directly to the product. Moreover even if the product is applied inside buildings, rats can live 3 to 11 days before dying. Therefore, they have the time to escape outside buildings and to be eaten by predators.

Primary and secondary poisoning calculations are carried out considering the 'in and around buildings' scenario from the EUBEES ESD PT14 as a worst case scenario in view of the fact that the product is applied inside buildings only.

2.8.4.1 PEC in surface water and sediment

Exposure of surface water and sediment after the treatment with rodenticides is only relevant for indoor application of liquid poisons, residues from mixing and cleaning (ESD PT14) when a release is foreseen via the STP. As NYNA D+ PATE is a solid form and is intended to be used indoor only, no indirect or direct exposure to surface water and sediment is expected.

2.8.4.2 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 a potential for rapid photo-oxidative degradation in the air (half-life about two hours). The exposure of air is therefore considered negligible for the application of NYNA D+ PATE biocidal product.

2.8.4.3 PEC in soil and groundwater

As NYNA D+ PATE is intended to be used indoor only, no exposure to soil and groundwater is expected.

2.8.4.4 Non compartment specific exposure relevant to the food chain (primary and secondary poisoning)

2.8.4.4.1 Primary poisoning

The risk assessment for the primary poisoning presented below was extracted from the Annex I inclusion dossier for the active substance considering that difenacoum concentration is identical in the product NYNA D+ PATE and in the representative product presented for the Annex I inclusion. Primary poisoning calculations are carried out

considering the ‘in and around buildings’ scenario from the EUBEES ESD PT14 as a worst case scenario in view of the fact that the product is applied inside buildings only.

According to ESD (Larsen, 2003), primary poisoning hazard to mammals and birds (both wild and domestic) can be considered small in the scenario “in and around buildings”. In use scenarios where difenacoum is placed in protected bait point, there is the risk for primary poisoning mainly for birds and mammals of equal size or smaller as the target rodents, which may be able to enter the bait stations. Also when target animals carry bait away from e.g. bait stations, non-target animals may be exposed.

Worst case exposure estimations are based on the equations and default values proposed by the ESD (Larsen, 2003). Some defaults parameters may be replaced by product-specific properties.

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 has access to difenacoum product. **The worst case Tier 1 PEC_{oral} is 50 mg/kg** (difenacoum present at 0.005% w/w in NYNA D+ PATE) and is used in quantitative risk assessment for the long-term situation.

According to ESD (Larsen, 2003) a Tier 2 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 animal is calculated with equation **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 (section 2.8.2.6) 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 used in calculations also for non-target animals as there is no other data available. Calculations for ETE and EC values for worst case and realistic worst case situations are presented in the table below. According to the guidance agreed at 23rd Competent Authority meeting, these values are used for qualitative risk assessment of primary poisoning in acute situation.

Table 2.8.4.4-1 : Expected concentrations of difenacoum in non-target animals in the worst case (Step 1[1]) and realistic worst case (Step 2[2]) for acute situations with and without elimination

Species		Body weight (g)	Daily mean food intake (dw) (g)	Rodenticide consumption (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	4563	600	2.28	1.37	1.64	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	*Vulpes vulpes*	5700	520 [5]	520	4.56	3.28	2.73	1.97
Representing General non-target mammal		5700	287[3]	287	2.5	1.5	1.8	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 ESD3.2.1. 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.

Calculations of the expected concentrations (EC) for 5 days exposure considering elimination are calculated according to ESD equation 21 as a worst case i.e. AV, PT and PD are set to 1. According to the guidance agreed at 23rd CA meeting, EC5 values are used for quantitative risk assessment of primary poisoning in the long-term situation.

Table 2.8.4.4-2 : Expected concentrations of difenacoum (EC5) in non-target animals for the long-term situations (worst case).

Species		Body weight(g)	Daily mean food intake (dw) (g)	Rodenti-cide con-sumption (g)	Expected concentration (EC_5) of a.i. in the animal after 5 days exposure, elimination taken into ac-count (mg/kg bw)
Dog	*Canis familiaris*	10000	456[3]	456	8.43
Pig	*Sus scrofa*	80000	2520[3] (600)[4]	600	0.52
Pig, young	*Sus scrofa*	25000	969[3] (600)[4]	600	1.57
Fox Representing General non-target mammal	*Vulpes vulpes*	5700 5700	520[5] 287[3]	520 287	5.95 3.33
Tree sparrow	*Passer montanus*	22	7.6	7.6	22.56
Chaffinch	*Fringilla coelebs*	21.4	6.42	6.42	19.58
Wood pigeon	*Columba palumbus*	490	53.1	53.1	7.05
Pheasant	*Phasianus colchicus*	953	102.7	102.7	7.04

1avoidance (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 ESD3.2.1. 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.

Among the anticoagulant poisoning incidents, dogs are common victims. The intoxication of dogs is easily detected as they live together with man. Intoxication incidents of wild animals may often remain unobserved. Small non-target rodents, such as voles, and small, granivorous birds can feed on rodenticidal baits because they can pass through the entrance hole of a bait station. Exposure may also arise if target animals carry bait away from the bait station. The domestic animals at risk are dog, pig and hen. Birds eating cereal and weed seeds like sparrows, pigeons and pheasants are possible wild species that may be at risk of primary poisoning.

2.8.4.4.2 Secondary poisoning

Secondary poisoning via the aquatic food chain

As no exposure of the aquatic compartment is foreseen with the use of NYNA D+ PATE inside buildings, no risk assessment for secondary poisoning through the aquatic food chain is required.

Secondary poisoning via the terrestrial food chain

As no exposure of the terrestrial compartment is foreseen with the use of NYNA D+ PATE inside buildings, no risk assessment for secondary poisoning through the terrestrial food chain is needed.

Secondary poisoning for the rodent-eating mammal or the rodent-eating bird

As secondary poisoning assessment according to the TGD part II considers the oral intake of a chemical only via fish or worms, another food chain rodenticide (bait) →rodent → rodent-eating mammal or rodent-eating bird is assessed in the EUBEES ESD PT14.

The risk assessment for the secondary poisoning presented below was extracted from the Annex I inclusion dossier for the active substance considering that difenacoum concentration is identical in the product NYNA D+ PATE and in the representative product presented for the Annex I inclusion. Secondary poisoning calculations are carried out considering the 'in and around buildings' scenario from the EUBEES ESD PT14 as a worst case scenario in view of the fact that the product is applied inside buildings only.

According to ESD (Larsen, 2003) document, for uses in and around buildings it is assumed that predators among mammals and birds may occur inside buildings or they may hunt rats in the immediate vicinity of buildings (parks and gardens or further away), also scavengers may search for food close to buildings and thus secondary poisoning through poisoned rats exists. Secondary poisoning hazard can only be ruled out completely when the rodenticide is used in fully enclosed spaces so that rodents cannot move to outdoor areas or to (parts of) buildings where predators may have access.

For estimation of secondary poisoning risk through poisoned rats, tiered approach is presented in the ESD:

- The Tier 1 assessment of secondary poisoning is based on the concentration in the predators or scavenger's food i.e. poisoned rodents (concentration in food); the predator is assumed to catch the rodent after last meal on day 5 or day 14.
- The Tier 2 assessment of long-term secondary poisoning is based on the expected concentration in predators compared to $PNEC_{oral}$ expressed as a daily dose; the predators accumulate difenacoum by feeding on poisoned target rodents during one day (rodents ate baits every day during 5 and 14 days).

Therefore, 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),

$$EC_n = \sum_{n=1}^{n-1} ETE \times (1 - El)^n$$

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 details in section 2.8.2.6. Results are presented in the following table.

Table 2.8.4.4-3 : 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.

Tier 1 PEC_{oral} for short term situation is calculated according to the equation 22 in ESD (Larsen, 2003):

PEC oral, predator = (ECn +ETE) x F_{rodent}

using value 1 for F_{rodent} (non-target animal consume 100% of their daily intake on poisoned rodents).

where:

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.

These values, presented in Table 2.8.4.4-4 below, are used for qualitative risk assessment of secondary poisoning in acute situation.

- Tier 1 PEC_{oral} for long term situation is calculated similar way, but the F_{rodent} is set to 0.5, which means that it is assumed that non-target animal consume 50 % of their daily intake on poisoned rodents. These values, presented in Table 2.8.4.4-4 below, are used for Tier 1 quantitative risk assessment of secondary poisoning in the long-term situation.

Table 2.8.4.4-4 : Predicted environmental concentrations of difenacoum in food of predator (PEC_{oral}) 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'	17.49	8.75	3.5
$PEC_{oral,predator}$ on day 14 'chronic'	8.74	4.37	1.75

- Tier 2 for long-term exposure: according to the CAR of difenacoum, 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 presented in the table 2.8.4.4-5 below are used for Tier 2 quantitative risk assessment of secondary poisoning in the long-term situation.

Table 2.8.4.4-5 : 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

2.8.5 Risk characterisation for the environment

Risk characterization for the environment is done quantitatively by comparing predicted environmental concentrations (PEC) and the concentrations below which effects on organism will not occur (PNEC) according to the guidance in Technical guidance document (TGD, 2003) and 'Emission scenario document for biocides used as rodenticides' (Larsen, 2003, ESD PT14).

The environmental risk characterization has been carried out for difenacoum.

2.8.5.1 Primary poisoning

Concentration of the bait is compared to the $PNEC_{oral}$ expressed as the concentration in food.

Table 2.8.5.1-1 : Tier 1 risk characterisation of primary poisoning.

	PEC mg/kg food	PNEC mg/kg food	PEC/PNEC
Birds	50	0.0005	100 000
Mammals	50	0.007	7 143

With a Tier 1 Approach, the risk for primary poisoning in birds and mammals is not acceptable.

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.

Table 2.8.5.1-2 : Tier 2 risk characterisation of primary poisoning

Species		PEC EC_5 µg/kg bw	$PNEC_{oral}$ µg/kg bw/d	PEC/PNEC
Dog	*Canis familiaris*	8 430	0.3	28 100
Pig	*Sus scrofa*	520	0.3	1 733
Pig, young	*Sus scrofa*	1 570	0.3	5 233
Fox	*Vulpes vulpes*	5 950	0.3	19 833
Fox, representing general non-target mammal		3 330	0.3	11 100
Tree sparrow	*Passer montanus*	22 560	0.1	225 600
Chaffinch	*Fringilla coelebs*	19 580	0.1	195 800
Wood pigeon	*Columba palumbus*	7 050	0.1	70 400
Pheasant	*Phasianus colchicus*	7 040	0.1	70 400

With a Tier 2 Approach, the risk for primary poisoning is not acceptable for the non-target animals.

The risk characterisation indicates a very high risk to non-target mammals and birds from direct eating of bait. Primary poisoning incidents can be minimised by preventing the access of non-target animals to the baits. 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 is stated at the EU level that 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.

Nevertheless, as the product NYNA D+ PATE is intended to be used indoor and in bait stations only, primary poisoning can therefore be considered negligible as domestic

animals can be kept away from the product, and wild animals other than rats and mice are not expected to be found inside buildings.

2.8.5.2 Secondary poisoning

The only relevant scenario of secondary poisoning in the case of an indoor application only is for the rodent-eating mammal or bird.

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 qualitative assessment indicates that birds are likely to survive and mammals are likely to die if they eat poisoned rats (Table 2.8.5.2-1). The species specific sensitivity differences or other aspects normally covered by the assessment factors are not taken into account in the qualitative assessment.

Table 2.8.5.2-1 : Qualitative assessment of acute secondary poisoning

	EC in rat on day 5 after last meal mg/kg	Birds LD50 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 assessment of secondary poisoning

The Tier 1 assessment of secondary poisoning is based on the concentration in the predator's or scavenger's food, i.e. poisoned rodents. The rodents are assumed to consume entirely the 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 baits in 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 catch the rodent after last meal on day 5 or day 14. Only resistant rodents are assumed to eat bait 14 day. The calculation of concentrations in rodents is explained in detail in Section 2.8.4.4.2. The $PNEC_{oral}$ is based on the highest concentration causing no effects in the test with long-term exposure. The derivations of PNECs are explained in Section 2.8.2.7.5.

Table 2.8.5.2-2 : Tier 1 risk characterization of secondary poisoning. Expected concentration in target rodents is compared to the $PNEC_{oral}$ expressed as concentration in food. Rodents are assumed to consume entirely bait (PD=1). Half of the predator's diet is poisoned rodents (F_{rodent}=0.5).

	PEC EC in rodent µg/kg	$PNEC_{oral}$ µg/kg food	PEC/PNEC
Rodents caught on day 5 after meal			
Birds	5760	0.5	11 520
Mammals	5760	7	823
Rodents caught on day 14 after meal			
Birds	8740	0.5	17 480
Mammals	8740	7	1 249

The Tier 1 risk characterization shows that there is an unacceptable risk for secondary poisoning and birds are at higher risk due to lower $PNEC_{oral}$ (Table 2.8.5.2-2).

Resistant rodents can feed on the poisoned baits longer and accumulate higher difenacoum residues than non-resistant rodents. Resistant rodents can continue to feed difenacoum up to two weeks, while the non-resistant rodents stop feeding after 5 days. Based on the calculations, the resistant rodents cause about 1.5 times higher risk for secondary poisoning of birds and mammals than non-resistant rodents.

Tier 2 assessment of secondary poisoning

In the Tier 2 assessment of long-term secondary poisoning the expected concentration in predators is compared to $PNEC_{oral}$ expressed as a daily dose. The predators accumulate difenacoum by feeding on poisoned target rodents during one day. The rodents are assumed to eat entirely the bait (PD = 1), whereas 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 baits in five or fourteen successive days. The susceptible rodents are assumed to stop feeding after 5 days, but resistant rodents are assumed to continue feeding until day 14. The calculation of expected concentrations is explained in detail in Section 2.8.4.4.2.

Table 2.8.5.2-3: Tier 2 risk characterization of secondary poisoning. The difenacoum 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

The Tier 2 risk characterization shows a high risk for secondary poisoning (Table 2.8.5.2-3). The $PNEC_{oral}$ expressed as a dose is approximately equal for birds and mammals, and the sensitivity of the species used in calculations is determined predominantly by the ratio of daily food consumption to body weight so that the higher ratio results in the higher risk. No data are available on the sensitivity of the example species (the species listed in Table 12 of the ESD) to difenacoum. Only one day exposure of predators is assumed in the ESD, but it is mentioned that predators could be exposed over several days. This would mean higher accumulation in predators, because daily elimination of difenacoum from the predators is assumed to be less than the ingested amount. On the other hand, it is unlikely that all worst case assumptions would materialize simultaneously in nature. It is likely that in the long-term exposure, the prey rodents do not eat only the bait and also the fraction of poisoned rodents in the predator's diet can be lower than 50%. The resistant rodents cause somewhat higher risk for predators than non-resistant rodents, but the difference is smaller than in the Tier 1 assessment.

The applicant has submitted two experimental studies on the secondary poisoning in Barn Owls. Tier 1 and Tier 2 risk characterization are recalculated for the Barn Owl on the basis of the measured concentrations in rats and mice with the experimental data provided in the Difenacoum Task Force Annex I inclusion dossier. The risks are significantly lower than with the ESD calculations however they are still considerably higher than 1 indicating an unacceptable risk for secondary poisoning of the Barn Owls.

A review of the available monitoring data was provided in the Difenacoum Task Force Annex I inclusion dossier to characterize the risk of secondary poisoning. Most of the incidents were due to misuse, abuse or unspecified use. Only few incidents resulted from approved use of difenacoum. However, like theoretical calculations and experimental results, the monitoring data clearly show that difenacoum poses an unacceptable risk for secondary poisoning. While all available information indicates risk, it does not tell the frequency of secondary poisoning incidents among wildlife.

However, considering the fact that NYNA D+ PATE is intended to be used indoor only, it can be assumed that, applying use restrictions (such as collecting dead rodents), the risk for secondary poisoning will be lower.

Nevertheless, in order to reduce the risk of secondary poisoning, it is very important to follow the use instructions of the rodenticide baits (see section 3). The risk reduction measures are considered in the section 2.9.

2.9 Measures to protect man, animals and the environment

The measures to protect man, animals and the environment are extracted from the Doc IIIB8 and updated according to the information submitted in the NYNA D+PATE dossier.

2.9.1 Recommended methods and precautions concerning handling, use, storage, transport or fire

Bait stations are provided to avoid the possibility for children and domestic animals to be in contact with the biocidal product. For professionals and non-professionals, the product is wrapped individually in heat-sealed paper sachet for preventing dermal and inhalation exposure. Size of containers is appropriate to intended uses to be done. Professional users have to be trained before using the biocidal product.

<u>Handling and use</u>
The product must be used in accordance with the label.
Appropriate protective clothes and gloves are recommended for users during handling and cleaning. Placing the baits in secured bait station out of the reach of children and domestic animals is necessary. The bait station must be secured with no possibility for children and domestic animals to open the bait boxes or to access to the bait stations. The bait station must not offer the possibility for rodents to take baits away in the nests. Collecting unconsumed baits and dead rodents must be done every week during the treatment.
Avoid exposure to high temperature and strong oxidizing agents.

Storage
Keep out of the reach of children and domestic animals; store away from food, drink and animal feeding stuff, and away from light. Keep container tightly closed in fresh and dry places.

Methods and precaution concerning transport
Not regulated.

Methods and precautions concerning fire
Suitable extinguishing media: foam and chemical powders. Water must not be used for environmental safety reasons.
Special protective equipment for fire-fighters: wear protective clothing and self-contained breathing apparatus.
Risk of toxic gases in fumes (carbon monoxide, carbon dioxide…)

2.9.2 Emergency measures in case of an accident

Personal precautions
Inhalation: no action should be necessary.
Ingestion: if swallowed, seek medical advice immediately and show container or leaflet. A treatment with vitamin K1 should be necessary during a long period.
Skin or eye contact: wash immediately with plenty of water.

Environmental precautions
Avoid uncontrolled disposal into the environment because of danger for non-target animals.
Do not throw the product on the ground, into a water course, into the sink or down the drain.
Any spillage should be cleared up immediately

2.9.3 Disposal considerations

Unconsumed products and packaging should be disposed according to national or local regulation.
Empty containers must not be reused.
The product is ready-to-use and applied directly in bait stations indoors only. The baits which have not been consumed by rodents and dead rodents are kept away by operators.
It is not expected that any direct release to soil compartment would occur as a direct result of the indoor application of NYNA D+ PATE. However, if a spill occurs, baits must be collected with a shovel and stored in hermetic containers and eliminated according to national or local regulation.

3 Proposal from authority in charge of the efficacy and risk assessment (ANSES) for the decision to be adopted by the competent authority in charge of the decision (French Ministry of Ecology)

This section is a proposal from the authority in charge of the efficacy and risk assessment (ANSES) for the decision to be adopted by the competent authority in charge of the decision (French Ministry of Ecology).
In case of inconsistency between the risk assessment and the decision, only the original and signed decision has a legal value. The decision specifies the terms and conditions to the making available on the market and use of the biocidal product.

The product NYNA D+ PATE has shown a sufficient efficacy for the control of mice *(Mus musculus)* and rats (*Rattus norvegicus* and *Rattus rattus*) inside buildings (private and public, including farm buildings).

Resistant strategies management has to be taken into account and difenacoum must not be used in areas where resistance to this substance is suspected.

The human health and environmental risk was assessed considering that NYNA D+ PATE is available in sachet and loaded in secured bait points.

Based on the assessment of data on the active substance and NYNA D+ PATE containing 0.005% difenacoum, human health risks for professional using the product are acceptable without gloves. However, gloves are recommended to prevent rodent-borne diseases.

The risk is also acceptable for non-professional users. However, accidental ingestion of baits poses a risk to infants. Adequate measures for protection and risk mitigation have to be applied during use to control especially the risk from secondary exposure.

No studies were conducted with NYNA D+ PATE for the environment part. The environmental risk assessment has been carried out by the French authority in charge of the risk assessment with data from the CAR of difenacoum. The environmental risk is considered as acceptable for the intended uses. The specific use restriction must be applied to reduce the risk for primary and secondary poisoning.

Specific use restriction and issues accounted for product labelling:

- The product must be applied inside building only.
- For professionals: Gloves have to be worn to help prevention against rodent-borne disease.
- The product must be supplied and applied in sachets.
- Apply strict hygiene measures: do not eat, drink or smoke during handling of the product and wash hands after use of the product.

- Use only in tamper-resistant bait stations. Tamper-resistant bait stations should be clearly marked to show that they contain rodenticides and that they should not be disturbed.
- The product and the sachet labels have to mention “Do not open the sachet”.
- The size of the package placed on the market should be proportionate to the duration of the treatment and to the user category..
- In order to prevent primary and secondary poisoning for children, for domestic and wild animals, bait point must be securely deposited, and placed in non accessible aeras.
- Unconsumed baits and dead rodents must be collected every week during the treatment, at least as often as when baits are checked and/or replenished. Dispose of dead rodents in accordance with local requirements.
- Authorisation holder should assure the availability of the bait box to professional users.
- Keep away from food, animal feedstuffs or drinking water.
- Do not clean the bait stations with water between two applications.
- Do not throw the product on the ground, into a water course, into the sink or down the drain and into the environment
- Remove all baits after treatment and dispose of them in accordance with local requirements.
- Store the product away from light
- The packaging must not be re-used or recycled.
- To avoid resistance and because of cross-resistances occurrence to second-generation anticoagulants,
    - the product label has to contain on resistance management for rodenticides.
    - The amount of bait per bait station and distances between bait stations must be respected. Products have always to be used in accordance with the label.
    - The treatment has to be alternated with active substances having different mode of action.
    - Integrated pest management (combination of chemical control, physical and hygienic measures) has to be taken into account.
    - The level of efficacy has to be monitored (periodic check), and the case of reduced efficacy has to be investigated for possible evidence of resistance.
    - Resistant management strategies have to be developed, and difenacoum must not be used in an area where resistance to this substance is suspected or established.

- The users should report straightforward to the registration holder any alarming signals which could be assumed to be resistance development.

Further information is required:

A 2-year storage stability study is required in post registration. The study should be performed with test items in quantity sufficient to overcome the heterogeneity problem. Intermediate results at one year have to be provided.

Reactivity toward heat-sealed paper sachet of 10g (the tested material should be clearly identified) is required in post registration too.

The authorization holder has to report any observed resistance incidents to the Competent Authorities or other appointed bodies involved in resistance management every two years.

Annex 0: Practical use of Biocides - PT14

This chart reflects the claim uses and the results of the risk assessment for each of them. Please refer to the decision/SPC for final authorised uses.

NYNA D+ PATE **Type of formulation (paste)**	**Target organism (rat, mice…)***	**User category (professional/non professional)***	**Area of use (sewers, in and around buildings, indoor only, open areas, waste dumps,…)***	**Dosage claimed expressed in g/bait point, for high and low infestation(if appropriate)**	**Dosage Validated expressed in g/bait point, for high and low infestation (if appropriate)**	**Time delay of the action of the product**	**Frequency and method of controls**	**Size(s) of the bait (g/bloc, g/grain, g/sachet, g/paste …)**	**Distance between 2 bait points, for high and low infestation (if appropriate)**	**Methods of application of the bait (ex: pre-filled secured bait box)**	**Package details : Individual packaging (yes/no)* **for more details please fulfill the column related to primary packaging and secondary packaging***	**Primary packaging : type : bulk, individual wrapping…/ nature: bucket, bottle, sachet…/ material: paper, polyethylene…/ sizes**	**Tertiary packaging**	**Conclusion of the efficacy and risk assessment**
NYNA D+ PATE Formulation : paste	Rats (*Rattus norvegicus* and *Rattus rattus)*	Professional	In the buildings	180 g	180 g	4-10 days	Once a week Over a period of 28 days for application,	10g/paste	secured bait point separated by 5-10 m	Sachets in secured bait	Yes	Primary packaging: Heat sealed paper sachet Secondary packaging: plastic heat-sealed bags 100 g - 1kg	Cardboard box 100g – 1kg Varnished iron circular box 200 – 600g Bucket (PP or PEHD) 2 – 18kg	Acceptable
	Mice (*Mus musculus*)	Professional	In the buildings	30 g	30 g	4-10 days	Once a week Over a period of 28 days for	10g/paste	secured bait point separated by 1-2 m	Sachets in secured bait	Yes	Primary packaging: Heat sealed paper sachet	Cardboard box 100g – 1kg	Acceptable

						application,					Secondary packaging: plastic heat-sealed bags 100 g - 1kg	Varnished iron circular box 200 – 600g Bucket (PP or PEHD) 2 – 18kg	
NYNA D+ PATE Formulation : paste	Rats (*Rattus norvegicus* and *Rattus rattus)*	Non Professional In the buildings	180 g	180 g	4-10 days	Once a week Over a period of 28 days for application,	10g/paste	secured bait point separated by 5-10 m	Sachets in secured bait	Yes	Primary packaging: Heat sealed paper sachet Secondary packaging: plastic heat-sealed bags 100 g - 1kg	Cardboard box 100g – 1kg Varnished iron circular box 200 – 600g Bucket (PP or PEHD) 2 – 18kg	Acceptable
	Mice (*Mus musculus*)	Non Professional In the buildings	30 g	30 g	4-10 days	Once a week Over a period of 28 days for application,	10g/paste	secured bait point separated by 1-2 m	Sachets in secured bait	Yes	Primary packaging: Heat sealed paper sachet Secondary packaging: plastic heat-sealed bags 100 g - 1kg	Cardboard box 100g – 1kg Varnished iron circular box 200 – 600g Bucket (PP or PEHD) 2 – 18kg	Acceptable

Annex 1: List of studies reviewed

List of <u>new data</u>[7] submitted in support of the evaluation of the active substance

Section No	Reference No	Author	Year	Title	Owner of data	Letter of Access		Data protection claimed	
						Yes	No	Yes	No
A2	CH-299-2009	Garofani S.	2009	Difenacoum technical: complete analysis of five batch samples	Activa	☒	☐	☒	☐
A2.7	CH-297-2009	Garofani S.	2009	Difenacoum technical: validation of the analytical method for the determination of the active ingredient content	Activa	☒	☐	☒	☐
A2.8	CH-298-2009	Garofani S.	2009	Difenacoum technical: validation of the analytical method for the determination of significant impurities content	Activa	☒	☐	☒	☐
A3.3	CH – 082/2010	Garofani S.	2010	Difenacoum technical: determination of the colour, odour and physical state	Activa	☒	☐	☒	☐
A4.2 (c)	CEMR-4470	Marshall L.	2009	Validation of a method for the determination of Difenacoum residues in sediment	Activa / PelGar Brodifacoum and Difenacoum Task Force	☒	☐	☒	☐
A4.2 (c)	CEMR-4469	Marshall L.	2009	Validation of a method for the determination of Difenacoum residues in animal Matrices (Liver and Muscle) and Crop matrix	Activa / PelGar Brodifacoum and Difenacoum Task Force	☒	☐	☒	☐

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

Section No	Reference No	Author	Year	Title	Owner of data	Letter of Access		Data protection claimed	
A4.2 (e)	CEMR-4469	Marshall L.	2009	Validation of a method for the determination of Difenacoum residues in animal Matrices (Liver and Muscle) and Crop matrix	Activa / PelGar Brodifacoum and Difenacoum Task Force	☒	☐	☒	☐

List of <u>new data</u> submitted in support of the evaluation of the biocidal product

Section No	Reference No	Author	Year	Title	Owner of data	Letter of Access		Data protection claimed	
						Yes	No	Yes	No
B3.1, 3.4, 3.6	10-920010-009	Demangel B.	2010	Physico chemical tests on NYNA D+ PATE	Triplan	☐	☒	☒	☐
B3.2, 3.3	09-920010-001	Da Costa C, Teiche A.	2010	Physico chemical tests on NYNA D+ PATE	Triplan	☐	☒	☒	☐
B3.5, 3.7	10-920010-010	Ferron N.	2010	Physico-chemical tests before and after accelerated storage procedure for 14 days at 54 ± 2℃ on NYNA D+ PATE in compliance with CIPAC MT 46.3 (CIPAC Handbok J – 2000)	Triplan	☐	☒	☒	☐
B4.1.1	10-920010-008	Ricau H	2010	Validation of an analytical method for the determination of difenacoum in NYNA D+ BLOC SP in compliance with CIPAC/3807R	Triplan	☐	☒	☒	☐
B4.1.2	10-920010-012	Ricau H	2010	Validation of an analytical method for the determination of difenacoum in NYNA D+ PATE in compliance with CIPAC/3807R	Triplan	☐	☒	☒	☐
B5.10.2./ 01	SB-2010-005	Barbieux S Grolleau G	2010	Efficacy laboratory study of NYNA D+, paste rodenticide containing 0.005% difenacoum with albino house mice (*Mus musculus*).	Triplan	☐	☒	☒	☐

Section No	Reference No	Author	Year	Title	Owner of data	Letter of Access		Data protection claimed	
B5.10.2./ 03	SB-2010-007	Barbieux S Grolleau G	2010	Efficacy field study for NYNA D+, paste rodenticide containing 0.005% difenacoum with black rats (*Rattus rattus*).	Triplan	☐	☒	☒	☐
B5.11	Published data	Pelz HJ *et al*	2005	The genetic basis of resistance to anticoagulants in rodents.	Published data	☐	☒	☐	☒
B5.11	Published data	Lasseur R et *al*	2006	Les rongeurs font de la résistance. Nuisibles et parasites	Published data	☐	☒	☐	☒
B5.11	Published data	Myllymäki A	1995	Anticoagulant resistance in Europe: Appraisal of the data from the 1992 EPPO questionnaire	Published data	☐	☒	☐	☒
B5.11	Published data	Kerins G M et al	2001	The interaction between the indirect Anticogulant Coumatetralyl and Calciferol (vitamin D3) in Warfarin-resistant rats (*Rattus norvegicus*)	Published data	☐	☒	☐	☒
B5.11	Published data	Desideri D *et al*	1978	Note préliminaire sur la mise en évidence à Marseille d'une résistance au coumafène chez *Rattus rattus*.	Published data	☐	☒	☐	☒
B6.1.1	TAO423-PH-10/0345	Colas S	2010	NYNA D+ BLOC SP evaluation of acute oral toxicity in rats – acute toxic class method.	Triplan	☐	☒	☒	☐

Section No	Reference No	Author	Year	Title	Owner of data	Letter of Access		Data protection claimed	
B6.1.2	TAD-PH-10/0345	Colas S	2010	NYNA D+ BLOC SP evaluation of acute dermal toxicity in rats.	Triplan	☐	☒	☒	☐
B6.2.1	IC-OCDE-PH-10/0345	Colas S	2010	NYNA D+ BLOC SP assessment of acute dermal irritation.	Triplan	☐	☒	☒	☐
B6.2.2	IO-OCDE-PH-10/0345	Colas S	2010	NYNA D+ BLOC SP assessment of acute eye irritation.	Triplan	☐	☒	☒	☐
B6.3	SMK-PH-10/0345	Colas S	2010	NYNA D+ BLOC SP Assessment of sensitizing properties on albino Guinea pigs maximization test according to Magnusson and Kligman.	Triplan	☐	☒	☒	☐

Annex 2: Analytical methods residues – active substance

Difenacoum

Date: 12/2011

Matrix, action levels, relevant residue and reference

matrix	limit	relevant residue	reference or comment
plant products	LOQ= 0.01mg/kg	Difenacoum	
food of animal origin	LOQ= 0.01mg/kg	Difenacoum	
soil	LOQ= 0.0214 µg/g	Difenacoum	
drinking water	LOQ = 0.05 µg/l	Difenacoum	
surface water	LOQ = 0.05 µg/l	Difenacoum	
air	Unnecessary due to the low vapour pressure of difenacoum		
body fluids / tissues	LOQ= 0.01mg/kg	Difenacoum	

Methods suitable for the determination of residues (monitoring methods)

Methods for products of plant origin

reference	matrix	LOQ (mg/kg)	principle	comment	owner
Marshall, L., 2009, Method Validation for the Determination of Difenacoum in Animal Matrices (Liver and Muscle) and Crop Matrix (Oilseed Rape), CEM Analytical Services Limited, Study CEMR-4469	Oil-seed rape	LOQ= 0.01mg/kg	*LC-MS/MS*		Activa / PelGar Brodifacoum and Difenacoum Task Force

Methods for foodstuffs of animal origin

reference	matrix	LOQ (mg/kg)	principle	comment	owner
Marshall, L., 2009, Method Validation for the Determination of Difenacoum in Animal Matrices (Liver and Muscle) and Crop Matrix (Oilseed Rape), CEM Analytical Services Limited, Study CEMR-4469	Meat	LOQ= 0.01mg/kg	*LC-MS/MS*		Activa / PelGar Brodifacoum and Difenacoum Task Force

Methods for soil

reference	LOQ (mg/kg)	principle	comment	owner
Morlacchini, M., 2006, Residues determination of Brodifacoum, Difenacoum and Bromadiolone in soil, CERZOO (Italy), Study CZ/05/002/Activa/Soil	LOQ= 0.0214 µg/g	*HPLC – UV-VIS*		Activa / PelGar Brodifacoum and Difenacoum Task Force

Methods for sediment

reference	LOQ (mg/kg)	principle	comment	owner
Marshall, L., 2009, Validation of a Method for the Determination of Difenacoum Residues in Sediment, CEM Analytical Services Limited, Study CEMR-4470	LOQ= 0.01mg/kg	*LC-MS/MS*		Activa / PelGar Brodifacoum and Difenacoum Task Force

Methods for drinking water and surface water

reference	matrix	LOQ (µg/l)	principle	comment	owner
Martinez M.P. 2005. Difenacoum Technical: Validation of the Analytical Method for the Determination of the Residues in Drinking, Ground and Surface waters, Test Laboratory of ChemService S.r.l. ChemService Study No. CH-288/2005	Water	LOQ = 0.05 µg/l	*HPLC – MS/MS*		Activa / PelGar Brodifacoum and Difenacoum Task Force

Methods for air

reference		LOQ (µg/m3)	principle	comment	owner
Unnecessary due to the low vapour pressure of difenacoum					

Methods for body fluids/tissue

reference	matrix	LOQ (mg/kg)	principle	comment	owner
Marshall, L., 2009, Method Validation for the Determination of Difenacoum in Animal Matrices (Liver and Muscle) and Crop Matrix (Oilseed Rape), CEM Analytical Services Limited, Study CEMR-4469	Liver	LOQ= 0.01mg/kg	*LC-MS/MS*		Activa / PelGar Brodifacoum and Difenacoum Task Force

Annexe 3: Efficacy of the Active Substance from its Use in the Product

Note that this table have been summarized by the applicant and FR CA had assessed it.

Test substance	Test organism(s)	Test conditions/concentration applied exposure time	Test results: effects, mode of action, resistance	Reference
NYNA D+ 0.005% difenacoum	Albino house mice (*Mus musculus)* 5 males and 5 females per lot (3 lots)	**Laboratory CEB n°1** Lot efficacy (no-choice food), Lot acceptance (free-choice food) Lot control animals. Intoxication duration: 3 days with daily measurements of mortality and consumption. Acclimation: 3 days in individual cage. At D0: - Control lot: - 25 g per animal of usual food - Acceptance lot: - 3 paste sachets (21.5 to 28.5 g) + 25 g of usual food per animal, - Efficacy lot :- 3 paste sachets (21.5 to 28.5 g) per animal 3 consecutive days with daily consumption measurements. Mortality was observed during 21 days from the first day of intoxication and noted every 24 hours.	In this study, the control animals' lot has been a good reference because operators noticed the increase of body weight within 21 days of food consumption. The efficacy lot's bait consumption is 25% to 55% lower than the controls' one. But in the acceptance lot we noticed that the bait was overwhelmingly preferred to usual food even the overall consumptions were close to the acceptance lot's ones. In both cases, **100% mortality** has been reached (average of 7.4 days for the no-choice test and 6.7 days for the free-choice one). Difenacoum efficacy has been confirmed and we think that the obtained results can be extrapolated to rats. No resistance is observed in this trial	Barbieux S Grolleau G SB-2010-005 (B5.10.1)
NYNA D+ 0.005% difenacoum	Black rats (*Rattus rattus)*	**Field study CEB n°2** The used method is relative and allows knowing the bait biocidal product efficacy on a rat's population without knowing the precise population size. Daily food consumptions are measured. 56 stations have been placed in a pig farm. Acclimation phase of mice to their new environment. Then: - Pre-baiting phase: 500 g grains per day per station (67 days). - Poisoning phase: 500 g baits per day and per bait	A field study conducted in a pig farm with black rats using paste sachets, containing 50 ppm difenacoum, has given excellent results: - Pre-baiting stage: 3734.7 g - Post-baiting stage : 211.7 g within 8 days of intoxication. **The assessed efficacy is 94.3%.** Because of the great rats' mistrust and high food competition (pig farm), the pre-baiting duration has been exceptionally long (67 days instead of 18 to 21 days for the brown rats). The tested bait has proven very effective; however we	Barbieux S Grolleau G SB-2010-007 (B5.10.2)

		station (5 days). - Post-baiting phase: 500 g grains oat per station and per day (11 days). Daily consumptions were recorded at interval of 24 hours at the beginning and the end of pre-baiting stage, at the end of post-baiting phase and during the two first days during poisoning phase.	cannot assess its acceptance because the rats stored the blocks. **Despite this low mortality rate, it is in adequacy with the results assessed in the laboratory with albino house mice and confirms the efficacy of the product** No resistance is observed in this trial	

Difenacoum

Threshold Limits and other Values for Human Health Risk Assessment

Date: 12/2011

Summary			
	Value	Study	SF
AEL long-term	0.0000011 mg/kg bw/day	Teratogenicity in rabbit	600
AEL medium-term	0.0000011 mg/kg bw/day	Teratogenicity in rabbit	600
AEL acute	0.0000011 mg/kg bw/day	Teratogenicity in rabbit	600

Inhalative absorption: 100%

Oral absorption: 68 %

Dermal absorption: 0.047 % for wax block bait (Activa Pelgar study) – 3 % for pellet and grain baits (Sorex study)

Classification	
with regard to toxicological data (according to the criteria in Dir. 67/548/EEC)	Current classification: T+ ; R28, R48/25 - N; R50/53 Proposed classification by the RMS: T+; R26/27/28, Repr. Cat. 1, R61 - T; R48/23/24/25 - N ; R50/53
with regard to toxicological data (according to the criteria in Reg. 1272/2008)	Current classification: Acute Tox 2, H300; STOT RE 1, H372 ; Aquatic Acute 1, H400; Acute chronic 1, H410 Proposed classification by the RMS: Acute Tox 2, H330, H310, H300; Repr. 1A, H360D; STOT RE 1, H372; Aquatic Acute 1, H400; Acute chronic 1, H410

NYNA D+ PATE

Date: 12/2011

General information
Formulation Type: paste
Active substance(s) (incl. content): 0.005% difenacoum

Acute toxicity, irritancy and skin sensitisation of the preparation (Annex IIIB, point 6.1, 6.2, 6.3)

Rat LD_{50} oral (OECD 420) > 2000 mg/kg bw
Rat LD_{50} dermal (OECD 402) > 2000 mg/kg bw
Rat LC_{50} inhalation (OECD 403): no study submitted
Skin irritation (OECD 404): non irritant
Eye irritation (OECD 405): non irritant
Skin sensitisation (OECD 406; Maximisation test): Study submitted but not acce

Acute toxicity tests:

Route	**Method Guideline**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD_{50}/LC_{50}**	**Remarks**	**Reference**
Oral	OECD 423	Sprague Dawley 6 Females	2000mg/kg bw	> 2000mg/kg bw	No mortality	Colas S. 2010
Dermal	OECD 402	Sprague Dawley 5/sex	2000mg/kg bw	> 2000mg/kg bw	No mortality Neither cutaneous nor systemic effects	Colas S. 2010

Skin irritation test:

Species	**Method**	**Average score 24 and 72 h**		**Reversibility yes/no**	**Result**	**Reference**
		Erythema	Oedema			
Albinos NZ rabbit 3 males	OECD 404 Semi-occlusive, 4h	0	0	na	Not irritant	Colas S. 2010

Eye irritation test:

Species	**Method**	**Average Score (24h, 48h, 72h)**				**Result**	**Reversibility yes/no**	**Remarks**	**Reference**
		Cornea	Iris	Redness Conjunctiva	Chemosis				
Albinos NZ rabbit 3 Males	OECD 405	0	0	1.7 0.7 1	1 0.3 0.3	Not irritant	Redness reversible at Day 3-4 Chemosis reversible at Day 2-3	Other findings: Slight corneal opacity in 1 animal at 1h, reversible at Day 1 Iris congestion at 1h, reversible at	Colas S. 2010

								Day 1	

Sensitisation test:

Species	Method	Number of animals sensitized/total number of animals	Result	Reference
Albino Guinea pig Treated group : 11 females	OECD 406: Maximization test Induction phases: 0.5 ml at 40% in distilled water by topic route Challenge phases: A cup containing the test item at 5 and 2.5% in distilled water	36 % (4/11) after the first challenge at 5% at 24 and 48 hours. No reaction at 72 hours. No reaction at 2.5 % 9 % (1/11) after the second challenge at 5% at 24 hours. No reaction at 48 hours. No reaction at 2.5 %	No skin sensitizing	Colas S. 2010 Not acceptable

Additional toxicological information (e.g. Annex IIIB, point 6.5, 6.7)	
Short-term toxicity studies	None
Toxicological data on active substance(s) (not tested with the preparation)	None
Toxicological data on non-active substance(s) (not tested with the preparation)	None
Further toxicological information	None

Classification and labelling proposed for the preparation with regard to toxicological properties (Annex IIIB, point 9)	
Directive 1999/45/EC	None
Regulation 1272/2008/EC	None

NYNA D+ PATE

Date: 12/2011

Exposure assessment

Exposure scenarios for intended uses (Annex IIIB, point 6.6)

Primary exposure of professionals – Control of rats

	Component	CAS	Potential Dermal Total [mg/kg/d]	Actual Dermal Total [mg/kg/d]	Inhalation Exposure [mg/m³]	Model
Sachet not considered: exposure during loading and cleaning (worst case)						
Tier 1 (without gloves)	Difenacoum	56073-07-5	$2.4x10^{-6}$	$2.4x10^{-6}$	negligible	Cefic study
Tier 2 (with gloves; penetration factor: 10%)	Difenacoum	56073-07-5	$2.4x10^{-6}$	$2.4x10^{-7}$	negligible	Cefic study
Sachet considered: exposure only during cleaning considered (reasonable case)						
Tier 1 (without PPE)	Difenacoum	56073-07-5	$3.4x10^{-8}$	$3.4x10^{-8}$	negligible	Cefic study

Primary exposure of professionals – Control of mice

	Component	CAS	Potential Dermal Total [mg/kg/d]	Actual Dermal Total [mg/kg/d]	Inhalation Exposure [mg/m³]	Model
Sachet not considered: exposure during loading and cleaning (worst case)						
Tier 1 (without gloves)	Difenacoum	56073-07-5	$4.3x10^{-7}$	$4.3x10^{-7}$	negligible	Cefic study
Sachet considered: exposure only during cleaning considered (reasonable case)						
Tier 1 (without PPE)	Difenacoum	56073-07-5	$3.4x10^{-8}$	$3.4x10^{-8}$	negligible	Cefic study

Risk assessment – Control of rats

Scenario	Component	CAS	AEL [mg/kg/d]	Absorption [%]		Inhal ext [mg/m^3]		Derm syst [mg/kg bw/d]		Risk
				inh	derm	Expo	%AEL	Expo	%AEL	
Sachet not considered: exposure during loading and cleaning (worst case)										
Tier 1 (without gloves)	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$2.4x10^{-6}$	217	Unacceptable
Tier 2 (with gloves; penetration factor: 10%)	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$2.4x10^{-7}$	22	Acceptable
Sachet considered: exposure only during cleaning considered (reasonable case)										
Tier 1 (without gloves)	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$3.4x10^{-8}$	3	Acceptable

Risk assessment – Control of mice

Scenario	Component	CAS	AEL [mg/kg/d]	Absorption [%]		Inhal ext [mg/m^3]		Derm syst [mg/kg bw/d]		Risk
				inh	derm	Expo	%AEL	Expo	%AEL	
Sachet not considered: exposure during loading and cleaning (worst case)										
Tier 1 (without gloves)	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$4.3x10^{-7}$	39	Unacceptable
Sachet considered: exposure only during cleaning considered (reasonable case)										
Tier 1 (without gloves)	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$3.4x10^{-8}$	3	Acceptable

Annex 7: Safety for non-professional operators and the general public

NYNA D+ PATE

Date: 12/2011

General information

Formulation Type: Paste

Active substance(s) (incl. content): Difenacoum (0.005%)

Difenacoum

Data base for exposure estimation

according to Appendix: Toxicology and metabolism – active substance/CAR

Exposure scenarios for intended uses (Annex IIIB, point 6.6)

Primary exposure: non-professional use
Secondary exposure, acute: child ingesting bait
Secondary exposure, chronic: none

Conclusion:

Exposure of non-professional users to the biocidal product containing difenacoum as active substance is considered acceptable, if the biocidal product is used as intended and all safety advices are followed.

The accidental ingestion of baits poses a risk to infants since the AEL is exceeded when infant ingests more than 0.3 mg of product per day (about 0.003% of a 10g paste).

Details for the exposure estimates:

Exposure assessment – Control of rats

	Component	CAS	Potential Dermal Total [mg/kg/d]	Actual Dermal Total [mg/kg/d]	Inhalation Exposure [mg/m³]	Model
Sachet not considered: exposure during decanting, loading and cleaning (worst case)						
Non professional	Difenacoum	56073-07-5	2.07×10^{-7}	2.07×10^{-7}	negligeable	Cefic study
Sachet considered: exposure only during cleaning considered (reasonable case)						
Non professional	Difenacoum	56073-07-5	1.1×10^{-8}	1.1×10^{-8}	negligeable	Cefic study

Exposure assessment – Control of mice

	Component	CAS	Potential Dermal Total [mg/kg/d]	Actual Dermal Total [mg/kg/d]	Inhalation Exposure [mg/m³]	Model
Sachet not considered: exposure during decanting, loading and cleaning (worst case)						
Non professional	Difenacoum	56073-07-5	$4.4x10^{-8}$	$4.4x10^{-8}$	negligeable	Cefic study
Sachet considered: exposure only during cleaning considered (reasonable case)						
Non professional	Difenacoum	56073-07-5	$1.1x10^{-8}$	$1.1x10^{-8}$	negligeable	Cefic study

Risk assessment - Control of rats

Scenario	Component	CAS	AEL [mg/kg/d]	Absorption [%]		Inhal ext [mg/m³]		Derm syst [mg/kg bw/d]		Risk
				inh	derm	Expo	%AEL	Expo	%AEL	
Sachet not considered: exposure during decanting, loading and cleaning (worst case)										
Non-professional	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$2.07x10^{-7}$	19	Acceptable
Sachet considered: exposure only during cleaning considered (reasonable case)										
Non-professional	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$1.1x10^{-8}$	1	Acceptable

Risk assessment - Control of mice

Scenario	Component	CAS	AEL [mg/kg/d]	Absorption [%]		Inhal ext [mg/m³]		Derm syst [mg/kg bw/d]		Risk
				inh	derm	Expo	%AEL	Expo	%AEL	
Sachet not considered: exposure during decanting, loading and cleaning (worst case)										
Non-professional	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$4.4x10^{-8}$	4	Acceptable
Sachet considered: exposure only during cleaning considered (reasonable case)										
Non-professional	Difenacoum	56073-07-5	$1.1x10^{-6}$	100	0.047	negligible	n.a	$1.1x10^{-8}$	1	Acceptable

Difenacoum

Date: 12/2011

Intended Use (critical application): Control of mice and rats
Active substance(s): Difenacoum
Formulation of biocidal product: Paste
Place of treatment: inside building (domestic, industrial and farm)

The product is a paste bait only used indoor in secured bait station. Collecting unconsumed baits and dead rodents must be done every week during the treatment so in these recommended conditions; no contamination is expected for feeding stuffs. Finally, according to the Assessment report on difenacoum, "*difenacoum baits should not be placed where food, feedingstuffs or drinking water could be contamined*".

The intended use descriptions of the difenacoum-containing biocidal products for which authorisation is sought indicate that these uses are not relevant in terms of residues in food and feed. No further data are required concerning the residue behaviour.

Product Assessment Report

Biocidal product assessment report related to product authorisation under Directive 98/8/EC

CONFIDENTIAL ANNEX

Formulation composition statement

NYNA D+ PATE

Triplan SA

December 2011

Internal registration/file no:	PB-10-00096
Authorisation/Registration no:	FR-2012-0003 (professional) / FR-2012-0051 (non-professional)
Granting date/entry into force of authorisation/ registration:	23 February 2012
Expiry date of authorisation/ registration:	31/03/2015 except where a decision of the European Commission extends the registration of the active substance
Active ingredient:	DIFENACOUM (CAS 56073-07-5)
Product type:	14 - Rodenticide

Competent Authority in charge of delivering the product authorisation:
French Ministry of Ecology
Department for Nuisance Prevention and Quality of the Environment
Chemical Substances and Preparation Unit
Grande Arche, Paroi Nord
92 055 La Défense cedex – FRANCE
autorisation-biocide@developpement-durable.gouv.fr

Authority in charge of the efficacy and risk assessment:
Anses - Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
Direction des Produits Réglementés
253 Avenue du Général Leclerc
94 701 Maisons-Alfort Cedex - FRANCE
biocides@anses.fr

Formulation composition statement

Name of the product : NYNA D+ PATE

Active Substance(s)

	Common Name	Chemical name	CAS number	Contents g/L or g/kg	Contents Other unit	Contents w/w (%)	Minimum purity
1	Difenacoum 2,5%	Premix (see below)	-	2	-	0,2	-

Co-formulant(s)

	Common Name	Chemical name	Function	CAS number	Contents g/kg	Contents Other unit	Contents w/w (%)	Substance of concern
2	Denatonium benzoate 10% + Monopropylene glycol	Premix (see below)		not applicable	0,4		0,04	No
3	Rapeseed oil	not applicable	adhesive	8002-13-9	220		22	No
4	BHT	2,6-ditert-butyl-4-methylphenol	Antioxidant	128-37-0	2		0,2	No
5	Sugar	not applicable	Sapidity	not	50,75		5,075	No

			agent	applicable				
6	Wheat flour	not applicable	carrier	not applicable	722,35		72,235	No
7	BLEU PHTALOFAST G	Copper, (29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32)-, (SP-4-1)-	Dyestuff	147-14-8	0,3		0,03	No
8	Mineral oil codex	White mineral oil codex (marcol 82)	Dispersant (pigment)	8042-47-5	1,2		0,12	No
9	Bronopol	2-bromo-2-nitropropane-1,3-diol	Preservative	52-51-7	1		0,1	No

Difenacoum 2,5% premix

					Contents			
	Common Name	**Chemical name**	**Function**	**CAS number**	**g/kg**	**Other unit**	**w/w (%)**	**Minimum purity**
1	Difenacoum	3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin	Active substance	56073-07-5	25	-	2,5	96,00%
								Substance of concern

2	Denatonium benzoate	phenylmethyl-[2- [(2,6-dimethylphenyl)amino]- 2-oxoethyl]-diethylammonium benzoate	Bittering agent	3734-33-6	5	-	0,5	No
3	Triethanolamine	-	Solvent	102-71-6	250	-	25	No
4	Polyethylene glycol 200	-	Solvent	25332-68-3	720	-	72	No

Denatonium benzoate 10% + Monopropylene glycol premix

					Contents			
	Common Name	**Chemical name**	**Function**	**CAS number**	**g/kg**	**Other unit**	**w/w (%)**	**Substance of concern**
1	Denatonium benzoate	phenylmethyl-[2- [(2,6-dimethylphenyl)amino]- 2-oxoethyl]-diethylammonium benzoate	Bittering agent	3734-33-6	100		10	No
2	Monopropylene glycol	propan-1,2-diol	Sapidity solvent	57-55-6	900		90	No