European Food Safety Authority

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CONCLUSION ON PESTICIDE PEER REVIEW

Peer review of the pesticide risk assessment of the active substance difenacoum[1]

Question No EFSA-Q-2008-391

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SUMMARY

Difenacoum is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004,[2] as amended by Regulation (EC) No 1095/2007.[3] This Regulation requires the European Food Safety Authority (EFSA) to organise upon request of the EU-Commission a peer review of the initial evaluation, i.e. the draft assessment report (DAR), provided by the designated rapporteur Member State and to provide within six months a conclusion on the risk assessment to the EU-Commission.

Finland being the designated rapporteur Member State submitted the DAR on difenacoum in accordance with the provisions of Article 22(1) of the Regulation (EC) No 2229/2004, which was received by the EFSA on 16 July 2007. The peer review was initiated on 3 March 2008 by dispatching the DAR for consultation of the Member States and the sole notifier Sorex Limited. Subsequently, the comments received on the DAR were examined and responded by the rapporteur Member State in the reporting table. This table was evaluated by the EFSA to identify the remaining issues. The identified issues as well as further information made available by the notifier upon request were evaluated in a series of scientific meetings with Member State experts in October 2008.

A final discussion of the outcome of the consultation of experts took place during a written procedure with the Member States in November-December 2008 leading to the conclusions as laid down in this report.

The conclusion was reached on the basis of the evaluation of the representative uses as a rodenticide as proposed by the notifier, which comprise manual application into protected bait boxes for rodent control. Full details of the representative use can be found in the attached endpoints.

[1] For citation purposes: Conclusion on pesticide peer review regarding the risk assessment of the active substance difenacoum. *EFSA Scientific Report* (2008) 218, 1-58.
[2] OJ L379, 24.12.2004, p.13.
[3] OJ L246, 21.9.2007, p.19.

Difenacoum consists of two diastereomeric pairs of enantiomers. A data gap was set for a new technical specification.

The representative formulated product for the evaluation was 'Neosorexa Pellets', a bait (ready-for-use) formulation (RB) containing 0.05 g/kg difenacoum.

Analytical methods as well as methods and data relating to physical, chemical and technical properties are available to ensure that quality control measurements of the plant protection products are possible, however a data gap was identified for storage stability study.

Monitoring methods to determine difenacoum residues in food/feed of plant and animal origin are not required, no MRLs are established. Adequate methods are available to monitor all compounds given in the respective residue definitions in soil and water. Adequate methods are available to monitor difenacoum residues in tissues, however a method for the determination of residues of difenacoum in body fluids (blood) was identified as a data gap.

With regard to its toxicological properties, difenacoum is a direct anticoagulant that interferes with the blood clotting mechanism by inhibiting the vitamin K epoxide reductase. The active substance is well absorbed following oral administration, and widely distributed within the body with the highest concentration in the liver. Based on the results of the acute toxicity studies, the proposed classification was **T+ R26/27/28 "Very toxic by inhalation, in contact with skin, and if swallowed"**. In repeated dose studies, no other toxic effect than reduced coagulation and haemorrhages were observed, leading to a short-term rat NOAEL of 0.03 mg/kg bw/day. In the *in vitro* genotoxicity studies, no gene mutation was induced in bacterial and mammalian cells, while two chromosome aberration tests gave positive results. As the three *in vivo* genotoxicity studies were negative, the overall conclusion is that difenacoum has no genotoxic potential. No multigeneration study was provided in the dossier. In the developmental studies with rats and rabbits, there was no evidence of teratogenicity. In rats, no developmental toxicity was observed at a dose maternally toxic. Foetal effects in rabbits were observed in both the test and control groups, and were concluded as not dose-related. However, the experts considered that difenacoum should be regarded as teratogenic based on the knowledge about analogous compounds (other antivitamin K anticoagulants in humans), and they agreed with the classification proposed by the Specialised Experts on Reproductive Toxicity (Ispra, 19-20 September 2006) i.e. **Reprotoxic Category 1, R61 "May cause harm to the unborn child"**.

The experts assumed that no contamination of crops would occur during the intended use, and concluded that the derivation of an acceptable daily intake and acute reference dose was not required. For the operator risk assessment, the agreed acceptable operator exposure level (AOEL) was 0.000017 mg/kg bw/day (or 17 ng/kg bw/day), based on the maternal NOAEL in the developmental rabbit study with the use of an overall safety factor of 300. The additional safety factor of 3 was justified by the severity of the toxicological effects of difenacoum, the higher potency of the second generation anticoagulants (such as difenacoum) compared to warfarin, and the much higher vulnerability of human foetuses to vitamin K deficiency compared to rodents. For the operator exposure assessment, the exposure estimates for the biocide use[4] were considered as a worst-case scenario, which gave an exposure of 52% of the AOEL without the use of personal protective equipment. No worker or bystander exposure

[4] evaluated under Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market

was expected due to the type of product assessed and the representative use in secured bait boxes.

Under the conditions of use as applied for (i.e. formulated bait in secure bait boxes), it is very unlikely that residues in food of plant or animal origin will occur. Therefore, it was concluded that the dietary consumer risk is negligible, and that data on the residue behaviour of difenacoum in plants and livestock animals are not required. No MRLs were proposed.

However, a situation has not been assessed where bait pellets are removed from bait boxes and hoarded by rodents because of their natural instinct. Depending on the treated area, this may lead to a situation where food or feed could become contaminated or where domestic animals might become exposed.

The consumer risk assessment is strictly based on the assumption of a 'no dietary exposure situation' for humans and livestock from the notified representative use, presuming that no contact of difenacoum with food, feed or drinking water will occur.

No reliable information is available on the route and rate of degradation of difenacoum in soil, though the information available indicates it has some persistence. The adsorption of difenacoum to soil would be expected to be pH-dependent at environmentally relevant pH, with lower adsorption expected at higher soil pH. Evidence from laboratory sieved soil column leaching studies indicates difenacoum would be expected to exhibit low mobility in soil including alkaline soils, though limitations in the design of experiments in three of the four soils investigated means it is not possible to conclude that difenacoum is immobile. Difenacoum is stable under conditions of sterile aqueous hydrolysis but undergoes rapid aqueous photodegradation. Information is not available on its behaviour in natural aerobic water/sediment systems. Though the available data are relatively limited, the available information is considered sufficient to complete an environmental exposure assessment at EU level for the applied for intended use, but only when formulated bait products are placed in secure bait boxes. When the product is used in this way, the potential for surface water body exposure was assessed as negligible. Also, when the product is used in this way, the potential for groundwater contamination by difenacoum above a toxicologically based concentration limit of 0.05 µg/L was considered to be low.

The acute, short-term and long-term risk to birds was assessed as high, if birds can get access to the baits. The acute risk of primary poisoning was assessed as low for larger mammals like dogs or pigs, but a high long-term risk was indicated. Risk mitigation measures are needed, which are proven to be efficient, to prevent birds and larger mammals gaining access to the baits (e.g. bait boxes). The risk to birds and mammals from secondary poisoning was assessed as high. It is more difficult to mitigate the risk from secondary poisoning of birds and mammals. The efficiency and applicability of risk mitigation in the context of the application in the field, such as removal of carcasses during and after the control campaign, is uncertain and would need some further consideration. A high risk was evident for small non-target mammals. No risk mitigation measures were proposed. It is unclear if the risk to small non-target mammals can be mitigated without reducing the efficacy of the product. Member States should be aware that it cannot be excluded that the suggested application of difenacoum in the field may also affect endangered/protected small mammal species potentially found in agricultural landscapes.

Difenacoum was very toxic to fish, aquatic invertebrates and algae. However, the risk to aquatic organisms was considered to be low because exposure of the aquatic environment was expected to be negligible from the applied for intended use.

The risk to bees, other arthropod species, earthworms, soil macro- and soil micro-organisms, terrestrial plants and biological sewage treatment plants was considered to be low because of negligible or low local point exposure.

Key words: difenacoum, peer review, risk assessment, pesticide, rodenticide

TABLE OF CONTENTS

BACKGROUND

Commission Regulation (EC) No 2229/2004 laying down the detailed rules for the implementation of the fourth stage of the work program referred to in Article 8(2) of Council Directive 91/414/EEC and amending Regulation (EC) No 1112/2002, as amended by Commission Regulation (EC) No 1095/2007, regulates for the European Food Safety Authority (EFSA) the procedure of evaluation of the draft assessment reports provided by the designated rapporteur Member State. Difenacoum is one of the 295 substances of the fourth stage, covered by the amended Regulation (EC) No 2229/2004 designating Finland as rapporteur Member State.

In accordance with the provisions of Article 22(1) of the Regulation (EC) No 2229/2004, Finland submitted the report of its initial evaluation of the dossier on difenacoum, hereafter referred to as the draft assessment report, received by the EFSA on 16 July 2007. Following an administrative evaluation, the draft assessment report was distributed for consultation in accordance with Article 24(2) of the Regulation (EC) 1095/2007 on 3 March 2008 to the Member States and to the sole notifier Sorex Limited, as identified by the rapporteur Member State.

The comments received on the draft assessment report were evaluated and addressed by the rapporteur Member State. Based on this evaluation, the EFSA identified and agreed on lacking information to be addressed by the notifier as well as issues for further detailed discussion at expert level.

Taking into account the requested information received from the notifier, a scientific discussion took place in expert meetings in October 2008. The reports of these meetings have been made available to the Member States electronically.

A final discussion of the outcome of the consultation of experts took place during a written procedure with the Member States in November-December 2008 leading to the conclusions as laid down in this report.

During the peer review of the draft assessment report and the consultation of technical experts no critical issues were identified for consultation of the Scientific Panel on Plant Protection Products and their Residues (PPR).

In accordance with Article 24c(1) of the amended Regulation (EC) No 2229/2004, this conclusion summarises the results of the peer review on the active substance and the representative formulation evaluated as finalised at the end of the examination period provided for by the same Article. A list of the relevant endpoints for the active substance as well as the formulation is provided in appendix A.

The documentation developed during the peer review was compiled as a peer review report comprising of the documents summarising and addressing the comments received on the initial evaluation provided in the rapporteur Member State's draft assessment report:

- the comments received,
- the resulting reporting table (revision 1-1, 18 June 2008),

as well as the documents summarising the follow-up of the issues identified as finalised at the end of the commenting period:

- the reports of the scientific expert consultation,
- the evaluation table (revision 2-1, 18 December 2008).

Given the importance of the draft assessment report including its addendum (compiled version of November 2008 containing all individually submitted addenda) and the peer review report with respect to the examination of the active substance, both documents are considered respectively as background documents A and B to this conclusion.

THE ACTIVE SUBSTANCE AND THE FORMULATED PRODUCT

Difenacoum is the ISO common name for 3-[(1*RS*,3*RS*;1*RS*,3*SR*)-3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin (IUPAC).

Difenacoum belongs to the class of coumarin rodenticides. Difenacoum is an indirect anticoagulant, which disrupts the normal blood-clotting mechanisms in the target animal, resulting in increased bleeding tendency and eventually, profound haemorrhage and death. Difenacoum is used in agriculture in plant protection situations for the control of rodents.

The representative formulated product for the evaluation was 'Neosorexa Pellets', a bait (ready-for-use) formulation (RB) containing 0.05 g/kg difenacoum, registered under different trade names in Europe.

The representative uses evaluated comprise manual application of measured amounts of product into protected bait boxes, at discrete locations throughout a rodent infested area, in plant protection situations in fields, in glasshouses and protection of crops stored in fields, for the control of rats (brown rat, *Rattus norvegicus* and black rat, *Rattus rattus*) and mice (*Mus domesticus/musculus*), in all EU countries. Protected bait boxes containing up to 200 g of product are used, at intervals of up to 10 metres, for rat control, and protected bait boxes containing up to 30 g of product are used for mouse control, at intervals of 1-2 metres. The number and timing of applications is dependent on the extent of the rodent infestation. An average rodent treatment should not continue beyond 35 days.

SPECIFIC CONCLUSIONS OF THE EVALUATION

1. Identity, physical/chemical/technical properties and methods of analysis

The minimum purity of the technical difenacoum could not be concluded on, as the PRAPeR 56 meeting of experts (October 2008) did not accept the technical specification for the active substance. No FAO specification exists.

Difenacoum consists of two diastereomeric pairs of enantiomers. The experts at the PRAPeR 56 meeting considered that the proposed specification is not supported by the 5-batch data, and a data gap was proposed for the applicant to provide a new specification in line with 5-batch data, including the ranges for the (1*RS*,3*RS)* pair (*trans*) to the (1*RS*,3*SR*) pair (*cis*) of isomers.

Clarification was also sought on the composition of the most recent batch, which had a lower purity and the content of some impurities increased, as well as an explanation of what happens with batches out-of-specification. The specification for the technical material should be regarded as provisional for the moment.

Besides the specification, the assessment of the data package revealed no issues that need to be included as critical areas of concern with respect to the identity, physical, chemical and technical properties of difenacoum or the respective formulation, however the following data gaps were identified:

- confirmatory data for the identity of three impurities in the technical material
- information concerning the purity of the starting materials
- surface tension according to EEC method A.5
- shelf-life study

The main data regarding the identity of difenacoum and its physical and chemical properties are given in appendix A.

Adequate analytical methods are available for the determination of difenacoum in the technical material and in the representative formulation (HPLC-UV), as well as for the determination of the respective impurities in the technical material (HPLC-UV).

Sufficient test methods and data relating to physical, chemical and technical properties are available to ensure that quality control measurements of the plant protection product are possible.

Monitoring methods to determine difenacoum residues in food/feed of plant and animal origin are not required, no MRLs are established.

LC-MS/MS methods, using positive chemical ionisation, are available to monitor residues of difenacoum in soil with LOQ of 0.01 mg/kg, and in surface and drinking/ground water with LOQ of 0.01 µg/L, respectively.

Analytical method for the determination of difenacoum residues in air was not submitted, however a monitoring method for air is not required in line with the proposal in section 4.3.

LC-MS/MS methods are available to monitor residues of difenacoum in meat and in tissues with LOQ of 0.01 mg/kg, however a data gap was identified for a method for the determination of residues of difenacoum in body fluids (blood).

2. Mammalian toxicity

Difenacoum was discussed at the PRAPeR 59 meeting of experts on mammalian toxicology (round 12, October 2008).

The proposed specification in addendum 1 to Volume 4 (August 2008) was not agreed by the PRAPeR 56 meeting of experts on identity, physical, chemical and technical properties (see section 1). Additionally, no detailed composition of the toxicological batches was available. However, the experts concurred that there was no concern regarding the impurities when taking account the high toxicity of the parent compound (which is originally proposed at a minimum purity of 96 % in the technical specification).

Under the biocide application[5], the active substance difenacoum has three notifiers and the rapporteur Member State is the same as for the pesticide application. The first notifier, Sorex Limited UK, was in possession of a full regulatory data package (according to Directive 98/8/EC), and has shared a number of studies under a legal agreement with a second notifier. However, no collaboration occurred with a third applicant. A complete new dossier has been evaluated by the rapporteur Member State, but the peer-review (written procedure) is not yet completed. For the pesticide authorisation, the only applicant was Sorex Limited UK, who made a lot of cross-references to the dossier for the biocide use. The meeting suggested that the rapporteur Member State shall communicate to the European Commission any further critical area of concern arising from the assessment of the data package for the third applicant under the biocide use.

The inhumane mode of action of difenacoum as a rodenticide was pointed out during the PRAPeR 59 meeting of experts, but this was also concluded to be a risk management issue.

[5] evaluated under Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market

2.1. Absorption, distribution, excretion and metabolism (toxicokinetics)

After oral administration in rats, difenacoum was rapidly absorbed. In the absence of bile excretion data, the experts agreed on the same oral absorption value as that adopted by the peer-review for difenacoum as a biocide, i.e. 82 % within 168 hours including the metabolites in faeces (considered as bioavailable since the liver is the major target organ). In the PRAPeR 59 meeting, it was noted that the third applicant for the use as a biocide had provided a new test (with bile excretion data) for the determination of the oral absorption value, but this was not part of the dossier under Council Directive 91/414/EC. The experts suggested that the rapporteur Member State would bring the results to the attention of the European Commission, if they lead to more critical endpoints.

Widely distributed, the highest concentration was found in the liver. The concentration in fat was relatively low, indicating that even with its high lipophilicity, difenacoum has a high affinity for specific binding sites in the liver, where a small percentage is accumulating after repeated exposure. The proposed metabolic pathway includes mainly glucuronidation of the 4-hydroxy group of the coumarin ring, and also hydroxylation of the aromatic rings. Up to five metabolites were found in the liver (0.4 to 11.4 % of the applied dose). Finally, the excretion of difenacoum occurs mainly via faeces (urine being only a minor route), with an initial rapid phase during the first 24 hours after dosing, but very slow on subsequent days.

2.2. Acute toxicity

In rat oral studies with the mixture *cis-trans*, the LD_{50} was 1.8 mg/kg bw in males and 2.6 mg/kg bw in females. A study with separate *cis-* and *trans-* isomers revealed that the *cis-*isomer is somewhat more toxic (male LD_{50} 1.17 mg/kg bw and female LD_{50} 1.6 mg/kg bw) than the *trans-* isomer (male LD_{50} 7.3 mg/kg bw and female LD_{50} 6.0 mg/kg bw).

After acute dermal exposure, a high toxicity was also observed in rats (LD_{50} 63 mg/kg bw with 95 % confidence limits of 34 to 85 mg/kg bw). Due to its low water solubility, the application of difenacoum on moistened skin is likely to have an effect on the systemic bioavailability of the compound. Therefore, the experts agreed with the rapporteur Member State to take into account the lower confidence limit for the LD_{50} of 34 mg/kg bw (below 50 mg/kg bw), and to propose the classification "Very toxic". It has to be noted that this classification had already been agreed in November 2006 by ECB (but not yet voted in an ATP), based on another study provided by the third applicant for the use as a biocide (and thus not available in the pesticide dossier).

In the acute studies by inhalation with rats, the LC_{50} was 3.6 to 5.8 µg/L/4h (head only, for both sexes). With regard to the skin and eye irritation tests, negative results were obtained as well as no effect of skin sensitisation in a maximisation test.

Based on these results, the proposed classification was **T+, R26/27/28 "Very toxic by inhalation, in contact with skin, and if swallowed"** (also agreed by ECB in November 2006, but not yet included in an ATP).

2.3. Short-term toxicity

After repeated oral administration in rats (90-day) and dogs (6-week), the adverse findings were related to the anticoagulant effect, leading to increased clotting time, haemorrhages in a wide range of tissues, and treatment related deaths.

For the 90-day rat study (Leuschner J, 2003), more detailed results were provided in the first revision of Volume 1 (August 2008). The proposed NOAEL of 0.03 mg/kg bw/day was confirmed by the meeting of experts, based on adverse effects at 0.1 mg/kg bw/day. A second 90-day rat study (Horner JM, 1991) was considered as not acceptable due to major methodological deficiencies (low number of animals, high mortality, and limited investigations).

In the 6-week oral dog study, considered as supplementary, the low dose of 0.01 mg/kg bw/day was a LOAEL based on changes in clotting times. As the study was terminated before scheduled completion and limited parameters were investigated, the results were considered as supplementary range-finding data. No conclusion could be drawn on the relative sensitivity of the dog in comparison to the rat.

Based on these results in rats after oral exposure (deaths at 0.1 mg/kg bw/day), the classification with R48 was proposed. Furthermore, based on the results of the acute toxicity studies and route-to-route extrapolation, the meeting of experts agreed with the rapporteur Member State's proposal to classify as **T; R48/23/24/25 "Toxic: Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed"**. It was noted that this classification had already been agreed under the ECB process in November 2006, but not yet included in an ATP.

2.4. Genotoxicity

Based on the results from *in vitro* studies (two Ames tests, a mouse lymphoma assay, two chromosome aberration tests, one with human lymphocytes and one with Chinese hamster lung cells), difenacoum did not induce gene mutations in bacteria or mammalian cells, but increased the number of chromosomal aberrations in cultured human and hamster cells.

Three *in vivo* studies were presented in the DAR, including tests for induction of micronuclei in bone marrow cells of rat and mouse, and an *in vivo/in vitro* UDS test with rat liver cells. All results were negative. Although there were clastogenic effects *in vitro*, the overall conclusion was that difenacoum has no potential for genotoxicity *in vivo*.

2.5. Long-term toxicity

No chronic or carcinogenic studies were provided in the dossier. The waiving of carcinogenicity studies in rodents was considered acceptable due to ethical reasons (rodents being the usual laboratory animals but also the target of difenacoum), the known mode of action (strong anticoagulant effect), the absence of genotoxic potential *in vivo*, and the current knowledge on analogous compounds (long-term administration of warfarin in humans as anti-clotting therapy for several decades showed no association with increased incidence of cancer). No classification for carcinogenic properties was considered necessary.

2.6. Reproductive toxicity

No toxicity study for the **fertility** parameters has been performed with difenacoum, due to ethical reasons, the known mode of action of difenacoum, and the possibility to use knowledge acquired with warfarin (an analogous compound). In the literature, there are no indications of impaired fertility associated with warfarin or vitamin K (hydroquinone) deficiency. It was concluded that there is currently no need to classify difenacoum for impaired fertility.

It was noted during the meeting that the third applicant for the use as a biocide had provided a rat multigeneration study with difenacoum, but this was not part of the dossier under Council Directive 91/414/EC. The experts suggested that the rapporteur Member State would bring this to the attention of the European Commission, if this results in more critical endpoints.

With regard to the testing for **teratogenicity**, difenacoum has been administered in rats and in rabbits in two developmental toxicity studies. In the rabbit study, the maternal NOAEL was 0.005 mg/kg bw/day based on increased coagulation times. The developmental NOAEL was 0.015 mg/kg bw/day (the highest dose tested) based on the absence of dose-related effects in foetuses. In the rat study, the maternal NOAEL was 0.03 mg/kg bw/day, and the developmental NOAEL was 0.09 mg/kg bw/day without evidence of embryotoxic or teratogenic potential.

The relationship between the placental transfer of difenacoum and the potential teratogenic effects was discussed by the meeting of experts. In the absence of data on the placental transfer, and based on the physico-chemical properties, difenacoum was assumed to pass the placenta. Additionally, the opinion of the Commission Working Group of Specialised Experts on Reproductive Toxicity (September 2006) was quoted. According to them, all anti-vitamin K rodenticides should collectively be regarded as human teratogens and classified as **Reprotoxic Category 1; R61 "May cause harm to the unborn child"** (this classification was still in discussion in ECB and should be finalized by EChA).

This proposal was supported by the rapporteur Member State and agreed during the meeting. It was also noted that more data are expected on the mechanism, and this will be submitted to the European Commission as soon as possible.

During the meeting, it was also mentioned that the third applicant for the use as a biocide had provided a new rabbit teratogenicity study with difenacoum, but this was not part of the dossier under Council Directive 91/414/EC. It was also suggested that the rapporteur Member State would bring the results to the attention of the European Commission, if they lead to more critical endpoints.

2.7. Neurotoxicity

In the absence of indications of neurotoxicity in the available studies, and due to the known mode of action of difenacoum, the waiving of neurotoxicity studies was accepted. Furthermore, the chemical structure of difenacoum is not similar or related to any compounds known to induce delayed neurotoxicity.

2.8. Further studies

No further toxicological data or assessment were provided or considered needed.

2.9. Medical data

During routine monitoring in workers producing the active substance and the formulated product, three rodenticide poisoning incidents occurred with successful recovery. It is not specified whether the poisonings were due to difenacoum, warfarin and/or brodifacoum. With the exception of these incidents, no adverse health effects induced by repeated and continual exposure to these anticoagulant rodenticides were observed. Cases of poisoning through contaminated food have also been reported for warfarin and brodifacoum (WHO, Environmental Health Criteria 175, 1995).

2.10. Acceptable daily intake (ADI), acceptable operator exposure level (AOEL) and acute reference dose (ARfD)

In the DAR, the maternal NOAEL of the rabbit developmental toxicity study (0.005 mg/kg bw/day) was proposed as the basis for the derivation of the reference values (ADI, AOEL and ARfD), with the application of a safety factor of 100 and an additional safety factor of 3. This was justified by the severity of the toxicological effects in the database, the higher potency of the second generation anticoagulants compared to warfarin, and the much higher vulnerability of human foetuses to vitamin K (hydroquinone) deficiency compared to rodents. Therefore, the proposed value for the ADI/AOEL/ARfD was 0.000017 mg/kg bw/day (17 ng/kg bw/day).

During the meeting, it was assumed that residues were unlikely to occur as a result of the intended uses (in bait boxes around the field margins), and therefore the derivation of an ADI and ARfD was not required (see also section 3.3).

With regard to the operator risk assessment, the proposed derivation by the rapporteur Member State (see above) was agreed by the meeting of experts, leading to an **AOEL of 0.000017 mg/kg bw/day** (17 ng/kg bw/day).

EFSA notes after PRAPeR 59 meeting of experts: in the DAR, based on the proposed ADI, a lower drinking water limit of 0.05 µg/L was established. This was not discussed by the experts, but is considered as agreed (This is not contentious since the same value was proposed and agreed for the AOEL).

EFSA notes after PRAPeR 59 meeting of experts: in the biocide evaluation procedure for the third applicant, the rapporteur Member State proposed an AOEL of 0.0000011 mg/kg bw/day (1.1 ng/kg bw/day) based on the maternal LOAEL of 0.001 mg/kg bw/day in the new rabbit developmental toxicity study, using an overall safety factor of 600 and a correction for oral absorption. However, currently the peer-review (written procedure) is not yet completed, thus it cannot be taken into account. The rapporteur Member State was recommended by the experts to highlight any further concern to the European Commission.

2.11. Dermal absorption

The results of an *in vitro* study with human skin were evaluated in the DAR. The study was performed with a representative product, but presented weaknesses related to insufficient analysis methods. Therefore, a conservative interpretation of the study led to a dermal absorption value of 3 %, being the sum of the limits of quantification in the receptor fluid and in the remaining skin after tape stripping (excluding the amount in the stratum corneum, also below the limit of quantification and considered as not bioavailable).

2.12. Exposure to operators, workers and bystanders

The representative plant protection product “Neosorexa Pellets” is a ready-for-use pellet bait containing 0.05 g difenacoum/kg. It will be used in bait boxes, in glasshouses and around crops, stored around the field margin. Up to 200 g of bait is used for rats and 30 g for mice. The size of the pellet packages are 500 g to 25 kg. Pellets are either loose, or packed in polypropylene sachets containing up to 100 g bait per sachet. Operator exposure may occur via dermal or inhalation route.

Operator exposure

Considering that professional pest controllers (for the use as a biocide) use rodenticides more often than farmers (for the use as PPP), it was agreed to consider the exposure estimates for the biocide use as a worst-case scenario for farmers.

As no valid model exists for this type of use, results of a field study during the use of Racumin ready bait (containing coumatetralyl), using five operators and 75th percentile values, were presented in the DAR. In this study, exposures associated with all activities involved in using a grain bait were monitored, including decanting material from a large container to a pail, filling and placing bait boxes, and clean-up and disposal of bait boxes. A summary of the operator exposure estimates (considering a bodyweight of 70 kg) is presented in the following table.

Operator exposure to difenacoum and comparison to the AOEL value (0.000 017 mg/kg bw/day)

	Inhalation exposure (mg/kg bw/day)	Dermal exposure (mg/kg bw/day)	Total systemic exposure (mg/kg bw/day)	% of AOEL
No PPE	1.5×10^{-7}	8.6×10^{-6}	8.8×10^{-6}	52
PPE[6]	1.5×10^{-7}	8.6×10^{-7}	1.0×10^{-6}	6

Consequently, the measured operator exposure level was below the AOEL (52%) even without personal protective equipment. However, the use of gloves is highly recommended due to the acute toxicity of the active substance.

EFSA notes after PRAPeR 59 meeting of experts: if the revised AOEL for the third applicant of the biocide review had to be taken into account for the pesticide authorisation, this would involve the use of PPE in order to have an operator exposure below the AOEL.

Worker and bystander exposure

In the DAR, it was stated that no worker or bystander exposure can occur due to the product type. Handling of dead rodents or contact with poisoned rodents was not included in the assessment, because the available scenarios were unrealistic.

Further consideration was given in the DAR to the indirect exposure of children as a potential cause of concern. It was noted that the biocide review concluded that the exposure of children could exceed the AOEL, and therefore specific risk mitigation provisions had been included for the biocide use.

However, the PRAPeR 59 meeting of experts concluded that given the product would be used in agriculture by farmers according to Good Agricultural Practices, the exposure of infants was not expected during the intended use in secured bait boxes.

3. Residues

Difenacoum was discussed at the PRAPeR 60 meeting of experts on residues (round 12, October 2008) in the context that the use pattern was just for bait products placed in secure bait boxes.

[6] PPE: gloves

3.1. Nature and magnitude of residues in plant

3.1.1. Primary crops

No data was submitted or required, as direct contact of difenacoum with plants or plant products, which could lead to any residues, is very unlikely under the applied for conditions of use.

3.1.2. Succeeding and rotational crops

No data was submitted or required, as the potential for soil exposure and subsequent plant uptake is very limited from the applied for intended use (see also to chapter 4).

3.2. Nature and magnitude of residues in livestock

No data was submitted or required. The notified representative use of difenacoum should not result in any dietary livestock exposure.

3.3. Consumer risk assessment

It is noted that no data were submitted to study and assess the residue behaviour of difenacoum in plants and livestock animals in order to define the relevant residues for dietary consumer risk assessment since the notified representative use of difenacoum is not expected to result in residues in food of plant or animal origin. Also for that reason toxicological reference values for oral exposure (ARfD and ADI) were not allocated for difenacoum in the peer review procedure (see paragraph 2.10). It was concluded that, under the conditions of use as applied for, the dietary consumer risk is negligible.

It should, however, be noted that a situation has not been assessed where difenacoum bait pellets are removed from bait boxes. Rodents are known to carry away and stockpile food because of their natural instinct. Depending on the treated area, this may lead to a situation where food or feed could become contaminated, or where domestic animals might become exposed.

The consumer risk assessment is strictly based on the assumption of a 'no dietary exposure situation' for humans and livestock from the notified representative use, presuming that no contact of difenacoum with food, feed or drinking water will occur.

3.4. Proposed MRLs

The applied for intended use (assessed in bait products placed in secure bait boxes) is unlikely to lead to any residues of difenacoum in agricultural commodities. No MRLs were proposed.

4. Environmental fate and behaviour

Difenacoum was discussed at the PRAPeR 57 meeting of experts for environmental fate and behaviour (October 2008). The discussions and agreements at the meeting were held in the context that the use pattern was just for bait products placed in secure bait boxes. Difenacoum has 2 chiral centres and thus consists of 4 diastereoisomers (2 enantiomer pairs). The methods of analysis used in the available environmental fate and behaviour studies did not resolve the enantiomers, therefore no information is available on the rate of breakdown or transformation

of the different individual enantiomers. The methods of analysis used were capable of resolving the diastereoisomer pairs. Results reported in the DAR differentiate results for the different diastereoisomer pairs by referring to them as *cis/trans* isomers. If no information is reported regarding results for *cis* and *trans* (in the DAR or section 4 of this conclusion), then any level for difenacoum reported relates to the sum of all isomers.

4.1. Fate and behaviour in soil

4.1.1. Route of degradation in soil

No satisfactory information on the route of degradation of difenacoum in soil was available. However, the view of the Member State experts was that because the potential for soil exposure from the applied for intended use was relatively limited, it was not necessary to require further data to address the route of degradation of difenacoum in soil. The use pattern applied for precludes direct contamination of soil by the formulated bait product (the bait is placed within bait boxes for rodents with the aim of precluding possible exposure to non-target mammals). Low levels of localised soil contamination will only occur when target animals have ingested the bait, and consequently urinate or defecate in the period until they have ingested a lethal dose. Information from rat metabolism studies indicates that about 40 % of the difenacoum consumed by target rodents would still be present as parent difenacoum in faeces. If it is assumed that 4 kg of the formulated bait might be set out in bait boxes per hectare (an estimate proposed by the rapporteur Member State in the meeting of experts and considered reasonable by the other experts), then this equates to 0.2 g difenacoum being set out per hectare. Using the value of 40 % difenacoum in faeces, this results in a very roughly estimated exposure per hectare of approximately 0.08 g difenacoum at the soil surface or in animal burrows.

4.1.2. Persistence of the active substance and their metabolites, degradation or reaction products

No satisfactory information on the rate of degradation of difenacoum in soil was available. However, the view of the Member State experts was that because the potential for soil exposure from the applied for intended use was relatively limited, it was not necessary to require further data to address the rate of degradation of difenacoum in soil. An indicative laboratory aerobic soil incubation experiment (20°C, 60% maximum water holding capacity (MWHC) soil moisture), that had deficiencies and does not satisfy regulatory requirements, is available. In a loamy sand soil with 2.8 % organic carbon (OC) content investigated in this experiment, a DT_{50} (pattern of decline not reported, value extrapolated beyond the study duration) of 439 days was reported. Though information was reported separately for residue levels of *cis* and *trans* isomers of difenacoum in soil extracts, the results were too variable to draw any conclusion of whether either diastereoisomer pair was preferentially degraded.

The peer review agreed on the PEC soil values presented in appendix A. It is acknowledged that these values represent concentrations that will occur in localised areas and are not concentrations that could be found over a whole field. It is also unusual that the concentrations are calculated following a spillage of small amounts of bait and thus represent accident and not the applied for intended use. It might be expected that concentrations for the assessed use (that will result from target organism urine and faeces) will be lower than those presented in appendix A.

4.1.3. Mobility in soil of the active substance and their metabolites, degradation or reaction products

Difenacoum has a measured pKa of 4.84 (20°C) and a water solubility that is pH dependent (range <0.05 mg/L at pH 4 to 61 mg/L at pH 9, pH 7 value 1.7 mg/L all at 20°C). Therefore, in the environmentally relevant pH range of soils, adsorption of difenacoum would be expected to be pH dependent, with adsorption being lower in alkaline soils.

No batch soil adsorption experiments were provided for difenacoum. Whilst a quantitative structure activity relationship (QSAR) calculation, that estimates adsorption, was provided, this value is only relevant for the non-dissociated form of difenacoum, which will not reflect the dissociation state of difenacoum in the normal pH range of most agricultural soils. Therefore, this QSAR value was not relied on, as it overestimates the soil adsorption potential of difenacoum in agricultural soils.

A satisfactory guideline laboratory aged soil column leaching study, where the soil column was loaded with radioactivity for which it was determined difenacoum accounted for 50.8 % of the radioactivity present, was available. This study indicated that for this pH 5.4 soil, radioactivity in the leachate collected from the bottom of the 30 cm column of sieved soil accounted for 0.44 % of the radioactivity added to the top of the column. Therefore for acidic (pH 5.4) soils there is good evidence for limited soil mobility of difenacoum.

Another soil column leaching study is also available (three soils investigated), where the range of soil pH tested was wider (pH 6.2, 7.5 and 7.6). In this study not aged difenacoum formulated as a bait was applied to the top of the 30 cm columns of sieved soil. The applicant made several clarifications available to the meeting of Member State experts that reassured the experts that these experiments provided information on the potential leaching of the active substance difenacoum and not just the formulated product that was investigated. These clarifications were that the constituents of the pellets used (primarily wheat flour) would mean the product had a neutral pH and that, when leached for 24 hours with deionised water, the pellets would have disintegrated relatively early on in the experiment. Only the leachates produced by the experiments (and not the soil columns) were analysed for difenacoum. No difenacoum was detected in the leachates of any of the three soils, though the limit of detection of the method used was relatively high at 6 µg/L of leachate. This limit of detection is only equivalent to 0.92 % of the quantity of difenacoum applied to the top of the columns[7]. The experts therefore concluded that this experiment indicated that even for alkaline soil pH there was evidence that the soil mobility of difenacoum was not very high. However, the high limit of quantification in the experiment did not allow a conclusion to be drawn that difenacoum would be immobile in soils of neutral or alkaline soil pH. See section 4.2.1 for further discussion on the potential for leaching and movement to surface water, and section 4.2.2 for further discussion on the potential for leaching to groundwater.

4.2. Fate and behaviour in water

4.2.1. Surface water and sediment

A satisfactory guideline sterile aqueous hydrolysis study indicated that difenacoum was stable to hydrolysis at pH 5 and 7, and hydrolysed slowly at pH 9 (estimated DT_{50} of 80 days at

[7] 0.24682 mg difenacoum was applied to the top of each of the soil columns and 0.38L of water was leached through each soil column. Vol. 1 Rev. 2 section 2.5.1.2 erroneously indicates that in these experiments the amount leached must be < 0.5 % of the applied difenacoum.

25°C, pattern of decline not reported). In another study, difenacoum was reported as being stable to hydrolysis at pH 9 even at a higher temperature of 50°C. Under the condition of a satisfactory sterile laboratory aqueous photolysis study (acetonitrile present as a solubilising agent), difenacoum was rapidly degraded (single first order DT_{50} estimated at 8.1 hours for natural midsummer sunlight in Scotland (55°N) at pH 7). No major (>10 % applied radioactivity (AR)) metabolites were formed. The *trans* enantiomer pairs were transformed faster than the *cis* enantiomer pairs.

No satisfactory information on the route and rate of degradation of difenacoum in aerobic natural sediment water systems was available. However, the view of the Member State experts was that the potential for soil exposure from the applied for intended use was relatively limited (see section 4.1.1), and the available information indicated that the soil mobility of difenacoum was not very high (see section 4.1.3). Therefore, the Member State experts agreed that it could be concluded that for the applied for intended uses the potential for exposure of natural surface water systems was negligible. Consequently, it was agreed that it was not necessary to require data to address the route and rate of degradation of difenacoum in natural aerobic sediment water systems.

Based on the results of ready biodegradability studies (OECD301D aerobic and ISO11734 anaerobic), difenacoum is classified as 'not readily biodegradable' according to the criteria of these tests.

4.2.2. Potential for ground water contamination of the active substance, their metabolites, degradation or reaction products

The Member State experts agreed that taking into account the applied for intended uses (direct contamination of soil is precluded, therefore low levels of localised soil contamination will only occur when target animals have ingested the bait, and consequently urinate or defecate in the period until they have ingested a lethal dose, see section 4.1.1) combined with the evidence that soil mobility of difenacoum was not very high (see section 4.1.3), it was possible using expert judgement to conclude that a groundwater concentration of 0.05 µg/L is unlikely to be exceeded. It was noted that toxicological considerations meant that a drinking water limit of <0.1 µg/L (i.e. 0.05 µg/L) was necessary for difenacoum (see section 2.10).

4.3. Fate and behaviour in air

The estimated vapour pressure of difenacoum ($1.9x10^{-11}$ Pa at 25°C) means that difenacoum would be classified under the national scheme of The Netherlands as very slightly volatile, indicating that losses due to volatilisation would not be expected from a formulated bait product. Calculations using the method of Atkinson for indirect photooxidation in the atmosphere through reaction with hydroxyl radicals resulted in an atmospheric half-life estimated at 2.08 hours (assuming an atmospheric hydroxyl radical concentration of $1.5x10^{6}$ radicals cm^{-3}) indicating that any small proportion of difenacoum that did volatilise would be unlikely to be subject to long-range atmospheric transport. Therefore EFSA proposes that a monitoring method for air is not required for the uses being assessed, as air exposure will be low for the formulation type being assessed.

5. Ecotoxicology

Difenacoum was discussed at the PRAPeR 58 meeting of experts for ecotoxicology (October 2008). The representative use evaluated was the use as a rodenticide against rats (*Rattus norvegicus*, *Rattus rattus*) and mice (*Mus musculus*/*domesticus*) in the field.

Under the biocide application, the active substance difenacoum has three notifiers, and the rapporteur Member State is the same as for the pesticide application. The first notifier, Sorex Limited UK, was in possession of a full regulatory data package (according to Directive 98/8/EC), and has shared a number of studies under a legal agreement with a second notifier. However, no collaboration occurred with a third applicant. A complete new dossier has been evaluated by the rapporteur Member State, but the peer-review is not yet completed. For the pesticide authorisation, the only applicant was Sorex Limited UK, who made a lot of cross-references to the dossier for the biocide use. It is suggested that the rapporteur Member State communicates to the European Commission any further critical area of concern arising from the assessment of the data package for the third applicant under the biocide use.

5.1. Risk to terrestrial vertebrates

Difenacoum is an anticoagulant interfering with the blood clotting mechanism. Lethal effects occur with some delay and depend also on external factors (injuries). The acute and short-term LD_{50} values used in the avian risk assessment were 56 mg a.s./kg bw and 3.5 mg a.s./kg bw/day. No effects on bird reproduction were observed up to 0.01 mg a.s./kg bw/day.

In the risk assessment it was assumed that birds satisfy their daily energy demand solely by feeding on the bait pellets. The acute TERs were less than 10 for small birds (*Passer montanus*, *Fringilla coelebs*), but exceeded the Annex VI trigger of 10 for larger birds (*Columba palumbus*, *Phasianus colchicus*). The short-term TERs were by more than one order of magnitude lower than the Annex VI trigger of 10, and the long-term TERs were more than 4 orders of magnitude lower than the trigger of 5. TERs based on AV and PT refinement of 0.9 and 0.8 were included in the first version of the DAR. The rapporteur Member State indicated that this was suggested as a higher tier risk assessment in the guidance document on biocidal uses. The outcome of the risk assessment was not changed by the proposed refinement. The experts rejected the proposed refinement, since it was not supported by data for the specific use of difenacoum. Subsequently, the refined risk assessment was deleted from the updated version of Volume 1 (rev. 2). The risk of primary poisoning of birds is high (particularly on the short-term and long-term time scale), if birds can get access to the baits. It was not clear from the information provided that this is ensured by the suggested application in protected bait boxes. Therefore, the experts suggested that risk mitigation measures are required at Member State level, which are proven to be efficient (e.g. the product must be applied in bait boxes).

Kestrel (*Falco tinnunculus*) was suggested as a focal species to assess the risk of secondary poisoning. The acute TER was calculated as 12.9 indicating a low risk to predatory birds, if they feed for one day on poisoned rats. However, a high short-term and long-term risk of secondary poisoning of predatory birds was identified in the DAR. The rapporteur Member State presented in the original DAR refined short-term TER calculations for five consecutive days, based on expected concentrations in kestrel immediately before a new meal of poisoned rats. It was assumed that the food of the rats consisted of 50 % of rodenticidal baits instead of 100%, and that only half of the food of kestrel consists of poisoned rats. Furthermore, it was suggested that the elimination rate of difenacoum in rats (40 %) can also be extrapolated to birds. The suggested refinement was not agreed by the Member State experts, since it was not

supported by data, and the higher concentrations in kestrel immediately after a meal were not covered by this calculation. A new calculation according to the suggestions of the experts without refinements, and considering the concentration after a new meal, was included in Volume 1 (rev 2). The calculated TERs were 0.16 (short-term) and >0.002 (long-term). Incidents of secondary poisoning of predatory birds were reported in the UK Wildlife Incident Investigation Scheme. The experts suggested that risk mitigation measures should be applied at Member State level, which are proven to be efficient in order to reduce the risk of secondary poisoning.

Acute toxicity endpoints of 1.8 mg a.s./kg bw, 50 mg a.s./kg bw and about 80 mg a.s./kg bw were observed in studies with rats, dogs and pigs. The long-term endpoint of 0.005 mg a.s./kg bw/day was derived from a study with rabbits.

It is evident that the risk to small mammals of similar size to the target organism is high. Therefore, no risk assessment for non-target small mammals was conducted by the rapporteur Member State. Instead, the risk to dogs and pigs, as potentially exposed pet/livestock animals was assessed. The acute TERs for dog and pig were greater than 10. It was noted that these are rather big animals, and higher body burdens are to be expected in smaller animals. The long-term TER was calculated in Volume 1 (rev. 2) as 0.005 by comparing the expected body burden after one meal (1 mg/kg bw) with the long-term endpoint of 0.005 mg a.s./kg bw/day. It can be concluded that there is a high long-term risk to mammals if they can access repeatedly the rodenticidal baits. However, the application in bait boxes should prevent larger mammals from accessing the baits.

The acute and long-term risk of secondary poisoning was assessed for weasel (*Mustela nivalis*). The acute TER was 0.4 based on a concentration in weasel of 4.52 mg difenacoum/kg bw after consumption of 100 % rats, which have consumed only rodenticidal baits, and on the acute endpoint of 1.8 mg a.s./kg bw. The long-term TER calculation in the original DAR was conducted in the same way as for birds (50 % of the weasel's diet consists of rats which consumed 50 % of rodenticidal baits, 40 % elimination rate). The concentration in weasel immediately before the next meal was calculated for five consecutive days and compared to the NOAEL of 0.005 mg a.s./kg bw/day. The suggested refinement steps (except the elimination rate of 40%) were not supported by data and hence were rejected by the Member State experts. The concentration in weasel was recalculated in Volume 1 (rev 2) taking into consideration only elimination of difenacoum. The long-term TER of 0.001 was calculated by comparison of the long-term endpoint and the expected concentration in weasel after one meal. The long-term risk to mammals from secondary poisoning was considered as high. Data were available which confirm accumulation of difenacoum in polecats (*Mustela putoris*).

The rapporteur Member State concluded in the original DAR that the risk of primary poisoning of non-target birds and mammals would be low on the basis of incident reports. This was not agreed by the Member State experts. In order to show that the risk to birds and mammals is low, it would be necessary to know the likelihood of an incident to be reported. The number of poisoning incidents, which were not reported, may be significantly higher.

The risk of bioaccumulation was calculated by the rapporteur Member State in the revised Volume 1 (rev. 2) according to Appendix III of SANCO/4145. The bioaccumulation factor (BAF) was calculated for fox to be 0.36, if the DT_{50} of 3 days from a bi-phasic depuration of difenacoum was applied. However, the half-life later in the depuration process of difenacoum is significantly longer with a DT_{50} of 118 days. A worst case estimate of the BAF for

difenacoum based on 118 days would significantly exceed the trigger of 1 (BAF of 14). It is considered more appropriate by EFSA to calculate the DT_{90} for the bi-phasic depuration of difenacoum, and to apply a conversion factor of 3.32 to convert the DT_{90} to a single first order $DT_{50,}$ which then can be used to calculate the bioaccumulation factor. After receipt of a comment on the conclusion, the EFSA plotted the dataset suggested by the applicant (DOC IIIA/Section 6.2, table A6 2-3, residues expressed as mmol equivalents difenacoum per g liver) using the First-Order Multi-Compartment model (FOMC). The resulting DT_{90} that accounts for the bi-phasic decline of difenacoum was 18.1 days. The corresponding single first order DT_{50} was 5.5 days, and the BAF of 0.65 for difenacoum was below the trigger of 1. However, the DT_{90} was 80.9 days if the calculation was based on percentage of radioactivity of dose in liver (DOC IIIA/Section 6.2, table A6 2-2). The corresponding single first order DT_{50} was 24.37 days and the BAF was 2.88 indicating a potential high risk of bioaccumulation. Some clarification on the underlying datasets is needed before a final conclusion can be drawn on the risk from bioaccumulation.

Overall, it was concluded that a high risk to birds and mammals from primary and secondary poisoning was identified. Risk mitigation measures are needed, which are proven to be efficient to prevent birds gaining access to the baits (e.g. bait boxes). It is more difficult to mitigate the risk from secondary poisoning of birds and mammals. The efficiency and applicability of risk mitigation in the context of the application in the field, such as removal of carcasses during and after the control campaign, is uncertain and would need some further consideration. A high risk was evident for small non-target mammals. No risk mitigation measures were proposed. It is unclear, if the risk to small non-target mammals can be mitigated without reducing the efficacy of the product. Member States should be aware that it cannot be excluded that the suggested application of difenacoum in the field may also affect endangered/protected small mammal species potentially found in agricultural landscapes.

5.2. Risk to aquatic organisms

Difenacoum was very toxic to fish, aquatic invertebrates and algae with an LC_{50} for fish of 0.064 mg a.s./L, EC_{50} for daphnids of 0.52 mg a.s./L, and E_bC_{50} for algae of 0.32 mg a.s./L. However, the risk to aquatic organisms was considered to be low, because exposure of the aquatic environment was expected to be negligible from the intended use of the rodenticide.

5.3. Risk to bees

No studies with bees were submitted. The risk to bees was considered to be low, since the recommended use of difenacoum was not expected to lead to any relevant exposure of bees.

5.4. Risk to other arthropod species

No studies with non-target arthropods were conducted. It is unlikely that the recommended use of difenacoum would lead to any significant exposure of non-target arthropods.

5.5. Risk to earthworms

No studies with earthworms were submitted. In the meeting of experts it was agreed that no studies with earthworms are required if the baits are applied in bait boxes without direct contact to the soil. If exposure of soil would occur, then it would be only limited point exposure, and the corresponding risk to earthworm populations would be low.

5.6. Risk to other soil non-target macro-organisms

No studies were required since exposure of soil-dwelling non-target macro-organisms was considered as negligible. If exposure of soil would occur, then it would be only limited point exposure, and the corresponding risk to populations of soil-dwelling non-target macro-organisms would be low.

5.7. Risk to soil non-target micro-organisms

No studies were required, since exposure of soil non-target micro-organisms was considered as negligible. If exposure of soil would occur, then it would be only limited point exposure, and the corresponding risk to soil non-target micro-organisms would be low.

5.8. Risk to other non-target-organisms (flora and fauna)

No studies were submitted with terrestrial plants. Exposure of plants was considered negligible.

5.9. Risk to biological methods of sewage treatment

No risk assessment was provided for biological sewage treatment plants. However, it was not expected that the recommended use of difenacoum would lead to any significant contamination of sewage treatment plants and hence the risk was regarded as low.

6. Residue definitions

6.1. Soil

Definition for risk assessment: difenacoum

Definition for monitoring: difenacoum

6.2. Water

6.2.1. Ground water

Definition for exposure assessment: difenacoum

Definition for monitoring: difenacoum

6.2.2. Surface water

Definition for risk assessment

in surface water: none

in sediment: none

Definition for monitoring: difenacoum

6.3. Air

Definition for risk assessment: difenacoum

Definition for monitoring: none required for the use assessed (formulated as a bait (ready to use))

6.4. Food of plant origin

Definition for risk assessment: none required for the use assessed

Definition for monitoring: none required for the use assessed

6.5. Food of animal origin

Definition for risk assessment: none required for the use assessed

Definition for monitoring: none required for the use assessed

6.6. Body fluids and tissues

Definition for monitoring: difenacoum

6.7. Overview of the risk assessment of compounds listed in residue definitions for the environmental compartments

6.7.1. Soil

Compound (name and/or code)	Persistence	Ecotoxicology
difenacoum	No reliable information available, but it has some persistence.	No studies available for soil-dwelling organisms. The risk was considered to be low because of localised point exposure.

6.7.2. Ground water

Compound (name and/or code)	Mobility in soil	>0.05 µg/L 1m depth for the representative uses	Pesticidal activity	Toxicological relevance	Ecotoxicological activity
difenacoum	Expected to be low based on evidence from soil column leaching studies.	No	Yes	Yes	Yes

6.7.3. Surface water and sediment

Compound (name and/or code)	Ecotoxicology
None (negligible exposure expected).	Difenacoum is very toxic to aquatic organisms. The risk was assessed as low assuming negligible exposure.

6.7.4. Air

Compound (name and/or code)	Toxicology
difenacoum	T+ R26 "Very toxic by inhalation" T R48/23 "Toxic: Danger of serious damage to health by prolonged exposure through inhalation"

LIST OF STUDIES TO BE GENERATED, STILL ONGOING OR AVAILABLE BUT NOT PEER REVIEWED

- A revised technical specification is required (relevant for all representative uses evaluated, data gap identified by PRAPeR 56 meeting of experts (October 2008), date of submission unknown; refer to chapter 1)
- Clarification of the composition of the most recent batch of the five-batch data, which had a lower purity and the content of some impurities increased, and an explanation of what happens with batches out-of-specification (relevant for all representative uses evaluated, data gap identified by PRAPeR 56 meeting of experts (October 2008), date of submission unknown; refer to chapter 1)
- Confirmatory data for the identity of three impurities in the technical material (relevant for all representative uses evaluated, data gap identified by PRAPeR 56 meeting of experts (October 2008), date of submission unknown; refer to chapter 1)
- Information concerning the purity of the starting materials (relevant for all representative uses evaluated, data gap identified by PRAPeR 56 meeting of experts (October 2008), date of submission unknown; refer to chapter 1)
- Surface tension according to EEC method A.5 (relevant for all representative uses evaluated, data gap identified by the rapporteur Member State, confirmed by PRAPeR 56 meeting of experts (October 2008), date of submission unknown; refer to chapter 1)
- Shelf-life study (relevant for all representative uses evaluated, data gap identified by PRAPeR 56 meeting of experts (October 2008), date of submission unknown; refer to chapter 1)
- Method for the determination of residues of difenacoum in body fluids (blood) (relevant for all representative uses evaluated, data gap identified by PRAPeR 56 meeting of experts (October 2008), date of submission unknown; refer to chapter 1)
- The risk from bioaccumulation needs to be addressed further (relevant for all representative uses evaluated, data gap identified by EFSA after the peer review, date of submission unknown; refer to section 5.1)

CONCLUSIONS AND RECOMMENDATIONS

OVERALL CONCLUSIONS

The conclusion was reached on the basis of the evaluation of the representative uses as rodenticide as proposed by the applicant, which comprise manual application of measured amounts of product into protected bait boxes, at discrete locations throughout a rodent infested area, in plant protection situations in fields, in glasshouses and protection of crops stored in fields, for the control of rats (brown rat, *Rattus norvegicus* and black rat, *Rattus rattus*) and mice (*Mus domesticus/musculus*), in all EU countries. The number and timing of applications is dependent on the extent of the rodent infestation.

The representative formulated product for the evaluation was 'Neosorexa Pellets', a bait (ready-for-use) formulation (RB) containing 0.05 g/kg difenacoum, registered under different trade names in Europe.

There is no agreed technical specification.

Analytical methods as well as methods and data relating to physical, chemical and technical properties are available to ensure that quality control measurements of the plant protection products are possible, however a data gap was identified for storage stability study.

Monitoring methods to determine difenacoum residues in food/feed of plant and animal origin are not required, no MRLs are established. Adequate methods are available to monitor all compounds given in the respective residue definitions in soil and water. Adequate methods are available to monitor difenacoum residues in tissues, however a method for the determination of residues of difenacoum in body fluids (blood) was identified as a data gap.

With regard to its toxicological properties, difenacoum is a direct anticoagulant that interferes with the blood clotting mechanism by inhibiting the vitamin K epoxide reductase. The active substance is well absorbed following oral administration, and widely distributed within the body with the highest concentration in the liver. Based on the results of the acute toxicity studies, the proposed classification was T+ R26/27/28 "Very toxic by inhalation, in contact with skin, and if swallowed". In repeated dose studies, no other toxic effect than reduced coagulation and haemorrhages were observed, leading to a short term rat NOAEL of 0.03 mg/kg bw/day. In the *in vitro* genotoxicity studies, no gene mutation was induced in bacterial and mammalian cells, while two chromosome aberration tests gave positive results. As the three *in vivo* genotoxicity studies were negative, the overall conclusion is that difenacoum has no genotoxic potential. No multigeneration study was provided in the dossier. In the developmental studies with rats and rabbits, there was no evidence of teratogenicity. In rats, no developmental toxicity was observed at a dose maternally toxic. Foetal effects in rabbits were observed in both the test and control groups, and were concluded as not dose-related. However, the experts considered that difenacoum should be regarded as teratogenic based on the knowledge about analogous compounds (other antivitamin K anticoagulants in humans), and they agreed with the classification proposed by the Specialised Experts on Reproductive Toxicity (Ispra, 19-20 September 2006) i.e. Reprotoxic Category 1, R61 "May cause harm to the unborn child".

The experts assumed that no contamination of crops would occur during the intended use, and concluded that the derivation of an acceptable daily intake and an acute reference dose was not required. For the operator risk assessment, the agreed AOEL was 0.000017 mg/kg bw/day (or 17 ng/kg bw/day), based on the maternal NOAEL in the developmental rabbit study with the application of an overall safety factor of 300. The additional safety factor of 3 was justified by the severity of the toxicological effects of difenacoum, the higher potency of the second generation anticoagulants (as difenacoum) compared to warfarin, and the much higher vulnerability of human foetuses to vitamin K (hydroquinone) deficiency compared to rodents. For the operator exposure assessment, the exposure estimates for the biocide use were considered as a worst-case scenario, which gave an exposure of 52 % of the AOEL without the use of personal protective equipment. No worker or bystander exposure was expected due to the product type and the representative use in secured bait boxes.

Under the conditions of use as applied for (i.e. formulated bait in secure bait boxes), it is very unlikely that residues in food of plant or animal origin will occur. Therefore, it was concluded that the dietary consumer risk is negligible, and that data on the residue behaviour of difenacoum in plants and livestock animals are not required. No MRLs were proposed.

However, a situation has not been assessed where bait pellets are removed from bait boxes and hoarded by rodents because of their natural instinct. Depending on the treated area, this may lead to a situation where food or feed could become contaminated, or where domestic animals might become exposed.

The consumer risk assessment is strictly based on the assumption of a 'no dietary exposure situation' for humans and livestock from the notified representative use, presuming that no contact of difenacoum with food, feed or drinking water will occur.

Limited information is available on the environmental fate and behaviour of difenacoum in soil, water and air. However, the available information is considered sufficient to complete an environmental exposure assessment at EU level for the applied for intended use, but only when formulated bait products are placed in secure bait boxes. When the product is used in this way, the potential for groundwater contamination by difenacoum above a toxicologically based concentration limit of 0.05 µg/L was assessed as low.

The risk to birds and mammals from primary and secondary poisoning was assessed as high. Risk mitigation measures are needed, which are proven to be efficient to prevent birds and larger mammals gaining access to the baits (e.g. bait boxes). It is more difficult to mitigate the risk from secondary poisoning. The efficiency and applicability of risk mitigation in the context of the application in the field, such as removal of carcasses during and after the control campaign, is uncertain and would need some further consideration. A high risk was evident for small non-target mammals. No risk mitigation measures were proposed. It is unclear if the risk to small non-target mammals can be mitigated without reducing the efficacy of the product. Difenacoum was very toxic to fish, aquatic invertebrates and algae. However, the risk to aquatic organisms was considered to be low, because exposure of the aquatic environment was expected to be negligible from the applied for intended use.

The risk to bees, to other arthropod species, earthworms, soil macro- and soil micro-organisms, terrestrial plants and biological sewage treatment plants was considered to be low because of negligible or low local point exposure.

PARTICULAR CONDITIONS PROPOSED TO BE TAKEN INTO ACCOUNT TO MANAGE THE RISK(S) IDENTIFIED

The bait formulated product to be placed only in secured bait boxes (see sections 2.12, 3, 4 and 5.1). The product should not be placed where food, feed or drinking water could become contaminated.

CRITICAL AREAS OF CONCERN

- Lack of specification for the technical material.
- Lack of a monitoring method for the determination of residues of difenacoum in body fluids (blood).
- A high risk to birds and mammals from primary and secondary poisoning. While the risk of primary poisoning can be mitigated by the use of secured bait boxes, it is unclear if the risk of secondary poisoning can be mitigated efficiently.

- A high risk to non-target small mammals which can enter the bait boxes (no risk mitigation proposed).

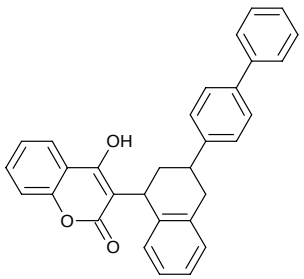
APPENDICES

APPENDIX A – LIST OF ENDPOINTS FOR THE ACTIVE SUBSTANCE AND THE REPRESENTATIVE FORMULATION

Identity, Physical and Chemical Properties, Details of Uses, Further Information

Active substance (ISO Common Name) ‡	Difenacoum
Function (*e.g.* fungicide)	Rodenticide
Rapporteur Member State	Finland

Identity (Annex IIA, point 1)

Chemical name (IUPAC) ‡	3-[(1*RS*,3*RS*;1*RS*,3*SR*)-3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin
Chemical name (CA) ‡	3-[3-(1,1'-biphenyl)-4-yl-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2*H*-1-benzopyran-2-one
CIPAC No ‡	514
CAS No ‡	[56073-07-5]
EC No (EINECS or ELINCS) ‡	259-978-4
FAO Specification (including year of publication) ‡	Not available
Minimum purity of the active substance as manufactured ‡	Open
Identity of relevant impurities (of toxicological, ecotoxicological and/or environmental concern) in the active substance as manufactured	None
Molecular formula ‡	$C_{31}H_{24}O_3$
Molecular mass ‡	444.5 g/mol
Structural formula ‡	

Physical and chemical properties (Annex IIA, point 2)

Melting point (state purity) ‡	211.0 - 215.0 °C (98.7 %)
Boiling point (state purity) ‡	No boiling point before start of decomposition (96.5 %)
Temperature of decomposition (state purity)	>300 °C (96.5 %)
Appearance (state purity) ‡	White fine powder (98.7 %)
Vapour pressure (state temperature, state purity) ‡	1.9×10^{-11} Pa at 25ºC estimation
Henry's law constant ‡	5.0×10^{-9} Pa m^3 mol^{-1} at pH 7 1.4×10^{-10} Pa m^3 mol^{-1} at pH 9
Solubility in water (state temperature, state purity and pH) ‡	< 0.05 mg/l at 20 °C, pH 4 (98.7 %) 1.7 mg/l at 20 °C, pH 7 (98.7%) 61 mg/l at 20 °C, pH 9 (98.7%)
Solubility in organic solvents ‡ (state temperature, state purity)	Solubility at 20 °C (96.3 %) acetone: 7.6 g/l propan-2-ol: 1.5 g/l ethyl acetate: 3.7 g/l toluene: 1.2 g/l methanol: 1.2 g/l n-hexane: 12.1 g/l dichloromethane: 19.6 g/l
Surface tension ‡ (state concentration and temperature, state purity)	Data required
Partition co-efficient ‡ (state temperature, pH and purity)	log $P_{O/W}$ = 7.6 (computer estimation method)
Dissociation constant (state purity) ‡	pKa = 4.84 at 20 °C (96.18 %)
UV/VIS absorption (max.) incl. ε ‡ (state purity, pH)	Dichloromethane solution at 23 °C: λ_{max} = 259.4 nm ε = 46600 l · mol^{-1} · cm^{-1} (98.7 %) λ_{max} = 310.6 nm ε = 17100 l · mol^{-1} · cm^{-1} (98.7 %)
Flammability ‡ (state purity)	Not highly flammable (96.18 %)
Explosive properties ‡ (state purity)	Not explosive (expert statement)
Oxidising properties ‡ (state purity)	Not oxidizing (96.18 %)

Summary of representative uses evaluated (*difenacoum*)*

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment (for explanation see the text in front of this section)			PHI (days) (m)	Remarks
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min/ max (k)	interval between applications (min)	g as/hL min – max (l)	water L/ha min – max	g as/ha min – max (l)		
To control rodent infestation beyond the field gate.	EU member states	Neosorexa Pellets	F G	Rats (brown rat, black rat) (*Rattus norvegicus, Rattus rattus*) Mice (house mouse) (*Mus domesticus/ musculus*)	RB	0.05 g/kg	**Note 1**	N/A	**Note 2**	**Note 3**	**Note 2**	N/A	**Note 2**	N/A	**Note 4** **[1]**

1 A high risk of primary and secondary poisoning of birds and mammals was identified. Substantial and efficient risk mitigation measures which are proven to be efficient are requried

Note 1 - The product is applied by manually placing measured amounts of product into protected bait points, at discrete locations throughout a rodent infested area.
Note 2 - Use as and when necessary. For rat control, protected bait points containing up to 200g of product are used, at intervals of up to 10 metres apart. For mouse control, protected bait points containing up to 30g of product are used, at intervals of 1-2 metres apart. An adequate number of baits points are placed in dry locations, protected from the weather and in appropriate positions to help prevent access by non-target animals
Note 3 **- Rodent control is undertaken by users in response to a rodent infestation. Rodenticidal products are used in the same manner whatever the geographical area or the climate, as the intended purpose for using the products is the same, i. e. to control rodent infestations. Therefore, the number and timings of applications is dependant on the presence of a rodent infestation. An average rodent treatment should not continue beyond 35 days.**
Note 4 - The product is ready-to-use. It is not intended to be diluted with any other substance or preparation prior to use. The product can be used indoors, around buildings, away from buildings. The method of

application is the same in each of these situations. Rodenticidal products are used in the same manner whatever the locality, geographical area or the climate.

* For uses where the column "Remarks" is marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s). (a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure) (b) Outdoor or field use (F), greenhouse application (G) or indoor application (I) (c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds (d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR) (e) GCPF Codes - GIFAP Technical Monograph No 2, 1989 (f) All abbreviations used must be explained (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench (h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated	(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).** (j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application (k) Indicate the minimum and maximum number of application possible under practical conditions of use (l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha (m) PHI - minimum pre-harvest interval

Methods of Analysis

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical as (analytical technique)	HPLC, UV 254
Impurities in technical as (analytical technique)	HPLC, UV 254
Plant protection product (analytical technique)	HPLC, UV 264

Analytical methods for residues (Annex IIA, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin	Not relevant
Food of animal origin	Not relevant
Soil	Difenacoum
Water surface	Difenacoum
drinking/ground	Difenacoum
Air	None required
Body fluids and tissues	Difenacoum

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	No MRL established.
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	No MRL established.
Soil (analytical technique and LOQ)	LC-MS/MS, LOQ = 0.01 mg/kg
Water (analytical technique and LOQ)	LC-MS/MS, LOQ = 0.01 µg/l (for surface and drinking/ground water)
Air (analytical technique and LOQ)	None required
Body fluids and tissues (analytical technique and LOQ)	LC-MS/MS, LOQ = 0.01 mg/kg for meat, tissues Method required for blood

Classification and proposed labelling with regard to physical and chemical data (Annex IIA, point 10)

	RMS/peer review proposal
Active substance	None

Impact on Human and Animal Health

Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA, point 5.1)

Rate and extent of oral absorption ‡	Rapid (peak level in blood at 4 h) and extensive: 82 % based on liver and other tissues, carcass, urine, CO_2, and the metabolized portion in faeces (168 h after dosing)
Distribution ‡	Widely distributed: liver > pancreas > gastrointestinal tract > kidney
Potential for accumulation ‡	Yes: 3.34 % of label persisted in the liver 182 days after dosing
Rate and extent of excretion ‡	Biphasic; half-lives of 3 and 118 days. Within seven days 37 to 55 % eliminated in faeces and 2 % in urine
Metabolism in animals ‡	24 to 36 % of the administered dose is as metabolites in faeces. 2 to 5 unidentified metabolites found in liver. Metabolism is assumed to drastically reduce the anticoagulant potential
Toxicologically relevant compounds ‡ (animals and plants)	Difenacoum
Toxicologically relevant compounds ‡ (environment)	Difenacoum

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral ‡	1.8 mg/kg bw	T+ R28
Rat LD_{50} dermal ‡	63 mg/kg bw (95 % confidence limits 34-85)	T+ R27
Rat LC_{50} inhalation ‡	3.646 to 5.848 µg/l/4 h, head-only	T+ R26
Skin irritation ‡	Non-irritant	
Eye irritation ‡	Non-irritant	
Skin sensitisation ‡	Non-sensitising (M & K)	

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect ‡	Blood coagulation / haemorrhage	
Relevant oral NOAEL ‡	0.03 mg/kg bw/day (90-day, rat)	T; R48/25

Relevant dermal NOAEL ‡	Waived based on scientific and animal welfare reasons: read-across from acute dermal toxicity and oral short term toxicity	T; R48/24
Relevant inhalation NOAEL ‡	Waived based on scientific and animal welfare reasons: read-across from acute inhalation toxicity and oral short term toxicity	T; R48/23

Genotoxicity ‡ (Annex IIA, point 5.4)

In vitro: positive result in two mammalian chromosome aberration tests. *In vivo*: Negative results in two micronucleus tests and in an *in vivo*/*in vitro* UDS test. Overall, difenacoum is unlikely to be genotoxic *in vivo*.	

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target/critical effect ‡	Waived based on scientific and animal welfare reasons	
Relevant NOAEL ‡	No data available.	
Carcinogenicity ‡	No data available.	

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction toxicity

Reproduction target / critical effect ‡	Waived based on scientific and animal welfare reasons	
Relevant parental NOAEL ‡	No data available.	
Relevant reproductive NOAEL ‡	No data available.	
Relevant offspring NOAEL ‡	No data available.	

Developmental toxicity

Developmental target / critical effect ‡	Rabbit: increased clotting time and haemorrhage in dams; no clear developmental toxicity in foetuses Rat: Haemorrhages in dams; no effects in foetuses Regardless of the submitted negative results, read-across from warfarin indicates cause of concern for developmental effects in humans.	T; Repr. Cat. 1; R61
Relevant maternal NOAEL ‡	Rabbit: 0.005 mg/kg bw/day Rat: 0.03 mg/kg bw/day	
Relevant developmental NOAEL ‡	Rabbit: 0.015 mg/kg bw/day for teratogenicity and embryotoxicity Rat: 0.09 mg/kg bw/day for teratogenicity and embryotoxicity	

Neurotoxicity (Annex IIA, point 5.7)

Acute neurotoxicity ‡	No evidence for neurotoxic potential from other studies	
Repeated neurotoxicity ‡	No data – not required	
Delayed neurotoxicity ‡	No data – not required	

Other toxicological studies (Annex IIA, point 5.8)

Mechanism studies ‡	No data available – not required
Studies performed on metabolites or impurities ‡	No data available – not required

Medical data ‡ (Annex IIA, point 5.9)

Routine monitoring of prothrombin times of workers producing the active substance and formulating products has been carried out for the last forty years. With the exception of three poisoning incidents (rodenticide not identified), routine monitoring has shown no clinical effects in any workers. There has been no evidence of allergy, sensitisation or any other abnormal effects induced by repeated and continual exposure to the anticoagulant rodenticides manufactured (difenacoum, warfarin, brodifacoum, flocoumafen).

Summary (Annex IIA, point 5.10)	Value	Study	Safety factor
ADI ‡	Not required for the intended use.		
AOEL ‡	0.000 017 mg/kg bw/day	Teratogenicity, rabbit, maternal effects	300
ARfD ‡	Not required for the intended use.		

Dermal absorption ‡ (Annex IIIA, point 7.3)

50 ppm Difenacoum Pellet Bait	3 % (*in vitro* study, human skin)

Exposure scenarios (Annex IIIA, point 7.2)

Operator	Exposure was below the AOEL (52 %) even without personal protective equipment.
Workers	Not relevant
Bystanders	Not relevant

Classification and proposed labelling with regard to toxicological data (Annex IIA, point 10)

	RMS/peer review proposal
Difenacoum	T; Repr. Cat. 1; R61 T+; R26/27/28 T; R48/23/24/25

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered	N.A.
Rotational crops	N.A.
Metabolism in rotational crops similar to metabolism in primary crops?	N.A.
Processed commodities	N.A.
Residue pattern in processed commodities similar to residue pattern in raw commodities?	N.A.
Plant residue definition for monitoring	N.A.
Plant residue definition for risk assessment	N.A.
Conversion factor (monitoring to risk assessment)	N.A.

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	N.A.
Time needed to reach a plateau concentration in milk and eggs	N.A.
Animal residue definition for monitoring	N.A.
Animal residue definition for risk assessment	N.A.
Conversion factor (monitoring to risk assessment)	N.A.
Metabolism in rat and ruminant similar (yes/no)	N.A.
Fat soluble residue: (yes/no)	N.A.

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

N.A.

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 Introduction)

N.A.

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

	Ruminant:	Poultry:	Pig:
	Conditions of requirement of feeding studies		
Expected intakes by livestock ≥ 0.1 mg/kg diet (dry weight basis) (yes/no - If yes, specify the	N.A.	N.A.	N.A.

level)			
Potential for accumulation (yes/no):	N.A.	N.A.	N.A.
Metabolism studies indicate potential level of residues ≥ 0.01 mg/kg in edible tissues (yes/no)	N.A.	N.A.	N.A.
	Feeding studies (Specify the feeding rate in cattle and poultry studies considered as relevant) Residue levels in matrices : Mean (max) mg/kg		
Muscle	N.A.	N.A.	N.A.
Liver	N.A.	N.A.	N.A.
Kidney	N.A.	N.A.	N.A.
Fat	N.A.	N.A.	N.A.
Milk	N.A.		
Eggs		N.A.	

Summary of residues data according to the representative uses on raw agricultural commodities and feedingstuffs (Annex IIA, point 6.3, Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region, field or glasshouse, and any other useful information	Trials results relevant to the representative uses (a)	Recommendation/comments	MRL estimated from trials according to the representative use	HR (c)	STMR (b)
N.A.						

(a) Numbers of trials in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17
(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the representative use
(c) Highest residue

Consumer risk assessment (Annex IIA, point 6.9, Annex IIIA, point 8.8)

ADI	-
TMDI (% ADI) according to WHO European diet	N.A.
TMDI (% ADI) according to national (to be specified) diets	N.A.
IEDI (WHO European Diet) (% ADI)	N.A.
NEDI (specify diet) (% ADI)	N.A.
Factors included in IEDI and NEDI	N.A.
ARfD	N.A.
IESTI (% ARfD)	N.A.
NESTI (% ARfD) according to national (to be specified) large portion consumption data	N.A.
Factors included in IESTI and NESTI	N.A.

Processing factors (Annex IIA, point 6.5, Annex IIIA, point 8.4)

Crop/ process/ processed product	Number of studies	Processing factors		Amount transferred (%) (Optional)
		Transfer factor	Yield factor	
N.A.				

Proposed MRLs (Annex IIA, point 6.7, Annex IIIA, point 8.6)

N.A.	-
.....	
.....	
.....	
.....	
.....	

When the MRL is proposed at the LOQ, this should be annotated by an asterisk after the figure.

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)

Mineralization after 100 days ‡	No satisfactory data available, not required for the applied for intended uses
Non-extractable residues after 100 days ‡	No satisfactory data available, not required for the applied for intended uses
Metabolites requiring further consideration ‡ - name and/or code, % of applied (range and maximum)	No satisfactory data available, not required for the applied for intended uses

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation ‡	Not applicable
Soil photolysis ‡	Not applicable

Rate of degradation in soil (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

Laboratory studies ‡

Parent	Aerobic conditions: No satisfactory data available, not required for the applied for intended uses						
Soil type	X[8]	pH	t. °C / % MWHC	DT_{50} /DT_{90} (d)	DT_{50} (d) 20°C pF2/10kPa	St. (r^2)	Method of calculation

Field studies ‡	Not performed.

pH dependence ‡ (yes / no) (if yes type of dependence)	No information available
Soil accumulation and plateau concentration ‡	Not applicable

Laboratory studies ‡

Parent	Anaerobic conditions	Not performed / Not applicable

[8] X This column is reserved for any other property that is considered to have a particular impact on the degradation rate.

Soil adsorption/desorption (Annex IIA, point 7.1.2)

Parent ‡ **No batch adsorption data available.**							
	OC %	pH	Kd (mL/g)	Koc	Kf (mL/g)	Kfoc (mL/g)	1/n
Arithmetic mean/median							
pH dependence, Yes or No			yes, lower adsorption expected at higher pH based on pKa and solubility in water endpoints				

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching ‡	Formulation study: YF6961 (Cereal-based pellet containing 0.005 % w/w difenacoum, pH of pellet unknown) Soils: 3 soil types (pH 6.2-7.6) Elution (mm): 200 mm/48 h Leachate: < 6 µg difenacoum/L (6 µg/L represents 0.92% of the difenacoum applied to the top of the column)
Aged residues leaching ‡	Aged for (d): 142 d Time period (d): 2.1 d Elution (mm): 200 mm Soil pH: 5.4
	Analysis of soil residues post ageing (soil residues pre-leaching): 50.8 % active substance, metabolites were not analysed Total residues/radioactivity retained in soil columns not reported
	Leachate: 0.44 % total residues/radioactivity in leachate
Lysimeter/ field leaching studies ‡	Not performed; Not applicable

PEC (soil) (Annex IIIA, point 9.1.3)

Parent Method of calculation	No direct soil application, therefore only initial PECs value were calculated
Total spillage of one bait to 1 m^2 soil area: - Bait 200 g, difenacoum content 0.005 % (w/w) ESD: open area scenario (25 % spillage)	0.13 mg/kg (Depth of soil layer: 5 cm, bulk density: 1.5 g/m^3) 0.346 mg/kg for soil volume of 0.0085 m^3 ("hot spot")

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolytic degradation of the active substance and metabolites > 10 % ‡	pH 5: stable at 25 °C
	pH 7: ca. 1000 days at 25 °C
	pH 9: ca. 80 days at 25 °C
Photolytic degradation of active substance and metabolites above 10 % ‡	pH 5: DT_{50} 3.3 h pH 7: DT_{50} 8.1 h pH 9: DT_{50} 7.3 h (Data generated in aqueous solution using Scotland local natural midsummer sunlight equivalent exposure periods) No degradation products >10% were found.
Quantum yield of direct phototransformation in water at Σ > 290 nm	Not applicable
Readily biodegradable ‡ (yes/no)	No

Degradation in water / sediment	Not performed, not applicable

PEC (surface water) and PEC sediment (Annex IIIA, point 9.2.3)

Parent	Not modelled, since the conventional models and methods used to predict PECsw for plant protection products are not applicable for rodenticide uses, primarily because of the lack of direct soil application. Any relevant contamination of surface waters is not expected to occur when using difenacoum in a rodenticide bait.

PEC (ground water) (Annex IIIA, point 9.2.1)

Method of calculation and type of study (*e.g.* modelling, field leaching, lysimeter)	Not modelled, since the use of a ready to use bait which is applied using baiting stations prevent any significant soil contamination. Taken also into account the low mobility of difenacoum it is reasonable to assume low risk for ground water contamination.

Fate and behaviour in air (Annex IIA, point 7.2.2, Annex III, point 9.3)

Direct photolysis in air ‡	Not applicable
Quantum yield of direct phototransformation	Not determined, not applicable
Photochemical oxidative degradation in air ‡	Model calculation (AopWin 1.91): DT_{50} 2.08 h (12 h, $c_{OH} = 1.5 \times 10^6$ molecules/cm³) DT_{50} 6.24 h (24 h, $c_{OH} = 0.5 \times 10^6$ molecules/cm³)
Volatilisation ‡	Vapour pressure 1.9×10^{-11} Pa at 25°C Henry's law constant 5×10^{-9} Pa m3/mol (based on water solubility of 1.7 mg/l, pH 7) Difencoum is not expected to volatilise to air in significant quantities.
Metabolites	Volatile metabolites have not been identified.

PEC (air)

$PEC_{(a)}$

Maximum concentration	Negligible

Residues requiring further assessment

Environmental occurring metabolite requiring further assessment by other disciplines (toxicology and ecotoxicology) or for which a groundwater exposure assessment is triggered.	Soil: difenacoum Surface Water: None for the use assessed Sediment: None for the use assessed Ground water: difenacoum Air: None for the use and product type assessed

Monitoring data, if available (Annex IIA, point 7.4)

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

Points pertinent to the classification and proposed labelling with regard to fate and behaviour data

Candidate for R53.

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Species	Test substance	Time scale	End point (mg/kg bw/day)	End point (mg/kg feed)
Birds				
Bobwhite quail	a.s.	Acute	56	
Mallard duck	a.s.	Short-term	3.5	18.9
Japanese quail	a.s.	Long-term	> 0.01	
Mammals				
Rat	a.s.	Acute	1.8	
Dog	a.s	Acute	50	
Pig	a.s.	Acute	Ca. 80	
~~Rat~~ Rabbit	a.s.	Long-term	0.005	

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

Since there is no scenario for rodenticidal use in the guidance document on risk assessment for birds and mammals (SANCO/4145/2000) the risk has been calculated based on the food consumption of small and large birds and for small and large mammals for a few example species.

Indicator species/Category	Time scale	ETE	TER	Annex VI Trigger
Primary poisoning (Birds) (Food bait)				
Small bird (e.g. tree sparrow)	Acute	17.3	**3.2**[2]	10
Large bird (e.g. wood pigeon)	Acute	5.4	10.4	10
Small bird (e.g. tree sparrow)	Short-term	17.3	**0.2**[2]	10
Large bird (e.g. wood pigeon)	Short-term	5.4	**0.65**[2]	10
Small bird (e.g. tree sparrow)	Long-term	17.3	**>0.0006**[2]	5
Large bird (e.g. wood pigeon)	Long-term	5.4	**>0.0019**[2]	5
Secondary poisoning (Birds) (Food poisoned rodents)				
Large bird (e.g. kestrel)	Acute	4.34	12.9 [3]	10
Large bird (e.g. kestrel)	Short-term	21.7	0.16 [3]	10
Large bird (e.g. kestrel)	Long-term	4.34	**>0.002** [3]	5

Indicator species/Category	Time scale	ETE	TER	Annex VI Trigger
Primary poisoning (Mammals) (Food bait)				
Small mammal	Acute		Clear risk, since target organism	10
Large mammal (e.g. dog)	Acute	1.0	50	10
Large mammal (e.g. dog)	Long-term	1.0	0.005	5
Secondary poisoning (Mammals) (Food poisoned rodents)				
Small mammal (e.g. weasel)	Acute	4.52	0.40 [4]	10
~~Large~~ Small mammal (e.g. weasel)	Long-term	4.52	0.001 [4]	5

[2] Risk considered to be lower in practice based on expert judgement: bait boxes designed to prevent accidental poisoning of birds, only incidental exposure should occur and if it happens the relatively large size of the pellet make them more unattractive than natural seeds for seed eating birds. The pellets are also coloured in order not to be attractive to birds.
[3] birds considered to feed on 100 % of poisoned rodents which are considered to feed 100 % on bait
[4] Mammals considered to feed on 100 % of poisoned rodents, which are considered to feed 100 % on bait

Bioaccumulation factor in terrestrial vertebrates	
Bioaccumulation factor (BAF)	0.36 calculated based on: α =0.82 F = 0.1(fox) DT_{50} = 3 days (k_2 = 0.23) This estimation was considered by EFSA a best-case approach. The depuration half life is bi-phasic – only at the beginning the depuration is rapid with a DT_{50} of 3 days, later it is 118 days. It is considered more appropriate to calculate the bi-phasic DT_{90} and to convert it to a single first order DT_{50} applying the factor of 3.32. Depending on the data set the DT_{90} would be 18.1d or 80.9 days with corresponding first order DT_{50} values of 5.5 and 24.37 days. The bioaccumulation factors are 0.65 and 2.88. A final conclusion can only be drawn after receipt of further explanation on the differences in the degradation patterns observed in the data sets provided in DOC IIIA/Section 6.2,

	tables A6 2-2 and A6 2-3.

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Laboratory tests ‡				
Fish				
Oncorhynchus mykiss	a.s.	96 hr (semi-static)	Mortality, LC_{50}	0.064 mg/L (mm)
Aquatic invertebrate				
Daphnia magna	a.s.	48 h (static)	Mortality, EC_{50}	0.520 mg/L (mm)
Algae				
Selenastrum capricornutum)	a.s.	72 h (static)	Biomass: E_bC_{50} Growth rate: E_rC_{50}	0.320 m/L (mm) 0.800 m/L (mm)
Microcosm or mesocosm tests Not required				

[1] indicate whether based on nominal ($_{nom}$) or mean measured concentrations ($_{mm}$). In the case of preparations indicate whether end points are presented as units of preparation or a.s.

Toxicity/exposure ratios for the most sensitive aquatic organisms (Annex IIIA, point 10.2)

Exposure of surface water from the use of difenacoum is considered to be insignificant since Neosorexa Pellets will always be in discretely placed bait trays and therefore the risk of aquatic exposure is insignificant. Therefore no TER values have been calculated. Nevertheless, the risk assessment performed under biocide directive 98/8/EC indicate low risk to water and sediment organisms.

Bioconcentration	
Bioconcentration factor (BCF) - estimated by calculation	BCFfish 35645 (calculated according to TGD method, Eq. 75, using estimated log Pow value of 7.6)
	BCFfish 9010 (calculated according to the EPA EPIWIN BCF estimation program, using log Pow value of 7.6)

	BCFearthworm 477 729 (calculated according to TGD method, Eq. 82d, using estimated log Pow value of 7.6)
	BCFearthworm 120 639 (calculated according to section 4.3 of document SANCO/4145/2000 using estimated log Pow value of 7.6)
Bio-accumulation in fish Depuration time(DT50) (DT90)	Waiving for non-submission of data is acceptable. However, using the calculated BCF values, an indication of the duration of the uptake phase was derived using the equations provided in OECD 305, Annex 4: - Uptake rate constant (K2) estimated to be 0.021 day-1, - time estimated to reach 80% of steady-state: 76 days, - time estimated to reach 95% of steady-state: 143 days. Therefore, these data suggest that steady-state may not be reached within the maximum duration of a study conducted according to the guidelines of OECD 305.
Level of metabolites (%) in organisms accounting for > 10 % of residues	-

Effects on honeybees (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

The recommended use of difenacoum as a rodenticide is not expected to result in any relevant exposure of honeybees, since difenacoum will be used in rodenticide baits in containers. Thus, for lack of any relevant exposure, testing for effects on honeybees is not considered relevant.

Effects on other arthropod species (Annex IIA, point 8.3.2, Annex IIIA, point 10.5)

The recommended use of difenacoum as a rodenticide is not expected to result in any relevant exposure of non-target arthropods, since difenacoum will be used in rodenticide baits in containers. Thus, for lack of any relevant exposure, testing for effects on non-target arthropods is not considered relevant.

Effects on earthworms, other soil macro-organisms and soil micro-organisms (Annex IIA points 8.4 and 8.5. Annex IIIA, points, 10.6 and 10.7)

The recommended use of difenacoum as a rodenticide is not expected to result in any relevant exposure of soil organisms, since difenacoum will be used in rodenticide baits in containers. Thus, for lack of any relevant exposure, testing for effects on soil organisms is not considered relevant.

Effects on non target plants (Annex IIA, point 8.6, Annex IIIA, point 10.8)

The recommended use of difenacoum as a rodenticide is not expected to result in any relevant exposure of non target plants, since difenacoum will be used in rodenticide baits in containers. Thus, for lack of any relevant exposure, testing for effects on non target plants is not considered relevant.

Effects on biological methods for sewage treatment (Annex IIA 8.7)

Test type/organism	end point
Activated sludge	Respiration inhibition; EC_{50} > 100 mg/L
Pseudomonas sp	Growth inhibition; EC_{50} > 2.3 mg/L

Ecotoxicologically relevant compounds (consider parent and all relevant metabolites requiring further assessment from the fate section)

Compartment	
soil	difenacoum
water	difenacoum
sediment	difenacoum
groundwater	difenacoum

Classification and proposed labelling with regard to ecotoxicological data (Annex IIA, point 10 and Annex IIIA, point 12.3)

	RMS/peer review proposal
Active substance	N; R50
	Cn > 2.5 %: N; R50-53 0.25 % < Cn < 2.5 %: N; R51-53 0.025 % < Cn < 0.25 %: R52-53 Cn <0.025%: not classified for the environmental hazard

	RMS/peer review proposal
Preparation	No classification; the content of difenacoum in Neusorexa Pellets is 0.005 % (w/w)

APPENDIX B – LIST OF ABBREVIATIONS

ε	decadic molar extinction coefficient
°C	degree Celsius (centigrade)
µg	microgram
µm	micrometer (micron)
a.s.	active substance
ADI	acceptable daily intake
AF	assessment factor
AOEL	acceptable operator exposure level
AR	applied radioactivity
ARfD	acute reference dose
ATP	Adaptation to Technical Progress (of the Council Directive 67/548/EEC)
AV	avoidance factor
BAF	Bioaccumulation factor
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstract Service
CI	confidence interval
CIPAC	Collaborative International Pesticide Analytical Council Limited
CL	confidence limits
d	day
DAA	days after application
DAR	draft assessment report
DAT	days after treatment
DM	dry matter
DT_{50}	period required for 50 percent disappearance (define method of estimation)
DT_{90}	period required for 90 percent disappearance (define method of estimation)
dw	dry weight
E_bC_{50}	effective concentration (biomass)
EC_{50}	effective concentration
ECB	European Chemical Bureau
EChA	European Chemical Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
ER_{50}	emergence rate/effective rate, median
ErC_{50}	effective concentration (growth rate)
EU	European Union
f(twa)	time weighted average factor
FAO	Food and Agriculture Organisation of the United Nations
FIR	Food intake rate
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FOMC	First-Order Multi-Compartment model
g	gram
GAP	good agricultural practice

GC	gas chromatography
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GS	growth stage
h	hour(s)
ha	hectare
hL	hectolitre
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HQ	hazard quotient
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
K_{foc}	Freundlich organic carbon adsorption coefficient
L	litre
LC	liquid chromatography
LC_{50}	lethal concentration, median
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
m	metre
M/L	mixing and loading
MAF	multiple application factor
mg	milligram
mL	millilitre
mm	millimetre
MRL	maximum residue limit or level
MS	mass spectrometry
MWHC	maximum water holding capacity
NESTI	national estimated short-term intake
ng	nanogram
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OC	organic carbon content
OM	organic matter content
PD	proportion of different food types
PEC	predicted environmental concentration
PEC_{air}	predicted environmental concentration in air
PEC_{gw}	predicted environmental concentration in ground water
PEC_{sed}	predicted environmental concentration in sediment
PEC_{soil}	predicted environmental concentration in soil
PEC_{sw}	predicted environmental concentration in surface water
pH	pH-value
PHI	pre-harvest interval
pK_a	negative logarithm (to the base 10) of the dissociation constant

P_{ow}	partition coefficient between n-octanol and water
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
PT	proportion of diet obtained in the treated area
QSAR	quantitative structure-activity relationship
r^2	coefficient of determination
RPE	respiratory protective equipment
SD	standard deviation
SFO	single first-order
STMR	supervised trials median residue
TER	toxicity exposure ratio
TER_A	toxicity exposure ratio for acute exposure
TER_{LT}	toxicity exposure ratio following chronic exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TWA	time weighted average
UDS	unscheduled DNA synthesis
UV	ultraviolet
W/S	water/sediment
WG	water dispersible granule
WHO	World Health Organisation
yr	year

APPENDIX C – USED COMPOUND CODE(S)

Code/Trivial name	Chemical name	Structural formula
N/A		