WHO SPECIFICATIONS AND EVALUATIONS FOR PUBLIC HEALTH PESTICIDES

BRODIFACOUM

3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4- tetrahydro-1-naphthyl]- 4-hydroxycoumarin

World Health Organization

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DISCLAIMER[1]

WHO specifications are developed with the basic objective of promoting, as far as practicable, the manufacture, distribution and use of pesticides that meet basic quality requirements.

Compliance with the specifications does not constitute an endorsement or warranty of the fitness of a particular pesticide for a particular purpose, including its suitability for the control of any given pest or its suitability for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular purpose and the content of the labelling instructions must be decided at the national or provincial level.

Furthermore, pesticides which are manufactured to comply with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable to their manufacture, sale, transportation, storage, handling, preparation and/or use.

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Additionally, WHO wishes to alert users to the fact that improper storage, handling, preparation and/or use of pesticides can result in either a lowering or complete loss of safety and/or efficacy.

WHO is not responsible, and does not accept any liability, for the testing of pesticides for compliance with the specifications, nor for any methods recommended and/or used for testing compliance. As a result, WHO does not in any way warrant or represent that any pesticide claimed to comply with a WHO specification actually does so.

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[1] This disclaimer applies to all specifications published by WHO.

INTRODUCTION

WHO establishes and publishes specifications[1] for technical material and related formulations of public health pesticides with the objective that these specifications may be used to provide an international point of reference against which products can be judged either for regulatory purposes or in commercial dealings.

From 2002, the development of WHO specifications follows the **New Procedure**, described in the "Manual for development and use of FAO and WHO specifications for pesticides." This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by WHO and the experts of the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS).

WHO specifications now only apply to products for which the technical materials have been evaluated. Consequently, from the year 2002 onwards, the publication of WHO specifications under the **New Procedure** has changed. Every specification consists now of two parts, namely the specifications and the evaluation report(s):

Part One: The Specification of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 9 of the above-mentioned manual.

Part Two: The Evaluation Report(s) of the pesticide, reflecting the evaluation of the data package carried out by WHO and the JMPS. The data are provided by the manufacturer(s) according to the requirements of chapter 3 of the above-mentioned manual and supported by other information sources. Evaluation reports include the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in chronological order to this report.

WHO specifications under the **New Procedure** do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other routes of manufacture. WHO has the possibility to extend the scope of the specifications to similar products but only when the JMPS has been satisfied that the additional products are equivalent to that which formed the basis of the reference specification.

Specifications bear the date (month and year) of publication of the current version. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.

[1] Publications available on the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website, https://extranet.who.int/pqweb/vector-control-products

PART ONE: SPECIFICATIONS

BRODIFACOUM

Brodifacoum Information

ISO common name

Brodifacoum (BSI, E-ISO, (m) F-ISO, ANSI)

Synonyms

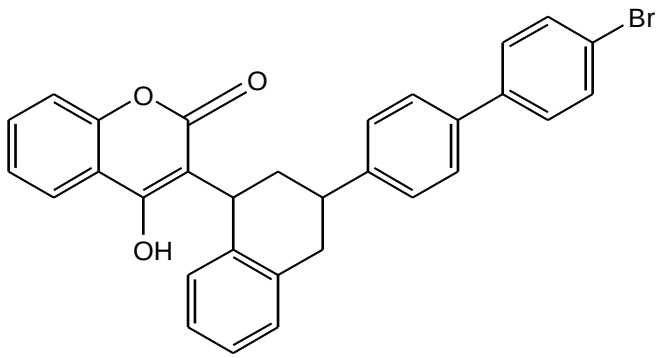
PP581

Chemical names

IUPAC 3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4- tetrahydro-1-naphthyl]- 4-hydroxycoumarin

CA 3-[3-(4’-bromo-[1,1’-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2*H*-1-benzopyran-2-one

Structural formula



Isomers of brodifacoum

cis isomer, a racemic mixture of (1*R*,3*S*) and (1*S*,3*R*) isomers
trans isomer, a racemic mixture of (1*R*,3*R*) and (1*S*,3*S*) isomers
cis:trans isomer ratio 50:50 to 80:20.

Molecular formula

$C_{31}H_{23}BrO_3$

Relative molecular mass

523.4 g/mol

CAS Registry number

56073-10-0

cis 72654-66-1
trans 72654-67-2

CIPAC number

370

EEC number

Annex I of Dir. 67/548/EEC Index # 607-172-00-1

EINECS 259-980-5

Identity tests

Retention time matches a reference standard under the analytical conditions described in the method of analysis (chromatographic separation with HPLC) for the formulated materials. Technical grade active ingredient can also be identified using IR spectroscopy in addition to the HPLC retention time matching with a reference standard.

Brodifacoum Technical Material

WHO specification 370/TC (December 2021*)

This specification, which is PART ONE of this publication, is based on evaluations of data submitted by the manufacturers whose names are listed in the evaluation reports (370/2014, 370/2021). This specification should be applicable to TC produced by these manufacturers, but it is not an endorsement of those products nor a guarantee that they comply with the specification. The specification may not be appropriate for TC produced by other manufacturers. The evaluation reports (370/2014, 370/2021), as PART TWO, form an integral part of this publication.

1 Description

The material shall consist of brodifacoum together with related manufacturing impurities in the form of white to pale cream powder and shall be free from visible extraneous matter and added modifying agents.

2 Active ingredient

2.1 **Identity tests** (370/TC/(M)/2, CIPAC Handbook O, p.13, 2017)

The active ingredient shall comply with an identity test, and where the identity remains in doubt, shall comply with at least one additional test.

2.2 **Brodifacoum content** (370/TC/(M)/3, CIPAC Handbook O, p.13, 2017)

The brodifacoum content shall be declared (not less than 950 g/kg), and when determined, the average measured content shall not be lower than the declared minimum content.

2.3 **Brodifacoum *cis:trans* isomer ratio** (370/TC/(M)/3, CIPAC Handbook O, p.13, 2017)

The brodifacoum *cis:trans* isomer ratio shall be declared, and when determined, the average measured ratio shall be in the range 50:50 to 80:20 (Note 1).

Note 1 The cis:trans isomer ratio does not affect the biological activity of the TC; therefore, it will not be considered for any future equivalence determination.

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* Specifications may be revised and/or additional evaluations may be undertaken.
Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website, https://extranet.who.int/pqweb/vector-control-products

Brodifacoum Bait (ready for use)

WHO specification 370/RB (December 2021*)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (370/2014). This specification should be applicable to relevant products of this manufacturer and those of any other formulators who use only TC from the evaluated sources. The specification is not an endorsement of those products nor a guarantee that they comply with the specification. The specification may not be appropriate for the products of other manufacturers who use TC from other sources. The evaluation report (370/2014), as PART TWO, forms an integral part of this publication.

1 Description

The material shall consist of solid blocks containing paraffin wax, palatability agents, pigments and technical brodifacoum, complying with the requirements of WHO specification 370/TC.

2 Active ingredient

2.1 **Identity tests** (370/RB/(M)/2, CIPAC Handbook O, p.16, 2017)

The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 **Brodifacoum content** (370/RB/(M)/3, CIPAC Handbook O, p.17, 2017)

The brodifacoum content shall be declared (mg/kg) and, when determined, the average measured content shall not differ from that declared by more than the following tolerances:

Declared content, mg/kg	Tolerance
above 25 up to 100 Note: the upper limit is included in the range	-20% to +30% of the declared content

3 Storage stability

3.1 **Stability at elevated temperature (MT 46.4, CIPAC Handbook P, p.232, 2021)**

After storage at 40 ± 2°C for 8 weeks, the determined average active ingredient content must not be lower than 90% relative to the determined average content found before storage (Note 1).

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* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website, https://extranet.who.int/pqweb/vector-control-products

Note 1 Samples of the formulation taken before and after the storage stability test may be analysed concurrently after the test in order to reduce the analytical error. In case the active ingredient content, after storage for 8 weeks at 40 ± 2°C, is below 90% of the initial analysis, the number of analytical samples should be increased (repetitions of the active ingredient content). This is due to the very low level of brodifacoum in the bait formulation as compared to formulations of other active ingredients and the difficulty in analysing such a low level of brodifacoum consistently in the bait matrix.

PART TWO: EVALUATION REPORTS

BRODIFACOUM

BRODIFACOUM

FAO/WHO Evaluation Report 370/2021

Recommendations

The Meeting recommended that:

i. The brodifacoum TC as proposed by Activa S.r.l. should be accepted as equivalent to the brodifacoum reference profile.
ii. The existing FAO specification for brodifacoum TC should be extended to encompass the technical material produced by Activa S.r.l.
iii. The existing WHO specification for brodifacoum TC should be extended to encompass the technical material produced by Activa S.r.l.

Appraisal

The Meeting considered data and supporting information submitted by Activa S.r.l. (Italy) (Activa)in 2019, 2020 and 2021 for the extension of the existing FAO and WHO specifications for brodifacoum TC. The data submitted were in accordance with the requirements of the Manual on development and use of FAO and WHO specifications for pesticides (2016, third revision of the first edition). The reference specifications and supporting data for brodifacoum TC and RB were submitted by Syngenta Crop Protection AG, and the FAO/WHO specifications were published in July 2015.

The Meeting was provided by Activa with commercially confidential information on the manufacturing process and five batch analysis data on all impurities present at or above 1 g/kg and their manufacturing limits in the TC.

Activa stated that the confidential data (manufacturing process, purity and impurity profile) submitted to FAO/WHO are identical to those submitted for registration in EU, Australia, Kenya, Lebanon, Serbia and South Africa. The Australian registration authorities (APVMA) confirmed that brodifacoum TC from Activa S.r.l. has been approved on 28 June 2018 for use in agriculture. The active substance brodifacoum was reviewed by the European Commission in 2016, on basis on applications submitted notably by Syngenta Crop Protection AG (reference profile) and Activa S.r.l. Brodifacoum was approved for use in biocidal products of product-type 14 as a rodenticide under regulation (EU) No 528/2012. The Meeting also noted that, in 2006, the active substance brodifacoum was not included in the Annex I to Council Directive 91/414/EEC for authorisations in plant protection products.

The manufacturing process of brodifacoum TC from Activa S.r.l. differs from this of the reference process (Syngenta Crop Protection AG). It consists of eight reaction steps, while this of the reference process consists of four reaction steps.

In the 5-batch GLP study initially submitted by Activa S.r.l. (batches manufactured in October 2011 and November 2011), the analytical method used for brodifacoum content was an in-house HPLC method that differs from the CIPAC method 370/TC/(M)/3 published in the Handbook O in 2017. Differences were observed in the extraction solvent, calibration, HPLC reversed phase column, elution type and detection wavelength. The in-house method for brodifacoum content was fully validated on its specificity, linearity of response, accuracy and precision. The FAO/WHO Manual recommends using standardised CIPAC methods when available,

which is the case for brodifacoum TC as referenced in the current specification. The Meeting therefore requested the manufacturer either to provide an analytical bridging study between the in-house and the CIPAC method either to provide a new 5-batch study using the CIPAC method for brodifacoum content.

The manufacturer provided a new 5-batch GLP study. In this study performed on batches of brodifacoum TC manufactured in October 2019, brodifacoum content was determined using the CIPAC method 370/TC/(M)/3 by reversed phase HPLC with UV detection at 266 nm and external standardisation. The content of brodifacoum was determined as the sum of both (cis and trans) diastereomers, and the cis/trans ratio was calculated from the fractions of the diastereomers. The manufacturing impurities were determined by reversed phase HPLC with UV detection at 275 nm. This method was fully validated on its specificity, linearity of response, accuracy (recoveries), precision and limits of detection (LOD) and quantification (LOQ). The identity of brodifacoum and significant impurities were confirmed by HPLC coupled with time-of-flight mass spectrometry detection (LC-TOF-MS). Water content was determined using the CIPAC method MT 30.5. The screening of the batches of brodifacoum TC using HPLC-DAD and LC-TOF-MS did not reveal any other significant impurities (higher than 1 g/kg) than those declared by the manufacturer. The Meeting noted that the brodifacoum content and the impurity profile of the 5 batches of the new study was very similar to this of the batches of the study initially submitted, which demonstrates that the in-house method provides similar results to the CIPAC method and also consistency in the manufacturing process.

The minimum purity of brodifacoum initially declared in the TC is 950 g/kg and complies with the existing FAO/WHO specification. The brodifacoum content in all 5 batches is higher than the specified limit, and the brodifacoum cis/trans isomer ratio is in the specified range of 50:50 to 80:20. Subsequently, the manufacturer amended the declared minimum purity of brodifacoum in their TC to 980 g/kg. No relevant impurities were declared. The mass balance range is 993–998 g/kg and similar to the reference profile of Syngenta Crop Protection AG (984–1000 g/kg). The maximum limits for the impurities were supported by the 5-batch data and are statistically justified. The 5-batch analysis study report indicates that no other significant impurity (each at or above 1 g/kg) was found in any of the 5 batches, and that there is no indication that the methods used missed any significant manufacturing-related impurity. The Meeting questioned the manufacturer on the possible presence of inorganic salts in the TC because several of them are used in the manufacturing process. Activa provided a GLP study report showing that the sulphated ash content measured according to the CIPAC method MT 29 is below 1 g/kg (0.2 g/kg), and therefore that the inorganic salts are correctly removed.

The manufacturer provided the Meeting with a bacterial reverse mutation test (Ames test) conducted with Salmonella typhimurium according to the OECD guideline 471 and complying with GLP priniciples. The manufacturer and the Meeting concluded that brodifacoum TC is non-mutagenic under the conditions of this test.

On basis of Tier-1 data provided by Activa (manufacturing process, purity/impurity profile, 5-batch analysis data, mutagenicity profile), the Meeting concluded that the brodifacoum TC from Activa should be considered as equivalent to the reference profile supporting the existing FAO and WHO specifications 370/TC (FAO/WHO evaluation report 370/2014).

The company provided the Meeting with summary data supported by GLP studies on the physical-chemical properties of pure brodifacoum TC, and also summary data on the toxicology profile of the brodifacoum TC, based on acute toxicity, irritation and skin sensitisation. These data were not considered by the Meeting, as it is not requested by the FAO/WHO Manual for equivalence assessment in Tier-1.

Additional action proposed by the Meeting

The Meeting also recommended updating the TC and RB specifications to align with the most recent versions of the specification templates in the Manual, in particular with the latest versions of CIPAC methods. They include:

- The reference to CIPAC methods for brodifacoum content and/or cis:trans isomer ratio that are now published in Handbook O for the TC and RB
- The replacement of method for accelerated storage stability MT 46.3 by MT 46.4 as published in CIPAC Handbook P for the RB
- The adaptation of some footnotes

Supporting Information
for
Evaluation Report 370/2021

Uses

Brodifacoum is a second-generation anticoagulant rodenticide. It is used in public health and agriculture against commensal rodent pests. It operates by disrupting the normal blood clotting mechanisms resulting in increased bleeding tendency, followed by eventual haemorrhaging and death.

Table 1. Chemical composition and properties of brodifacoum technical material (TC)

Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data	Confidential information supplied and held on file by FAO and WHO. Mass balances were 99.3-99.8% with no unknowns.
Declared minimum brodifacoum content	980 g/kg (cis/trans isomer ratio in the range 50:50 to 80:20)
Relevant impurities ≥ 1 g/kg and maximum limits for them	None
Relevant impurities **<** 1 g/kg and maximum limits for them	None
Stabilisers or other additives and maximum limits for them:	None

Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature range of the TC	235.8°C	100%	EEC method A.1	ENV5808/120140
Solubility in organic solvents	5.89 g/l in toluene at 20°C 29-34 g/l in dichloromethane at 20°C 10.1 g/l in ethyl acetate at 20°C 1.61 g/l in methanol at 20°C 21.2 g/l in acetone at 20°C 8.90 x 10^{-3} g/l in n-hexane at 20°C	99.7%	EEC method A.6 CIPAC MT 181	2109/0002

Annex 1: Hazard Summary Provided by the Proposer

Toxicological summaries

Notes:

i. The proposer confirmed that the toxicological data included in the summary below were derived from brodifacoum having impurity profiles similar to those referred to in the table above.
ii. The conclusions expressed in the summary below are those of the proposer, unless otherwise specified.

Table A. Mutagenicity profile of brodifacoum technical material based on bacterial *in vitro* tests

Species	Test	Purity %	Conditions and guideline	Result	Reference
Salmonella typhimurium TA 1535, TA 1537, TA 102, TA 98 and TA 100	Bacterial reverse mutation test (Ames test)	99.9 % w/w	OECD 471 0.15 - 5000 µg/plate (+/- S9)	Non-mutagenic	1558/006
Human lymphocytes	*In-vitro* chromosome aberration	99.9 % w/w	OECD 473 0.2, 0.4 µg/ml (-S9) 10 µg/ml (+S9)	Non-clastogenic	1558/003
L5178Y TK+/- mouse lymphoma mutation assay	*In-vitro* mammalian gene mutation	99.4 % w/w	OECD 490:2016 Exp 1: 3.91 - 62.5 µg/ml (+/- S9) Exp 2: 0.98 - 15.625 µg/ml (+/- S9)	Non-mutagenic	20.507765.0001

Annex 2: References

Study number	Author(s)	year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
CH-222/2013	Paronuzzi Ticco S.	2013	Brodifacoum technical: complete analysis of five batch samples. CH-222/2013. GLP. ChemService. Unpublished.
CH-221/2013	Paronuzzi Ticco S.	2013	Brodifacoum technical: validation of the analytical method for the determination of the significant impurities content. CH-221/2013. GLP. ChemService. Unpublished.
CH-606/2008	Garofani S.	2009	Brodifacoum technical: validation of the analytical method for the determination of the active ingredient content. CH-606/2008. GLP. ChemService. Unpublished.
CH-0204/2020	Rigamonti E	2020	Brodifacoum technical: complete analysis of five batch samples. CH-0204/2020. GLP. ChemService. Unpublished.
CH-0100/2021	Rigamonti E	2021	Brodifacoum technical: Determination of the Sulphated Ash. CH-0100/2021. GLP. ChemService. Unpublished.
2109/0002	D F White D M Mullee	2006	Determination of physico-chemical properties. 2109/0002. GLP. SafePharma Laboratories. Unpublished.
ENV5808/120140	R M Drake	2003	Determination of the Melting Point and Boiling Point of Brodifacoum technical. ENV5808/120140. GLP. Chemex. Unpublished.
1558/003	N P Wright	2003	Brodifacoum: Chromosome aberration test in Human Lymphocytes in vitro. 1558/003. GLP. SafePharma Laboratories. Unpublished.
1558/004	R Durward	2004	Brodifacoum: L5178Y TK +/- Mouse Lymphoma Assay. 1558/004. GLP. SafePharma Laboratories. Unpublished.
1558/006	P W Thompson	2004	Brodifacoum: reverse mutation assay “Ames Test” using *Salmonella typhimurium*. 1558/006. GLP. SafePharma Laboratories. Unpublished.
2109/0004	A Sanders	2006	Local Lymph node assay in the mouse. 2109/0004. GLP. SafePharma Laboratories. Unpublished.
20.507765.0001 (with amendments N° 1 and 2)	Licitra F.	2020 2021	Brodifacoum: L5178Y TK +/- Mouse Lymphoma Assay (MLA) according to OECD 490:2016 on BRODIFACOUM, batch n° 041605. 20.507765.0001. GLP. Chelab. Unpublished.

BRODIFACOUM

FAO/WHO Evaluation Report 370/2014

Recommendations

The Meeting recommended the following:

i. The existing WHO specifications for brodifacoum TC (WHO/SRoT/1.R1, August 2009) and RB (WHO/IS/7.Ro1.1.R3, August 2009) should be withdrawn.
ii. The revised specifications for brodifacoum TC and RB, proposed by Syngenta Crop Protection AG, and as amended by the Meeting, should be adopted by FAO and WHO, and published under the category of specifications under the new procedure.

Appraisal

Draft specifications and supporting data were provided by Syngenta Crop Protection AG in 2013 and evaluated by the Meeting in support of new FAO and WHO specifications for brodifacoum TC and RB.

Brodifacoum is a second-generation anticoagulant rodenticide. It is used in public health and agriculture against commensal rodent pests. It operates by disrupting the normal blood clotting mechanisms resulting in increased bleeding tendency, followed by eventual haemorrhaging and death.

Brodifacoum is not under patent.

Brodifacoum was evaluated by WHO in 2009 under the old procedure. The 2009 WHO published specifications for brodifacoum TC, CB and RB replaced the WHO specifications published in 1999.

Brodifacoum was also evaluated by the US EPA in 2008 and the European Commission in 2010. Brodifacoum was included in Annex I of Directive 98/8/EC in 2010 with a minimum purity of 950 g/kg.

Brodifacoum has two chiral centres. Brodifacoum exists as cis- and trans-isomers, where the cis-isomer is a racemic mixture of (1R,3S) and (1S,3R) and the trans-isomer is a racemic mixture of (1R,3R) and (1S,3S). The minimum purity of 950 g/kg refers to the sum of cis and trans isomers. The cis:trans isomer ratio range for the technical material has been specified as 50:50 to 80:20.

The meeting noted that a letter of access remained outstanding and that Syngenta had not yet submitted their new 5-batch analysis to a national regulatory authority confirming that the confidential data on the manufacturing process and declaration of composition submitted to the WHO/FAO were the same as those submitted to a national regulatory authority. However, the meeting also noted that the proposed specification is identical to the Syngenta specification that was accepted under the EU biocides review process (the EU biocide submission was based on an older 5-batch from the same source). The Meeting agreed that although strictly speaking the same data was not presented to both JMPS and the EU biocides review process, a letter of access will not be required in this specific case because the method of manufacturing is the same and the specification has not changed.

Syngenta Crop Protection AG brodifacoum is currently registered in Europe, Australia, New Zealand, Canada, Africa, North America and South America.

Syngenta informed the Meeting that it was not their intention to support the CB specification.

Syngenta provided physical and chemical data for pure and technical brodifacoum. Brodifacoum is a creamy white substance. It is not considered to be volatile (<10-9 Pa at 20oC) and has a melting point of 232oC with decomposition. It is relatively insoluble in water at 20oC and pH 7.4 (solubility of 0.24 mg/l). Brodifacoum is reasonably soluble in organic solvents and is expected to bioaccumulate (log Pow = 8.5). It is considered to be relatively stable to hydrolysis at all environmentally relevant pHs (DT50 at 25oC = 173 days at pH 5, 300 days at pH 7, and stable at pH 9). It undergoes rapid photolysis (test pH and temperature not mentioned) with a DT50 < 7 hours. The pKa was not experimentally determined due to the low solubility of brodifacoum in water.

Physical-chemical data are not available for the individual diastereomers and the company did not want to commission new experimental tests due to the toxicity of the active ingredient. The company provided significant evidence that they had gone to every extent possible in order to obtain useful information from the open literature or predictive models – no useful information could be found with respect to the physical-chemical properties of the individual diastereomers.

The Meeting was provided with commercially confidential information in relation to the proposed technical specification, the manufacturing process and the supporting 5-batch analysis. Mass balances were between 98.4 - 100.0% w/w in the 5-batch data and no unidentified impurities greater than 1 g/kg were reported. Syngenta proposed a minimum active ingredient content of 950 g/kg in the technical material as manufactured with a cis:trans isomer ratio range of 50:50 to 80:20. The meeting noted that the brodifacoum isomers exhibit similar biological activity and therefore the cis:trans isomer ratio will not be used for equivalency determination in the future.

There are no relevant impurities in the technical material as manufactured.

Syngenta used fully validated methods of analysis for the analysis of active ingredient content, isomer ratio, and impurities in their supporting 5-batch analysis. The Meeting noted that the method of analysis for determining the active ingredient content and isomer ratio in the 5-batch analysis is not the same method of analysis as the CIPAC method accepted as provisional in 2014 . The 5-batch method and the CIPAC method use different chromatographic conditions. However, Syngenta provided a bridging study to show that the 5-batch method and the CIPAC method give comparable results.

It should be noted that the current FAO/WHO specification manual (November 2010 - second revision of the first edition) does not contain a template for RB specifications. However, Syngenta included a storage stability clause for active ingredient content in their RB specification proposal and the RB specification was considered acceptable.

Supporting Information
for
Evaluation Report 370/2014

Physico-chemical properties of brodifacoum

Table 1. Physico-chemical properties of pure brodifacoum

Parameter	Value(s) and conditions	Purity %	Method reference (and technique if the reference gives more than one)	Study number
Vapour pressure	$<<10^{-6}$ Pa at 20°C	98.7	OECD 104, by extrapolation	PP581_0007 (1991)
Melting point	232°C with decomposition	98.7	OECD102	PP581_0007 (1991)
Boiling point and/or temperature of decomposition	Not determined. The substance decomposes above 232°C.	-	-	PP581_0007 (1991)
Solubility in water	pH 5.2 : 0.0038 x 10^{-3} g/l (20 °C) pH 7.4 : 0.24 x 10^{-3} g/l (20°C) pH 9.3 : 10 x 10^{-3} g/l (20°C)	97.4	OECD 105	PP581_0007 (1991)
Octanol/water partition coefficient	log P_{OW} = 8.5 log P_{OW} = 6.2	-	Calculated CLOGP algorithm Estimate from K_{oc}	PP581_0009 (1990)
Hydrolysis characteristics	Half life = 173 day at 25°C at pH 5 Half life = 300 day at 25°C at pH 7 Half life = Stable at 25°C at pH 9	97.9	OECD 111	PP581_0189 (1995)
Photolysis characteristics	Open water (minutes): 60 (summer), 366 (winter), 78 (summer) Clear sky (minutes): 23 (summer), 143 (winter), 30 (spring) pH not mentioned	100	OPPTS 835.2210	PP581_0441 (2004)
Dissociation characteristics	pK_a not determined due to very low water solubility.	-	-	PP581_10455 (2008)
Solubility in organic solvents	Solubility in organic solvents is only available for the Technical Grade Active Ingredient	-	-	-

Table 2. Chemical composition and properties of brodifacoum technical material (TC)

Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data	Confidential information supplied and held on file by FAO and WHO. Mass balances were 98.4-100.0% with no unknowns.			
Declared minimum brodifacoum content	950 g/kg (cis/trans isomer ratio of 50:50 to 80:20)			
Relevant impurities ≥ 1 g/kg and maximum limits for them	None			
Relevant impurities < 1 g/kg and maximum limits for them	None			
Stabilisers or other additives and maximum limits for them	None			
Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature range of the TC	Melts with decomposition 201-205°C	92.5	OECD 102	PP581_0006 (1991)
Solubility in organic solvents	Solubility at 20°C (g/l) Hexane 0.088 Toluene 7.2 Dichloromethane 50 Acetone 23 Ethyl acetate 12 Acetonitrile 3.2 Methanol 2.7	92.5	OECD 105	PP581_0006 (1991)

Formulations and co-formulated active ingredients

The main formulation type available is the RB. This formulation is registered and sold in many countries throughout the world. Brodifacoum is not co-formulated with other pesticides.

Methods of analysis and testing

The analytical method for the active ingredient (including identity tests) is based on LC with UV detection at 254 nm using an external standard. A CIPAC collaborative study of the method was initiated in 2013; and the results of the study were presented to CIPAC in 2014. The method has been accepted as a provisional CIPAC method. Identity is based upon comparing retention time and IR spectra with that of a characterised reference material.

The analytical method for the determination of *cis:trans* isomer ratio is based on HPLC with UV detection at 254 nm using an external standard.

The method(s) for determination of impurities are based on HPLC with UV detection at 254 nm, using an external standard.

Test methods for determination of physico-chemical properties of the technical active ingredient were essentially OECD and EPA methods, while those for the formulations were CIPAC methods, as indicated in the specifications.

Physical properties

The FAO/WHO Manual does not contain specification guidelines for RB formulations, however the active ingredient content remains stable in the formulation after accelerated storage.

Containers and packaging

No special requirements for containers and packaging have been identified.

Expression of the active ingredient

The active ingredient is expressed as brodifacoum.

Annex 1: Hazard Summary Provided by the Proposer

Note:

(i) The proposer confirmed that the toxicological and ecotoxicological data included in the summary below were derived from brodifacoum having impurity profiles similar to those referred to in the table 2 above.
(ii) The conclusions expressed in the summary below are those of the proposer, unless otherwise specified.

Table A. Toxicology profile of brodifacoum technical material, based on acute toxicity, irritation and sensitisation

Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
Rat (m, f)	Acute Oral LD_{50} (OECD 401)	96.1	14 day observation period Dose levels (mg/kg): 0.25, 0.5 (m,f) 0.35 (m), 0.75 (f)	LD_{50} = 0.49 mg/kg (0.418 mg/kg males, 0.561 mg/kg females)	PP581_0077 (1993)
Rat (m, f)	Acute Dermal LD_{50} (OECD 402)	95.6	14 day observation period Dose levels: 1, 10, 500 mg/kg	LD_{50} = 4.1 mg/kg (5.21 mg/kg males, 3.16 mg/kg females)	PP581_0075 (1991)
Rat (m, f)	Acute Inhalation LC_{50} (OECD 403)	96.1	4 h exposure (nose only) 14 day observation period Nominal: 0.5, 1.5, 5 µg/l Analytical: 0.69, 1.72, 4.4 µg/l	LC_{50} = 3.96 µg/L (4.86 µg/L males, 3.05 µg/L females)	PP581_0079 (1993)
Rabbit (f)	Skin irritation (OECD 404)	92.5	Observations 1-72 hours Dose: 0.25 ml, 0.5% w/v /animal	Non-irritant	PP581_0380 (1978)
Rabbit (f)	Eye irritation (OECD 405)	92.5	Observations 1h-7 day Dose: 100 mg/eye	Non-irritant	PP581_0380 (1978)
Guinea-pig (m, f)	Skin sensitisation Buehler (OECD 406)	96.1	Observations 24-48 h. Induction: 1%, 1%, 0.1% BFC Challenge: 0.1% 0.05% BFC	Skin sensitiser	PP581_0089 (1996)

Brodifacoum is of high acute toxicity if swallowed, inhaled or in contact with skin (WHO classification extremely hazardous, class Ia). It is not a skin or eye irritant but is a skin sensitiser.

Table B. Toxicology profile of brodifacoum technical material, based on repeated administration (subacute to chronic)

Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
Rat (m,f)	Short term toxicity	92.5	OECD 408 90 d dietary oral Rat Wistar Dose Levels: 0, 0.02, 0.08 ppm	NOAEL = 0.001 mg/kg/d	PP581_0119 PP581_0120 (1984)
Dog (m,f)	Short term toxicity	96.1	OECD 409 6-week oral capsule Beagle Dog Dose levels: 0, 0.0001, 0.0003, 0.001, 0.003, 0.01 mg/kg bw/day	NOAEL = 0.003 mg/kg/d	PP581_0114 (1997)

	Waiver for long term and multigeneration studies				PP581_0409 (2004)
	Waiver for repeat dose and sub-chronic dermal inhalation studies				PP581_0410 (2004)
Rat (F)	Developmental toxicity	92.5	OECD 414 Dose levels: 0, 0.001, 0.01, 0.02 mg/kg bw/day	Maternal = 0.001 mg/kg/bw/d Developmental = 0.02 mg/kg/bw/d	PP581_0125 (1980) PP581_0123 (1991) PP581_0376 (1991) PP581_0377 (1980)
Rabbit (F)	Developmental toxicity	92.5	OECD 4140, 0.001, Dose levels: 0.002, 0.005 mg/kg bw/day	Maternal = 0.002 mg/kg/d Developmental = 0.005 mg/kg/d	PP581_0344 (1980) PP581_0346 (1991) PP581_0345 (1991)

Table C. Mutagenicity profile of brodifacoum technical material based on *in vitro* and *in vivo* tests

Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
Bacterial gene mutation (Salmonella)	Ames	96.0	1.6-5000 µg/plate (+/- S9) 0.064 µg/plate (+/- S9) for TA1538 and TA100 OECD 471	Negative	PP581_0383 (1984)
Human lymphocytes	*In-vitro* cytogenetics	97.6	5, 10, 50 µg/ml (+/- S9) OECD 473	Negative	PP581_0335 (1990)
Human lymphocytes	*In-vitro* chromosome abberation	96.0	1, 10, 100, 1000 µg/ml (+/- S9) OECD 473	Negative	PP581_0129 (1984)
L5178Y mouse lymphoma cells	*In-vitro* mammalian gene mutation	96.0	Exp 1: 3.9-62.5 µg/ml (+/- S9) Exp 2: 8-128 µg/ml (+/- S9) Exp 3: 47.5-112.5 µg/ml (+/- S9) OECD 476	Negative	PP581_0384 (1984)
HeLa cells	*In-vitro* Unscheduled DNA synthesis	96.0	1, 10, 100, 1000 µg/ml (+/- S9)	Negative	PP581_0128 (1984)
Mouse	*In-vivo* micronucleus	96.0	0.187 and 0.3 mg/kg OECD 474	Negative	PP581_0130 (1984)

Brodifacoum was tested for different endpoints including gene mutation, chromosome aberration and DNA-damage in bacteria and in mammalian cells in-vitro and in-vivo. No mutagenic effects were noted in any test.

Table D. Ecotoxicity profile of brodifacoum technical material

Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
Anas platyrhynchos Mallard Duck	Acute oral	97.6	USA EPA 163.71-1 28 days observation Dose levels: 0, 0.1, 0.2, 0.25, 0.80, 1.40, 2.00, 2.60, 3.20, 3.80, 4.40 5 mg/kg	LD_{50} = 0.31 mg/kg	PP581_0205 (1980)
Coturnix japonica Japanese quail	Acute oral	92.5	USA EPA 14 days observation Dose levels: 7, 8.2, 10, 12.8, 16.6, 22.9 mg/kg	LD_{50} = 11.6 mg/kg	PP581_0340 (1977)
Chicken	Acute oral	92.5	USA EPA 14 days observation Dose levels: 7, 4.5, 9, 18, 36, 72 mg/kg	LD_{50} = 4.5 mg/kg	PP581_0213 (1977)
Phasianus colchicus Ring necked pheasant	Acute oral	96	USA EPA 71-1 (1982) 35 days observation Dose levels: 0, 0.038, 0.080, 0.155, 0.345, 0.650 mg/kg	LC_{50} = 0.545 mg/kg	PP581_0210 (1986)
Larus atricilla Laughing gull	Acute dietary	Not stated	OECD 205 5-day dosing, 35 day observation Dose levels: 0, 0.72, 1.62, 3.41, 7.26, 14.02 ppm in diet	LC_{50} = 0.72 mg/kg diet	PP581_0336 (1979)
Larus atricilla Laughing gull	Acute dietary	Not stated	OECD 205 5-day dosing, 35 day observation Dose levels: 0, 0.13, 0.34, 0.84, 2.10, 5.26 ppm in diet	LC_{50} = 1.6 mg/kg diet	PP581_0208 (1979)
Anas platyrhynchos Mallard Duck	Acute dietary	94	OECD 205 5-day dosing, 35 day observation Dose levels: 1, 1.78, 3.16, 5.62, 10.0, 17.8, 31.6, 56.2, 100 ppm in diet	LC_{50} = 2.7 mg/kg diet	PP581_0203 (1978)
Oncorhynchus mykiss Rainbow trout	Acute	Not stated	96 hour exposure under flow through conditions/freshwater OECD 203 Test concentrations: 0.0092, 0.0110, 0.0215, 0.023, 0.029, 0.055, 0.103, 0.182 mg/l	LC_{50} = 0.04 mg/l	PP581_0238 (1976)
Lepomis macrochirus Bluegill sunfish	Acute	Not stated	96 hours exposure under flow-through conditions/ freshwater OECD 203 Test concentrations: 0, 0.022, 0.033, 0.047, 0.068, 0.1, 0.15, 0.22, 0.33, 0.68 mg/l	LC_{50} = 0.165 mg/l (based on nominal concentrations)	PP581_0240 (1976)
Daphnia magna Water Flea	Acute	95%	48 hours exposure under static renewal conditions/ freshwater OECD 202 Test concentrations: 0, 0.13, 0.25, 0.5, 1.0, 2.0, 4.0 mg/l	EC_{50} = 0.45 mg/l	PP581_0440 (2003)
Selenastrum capricornutum Fresh water green alga	Growth inhibition	95%	72 hours exposure under static conditions/ freshwater OECD 201	E_rC_{50} = 0.27 mg/l E_bC_{50} =0.06 mg/l	PP581_0439 (2003)

Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
			Test concentrations: 0, 0.032, 0.056, 0.1, 0.18, 0.32 mg/l		
Eisenia foetida Earthworm	Acute toxicity, mortality / behaviour	95%	14 days exposure OECD 207 Soil concentration: 0, 318, 556, 994 mg/kg dry soil	LC_{50} > 994 mg/kg dry soil	PP581_0438 (2005)
Aerobic bacteria Sewage treatment plant sludge	Activated sewage sludge respiration inhibition	95.6	30 minutes contact time; OECD 209; test concentration: 100 mg/l	IC_{50} > 100 mg/l	PP581_10461 (2001)

Brodifacoum is of high acute toxicity to birds, fish, aquatic invertebrates and algae, but is of low toxicity to earthworms and aerobic sewage sludge bacteria.

EU classification 1272/2008, Annex Vi, Table 3.1

Classification		**Labelling**		
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Pictogram Signal Word Code(s)**	**Hazard Statement Code(s)**	**Suppl. Hazard statement code(s)**
Acute Tox. 1 Acute Tox. 2 * STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H310 H300 H372 H400 H410	GHS06 GHS08 GHS09 Dg	H310 H300 H372 ** H410	-
Specific Concentration Limits and M Factors				
Concentration		**Classification**		
None		None		
Pictogram(s)				
				
Skull and crossbones		Health Hazard		Environment

Regulation (EC) No 1272/2008, Annex VI, Table 3.2

Classification	Risk Phrases	Safety Phrases	Indication(s) of danger
T+; R27/28 T; R48/24/25 N; R50-53	27/28 48/24/25 50/53	1/2 36/37 45 60 61	T+ N
Concentration Limits			
Concentration		**Classification**	
None		**None**	
Symbols			
			
Very Toxic		Dangerous for the Environment	

Annex 2: References

Study number	Author(s)	Year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study
PP581_0006	SYT	1991	Brodifacoum TGAI: Physicochemical Data File.
PP581_0007	SYT	1991	Pure Brodifacoum: Physico-chemical Data File.
PP581_0009	SYT	1990	Brodifacoum: Octanol-Water Partition Coefficient.
PP581_0075	SYT	1991	Brodifacoum Technical: Acute Dermal Toxicity in the rat.
PP581_0077	SYT	1993	Brodifacoum Technical: Acute Oral Toxicity in the rat.
PP581_0079	SYT	1993	Brodifacoum Technical: 4-Hour Acute Inhalation Toxicity Study in the rat.
PP581_0089	SYT	1996	Brodifacoum: Skin sensitisation in the guinea-pig.
PP581_0114	SYT	1997	Brodifacoum: 6 week oral toxicity study in dogs.
PP581_0119	SYT	1984	Brodifacoum: 90-day Feeding Study in Rats.
PP581_0120	SYT	1984	Brodifacoum: 90-day Feeding Study in Rats, Individual Animal Data Supplement.
PP581_0123	SYT	1991	Brodifacoum: Tetratogenicity Study in the Rat, 1st amendment.
PP581_0125	SYT	1980	Brodifacoum: Tetratogenicity Study in the Rat.
PP581_0128	SYT	1984	Study of the Capacity of the Test Article Brodifacoum to Induce Unscheduled DNA Synthesis in Cultured HeLa Cells (Autoradiographic Method).
PP581_0129	SYT	1984	InVitro Study of Chromosome Abberation Induced by the Test Article Brodifacoum in Cultured Human Lymphocytes.
PP581_0130	SYT	1984	An Evaluation of Brodifacoum in the Mouse Micronucleus Test.
PP581_0189	SYT	1995	Brodifacoum: Aqueous Hydrolysis in pH5, pH7 and pH9 Solutions at 25°C.
PP581_0203	SYT	1978	Forty-Day Dietary LC50 - Mallard Duck - Technical Brodifacoum.
PP581_0205	SYT	1980	The Acute Oral Toxicity (LD50) of Brodifacoum to the Mallard Duck.
PP581_0208	SYT	1979	Forty-Day Dietary LC50 - Laughing Gull - Masticated Rodent Tissue containing PP581.
PP581_0210	SYT	1986	The Acute Oral Toxicity of Brodifacoum to the Ring-Necked Pheasant.
PP581_0213	SYT	1977	The Acute Oral Toxicity (LD50) of PP581 to the Chicken.
PP581_0238	SYT	1976	Determination of the Acute Toxicity of PP581 to Rainbow Trout (Salmo Gairdneri).
PP581_0240	SYT	1976	Determination of the Acute Toxicity of PP581 to Bluegill Sunfish (Lepomis Macrochirus).
PP581_0335	SYT	1990	Brodifacoum: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes.
PP581_0336	SYT	1979	Forty Day LC50 - Laughing Gull technical Brodifacoum.
PP581_0340	SYT	1977	The Acute Oral Toxicity (LD50) of PP581 to the Japanese Quail.
PP581_0344	SYT	1980	Brodifacoum: Teratogenicity Study in the Rabbit.

Study number	Author(s)	Year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study
PP581_0345	SYT	1991	Brodifacoum: Teratogenicity Study in the Rabbit, Individual Foetal Data Supplement.
PP581_0346	SYT	1991	Brodifacoum: Teratogenicity Study in the Rabbit, 1st amendment.
PP581_0376	SYT	1991	Brodifacoum: Teratogenicity Study in the Rat, Individual Foetal Data Supplement.
PP581_0377	SYT	1991	Brodifacoum: Teratogenicity Study in the Rat, Individual Animal Data Supplement.
PP581_0380	SYT	1978	Brodifacoum : Skin and Eye Irritation (Rabbit).
PP581_0380	SYT	1978	Brodifacoum : Skin and Eye Irritation (Rabbit).
PP581_0383	SYT	1984	Brodifacoum - An Evaluation in the Salmonella Mutagenicity Assay.
PP581_0384	SYT	1984	Brodifiacoum: Assessment of mutagenic potential using L5178Y mouse lymphoma cells.
PP581_0409	SYT	2004	Brodifacoum: Waiver Requests for Long Term and Multigeneration Studies.
PP581_0410	SYT	2004	Brodifacoum: Waiver Requests for Repeat Dose and Sub-Chronic Dermal and Inhalation Studies.
PP581_0438	SYT	2005	The toxicity to Eisenia foetida foetida of Brodifacoum.
PP581_0439	SYT	2003	The growth inhibition of the Alga Selanastrum capricornutum by Brodifacoum Technical.
PP581_0440	SYT	2003	The toxicity to Daphnia magna of Brodifacoum technical.
PP581_0441	SYT	2004	Determination of the direct Photolysis Rate in Water by Sunlight of Brodifacoum.
PP581_10455	SYT	2008	Calculation of the partion coefficient at different temperatures.
PP581_10461	SYT	2001	Activated Sludge Respiration Inhibition test with Brodifacoum.
PP581_10645	Malek J.	2013	Brodifacoum Technical (PP581) - Analysis of Five Representative Batches Produced at Pentagon Fine Chemicals ltd, Widnes, Cheshire, UK. Report No. TK0219143. GLP. Syngenta Crop Protection.