European Commission

Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

BLOOD MEAL

Volume 3 – B.9 (PPP) – Certosan

Rapporteur Member State: Austria
Co-Rapporteur Member State: Lithuania

Version History

When	What
2018/02	Original dossier submission by applicant
2018/04	Revised dossier submission by applicant
2018/12	Draft RAR by RMS AT
2019/02	Draft RAR by RMS AT after commenting by Co-RMS LT

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B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

This document contains the ecotoxicological risk assessment for the active substance **Blood meal** and the product **Certosan** (99.8% w/w Blood meal), which is a wettable powder (WP) formulation. The representative formulation Certosan is intended as game repellent and will be used as protection coating on the outer surface of trees.

This document supports the application for renewal of the regulatory approval of **Blood meal** under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012. This document presents ecotoxicological studies and risk assessments of the plants protection product **Certosan**.

This document covers data and risk assessments which were not part of the original dossier and which are necessary to reflect changes:

- In requirements under Commission Regulation (EU) No 283/2013, and the associated Annex, which repeals Commission Regulation (EU) No 544/2011 which, under Regulation (EC) 1107/2009, replaced the requirements of Annex II to Directive 91/414/EEC
- In scientific and technical knowledge since the approval or last renewal of the approval
- To representative uses

Where the conclusions of the EU review had specific areas of concern on Blood meal, new data and/or reviews and/or risk assessments have been provided. Where additional and/or new data on Blood meal are provided, a justification has been included. In addition, a justification has been given if new data are required but none were provided.

For the renewal of the active substance Blood meal and the representative formulation **Certosan** (99.8% w/w Blood meal) in Annex I, data to support the application for inclusion regarding ecotoxicology is provided in the following section. This renewal assessment report (RAR) contains summaries of studies on the product **Certosan**, which were not available at the time of the Annex I of **Blood meal** inclusion and were therefore, not evaluated during the first EU review of this compound. In addition, all studies, which were already submitted for the Annex I inclusion, were re-evaluated according to the current valid test guidelines and were summarised in the RAR (study title box is fully greyed out).
Studies, which were submitted for the first EU peer-review of the active substance **Blood meal** but are no longer a data requirement according to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) are briefly summarised (text in *italic*).

In the case where published literature is used to **scientifically justify or support** why a study was not deemed necessary to be conducted or as supporting information, only a superscript is referenced in the text, while full bibliographical information can be found in a respective footnote. Relevant literature from the EFSA- compliant literature search, which has to be evaluated on full-text level, is discussed under the respective data point under point Vol. 3 CA B9 B.9.10.1.

B.9.0. INTRODUCTION

Blood meal was included in the Annex I of Directive 91/414 under Inclusion Directive 2008/127/EC RMS for assessment of Blood meal was Belgium. The Regulation (EU) No 1107/2009 repealed and replaced the Directive 91/414/EEC and the active substance Blood meal is deemed to be approved under that Regulation and included in the Annex to Regulation (EC) No 540/2011 amended by Commission Implementing Regulation (EU) No 369/2012 and Commission Implementing Regulation (EU) 2017/195.

Blood meal was included in Annex I under provision as use in game repellent. The SANCO report for Blood meal (SANCO/2604/08 - rev 1-4 dated 11th July 2014) and Peer review document EFSA 2011 (EFSA Journal 2011;9(10):2394) are considered to provide the relevant information for the re-registration of Blood meal. The formulated product Certosan contains 99.8 % blood meal and is therefore identical with the active substance. Data obtained with the product can be used also for the active substance Blood meal.

The product Certosan is a game repellent and will be used as protection coating on the outer surface of trees. Certosan dries off to a layer and unfolds its action by its deterrent smell and taste. The product leads to a reduction of losses caused by game biting of trees during all-season.
Certosan will be mixed with water and then be painted or sprayed onto trees. A dipping application of the whole plant is also be used. The application of Certosan in forestry or orchards is not comparable with usual agricultural applications as only the individual plants will be treated. Only in nurseries young trees will be treated with tractor mounted spray equipment. The product is not used on plant parts which serve as nourishment for humans or animals.

The use pattern for the representative formulation Certosan (99.8% w/w Blood meal) is summarised in Table 9.0-1.

Table 9.0-1 : Intended application pattern for Certosan

1	2	3	4	5	6	7	8	9	10	11	12	13
Use-No.	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**			**Application rate**			**PHI** (days)	**Remarks:**
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg a.s. / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. g safener/ synergist per ha
1	Central North	Deciduous and coniferous trees in forestry	F	Game repellent	Coating with brush, Spraying or dipping individual plants, entire plants	all season	a) 1 b) 1	a) 19.8 b) 19.8	a) 19.8 b) 19.8	80-400	-	-
2	Central North	Trees in orchards	F	Game repellent	Coating with brush, Spraying or dipping individual plants, entire plants	all season	a) 1 b) 1	a) 19.8 b) 19.8	a) 19.8 b) 19.8	80-400	-	-
3	Central North	Ornamental plants	F	Game repellent	Coating with brush, Spraying or dipping individual plants, entire plants	all season	a) 1 b) 1	a) 19.8 b) 19.8	a) 19.8 b) 19.8	80-400	-	-
4	North	Deciduous and coniferous trees in forestry Agriculture and garden	F	Game repellent	Coating with brush, Spraying or dipping individual plants, entire plants	all season	a) 1 b) 1	a) 20 b) 20	a) 19.96 b) 19.96	5-15	-	-
5	North	Deciduous and coniferous trees in forestry Agriculture and garden	F	Vole	Coating with brush, Spraying or dipping individual plants, entire plants	all season	a) 1 b) 1	a) 20 b) 20	a) 19.96 b) 19.96	5-15	-	-

1	2	3	4	5	6	7	8	9	10	11	12	13
Use-No.	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/ synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg a.s. / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
6	Central North	Deciduous and coniferous trees in forestry Agriculture and garden	F	Vole	Spraying individual plants; entire plants	all season	a) 1 b) 1	a) 20 b) 20		5-15	-	-

F… Field use

Table 9.0-2 : Substances and metabolites of environmental relevance (structure, synonyms and codes)

Code	IUPAC name	Compound found in	Structural formula
Blood meal	Not applicable (CAS no.: 90989-74-5)	Environment (soil, surface water)	Not applicable
No relevant metabolites.			

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

Toxicity of the active substance

As reported in Vol. 3 CA B9 B.9.1.1 the waiver for standard toxicity studies on birds is considered acceptable taking into account that Blood meal, consisting mainly out of protein, can serve as food item of omnivorous birds or as a repellent for herbivorous birds. Thus adverse effects posed by Blood meal are considered unlikely and the data requirement is considered sufficiently addressed.

Toxicity of the formulated product

No acute effect studies on birds with the product Certosan were provided by the applicant. The representative product Certosan consists of 99.8% Blood meal (please refer to Volume 4). Therefore, toxicity of safeners, synergists and co-formulants posed by Certosan is not to be expected and the data requirement is considered sufficiently addressed.

Table 9.1-1 summarises all available toxicity endpoints of birds for Blood meal and the representative formulation Certosan.

Table 9.1-1: Toxicity of Blood meal and Certosan to birds

Test species	Test substance	Test design	End point	Toxicity	Reference
	Blood meal			No data	
	Certosan (99.8% a.s. w/w)			No data	

Bold values were used for the risk assessment.

Endocrine disruption

The available information in the ecotoxicology section indicates that Blood meal can be considered unlikely to exhibit endocrine disrupting properties. Please refer to Vol. 3 CA B9 under point B.9.1.5.

Metabolites of Blood meal

There are no ecotoxicologically relevant metabolites.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Toxicity of the active substance

As reported in Vol. 3 CA B9 B.9.1.2 the waiver for standard toxicity studies on mammals is considered acceptable taking into account that Blood meal, consisting mainly out of protein, can serve as food item of omnivorous mammals or as a repellent for herbivorous mammals. Thus adverse effects posed by Blood meal are considered unlikely and the data requirement is considered sufficiently addressed.

Toxicity of the formulated product

No acute effect studies on mammals with the product Certosan were provided by the applicant. The representative product Certosan consists of 99.8% Blood meal (please refer to Volume 4). Therefore, toxicity of safeners, synergists and co-formulants posed by Certosan is not to be expected and the data requirement is considered sufficiently addressed.

Additionally, Certosan is considered to be a non-toxic game repellent which dries off to a water-insoluble coating after painting directly onto the target parts of the plants. Theoretical consumption would be if game

nibbled on the shoots, but this will not be considered being harmful since Blood meal is used in food as additive for human consumption.

Table 9.1-2 summarises all available toxicity endpoints of mammals for Blood meal and the representative formulation Certosan.

Table 9.1-2: Toxicity of Blood meal and Certosan to mammals

Test species	Test substance	Test design	End point	Toxicity	Reference
	Blood meal			No data	
	Certosan (99.8% a.s. w/w)			No data	

Bold values were used for the risk assessment.

Endocrine disruption

The available information in the ecotoxicology section indicates that Blood meal can be considered unlikely to exhibit endocrine disrupting properties. Please refer to Vol. 3 CA B9 under point B.9.1.5.

Metabolites of Blood meal

There are no ecotoxicologically relevant metabolites.

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

The risk assessment for birds and mammals was conducted according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009;7(12):1438).

Blood meal consists mainly out of denaturated protein, the formulated product Certosan (99.8% Blood meal) has a non-toxic mode of action and is also considered to be non-toxic by itself.
According to the applicant the following quality criteria are applied to the active substance:

- *Food grade quality Blood collected in authorized slaughterhouses*
- *Destruction of pathogens and protein denaturation occur during Blood processing*
- *Blood of porcine origin*

No toxicity from the active substance Blood meal and the formulated product Certosan is expected to birds and other terrestrial vertebrates. Blood meal can be considered as possible food source for omnivorous/carnivorous terrestrial vertebrates, and is intended to act as a repellent for herbivorous terrestrial vertebrates. Therefore a potential risk to birds and mammals (including the consumption of unintentional oversprayed feed items following spray applications) is considered as low and the calculation of TER values is not considered necessary.

B.9.2.1. Risk assessment for birds

The calculation of acute and long-term toxicity/exposure ratios is not considered necessary, please refer to B9.2.

Risk from bioaccumulation and food chain behaviour

- Secondary poisoning

Not considered relevant, please refer to B9.2.

- Food chain from earthworm to earthworm-eating birds

Not considered relevant, please refer to B9.2.

- Food chain from fish to fish-eating birds
Not considered relevant, please refer to B9.2.

- Biomagnification in terrestrial food chains
Not considered relevant, please refer to B9.2.

Risk from consumption of contaminated water
Not considered relevant, please refer to B9.2.

Consideration of metabolites

- Screening level risk assessment for metabolites
There are no ecotoxicologically relevant metabolites. Thus, no risk assessment for metabolites is considered necessary.

- Bioaccumulation risk assessment for metabolites
There are no ecotoxicologically relevant metabolites. Thus, no risk assessment for metabolites is considered necessary.

Identification of endocrine disrupting properties
There is no indication that Blood meal acts as an endocrine disruptor.
Please refer for more details to Vol. 3 CA B9 under point B.9.1.5.

B.9.2.2. Risk assessment for mammals

The calculation of acute and long-term toxicity/exposure ratios is not considered necessary, please refer to B9.2.

Risk from bioaccumulation and food chain behaviour

- Secondary poisoning
Not considered relevant, please refer to B9.2.

- Food chain from earthworm to earthworm-eating mammals
Not considered relevant, please refer to B9.2.

- Food chain from fish to fish-eating mammals
Not considered relevant, please refer to B9.2.

- Biomagnification in terrestrial food chains
Not considered relevant, please refer to B9.2.

Risk from consumption of contaminated water
Not considered relevant, please refer to B9.2.

Consideration of metabolites

- Screening level risk assessment for metabolites
There are no ecotoxicologically relevant metabolites. Thus, no risk assessment for metabolites is considered necessary.

- Bioaccumulation risk assessment for metabolites

There are no ecotoxicologically relevant metabolites. Thus, no risk assessment for metabolites is considered necessary.

Identification of endocrine disrupting properties

There is no indication that Blood meal acts as an endocrine disruptor.
Please refer for more details to Vol. 3 CA B9 under point B.9.1.5.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

Table 9.3-1 summarises all available aquatic toxicity endpoints for Blood meal and the representative formulation Certosan (99.8% Blood meal).

Toxicity of the active substance

There are no toxicity endpoints on fish, aquatic invertebrates and algae available for the pure active substance Blood meal. However these standard toxicity studies are provided with the formulation Certosan (99.8% Blood meal) by [redacted] for the renewal of Blood meal and are presented below. The studies are considered to address the active substance data requirement sufficiently, please refer to Vol. 3 CA B9 B.9.2.

Table 9.3-1: Toxicity data of Blood meal and Certosan for aquatic species

Group	Test substance	Time scale (Test type)	Endpoint	Toxicity [mg a.s./L]	Reference
Fish					
Rainbow trout (*Oncorhynchus mykiss*)	Certosan (99.8 % Blood meal)	Acute, 96 hr (semi static)	Mortality, LC_{50}	> 33.5 $_{(mm)}^{2}$	[redacted]
		Long-term	No data		
Aquatic invertebrates					
Daphnia magna	Certosan (99.8 % Blood meal)	Acute, 48 hr (semi static)	Mortality, EC_{50}	> 62.4 $_{(mm)}^{2}$	Muckle, M. (2013b)
		Long-term	No data		
Sediment-dwelling organisms					
No data.					
Algae					
Desmodesmus subspicatus	Certosan (99.8 % Blood meal)	72 hr (static)	Growth rate: Biomass Integral (AUC^{1}): Yield:	E_rC_{50} > 59 $_{(mm)}^{2}$ E_rC_{10} = 3.7 $_{(mm)}$ NOE_rC < 6 $_{(mm)}$ * E_bC_{50} > 59 $_{(mm)}^{2}$ E_bC_{10} = 1.4 $_{(mm)}$ NOE_bC < 6 $_{(mm)}$ * E_yC_{50} = 16.4 $_{(mm)}$ E_yC_{10} = 1.1 $_{(mm)}$ NOE_yC < 6 $_{(mm)}$*	Muckle, M. (2013c)
Aquatic macrophyts					
No data submitted. Not required.					
Further testing on aquatic organisms					
Not required.					

Potential endocrine disrupting properties
No ED potential indicated.

(nom)…nominal concentration; (mm)…mean measured concentration
* … the two lowest test concentrations (nominal 4.6 & 10 mg/L) could not be analytically verified
[1] AUC…Area Under the Curve
[2] Limit of solibility

Metabolites of Blood meal
There are no ecotoxicologically relevant metabolites.

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

No toxicity studies on aquatic organisms were submitted for Annex I inclusion of Blood meal, a data gap was identified by EFSA during the former evaluation (EFSA Journal 2011;9(10):2394). Therefore, conduction of acute aquatic toxicity tests with fish, invertebrate and algae is principally required in order to allow classification.

Effect studies on aquatic organisms for Certosan (99.8% Blood meal) were conducted by [redacted]

B.9.3.1.1. Acute toxicity to fish

Acute toxicity of the formulated product to fish

Data point addressed:	KCP 10.2.1/01 (Vol. 3 B9 CP B.9.3.1.1)
Reference:	KCP 10.2.1/0001
Author(s) (year):	[redacted]
Title:	Determination of the acute toxicity of Blood Meal (Certosan) against *Oncorhynchus mykiss* following EU-method C.1 resp. OECD Guideline 203
Laboratory report / project Number (Doc No.):	Study No.: 13022801G503
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD Guideline 203 (1992)
Deviations:	Yes – see RMS comment below
GLP:	Yes
Acceptability:	**Yes**
Already EU evaluated?	No

Summary and conclusion
The acute toxicity of Certosan (blood meal) to rainbow trout was determined according to the EU-method C.1 (Commission Regulation (EC) No. 440/2008, adopted 31. May 2008) resp. OECD guideline for Testing of Chemical No. 203, adopted 17. July 1992 in a semi-static limit test design. The duration of the test was 96 h and 7 test organisms were exposed to the solution and to controls. Blood meal did not cause any effects on rainbow trout after 96 h when tested with the saturated solution prepared of 100 mg/L test item, therefore no classification is required. Blood meal is not toxic to fish.
At the beginning and at the end of the test and at each medium renewal, the content of the test item in the test solutions was determined by calculation from content of organic carbon (48.71 %) and DOC measurement in the fresh and in the old solution. The correlation between nominal and measured concentration was poor. This was caused by the limited solubility of the test item in the test medium. The recovery after 24 hours ranged from 54

% to 76 % of the measured start concentration. Therefore, the determination of the biological results was based on the geometric mean of the measured concentrations.

Test item: [copied from the study report by RMS]

Designation in Test Facility:	13022801G
Date of Receipt:	28. Feb. 2013
Condition at Receipt:	room temp., in proper conditions

6.1.1 Specification

The following information concerning identity and composition of the test item was provided by the sponsor.

Name	Blood Meal (Certosan)
Batch no.	94015FO/1
Appearance	dark red brown powder
Composition	wettable powder; 99.8% blood meal, [redacted]
CAS No.	68911-49-9
EINECS-No.	272-771-3
Molecular formula	not stated
Molecular weight	not stated
Purity	99.8 %
Homogeneity	not stated
Vapour pressure	unknown
Stability	H_2O; EtOH; DMSO; acetone; CH_3CN: unknown
Solubility	H_2O; EtOH; DMSO; acetone; CH_3CN: unknown
Production date	not stated
Expiry date	15. Jul. 2015
Storage	room temperature 20 ± 5 °C

Test system: [copied from the study report by RMS]

6.2.1 Specification

Species	*Oncorhynchus mykiss* HAMILTON-BUCHANAN
Age	sexually immature young fish, length 2 ± 1 cm

6.2.2 Origin

The animals were obtained from a commercial supplier. Before being used for the test, the fish were kept for 12 days under test conditions. During this period, mortality didn't surpass 5 %.

Supplier:	Forellenzucht Schneider, D-67734 Katzweiler
Date of arrival:	12. Jul. 2013

6.2.3 Husbandry

Oncorhynchus mykiss is routinely used for toxicity tests. The test fish were kept following SOP 115 005 03 in the current edition.

Before being used for the test, the fish are kept for ten days under test conditions. During this period, no mortality could be observed.

vessels	glass aquaria
medium	chlorine-free tap water with a copper concentration below 0.01 mg/L (composition see annex, page 19)
food	three times a day; trout food
changing of medium	flow through conditions
photo period	12/12 hours, using neon tubes
temperature	19 - 21 °C

Preparation: [copied from the study report by RMS]

The water-accommodated fraction was prepared for the test. This was done by weighing the load of 100 mg/L ± 10% adding the corresponding amount of dilution water and shaking gently (approximately 100 rpm) for 24 ± 1 hours. Because membrane filtration of the test solution was not possible, undissolved particles were removed using a test sieve with a mesh size of 50 µm.

At the beginning and every 24 hours, four Schott flasks, each containing 2 L test solution, were prepared.

Experimental conditions: [copied from the study report by RMS]

24 hours before the start of the test, food was withheld from the designated test fish.	
Date:	22. - 26. Jul. 2013
Treatments	100 mg/L nominal concentration
Test design	semi-static
Medium renewal	every 24 ± 1 hours
Duration	96 hours
Loading	1 fish/L
Vessels:	glass aquaria, maximal volume 10 L
Aeration:	accomplished with glass tubes, frequency of bubbles 1/s
Feeding:	none
Photo period:	12/12 hours using neon tubes
Temperature:	15.1 – 17.0 °C
pH adjustment:	none
Replicates:	one vessel, each containing 7 L test solution and 7 fish
Control:	one vessel, containing 7 L dilution water and 7 fish

Analytical method: [copied from the study report by RMS]

The content of the test item Blood Meal (Certosan) in the test solutions was determined by calculation from the carbon content of the test item (48.71 %, based on elemental analysis) and DOC measurement following SOP 118 009 02 in the current edition.

DOC (dissolved organic carbon) was determined as TC (total carbon) minus IC (inorganic carbon) in the filtrated (0.45 µm) test solutions.

6.5.1 Analytical Instrument

Specification:	TOC multi N/C 2100S
Serial number:	N5-108/G
Producer:	Analytik Jena AG
Calibration interval:	weekly, QC samples measured daily
Calibration method:	Calibration function using standards of different concentrations (see chapter page 20).

All data is archived following GLP regulations.

The following results for the test item Certosan (99.8% Blood meal) could be determined:
[copied from the study report by RMS]

Parameter	Value	95%-confidence-interval
96h NOEC	34 mg/L	not determinable
96h LC50	> 34 mg/L (limit of solubility)	not determinable
96h LC100	> 34mg/L (limit of solubility)	not determinable

Validity criteria	- The mortality in the control may not exceed one fish at the end of the test, was actually 0 - Constant conditions were maintained, the pH-value in the test solutions did not vary more than 1 unit during the test, the temperature was in the range for rainbow trout (13 – 17°C) and did not vary more than 2°C. - The dissolved oxygen concentration must be at least 60% throughout the test, was actually > 65% (6.5 mg/L) - The concentration of the test substance was satisfactorily maintained (see analytical recovery part below).
Analytic recovery results [copied from the study report by RMS]	At the beginning and at the end of the test and at each medium renewal in the fresh and the old solution, the content of the test item in the test solutions was determined by calculation from content of organic carbon (48.71 %) and DOC measurement. The measured concentrations showed poor correlation with the nominal concentrations. This was caused by the limited solubility of the test item in the test medium. The recoveries after 24 hours were in a range of 54 % - 76 % of the measured concentration 24 hours before. Therefore, the determination of the biological results was based on the geometric mean of the measured concentrations. Geometric mean is calculated by multiplication of the n participating concentrations and taking the n^{th} root. The measured concentrations can be found in the following tables.

Table 8.3-a Measured DOC Concentrations

Nominal Conc.	Measured DOC 0h	Measured DOC 24h old	Measured DOC 24h new	Measured DOC 48h old	Measured DOC 48h new	Measured DOC 72h old	Measured DOC 72h new	Measured DOC 96h
mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
0	0.34	1.92	0.32	1.29	0.05	2.01	4.30	5.89
100	24.99	20.75	23.36	16.67	19.69	12.63	19.27	15.75

Table 8.3-b Measured DOC Concentrations minus Blank values

Nominal Conc.	Measured DOC 0h - blank	Measured DOC 24h old - blank	Measured DOC 24h new - blank	Measured DOC 48h old - blank	Measured DOC 48h new - blank	Measured DOC 72h old - blank	Measured DOC 72h new - blank	Measured DOC 96h new - blank
mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
100	24.7	18.8	23.0	15.4	19.6	10.6	15.0	9.9

Table 8.3-c Measured Concentrations test item

Nominal Conc.	Measured Conc.Test Item 0h	Measured Conc. Test Item 24h old	Measured Conc. Test Item 24h new	Measured Conc. Test Item 48h Old	Measured Conc. Test Item 48h new	Measured Conc. Test Item 72h Old	Measured Conc. Test Item 72h new	Measured Conc. Test Item 96h
mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
100	50.6	38.7	47.3	31.6	40.3	21.8	30.7	20.2

Table 8.3-d Recovery and Geometric Mean

Nominal Conc.	Recovery 0h - 24 h	Recovery 24h - 48 h	Recovery 48h - 72 h	Recovery 72h - 96 h	Geometric mean
mg/L	%	%	%	%	mg/L
100	76%	67%	54%	66%	33.5

Observed effects [copied from the study report by RMS]

Nom. Conc. in mg/L	Total of fish used	dead fish							
		24 h		48 h		72 h		96 h	
		new	cum.	new	cum.	new	cum.	new	cum.
0	7	0	0	0	0	0	0	0	0
100	7	0	0	0	0	0	0	0	0

No abnormal behaviour of the fish was observed.

KCP 10.2.1/0001	**Comment RMS:** The study is relevant but not fully reliable (see deviations below). The validity criteria according OECD 203 (1992) are met. RMS still consideres the study acceptable for the use in the risk assessment and to address the data requirement. Deviation to the Test Guideline: - The fish used in the test were to small, instead of 5 cm ± 1 cm the fish had a length of 2 cm ± 1 cm. The weight of the fish was not measured, i.e. the loading in g fish/litre could not be determined. The smaller fish size does not allow to consider the study fully reliable. No mortality occurred in the acute limit test, therefore the endpoint is confirmed: **LD_{50} > 33.5 mg a.s./L (mm)**

Overall RMS conclusion – Acute toxicity to fish:

Table 9.3.1-1 summarises the results of the available acute toxicity study conducted with Certosan ([redacted]). The relevant endpoint to be used for the acute risk assessment of Certosan (99.8% Blood meal) is the 96-hours LC_{50} > 33.5 mg a.s./L.

Table 9.3.1-1: Acute toxicity of Certosan to fish

Group	Test substance	Time scale	Endpoint	Endpoint	Reference

				[mg a.s./L]	
Rainbowtrout (*Oncorhynchus mykiss*)	Certosan (99.8% Blood meal)	Acute, 96 h, semi static	Mortality, LC_{50}	> 33.5 (mm)	[redacted]

B.9.3.1.2. Acute toxicity to aquatic invertebrates

Acute toxicity of the formulated product to aquatic invertebrates

Data point addressed:	KCP 10.2.1/02 (Vol. 3 B9 CP B.9.3.1.2)
Reference:	KCP 10.2.1/0002
Author(s) (year):	Muckle, M. (2013b)
Title:	Determination of short term toxicity of Blood Meal (Certosan) against *Daphnia magna* STRAUS according to OECD 202 resp. EU C.2
Laboratory report / project Number (Doc No.):	Study No.: 13022801G201
Testing facility:	Laus GmbH, Kirrweiler, Germany
Published:	No
Test guideline used:	OECD Guideline 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	**Yes**
Already EU evaluated?	No

Summary and conclusion

The acute toxicity of Certosan (99.8% Blood meal) to *Daphnia magna* STRAUS was determined according to the EU-method C.2 (Commission Regulation (EC) No. 440/2008, adopted 31. May 2008) resp. OECD guideline for Testing of Chemical No. 202, adopted 13. April 2004.

The study was performed as a limit test at the concentration containing 100 mg/L. Twenty daphnia were exposed to the test item for 48 hours in a semi-static test system. After 24 and 48 hours, the immobilised daphnia were counted.

The treatment showed no toxicity. None of the animals were immobilised in the control.

Potassium dichromate $K_2Cr_2O_7$ (CAS No. 7778-50-9) was used as positive control in a current reference study (201301R201). The value was determined as 1.2 mg/L, lying within the demanded range of 0.6 – 1.7 mg/L.

At the beginning and at the end of the test, the content of the test item in the test solutions was calculated using DOC-determination. The recovery after 48 hours ranged from 97 to 120 % of the start concentration, the correlation between nominal and measured concentration was poor. This was caused by the limited solubility of the test item in the test medium. Therefore, the determination of the biological results was based on the geometric mean of the measured concentrations.

Test item: [copied from the study report by RMS]

Designation in Test Facility:	13022801G
Date of Receipt:	28. Feb. 2013
Condition at Receipt:	room temp., in proper conditions

6.1.1 Specification

The following information concerning identity and composition of the test item was provided by the sponsor.

Name	Blood Meal (Certosan)
Batch No.	94015FO/1
Appearance	dark red brown powder
Composition	wettable powder; 99.8% blood meal, [redacted]
CAS-No.	68911-49-9
EINECS-No.	272-771-3
Molecular formula	not stated
Molecular weight	not stated
Purity	99.8 %
Volatility	unknown
Homogeneity	not stated
Solubility	H_2O; EtOH; DMSO; acetone; CH_3CN: unknown
Production date	not stated
Expiry date	15. Jul. 2015
Storage	room temperature 20 ± 5 °C
Stability	H_2O; EtOH; DMSO; acetone; CH_3CN: unknown
Hazard information	not stated
R-phrases	not stated
S-phrases	S2: Keep out of the reach of children. S13: Keep away from food, drink and animal feeding stuffs. S20/21: When using do not eat, drink or smoke.

Test system: [copied from the study report by RMS]

6.3.1 Specification

Species	*Daphnia magna*
Variety	STRAUS
Strain	Berlin
Sex	female
Age	between 0 and 24 hours
Origin	Umweltbundesamt Berlin
In-house breeding since	27. Sep. 2007

Selection of the test system was made following the proposal of the guidelines.

6.3.2 Animal Husbandry

Daphnia magna is bred in the LAUS GmbH throughout the year. The animals are kept for the use in toxicity tests. They multiply by parthenogenesis, thus being genetically identical. The husbandry is performed similar to the method described in EN ISO 6341, following SOP 115 002 01 ("Zucht und Hälterung von *Daphnia magna* STRAUS"), edition 11, adopted 15. Jan. 2013.

Vessels	preserving glasses, nominal volume 2 L
Medium	M4-Medium (recipe of ELENDT), composition see annex
Food	unicellular green algae (*Desmodesmus subspicatus*)
Medium renewal	twice a week
Photo period	16/8 hours, using neon tubes
Temperature	20 ± 2 °C

Preparation: [copied from the study report by RMS]

The water-accommodated fraction was prepared for the test. This was done by weighing the load of 101.3 mg/L (start of the test) resp. 100.2 mg/L (medium renewal after 24 hours), adding the corresponding amount of dilution water and shaking gently (approximately 100 rpm) for 24 ± 1 hours. The resulting solution was filtrated through 0.45 µm nylon filters.

Experimental conditions: [copied from the study report by RMS]

Date of performance	09. – 11. Jul. 2013
Treatments	100 mg/L
Temperature	21.8 – 22.1 °C
Duration	48 hours
Observation times	24 and 48 hours
Feeding	none
Lighting	diffuse lighting
Test vessels	glass beakers, nominal volume 50 mL, tall shape
Replicates	four vessels, each containing 20 ± 5 mL test solution and five daphnia
Control	four vessels, each containing 20 ± 5 mL dilution water and five daphnia

Analytical method: [copied from the study report by RMS]

The content of the test item Blood Meal (Certosan) in the test solutions was determined by calculation from the content of organic carbon of the test item (48.71 %, based on elemental analysis) and DOC measurement following SOP 118 009 02 in the current edition.

DOC (dissolved organic carbon) was determined as TC (total carbon) minus IC (inorganic carbon) in the filtrated (0.45 µm) test solutions.

6.7.1 TOC multi N/C 2100 S

Specification:	TOC multi N/C 2100S
Serial number:	N5-108/G
Producer:	Analytik Jena AG
Calibration interval:	weekly, QC samples measured daily
Calibration method:	Calibration function using standards of different concentrations (see chapter Annex 3: Calibration Data page 21)

All data is archived following GLP regulations.

The following results were determined for the test item Certosan (99.8% Blood meal) (species: *Daphnia magna*): [copied from the study report by RMS]

Parameter	Value	95%-confidence interval
NOEC 24h	≥ 62 mg/L	not determinable
24h $EC50_i$	> 62 mg/L	not determinable
NOEC 48h	≥ 62 mg/L	not determinable
48h $EC50_i$	> 62 mg/L	not determinable

Validity criteria [copied from the study report by RMS]

- The 24h-$EC50_i$ of $K_2Cr_2O_7$ should lie between 0.6 and 1.7 mg/L.
  The 24h-$EC50_i$ of $K_2Cr_2O_7$ was determined as 1.2 mg/L in a separate GLP study.
- Immobilisation in the controls may not exceed 10 %.
  Immobilisation in the controls was 0 %.
- The concentration of dissolved oxygen at the end of the test must be at least 3 mg/L.
  The lowest concentration of dissolved oxygen at the end of the test was 8.0 mg/L.
- The pH-value in the test solutions should not vary more than 1 unit during the test.
  The highest variation was 0.1 units.

Analytic recovery results [copied from the study report by RMS]

The measured concentration were in a range of 56 - 67 % of the nominal concentration. The recoveries after 24 hours were 120 % resp. 97% of the measured concentration 24 hours before.

Therefore, the determination of the biological results was based on the geometric mean of the measured concentrations. Geometric mean is calculated by multiplication of the n participating concentrations and taking the n^{th} root.

The measured concentrations for treatment and control are given in the following table:

Table 8.3-a Measured DOC Concentrations

Nominal Concentration test item	Measured DOC t = 0 h	Measured DOC t = 24 h old	Measured DOC t = 24 h new	Measured DOC t = 48 h
mg/L	mg/L	mg/L	mg/L	mg/L
0	1.65	0.92	1.19	1.33
100	28.93	33.69	32.62	31.71

old = Medium made 24 hours before medium renewal
new = freshly prepared medium for medium renewal

Table 8.3-b Measured DOC Concentrations minus Blank values

Nominal Concentration test item	Measured DOC t = 0 h – Blank value	Measured DOC t = 24 h old – Blank value	Measured DOC t = 24 h new– Blank value	Measured DOC t = 48 h – Blank value
mg/L	mg/L	mg/L	mg/L	mg/L
0	0	0	0	0
100	27.28	32.77	31.43	30.38

Table 8.3-c Measured Concentrations test item

Nominal Concentration test item	Measured Concentration t = 0 h	Measured Concentration t = 24 h old	Measured Concentration t = 24 h new	Measured Concentration t = 48 h
mg/L	mg/L	mg/L	mg/L	mg/L
0	--	--	--	--
100	56.00	67.27	64.52	62.37

Table 8.3-d Percentage of Nominal Concentrations and Recovery

Nominal Concentration Test Item	% of Nominal Concentration t = 0h	% of Nominal Concentration t = 24h old	% of Nominal Concentration t = 24h new	% of Nominal Concentration t = 48h
mg/L	%	%	%	%
0	--	--	--	--
100	56	67	65	62

Table 8.3-e Percentage of Nominal Concentrations and Recovery

Nominal Concentration test item	Recovery 0 h - 24 h	Recovery 24 h - 48 h	Geometric mean of measured concentration
mg/L	%	%	mg/L
0	--	--	--
100	120	97	62.40

Observed effects [copied from the study report by RMS]

In treatment and control, none of the daphnia died or showed any signs of abnormal behaviour throughout the test (see table below).

Table 8.1-a Immobilities

Nominal Concentration in mg/L	Immobility 24 hours					Immobility 48 hours				
	abs.				in %	abs.				in %
0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0

KCP 10.2.1/0002	**Comment RMS:** The study is relevant and reliable. The validity criteria according OECD 202 (2004) are met. No immobilisation occurred in the acute limit test, therefore the endpoint is confirmed: **EC_{50} > 62.4 mg a.s./L (mm)**

Overall RMS conclusion – Acute toxicity to aquatic invertebrates

Table 9.3.1-2 summarises the result of the available acute invertebrate toxicity study conducted with Certosan (Muckle, 2013b). The relevant endpoint to be used for the acute risk assessment of Certosan is the 48-hours EC_{50} > 62.4 mg a.s./L, based on mean measured test concentrations.

Table 9.3.1-2: Acute toxicity of Certosan to aquatic invertebrates

Group	Test substance	Time scale	Endpoint	Endpoint [mg a.s./L]	Reference
Daphnia magna	Certosan (99.8% Blood meal)	Acute, 48 hr, semi static	Mortality, EC_{50}	> 62.4 (mm)	Muckle (2013b)

B.9.3.1.3. Effects on growth of green algae

Effects of the formulated product on aquatic algae

Data point addressed:	KCP 10.2.1/03 (Vol. 3 B9 CP B.9.3.1.3)
Reference:	KCP 10.2.1/0003
Author(s) (year):	Muckle, M. (2013c)
Title:	Determination of the toxicity of Blood Meal (Certosan) in *Desmodesmus subspicatus* according to OECD 201 resp. EU C.3
Laboratory report / project Number (Doc No.):	Study No.: 13022801G301
Testing facility:	Laus GmbH, Kirrweiler, Germany
Published:	No
Test guideline used:	OECD Guideline 201 (2011)
Deviations:	No
GLP:	Yes
Acceptability:	**Yes**
Already EU evaluated?	No

Summary and conclusion

The toxicity of Blood meal to the unicellular freshwater green algae *Desmodesmus subspicatus* was determined according to the EU-method C.3 (Commission Regulation (EC) No. 440/2008, adopted 24. August 2009) resp. OECD guideline for Testing of Chemical No. 201, adopted 23. March 2006, Annex 5 corrected 28. July 2011. The incubation time was 72 hours.

Significant inhibition of algal growth was observed in treatments 22; 46 and 100 mg/L (nominal concentrations). The inhibition values in the two highest concentrations were in the same range.

At the start and at the end of the test, the content of the test item in the test solutions was estimated using DOC analysis. In the two lowest concentrated treatments, the DOC was in a similar range as in the control. The lowest concentrated treatment was not used for evaluation of the results. The recovery of the DOC concentrations after 72 hours was in a range of 0 and 83 % of the measured start concentration. Therefore, the determination of the results was based on the means of the measured DOC concentrations for treatments 22 - 100 mg/L and the measured start concentration for treatment 10 mg/L.

The EC_{50}s of potassium dichromate were tested in a separate reference test. The values lay within the normal range of the laboratory.

Test item: [copied from the study report by RMS]

Designation in Test Facility:	13022801G
Date of Receipt:	28. Feb. 2013
Condition at Receipt:	room temp., in proper conditions

6.1.1 Specification

The following information concerning identity and composition of the test item was provided by the sponsor.

Name	Blood Meal (Certosan)
Batch No.	94015FO/1
Appearance	dark red brown powder
Composition	wettable powder; 99.8% blood meal, [redacted]
CAS-No.	68911-49-9
EINECS-No.	272-771-3
Molecular formula	not stated
Molecular weight	not stated
Purity	99.8 %
Volatility	unknown
Homogeneity	not stated
Solubility	H_2O; EtOH; DMSO; acetone; CH_3CN: unknown
Production date	not stated
Expiry date	15. Jul. 2015
Storage	room temperature 20 ± 5 °C
Stability	H_2O; EtOH; DMSO; acetone; CH_3CN: unknown
Hazard information	not stated
R-phrases	not stated
S-phrases	S2: Keep out of the reach of children. S13: Keep away from food, drink and animal feeding stuffs. S20/21: When using do not eat, drink or smoke.

Test system: [copied from the study report by RMS]

Selection of the test system was made following the proposal of the guidelines.

6.3.1 Specification

Unicellular freshwater green alga.

Genus, Species	*Desmodesmus subspicatus*
Strain	CHODAT
Family	*Scenedesmacea*
Order	*Sphaeropleales*

6.3.2 Origin and Culture

The culture of *Desmodesmus subspicatus* was obtained in Jan. 2013 by MBM Sciencebridge GmbH (Institut für Pflanzenphysiologie of Universität Göttingen). The algae are kept as stock culture on solid agar at 7 °C. From the stock culture, a permanent culture was prepared. From an aliquot of the permanent culture, the pre-culture was prepared.

Preparation: [copied from the study report by RMS]

Before each experiment, the water-accommodated fractions (WAF) of the concentrations to be tested were prepared. This was done by weighing the nominal load, adding the appropriate amount of nutrient medium (demineralised water enriched with minerals but without algae) and shaking gently (approximately 100 rpm) for 24 hours. After membrane filtration, the solutions were used to prepare the treatments.

Experimental conditions: [copied from the study report by RMS]

Date:	16. – 19. Jul. 2013
Treatments tested:	4.6 / 10 / 22 / 46 / 100 mg/L nominal concentration
Number of replicates:	six replicates for the control three replicates for each treatment
Vessels:	glass flasks total volume 65 mL
Duration:	72 hours
Temperature:	23 – 24 °C
Lighting:	5800 Lux
Control:	nutrient medium and alga
Treatments:	test solution and alga

Analytical method: [copied from the study report by RMS]

The content of the test item Blood Meal (Certosan) in the test solutions was determined by calculation from the carbon content of the test item (48.71 %, based on elemental analysis) and DOC measurement following SOP 118 009 02 in the current edition.

DOC (dissolved organic carbon) was determined as TC (total carbon) minus IC (inorganic carbon) in the filtrated (0.45 µm) test solutions.

6.6.1 Analytical Instrument

Specification:	TOC multi N/C 2100S
Serial number:	N5-108/G
Producer:	Analytik Jena AG
Calibration interval:	weekly, QC samples measured daily
Calibration method:	Calibration function using standards of different concentrations (see chapter 17, page 33)

All data is archived following GLP regulations.

The following results were determined for the test item Certosan (99.8% Blood meal) (species: *Desmodesmus subspicatus*):
[copied from the study report by RMS]

Parameter	Value	95%-confidence-interval
NOEC (Growth Rate) 72 h	1.6 mg/L	not determinable
NOEC (AUC) 72 h	1.6 mg/L	not determinable
NOEC (Yield) 72 h	1.6 mg/L	not determinable
LOEC (Growth Rate) 72 h	6.0 mg/L	not determinable
LOEC (AUC) 72 h	6.0 mg/L	not determinable
LOEC (Yield) 72 h	6.0 mg/L	not determinable
72h E_rC10	6.1 mg/L	2.2 – 10 mg/L
72h E_bC10	0.92 mg/L	< 4.7 mg/L
72h E_yC10	1.0 mg/L	< 2.6 mg/L
72h E_rC50	> 59 mg/L (limit of solubility)	--
72h E_bC50	> 59 mg/L (limit of solubility)	--
72h E_yC50	17 mg/L	9.1 – 30 mg/L

Validity criteria [copied from the study report by RMS]

11.1 Growth

11.1.1 Criterion Increase Factor

The cell concentration in the control should increase by a factor of at least 16 within 72h.

11.1.2 Criterion Daily Growth Rates

Mean coefficient of variation of daily growth rates should be 35% at the most.

Coefficient of variation of average growth rate during the whole test period should be 7% at the most.

11.1.3 Values and Assessment

The daily growth rates of the controls were calculated. Means, standard deviations and coefficients of variation were determined. Values and assessment can be found in the following tables.

Table 11.1-a Daily Growth Rates of the Controls

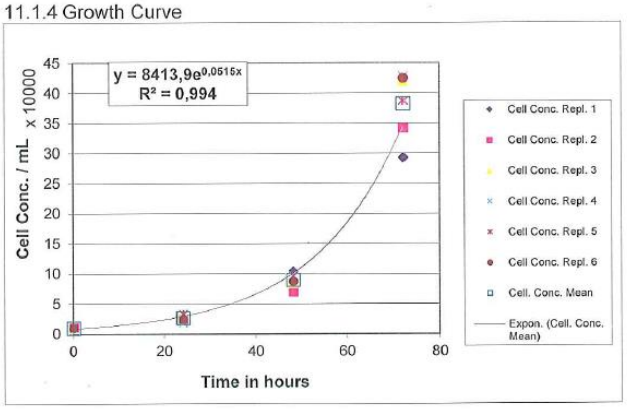
Growth rates	days 0 – 1	days 1 – 2	days 2 – 3	CV of sectional daily growth rates	days 0 – 3
Replicate 1	1.089	1.319	1.034	13%	1.15
Replicate 2	0.914	1.091	1.592	29%	1.20
Replicate 3	1.237	0.950	1.615	26%	1.27
Replicate 4	0.673	1.657	1.493	41%	1.27
Replicate 5	1.237	1.087	1.398	13%	1.24
Replicate 6	0.890	1.336	1.589	28%	1.27
Mean	1.007	1.240	1.453	**25%**	1.233
Standard deviation	0.222	0.253	0.221		0.051
CV	22%	20%	15%		**4%**

CV = Coefficient of Variation

Table 11.1-b Assessment

Parameter	Validity criteria	Observed value	Assessment
Increase factor biomass	factor 16 in 72 h	41	Valid
Mean coefficient of variation of daily growth rates	max. 35%	25%	Valid
Coefficient of variation of average growth rate during the whole test period	max. 7%	4%	Valid

11.1.4 Growth Curve



During the test, exponential growth was given.

11.2 Change of pH

The pH of the control shouldn't change by more than 1.5 units. The change was 0.6 units.

Analytic recovery results [copied from the study report by RMS]

Due to the insufficient solubility of the test item, the correlation between nominal and measured concentration was poor. In the two lowest concentrated treatments, the DOC was in a similar range as in the control. The lowest concentrated treatment was not used for evaluation of the results.

The recovery after 72 hours was in a range of 0 and 83 % of the start concentration. The poor recovery, especially in the lower concentrated treatments may be caused by the presence of the alga cells (ingestion and / or adsorption of test item). Therefore, the determination of the results was based on the geometric means of the measured concentrations for treatments 22 - 100 mg/L and on the measured start concentration for treatment 10 mg/L.

Geometric mean is calculated by multiplication of the n participating concentrations and taking the n^{th} root.

The details are given in the following table:

Table 9.4-a Measured Concentrations

Nominal Concentration Test Item	Measured DOC t = 0 h	Measured DOC t = 72 h	Net DOC t = 0 h	Net DOC t = 72 h
mg/L	mg/L	mg/L	mg/L	mg/L
0	2.88	3.42	--	--
4.6	1.72	1.61	--	--
10	3.64	2.75	0.76	--
22	6.98	5.49	4.1	2.07
46	14.83	12.91	11.95	9.49
100	34.43	29.71	31.55	26.29

Note: Net DOC refers to DOC treatment minus DOC blank.

Table 9.4-b Calculated Test Item Concentrations

Nominal Concentration Test Item	Calculated Concentration Test Item t = 0 h	Calculated Concentration Test Item t = 72 h	Geometric Mean Calculated Concentration Test Item
mg/L	mg/L	mg/L	mg/L
4.6	--	--	--
10	1.56	--	--
22	8.42	4.25	6.0
46	24.53	19.48	21.9
100	64.77	53.97	59.1

Table 9.4-c Percentage of Nominal Concentrations and Recovery

Nominal Concentration Test Item	Nominal Concentration Test Item t = 0 h	Nominal Concentration Test Item t = 72 h	Recovery after 72 h
mg/L	%	%	%
4.6	0	0	0
10	16	0	0
22	38	19	50
46	53	42	79
100	65	54	83

Observed effects [copied from the study report by RMS]

9.1 Cell Numbers

The cell numbers were determined via microscopical counting. Cell numbers of individual replicates are given in the annex, page 28. The means and standard deviations of the cell numbers of the control and the treatments are presented in the following table:

Table 9.1-a Cell Number/mL Main Study

Nominal Concentration in mg/L	Parameter	Cell Number/mL			
		0 h	24 h	48 h	72 h
0	Mean	9352	26111	89167	381852
0	SD	0	5600	12125	54793
4.6	Mean	9352	12222	83148	332222
4.6	SD	0	962	8504	55322
10	Mean	9352	22593	83704	395185
10	SD	0	2740	10604	53798
22	Mean	9352	22222	102222	277778
22	SD	0	11097	6939	13517
46	Mean	9352	37963	70370	125185
46	SD	0	17962	7804	3572
100	Mean	9352	34444	99444	140741
100	SD	0	4938	30292	54167

SD = Standard Deviation

	9.5 Area under the Curve, Growth Rate, Yield From the cell numbers, the growth rate µ, the area under the curve AUC and the yield were calculated. The means and standard deviations at the end of the test are given in the following table: Table 9.5-a Growth Rate µ, Area under the Curve AUC, Yield Main Study

Nom. Concentration in mg/L	Meas. Concentration in mg/L		Growth Rate [day^{-1}]	AUC [Cell Concentration/mL*day]	Yield [Cell Concentration/mL]
0	--	Mean	1.23	282824	372500
		SD	0.05	28108	54793
4.6	--	Mean	1.19	238102	322870
		SD	0.06	37091	55322
10	*1.6	Mean	1.25	280509	385833
		SD	0.05	36214	53798
22	6	Mean	1.13	239954	268426
		SD	0.02	3889	13517
46	22	Mean	0.86	147546	115833
		SD	0.01	15579	3572
100	59	Mean	0.89	180880	131389
		SD	0.13	50443	54167

SD = Standard Deviation
*measured start concentration

9.6 Inhibition

The following mean inhibition values were calculated for the growth rate µ, the area under the curve AUC and the yield. Individual inhibition values are given in the annex.

Table 9.6-a Inhibition Values Main Study

Nom. Concentration in mg/L	Meas. Concentration in mg/L	% Inhibition		
		Growth Rate µ	Area under the Curve AUC	Yield
0	--	0	0	0
4.6	--	3.78	15.81	13.32
10	*1.6	-1.00	0.82	-3.58
22	6	8.37	15.16	27.94
46	22	29.90	47.83	68.90
100	59	28.02	36.05	64.73

*measured start concentration

KCP 10.2.1/0003	**Comment RMS:** The study is relevant and reliable. The validity criteria according OECD 201 (2011) are met. RMS recalculated the endpoints (based on geometric mean measured concentrations) using ToxRat Professional (OECD 201 masterbook with default settings). For the growth rate the default setting terminated without finding an appropriate dose-response model, therefore RMS refined recalculated this parameter with a 3 parameter normal CDF:

%Inhibition of growth rate caused by the test item after 0 - 72 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,233	0,0509	6	
0,500	1,187	0,0586	3	3,8
0,500	1,246	0,0474	3	-1,0
6,000	1,130	0,0160	3	8,4
22,000	0,865	0,0096	3	29,9
59,000	0,888	0,1256	3	28,0

Please note: since in the two lowest test concentrations no test item could be verified by analytical measurements, the Limit of Detection (0.5 mg/L) was used instead. RMS is aware that this is not ideal.

Estimated parameters of the 3-param. normal CDF

Estimated parameters of the 3-param. normal CDF with growth rate at 0 - 72 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	1,243	0,029	1,181	1,305	42,395	<0.001
b1	0,570	0,282	-0,023	1,163	2,021	0,029
b2	1,447	0,356	0,699	2,195	4,064	<0.001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)
R^2: 0,821; adjusted R^2: 0,801
Residual standard error: 0,07608
Akaike Criterion (AIC): -79,926

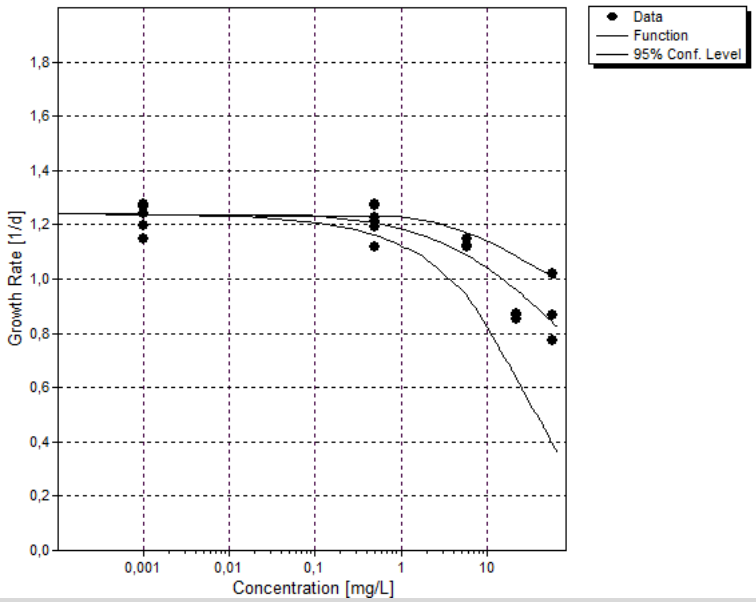
Source	SS	df	MSS	F	p(F)
Regression	0,452	2	0,226	39,045	<0.001
Residuals	0,104	18	0,006		
- Lack of Fit	0,048	3	0,016	4,210	0,024
- Pure Error	0,057	15	0,004		
Total	0,551	20			

Point estimates from the 3-param. normal CDF

Point estimates from the 3-param. normal CDF with growth rate at 0 - 72 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	3,715	16,089	265,613
lower 95%-cl	0,949	3,885	23,227
upper 95%-cl	14,539	70,179	2748,001

n.d.: not determined due to mathematical reasons
The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs).



Williams Multiple Sequential t-test Procedure

Comparison of treatments with "Control" by the t test procedure after Williams with growth rate at 0 - 72 h: Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*': critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	1,233	0,0614						
0,500	1,187	0,0614	15	1,216	-6,2	-0,39	-1,75	-
0,500	1,246	0,0614	15	1,216	-6,4	-0,39	-1,83	-
6,000	1,130	0,0614	15	1,130	-6,5	-2,38	-1,85	+
22,000	0,865	0,0614	15	0,876	-6,5	-8,23	-1,86	+
59,000	0,888	0,0614	15	0,876	-6,6	-8,23	-1,87	+

+: significant; -: non-significant

A NOEC of 0,500 mg/L is suggested by the program.

Deviation from the test guideline:
- A higher initial cell number was used in the study. Instead of 2000 – 5000 cells/ml a cell number of 9352 cells/ml was used. However since all validity criteria are met, this deviation is not considered to negatively influence the outcome of the study.
- The two lowest test concentrations (nominal 4.6 & 10 mg/L) could not be analytically verified.

The following toxicity endpoints (72h) are confirmed:

E_rC_{50} > 59 mg a.s./L (mm)
E_bC_{50}* > 59 mg a.s./L (mm)
E_yC_{50} = 16.4 mg a.s./L (mm) – 95%-CL: 1.4 – 177.1

The NOECs are determined as follows:
NOE_rC < 6 mg a.s./L (mm)
NOE_bC* < 6 mg a.s./L (mm)
NOE_yC < 6 mg a.s./L (mm)

The EC_{10} values are determined as follows:
E_rC_{10} = 3.7 mg a.s./L (mm) – 95%-CL: 0.95 – 14.54
E_bC_{10}* = 1.4 mg a.s./L (mm) – 95%-CL: 0.03 – 55.9
E_yC_{10} = 1.1 mg a.s./L (mm) – 95%-CL: 0.2 – 8.9

*** Biomass is measured as integral (Area under the Curve)**

Overall RMS conclusion – Effects on algal growth

Table 9.3.1-3 summarises the result of the available effect study on algae conducted with Certosan (Muckle, 2013c). The relevant endpoint to be used for the risk assessment of Certosan is the 72-hours E_rC_{50} > 59 mg a.s./L, based on mean measured test concentrations.

Table 9.3.1-3: Acute toxicity of Certosan to algae

Group	Test substance	Time scale	Endpoint	Toxicity [mg a.s./L]	Reference
Desmodesmus subspicatus	Certosan (99.8% Blood meal)	72 hr, static	E_rC_{50} E_bC_{50} E_yC_{50}	> 59 mg a.s./L (mm) > 59 mg a.s./L (mm) 16.4 mg a.s./L (mm)	Muckle (2013c)

			NOE_rC NOE_bC NOE_yC	< 6 mg a.s./L (mm) < 6 mg a.s./L (mm) < 6 mg a.s./L (mm)	
			E_rC_{10} E_bC_{10} E_yC_{10}	3.7 mg a.s./L (mm) 1.4 mg a.s./L (mm) 1.1 mg a.s./L (mm)	

B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No long-term and chronic toxicity studies on fish, aquatic invertebrates or sediment-dwelling organisms were submitted, neither with the representative formulation Certosan, nor with the active substance Blood meal (please refer to Vol. 3 CA B9 B.9.2.2 and B.9.2.5).

The representative product Certosan consist out of 99.8% Blood meal (please refer to Volume 4). Therefore, toxicity of safeners, synergists and co-formulants posed by Certosan is not to be expected. A waiver for the performance of long-term and chronic toxicity studies on fish and aquatic invertebrates is considered acceptable. For targeted application methods (coating with brush, dipping) an exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the available acute toxicity studies with the formulation Certosan (99.8% Blood meal) on fish and *Daphnia* as well as the effect data on algae are considered sufficient to demonstrate a low concern and to address the data requirement. Even if the active substance Blood meal accidentally enters the surface water, it should be taken in account that Blood meal is commonly used as fish feed.

Therefore in conclusion, adverse long-term effects to fish, aquatic invertebrates and sediment dwelling organisms posed by Blood meal and the formulation Certosan are considered unlikely and the data requirement was sufficiently addressed.

B.9.3.3. Further testing on aquatic organisms

Not considered necessary.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The risk assessment was carried out according to EFSA Journal 2013;11(7):3290.

For targeted application methods (coating with brush, dipping) an exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the available acute toxicity studies with the formulation Certosan (99.8% Blood meal) on fish and *Daphnia* as well as effect data on algae are considered sufficient to demonstrate a low concern. Nevertheless a worst case risk assessment based on the PEC/RAC ratio is presented below.

Exposure
Aquatic organisms may be exposed to Blood meal as a consequence of the accidental entry of the compound into the environmental compartments by run-off or drift events. However, these events are highly unlikely, as the common application technique is applying the formulated product directly on the individual trees or parts of trees. Therefore contamination of the environment under good working practice is considered unlikely to occur. If applied under weather conditions as recommended, accidental entry into water systems should be minimal and

of no safety concern. Even if the active substance Blood meal is accidentally got into surface water, it should be taken in account that Blood meal is commonly used as fish feed. Nevertheless RMS calculated PEC_{SW} values using FOCUS Step 1 and 2. The application pattern was set to "no interception", "North Europe, October - February" and the crop type was set to "hand held application, crop <50 cm" and "hand held application, crop > 50 cm" (please refer to Vol. 3 CP B8 B.8.5). The initial worst-case PEC_{SW} values for STEPs 1-2 were used in the risk assessment below.

Table 9.3.3-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Blood meal for each organism group based on FOCUS Steps 1 & 2 calculations for the use of Certosan

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		> 33500	> 62400	> 59000
AF		100	100	10
RAC (µg/L)		> 335	> 624	5900
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
Hand held application, crop < 50 cm	6760	**< 20.2**	**< 10.8**	**< 1.1**
Hand held application, crop > 50 cm	7110	**< 21.2**	**< 11.4**	**< 1.2**
Step 2				
Hand held application, crop < 50 cm	3460	**< 10.3**	**< 5.5**	< 0.6
Hand held application, crop > 50 cm	3810	**< 11.4**	**< 6.1**	< 0.7

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

The risk assessment indicates an acceptable risk for algae at FOCUS Step 2. However, a potential acute risk for fish and *Daphnia* is indicated at FOCUS Step 1 & 2 (i.e. PEC/RAC ratio > 1). This potential risk is considered to be acceptable since worst case assumptions are reflected in this risk assessment. No mortality/immobility of the test organisms occurred at the highest tested concentrations (which is the limit of solubility in the respective study) and no interception was taken into account for the PEC calculation. Moreover it should be taken in account that Blood meal is commonly used as fish feed.

Therefore overall the risk to aquatic organisms is considered to be low.

Metabolites of Blood meal

There are no ecotoxicologically relevant metabolites.

B.9.4.1. Bioconcentration and secondary poisoning

No risk assessment on food chain behaviour is considered necessary.

B.9.5. EFFECTS ON ARTHROPODS

B.9.5.1. Effects on bees

Toxicity of the active substance to bees

Please refer to the acute contact and oral toxicity formulation study with Certosan (99.8% Blood meal) by Kleiner (1996b) from the former evaluation of Blood meal. This study is considered to address the data requirement sufficiently.

Table 9.5-1 summarises all available toxicity endpoints of bees for Blood meal and the representative formulation Certosan.

Table 9.5-1: Toxicity endpoints of Blood mean and Certosan in bees

Species	Test substance	Time scale/type of study	End point	Toxicity	Reference
Acute					
Apis mellifera	Certosan (99.8% Blood meal)	Acute (48 h)	Oral toxicity (LD_{50})	> 198 µg product/bee	Kleiner, R. (1996b)
Apis mellifera	Certosan (99.8% Blood meal)	Acute (48 h)	Contact toxicity (LD_{50})	> 200 µg product/bee	Kleiner, R. (1996b)
Chronic					
No data					
Bee brood development					
No data					
Sub-lethal effects					
No data					

B.9.5.1.1. Acute toxicity to bees

Acute oral & contact toxicity of the formulated product to bees

Data point addressed:	KCP 10.3.1.1.1 & KCP 10.3.1.1.2 (Vol. 3 CP B9 B.9.5.1.1)
Reference:	KCA 8.3.1.1.1/0001
Author(s) (year):	Kleiner, R., M. (1996b)
Title:	Testing toxicity to Honeybee – Apis mellifera (laboratory) according to EPPO Guideline No. 170
Laboratory report / project Number (Doc No.):	Study No.: 96 10 48 027
Testing facility:	BioChem GmbH Karlsruhe, Labor Cunnersdorf, Germany
Published:	No
Test guideline used:	EPPO Guideline 170 (1992) but evaluated by RMS according to OECD TG 213 (1998)

Deviations:	No
GLP:	Yes
Acceptability:	**Yes**
Already EU evaluated?	**Yes**

Summary and conclusion

Two experiments according to EPPO Guideline No. 170, each with duration of 48 h, were conducted to determine the LD_{50} of the test substance Certosan to the honeybee *Apis mellifera* L. in the oral toxicity test and in the contact toxicity test.

Exposure to Certosan showed no effect on honey bee mortality (0 %) up to the dose of 198 µg formulation /bee in the oral toxicity and negligible effects up to a dose of 200 µg formulation/bee in the contact toxicity test after 48 hours. A calculation of the LD_{50} of the test substance is inapplicable. Certosan can be classified as harmless to the honeybee *Apis mellifera* L.

Test item: [copied from the study report by RMS]

Product name:	CERTOSAN
Code No.:	FLU-00194-P-O-WP
Batch No.:	95013G93
Production date:	13.07.93
Formulation:	wettable powder (WP)
Active ingredients/content:	blood meal: protein 87 % water 5 % salts 5 % fat 3 % figures given above may vary ± 3 units
Water solubility:	suspensible
Stability under test conditions:	no information
Storage conditions:	room temperature, dry
Classification:	designation according to "Gefahrstoffverordnung": not required
Field of use:	game repellent
Highest recommended dose:	20 kg/ha in 200 l/ha of water (= 10 % w/v)
Dose in the test:	concentration series
Further remarks:	"Gyllebo Plantskydd Product information" of 01.10.93; "Vorläufige Gebrauchsanweisung von Certosan"; "Veterinary certificate 33-456/94" of 20.03.95

Reference item:

Name:	Dimethoate EC 400
Batch No.:	94-2
Formulation:	EC 400
Active ingredient/content:	Dimethoate 393.3 g/l (according to certificate PCP 03827)
Density (20 °C):	1.073 g/cm^3

Test system: [copied from the study report by RMS]

Test organism:	honeybee - *Apis mellifera* L. (workerbees of a colony in good health)
Origin of the test animals:	purchase from the bee-keeper Mr Herbert Weimann, Merkwitzer Str. 21 a, 04448 Gottscheina on May 17, 1996

The honeybees were reared in the hive up to the use for testing and acclimatized to the climatic conditions in the test room for 1-2 hours before application. After that 10 bees were transferred to each test cage.

Test conditions

Test cages:	disposable cages of cardboard with holes in the bottom for ventilation and a glass plate in front for observation of the bees (dimensions, inside: 80 mm x 45 mm x 65 mm)
Number of honeybees/cage:	10
Number of cages/concentration:	3
Number of honeybees/concentration:	30
Feeding:	continuously during the test
Food:	candy (mixture of sugar and honey - 3:1 w/w) and water

Climatic conditions (test room)

Temperature:	24-26 °C (according to study plan: (25±2) °C)
Relative humidity:	55-82 % (according to study plan: about 60-70 %)

Short-term deviations of the relative humidity in the test room, owing to a technical fault, were not considered to have any effect on the test results.

Both registered:	continuously by a thermohygrograph
Illumination:	diffuse artificial light during 8 hours (ca 100 lx)
Test duration:	48 hours

Preparation: [copied from the study report by RMS]

Treatment	Product name	Concentration
1a Control (oral)	sucrose solution	
1b Control (contact)	Extravon *) (aqueous solution)	0.1 % v/v
2 Reference substance	Dimethoate EC 400	*oral toxicity test (sucrose solution):* 0.001 %, 0.0012 %, 0.0014 %, 0.0016 %, 0.0018 %, 0.002 % w/v
		contact toxicity test (0.1 % aqueous Extravon solution): 0.00313 %, 0.00625 %, 0.0125 %, 0.025 %, 0.05 %, 0.1 % w/v
3 Test substance	CERTOSAN	*oral toxicity test (sucrose solution):* 0.5 %, 1.0 % w/v
		contact toxicity test (0.1 % aqueous Extravon solution): 2 %, 4 % w/v

*) surfactant Extravon (derivate of polyglycol ether)

Course of the trial: [copied from the study report by RMS]

Young workerbees of the honeybee *Apis mellifera* L. were treated with varied concentrations (concentration series) of the test substance and of the reference substance, respectively. The treated bees were kept under controlled climatic conditions and assessed for toxic effects up to 48 hours. Two different treatments were used permitting the evaluation of toxic feeding effects and contact effects of the test substance and the reference substance.
Mortality values were used to provide a regression line and calculate the median lethal dose value (LD_{50}) expressed in µg of the substance per bee.

At the beginning of the test 10 workerbees were transferred in glass tubes from the hive to each cage, without anaesthetizing. In the oral toxicity test, the bees were not fed for a period of 1-2 hours prior to test initiation.
The preparation of the test solution was performed according to point 2.4.1.

Oral toxicity test

The bees were fed with a defined quantity of a 50 % aqueous sucrose solution that contained varied concentrations (concentration series) of the test substance.
The test was performed by way of group feeding: ca. 0.2 ml (= 0.251 g) of the test solution were offered to ten bees per cage. After consumption of the test solution the food tube was reweighed to control the quantity of the test solution consumed.

Contact toxicity test

The compounds were dispersed in 0.1 % aqueous solution of the surfactant Extravon.
Bees anaesthetized with CO_2 were treated individually by topical application with an Eppendorf Micropipette. 5 µl of the test solution or 1 µl of the reference solution were dosed to the thorax of each bee. In the control variant 5 µl of the 0.1 % aqueous Extravon solution was applied.

After application, the treated bees were put back into the test cages, which were supplied with water and food tubes (with candy) and transferred to the test room.

Results: [copied from the study report by RMS]

The results are summarized in the table of results on page 12 (see also appendices - tables and diagrams).

In the oral toxicity test the most of substance sucrose solutions was consumed in each concentration (see appendix 2.1).

For controlling the reproducibility of the test system the reference substance was tested at a concentration range of 0.001 % up to 0.002 % in the oral toxicity test and of 0.00313 % up to 0.1 % in the contact toxicity test.

Regarding the reference substance Dimethoate EC 400 following median lethal dose values (LD_{50}) were determined:

Validity criteria [copied from the study report by RMS]	Mortality in the controls: The mortality in the control was 0 %. Consequently the test accomplished the validity criterion (mortality of the control ≤ 15 %). The test substance CERTOSAN caused no mortality (0 %) up to a dose of 198 µg formulation/bee in the oral toxicity test and negligible mortality (10 %) up to a dose of 200 µg formulation/bee in the contact toxicity test. Thus a calculation of the median lethal dose value (LD_{50}) of the test substance was inapplicable. No behavioural anomalies were observed. [note by RMS: OECD TG 2013 & 214: control mortality must not exceed 10%, which is met] Toxicity of the toxic standard: Oral toxicity: LD_{50} (24h) = 0.32 µg Dimethoate EC 400/bee equivalent to **0.12 µg Dimethoate /bee** (based on 36.7% a.s. content in Dimethoate EC 400)

	Contact toxicity: LD_{50} (24h) = 0.38 µg Dimethoate EC 400/bee equivalent to **0.14 µg Dimethoate /bee** (based on 36.7% a.s. content in Dimethoate EC 400)
Observed effects [copied from the study report by RMS]	Reference item: Test: LD_{50} (µg/bee) Dimethoate product Oral toxicity test: 24 h 0.32; 48 h after application 0.31 Contact toxicity test: 24 h 0.38; 48 h after application 0.29 In the reference variant apathy, uncontrollable motions and dorsal position of the affected bees could be observed before dying, 24 h and 48 h after application the surviving bees exhibited no behavioural anomalies. Test item: *a) Oral toxicity test (group feeding)* control (sucrose solution): 24 h 0; 48 h 0 test substance CERTOSAN: 0.5 % / 99 µg formulation/bee: 24 h 0; 48 h 0 test substance CERTOSAN: 1.0 % / 198 µg formulation/bee: 24 h 0; 48 h 0 reference substance Dimethoate EC 400: 0.001 % / 0.2: 24 h 3; 48 h 7 0.0012 % / 0.24: 24 h 20; 48 h 27 0.0014 % / 0.27: 24 h 23; 48 h 27 0.0016 % / 0.32: 24 h 37; 48 h 43 0.0018 % / 0.35: 24 h 63; 48 h 70 0.002 % / 0.4: 24 h 83; 48 h 83 *) calculated with the fed quantity of test solution (see appendix 2.1) *b) Contact toxicity test (topical application)* control (0.1 % aqueous Extravon solution): 24 h 0; 48 h 0 test substance CERTOSAN: 2 % / 100 µg formulation/bee: 24 h 3; 48 h 7 test substance CERTOSAN: 4 % / 200 µg formulation/bee: 24 h 0; 48 h 10 reference substance Dimethoate EC 400: 0.00313 % / 0.0313: 24 h 3; 48 h 3 0.00625 % / 0.0625: 24 h 7; 48 h 7 0.0125 % / 0.125: 24 h 3; 48 h 17 0.025 % / 0.25: 24 h 23; 48 h 33 0.05 % / 0.5: 24 h 70; 48 h 80 0.1 % / 1.0: 24 h 87; 48 h 90
Conclusion:	

Exposure to CERTOSAN showed no effect (0 %) on honeybee mortality up to a dose of 198 µg formulation/bee in the oral toxicity test and negligible effects up to a dose of 200 µg formulation/bee in the contact toxicity test after 48 hours according to the EPPO Guideline No. 170.
A calculation of the LD_{50} of the test substance was inapplicable.
No behavioural anomalies were observed in comparison to the control.

Oral toxicity: LD_{50} > 198 µg product/bee
Contact toxicity: LD_{50} > 200 µg product/bee

KCA 8.3.1.1.1/0001	**Comment RMS:** The study is relevant and reliable. The validity criteria according OECD TG 213 (1998) and OECD TG 214 (1998) are met. It is noted, that the study by Kleiner (1996b) was performed before OECD TG 213 & 214 were published. Instead the study was performed according to EPPO Guideline No. 170 (1992), however it is noted that the OECD Test guidelines are based upon the EPPO Guideline. Note: This study was already included in the first Annex I inclusion and is now re-evaluated by RMS AT. The toxicity endpoints are confirmed: **Oral toxicity: LD_{50} > 198 µg product/bee** **Contact toxicity: LD_{50} > 200 µg product/bee**

Overall RMS conclusion – Acute toxicity to bees:
Table 9.5.1-1 summarises the results of the available acute toxicity study conducted with Certosan (Kleiner, 1996b). The relevant endpoint to be used for the acute risk assessment of Certosan (99.8% Blood meal) is the 48-hours LD_{50} of > 198 µg product/bee for oral toxicity and > 200 µg product/bee for contact toxicity.

Table 9.5.1-1: Acute toxicity of Certosan to bees

Species	Test substance	Time scale/type of study	End point	Toxicity	Reference
Acute					
Apis mellifera	Certosan (99.8% Blood meal)	Acute (48 h)	Oral toxicity (LD_{50})	> 198 µg product/bee	Kleiner, R. (1996b)
Apis mellifera	Certosan (99.8% Blood meal)	Acute (48 h)	Contact toxicity (LD_{50})	> 200 µg product/bee	Kleiner, R. (1996b)

B.9.5.1.2. Chronic toxicity to adult bees

Chronic toxicity of the formulated product to adult bees
No chronic toxicity studies on bees with Blood meal or the representative formulation Certosan (99.8% Blood meal) were submitted for neither Annex I inclusion of Blood meal nor are considered necessary for re-evaluation. A waiver is requested since Blood meal is not considered to be an attractive food source for bees,

therefore chronic exposure to bees is considered negligible and the data requirement is considered sufficiently addressed. Please refer to Vol. 3 CA B9 B.9.3.1.2.

B.9.5.1.3. Effects on honeybee development and other honeybee life stages

Please refer to B.9.5.1.2.

B.9.5.1.4. Cage and tunnel tests

Not considered necessary, please refer to B.9.5.1.2.

B.9.5.1.5. Field tests

Not considered necessary, please refer to B.9.5.1.2.

B.9.5.2. Effects on non-target arthropods other than bees

Toxicity of the active substance to NTAs

Please refer to the studies on *Poecilus curpeus* and *Pardosa spp.* with Certosan (99.8% Blood meal) by Kleiner (1996a & 1996c) from the former evaluation of Blood meal. There studies are presented below and are considered to address the data requirement sufficiently.

Table 9.5-2 summarises all available toxicity endpoints of non-target arthropods other than bees for Blood meal and the representative formulation Certosan.

Table 9.5-2: Toxicity endpoints of Blood meal and Certosan in non-target arthropods (other than bees)

Species	Test substance	Time scale/type of study	End point	Toxicity	Reference
Poecilus curpeus	Certosan (99.8% Blood meal)	moistened sand, 14 d (2D)	LR_{50}	> 40 kg product/ha	Kleiner, R. (1996a)
Pardosa spp.	Certosan (99.8% Blood meal)	moistened sand, 14 d (2D)	LR_{50}	> 40 kg product/ha	Kleiner, R. (1996c)

Toxicity of the formulated product to NTAs

B.9.5.2.1. Effect study on *Aphidius rhopalosiphi*

The applicant submitted the following statement:
"Aphidius rhopalosiphi and Typhlodromus pyri are the two standard indicator species. No tests are available here for these species. However, supportive tests with the additional species Poecilus cupreus and Pardosa spp. are presented which are indicating that there would be no harmful effects on non-target arthropods.
Blood meal (Certosan) will be applied directly to plant parts or individual plants. No broadcast use is intended. The supportive tests show that direct contact with blood meal is not harmful to single arthropods.
Blood meal was evaluated already and RMS Belgium in 2008 and the EFSA in 2011 do not insist on tests with Aphidius and Typhlodromus and concluded on the available data that the risk from blood meal and the formulation Certosan to non-target arthropods is low."

Comment RMS:

The waiver/argumentation above for standard toxicity studies on non-target arthropods (other than bees) presented above is considered acceptable by RMS.

B.9.5.2.2. Effect study on *Typhlodromus pyri*

Please refer to B.9.5.2.1.

B.9.5.2.3. Effect study on *Poecilus curpeus*

Data point addressed:	KCP 10.3.2.1 (Vol. 3 CP B9 B.9.5.2.3)
Reference:	KCA 8.3.2.1/0001
Author(s) (year):	Kleiner, R., M. (1996a)
Title:	Testing toxicity to beneficial arthropods Carabid beetle – *Poecilus cupreus* L. according to BBA Guideline VI, 23-2.1.8 (1991)
Laboratory report / project Number (Doc No.):	Study No.: 96 10 48 026
Testing facility:	BioChem GmbH Karlsruhe, Labor Cunnersdorf, Germany
Published:	No
Test guideline used:	BBA Guideline VI, 23-2.1.8 (1991) but evaluated by RMS according to IOBC TG Heimbach et al. (2000)
Deviations:	No
GLP:	Yes
Acceptability:	**Yes**
Already EU evaluated?	Yes

Summary and conclusion

The effect of the test substance Certosan to adults of the carabid beetle *Poecilus cupreus* L. was tested over a period of 14 days after spraying the product at a dose of 40 kg/ha in 400 L water /ha (= 10 % w/v) onto the substrate (moistened sand) and the beetles in the test cages.

The test substance Certosan caused a negligible mortality of the beetles (3.3 % corrected by SCHNEIDER-ORELLI) and a slight increase of the feeding rate compared to the control. The relative decrease of beneficial effectivity calculated according to OVERMEER & VAN ZON was E = -4.7 %.

Therefore, Certosan can be classified as harmless to the carabid beetle *Poecilus cupreus* L. up to the dose tested in the laboratory test.

Test item: [copied from the study report by RMS]

Product name:	CERTOSAN
Code No.:	FLU-00194-P-O-WP
Batch No.:	95013G93
Production date:	13.07.93
Formulation:	wettable powder (WP)
Active ingredients/content:	blood meal: protein 87 % water 5 % salts 5 % fat 3 % figures given above may vary ± 3 units
Water solubility:	suspensible
Stability under test conditions:	no information
Storage conditions:	room temperature, dry
Safety precautions:	designation according to "Gefahrstoffverordnung": not required
Field of use:	game repellent
Highest recommended dose:	20 kg/ha in 200 l/ha of water (= 10 % w/v)
Dose in the test:	40 kg/ha in 400 l/ha of water (= 10 % w/v)
Further remarks:	"Gyllebo Plantskydd Product information" of 01.10.93; "Vorläufige Gebrauchsanweisung von Certosan"; "Veterinary certificate 33-456/94" of 20.03.95

Reference item:

Trade name:	Afugan 30 EC
Batch No.:	C07143158
Formulation:	EC 30
Active ingredient/content:	Pyrazophos 294 g/l (30.3 % w/w according to certificate AZ 06334)
Density (20 °C):	0.980 g/cm^3

Test system: [copied from the study report by RMS]

Test organism:	carabid beetle - *Poecilus cupreus L.*
Origin of the test animals:	laboratory rearing of BBA Braunschweig
Origin of the beetles used in the test:	reared in the laboratory of the testing facility
Age of the beetles:	4-5 weeks
Test conditions	
Test cages:	cages of plastics (Bellaplast) (18.3 cm x 13.6 cm x 6.4 cm)
Test substrate:	quartz sand (Hohenbockaer Quarzsand)
Substrate/test cage:	250 g
Number of beetles/replicate (= 1 cage):	6 (3 females and 3 males)
Number of replicates/variant:	5
Number of beetles/variant:	30
Feeding during the test:	at the day of application and 1, 2, 4, 7 and 11 days after that
Prey:	onion fly (*Delia antiqua*) 2 pupae/beetle/feeding time
Climatic conditions (test room):	
Temperature:	20-24 °C
Relative humidity:	67-80 %
Both registered:	continuously by a thermohygrograph
Illumination:	light period: 16 h light intensity: ca 1000 lx

Test duration: 14 days

Preparation: [copied from the study report by RMS]

Variant	Product name	Dose/Concentration
1 Control	deionized water	400 l/ha
2 Reference substance	Afugan 30 EC	0.8 l/ha in 400 l/ha of water (= 0.2 % v/v = 0.196 % w/v)
3 Test substance	CERTOSAN	40 kg/ha in 400 l/ha (= 10 % w/v)

The exactly measured amounts of the substances were mixed with deionized water without addition of solubility mediators, immediately before application.
The treatments were applied to the test cages in an automatic application cabin to ensure a standard high level of uniform deposit (400 l/ha = 40 ml/m²).
The quantity of the test solution per area was checked up by reweighing of glass plates (4.9 cm x 4.9 cm) placed at representative spots of the application cabin. The glass plates were weighed before and immediately after application. These glass plates were used to determine the accuracy of the treatment application. The dose rate was 99 % of the nominal dose (396 l/ha = 39.6 kg test substance/ha).

Course of the trial: [copied from the study report by RMS]

Three days before treatment 3 females and 3 males were placed in each test cage without food. The cages were filled with moistened sand covering the bottom.
Immediately before the treatment the beetles were inspected, anomalous ones were replaced and after moistening the sand fly pupae were added as food supply.
The test solutions were applied to the cages in an automatic application cabin if there were all beetles on the surface of the sand.
After application the cages were closed with gauze covers and incubated in an air-conditioned room for 14 days.
After 1, 2, 4, 7 and 11 days the food was changed (2 pupae/beetle) and the sand moistened. Dead and damaged beetles were counted and the feeding rate was determined by controlling the fed fly pupae.
14 days after application the final assessment was accomplished by counting of living and dead beetles, including the beetles present in the sand, and by counting of the fed fly pupae.

Results: [copied from the study report by RMS]

The mortality in the control was 0 %. Consequently the test accomplished the validity criterion (mortality in the control ≤ 10 %).
The substance CERTOSAN was tested at a dose of 40 kg/ha in 400 l/ha of water (= 10 % w/v).

The results are summarized in the tables 1 and 2 of the appendix.

The corrected mortality according to SCHNEIDER-ORELLI was 3.3 %.
The feeding rate showed no decrease in comparison with the control variant.
No behavioural anomalies were observed.

The relative decrease of beneficial effectivity calculated according to OVERMEER & VAN ZON was

E = -4,7 %.

Validity criteria [inserted by RMS]	Maximum mortality rate in the control must not exceed 2 beetles (6.7%) for a test design with 5 replicates consisting of 6 beetles each. Actually was in the study: 0%. The mortality rate in the reference item should range at 65% ± 35% after 2 weeks (corrected for control mortality). Actually was in the study: 100%

Observed effects [copied from the study report by RMS]

Mortality

Application date:	15.04.96		
Variant:	**Test substance**	**Control**	**Reference substance**
	CERTOSAN	deionized water	Afugan 30 EC
Dosage:	40 kg/ha		0.8 l/ha
Spray volume:	400 l/ha	400 l/ha	400 l/ha

Number of dead beetles

time after application	test substance		control		reference substance	
	♂	♀	♂	♀	♂	♀
number of tested beetles:	15	15	15	15	15	15
2 h	0	0	0	0	0	0
4 h	0	0	0	0	0	0
6 h	0	0	0	0	0	0
1 d	0	0	0	0	0	0
2 d	0	1	0	0	15	15
4 d	0	0	0	0	0	0
7 d	0	0	0	0	0	1
11 d	0	0	0	0	0	0
14 d	0	0	0	0	0	0
total	0	1	0	0	15	15
per cent	0	3.3	0	0	100	100

Feeding rate

Application date:	15.04.96		
Variant:	**Test substance**	**Control**	**Reference substance**
	CERTOSAN	deionized water	Afugan 30 EC
Dosage:	40 kg/ha		0.8 l/ha
Spray volume:	400 l/ha	400 l/ha	400 l/ha

Number of fed pupae

time after application	test substance	control	reference substance
1 d	33	34	11
2 d	15	11	0
4 d	27	26	0
7 d	36	36	0
11 d	39	36	0
14 d	35	28	0
total	185	171	11

Conclusion:

The test substance CERTOSAN caused negligible mortality of the carabid beetles. The relative decrease of beneficial effectivity was less than 30 %.

Therefore, using the IOBC categories, CERTOSAN can be classified as harmless for the carabid beetle *Poecilus cupreus* L. up to the dose tested in the laboratory test.

Mortality: LR_{50} > 40 kg product/ha

KCA 8.3.2.1/0001	**Comment RMS:** The study is relevant and reliable. The validity criteria according IOBC TG Heimbach et al. (2000) are met. It is noted, that the study by Kleiner (1996a) was performed before IOBC TG Heimbach et al. (2000) was published. Instead the study was performed according to BBA Guideline VI, 23-2.1.8 (1991), however it is noted that the test design is essentially the same. Note: This study was already included in the first Annex I inclusion and is now re-evaluated by RMS AT. The toxicity endpoint is confirmed: **Mortality (14d): LR_{50} > 40 kg product/ha (actually 3.3% at 40 kg product/ha)**

Overall RMS conclusion – Effects on *Poecilus curpeus*

Table 9.5.2-1 summarises the result of the available effect study on *Poecilus curpeus* conducted with Certosan (Kleiner, 1996a). The relevant endpoint to be used for the risk assessment of Certosan is the LR_{50} > 40 kg product/ha.

Table 9.5.2-1: Toxicity of Certosan to *Poecilus curpeus*

Species	Test substance	Time scale/type of study	End point	Toxicity	Reference
Poecilus curpeus	Certosan (99.8% Blood meal)	moistened sand, 14 d (2D)	LR_{50}	> 40 kg product/ha	Kleiner, R. (1996a)

B.9.5.2.4. Effect study on *Pardosa spp.*

Data point addressed:	KCP 10.3.2.1 (Vol. 3 CP B9 B.9.5.2.3)
Reference:	KCA 8.3.2.2/0001
Author(s) (year):	Kleiner, R., M. (1996c)
Title:	Testing toxicity to beneficial arthropods Spider – *Pardosa* spp. (laboratory) according to BBA Guideline (Proposal 1994)
Laboratory report / project Number (Doc No.):	Study No.: 96 10 48 080
Testing facility:	BioChem GmbH Karlsruhe, Labor Cunnersdorf, Germany
Published:	No
Test guideline used:	BBA Guideline (Proposal 1994) but evaluated by RMS according to IOBC TG Heimbach et al. (2000)
Deviations:	No
GLP:	Yes
Acceptability:	**Yes**
Already EU evaluated?	Yes

Summary and conclusion

The effect of the test substance Certosan to adults of the spider *Pardosa spp.* was tested over a period of 14 days after spraying the product at a dose of 40 kg/ha in 400 L water /ha (= 10 % w/v) onto the substrate (moistened sand) and the spiders in the test cages.

Exposure to Certosan caused negligible mortality to the spiders (5 % corrected according to SCHNEIDER-ORELLI) and increased the feeding rate slightly compared with the control. The relative decrease of beneficial effectivity according to OVERMEER & VAN ZON was E = -4.3 %.

Test item: [copied from the study report by RMS]

Product name:	CERTOSAN
Code No.:	FLU-00194-P-O-WP
Batch No.:	950 13G93
Production date:	13.07.93
Shelf life:	48 months
Formulation:	wettable powder (WP)
Active ingredients/content:	blood meal: protein 87 % water 5 % salts 5 % fat 3 % figures given above may vary ± 3 units
Water solubility:	suspensible
Stability under test conditions:	no information
Storage conditions:	room temperature, dry
Safety precautions:	designation according to "Gefahrstoffverordnung": not required
Field of use:	game repellent
Highest recommended dose:	20 kg/ha in 200 l/ha of water (= 10 % w/v)
Dose in the test:	40 kg/ha in 400 l/ha of water (= 10 % w/v)
Further remarks:	"Gyllebo Plantskydd Product information" of 01.10.93; "Vorläufige Gebrauchsanweisung von Certosan"; "Veterinary certificate 33-456/94" of 20.03.95

Reference item:

Trade name:	Thiodan 35 EC
Batch No.:	C0235008
Active ingredient/content:	Endosulfan 33.0 % w/w (according to certificate)
Formulation:	EC 35
Density (20 °C):	ca. 1.07 g/cm^3

Test system: [copied from the study report by RMS]

Test organism:	wolf spider - *Pardosa spp.*
Origin of the spiders used in the test:	field population (Cunnersdorf) - October 1996
Prey:	onion fly (*Delia antiqua*), reared in the laboratory
Test conditions	
Test cages:	plastic cages (Bellaplast), 11.5 cm x 11.5 cm x 6.0 cm, with gauze covers, (plastic wated fibre glass gauze with a mesh width of 1.4 mm x 1.4 mm, with Fluon painted brims)
Test substrate:	quartz sand (Hohenbockaer Quarzsand) (suppliers: Hohenbockaer Quarzwerke GmbH)
particle size:	0.1 mm-0.5 mm
sand layer:	ca 1 cm height
weight:	125 g dry sand
water addition:	23±1 ml
moisture of sand:	ca 70 % of WHC_{max} (max. water holding capacity)
moistening of sand:	each 2 to 4 days (weight control)
Number of spiders/cage (= 1 replicate):	1 female or 1 male
Number of replicates/variant:	10 + 10
Number of spiders/variant:	20 (10 females + 10 males)
Food:	onion fly (*Delia antiqua*) 1-2 adult flies/spider/feeding time
Climatic conditions (test room)	
Temperature:	18-22 °C (according to study plan: (20±2) °C)
Relative humidity:	70-79 % (according to study plan: (80±10) %)
Both registered:	continuously by a thermohygrograph
Illumination:	light period: 16 h/day light intensity: ca 1000 lx
Test duration:	14 days

Preparation: [copied from the study report by RMS]

Variant	Product name	Dose/Concentration
1 Control	deionized water	400 l/ha
2 Reference substance	Thiodan 35 EC	0.085 l/ha (30 g a.i./ha) in 400 l/ha of water (= 0.0213 % v/v = 0.0228 % w/v)
3 Test substance	CERTOSAN	40 kg/ha in 400 l/ha of water (= 10 % w/v)

The exact measured amounts of the substances were miscibled with deionized water without addition of solubility mediators, immediately before application.
The treatments were applied to the test cages in an automatic application cabin, to ensure a standard high level of uniform deposit on the surface of the sand (400 l/ha = 40 ml/m^2).

Course of the trial: [copied from the study report by RMS]

The test spiders were kept for 5 days under laboratory conditions at (20±2) °C to become acclimatized. Three days before treatment one female or one male was placed into each test cage with moistened sand covering the bottom and with Fluon painted brims. They were held without food.
Immediately before the treatment, the spiders were inspected, anomalous ones were replaced by animals of the same sex. The sand was moistened with deionized water (ca 70 % of max. water holding capacity).
The treatments were applied to the cages with the spiders (without gauze covers) in an automatic application cabin. Bellaplast cages without bottoms were hung in the test cages in order to avoid contamination of the brim of the test cage and were removed after the spraying.
Immediately after application, 2 onion flies (*Delia antiqua*) were added as food supply to each spider and the cages were closed with the gauze covers.
After a waiting period of 2 hours (at 20 °C in a well-ventilated room) the cages were incubated in an air-conditioned room (20 °C, 16 h/8 h light/dark) for 14 days. Every 1, 2 or 3 days food was changed and every 2, 3 or 4 days the sand was moistened evenly (ca. 70 % of WHC_{max}) without moistening the spiders.

Results: [copied from the study report by RMS]

The mortality in the control was 0 %. Consequently the test accomplished the validity criterion (mortality in the control ≤ 10 %).

The substance CERTOSAN was tested at a dose of 40 kg/ha in 400 l/ha of water (= 10 % w/v).

The results are summarized in the tables (appendices 1-3).

The mortality of the test variant was 5 %, whereas the reference substance caused 80 % mortality.
The feeding rate per spider was slightly increased in the CERTOSAN variant in comparison with the control variant.
No behavioural anomalies were observed.

The relative decrease of beneficial effectivity calculated according to OVERMEER & VAN ZON (1982) was

E = -4.3 %

Validity criteria [inserted by RMS]	Maximum mortality rate in the control must not exceed 1 spider beetles (3.9%) for a test design with 26 spiders (i.e. "replicates"). Actually was in the study: 0%. (10 female and 10 male spiders were used in the reference substance treatment) The mortality rate in the reference item should range at 65% ± 35% after 2 weeks (corrected for control mortality). Actually was in the study: 80% (10 female and 10 male spiders were used in the reference substance treatment)
Observed effects [copied from the study report by RMS]	Mortality:

Test organism:	*Pardosa spp.* - adults		
Application date:	04.11.96		
Variant:	Test substance	Control	Reference substance
	CERTOSAN		**Thiodan 35 EC**
Dosage:	40 kg/ha		0.085 l/ha
Spray volume:	400 l/ha	400 l/ha	400 l/ha

Number of dead spiders

time after application	test substance		control		reference substance	
	♀	♂	♀	♂	♀	♂
number of tested spiders:	10	10	10	10	10	10
2 h	0	0	0	0	0	0
4 h	0	0	0	0	0	0
6 h	0	0	0	0	0	0
1 d	0	0	0	0	5	8
2 d	0	0	0	0	1	0
3 d	0	0	0	0	0	0
4 d	0	0	0	0	1	1
7 d	1	0	0	0	0	0
9 d	0	0	0	0	0	0
11 d	0	0	0	0	0	0
14 d	0	0	0	0	0	0
total	1	0	0	0	7	9
per cent	5		0		80	

Feeding rate:

Test organism:	*Pardosa spp.* - adults		
Application date:	04.11.96		
Variant:	Test substance	Control	Reference substance
	CERTOSAN		**Thiodan 35 EC**
Dosage:	40 kg/ha		0.085 l/ha
Spray volume:	400 l/ha	400 l/ha	400 l/ha

Number of eaten flies/10 spiders

time after application	test substance		control		reference substance	
	♀	♂	♀	♂	♀	♂
1 d	9	9	11	13	0	0
2 d	9	6	7	7	3	0
3 d	5	1	8	4	0	2
4 d	9	7	5	5	3	0
7 d	12	13	10	10	3	1
9 d	5	7	6	7	1	1
11 d	5	10	8	5	2	0
14 d	10	17	10	6	3	0
total	64	70	65	57	15	4
total (♀ + ♂)	134		122		19	

Conclusion:

Exposure to CERTOSAN caused negligible mortality of the spiders at the dose tested. There was a slight relative increase of the feeding capacity.

The calculated reduction of beneficial effectivity was E = -4.3 %.

Therefore, using the IOBC categories, CERTOSAN can be classified as harmless for the wolf spider *Pardosa spp.* at the dose tested in this laboratory test.

Mortality: LR_{50} > 40 kg product/ha

KCA 8.3.2.2/0001	**Comment RMS:** The study is relevant and reliable. The validity criteria according IOBC TG Heimbach et al. (2000) are met. It is noted, that the study by Kleiner (1996c) was performed before IOBC TG Heimbach et al. (2000) was published. Instead the study was performed according to BBA Guideline (Proposal 1994), however it is noted that the test design is essentially the same. Note: This study was already included in the first Annex I inclusion and is now re-evaluated by RMS AT.

	The toxicity endpoint is confirmed: **Mortality (14d): LR_{50} > 40 kg product/ha (actually 5% at 40 kg product/ha)**

Overall RMS conclusion – Effects on *Pardosa spp.*
Table 9.5.2-2 summarises the result of the available effect study on *Pardosa spp.* conducted with Certosan (Kleiner, 1996c). The relevant endpoint to be used for the risk assessment of Certosan is the LR_{50} > 40 kg product/ha.

Table 9.5.2-3: Acute toxicity of Certosan to *Pardosa spp.*

Species	Test substance	Time scale/type of study	End point	Toxicity	Reference
Pardosa spp.	Certosan (99.8% Blood meal)	moistened sand, 14 d (2D)	LR_{50}	> 40 kg product/ha	Kleiner, R. (1996c)

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

B.9.6.1. Risk assessment for honey bees

The risk assessment for honey-bees addresses both the EFSA Bee Guidance Document (EFSA Journal 2013;11(7):3295) and the Terrestrial Ecotoxicology GD (SANCO/10329/2002).

The use pattern involves treatments on deciduous and coniferous trees in forestry, trees in orchards and ornamental plants at a maximum single application rate of 19.96 kg a.s./ha.

For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying by e.g. knapsack sprayer) exposure can not be excluded, however the use of Certosan (99.8% Blood meal) is considered to pose a low risk to bees based on the available acute toxicity data. Therefore, further calculations regarding the risk of bees following the exposure to Certosan were not considered necessary.

B.9.6.2. Risk assessment of non-target arthropods other than bees

The risk assessment was addressed according to the ESCORT 2 Guidance Document (2000).

The use pattern involves treatments on deciduous and coniferous trees in forestry, trees in orchards and ornamental plants at a maximum single application rate of 19.96 kg a.s./ha.

For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying by e.g. knapsack sprayer) exposure can not be excluded, however the use of Certosan (99.8% Blood meal) is considered to pose a low risk to non-target arthropods based on the available toxicity data. No effects > 50% were observed up to a dose of 40 kg product/ha (equivalent to 39.96 kg a.s./ha) for the soil-dwelling arthropods *Poecilus cupreus* and *Pardosa* spp. Therefore, further calculations regarding the risk of non-target arthropods following the exposure to Certosan were not considered necessary.

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.7.1. Earthworms

Active substance data

No sublethal toxicity studies with earthworms are available for Blood meal, the waiver for standard toxicity studies on sub-lethal effects on earthworms is considered acceptable (please refer to Vol. 3 CA B9 B.9.4.1). For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the use of Blood meal as fertiliser and the natural mineralisation of Blood meal (consisting to >80% out of protein) in soil is considered sufficient to demonstrate a low concern to earthworms and to address the data requirement.

Table 9.7.1-1 summarises all available toxicity endpoints of earthworms for Blood meal and the representative formulation Certosan.

Table 9.77.1-1: Toxicity endpoints of Blood meal in earthworms

Species	Test substance	Time scale	End point	Toxicity	Reference
Earthworm	Blood meal/ Certosan			No data	

B.9.7.1.1. Sub-lethal effects to earthworms

Formulated product data

No studies with the EU representative formulation Certosan have been conducted with earthworms. The representative product Certosan consists out of 99.8% Blood meal (please refer to Volume 4). Therefore, toxicity of safeners, synergists and co-formulants posed by Certosan is not to be expected and the data requirement is considered sufficiently addressed based on the information on the active substance.

B.9.7.1.2. Field studies with earthworms

Formulated product data

Not considered necessary, please refer to B.9.7.1.1.

B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Active substance data

No toxicity studies with soil organisms other than earthworms are available for Blood meal, the waiver for standard toxicity studies on non-target soil organisms is considered acceptable (please refer to Vol. 3 CA B9 B.9.4.1). For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the low toxicity demonstrated in bees and other non-target arthropods, the use of Blood meal as fertiliser and the natural mineralisation of Blood meal (consisting to >80% out of protein) in soil is considered sufficient to demonstrate a low concern to non-target soil organisms and to address the data requirement.

Table 9.7.2-1 summarises all available toxicity endpoints of earthworms for Blood meal and the representative formulation Certosan.

Table 9.77.2-2: Toxicity endpoints of Blood meal and Certosan in non-target soil organisms other than earthworms

Species	Test substance	Time scale/type of endpoint	End point	Toxicity	Reference
Folsomia candida	Blood meal/ Certosan			No data	
Hypoaspis aculeifer	Blood meal/ Certosan			No data	

B.9.7.2.1. Species level testing

Formulated product data

No studies with the EU representative formulation Certosan have been conducted with non-target soil meso- and macrofauna other than earthworms. The representative product Certosan consists out of 99.8% Blood meal (please refer to Volume 4). Therefore, toxicity of safeners, synergists and co-formulants posed by Certosan is not to be expected and the data requirement is considered sufficiently addressed based on the information on the active substance.

B.9.7.2.2. Higher tier testing

Not considered necessary, please refer to B.9.7.2.1.

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

The risk assessment for soil organisms was addressed according to the Terrestrial Ecotoxicology GD (SANCO/10329/2002).

B.9.8.1. Risk assessment for earthworms

For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the use of Blood meal as fertiliser and the natural mineralisation of Blood meal (consisting to >80% out of protein) in soil is considered sufficient to demonstrate a low risk to earthworms and to address the data requirement. The calculation of Toxicity/Exposure Ratios (TERs) is therefore not considered necessary.

No negative effects of the active substance Blood meal and the product Certosan on earthworms are expected. Further it should be noted, that Blood meal is a fertiliser in organic farming (refer to the EU-Regulation No. 1069/2009) and the application rate is multiple compared to the use of Certosan (up to 2500 kg fertiliser/ha[1]).

B.9.8.2. Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the low toxicity demonstrated in bees and other non-target arthropods, the use of Blood meal as fertiliser and the natural mineralisation of Blood meal (consisting to >80% out of protein) in soil is considered sufficient to demonstrate a low risk to non-target soil organisms other than earthworms and to address the data requirement. The calculation of Toxicity/Exposure Ratios (TERs) is therefore not considered necessary.

[1] https://styriafert.at/downloads/StyriaFert-Datenblatt-NPK-screen.pdf

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Active substance data

No studies were submitted by the notifier to address the effects on soil nitrogen transformation, the waiver is considered acceptable (please refer to Vol. 3 CA B9 B.9.5). For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the use of Blood meal as fertiliser and the natural mineralisation of Blood meal (consisting to >80% out of protein) in soil are considered sufficient to demonstrate a low concern to soil nitrogen transformation and to address the data requirement.

Table 9.9-1 summarises all available toxicity endpoints on soil nitrogen transformation for Blood meal and the representative formulation Certosan.

Table 9.99-1: Toxicity endpoints of Blood meal on soil nitrogen transformation

Test type	Test substance		Effect	Reference
Nitrogen transformation	Blood meal / Certosan (99.8% Blood meal)		No data	

Formulated product data

No studies with the EU representative formulation Certosan have been conducted to assess the effects on soil nitrogen transformation. The representative product Certosan consists out of 99.8% Blood meal (please refer to Volume 4). Therefore, toxicity of safeners, synergists and co-formulants posed by Certosan is not to be expected and the data requirement is considered sufficiently addressed based on the information on the active substance.

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

The risk assessment for soil nitrogen transformation was addressed according to the Terrestrial Ecotoxicology GD (SANCO/10329/2002).

For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the use of Blood meal as fertiliser and the natural mineralisation of Blood meal (consisting to >80% out of protein) in soil is considered sufficient to demonstrate a low risk to soil nitrogen transformation and to address the data requirement. Therefore a further consideration of a risk assessment is not considered necessary.

No negative effects of the active substance Blood meal and the product Certosan on soil microbial activity are expected. Further it should be noted, that Blood meal is a fertiliser in organic farming (refer to the EU-Regulation No. 1069/2009) and the application rate is multiple compared to the use of Certosan (up to 2500 kg fertiliser/ha[2]).

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

Active substance data

No studies were submitted by the notifier to address the effects on non-target plants, the waiver for standard toxicity studies is considered acceptable (please refer to Vol. 3 CA B9 B.9.6). For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be fully excluded, however the use of Blood meal as fertiliser, the natural mineralisation of Blood meal (consisting to >80% out of protein) in soil and the absence of phytotoxic

[2] https://styriafert.at/downloads/StyriaFert-Datenblatt-NPK-screen.pdf

effects in the efficacy section (please refer to Vol. 3 CP B3 B.3.11) is considered sufficient to demonstrate a low concern to non-target plants and to address the data requirement.

Table 9.10-1 summarises all available toxicity endpoints of non-target terrestrial plants for Blood meal and the representative formulation Certosan.

Table 9.8.2-1: Toxicity endpoints of Blood meal on non-target terrestrial plants

Species	Test substance	ER_{50} vegetative vigour (g a.s./ha)	ER_{50} emergence (g a.s./ha)	Reference
	Blood meal/ Certosan (99.8% Blood meal)	No data	No data	

B.9.11.1. Summary of screening data

Formulated product data

No studies with the EU representative formulation Certosan have been conducted to assess the effects on non-target plants. The representative product Certosan consists out of 99.8% Blood meal (please refer to Volume 4). Therefore, toxicity of safeners, synergists and co-formulants posed by Certosan is not to be expected and the data requirement is considered sufficiently addressed based on the information on the active substance.

B.9.11.2. Testing on non-target plants

Not considered necessary, please refer to B.9.11.1.

B.9.11.3. Extended laboratory studies on non-target plants

Not considered necessary, please refer to B.9.11.1.

B.9.11.4. Semi-field and field tests on non-target plants

Not considered necessary, please refer to B.9.11.1.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

The risk assessment for non-target plants was addressed according to the Terrestrial Ecotoxicology GD (SANCO/10329/2002).

For targeted application methods (coating with brush, dipping) a relevant exposure is considered to be negligible. For less targeted application methods (i.e. spraying) exposure can not be excluded, however the use of Blood meal as fertiliser and the natural mineralisation of Blood meal (consisting to >80% out of protein) in soil is considered sufficient to demonstrate a low risk to non-target plants and to address the data requirement. Therefore a further consideration of a risk assessment is not considered necessary.

Further no signs of phytotoxicity of the test product were visible on coniferous and deciduous trees as well as on fruit trees or ornamental plants with the intended dose rate as well as with 2-3 times higher dose rates (assessed on forests tree species only), please refer to Vol. 3 CP B3 B.3.11. Hence, no negative effects of the active substance Blood meal and the formulation Certosan on non-target plants are expected. Further it should be noted,

that Blood meal is a fertiliser in organic farming (refer to the EU-Regulation No. 1069/2009) and the application rate is multiple compared to the use of Certosan (up to 2500 kg fertiliser/ha[3]).

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No other data concerning effects of the active substance Blood meal or the formulated product Certosan to other terrestrial non-target organisms are available and are not a mandatory requirement.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Not relevant, please refer to B.9.13.

B.9.15. MONITORING DATA

Monitoring data concerning effects of the active substance and the formulated product to non-target organisms are not available and are not a mandatory requirement.

B.9.16. REFERENCES RELIED ON

Literature search:
A literature search was performed for the active substance Blood meal, please refer to Vol. 3 CA B9 under point B.9.10.

List of data submitted by the applicant and relied on:

Data point	Author(s)	Year	Title Doc. No., (prev. used Doc. No.), (Report No.) Source (where different from company) GLP or GEP status, Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N
KCP 10.2.1/01	[redacted]	2013	Determination of the acute toxicity of Blood Meal (Certosan) against *Oncorhynchus mykiss* following EU-Method C1. resp. OECD Guideline 203 [redacted] Study No. 13022801G503 GLP: yes unpublished	Y	Y	new study	Flügel GmbH	N

[3] https://styriafert.at/downloads/StyriaFert-Datenblatt-NPK-screen.pdf

Data point	Author(s)	Year	Title Doc. No., (prev. used Doc. No.), (Report No.) Source (where different from company) GLP or GEP status, Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N
KCP 10.2.1/02	Muckle, M.	2013b	Determination of short term toxicity of Blood Meal (Certosan) against *Daphnia magna* STRAUS according to OECD 202 resp. EU C.2 LAUS GmbH Study No. 13022801G201 GLP: yes unpublished	N	Y	new study	Flügel GmbH	N
KCP 10.2.1/03	Muckle, M.	2013c	Determination of the toxicity of Blood Meal (Certosan) in *Desmodesmus subspicatus* according to OECD 201 resp. EU C.3 LAUS GmbH Study No. 13022801G301 GLP: yes unpublished	N	Y	new study	Flügel GmbH	N
KCP 10.3.1.1.1 & KCP 10.3.1.1.2	Kleiner, R	1996b	Testing toxicity to Honeybee – *Apis mellifera* L. (laboratory) according to EPPO Guideline No. 170 BioChem – Labor für biologische und chemische Analytik GmbH Study No. 96 10 48 027 GLP: yes unpublished	N	N	-	Flügel GmbH	Y
KCP 10.3.2.1	Kleiner, R	1996a	Testing toxicity to beneficial arthropods Carabid beetle – *Poecilus cupreus* L. according to BBA Guideline VI, 23-2.1.8 (1991) BioChem – Labor für biologische und chemische Analytik GmbH Study No. 96 10 48 026 GLP: yes unpublished	N	N	-	Flügel GmbH	Y
KCP 10.3.2.1	Kleiner, R	1996c	Testing toxicity to beneficial arthropods Spider – *Pardosa spp.* (loboratory) according to BBA Guideline (Proposal 1994) BioChem – Labor für biologische und chemische Analytik GmbH Study No. 96 10 48 080 GLP: yes unpublished	N	N	-	Flügel GmbH	Y

na = not applicable / ni = not indicated / nr = not relevant