REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: Salaman 510

Product name(s): **FOSIKA**

Chemical active substance:

potassium phosphonates (510 g/L, expr. as phosphorous acid)

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Lainco, S.A. /Exclusivas Sarabia S.A / Biovert S.L.

Submission date: October 2021

MS Evaluation date: July 2022

MS Finalisation date: dd/mm/yyyy

Version history

When	What
October 2021	Application for the first approval of the product's code SALAMAN 510 in Poland.
July 2022	Version evaluated by zRMS Poland

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
					Application				**Application rate**					**Conclusion**						
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	L f.p./ha a) min-max. rate per appl. b) min-max. total rate per crop/season	kg a.s./ha a) min-max. rate per appl. b) min-max. total rate per crop/season	Water L/ha min/max	PHI (days)	Remarks: e.g. g safener/ synergist per ha	Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Pome fruit	F	*Venturia inaequalis* *Venturia pyrina*	Foliar spray	BBCH 53-81	a) 3 b) 3	5	a) 1.50-2.50 b) 4.50-7.50	a) 0.765-1.275 b) 2.295-3.825	500-1000	35	-							

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1.

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application.

Explanation for column 15 – 21 "Conclusion"

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

Remarks table:

(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
(6) Method, e.g. high-volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(8) The maximum number of application possible under practical conditions of use must be provided
(9) Minimum interval (in days) between applications of the same product.
(10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under "application: method/kind".
(13) PHI - minimum pre-harvest interval
(14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

The ecotoxicological risk assessment of the formulation was performed according to the requirements of Regulation (EC) No 1107/2009. Appropriate endpoints from the EU conclusions for the active substance was used for the intended use patterns. In cases where deviations from the EU agreed endpoints were considered appropriate (for example when additional studies are provided), such deviations were highlighted and justified accordingly.

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

The calculated TER_A and TER_{LT} values are above the triggers of 10 and 5 respectively, indicating no unacceptable risk to birds and mammals following application of Salaman 510 according to the proposed use pattern.

No unacceptable effects to birds and mammals through drinking water are expected following application of Salaman 510 according to the proposed use pattern.

No unacceptable effects to fish-eating and earthworm-eating birds and mammals are expected following application of Salaman 510 according to the proposed use pattern.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

No unacceptable risk is expected for aquatic organisms for the intended uses of Salaman 510.

To protect aquatic organisms, respect an unsprayed buffer zone of 3 m to surface water bodies.

9.1.1.3 Effects on bees (KCP 10.3.1)

No unacceptable risk is expected for bees for the intended uses of Salaman 510.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

No unacceptable risk is expected for arthropods other than bees for the intended uses of Salaman 510.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4)

The proposed use of Salaman 510 poses no unacceptable risk to non-target soil meso- and macrofauna. The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

9.1.1.6 The chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to potassium phosphonates (as phosphorous acid) are greater than the trigger of 5, indicating that the risk to earthworms and other non-target soil organisms (meso- and macrofauna) is acceptable

following use of Salaman 510 according to the proposed use pattern.

9.1.1.7 Effects on soil microbial activity (KCP 10.5)

The proposed use of Salaman 510 poses no unacceptable risk to soil microbial activity.

9.1.1.8 Effects on non-target terrestrial plants (KCP 10.6)

The proposed use of Salaman 510 poses no unacceptable risk to non-target plants.

9.1.1.9 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Tests on other non-target species are not required.

9.1.2 Grouping of intended uses for risk assessment

The details concerning the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011) are reported in the corresponding parts of the dRR.

9.1.3 Consideration of metabolites

The experts of the PRAPeR 09 expert meeting assumed that given the elementary nature of this active substance (potassium phosphonate) only transformation of the potassium phosphonate salts into phosphonic acid is expected in plants and agreed that the available data from the public literature were sufficient to address the uptake and metabolism of potassium phosphonates in plants.

The proposed residue definition for monitoring and risk assessment is phosphonic acid and its salts expressed as phosphonic acid (*EFSA Journal 2012*;10(12);2963).

9.2 Effects on birds (KCP 10.1.1)

zRMS Comments:	The acute and long-term risk for birds due to the use of Salaman 510 in pome fruits was submitted. The active substance name was corrected to phosphonic acid. The screening assessment for acute and reproductive risk were submitted using the phosphonic acid. **Acute risk assessment** The used endpoint for acute risk assessment LD_{50} > 2250 mg a.s./kg bw was agreed at the EU level. The TER_A value for birds is above the trigger value of 10 and an acceptable acute risk for birds can be concluded. **Long-term risk assessment** The recommended by EFSA (2012) corrected endpoint NOEC of 149.04 mg a.s./kg bw/d was used in long-term risk assessment. This corrected value does not affect the final conclusion. The TER_{LT} value for birds is above the trigger value of 5 indicating an acceptable long-term risk for birds.

	The risk assessment of secondary poisoning was not required (log Pow <3). The risk is acceptable if product Salaman 510 is applied in accordance with proposed pattern use.

9.2.1 Toxicity data

Avian toxicity studies have been carried out with potassium phosphonates. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of Salaman 510 were not evaluated as part of the EU assessment of potassium phosphonates. However, the provision of further data on Salaman 510 is not considered essential, because the toxicity of the plant protection product can be predicted on the basis of the data for the active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Potassium phosphonate (expressed as H_3PO_3)	Acute toxicity	**LD_{50}: >2250 mg phosphonic acid / kg bw**	*EFSA Journal 2012*;10(12);2963 (peer review potassium phosphonates)
		Dietary toxicity (short term)	LC_{50} (bobwhite quail) > 5620 mg/kg feed (> 1335 mg phosphonic acid /kg feed LC_{50} (mallard duck) > 5620 mg/kg feed (> 2363 mg phosphonic acid /kg feed	*EFSA Journal 2012*;10(12);2963 (peer review potassium phosphonates)
		Reproductive toxicity (long term)	**NOEC = 149.04** ~~**NOAEL: 149.9**~~ **mg /kg bw**	*EFSA Journal 2012*;10(12);2963 (peer review potassium phosphonates)

9.2.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (*EFSA Journal 2009*; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

9.2.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following table.

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of Salaman 510 in pome fruit trees (representative group: orchards)

Intended use	POME FRUIT TREES (representative group: orchards)
Active substance/product	potassium phosphonates

Application rate (g a.s./ha)	3 x 1.275 kg a.s./ha (interval between applications: 5 days)				
Acute toxicity (mg/kg bw)	**LD_{50} >2250 mg H_3PO_3/kg bw**				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_A**
All stages	Small insectivorous bird	46.8	1.8	107.41	20.9
Reprod. toxicity (mg/kg bw/d)	**NOEC: 149.04 mg H_3PO_3/kg bw ~~NOEL 149.9 mg/kg bw~~**				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{LT}**
All stages	Small insectivorous bird	18.2	2.2*0.53	27.06	5.5

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Conclusions

Based on the screening assessments, the TER_A and the TER_{LT} values are above the trigger values of 10 and 5, respectively for pome fruits. Therefore, the application of Salaman 510 in pome fruit trees presents no acute or long-term risk to birds.

9.2.2.2 Higher-tier risk assessment

No further acute refinement is required.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of *EFSA/2009/1438*).

Leaf scenario

Since Salaman 510 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario is not to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (K_{oc} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{oc} ≥ 500 L/kg).

For Potassium phosphonate, the ratio of highest application rate for pome fruits (1275 g a.s./ha) to lowest relevant endpoint (~~NOAEL = 149.9~~ NOEC: 149.04 mg a.s./kg bw/d) is 8.5. As the $K_{f,OC}$ for Potassium phosphonate is 972 mL/g (*EFSA Journal 2012*;10(12): 2963**)**, the risk can be considered acceptable without the need of further calculations.

Effective application rate (g/ha) = 1275
Acute toxicity (mg/kg bw) > 2250 quotient = 0.56
Reprod. toxicity (mg/kg bw/d) = ~~149.9~~ 149.04 quotient = 8.5

No unacceptable effects to birds through drinking water are expected following application of Salaman 510 according to the proposed use pattern.

9.2.2.4 Effects of secondary poisoning

According to the EFSA guide "Risk Assessment for Birds and Mammals", *EFSA Journal 2009*; 7(12):1438, substances with a Log P_{ow} greater than 3 have potential to bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

Potassium phosphonate is an inorganic substance with a very low solubility in organic solvents and totally miscible with water. Therefore, the assessment of secondary poisoning is not necessary.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.2.3 Risk assessment for baits. pellets. granules. prills or treated seed

Not relevant.

9.2.4 Overall conclusions

The calculated TER_A and TER_{LT} values are above the triggers of 10 and 5 respectively, indicating no unacceptable risk to birds following application of Salaman 510 according to the proposed use pattern.

No unacceptable effects to birds through drinking water are expected following application of Salaman 510 according to the proposed use pattern.

No unacceptable effects to fish-eating and earthworm-eating birds are expected following application of Salaman 510 according to the proposed use pattern.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

zRMS Comments:	The acute and long-term risk for mammals due to the use of Salaman 510 in pome fruits was submitted. The screening assessment for acute and reproductive risk were submitted using the phosphonic acid. The screening and Tier 1 assessment for acute and reproductive risk were submitted. The submitted acute and long-term risk assessment for growth stage BBCH < 53 was not evaluated as intended use considers BBCH 53 – 81. **Acute risk assessment** The used endpoint for acute risk assessment LD_{50} > 1736 mg a.s./kg bw was agreed at the EU level. The TER_A value for mammals is above the trigger value of 10 at Tier 1 step indicating an acceptable acute risk for mammals. **Long-term risk assessment** The recommended by EFSA (2012) endpoint NOAEL = 302.9 mg a.s./kg bw/d was used in long-term risk assessment. The TER_{LT} value for mammals is above the trigger value of 5 at Tier 1 step indicating an acceptable long-term risk for mammals.

	The risk assessment of secondary poisoning was not required (log Pow <3). The risk is acceptable if product Salaman 510 is applied in accordance with proposed pattern use.

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with potassium phosphonates. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of Salaman 510 were not evaluated as part of the EU assessment of potassium phosphonates. However, the provision of further data on the formulation Salaman 510 is not considered essential, because the toxicity of the plant protection product can be predicted on the basis of the data for the active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
SD Rat	Potassium phosphite (equiv. to 38% w/w in H_3PO_3)	Acute toxicity	LD_{50} >2000 mg/kg bw (equivalent to > 760 mg H_3PO_3/kg bw)	Richeux (2012)
CD Rat	Phosphorous acid		LD_{50}: 2950 mg/kg bw	Pasquet. Mazuret (1977)
CD Rat	Disodium phosphite (equiv. to 65% in H_3PO_3)		LD_{50}: 5300 mg/kg bw (equivalent to 3450 mg H_3PO_3/kg bw)	Pasquet. Mazuret (1977)
SD Rat	Potassium salts of phosphorous acid (equiv. to 61% in H_3PO_3)		LD_{50}: 3624 mg/kg bw (equivalent to 2210 mg H_3PO_3/kg bw)	*EFSA Scientific Report (2005) 54.* 1-79 (Wilson, 1995)
--	Phosphonic acid/ ~~Potassium phosphonates~~		**LD_{50}: 1736 mg/kg bw**	Peer Review of potassium phosphonates
Rat	Potassium phosphonates		LD_{50}: 5000 mg as/kg bw (equivalent to 1736 mg H_3PO_3/ kg bw)	*EFSA Journal 2012;10(12):2963*
	Fosetyl-Al (equivalent to 69% in H_3PO_3)	Reproductive toxicity (Long term)	**NOEC:** 439 mg Fosetyl-Al/kg bw (equivalent to **302.9 mg H_3PO_3/ kg bw)**	*EFSA Journal 2012;10(12):2963* (Palmer & Bottomley, 1981)

9.3.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (*EFSA Journal 2009; 7(12): 1438*; hereafter referred to as EFSA/2009/1438).

9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of Salaman 510 in pome fruit trees (representative group: orchards)

Intended use	POME FRUIT TREES (representative group: orchards)				
Active substance/product	potassium phosphonates				
Application rate (g a.s./ha)	3 x 1.275 kg a.s./ha (interval between applications = 5 days)				
Acute toxicity (mg/kg bw)	**LD_{50}: 1736 mg H_3PO_3/kg bw**				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
All stages	Small herbivorous mammal	136.4	1.8	313.04	**5.5**
Reprod. toxicity (mg/kg bw/d)	**NOEL: 302.9 mg H_3PO_3/kg bw ~~mg/kg bw/d~~**				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
All stages	Small herbivorous mammal	72.3	2.2 x 0.53	107.48	**2.82**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-3: Tier I assessment of the acute and long-term/reproductive risk for mammals due to the use of Salaman 510 in pome fruit trees (representative group: orchards)

Intended use	POME FRUIT TREES (representative group: orchards)				
Active substance/product	potassium phosphonates				
Application rate (g a.s./ha)	3 x 1.275 kg a.s./ha (interval between applications = 5 days)				
Acute toxicity (mg/kg bw)	**LD_{50}: 1736 mg H_3PO_3/kg bw**				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Application crop directed BBCH ≥ 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	10.5	1.8	24.11	72.0
Application crop directed BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	40.9	1.8	93.84	18.5
Application crop directed BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	5.2	1.8	11.93	145.5
~~Application crop directed BBCH 10- 19~~	~~Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs~~	~~28.1~~	~~1.8~~	~~64.54~~	~~26.9~~
~~Application crop directed BBCH 10- 19~~	~~Small herbivorous mammal "vole Grass + cereals 100% grass~~	~~109.2~~	~~1.8~~	~~251.59~~	~~**6.9**~~
~~Application crop directed BBCH 10- 19~~	~~Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods~~	~~13.8~~	~~1.8~~	~~31.68~~	~~54.8~~
~~Application crop directed BBCH 20- 40~~	~~Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs~~	~~21.1~~	~~1.8~~	~~48.49~~	~~35.8~~
~~Application crop directed BBCH 20- 40~~	~~Small herbivorous mammal "vole Grass + cereals 100% grass~~	~~81.9~~	~~1.8~~	~~188.70~~	~~**9.2**~~
~~Application crop directed BBCH 20- 40~~	~~Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods~~	~~10.3~~	~~1.8~~	~~23.65~~	~~73.4~~
Fruit stage BBCH 71-79 currants	Frugivorous mammal "dormouse" larger fruits 100% fruit	47.9	1.8	109.87	15.8

Reprod. toxicity (mg/kg bw/d)	**NOEL: 302.9 mg H_3PO_3/kg bw ~~mg/kg bw/d~~**				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Application crop directed BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs	4.3	2.2 x 0.53	6.39	47.4
Application crop directed BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	2.2 x 0.53	32.22	9.4
Application crop directed BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	2.2 x 0.53	3.42	88.6
~~Application crop directed BBCH 10- 19~~	~~Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs~~	~~11.5~~	~~2.2 x 0.53~~	~~17.11~~	~~17.7~~
~~Application crop directed BBCH 10- 19~~	~~Small herbivorous mammal "vole Grass + cereals 100% grass~~	~~57.8~~	~~2.2 x 0.53~~	~~86.54~~	~~**3.5**~~
~~Application crop directed BBCH 10- 19~~	~~Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods~~	~~6.2~~	~~2.2 x 0.53~~	~~9.21~~	~~32.9~~
~~Application crop directed BBCH 20- 40~~	~~Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs~~	~~8.6~~	~~2.2 x 0.53~~	~~12.78~~	~~23.7~~
~~Application crop directed BBCH 20- 40~~	~~Small herbivorous mammal "vole Grass + cereals 100% grass~~	~~43.4~~	~~2.2 x 0.53~~	~~64.45~~	~~**4.7**~~
~~Application crop directed BBCH 20- 40~~	~~Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods~~	~~4.7~~	~~2.2 x 0.53~~	~~6.98~~	~~43.4~~
Fruit stage BBCH 71-79 currants	Frugivorous mammal "dormouse" larger fruits 100% fruit	22.7	2.2 x 0.53	33.66	9.0

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Conclusions

Based on the tier 1 assessments, the TER_A and the TER_{LT} values are above the trigger values of 10 and 5, respectively for pome fruits at intended BBCH 53-81. Therefore, the application of Salaman 510 in pome fruit trees presents no acute or long-term risk to mammals.

9.3.2.2 Higher-tier risk assessment

The TER_A and the TER_{LT} values are below the trigger values of 10 and 5, respectively for voles at BBCH 10-40. However, as the TER_A and the TER_{LT} values are above the trigger values when the Salaman 510 product is intended to use (BBCH 53-81), a higher-tier risk assessment is not required for this species.

9.3.2.3 Drinking water exposure

When necessary. the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

Since Salaman 510 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario is not to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

For Potassium phosphonate, the ratio of highest application rate (1275 g a.s./ha) to lowest relevant endpoint (NOAEL = 302.9 mg a.s./kg bw/d) is 4.2. As the $K_{f,OC}$ for Potassium phosphonate is 972 mL/g (EFSA Journal 2012;10(12): 2963**)**, the risk can be considered acceptable without the need for further calculations.

Effective application rate (g/ha)	=	1275		
Acute toxicity (mg/kg bw)	=	1736	quotient	= 0.73
Reprod. toxicity (mg/kg bw/d)	=	302.9	quotient	= 4.2

No unacceptable effects to mammals through drinking water are expected following application of Salaman 510 according to the proposed use pattern.

9.3.2.4 Effects of secondary poisoning

According to the EFSA guide "Risk Assessment for Birds and Mammals", *EFSA Journal 2009; 7(12):1438*, substances with a Log P_{ow} greater than 3 have potential to bioaccumulation and should be assessed for the risk of biomagnifications in terrestrial food chains.

Potassium phosphonate is an inorganic substance with a very low solubility in organic solvents and totally miscible with water. Therefore, the assessment of secondary poisoning is not necessary.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits. pellets. granules. prills or treated seed

Not relevant.

9.3.4 Overall conclusions

The TER_A and the TER_{LT} values are above the trigger values of 10 and 5, respectively for all intended uses. Therefore, the application of Salaman 510 presents no acute or long-term risk to mammals.

No unacceptable effects to mammals through drinking water are expected following application of Salaman 510 according to the proposed use pattern.

No unacceptable effects to fish-eating and earthworm-eating mammals are expected following application of Salaman 510 according to the proposed use pattern.

9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

No relevant data is presented as they are considered not necessary.

9.5 Effects on aquatic organisms (KCP 10.2)

zRMS Comments:

The acute and long-term risk for active substance for aquatic organisms based on FOCUS PECsw assessment submitted by the Applicant was accepted and corrected by zRMS, if necessary.

The risk assessment based on EXPOSIT 3 was not evaluated.

The endpoints for active substances used in the risk assessment submitted by the Applicant were agreed at the EU level (EFSA, 2012).

The endpoints for active substance were recalculated as an equivalent from formulation endpoints (Table 9.5-1). Based on recalculated endpoints it can not be excluded that the formulation Salaman 510 is more toxic than active substance (phosphonic acid).
In case of algae, the lower endpoint E_yC_{50} = 33.8 mg Salaman 510/L (equivalent to 12.8 mg H_3PO_3/L) was used in the risk assessment.
The risk assessment for recalculated endpoints is provided in Table 9.5-2A and Table 9.5-3A.

Additionally, the phosphate ions were taken into consideration. As these compounds are non-organic the DT_{50} of 1000 d in soil was used in Step 1 and 2 PECsw assessment. In this approach, the PEC/RAC is slightly over 1 if formulation is applied in October-February. The phosphates are inorganic compounds and the Step 3 assessment for PECsw is not relevant. Considering the reduction of the phosphates concentration in water it is recommended to apply a non-spray buffer strip of 3 m (as recommended for pome fruits) This should be decided at the Member State level in accordance with national requirements.

Formulation
For formulation, the risk assessment was corrected using the RAC > 380 µg/L

Crop	Application rate g product./ha	No spray buffer (m)	Max PEC_{sw} (µg/L)	PEC/RAC
Pome fruits	3625	3	158.8	< 0.42

No additional mitigation is required.

Note: Taking into account the lower formulation based endpoints for fish acute toxicity, invertebrate acute toxicity sediment dwelling prolonged toxicity, the risk was re-assessed. Member States may consider the relevant approach in accordance with their own national requirements.

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with Phosphonic acid. Full details of these studies are provided in the respective EU DAR and related documents. See endpoints in Table 9.5-1.

Studies to determine the effects on aquatic organisms of Salaman 510 are listed in Appendix 1 and summarised in Appendix 2. Please, refer to Table 9.5-1, below.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms –

phosphonic acid and Salaman 510

Species	Substance	Exposure System	Results	Reference
Acute toxicity to fish				
Oncorhynchus mykiss	Phosphonic acid	96 h. s	LC_{50}: **>118 mg H_3PO_3/L**	Phosphonic acid *EFSA Journal 2012; 10(12):2963*
Oncorhynchus mykiss	Salaman 510	96 h. s	LC_{50}: >100 mg Salaman 510 /L Phosphonic acid equivalent: LC_{50}: **>38 mg H_3PO_3/L** Mortality: 0%	
Chronic toxicity to fish				
Oncorhynchus mykiss	Salaman 510	28 d. s	NOEC > 40 mg Salaman 510 /L Phosphonic acid equivalent: **>15.2 mg H_3PO_3/L**	
Acute toxicity to aquatic invertebrates				
Daphnia magna	Phosphonic acid	48 h. s	EC_{50} (mortality, static): **>118 mg H_3PO_3/L**	Phosphonic acid EFSA Journal 2012; 10(12):2963
Daphnia magna	Salaman 510	48 h. s	EC_{50} / NOEC (mortality, static): >100 mg Salaman 510 /L Phosphonic acid equivalent: EC_{50} / NOEC (mortality): **>38 mg H_3PO_3/L**	KCP 10.2.1/02 (2012) Pupp. A. & Wydra. V.
Chronic toxicity to aquatic invertebrates				
Daphnia magna	Fosetyl-Al	21 d. s	NOEC (reproduction): 17 mg as/L. Expressed as H_3PO_3 equivalent: **11.7 mg H_3PO_3/L** (Considering a 69% of H_3PO_3 in fosetyl-Al)	KCP 10.2.2/02 (1996) Sewell. I.G.
Daphnia magna	Phosphonic acid	21 d. s	NOEC (reproduction): 100 mg H_3PO_3/L	Phosphonic acid EFSA Journal 2012; 10(12):2963 Dengler, D, (2001)
Daphnia magna	Salaman 510	21 d. s	EC_{50} (reproduction): >100 mg Salaman 510 /L NOEC (reproduction/mortality): 100 mg Salaman 510 /L Phosphonic acid equivalent: NOEC **>38 mg H_3PO_3/L**	KCP 10.2.2/03 (2014) Zawadsky, C
Toxicity to Chironomids				
Chironomus riparius	Phosphonic acid	28 d. s	NOEC **>100 mg H_3PO_3/L**	Phosphonic acid *EFSA Journal 2012; 10(12):2963*
Chironomus riparius	Salaman 510	28 d. s	NOEC >100 mg Salaman 510 /L Phosphonic acid equivalent: NOEC **>38 mg H_3PO_3/L**	KCP 10.2.2/04 (2013) Pupp. A. & Wydra. V.
Toxicity to algae				
Scenedesnus subcapitatus	Phosphonic acid	72 h. s	E_bC_{50}: 146.7 mg H_3PO_3/L	Phosphonic acid *EFSA Journal 2012; 10(12):2963*
Pseudokirchneriella subcapitata	Salaman 510	72 h. s	Growth E_rC_{50} >100 mg Salaman 510 /L Phosphonic acid equivalent: Growth E_rC_{50}: >38 mg H_3PO_3/L Yield E_yC_{50}: 33.8 mg Salaman 510/L Yield E_yC_{50}: **12.8 mg H_3PO_3/L**	KCP 10.2.1/03 (2013) Pupp. A. & Wydra. V.

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations.
* End-points used in the risk assessment are highlighted in bold.

9.5.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Studies were carried out for the Salaman 510 product until at maximum concentration of 100 mg test item/L. However, considering that effects on mortality are absent even at the highest tested level (100 mg test item/L), the agreed endpoints of the active substance were used in the risk assessment.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009". as provided by the Commission Services (SANTE-2015-00080. 15 January 2015).

The relevant global maximum FOCUS Step 1and 2 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in Table 9.5-2 and Table 9.5-3.

Potential risk of eutrophication

The inclusion directive for Potassium Phosphonates states that member states shall pay particular attention to the risk of eutrophication of surface water.

The use of potassium phosphonates leads to addition of phosphorous in the environment, high phosphorous content in surface waters causes eutrophication at moderate to higher temperatures that means exaggerate algae growth accompanied with a decline of dissolved oxygen. Therefore, an evaluation of the potential risk of eutrophication of surface water (OECD, 1982) should by performed.

It has evaluated the potential risk of eutrophication following the use of Salaman 510 (potassium phosphonates).

FOCUS models at Step 3 and Step 4 are not suitable to describe the run-off and drainage of inorganic compounds. Therefore, runoff and drainage PEC_{SW} values for the phosphate ion was calculated separately using the EXPOSIT 3 (German model), as it is considered that one of the main routes of entry phosphorous to surface water is runoff.

Based on the EXPOSIT 3 PEC_{SW} value the limit of annual average concentration of 35 µg/L is not exceed. Please refer to Table 8.9-5 of Section B8.

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}. PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given for each FOCUS scenario and each organism group.

Table 9.5-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PHOSPHONIC ACID for each organism group based on FOCUS Steps 1&2 calculations for the use of Salaman 510 in pome fruits (FOCUS scenario: pome fruits, early applns.) following multiple applications

~~Group~~		~~Fish acute~~	~~Fish prolonged~~	~~Inverteb. acute~~	~~Inverteb. prolonged~~	~~Algae~~	~~Sed. dwell. prolonged~~
~~Test species~~		~~*Oncorhynchus mykiss*~~	~~*Oncorhynchus mykiss*~~	~~*Daphnia magna*~~	~~*Daphnia magna*~~	~~*Pseudokirchn. subcapitata*~~	~~*Chironomus riparius*~~
~~Endpoint~~		~~LC_{50}~~	~~NOEC~~	~~LC_{50}~~	~~NOEC~~	~~E_rC_{50}~~	~~NOEC~~
~~(µg/L)~~		~~> 118000~~	~~> 15200~~	~~> 118000~~	~~11700~~	~~> 38000~~	~~> 100000~~
~~AF~~		~~100~~	~~10~~	~~100~~	~~10~~	~~10~~	~~10~~
~~RAC (µg/L)~~		~~> 1180~~	~~> 1520~~	~~> 1180~~	~~1170~~	~~> 3800~~	~~> 10000~~
~~FOCUS Scenario~~	~~$PEC_{gl\ max}$ (µg/L)~~						
~~Step 1~~							
	~~1460~~	~~**< 1.23**~~	~~< 0.96~~	~~**< 1.23**~~	~~**1.24**~~	~~< 0.38~~	~~< 0.14~~
~~Step 2 (N-EU)~~							
~~October-February~~	~~351.32~~	~~< 0.29~~	~~< 0.23~~	~~< 0.29~~	~~0.30~~	~~< 0.09~~	~~< 0.03~~
~~March-May~~	~~284.34~~	~~< 0.24~~	~~< 0.18~~	~~< 0.24~~	~~0.24~~	~~< 0.07~~	~~< 0.02~~
~~June-September~~	~~223.45~~	~~< 0.18~~	~~< 0.14~~	~~< 0.18~~	~~0.19~~	~~< 0.05~~	~~< 0.02~~

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

Table 9.5-3A: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PHOSPHONIC ACID for each organism group based on FOCUS Steps 1&2 calculations for the use of Salaman 510 in pome fruits (FOCUS scenario: pome fruits, early applns.) following multiple applications and using the corrected endpoints for active substance.

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_yC_{50}	NOEC
(µg/L)		> 38000	> 15200	> 38000	11700	12800	> 38000
AF		100	10	100	10	10	10

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
RAC (µg/L)		> 380	> 1520	> 380	1170	1280	> 3800
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	1460	**< 3.84**	< 0.96	**< 3.84**	**1.24**	**1.14**	< 0.38
Step 2 (N-EU)							
October-February	351.32	< 0.92	< 0.23	< 0.92	0.30	0.27	< 0.09
March-May	284.34	< 0.75	< 0.18	< 0.75	0.24	0.22	< 0.08
June-September	223.45	< 0.59	< 0.14	< 0.59	0.19	0.17	< 0.06

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PHOSPHATE ION for each organism group based on FOCUS Steps 1&2 calculations for the use of Salaman 510 in pome fruits (FOCUS scenario: pome fruits, late applns.) following multiple applications

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Chironomus riparius*
Endpoint		LC_{50}	NOEC	LC_{50}	NOEC	E_rC_{50}	NOEC
(µg/L)		> 118000	> 15200	> 118000	11700	> 38000	> 100000
AF		100	10	100	10	10	10
RAC (µg/L)		> 1180	> 1520	> 1180	1170	> 3800	> 10000
FOCUS Scenario	$PEC_{gl\ max}$ (µg/L)						
Step 1							
	1720	**< 1.4**	**< 1.13**	**< 1.4**	**1.47**	< 0.45	< 0.17
Step 2 (N-EU)							
October-February	420.97	< 0.35	< 0.27	< 0.35	0.35	< 0.11	< 0.03
March-May	339.90	< 0.24	< 0.19	< 0.24	0.24	< 0.07	< 0.02
June-September	226.20	< 0.19	< 0.14	< 0.19	<0.19	< 0.06	< 0.02

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

Table 9.5-5A: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for PHOSPHATE ION for each organism group based on FOCUS Steps 1&2 calculations for the use of Salaman 510 in pome fruits (FOCUS scenario: pome fruits, late applns.) following multiple applications

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*	*Chironomus riparius*
Endpoint		LC_{50}	NOEC	LC_{50}	NOEC	E_rC_{50}	NOEC
(µg/L)		> 38000	> 15200	> 38000	11700	12800	> 38000
AF		100	10	100	10	10	10
RAC (µg/L)		> 380	> 1520	> 380	1170	1280	> 3800

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	1720	**< 4.53**	**< 1.13**	**< 4.53**	**1.47**	**1.34**	< 0.17
Step 2 (N-EU)							
October-February	420.97	**< 1.11**	< 0.27	**< 1.11**	0.35	0.33	< 0.11
March-May	339.90	< 0.89	< 0.19	< 0.89	0.24	0.27	< 0.09
June-September	226.20	< 0.60	< 0.14	< 0.60	<0.19	0.18	< 0.06

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

9.5.3 Overall conclusions

Based on the calculated concentrations of Potassium Phosphonates in surface water (FOCUS STEP 1&2), the calculated TER values for the acute and long-term risk resulting from an exposure of aquatic organisms to Potassium Phosphonates according to the GAP of the formulation SALAMAN 510, indicate an acceptable risk for aquatic organisms due to the intended use of SALAMAN 510 in apple and pear according to the label.

The inclusion directive for Potassium Phosphonates states that member states shall pay particular attention to the risk of eutrophication of surface water.

Based on the EXPOSIT 3 calculations this limit of 35 µg/L indicate phosphorous levels in still waters (OECD, 1982) is not exceed. However, no additional entries as those according to the evaluated use pattern and good agricultural practice are acceptable.

Based on the EXPOSIT 3 PEC_{SW} value the limit of annual average concentration of 35 µg/L is not exceed. Please refer to Table 8.9-5 of Section B8.

However, additional labelling with precautionary measures is recommended:

Do not contaminate water with the product or its container [Do not clean application equipment near surface water / Avoid contamination via drains from farmyards and roads].

To protect aquatic organisms, respect an unsprayed buffer zone of 3 m to surface water bodies.

9.6 Effects on bees (KCP 10.3.1)

zRMS Comments:	The submitted risk assessment for bees was conducted in line with SANCO, 2002 and EFSA, 2013. New studies for acute and chronic toxicity were submitted and accepted. The oral and contact risk caused by formulation is acceptable (Q_{HO}, Q_{HC} values are below the trigger value of 50). The endpoints for acute oral and contact toxicity were corrected in accordance with submitted study report. The hazard quotients are below the trigger value, indicating that the formulation poses an acceptable acute and chronic risk to bees. The semi-field study with Salaman 510 was also submitted and accepted (please refer to A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees). The study results confirm that risk for bees after application of formulation Salaman 510 is acceptable. The risk assessment based on EFSA, 2013, was not evaluated as this guidance still has not been agreed. Its relevance could be decided at the Member State level.

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with phosphonic acid. Full details of these studies are provided in the EU DAR and related documents.

Effects on bees of formulation were not evaluated as part of the EU assessment of phosphonic acid. As a LDD_{50} value could not be obtained in the study Ansolini, T. (2012), a new study is provided. Moreover, as the study of effects on larvae (Ansaloni, T; 2013) was completed at D8, it does not cover the potential effects on emergence, and it is not considered sufficient to address the requirement of Regulation EU No 284/2013

on development of honeybees. Therefore, a new larvae chronic study has been also performed. Finally, a semi-field study (OECD 75) to evaluate side effects on the brood of honey bees has been also conducted. The dose rates applied (2*8.5 L formulated product/ha) in the semi-field study is higher than the application rate intended for pome fruits (3*2.5 L formulated product/ha).

New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	SALAMAN 510	Acute Oral	LD_{50} = 0.177µL f.p./bee ~~(> 100 µg Potassium phosphite/bee)~~ **(> 99.68 µg Potassium phosphite/bee)**	KCP 10.3.1.1.1/01 Ansaloni, T. (2012)
		Acute Contact	LD_{50} = 0.177µL f.p./bee ~~(> 99.68 µg Potassium phosphite/bee)~~ **(> 100 µg Potassium phosphite/bee)**	KCP 10.3.1.1.1/01 Ansaloni, T. (2012)
		Chronic Adults	LDD_{50} > 0.047µL f.p./bee/day (> 24.22 µg Potassium phosphite /bee/day) NOEDD = 0.047µL f.p./bee/day (24.22 µg Potassium phosphite /bee/day)	KCP 10.3.1.2/01 Ansaloni, T. (2016)
			LDD_{50} 168.29µf f.p./bee/day **(59.19 µg Potassium phosphite /day)**	KCP 10.3.1.2/02 Ansaloni, T. (2021)
		Chronic Larvae	D8-LD_{50} /NOED > 0.290µL f.p./larva (> 150 µg ~~Potassium phosphite~~ Phosphorous acid /larva)*	KCP 10.3.1.3/01 Ansaloni, T. (2013)
			D22-LD_{50} /NOED ≥ 545.88µL f.p./larva **(≥ 192.00 µg Potassium phosphite /larva)**	KCP 10.3.1.3/02 Ansaloni, T. (2021)
		Brood of honeybee's study OECD75	2 applic. x 8.75 L f.p./ha did not cause any treatment-related adverse effect on honey bee brood development	KCP 10.3.1.6/01 Gimeno, I. (2021)

* The study of effects on larvae is not considered sufficient to address the requirement of Regulation EU No 284/2013 on development of honeybees since the study is completed at D8 and does not cover the potential effects on emergence

9.6.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" as provided by the Commission Services (SANCO/10329/2002 rev.2 (final). October 17. 2002).

The bee risk assessment has been also conducted in line with the EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees) (EFSA Journal 2013; 11(7):3295). This Guidance is still under revision and therefore, it is not binding in the moment of the submission of this dossier.

The risk assessment presented (SANCO/10329/2002 rev.2 (final). October 17. 2002) is still in force. The EFSA guidance is still a draft, and it is not of obligatory compliance and therefore, the risk assessment

according to this draft guidance is only presented for information.

In accordance with the technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA supporting publication 2015:EN-924), for bumble bees and solitary bees, currently it cannot be recommended to routinely perform a risk assessment. As no data for bumble bees or solitary bees was available, no risk assessments for these species were performed.

To achieve a concise risk assessment, the risk envelope approach is applied.

9.6.2.1 Hazard quotients for bees

Salaman 510 is intended to use as a fungicide in pome fruit trees. The maximum recommended application dose for the intended crops is 2.5 L Salaman 510/ha (equivalent to 1.275 kg potassium phosphonates/ha).

The acute risk of Salaman 510 to honeybees was assessed according to SANCO/10329/2002 rev.2 (final), from hazard quotients between toxicity endpoints estimated from acute oral and contact studies with Salaman 510 and the maximum single application rate of 1.275 kg potassium phosphonates/ha in pome fruits. Moreover, acute and chronic risk assessment was conducted in line with the EFSA Guidance Document for all intended crops.

Table 9.6-2: First-tier assessment of the risk for bees due to the use of Salaman 510 in pome fruit trees (SANCO/10329/2002 rev.2 (final). October 17. 2002).

Intended use	Pome fruits		
Active substance	Potassium phosphonates		
Application rate (g/ha)	3 × 1275 g a.s./ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}. Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 100	1275	< 12.75
Contact toxicity	> 99.68		< 12.79
Product	Salaman 510		
Application rate (g/ha)	3 × 2.5 L f.p./ha		
Test design	**LD_{50} (lab.) (µL/bee)**	**Single application rate (L/ha)**	**Q_{HO}. Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 0.177	2.5	< 14.12
Contact toxicity	> 0.177		< 14.12

Q_{HO}. Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

According to the "Guidance Document on Terrestrial Ecotoxicology" as provided by the Commission Services (SANCO/10329/2002 rev.2 (final). October 17. 2002), the HQ values based on the acute oral/contact LD_{50} are below the trigger of 50, showing an acceptable risk to bees after the application of Salaman 510.

Risk assessment with this endpoint and acute endpoints presented above, is performed according to the EFSA Bee Guidance Document (EFSA, 2013).

Table 9.6-3: Screening assessment of the acute contact risk for bees (EFSA Guidance Document)

Crop scenario	Application rate (g a.s./ha)	Toxicity LD_{50} contact (µg a.s./bee)	Hazard Quotient (HQ)	Trigger value
Pome fruits	1275	>100	<12.75	> 42

HQ values in **bold** breach the relevant trigger.

Table 9.6-4: Screening assessment of the acute oral risk for bees (EFSA Guidance Document)

Crop scenario	Application rate (g a.s./ha)	Toxicity LD_{50} oral (µg a.s./bee)	Calculation factor (Ef*SV)	Exposure Toxicity Ratio (ETR)	Trigger value
Pome fruits	1275	> 99.68	7.6	0.10	< 0.2

ETR values in **bold** breach the relevant trigger.

Table 9.6-5: Screening assessment of the chronic risk for adult bees (EFSA Guidance Document)

Crop scenario	Application rate (g a.s./ha)	Toxicity LDD_{50} (µg a.s./bee/day)	Calculation factor (Ef*SV)	Exposure Toxicity Ratio (ETR)	Trigger value
Pome fruits	1275	59.19	7.6	**0.164**	< 0.03

ETR values in **bold** breach the relevant trigger.

Table 9.6-6: Screening assessment of the chronic risk for bee larvae (EFSA Guidance Document)

Crop scenario	Application rate (g a.s./ha)	Toxicity $NOED_{oral}$ (µg a.s./bee)	Calculation factor (Ef*SV)	Exposure Toxicity Ratio (ETR)	Trigger value
Pome fruits	1275	≥ 192	4.4	0.03	< 0.2

ETR values in **bold** breach the relevant trigger

The screening assessments of the risks to bees from the use of potassium phosphonates indicates that:

1.- Acceptable acute (oral and contact) risk for bees when the product is applied on the intended use (pome fruit trees).

2.- There may be unacceptable chronic risks for adult bees.

3.- Acceptable chronic risk for larvae bees when the product is applied in the intended use (pome fruit trees).

Therefore, refinement of the assessment in a 1st tier assessment is presented in the tables below for adult bees.

First tier risk assessment

When concern has been raised regarding the potential risk to bees from the consumption of pollen and nectar in the screening assessment, the initial step of the Tier I risk assessment is to refine the exposure estimate used in the above calculations. In order to do this, it is necessary to consider all the appropriate routes of exposure:

- risk from foraging on the treated crop
- risk from foraging on weeds in the treated field
- risk from foraging in the field margin
- risk from foraging on an adjacent crop
- risk from foraging the following year on the same crop

Each route of exposure is considered in turn below.

Tier I assessment for oral route of exposure – foraging on the treated crop

Table 9.6-7: First tier assessment for oral route of exposure – foraging on treated crops

Category	Scenario	BBCH	SV	Honeybee	
				ETR	trigger
Application rate: 1275 g a.s./ha					
chronic	treated crop	40 - 69	8.2	**0.127**	0.03
chronic	treated crop	≥ 70	0	<0.03	0.03

The ETR oral values for adult honeybee are greater than the trigger value at BBCH 40-69 indicating an unacceptable risk to bees foraging this treated crop at this stage.

Tier I assessment for oral route of exposure – foraging on weeds in the treated field

Table 9.6-8: First tier assessment for oral route of exposure – foraging on weeds (already emerged) in the treated field

Category	Scenario	BBCH	Ef	SV	Honeybee	
					ETR	trigger
Application rate: 1275 g a.s./ha						
chronic	weeds	40 - 69	0.3	2.9	0.013	0.03
chronic	weeds	≥ 70	0.3	2.9	0.013	0.03

The ETR oral values for adults are below the trigger value indicating an acceptable risk to bees foraging the flowering weeds at this stage.

Tier I assessment for oral route of exposure – foraging in the field margin

Table 9.6-9: First tier assessment for oral route of exposure - foraging in the field margin

Category	Scenario	BBCH	Ef	SV	TWA	Honeybee	
						ETR	trigger
Application rate: 1275 g a.s./ha							
chronic	field margin	40 - 69	0.097	2.9	0.72	0.004	0.03
chronic	field margin	≥ 70	0.097	2.9	0.72	0.004	0.03

The ETR oral values for adults are lower than the trigger value indicating an acceptable risk to bees foraging in the field margin.

Tier I assessment for oral route of exposure – foraging on the adjacent crop

Table 9.6-10: First tier assessment for oral route of exposure - foraging on the adjacent crop

Category	Scenario	BBCH	Ef	SV	TWA	Honeybee	
						ETR	trigger
Application rate: 1275 g a.s./ha							
chronic	adjacent crop	40 - 69	0.066	5.8	0.72	0.006	0.03
chronic	adjacent crop	≥ 70	0.066	5.8	0.72	0.006	0.03

The ETR oral values for adults are lower than the trigger value indicating an acceptable risk to bees foraging in flowering in adjacent crops.

Tier I assessment for oral route of exposure – foraging the following year on the same crop

Table 9.6-11: First tier assessment for oral route of exposure - foraging the following year on the same crop

Category	Scenario	BBCH	Ef	SV	TWA	Honeybee	
						ETR	trigger
Application rate: 1275 g a.s./ha							
chronic	next crop	40 - 69	1	0.54	0.72	0.008	0.03
chronic	next crop	≥ 70	1	0.54	0.72	0.008	0.03

The ETR oral values for adults are lower than the trigger value indicating an acceptable risk to bees foraging the following year on the same crop.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test. field studies)

A Semi-Field Study (S21-00857) to Evaluate Side Effects on the Brood of Honeybees (*Apis mellifera* L.) in Rapeseed (Brassica napus L.) following the OECD guidance document No. 75, (2007) and partial integration of recommendations by EFSA (2013) is presented.

Salaman 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid) was applied in two applications, at a target rate corresponding to 8.75 L formulated product/ha each, one 25 days before full-flowering and the second one at full-flowering (BBCH 64-65) in Rapeseed (*Brasica napus* L.) during daily honeybee foraging activity.

The effects on honeybee colonies under confined (semi-field) conditions considering mortality, flight intensity, behavior, colony strength, amount of brood and brood cell development were evaluated.

No test item (T) related adverse effects on adult honeybee mortality compared to the control group (C) resulted in the mean mortality during the confinement period from 0DAA2 to 7DAA2. On the other hand, T showed significantly differences on adult honeybee mean mortality compared to the control group (C) during the post-application period from 0DAA2 to 28DAA2. However, mean mortality data in this period was under the natural colony loss and did not impact on the other parameters evaluated.

No test item related adverse effects on mortality of larvae and pupae were observed in Test Item (T).

No reduction in foraging activity was seen in Test Item (T) throughout the study.

No unusual behaviour was observed in Test Item (T).

The quantitative assessment of brood development in individually marked cells performed in this study revealed that Salaman 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid) at a target rate of 8.75 L formulated product/ha and after two applications did not cause any treatment-related adverse effect on honeybee brood development at the end of the observation period (BFD22).

On the other hand, brood development in individually marked cells of the reference item group showed a statistically significant differences for both brood and compensation indices as well as the brood termination rate compared to the control group in all of the assessment performed. Moreover, statistically significant effect in the mean number of dead larvae and pupae resulted when compared the reference item to the control group in the monitoring period from 8DAA2 to 28DAA2. Therefore, sensitivity of the test was proven.

No test item related adverse effects on colony strength or on the development of the brood and food storage area were observed in T.

Analytical verification confirmed that the honeybees were adequately exposed to the test item.

9.6.3 Effects on bumble bees

No data/information available.

9.6.4 Effects on solitary bees

No data/information available.

9.6.5 Overall conclusions

Based on the risk assessment for honeybees according to SANCO/10329/2002 and EFSA bee GD, an acceptable risk to bees can be concluded.

A Semi-Field Study (S21-00857) to Evaluate Side Effects on the Brood of Honeybees (*Apis mellifera* L.) following the OECD guidance document No. 75, (2007) has been performed. No test item (T) related adverse effects on adult honeybee mortality compared to the control group (C) resulted during the confinement period from 0DAA2 to 7DAA2. On the other hand, Test item (T) showed significantly differences on adult honeybee mortality compared to the control group (C) during the post-application period from 0DAA2 to 28DAA2. However, mean mortality data in this period was under the natural colony loss and did not impact on the other parameters evaluated.

Moreover, the quantitative assessment of brood development in individually marked cells performed in the study revealed that Salaman 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid) at a target rate of 8.75 L formulated product/ha and after two applications did not cause any treatment-related adverse effect on honeybee brood development at the end of the observation period (BFD22).

9.7 Effects on arthropods other than bees (KCP 10.3.2)

zRMS Comments:	The submitted risk assessment based on the "Guidance Document on Terrestrial Ecotoxicology" (2002) and ESCORT 2 (2001) was accepted. New studies for formulation were submitted. The laboratory study 2D and 3D and field study were evaluated and accepted for the risk assessment. **In field risk**. The hazard quotients are below the trigger value (HQ ≤ 1) for all species. **Off-field risk**. The hazard quotients are below the trigger value (HQ ≤ 1) for all species. The HQ value is below the trigger of 1, indicating that the active substance and formulation pose an acceptable risk to arthropods other than bees. The risk to arthropods other than bees is acceptable if the Salaman 510 is applied in accordance with proposed intended uses.

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with potassium phosphonates. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of formulation were not evaluated as part of the EU assessment of potassium phosphonates. These studies are listed in Table 9.7-1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Chrysoperla carnea	Potassium phosphonate	Laboratory test	5.75 L FP/ha = Mortality: ~~3~~ 6.7% Fertility (mean hatching rate): 96.52%. 20.1250 L FP/ha = Mortality: ~~28~~ 40% Fertility (mean number of eggs per female and day): 7.33 eggs per female, below the trigger value of 15 eggs per female. No effects were observed on fertility with 100% as hatching rate.	KCP 10.3.2/01 Luna (2013)
Typhlodromus pyri		Extended laboratory test	LR_{50} >24 L SALAMAN/ha equivalent to > **13538 g H_3PO_3/ha**	KCP 10.3.2/02 Luna (2013)
Aphidius rhopalosiphi			LR_{50} >24 L SALAMAN/ha equivalent to > **13538 g H_3PO_3/ha**	KCP 10.3.2/03 Luna (2013)
Chrysoperla carnea			LR_{50} >11352 g H_3PO_3/ha	KCP 10.3.2/05 Luna (2013)

Species	Substance	Exposure System	Results	Reference
Euseius stipulatus		Field test	Citrus. 3 appl. (11351 g/ha) No effects.	KCP 10.3.2/04 Luna (2013)

9.7.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final). October 17. 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of Salaman 510 in pome fruits

Intended use	Pome fruits		
Active substance/product	Potassium phosphonates / Salaman 510		
Application rate (g a.s./ha)	3 × 1275 g a.s./ha		
MAF	2.3		
Test species Tier I Tier II	**LR_{50} (lab.) (g a.s./ha)**	**$PER_{in\text{-}field}$ (g a.s./ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2 (Tier I) criterion: HQ ≤ 1 (Tier II)**
Typhlodromus pyri (Tier II)	>13538	2932.5	<0.21
Aphidius rhopalosiphi (Tier II)	>13538		<0.21
Chrysoperla carnea (Tier I)	>11352		<0.25

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available. it should be considered in place of the rate with ≤ 50 % effect.

The in-field HQ values for exposure to maximum residues on leaves for the *T. pyri, A. rhopalosiphi* and *Chrysoperla carnea* are below the trigger value of 1, indicating that Salaman 510 does not pose an unacceptable risk to non-target arthropods in in-field areas.

A field study was performed to assess the effects of the crop protection product "SALAMAN 510" (Potassium phosphite 510 g/L, SL) on the predatory mite *E. stipulatus* population density in citrus orchards. After three applications of "SALAMAN 510" (Potassium phosphite 510 g/L, SL) applied at the rate of 8.75 L/ha with an interval of 20 days, are not expected a reduction in phytoseiid mite populations.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of Salaman 510 in pome fruits

Intended use	Pome fruits				
Active substance/product	Potassium phosphonates / Salaman 510				
Application rate (g/ha)	3 x 1275 g a.s./ha				
MAF	2.3				
vdf	10 (Tier I) / 1 (Tier II)				
Test species Tier I	**LR_{50} (lab.) (g a.s./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2 (Tier I) criterion: HQ ≤ 1 (Tier II)**
Typhlodromus pyri (Tier II)	>13538	23.96 % (Early applns.)	702.62	5	<0.26
Aphidius rhopalosiphi (Tier II)	>13538				<0.26
Chrysoperla carnea (Tier I)	>11352			10	<0.61

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available. it should be considered in place of the rate with ≤ 50 % effect.

The off-field HQ values for *T. pyri*, *A. rhopalosiphi* and *Chrysoperla carnea* fall below the trigger value of 1, indicating that Salaman 510 does not pose an unacceptable risk to non-target arthropods in off-field areas.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The risk to non-target arthropods is assessed using the approach recommended in the published *ESCORT 2 document (*Candolfi *et al.* 2001)[1] and the *EC Guidance Document on Terrestrial Ecotoxicology*.

The in-field and off-field HQ values fall below the trigger value for *Typhlodromus pyri*, *Aphidius rhopalosiphi* and *Chrysoperla carnea*, indicating that Salaman 510 does not pose an unacceptable risk to non-target arthropods in in-field and off-field areas.

A reduction in phytoseiid mite populations is not expected after three applications of "SALAMAN 510" (Potassium phosphite 510 g/L, SL) applied at the rate of 8.75 L/ha with an interval of 20 days.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

zRMS Comments:	New studies were submitted and accepted. The PECs accum value for phosphonic acid was used (the highest value for 5 cm tillage

[1] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' from the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

	depth as a worse case) for acute and long-term risk assessment (see Section 8. Fate and behavior). The use of higher PECs value does not affect the final conclusion. The risk assessment for formulation was added by evaluator. The risk is acceptable as the TER_A and TER_{LT} values for active substance are above the trigger value of 10 and 5, respectively. An acceptable risk to non-target soil organisms meso- and macrofauna is expected if the application of the Salaman 510 is in accordance with proposed use pattern.

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with Phosphonic acid. See endpoints in Table 9.8-1.

Studies to determine the effects on earthworms and other non-target soil organisms (meso- and macrofauna) of Salaman 510 are listed in Appendix 1 and summarised in Appendix 2 (only for information). Please, refer to Table 9.8-1, below.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Potassium phosphonate	Acute	LC_{50} > **1000 mg/kg soil dw**	*EFSA Journal 2012;10(12):2963*
Eisenia fetida		Mixed into substrate 56 d. chronic 10 % peat content	NOEC: 315.2 mg test item/kg dw soil (**119.8 mg H_3PO_3 a.s./kg soil dw)**	KCP 10.4.1.1/01 Ansaloni, T. (2012)
Folsomia candida		Mixed into substrate 29 d. chronic 5 % peat content	LC_{50}: >1000 mg test item/kg dry soil (**384.36 mg phosphorous/kg soil dw)** NOEC: 555.56 mg test item/kg dry soil (**213.53 mg phosphorous/kg soil dw)**	KCP 10.4.2.1/01 Luna, F. (2015)
Hypoaspis aculeifer		Mixed into substrate 19 d. chronic 5 % peat content	NOEC = 1000 mg test item/kg dry soil (**384.36 mg phosphorous/kg soil dw)**	KCP 10.4.2.1/02 Ansaloni, T. (2016)

9.8.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final). October 17. 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2. and Table 8.7-3. According to the assessment of environmental-fate data. multi-annual accumulation in soil is considered for potassium phosphonates (as phosphorous acid).

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of Salaman 510 in pome fruits, without interception (0%), following multiple applications and 5 cm tillage depth (worst case)

Intended use	Pome fruits		
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg a.s./kg soil dw)**	**$PEC_{soil\ accumulation}$ (mg a.s/kg soil dw)**	**TER_a (criterion TER ≥ 10)**
H_3PO_3	> 1000	~~5.486~~ 6.912	~~>182.28~~ > 144.7
Chronic effects on earthworms			
Product/active substance	**NOEC (mg a.s./kg soil dw)**	**$PEC_{soil\ accumulation}$ (mg a.s/kg soil dw)**	**TER_{lt} (criterion TER ≥ 5)**
H_3PO_3	119.80	~~5.486~~ 6.912	~~21.83~~ 17.3
Salaman 510	315.2	4.833	65.2
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg a.s./kg soil dw)**	**$PEC_{soil\ accumulation}$ (mg a.s/kg soil dw)**	**TER_{lt} (criterion TER ≥ 5)**
H_3PO_3 (*Folsomia candida*)	213.53	~~5.486~~ 6.912	~~38.92~~ 30.9
Salaman 510	555.6	4.833	115
H_3PO_3 (*Hypoapsis aculeifer*)	384.36	~~5.486~~ 6.912	~~70.06~~ 55.6
Salaman 510	1000	4.833	207

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to potassium phosphonates (as phosphorous acid) are greater than the trigger of 5, indicating that the risk to earthworms and other non-target soil organisms (meso- and macrofauna) is acceptable following use of Salaman 510 according to the proposed use pattern.

9.9 Effects on soil microbial activity (KCP 10.5)

zRMS Comments:	The submitted risk assessment considering the active substance for soil microorganisms was accepted. The used endpoints were agreed at the EU level. The risk assessment for formulation was added by the evaluator. The risk for soil microorganisms is acceptable.

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with Phosphonic acid. See endpoints in Table 9.9-1.

Studies to determine de effects on soil microorganisms of Salaman 510 are listed in Appendix 1 and summarised in Appendix 2. Please, refer to Table 9.9-1, below.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N - mineralisation	Potassium phosphonates	28 d, aerobic soil type	Deviation < 25% of nitrogen turnover and rate of nitrate transformation after 28 days at 7.87 and 78.67 mg LBG-0134F /kg soil (2.70 and **26.99** mg phosphonic acid equivalent/kg soil)	*EFSA Journal 2012;10(12):2963*
C - mineralisation	Potassium phosphonates	14 d, aerobic soil type	Deviation < 25% of respiration rate after 28 days at 7.87 and 78.67 mg LBG-0134F /kg soil (2.70 and **26.99** mg phosphonic acid equivalent/kg soil)	*EFSA Journal 2012;10(12):2963*
N/C - transformation test	Salaman 510 (510 g phosphorus acid/L)	28 d, aerobic soil type	Deviation < 25% of respiration activity, soil nitrate content and soil nitrate formation rate of soil microflora when applied up to 142.11 mg Salaman 510 (510 g/L Phosphorus acid)/kg soil dry weight.	KCP 10.5/01 Hammesfahr, U. (2013)

9.9.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final). October 17. 2002).

The relevant PEC_{SOIL} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate). Chapter 8.7.2. and Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of Salaman 510 in pome fruits, without interception (0%), following multiple applications (worst case)

Intended use	Pome fruits		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg a.s./kg soil dw)**	**Initial PEC_{soil} (mg a.s./kg soil dw)**	**Risk acceptable?**
H_3PO_3	26.99 (at 28 d)	5.011 and 6.912	yes
SALAMAN 510 (recalculated for active substance)	54.6 (at 28 d)	4.833	yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**Initial PEC_{soil} (mg a.s./kg soil dw)**	**Risk acceptable?**
H_3PO_3	26.99 (at 28 d)	5.011 and 6.912	Yes
SALAMAN 510 (recalculated for active substance)	54.6 (at 28 d)	4.833	yes

9.9.3 Overall conclusions

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The risk of phosphorous acid to soil micro-organisms was evaluated by comparison of no-effect concentrations, derived from laboratory tests, with PEC_{SOIL}.

Effects at expected soil concentrations for proposed uses of phosphorous acid are below the triggers of 25%, indicating that the risk to soil micro-organisms is acceptable following use of Salaman 510 according to the proposed use pattern.

9.10 Effects on non-target terrestrial plants (KCP 10.6)

zRMS Comments:	The submitted justification for non-target terrestrial plants was accepted. As the application rate of max 2.50 L formulation/ha is much lower than ER_{50} for vegetative vigour and seedling emergence (23.63 L formulation/ha), the risk for non-target terrestrial plants is acceptable.

9.10.1 Toxicity data

Studies to determine the effects on non-target terrestrial plants of Salaman 510 are listed in Appendix 1 and summarised in Appendix 2. Please, refer to Table 9.10-1, below.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
All species tested: *Solanum lycopersicon, Cucumis sativus, Lactuca sativa, Pisum sativum, Brassica oleracea, Daucus carota, Brassica napus, Lolium perenne, Zea mays* and *Allium cepa*	Salaman 510	21 d Vegetative vigour	ER_{50} vegetative vigour >**23.63 L/ha**	KCP 10.6.2/01 (2013) Gimeno
		21 Seedling emergence	ER_{50} emergence: >**23.63 L/ha**	KCP 10.6.2/02 (2013) Gimeno

9.10.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

To achieve a concise risk assessment, the risk envelope approach is applied.

Limit tests at rates up to 23.63 L/ha were conducted with formulation and effects were below the critical threshold as defined by the "Guidance Document on Terrestrial Ecotoxicology". (SANCO/10329/2002 rev.2 final. 2002). The limit test rates exceed the highest field application rate in intended uses and are thus considered an indicator for an acceptable risk.

9.10.2.2 Tier-2 risk assessment (based on dose-response data)

Not relevant.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

It can therefore be concluded that the proposed use of Salaman 510 poses no unacceptable risk to non-target plants.

9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

There are no other relevant data for the active substance or product on organisms in the environment generated from monitoring schemes.

9.13 Classification and Labelling

Based on the available aquatic toxicity data for the active substance and the formulated product, a classification is not required.

SP1	Do not contaminate water with the product or its container. Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads.
SPe3	To protect aquatic organisms, respect an unsprayed buffer zone of 3 m to surface water bodies.

The proposed classifications for SALAMAN 510 according to Regulation 1272/2008 are given below:

Hazard Symbol: No

Indication of danger: No

Risk Phrases: No

Safety Phrases:

P501	Dispose of contents/container according to national regulations

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	…	2012a	Acute Toxicity of Salaman 510 (510 g/L phosphorous acid) to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-hour Static Limit test … GLP Unpublished	Y	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.2.1/02	Pupp, A. and Wydra V.	2012b	Acute Toxicity of Salaman 510 (510 g/L phosphorous acid) to *Daphnia magna* in a Static 48-hour Immobilization Limit test IBACON Final Report Nr.- 65672220 GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.2.1/03	Pupp, A. and Wydra V.	2013a	Toxicity of Salaman 510 (510 g/L phosphorus acid) to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test IBACON Final Report Nr.- 65671210 GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.2.2/01	[redacted]	2013b	Toxicity of Salaman 510 (510 g/L phosphorous acid) to Rainbow Trout (*Oncorhynchus mykiss*) in a Prolonged Semi Static Test over 28 Days … GLP Unpublished	Y	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.2.2/03	Zawadsky	2014	Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Reproduction Test) EUROFINS Final Report Nr.- S14-00233 GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.2/04	Pupp, A. and Wydra V.	2013c	Effects of Salaman 510 (510 g/L phosphorous acid) on the Development of Sediment Dwelling Larvae of *Chironomus riparius* in a Sediment-Water System – exposed via spiked Water IBACON Final Report Nr.- 65676250 GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.1.1.1/01	Ansaloni, T.	2012	Acute oral and contact toxicity of "SALAMAN 510" (Potassium phosphite 510g/L, as Phosphorous acid) on honeybees (*Apis mellifera* L.) TRIALCAMP Study Nr.- TRC12-018BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.1.2/01	Ansaloni. T.	2016	Chronic toxicity of "SALAMAN 510" (Potassium phosphite 510 g/L. as phosphorous acid) on honeybees (*Apis mellifera* L.) Trial report no.: TRC16-088BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.1.2/02	Ansaloni. T.	2021	SALAMAN 510: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee (*Apis mellifera* L.) under Laboratory Conditions). Trial report no.: S20-08782 Trialcamp S.L.U. GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.1.3/01	Ansaloni. T.	2016	Toxicity of "SALAMAN 510" (Potassium phosphite 510g/L. as Phosphorous acid). SL on honeybee larvae (*Apis mellifera* L.) after repeated exposure under laboratory conditions Trial report no.: TRC16-202BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.1.3/02	Ansaloni. T.	2021	SALAMAN 510: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions. Trial report no.: S20-08783 TrialCamp S.L.U. GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.6/01	Gimeno, I	2021	SALAMAN 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid): A Semi-Field Study to Evaluate Side Effects on the Brood of Honey Bees (*Apis mellifera* L.) in Rapeseed (*Brassica napus* L.) in Spain in 2021. Trial report no.: S21-00857 TrialCamp S.L.U. GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.2/01	Luna, F.	2013a	Side-effects of the product "SALAMAN 510" (Potassium phosphite 510 g/L, as Phosphorous acid) on *Chrysoperla carnea* (Neuroptera: Chrysopidae) under laboratory conditions**.** TRIALCAMP Study Nr.- TRC12-286BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.2/02	Luna, F.	2013c	An extended laboratory test to determine the LR_{50} of the product "SALAMAN 510" (Potassium phosphite 510g/L, as Phosphorous acid) on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) TRIALCAMP Study Nr.- TRC12-015BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.2/03	Luna, F.	2013b	An extended laboratory test to determine the LR_{50} of the product "SALAMAN 510" (Potassium phosphite 510 g/L, as Phosphorous acid) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) TRIALCAMP Study Nr.- TRC12-014BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.2/04	Luna, F.	2013e	Side-effects of the formulated product "SALAMAN 510" (Potassium phosphite 510 g/L, SL) on the predatory mite, *Euseius stipulatus* (Athias-Henriot) (Acari: Phytoseiidae) in citrus under field conditions. TRIALCAMP Study Nr.- TRC12-283BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.3.2/05	Luna, F.	2013d	Side-effects of the product "SALAMAN 510" (Potassium phosphite 510g/L, as Phosphorous acid) on *Chrysoperla carnea* (Neuroptera: Chrysopidae) under extended laboratory conditions. TRIALCAMP Study Nr.- TRC12-156BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.1/01	Ansaloni, T.	2012	A laboratory test to determine the chronic (sub-lethal) effects of "SALAMAN 510" (Potassium phosphite 510g/L, as Phosphorous acid) to the earthworm *Eisenia foetida* (Oligochaeta: Lumbricidae) TRIALCAMP Study Nr.- TRC 11-295BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.4.2.1/01	Luna. F.	2015	Effects of the formulation "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) on the non-target soil arthropod. *Folsomia candida* (Collembola. Isotomidae) Trial report no.: TRC13-297BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.4.2.1/02	Ansaloni. T.	2016	Side-effects of "SALAMAN 510" (POTASSIUM PHOSPHITE 510G/L. AS PHOSPHOROUS ACID) on the predatory mite. *Hypoaspis* (*Geolaelaps*) aculeifer Canestrini (Acari: Laelapidae) under laboratory conditions. Trial report no.: TRC13-298BA GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.5/01	Hammesfahr, U.	2013	Effects of SALAMAN 510 (510 g/l Phosphorus acid) on the Activity of the Soil Microflora in the Laboratory IBACON Project Nr.- 65677080 GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.6.2/01	Gimeno C.	2013a	Effects of the formulated product "SALAMAN 510" (Potassium phosphite 510 g/L SL) on Vegetative Vigour of terrestrial non-target plants TRIALCAMP Study Nr.- TRC12-012BP GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 10.6.2/02	Gimeno C.	2013b	Effects of the formulated product "SALAMAN 510" (Potassium phosphite 510 g/L SL) on Seedling Emergence and Seedling growth TRIALCAMP Study Nr.- TRC12-011BP GLP Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.2/02	Sewell I.G.	1996	Fosetyl Al: *Daphnia magna* reproduction test Report Nr. 282/480 (R014229) GLP, Unpublished	N	-

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates or effects on aquatic algae and macrophytes

zRMS Comments:	The submitted study was accepted. The validity criteria were met: • no died fish were observed in the control up to end of the study; • the dissolved oxygen concentration in the test media did not fall below 90 % of air saturation value during the test. • No deviations were noted.

Table 1. Observed Mortality of unfed Rainbow Trout (*Oncorhynchus mykiss*) exposed to Salaman 510 (510 g/L phosphorous acid) for 96 hours

	Mortality					
Nominal concentration [mg test item/L]	Exposure Time [h]					
	0	2	24	48	72	96
	# mort	# mort	# mort	# mort	# mort	# mort
Control	0	0	0	0	0	0
100	0	0	0	0	0	0
LC_{50} [mg/L]	-	> 100	> 100	> 100	> 100	> 100
95% C.I.	-	n.d.	n.d.	n.d.	n.d.	n.d.

mort: Number of dead fish
n.d.: not determinable
CI.: Confidence interval
Values refer to nominal test concentrations.

Based on the test results the 96-hour LC_{50} of Salaman 510 for rainbow trout (*Oncorhynchus mykiss*) was determined to be higher than 100 mg test item/L based on nominal concentrations (equivalent to 38 mg H_3PO_3/L);

Report:	KCP 10.2.1/01
Authors (year)	
Title:	Acute Toxicity of SALAMAN 510 (510 g/L phosphorous acid) to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-hour Static Limit test
Document No:	IBACON Study report
Guidelines:	Commission Regulation (EC) No 440/2008. Annex. Part C. C.1.: "Acute Toxicity for Fish". Official Journal of the European Union. May 30. 2008
GLP:	Yes
Deviations	--

Material and Methods:

Test Item

SALAMAN 510 (510 g/L phosphorus acid); batch no.: 113 2015; purity: 564.1 g/L (analytical); 510 g/L (nominal).

Test Species

Juvenile rainbow trout (*Oncorhynchus mykiss)* mean length: 5.5 cm ± 0.2 cm; source: Forellenzuchtbetrieb Störk, 88348 Bad Saulgau, Germany.

Test Design

This study encompassed two treatment groups (one test item concentration at nominal 100 mg/L and one control) each containing 7 individuals. The acute toxicity to unfed juvenile rainbow trout was determined in an aerated. static. 96-hour test. The test fish were observed after approximately 2. 24. 48. 72- and 96-hours' test duration for sublethal effects and mortality. The samples collected at start and after 96 hours were analysed *via* ion chromatography with conductivity detection (IC-CD).

Endpoints

NOEC after 96 h. LOEC after 96 h; LC50: lethal concentration producing 50 % mortality after 96 h of exposure.

Test Concentration

100 mg test item/L and a control.

Test Conditions

Water temperature: 13°C; pH value: 7.8 to 8.0; dissolved oxygen concentration: 90 to 100% of the air saturation value; photoperiod: 16 h light - 8 h dark; light intensity: 750 to 1150 lux and thus were within the ranges requested by guideline OECD 203.

Results

Biological test results

In the control and the only test concentration of nominal 100 mg test item/L. all fish survived until the end of the experiment and showed no sublethal effects during the exposure time.

All biological results are listed in Table 10.2.1-11.

Table 10.2.1-1 Observed Mortality of unfed Rainbow Trout (*Oncorhynchus mykiss*) exposed to SALAMAN 510 (510 g/L phosphorous acid) for 96 hours

Mortality						
Nominal concentration [mg test item/L]	**Exposure time [h]**					
	0	**2**	**24**	**48**	**72**	**96**
	# mort	**# mort**	**# mort**	**# mort**	**# mort**	**# mort**
Control	0	0	0	0	0	0
100	0	0	0	0	0	0
LC50 [mg/L]	-	>100	>100	>100	>100	>100
95% C.I.	-	n.d.	n.d.	n.d.	n.d.	n.d.

Analytical results

The quantification of the test item SALAMAN 510 (510 g/L phosphorous acid) was performed using ion chromatography with conductivity detection (IC-CD).

At the start of the test just before introduction of the fish 101 % of the nominal test concentrations were found. After 96 hours' test duration 99 % of the nominal values were determined. Thus. during the test period of 96 hours the fish were exposed to a mean of 100 % of nominal. Therefore. all reported results are related to nominal concentrations of the test item.

Conclusion

Based on the test results the 96-hour LC_{50} of SALAMAN 510 (510 g/L phosphorous acid) for rainbow trout (*Oncorhynchus mykiss*) was determined to be higher than 100 mg test item/L based on nominal concentrations. Also. the 96-hour LC_{100} was determined to be higher than 100 mg test item/L and the LC_0 was determined to be at least 100 mg test item/L. both values also based on nominal concentrations.

LC_{50} – 96 h > 100 mg/L (> 38 mg H_3PO_3/L)

* * * * *

zRMS Comments:	The submitted study was accepted. The validity criteria were met: • no daphnid showed signs of disease or stress; no immobilization was observed; • the dissolved oxygen concentration was ≥ 8.2 mg O_2/L in the control and test vessels at the end of the test. No deviations were noted.

Table 3. Influence of Salaman 510 (510 g/L phosphorous acid) on the Mobility of *Daphnia magna*

Nominal Concentration [mg test item/L]	No. of daphnids tested	No. of immobilised daphnids after 24 h	No. of immobilised daphnids after 48 h	% of immobilised daphnids after 24 h	% of immobilised daphnids after 48 h
Control	20	0	0	0	0
100	20	0	0	0	0

The following endpoints were derived:

- EC_{50} > 100 mg Salaman 510/L (equivalent to 38 mg H_3PO_3/L);
- NOEC > 100 mg Salaman 510/L (equivalent to 38 mg H_3PO_3/L).

Report:	KCP 10.2.1/02
Authors (year)	Pupp. A. and Wydra V. (2012)b
Title:	Acute Toxicity of SALAMAN 510 (510 g/L phosphorous acid) to *Daphnia magna* in a Static 48-hour Immobilization Limit test
Document No:	IBACON Study report Nº 65672220
Guidelines:	Commission Regulation (EC) No 440/2008. Annex. Part C. C.2.: "*Daphnia* sp. Acute Immobilisation Test". Official Journal of the European Union (EN). dated May 30. 2008
GLP:	Yes
Deviations	--

Methods

Young daphnids (< 24 hours old) were exposed in a static test for 48 hours to test water containing the test item at the concentration of nominal 100 mg test item/L. This limit test was performed in compliance with the test guidelines to demonstrate that the test item has no toxic effect on daphnids up to at least this concentration.

The test method of application and the test species *Daphnia magna* are recommended by the test guidelines.

The purpose of the analytical part of this study was to verify the concentration of the test item in the test water.

Results

After 48 hours of exposure no immobilization of the test animals was observed in the control and the only test concentration of nominal 100 mg test item/L.

Table 10.2.1-2: Summary of biological results

Nominal concentration [mg test item/L]	**% of immobilized daphnids after**	
	24 hours	**48 hours**
Control	0	0
100	0	0
EC_{50} [mg/L]	>100	>100
95% CI [mg/L]	n.d.	n.d.
NOEC [mg/L]	≥100	≥100
LOEC [mg/L]	>100	>100

The quantification of the test item SALAMAN 510 (510 g/L phosphorous acid) was performed using Ion Chromatography with Conductivity Cell (IC-CD).

At the start of the test 102 % of the nominal test concentration was found. Therefore, correct dosing could be demonstrated. After 48 hours' test duration. 102 % of the nominal value was. During the test the daphnids were exposed to a mean of 102 % of nominal. Therefore. all reported results refer to nominal concentrations.

Conclusion

The toxic effect of the test item SALAMAN 510 (510 g/L phosphorous acid) to *Daphnia magna* was assessed in a static limit-test. The 48-hour EC_{50} value could not be calculated due to the absence of toxicity and was therefore determined to be > 100 mg test item/L. The 48-hour NOEC was determined to be ≥ 100 mg test item/L and the 48-hour LOEC was determined to be > 100 mg test item/L.

EC_{50} – 48 h > 100 mg/L (nominal concentration) (>38 mg H_3PO_3/L)

NOEC - 48 h ≥ 100 mg/L (nominal concentration) (≥ 38 mg H_3PO_3/L)

*** * * * ***

zRMS Comments:	The submitted study was accepted. The validity criteria were met: • Cell Density: increase in Control Cultures: 180-fold increase within 72 hours; • Coefficient of Variation of Daily Growth Rates in Control Cultures: 21.6 %; • Coefficient of Variation of Average Growth between Control Replicates: 1.6 %. No deviations were noted. The following endpoints based on biomass dry weight were derived: • E_rC_{50} >100 mg Salaman 510/L (equivalent to 38 mg H_3PO_3/L); • E_yC_{50} = 33.8 mg Salaman 510/L (equivalent to 12.8 mg H_3PO_3/L). • NOEC = 3.2 mg Salaman 510/L (equivalent to 38 mg H_3PO_3/L).

Report:	KCP 10.2.1/03
Authors (year)	Pupp A. and Wydra V. (2013)a
Title:	Toxicity of SALAMAN 510 (510 g/L phosphorus acid) to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test
Document No:	IBACON Study report Nº 65671210
Guidelines:	Commission Regulation (EC) No 761/2009. Annex. Part C. C.3.: "Freshwater Algae and Cyanobacteria. Growth Inhibition Test". Official Journal of the European Union (EN). dated August 24. 2009
GLP:	Yes
Deviations	--

Methods

Exponentially growing cultures of this unicellular green algal species were exposed to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 72 hours and thus over several algal generations.

The test method of application and the test system are recommended by the test guidelines and *Pseudokirchneriella subcapitata* is one of the recommended test species.

The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium.

This study encompassed 6 treatment groups (5 dose rates of the test item. control) with three replicates per test concentration and six replicates for the control. At test start 50 mL of the test concentrations were inoculated with 5000 algal cells per mL test medium and defined volumes of the algal suspensions were sampled after 24. 48 and 72 hours for determination of cell densities by spectrophotometrical measurement.

The samples collected at start and after 72 hours were analysed *via* ion chromatography with conductivity detection (IC-CD).

Results

The quantification of the test item SALAMAN 510 (510 g/L phosphorous acid) was performed using ion chromatography with conductivity detection (IC-CD).

At the start of the test 104 % of the nominal test concentrations were found (mean value of test media of 10 to 100 mg test item/L). After 72 hours of test duration 101 % of the nominal values were determined (mean value of test media of 10 to 100 mg test item/L). Thus. during the test period of 72 hours the algae were exposed to a mean of 102 % of nominal. Therefore. all reported results are related to nominal concentrations of the test item.

10.2.1-3: Biological results

Parameter	**Yield [mg test item/L]**	**Growth rate [mg test item/L]**
72-hours EC_{50} 95% conf. interval	33.8 27.2 – 42.7	>100 n.d.
72-hours EC_{20} 95% conf. interval	7.03 4.71 – 9.43	76.9 63.7 – 96.0
72-hours EC_{10} 95% conf. interval	3.09 1.74 – 4.63	21.2 15.7 – 26.7
72-hours NOEC 72-hours LOEC	3.2 10	3.2 10

n.d.= not determinable
values refer to nominal test concentrations

Conclusion

The influence of SALAMAN 510 (510 g/L phosphorus acid) on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* was assessed in a static dose-response test. The 72-hour E_rC_{50} value was calculated to be > 100 mg test item/L and the 72-hour E_yC_{50} was calculated to be 33.8 mg test item/L. The 72-hour NOE_rC and the 72-hour NOE_yC were determined to be 3.2 mg test item/L and the associated 72-hour LOE_rC and LOE_yC were determined to be 10 mg test item/L.

E_rC_{50} – 72h > 100 mg/L (38 mg H_3PO_3/L)

E_yC_{50} – 72h = 33.8 mg/L (12.8 mg H_3PO_3/L)

NOEC – 72h = 3.2 mg/L (1.2 mg H_3PO_3/L)

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish aquatic invertebrates and sediment dwelling organisms

zRMS Comments:	The submitted study was accepted; the study has not been not evaluated at the zonal level (Central zone) before this submission. The validity criteria were met: • one died fish was observed in the control up to end of the study; the mean body weight of the control fish increased by a factor of 1.76 corresponding to 76 % after 28 days compared to the mean initial weight; • the oxygen concentration in the test media did not fall below 77 % of air saturation

value during the study;

- the water temperature was 13 - 14 °C and did not differ by more than ± 1 °C between test chambers at any time during the test. Thus, the temperature was within the temperature range of 2 °C given in the study plan.

No deviations were noted.

The juvenile rainbow trout were exposed in a semi static test to aqueous test media containing the test item at various concentrations under defined conditions. The recorded effects were mortality, symptoms of intoxication and, at the start and the end of the test, the growth parameters body weight and length of surviving fish.

Table 5. Mortality, Number of Dead Fish at the Respective Experimental Days

Nominal test concentration [mg test item/L]	Control	1.0	2.6	6.4	16	40
Exposure Time [day]						
0	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0
11	1	0	0	0	0	0
12	1	0	0	0	0	0
13	1	0	0	0	0	0
14	1	0	0	0	0	0
15	1	0	0	0	0	0
16	1	0	0	0	0	0
17	1	0	0	0	0	0
18	1	0	0	0	0	0
19	1	0	0	0	0	0
20	1	0	0	0	0	0
21	1	0	0	0	0	0
22	1	0	0	0	0	0
23	1	0	0	0	0	0
24	1	0	0	0	0	0
25	1	0	0	0	0	0
26	1	0	0	0	0	0
27	1	0	0	0	0	0
28	1	0	0	0	0	1
# dead fish	1	0	0	0	0	1

Table 6. Sub-lethal Effects, Number of Fish with Sub-Lethal Effects

Nominal test concentration [mg test item/L]		Control	1.0	2.6	6.4	16	40
Exposure Time [day]	Symptoms						
0	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
1	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
2	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
3	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
4	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
5	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
6	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
7	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
8	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
9	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
10	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
11	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
12	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
13	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
14	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-

: Number of fish with sublethal effects
des: description

Table 6. Sub-lethal Effects, Number of Fish with Sub-Lethal Effects (continued)

Nominal test concentration [mg test item/L]		Control	1.0	2.6	6.4	16	40
Exposure Time [day]	Symptoms						
15	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
16	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
17	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
18	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
19	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
20	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
21	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
22	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
23	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
24	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-
25	#	0	0	0	0	0	1
	des.	-	-	-	-	-	DC, SV
26	#	0	0	0	0	0	1
	des.	-	-	-	-	-	SV
27	#	0	0	0	0	0	1
	des.	-	-	-	-	-	DC, SV, FF
28	#	0	0	0	0	0	0
	des.	-	-	-	-	-	-

: Number of fish with sublethal effects
des: description
DC: dark colouration
SV: strong ventilation
FF: fins clearly shorten or frayed out at the border

Based on the test results the 28 days NOEC of Salaman 510 for rainbow trout (*Oncorhynchus mykiss*) was determined to be ≥ 40 mg Salaman 510/L based on nominal concentrations (equivalent to 15.2 mg H_3PO_3/L);

The studies summarized in this point has been already submitted for the authorization of the product.

Report:	KCP 10.2.2/01
Authors (year)	
Title:	Toxicity of SALAMAN 510 (510 g/L phosphorous acid) to Rainbow Trout (*Oncorhynchus mykiss*) in a Prolonged Semi Static Test over 28 Days
Document No:	IBACON Study report
Guidelines:	OECD Guideline for Testing of Chemicals. Section 2. No. 215: „Fish. Juvenile Growth Test“. January 21. 2000.
GLP:	Yes
Deviations	--

Methods

Juvenile Rainbow Trout were exposed in a semi static test to aqueous test media containing the test item at various concentrations under defined conditions. The recorded effects were mortality. symptoms of

intoxication and. at the start and the end of the test. the growth parameters body weight and length of surviving fish.

The used method of application is recommended by the test guidelines. and also Rainbow Trout is one of the fish species recommended by the international test guidelines of the OECD.

The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium.

Test concentrations: 40. 16. 6.4. 2.6 and 1.0 mg test item/L and a control.

The samples of the test medium were analysed for phosphorous acid via ion chromatography with conductivity detection (IC-CD).

Results

The quantification of the test item SALAMAN 510 (510 g/L phosphorous acid) was performed using ion chromatography with conductivity detection (IC-CD).

In the freshly prepared test media 87% of the nominal test concentrations and in the aged test media 91% of the nominal values were found (average of test concentrations of nominal 6.4 to 40 mg test item/L. respectively).

Therefore. all reported results are related to nominal concentrations of the test item.

Conclusions

Based on the test results the 28-days NOEC for mortality of the test item SALAMAN 510 (510 g/L phosphorous acid) for Rainbow Trout (*Oncorhynchus mykiss*) was determined to be at least 40 mg test item/L. based on nominal test concentrations. The 28-days NOEC for weight and growth rate was determined to be at least 40 mg test item/L. both values also based on nominal test concentration. The 28-days LOEC and the 28-days LLC were determined to be higher than 40 mg test item/L. the highest nominal concentration tested.

NOEC – 28 d >40 mg test item/L (nominal concentrations)

NOEC – 28 d >15.2 mg H_3PO_3/L

*** * * * ***

zRMS Comments:	The submitted study was not evaluated as it concerns other product.

~~**Report:**~~	~~KCP 10.2.2/02~~
~~**Authors (year)**~~	~~Sewell I.G. (1996)~~
~~**Title:**~~	~~Fosetyl Al: *Daphnia magna* reproduction test~~
~~**Document No:**~~	~~Study report Nº 282/480 (R014229)~~
~~**Guidelines:**~~	~~OECD no 202/II~~
~~**GLP:**~~	~~Yes~~
~~**Deviations**~~	~~–~~

~~**Methods**~~

~~The study was carried out at nominal concentration of 1.0 – 3.2 – 10 – 32 and 100 mg/L (purity: 959 g/kg) over a period of 21 d. The test was conducted with four replicates per treatment level (10 daphnids per replicate).~~

~~**Results**~~

~~Analytical verification showed initial measured concentration to be near nominal. Analysis of expired test media revealed a marked and highly variable decline of the test levels in the period between media renewal. The results of this test were expressed based on the following mean measured concentrations:~~

~~0.47 – 1.97 – 4.54 – 17.0 and 88.6 mg/L. respectively. At d 7 first juveniles appeared at all treatment levels below 100 mg/L. at 88.6 mg/L. 100 % mortality of parental daphnids occurred by d 5. Neither adverse effects of the test substance on the survival or growth of parental generation daphnids, nor effects on reproduction were observed at concentration up to and including 17.0 mg/L. Up to 17 mg/L. the daphnids appeared to have the same colour and the same size as those from the control replicate.~~

~~**EC_{50} – 21 d = 39 mg a.s./L (mean measured concentrations; CI 95%: 17- 89 mg/L)**~~

~~**NOEC – 21 d = 17 mg a.s./L (mean measured concentrations)**~~

*** * * * ***

zRMS Comments:

The submitted study was accepted.
All validity criteria of the OECD test guideline No. 211 were fulfilled:

- the mortality of parent animals did not exceed 20% at the end of the test in the control;
- the mean number of live offspring produced per parent animal alive at the end of the test was ≥ 60 in the control.

Not significant deviation was noted: total hardness was above 14° dH at three assessment dates (frm the procedural reason).

Mortality of adults. Mortality of adult daphnids was observed but did not follow a monotonous concentration-effect curve (or any other explainable pattern) and thus is not considered to be concentration-related. No mortality of adult Daphnia above the allowed control mortality was observed in the control and all test item concentrations.
The NOEC for mortality of adult Daphnia was therefore settled at the highest test item concentration of 100 mg/L. The LOEC for mortality was assumed to be > 100 mg/L.
The LC_{50} (after 21 days) was determined to be > 100 mg/L (equivalent to 38 mg H_3PO_3/L).

Table 3: Mortality rates of adult animals for every day (% of initial numbers)

Conc.	Day										
	1	2	3	4	5	6	7	8	9	10	11
	Mortality [%]										
Control	0	0	0	0	0	0	0	0	0	0	0
6.25 mg/L	0	0	0	0	0	0	0	0	0	0	0
12.5 mg/L	0	0	0	0	0	0	0	0	0	0	0
25.0 mg/L	0	0	0	0	0	0	0	0	10	10	10
50.0 mg/L	0	0	0	0	0	0	0	0	0	0	0
100 mg/L	0	0	0	0	0	0	0	0	0	0	0
Conc.	**Day**										
	12	13	14	15	16	17	18	19	20	21	
	Mortality [%]										
Control	0	0	0	0	0	0	0	0	0	0	
6.25 mg/L	0	0	0	0	0	0	0	0	10	20	
12.5 mg/L	0	0	10	10	10	10	10	10	10	10	
25.0 mg/L	10	10	10	10	10	10	10	10	10	10	
50.0 mg/L	0	0	0	0	0	0	0	10	10	10	
100 mg/L	0	0	0	0	0	0	0	0	0	0	

Reproduction
On the basis of the sum of living offspring produced by parent Daphnia alive at the end of the test, the number of offspring was not significantly inhibited up to and including the test item concentration of 100 mg/L. A non-significant inhibition of reproduction of max. 2.3 % was observed at the test item concentration of 50.0 mg/L. The NOEC for reproduction was determined to be 100 mg/L. The LOEC and EC_{50} were therefore

determined to be > 100 mg/L (equivalent to 38 mg H_3PO_3/L).

Table 4: Number of adult Daphnia and offspring (day 8 - 21)

Conc./Day	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Σ
Control															
Adults	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
Offspring alive	0	40	30	45	0	224	89	0	259	121	0	146	221	62	1237
Offspring dead	0	1	6	0	0	0	1	0	0	1	0	0	0	0	9
6.25 mg/L															
Adults	10	10	10	10	10	10	10	10	10	10	10	10	9	8	
Offspring alive	0	1	41	35	33	124	106	75	130	127	108	94	177	72	1123
Offspring dead	0	13	14	0	7	0	13	2	0	2	0	0	26	0	77
12.5 mg/L															
Adults	10	10	10	10	10	10	9	9	9	9	9	9	9	9	
Offspring alive	0	1	11	27	122	93	125	104	143	73	54	232	136	0	1121
Offspring dead	0	31	12	3	0	0	0	0	1	9	0	0	0	0	56
25.0 mg/L															
Adults	10	9	9	9	9	9	9	9	9	9	9	9	9	9	
Offspring alive	0	0	38	17	28	192	75	0	270	85	0	178	228	0	1111
Offspring dead	0	0	15	11	0	0	4	0	0	0	0	0	0	0	30
50.0 mg/L															
Adults	10	10	10	10	10	10	10	10	10	10	10	9	9	9	
Offspring alive	0	15	18	11	59	171	70	45	233	90	69	193	118	84	1176
Offspring dead	0	4	25	1	0	0	0	0	3	0	0	0	0	0	33
100 mg/L															
Adults	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
Offspring alive	0	3	23	7	124	133	61	58	282	45	110	141	194	52	1233
Offspring dead	0	35	27	10	0	0	0	0	0	0	1	0	0	0	73

Table 1: Effect concentration of Daphnia exposed to Salaman 510

	Salaman 510 (nominal)
NOEC (mortality & reproduction)	100 mg/L
LOEC (mortality)	> 100 mg/L
LOEC (reproduction)	> 100 mg/L
EC_{50} (reproduction)	> 100 mg/L
LC_{50} (mortality of adults)	> 100 mg/L

Report:	KCP 10.2.2/03
Authors (year)	Zawadsky. C. (2014)
Title:	Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Reproduction Test)
Document No:	EUROFINS study report Nº S14-00233
Guidelines:	OECD 211 (2012)
GLP:	Yes
Deviations	--

Method

SALAMAN 510. Batch number: 1132015; active substance (a.s.): monopotassium phosphite. content of a.s. (analysed): 570.7 g/L (phosphorous acid equivalent). Test species: *Daphnia magna* Straus. Clone V. aged less than 24 hours. 10 Daphnia per test item concentration and the untreated control were exposed to the test item for 21 days. The results were evaluated in a semi-static test with nominal concentrations of 0. 6.25. 12.5. 25.0. 50.0 and 100 mg/L and renewal of test solutions every Monday. Wednesday and Friday. Assessments of immobilisation and other effects were performed each day. Offspring was counted and removed daily after appearance of first brood. Test item concentrations were verified by analyzing the control and all concentration levels at six representative sampling dates from fresh and aged samples. Temperature. pH-value and oxygen concentration were measured in the control and each test item concentration at test start and in the control and the lowest and highest test item concentration at each renewal of the test solutions. Endpoints reported are EC_{50}/LC_{50}. NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) for reproduction and mortality of adult daphnids.

Results

Mortality of Adults

Mortality of adult daphnids was observed but did not follow a monotonous concentration-effect curve (or any other explainable pattern) and thus is not considered to be concentration-related. No mortality of adult Daphnia above the allowed control mortality was observed in the control and all test item concentrations.

The **NOEC** for mortality of adult Daphnia was therefore settled at the highest test item concentration of **100 mg/L**. The **LOEC** for mortality was assumed to be **> 100 mg/L**.

The **LC_{50}** (after 21 days) was determined to be **> 100 mg/L**.

Reproduction

On the basis of the sum of living offspring produced by parent Daphnia alive at the end of the test. the number of offspring was not significantly inhibited up to and including the test item concentration of 100 mg/L. A non-significant inhibition of reproduction of max. 2.3 % was observed at the test item concentration of 50.0 mg/L. The **NOEC** for reproduction was determined to be **100 mg/L**. The **LOEC** and **EC_{50}** were therefore determined to be **> 100 mg/L**.

The first offspring in the control and in the test item concentrations of 6.25. 12.5. 50.0 and 100 mg/L was observed on day 9 and in the test item concentration of 25.0 mg/L on day 10.

Conclusions

No toxic effects were observed up to 100 mg/L.

The **EC_{50} for inhibition of reproduction (21 d)** was assumed to be **> 100 mg/L.** The **NOEC (21 d) for inhibition of reproduction** was determined to be **100 mg/L**. The **LOEC (21 d) for inhibition of reproduction** was assumed to be **> 100 mg/L**.

The **LC_{50} (21d)** was assumed to be **> 100 mg/L.** The **NOEC (21 d) for mortality of adults** was observed at **100 mg/L**. The **LOEC (21 d) for mortality of adults** was assumed to be > **100 mg/L**.

* * * * *

zRMS Comments:

The submitted study was accepted.
The validity criteria were met:

- the emergence in the control was 94.2 % at the end of the test. Main emergence to adults of *Chironomus riparius* occurred between day 13 and 19 after insertion of the larvae ;
- during the whole test the oxygen concentration was ≥ 54 % of the air saturation value at the temperature of 19 to 21 °C and the pH of overlying water was in the range of 7.3 to 8.5 in all test vessels.
- The water temperature was 19 to 21 °C and therefore did not differ by more than ± 1.0 °C.

A not significant deviation was noted: The oxygen concentration of the overlaying water was 54 to 109 %. Following the 24 hours period of stopped aeration after introduction of larvae at Day -1, the oxygen concentration of the overlaying water in the 1st, 2nd, 5th and 6th replicate of the control and in the 1st and 2nd replicate of the only test concentration of nominal 100 mg test item/L dropped slightly below 60 % at test start (= Day 0). Since aeration was supplied again directly after measurement and an emergence of 85 to 100 % occurred and thus, no significantly reduced emergence was determined in the replicates concerned.

Table 2. Mean Development Rate and Emergence Rate for the Control and the only Test Item Group of nominal 100 mg test item/L

Treatment [mg test item/L]	Emergence Rate [% emerged midges]			Development Rate		
Control	94.2	±	5.8	0.065	±	0.002
100	95.8	±	5.8	0.067	±	0.002

values represent means and standard deviation from all replicates with 20 larvae each

Table 5. Emergence Rate for the Control and the only Test Item Group of nominal 100 mg/L

Control	Replicates					
	1	2	3	4	5	6
introduced larvae	20	20	20	20	20	20
total emerged midges	20	17	18	20	19	19
ER	1.00	0.85	0.90	1.00	0.95	0.95
ER [%]	100.0	85.0	90.0	100.0	95.0	95.0

100 mg/L	Replicates					
	1	2	3	4	5	6
introduced larvae	20	20	20	20	20	20
total emerged midges	20	19	20	19	17	20
ER	1.00	0.95	1.00	0.95	0.85	1.00
ER [%]	100.0	95.0	100.0	95.0	85.0	100.0

Table 6. Developmental Rate for the Control and the only Test Item Group of nominal 100 mg/L

Control

	day of exposure	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Total
Replicates	1	0.000	0.000	0.030	0.007	0.013	0.009	0.000	0.003	0.000	0.002	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.066
	2	0.000	0.000	0.035	0.008	0.011	0.004	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067
	3	0.000	0.009	0.004	0.015	0.014	0.007	0.010	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.064
	4	0.000	0.008	0.011	0.003	0.010	0.003	0.014	0.008	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.062
	5	0.000	0.008	0.016	0.015	0.007	0.003	0.006	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.066
	6	0.000	0.017	0.023	0.004	0.003	0.000	0.015	0.003	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.067

100 mg test item/L

	day of exposure	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Total
Replicates	1	0.000	0.024	0.007	0.010	0.019	0.003	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.069
	2	0.000	0.004	0.027	0.007	0.007	0.006	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.066
	3	0.004	0.008	0.007	0.014	0.006	0.012	0.006	0.003	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.065
	4	0.000	0.004	0.012	0.015	0.010	0.006	0.015	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065
	5	0.000	0.019	0.013	0.004	0.008	0.011	0.000	0.010	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067
	6	0.004	0.024	0.007	0.003	0.006	0.012	0.003	0.005	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.069

The following endpoints were derived:

- 28 d NOEC ≥ 100 mg Salaman 510/L (equivalent to 38 mg H_3PO_3/L);
- 28 d EC_{50} > 100 mg Salaman 510/L (equivalent to 38 mg H_3PO_3/L);
- 28 d LOEC > 100 mg Salaman 510/L (equivalent to 38 mg H_3PO_3/L).

Report:	KCP 10.2.2/04
Authors (year)	Pupp A. and Wydra V. (2013)
Title:	Effects of SALAMAN 510 (510 g/L phosphorous acid) on the Development of Sediment Dwelling Larvae of *Chironomus riparius* in a Sediment-Water System – exposed via spiked Water
Document No:	IBACON Study report Nº 65676250
Guidelines:	OECD Guideline for the Testing of Chemicals 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water". adopted April 13. 2004
GLP:	Yes
Deviations	--

Methods

First instar larvae of *Chironomus riparius* were exposed to the test item for 28 days to assess the impact on full maturation of the larvae to adult midges. The larvae were exposed in a sediment-water system containing the test item only at the nominal test concentration of 100 mg/L.

Therefore. the water phase was spiked with the test item to simulate the presence of SALAMAN 510 (510 g/L phosphorous acid) in the water.

This limit-test was performed in compliance with the test guidelines in order to demonstrate that the test item has no toxic effects on the chironomids up to this concentration.

The endpoints of the study are the number of adult midges emerged (emergence rate) and the time to emergence (development rate).

The used method of application is recommended by the test guidelines. and also *Chironomus riparius* is a preferred freshwater aquatic insect species recommended by the test guidelines of the OECD.

The only concentration tested was nominal 100 mg test item/L. Additionally; a control was tested in parallel (test water without addition of the test item). Thus. a limit test was performed in accordance with OECD Guideline 219 to demonstrate that the test item has no toxic effect on the test animals up to this concentration.

The quantification of the test item was performed using ion chromatography (IC-method).

Results

Table 10.2.2-1 Summary of analytical results

Sample description		Age	5 of	RDS	n
[mg test item/L]	compartment	[day]	nominal [1]	[%]	
control	overlying water	0/28	n.a.	n.a.	1
control	pore water	0/28	n.a.	n.a.	1
control	sediment	0/28	n.a.	n.a.	1
100	overlying water	0	82	3	2
100	pore water	0	0	n.a.	0
100	sediment	0	n.a.	n.a.	0
100	overlying water	28	67	4	2
100	pore water	28	4	0	2
100	sediment	28	6	0	2

At the test start concentrations of the test item close to the nominal concentrations were found in the overlying water. About 82 % of the test item was found in the overlying water. whereas the concentrations in the pore water and sediment were negligible. In the aged test media. the amount of the test item in the overlying water was 67 %. and the concentrations in the pore water and sediment were 4 % and 6 % of nominal. respectively. Since the dose verification could be performed. all biological endpoints were related to nominal concentration.

NOEC – 28 d > 100.0 mg test item/L (38 mg H_3PO_3/L)

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The submitted study was accepted. **Oral test**. The validity criteria were met – no mortality in the control groups was observed (recommended ≤ 10 %); The following endpoint was derived: 48 h oral LD_{50} > 0.177 µL form./bee; equivalent for > 99.68 µg a.s./bee. **Contact test**. The validity criteria were met – no mortality in the control groups was observed (recommended ≤ 10 %); The following endpoint was derived: 48 h oral LD_{50} > 0.177 µL form./bee; equivalent for > 100.0 µg a.s./bee. These endpoints were used in risk assessment.

Report:	KCP 10.3.1.1.1/01
Authors (year)	Ansaloni. T. (2012)
Title:	Acute oral and contact toxicity of "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) on honeybees (*Apis mellifera* L.)
Document No:	TRIALCAMP Study report TRC12-018BA
Guidelines:	OCDE Guidelines no 213 and 214 (1998)
GLP:	Yes
Deviations	--

Objective of the study

A study was carried out under laboratory conditions with the objective to determine the acute oral and contact toxicity of Potassium phosphite 510 g/L. as Phosphorous acid. to adult worker honeybees. Based on non-GLP range finding the tests were performed at the limit dose of 100µg Potassium phosphite/bee. The study followed OECD guidelines 213 and 214 and was conducted under study code TRC12-018BA.

Methods

Test item

Potassium phosphite 510 g/L, as Phosphorous acid. (SE12057/02), batch 1132015. purity 38.0% w/w (564.1 g/L). density 1.4846 g/mL, expiry 4 August 2013.

Test organism

Apis mellifera L. Var. *Iberica*; adult worker's honeybees collected from a healthy queen-right colony sourced from a commercial apiary. Honeybees were collected the day prior to testing and were maintained under test conditions in holding cages.

Test design

Contact***:*** A limit test was performed at 100 µg Potassium phosphite/bee. A water only treated control was included in the test. There were five replicates for both the test item and control treatments each with 10 honeybees. Honeybees were anaesthetised and were each dosed with 2 µL of test solution. or deionised water in the controls. which was applied to the dorsal thorax by micro-applicator. Honeybees were maintained in test cages of 10 bees and were supplied with untreated sucrose solution (50% w/w) as a food source.

Oral***:*** Before being used for the oral toxicity test the honeybees were starved for about two hours and fifteen minutes. The test item was mixed with sucrose solution (50% w/w) to achieve the required concentration. Replicates were group fed with one feeder per cage containing 200 µL of test solution. as honeybees share food each is assumed to consume 20 µL/bee. Feeders were weighed prior to dose addition. and once fully consumed (or after a maximum of 6 hours) the feeder was removed and replaced

with a feeder containing untreated sucrose solution. If the dose was not fully consumed the feeder was re-weighed and the dose consumed calculated. The control was treated with untreated sucrose solution only.

Assessments

In both the contact and oral tests honeybees were observed at approximately 4. 24 and 48 hours. mortality and behavioural effects were assessed. Dead bees were not removed from the test chambers until the test was finished.

Toxic reference treatment

In both studies as a toxic standard reference product. Dimethoate (Dimethoate 40% EC) was applied at 4 dose rates covering the LD_{50} range. Four replicate cages were tested per concentration each with 10 honeybees. Procedures followed those described above for the test item.

Results

Contact***:*** Mean mortality in the control of the contact test was 0.00% up to 48 hours after the application. Mean mortality of honeybees applied topically with the test product at 100 µg Potassium phosphite/bee. was 0.00% throughout the test.

Oral***:*** Mean consumption in the control group of the oral test was 95.63% of the offered diet. Mean mortality in the control of the oral test was 0.00% throughout the test.

Consumption of the bees exposed to the test product was 99.68% of the offered diet (99.68 µg/bee of the active substance Potassium phosphite. equal to 0.177 µL formulated product/bee). Mean mortality of honeybees dosed orally with the test product was 0.00% throughout the test.

Reference treatment***:*** The estimated LD_{50}-values for the reference product Dimethoate were within the Guideline specific range for both the oral and the contact test (oral LD_{50} = 0.190 µg a.s./bee at 24h. contact LD_{50} = 0.175 µg a.s./bee at 24h) and therefore confirmed the sensitivity of the test organism and the test conditions.

The oral and contact LD_{50} values are reported in the following tables:

Oral Test	LD_{50} (a.s.)	LD_{50} (formulated product)
Test product 24h	> 99.68 µg a.s./bee	> 0.177 µL formulated product/bee
Test product 48h	> 99.68 µg a.s./bee	> 0.177 µL formulated product/bee
Dimethoate 40% 24h	0.190 µg a.s./bee	4.61E-04 µL formulated product/bee
Dimethoate 40% 48h	0.177 µg a.s./bee	4.31E-04 µL formulated product/bee

Contact Test	LD_{50} (a.s.)	LD_{50} (formulated product)
Test product 24h	> 100.00 µg a.s./bee	> 0.177 µL formulated product/bee
Test product 48h	> 100.00 µg a.s./bee	> 0.177 µL formulated product/bee
Dimethoate 40% 24h	0.175 µg a.s./bee	4.25E-04 µL formulated product/bee
Dimethoate 40% 48h	0.156 µg a.s./bee	3.79E-04 µL formulated product/bee

Conclusion

Contact LD_{50}-values at 24 and 48h after the application of the formulated product were greater than the limit dose of 100 µg Potassium phosphite/bee (equivalent to 0.177 µL formulated product/bee).

Oral LD_{50}-values at 24 and 48h after dosing with the formulated product were greater than the consumed dose of 99.68 µg Potassium phosphite/bee (equivalent to 0.177 µL formulation/bee).

No signs of intoxication were observed both in the contact and the oral test at any of the assessments.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

See above. point KCP 10.3.1.1.1.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was evaluated and accepted. The validity criteria were met. No significant deviations from the guidelines were noted. The following endpoints were derived: LDD_{50} > 0.047µL f.p./bee/day equivalent to > 24.22 µg Potassium phosphite /bee/day). NOEDD = 0.047µL f.p./bee/day equivalent to 24.22 µg Potassium phosphite /bee/day).

Report:	KCP 10.3.1.2/01
Authors (year)	Ansaloni. T. (2016)
Title:	Chronic toxicity of "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) on honeybees (*Apis mellifera* L.)
Document No:	TRIALCAMP Study report TRC16-088BA
Guidelines:	OECD Guideline nº 213 (1998)
GLP:	Yes
Deviations	--

Introduction:

A study was carried out under laboratory conditions with the objective to determine the chronic toxicity of "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) to adult worker honeybees. The test was performed with a range of five doses with a spacing factor of 1.8. The study followed the CEB (2012) method. adaptions of OECD Guidelines nº 213 (1998). publications of Decourty *et al*. (2005) and Suchail *et al*. (2001). recommendations of the German ring test group (2013) and EPPO 170 and was conducted under study code TRC16-088BA.

Materials and methods

Test product

"SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid). batch 1511066. purity for Phosphorous acid 51.7% w/v (517 g/L). density 1.4475 g/ml. expiry 1st June 2017.

Test species

Apis mellifera L.; young adult worker honeybees (≤ 24h old) collected from healthy queen-right colony sourced from a commercial apiary. Honeybees were collected shortly after emergence two days prior to the first application and were maintained under test conditions in holding cages throughout the study.

Test design

Five doses in a geometric series (factor of 1.8) of the test product were assessed: 14.29. 25.72. 46.30. 83.33 and 150.00 µg Phosphorous acid/bee/day. Each test dose was prepared daily from a stock solution prepared by mixing a defined amount of test product with a defined amount of a 50% w/v aqueous sucrose solution. A control group. with untreated sucrose solution only (50% w/v). and the reference product Dimethoate 40% EC at a daily dose of 0.107 µg a.s./bee/day were concurrently tested. Five replicates per treatment each enclosing at least ten bees. were group fed with one feeder per cage containing 1000 µl of test solution. thus providing 100 µl of test solution per bee. Feeders were weighed prior to their placement in the test cages and were changed on a daily basis with new feeders containing fresh test solutions. When removed. each feeder was re-weighed, and the mean dose consumed per bee

was calculated taking in account the surviving individuals at the moment of replacement. Five additional cages with syringes with the feeding solution but no bees were maintained in the climatic chamber. Syringes of these additional cages were changed daily in concomitance with the test syringes and were weighed before and after each replacement for the calculation of sucrose solution evaporation. Daily consumption of the test solutions (control and treatments with the test and the reference products) were adjusted taking in account the daily evaporation.

Assessments

Honeybees were observed daily at approximately the same time (when the feeders were changed) for mortality and behaviour assessments. Dead bees were removed from the test units.

Statistic

Mean daily consumption for each dose of the test substance was compared with the control by means of a non-parametric pair-wise test (Mann-Whitney exact test; $\alpha = 0.05$). For the determination of the cumulative NOED at 10 days (No Observed Effect Concentration. cumulative over ten days of exposure). mortalities of each dose of the test substance were compared with the control mortality by means of a non-parametric pair-wise test (Mann-Whitney exact test; $\alpha = 0.05$).

Results

Diet consumption and mortality: Mean daily consumption in the control group was 17.37 µL/bee of the offered diet. Mean cumulative mortality in the control after the ten days of exposure was 8.00%.

Mean daily consumption of the bees exposed to the test product ranged between 17.34 µL/bee (T2 = 25.72 µg Phosphorous acid/bee/day) and 18.40 µL/bee (T3 = 46.30 µg Phosphorous acid/bee/day). As a consequence. the recalculated daily consumed doses ranged between 2.59 µg Phosphorous acid/bee/day and 24.22 µg Phosphorous acid/bee/day. Mean cumulative consumption (consumption over the ten days dosing period) ranged between 25.89 µg Phosphorous acid/bee and 242.17 µg Phosphorous acid/bee. Mean daily consumption (in µl solution/bee/day) observed for all the assayed test product concentrations was not statistically significantly different than mean daily consumption of the control group (Mann-Whitney exact test. $\alpha = 0.05$).

Mean cumulative mortality of the honeybees dosed orally with the test product for ten consecutive days' range between 0.00% (T2. 4.46 consumed µg Phosphorous acid/bee/day) and 12.00% (T4. 14.39 consumed µg Phosphorous acid/bee/day). Estimated LDD_{50} (Lethal Dietary Dose) was higher than the highest consumed dose of 24.22 µg Phosphorous acid/bee/day. Based on the mortality data. the NOEDD (No Observed Effect Dietary Dose) was determined to correspond the highest consumed dose of 24.22 µg Phosphorous acid/bee/day (Mann-Whitney exact test. $\alpha = 0.05$). corresponding to 0.047 µl consumed formulated product/bee/day.

Symptoms of intoxication were observed for few of the bees exposed to some of the test product concentrations starting on the second day of dosing and the percentage of affected bees over the surviving individuals started to progressively increase in time between the assessments at day 8 and day 10 for all doses. Symptoms observed throughout the study were mainly apathy (little response to an external stimulus. i.e. a gentle air blow) and affected bees (lack of coordination). By the end of the study (day 10) the percentage of affected bees (reduced coordination) based on the surviving individuals ranged between 2.04% at the lowest dose (T1. 2.59 consumed µg Phosphorous acid/bee/day) and 67.39% at the highest dose (T5 = 24.22 consumed µg Phosphorous acid/bee/day). Overall. two control individuals were observed showing abnormal behaviour. one moribund on assessment day 2 and one affected on assessment day 9.

Conclusion

Treated diet with "SALAMAN 510" (Potassium phosphite 510 g/L as Phosphorous acid) did not result in a statistically significantly different food consumption at any of the test item doses when compared to the untreated control.

Sublethal effects (i.e. apathy and affected bees) were observed throughout the exposure phase for some of the test product doses. and symptoms of intoxication progressively increase in time between the

assessments at day 8 and day 10 for all doses. ranging from 2.04% to 67.39% of the surviving individuals after 240h chronic exposure.

The estimated consumed chronic LDD_{50}-value (Lethal Dietary Dose that kills 50% of the exposed individuals) for "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) corresponded to the mean consumed dose of 24.22 µg Phosphorous acid/bee/day. equal to 0.047 µl consumed formulated product/bee/day.

Based on the mortality data. the NOEDD at 10 days was determined to be 24.22 µg Phosphorous acid/bee/day. equal to 0.047 µl consumed formulated product/bee/day.

	LDD_{50}		**NOEDD**	
	formulated product	**Phosphorous acid***	**formulated product**	**Phosphorous acid***
Endpoints	>0.047 µL	>24.22 µg	0.047 µL	24.22 µg

* Analytical content

The results obtained with the toxic reference substance confirmed the sensitivity of the bees under the conditions of the oral test.

* * * * *

Comments of zRMS:	The study was evaluated and accepted. The validity criteria were met. No significant deviations from the guidelines were noted. The following endpoints were derived: LDD_{50} = 168.29 µg formulation/bee/d, equivalent to 59.19 µg Potassium phosphite /d; NOEDD < 105.10 µg formulation/bee/d, equivalent to 36.96 µg Potassium phosphite /d; LC_{50} = 9556.13 mg test item/kg food, equivalent to 3361.12 mg Potassium phosphite /d; NOEC < 5572.55 mg test item/kg food, equivalent to 1960 mg Potassium phosphite /d.

Report:	KCP 10.3.1.2/02
Authors (year)	Ansaloni. T. (2021)
Title:	SALAMAN 510: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee (*Apis mellifera* L.) under Laboratory Conditions.
Document No:	TRIALCAMP Study report S20-08782
Guidelines:	OECD Guideline nº 245 (2017)
GLP:	Yes
Deviations	• Amendment 1: Inclusion of the Analytical Phase Plan E20112. • Amendment 2: Correction the formulation of the Test Item. • Amendment 3: Correction of the name of one Sponsor, distribution of the Study Plan, Amendments and Final Report and of the cross-reference to one of the Study Plan sections. The reported amendments had no impact on the outcome of the study.

Introduction:

The study objective was to determine the effects of SALAMAN 510 on the honey bee *Apis mellifera* L. from chronic oral exposure. To estimate the Median Lethal Dietary Dose (LDD_{50}), the respective Median Lethal Concentration (LC_{50}), the daily Lethal Dietary Doses and the Lethal Concentrations that caused 10 and 20% mortality ($LDD_{10/20}$/$LC_{10/20}$), the No Observed Effect Daily Dose (NOEDD) and the respective No Observed Effect Concentration (NOEC) values were determined where possible.

Material and methods

Test item

"SALAMAN 510" (Potassium phosphonate 510 g/L. as Phosphorous acid), batch 2008011, purity for Phosphorous acid 51.0% w/v, density 1.45 g/mL, expiry 20th February 2024.

Reference item

"BAS 152 65 I" (Dimethoate 400 g/L EC), batch 10248664A, purity for dimethoate: 400 g/L (nominal), 414 g/L (analytical), density 1.062 g/mL, expiry 1st March 2021.

Test species

Young adult worker bees (newly hatched; 1 to 2 days old) of *Apis mellifera* L. (Hymenoptera, Apidae) were used as test organisms. They were obtained from healthy colony of beehives sited in a commercial apiary near Trialcamp facilities. Bees were foraging on wild flowers.

Test design

Ten days dose response test including one untreated control group, 5 test item concentrations, one concentration of the reference item; 5 replicates with 10 bees each per treatment group. Mortality and behavioural abnormalities were assessed daily over the test duration.

Five additional test units without bees with full food syringes containing pure 50 % (w/v) aqueous sucrose solution for evaluation of the evaporation.

Test concentrations: 1 control group, 5 test item groups with 1960.00, 2940.00, 4410.00, 6615.00 and 9922.50 mg a.s./kg feeding solution, 1 reference item group with 0.90 mg dimethoate/kg feeding solution.

Application code	Timing	Treatment group	Code	Concentration		Application Volume
				[mg a.s./L f.s.][a]	[mg a.s./kg f.s.]	
A1 to A10	D0 to D9	Test Item SALAMAN 510	T1	2332.40	1960.00	0.1 mL/bee
			T2	3498.60	2940.00	
			T3	5247.90	4410.00	
			T4	7871.85	6615.00	
			T5	11807.77	9922.50	
		Reference Item Dimethoate	R[b]	1.07	0.90	

a.s.: active substance (potassium phosphonate, as phosphorous acid); T: test item treated group; A: Application; D: Day; f.s.: feeding solution.
[a] Based on the feeding solution (50% w/v aqueous sucrose solution) density (1.19 g/mL).
[b] For the reference item, the value indicates the amount of active substance (Dimethoate).

Assessments

Mortality and behavioural abnormalities were recorded every 24 hours (± 2 hours) before each application (start of feeding). At each assessment time, dead bees were removed for sanitary reasons. Behavioural abnormalities were recorded according to the following categories:

a = affected (bees still upright and attempting to walk but showing signs of reduced coordination),
ap = apathetic (bees show only low or delayed reactions to stimulation, e.g. light or blowing; bees are sitting motionless in the unit or are able to walk but not correctly),
c = cramps (bees contracting abdomen or entire body),
m = moribund (bees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation; e.g. light or blowing; bees may recover but usually die),
v = vomiting.

Endpoints

LC_{10}/LDD_{10}, LC_{20}/LDD_{20}, LC_{50}/LDD_{50} and NOEC/NOEDD on exposure at day 10, where possible.

Test conditions

- Air temperature: Min / Max: 32.8 / 34.1 °C (target 33 ± 2 ºC)

- Relative air humidity: Min / Max: 38.2 %* / 60.1 % (target 50 – 70 %)

* Punctual temperature (less than 1 hour)

- Photoperiod: 24 h darkness, except during application and assessments.

- Exposure to light: constant darkness except during feeding and assessments.

Statistics

Statistical calculations were made with the statistical program ToxRatPro Version 3.3.0.

Step-down Cochran-Armitage Test Procedure was used to calculate the 10-Day, NOED / NOEC and LOEC / LOED values.

The LDD_x/LC_x endpoints were calculated by Weibull analysis using linear max. likelihood regression.

Results

Validity Criteria of the Study

The study is considered valid because:

• Mean mortality in the control group was ≤ 15% at the end of the test (actual 8.0 %).

• Mean mortality in the reference item group was ≥ 50 % at the end of the test (actual 100.0 %)

Food Consumption and Uptake of Test item

The overall mean daily consumption of feeding solution was 20.0 mg/bee/day in the control group C. The overall mean daily consumption of feeding solution for the test item concentrations of 1960.00, 2940.00, 4410.00, 6615.00 and 9922.50 mg a.s./kg feeding solution was 18.9, 19.4, 16.0, 13.2 and 10.9 mg/bee/day, respectively.

The overall mean daily consumption of feeding solution in the reference item treatment group was 15.1 mg/bee/day.

The mean daily uptake for the test item concentrations of 1960.00, 2940.00, 4410.00, 6615.00 and 9922.50 mg a.s./kg feeding solution was 36.96, 57.12, 70.75, 87.28 and 108.44 µg a.s./bee/day, respectively, corresponding to cumulative doses of 369.65, 571.21, 707.49, 837.87 and 802.48 µg a.s./bee, respectively.

Consumption of feeding solution per day in the control, the test item and reference item groups over the 10 day test period

	Amount of test solution consumed per bee (mean; mg)									
TRT	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
C	21.0	21.5	12.8	22.0	23.8	16.6	19.3	22.1	16.9	23.8
T1	16.8	21.1	12.9	23.5	16.6	14.3	20.9	18.5	16.0	28.0
T2	22.7	14.6	21.3	21.9	16.0	24.6	17.6	19.6	15.7	20.3
T3	15.5	15.2	19.1	21.6	19.2	12.2	14.2	14.3	14.0	15.2
T4	12.9	16.0	14.0	21.7	13.4	18.6	10.6	14.0	5.3	0.5
T5	15.7	11.7	15.2	16.1	11.2	7.0	3.3	3.5	0.0	n.s.
R	20.5	15.7	9.5	21.7	15.2	13.6	17.6	6.2	2.4	n.s.

n.s. no surviving individuals

Accumulated mean uptake of solution or active substance in the test item and reference item groups

	Mean Consumed solution			Average consumed dose (µg a.s./bee)	
TRT	**(mg/bee/day)**	**SD**	**SE**	**Daily**	**Cumulative (10 days)**
C	20.0	8.0	1.1	--	--

T1	18.9	6.9	1.0	36.96	369.65
T2	19.4	6.0	0.9	57.12	571.21
T3	16.0	5.7	0.8	70.75	707.49
T4	13.2	7.0	1.0	87.28	837.87
T5	10.9	7.0	1.1	108.44	802.48 [a]
R	15.1	9.9	1.6	0.014	0.106 [a]

[a] Over 9 days of exposure

Cumulative Mortality and Behavioural Abnormalities

In the test item groups of 36.96, 57.12, 70.75, 87.28 and 108.44 consumed µg active substance/bee/day cumulative mortalities of 20.0, 40.0, 82.0, 100.0, and 100.0 % were observed, respectively, at the final assessment after 10 days of exposure.

Cumulative and corrected cumulative mortality in the control, the test item and reference item treatment groups

Treatment	Concentration (mg a.s./kg feeding solution)	Total number of bees dosed	Final Mortality (cumulative %)	SE
C	--	50	8.00	3.74
T1	1960.00	50	20.00	8.37
T2	2940.00	50	40.00	6.32
T3	4410.00	50	82.00	5.83
T4	6615.00	50	100.00	0.00
T5	9922.50	50	100.00	0.00
R	0.90	50	100.00	0.00

Symptoms of intoxication were observed starting on D4, with two affected bees in treatments T3 and T4 and 4 affected bees in treatment T5. By the end of the test (D10), one affected bee was observed in treatments T1 and T3 and three affected bees were observed in treatment T2. No affected bees were observed in in the control group C throughout the test.

Behavioural Abnormalities

Treatment	Concentration (mg a.s./kg feeding solution)	% of affected bees									
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
C	--	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T1	1960.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	2.5
T2	2940.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.8	5.3	10.0
T3	4410.00	0.0	0.0	0.0	4.3	4.7	15.4	6.1	15.0	21.4	11.1
T4	6615.00	0.0	0.0	0.0	4.3	13.2	27.6	15.8	36.4	66.7	n.s.
T5	9922.50	0.0	0.0	0.0	12.1	27.6	28.6	100.0	100.0	n.s.	n.s.

n.s. no surviving individuals

Detailed information about behavioural abnormalities in the control group and test item treatment groups are presented in **Błąd! Nie można odnaleźć źródła odwołania.** and **Błąd! Nie można odnaleźć źródła odwołania.**, **Błąd! Nie można odnaleźć źródła odwołania.**.

Statistical results and endpoints of the study

The NOEC for mortality after 10 days of continuous exposure was determined to be < 1960.00 mg a.s./kg feeding solution. The NOEDD, based on the actual consumption of the feeding solutions, was determined to be < 36.96 µg a.s./bee/day

After 10 days of continuous exposure, the LC_{50} was determined to be 3361.12 mg a.s./kg feeding solution. The LDD_{50}, based on the actual consumption of the feeding solutions, was determined to be 59.19 µg a.s./bee/day.

The LC_{10}/LC_{20} values were determined to be 1870.40 and 2362.29 mg a.s./kg feeding solution, respectively. The 10-day LDD_{10}/ LDD_{20} values were determined to be 39.71 and 46.55 µg a.s./bee/day, respectively.

The endpoints are shown in the table below.

	µg/bee/day	
	test item [1]	**Potassium phosphonate**
NOEDD [2]	< 105.10	< 36.96
LDD_{10} (95% CI) [3]	112.90 (94.36 – 126.26)	39.71 (33.19 – 44.41)
LDD_{20} (95% CI) [3]	132.35 (116.14 – 143.95)	46.55 (40.85 – 50.63)
LDD_{50} (95% CI) [3]	168.29 (157.31 – 177.27)	59.19 (55.33 – 62.35)
	mg/kg feeding solution	
	test item [3]	**Potassium phosphonate**
NOEC [2]	< 5572.55	< 1960.00
LC_{10} (95% CI) [3]	5317.80 (4126.25 – 6222.26)	1870.40 (1451.30 – 2188.52)
LC_{20} (95% CI) [3]	6716.31 (5606.04 – 7548.98)	2362.29 (1971.78 – 2655.16)
LC_{50} (95% CI) [3]	9556.13 (8706.14 – 10340.38)	3361.12 (3062.16 – 3636.96)

[1] Calculated on the basis of the analytical content
[2] Step-down Cochran-Armitage Test Procedure (α = 0.050; one-sided greater)
[3] Probit analysis Using linear max. likelihood regression

Conclusions

The LDD_{10}-value (Lethal Dietary Dose that kills 10 % of exposed individuals) for SALAMAN 510 was estimated to be 39.71 µg a.s./bee/day. The LC_{10}-value (Lethal Concentration that kills 10 % of exposed individuals) was estimated to be 1870.40 mg a.s./kg feeding solution.

The LDD_{20}-value (Lethal Dietary Dose that kills 20 % of exposed individuals) for SALAMAN 510 was estimated to be 46.55 µg a.s./bee/day. The LC_{20}-value (Lethal Concentration that kills 20 % of exposed individuals) was estimated to be 2362.29 mg a.s./kg feeding solution.

The LDD_{50}-value (Lethal Dietary Dose that kills 50 % of exposed individuals) for SALAMAN 510 was estimated to be 59.19 µg a.s./bee/day. The LC_{50}-value (Lethal Concentration that kills 50 % of exposed individuals) was estimated to be 3361.12 mg a.s./kg feeding solution.

The NOEDD (No Observed Effect Dietary Dose), based on actual consumption of the feeding solutions, was determined to be lower than the consumed dose of 36.96 µg a.s./bee/day. After 10 days the NOEC (No Observed Effect Concentration) was determined to be lower than 1960.00 mg a.s./kg feeding solution.

The results obtained with the toxic reference item, dimethoate, confirmed the sensitivity of the test system.

A 2.3.1.3 KCP 10.3.1.3 Effects on honeybee development and other honeybee life stages

Comments of zRMS:	The study was evaluated and accepted. The study was conducted for 8 days. The validity criteria were met. No significant deviations from the guidelines were noted. The following endpoints for developmental period were derived: 120 h LD_{50} > 0.290 µL test item./larva, equivalent to > 150 µg Phosphorous acid /larva; NOED = 0.290 µL test item./larva, equivalent to > 150 µg Phosphorous acid /larva;

Report:	KCP 10.3.1.3/01
Authors (year)	Ansaloni. T. (2016)
Title:	Toxicity of "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid). SL on honeybee larvae (*Apis mellifera* L.) after repeated exposure under laboratory conditions.
Document No:	TRIALCAMP Study report TRC16-202BA
Guidelines:	OECD Guideline nº 237 (2013)
GLP:	Yes
Deviations	--

Study objective

A study was carried out under laboratory conditions with the objective to determine the toxicity of "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) to honeybees' larvae after repeated exposure. Based on non-GLP range finding the test was performed with a range of five doses. The study followed the OECD guideline 237 with modifications of the dose administration (repeated dosing based on OECD Draft Guidance Document "Honeybee (*Apis mellifera*) Larval Toxicity Test. Repeated Exposure" (2014) and was conducted under study code TRC16-202BA.

Materials and methods

Test item

"SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid). SL. batch 1511066. purity for Phosphorous acid 51.7 % w/v (517 g/L). density 1.4475 g/ml. expiry June 1st. 2017.

Test organisms

Apis mellifera L. Var. Iberica; larvae of honeybees collected from a healthy queen-right colony sourced from a commercial apiary. Honeybee larvae at the stage L1 were selected from three different colonies and individually placed into cellular well-plates where they were fed with a standardized amount of artificial diet.

Selection of test larvae***:*** Queens of a minimum of three colonies were confined within an empty comb or a comb with emerging worker bees and empty cells of their own colony with an exclusion cage 4 days before the beginning of the test (D -3). At Day -2 (D -2). and within a maximum of 30 hours after confinement. the queens were released after checking the presence of fresh laid eggs. The comb with the eggs was left in the cage near the brood combs until hatching (D1), when the first instar (L1) larvae were taken from the combs and individually placed in well-plates under controlled conditions.

Test design

Test Units

Larvae were reared in sterilised crystal polystyrene grafting cells placed individually into a well of a 48-good plate. The plates were placed into a hermetic Plexiglass desiccator with water saturated atmosphere. The desiccator was placed into an incubator with forced ventilation at 33-35 ºC and water saturated atmosphere for the duration of the test.

Diet composition

All larvae were fed once a day with the exception of D2. Three different diets. adapted to the needs of each larval stage. were prepared during the test: Diet A (D1. 20 µL/larva): 44.25% weight of fresh royal jelly. 44.25% weight of deionized water. 0.90% weight of yeast extract. 5.30% weight of glucose and 5.30% weight of fructose. Diet B (D3. 20 µL/larva): 42.95% weight of fresh royal jelly. 42.95% weight of deionized water. 1.30% weight of yeast extract. 6.40% weight of glucose and 6.40% weight of fructose. Diet C (D4 to D6): 83.333% weight of fresh royal jelly. 30% weight of deionized water. 2.00% weight of yeast extract. 9.00% weight of glucose and 9.00% weight of fructose. The following volumes of diet were administered on days D4 to D6: D4 = 30 µl. D5 = 40 µl. D6 = 50 µl.

Application of the test substance

Five doses of the test item were assessed daily for four consecutive days (D3 to D6). Each test dose was prepared daily from a fresh stock solution obtained by mixing a defined amount of the test item with a defined amount of de-ionized water. Aliquots for the preparation of the daily doses were extracted from the stock solution and were mixed with a fixed amount of the corresponding diet. The volume of each test solution corresponded to 10% of the final diet volume. To maintain constant concentrations in terms of µg a.s./mL diet/day. daily doses increased progressively in accordance to the increasing volume of diet administered each day. The final cumulative doses (total of four applications) were of 14.289. 25.720. 46.296. 83.333 and 150.000 µg Phosphorous acid /larva. On D3. sixteen well-fed larvae from each of the three colonies (48 larvae per treatment) were selected for each treatment and dosed with 20 µl of the corresponding diet (diet B) containing the test solution with the corresponding concentration. Administration of the selected doses of test item continued on a daily basis until day 6 with the corresponding diets. Mixing of the test solution with the diet was performed just before administration.

Assessments

Mortality was assessed and recorded at feeding time at D4. D5. D6. D7 and D8. An immobile larva or a larva that did not react to the contact with the grafting tool was noted as dead. Dead larvae were removed at each assessment and anomalies in behaviour were recorded. On D8. the presence of uneaten food was qualitatively recorded.

Toxic reference treatment

A toxic standard reference product. Dimethoate (Dimethoate 40% EC) was applied at a constant concentration of 40 mg a.s./kg diet/day on forty-eight larvae on the same days the test item was applied. Procedures followed those described above for the test item with the difference that no dilution of the stock solution was made for the extraction of the aliquots needed for the preparation of the test dose.

***Statistics*:** For mortality data at 120 hours. a Chi-square test (Fisher's exact test. $\alpha = 0.05$) was performed for comparison of the results of each test concentration with the control. Statistics was performed using the statistical software SPSS 19. SPSS© Inc. 1989-2010.

Results

Mean mortality in the control was 2.08% 120 hours after the first application (D8).

Mean mortality of honeybees' larvae dosed orally with the test item ranged between 0.00% (T4 = 83.333 µg Phosphorous acid/larva/developmental period) and 6.25% (T5 = 150.000 µg Phosphorous acid / larva / developmental period) 120 hours after the first application.

The estimated 120 hours LD_{50}-value is reported in the following table.

Oral Test	**LD*$_{50}$ (developmental period)**	
	Phosphorous acid	**Test product**
Test item 120h (D3 to D8)	> 150 µg/larva	> 0.290 µL/larva

* LD: Lethal Dose

No significant difference in per cent mortality at 120 hours was observed between any of the tested doses with the formulation SALAMAN 510 and the control. Therefore. cumulative NOED (No Observed Effect Dose over 5 days after the first application. cumulative dosing) corresponded to a cumulated dose of 150.000 µg Phosphorous acid/larva at 120 hours after the first application. equivalent to 0.290 µl formulated product/larva (CoA content).

Hours after the first application	**NOED (developmental period)**	
	Phosphorous acid	**Test product**
120	150 µg/larva	0.290 µL/larva

At the 120 hours' assessment. 2 larvae both in the control group and treatment T4 (83.333 µg Phosphorous acid/larva/developmental period) had not completely consumed the diet and were underdeveloped. At the same assessment. two malformed larvae were observed in treatment T5 (150.000 µg Phosphorous acid/larva/developmental period).

Reference treatment: Corrected mortality observed in the larvae exposed to the reference product was 72.34% at 120 hours after dosing.

Conclusions

The estimated LD_{50}-value for "SALAMAN 510" (Potassium phosphite 510 g/L as Phosphorous acid). SL was higher than a cumulative (over 4 days of application) dose of 150 µg Phosphorous acid/larva 120 hours after dosing. equivalent to 0.290 µl formulated product/larva (CoA content).

A cumulative dose of 150 µg Phosphorous acid /larva (equivalent to 0.290 µl formulated product/larva. CoA content) resulted in a NOED at the end of the study (No Observed Effect Dose over the 4 days of exposure. cumulative dosing. at 120 hours after the first application).

The results obtained with the toxic reference substance confirmed the sensitivity of the test system (bees' larvae) under the test conditions.

* * * * *

zRMS Comment	The study was accepted. The study was performed in accordance with GLP requirements and OECD 239 guidelines. The validity criteria were met. Some deviations from the guidelines were noted, but they had no negative impact on study results. The following endpoints for developmental period were derived: These endpoints are acceptable for the risk assessment.

Endpoints	Concentration		Dose	
	mg t.i./kg diet	mg a.i./kg diet	µg t.i./larva	µg a.i./larva
NOEC/NOED	≥ 3544.69	≥ 1246.76	≥ 545.88	≥ 192.00
LOEC/LOED	> 3544.69	> 1246.76	> 545.88	> 192.00
EC_{10} / ED_{10}	n.d.	n.d.	n.d.	n.d.
EC_{20} / ED_{20}	n.d.	n.d.	n.d.	n.d.
EC_{50} / ED_{50}	> 3544.69	> 1246.76	> 545.88	> 192.00

Report:	KCP 10.3.1.3/02
Authors (year)	Ansaloni. T. (2021)
Title:	SALAMAN 510: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions.
Document No:	TRIALCAMP Study report SC20-08783
Guidelines:	ENV/JM/MONO (2016) 34: Guidance Document on Honey bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure (OECD 239).
GLP:	Yes
Deviations	- Recorded temperature in the incubator was slightly above 35 ºC (maximum 35.1 ºC) during most of the D7 – D15 period. - Recorded Relative Humidity in the incubator was slightly above 85 % (maximum 86.7 %) during approximately 19 hours and 30 minutes in the D7 – D15 period. - Storing temperature of the diet was slightly above 5 ºC (maximum 5.4 ºC) during relatively short periods of time (maximum 3 hours). The aforementioned deviations had no negative impact on the outcome of the study.

Study Objective

The objective of this study was to determine the effects of SALAMAN 510 on honey bee (*Apis mellifera* L.) larvae from repeated exposure, specifically to determine the No Observed Effect Dose/Concentration (NOED/NOEC), the Lowest Observed Effect Dose/Concentration (LOED/LOEC), the Median Effect Dose/Concentration (ED_{50}/EC_{50}) and any EDx/ECx (i.e., ED_{10}/EC_{10} and ED_{20}/EC_{20}) for adult emergence (from D3 to D22), where possible.

Materials and methods

Test item

"SALAMAN 510" (Potassium phosphonate 510 g/L. as Phosphorous acid), batch 2008011, purity for Phosphorous acid 51.0% w/v, density 1.45 g/mL, expiry 20th February 2024.

Reference item

"BAS 152 I" (Dimethoate 98.2% w/w), batch COD-002332, expiry 23rd January 2022.

Test species

First instar larvae (L1) of the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae), were used as test organisms. The larvae were originated from three different bee hives sited in a commercial apiary from the in-house test facility stock near Trialcamp facilities. Bees were foraging on wild flowers. Bee hive exploitation is registered in the Local Government Administration under the official number 176-V-026.

Test design

The study was conducted as a dose response test with a duration of 21 days from grafting on day 1 (D1) to the final assessment on day 22 (D22). It comprised 1 control group (C), 5 test item groups (T1 – T5) with five different concentrations of 540.88, 865.40, 1384.64, 2215.43 and 3544.69 mg t.i./kg diet, equivalent to 190.24, 304.38, 487.01, 779.22 and 1246.76 mg a.s./kg diet a. Based on the cumulative application volume of 140 µL/larva, the corresponding doses were 83.30, 133.27, 213.24, 341.18 and 545.88 µg t.i./larva, equivalent to 29.30, 46.88, 75.00, 120.00 and 192.00 µg a.s./larva. One reference item group (R) with 48 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.39 µg dimethoate/larva, was also included in the study.

For each treatment group, 48 larvae from three different hives were tested. Each hive corresponded to one replicate; 16 larvae from each replicate were used.

Test Units

Larvae were transferred into crystal polystyrene grafting cells (NICOTPLAST) having a diameter of 9 mm. Cells were initially sterilised by submersion in ethanol 70 % (v/v) for 30 min, and were subsequently dried. Each cell was placed into a well of a sterile 48-well cellular culture plate (Greiner Bio One), and the set up was sterilised by exposure to Ultra-violet light during 15 minutes. The open plates of the control groups, all test item treatment groups and the reference item group were placed into hermetically sealed Plexiglas desiccators, containing a dish filled with a saturated potassium sulphate (K_2SO_4) solution in order to keep a water saturated atmosphere from day 1 until day 7. On day 7, the well plates were transferred to another Plexiglas desiccator, containing a dish with a saturated sodium chloride (NaCl) solution in order to maintain a slightly lower relative humidity until day 15. On day 15, each plate was transferred into an emergence box (18 x 13 x 7 cm, all approximate) in an incubator. Bees that emerged in the emergence box had access to aqueous sucrose solution *ad libitum*.

Test Conditions

Summary of the climatic conditions during the test

Climatic Conditions under the Test		D1-D7	D7-D15	D15-D22
Temperature [ºC]	Max.	35.0	35.1*	34.7
	Min.	34.3	34.3	34.3
Relative Humidity [%]	Max.	100.0	100.0**	68.3
	Min.	55.1*	39.4**	51.9

* Deviation described in section 4
** Minimum punctual value caused by he opening of the desiccator and recorded as short term deviation

Application

Test concentrations and doses

Based on the study Monitor requirements, 5 test item concentrations were tested. Calculations were made from the cumulative dose of 192.00 µg a.s./larva with a constant spacing factor of 1.6. Calculations were made with all the decimals, so there may be slight differences with the calculations made by hand.

A reference test was conducted concurrently with the definitive test at a single concentration of 52.80 mg dimethoate/L diet (equivalent to 7.39 µg dimethoate/larva, cumulative). The results of the reference item were used to evaluate the sensitivity of the larvae to a known toxicant.

Application Schedule

App. code	Treatment group	Code	Concentration		Dose [µg t.i./larva] [b]				
			[mg t.i./L diet]	[mg t.i./kg diet] [a]	D3	D4	D5	D6	Total
A1 – A4	Control (Untreated diet)	C	-	-	-	-	-	-	-
	Test item SALAMAN 510	T1	594.96	540.88	11.90	17.85	23.80	29.75	83.30
		T2	951.94	865.40	19.04	28.56	38.08	47.60	133.27
		T3	1523.11	1384.64	30.46	45.69	60.92	76.16	213.24
		T4	2436.98	2215.43	48.74	73.11	97.48	121.85	341.18
		T5	3899.16	3544.69	77.98	116.97	155.97	194.96	545.88
	Reference item (dimethoate) [c]	R	52.80	48.00	1.06	1.58	2.11	2.64	7.39

t.i.: test item (SALAMAN 510); C: control group; T: test item; R: reference item treated group
[a] based on the diet density (1.1 g/mL)
[b] Based on the application volume/day (20 µL/larva at D3; 30 µL/larva at D4; 40 µL/larva at D5; 50 µL/larva at D6) and the cumulative volume of 140 µL/larva (total).
[c] For the reference item, the values indicate the amount of active substance (dimethoate)

The corresponding nominal values, according to the available certificate of analysis, were as follows:

Application Schedule (active substance, Potassium phosphonate)

App. code	Treatment group	Code	Concentration		Dose [µg a.s./larva] [b]				
			[mg a.s./L diet]	[mg a.s./kg diet] [a]	D3	D4	D5	D6	Total
A1 – A4	Test item SALAMAN 510	T1	209.26	190.24	4.19	6.28	8.37	10.46	29.30
		T2	334.82	304.38	6.70	10.04	13.39	16.74	46.88
		T3	535.71	487.01	10.71	16.07	21.43	26.79	75.00
		T4	857.14	779.22	17.14	25.71	34.29	42.86	120.00
		T5	1371.43	1246.75	27.43	41.14	54.86	68.57	192.00

a.s. active substance (Potassium phosphonate)
[a] based on the diet density (1.1 g/mL)
[b] Based on the application volume/day (20 µL/larva at D3; 30 µL/larva at D4; 40 µL/larva at D5; 50 µL/larva at D6) and the cumulative volume of 140 µL/larva (total)

Assessments

Assessment of larval mortality was conducted on D4, D5 and D6 before feeding and on D7 and D8. With the assistance of a stereo microscope, larvae were recorded as dead if no respiration (movement of spiracles) was observed. On D8, during the assessment of mortality, the presence of uneaten food was qualitatively recorded. Assessment of mortality during the pupation phase was conducted on day D15 and assessment of emergence was conducted on D22. At each assessment time, dead larvae and pupae were removed for sanitary reasons.

Statistical Evaluation of Results

Statistical calculations were made with MS Excel 2016 and the statistical program ToxRatPro® Version 3.3.0.

A Chi² 2x2 Table Test with Bonferroni Correction ($\alpha = 0.05$, one sided greater) was used to determine the NOEC / NOED and the LOEC / LOED values.

Since no statistically significant dose/response was found, no valid EC_{10} /ED_{10} and EC_{20}/ED_{20} with 95 % Confidence Limits could be determined. Since mean corrected mortality was < 50 % in all treatments with the test item, no regression analysis for the endpoints and EC_{50}/ED_{50} was performed.

Results

Validity Criteria of the Study

The study was considered valid since validity criteria for both control and reference item were met.

Control — The cumulative larval mortality from day 3 (D3) until day 8 (D8) was ≤ 15 % across all replicates (actual mean value 2.08 %). On day 22 (D22) the adult emergence rate was ≥ 70 % across all replicates (actual mean value 89.58 %).

Reference — The cumulative larval mortality was ≥ 50 % across all replicates on Day 8 (D8) (actual mean value 77.08 %).

Results of the Test

A summary of the mortality results over the test period is presented in the following tables.

Effects of SALAMAN 510 on honey bee (*Apis mellifera* L.) larvae from repeated exposure

Treatment Group	Cumulative Mortality [%]						
[mg t.i./kg diet]	D4	D5	D6	D7	D8	D15	D22
C [0]	0.00	0.00	0.00	0.00	2.08	8.33	10.42
T1 [540.88]	4.17	4.17	6.25	6.25	6.25	14.58	16.67
T2 [865.40]	0.00	2.08	2.08	2.08	6.25	18.75	20.83
T3 [1384.64]	2.08	4.17	4.17	6.25	6.25	16.67	18.75
T4 [2215.43]	2.08	2.08	2.08	4.17	6.25	18.75	25.00
T5 [3544.69]	0.00	0.00	2.08	2.08	2.08	22.92	25.00
R [48.00] [a]	37.50	66.67	72.92	72.92	77.08	87.50	97.92

[a] For the reference item, the values indicate the amount of active substance (dimethoate)
t.i.: test item (SALAMAN 510)

Effects of SALAMAN 510 on honey bee (*Apis mellifera* L.) larvae from repeated exposure (corrected mortality)

Treatment Group	Corrected Mortality [%][a]						
[mg t.i./kg diet]	D4	D5	D6	D7	D8	D15	D22
T1 [540.88]	4.17	4.17	6.25	6.25	4.26	6.82	6.98
T2 [865.40]	0.00	2.08	2.08	2.08	4.26	11.36	11.63
T3 [1384.64]	2.08	4.17	4.17	6.25	4.26	9.09	9.30
T4 [2215.43]	2.08	2.08	2.08	4.17	4.26	11.36	16.28

T5 [3544.69]	0.00	0.00	2.08	2.08	0.00	15.91	16.28

[a] Corrected for control mortality according to Abbott modified by Schneider-Orelli
t.i.: test item (SALAMAN 510)

Mortality during pupation phase (D8-D22) and emergence rate (D22)

Treatment Group [mg t.i./kg diet]	Mortality D8-D15 [%]	Mortality D15-D22 [%]	Mortality D8-D22 [%]	Emergence D22 [%]
C [0]	6.38	2.27	8.51	89.58
T1 [540.88]	8.89	2.44	11.11	83.33
T2 [865.40]	13.33	2.56	15.56	79.17
T3 [1384.64]	11.11	2.50	13.33	81.25
T4 [2215.43]	13.33	7.69	20.00	75.00
T5 [3544.69]	21.28	2.70	23.40	75.00

t.i.: test item (SALAMAN 510)

Mean corrected cumulative larval mortality on day 8 (D8) of the test item treatment groups of 540.88, 865.40, 1384.64, 2215.43 and 3544.69 mg t.i./kg diet was 4.26, 4.26, 4.26, 4.26 and 0.00 %, respectively.

Mean corrected cumulative larval mortality on day 15 (D15) of the test item treatment groups of 540.88, 865.40, 1384.64, 2215.43 and 3544.69 mg t.i./kg diet was 6.82, 11.36, 9.09, 11.36 and 15.91 %, respectively.

Mean corrected cumulative larval mortality on day 22 (D22) of the test item treatment groups of 540.88, 865.40, 1384.64, 2215.43 and 3544.69 mg t.i./kg diet was 6.98, 11.63, 9.30, 16.28 and 16.28 %, respectively. Emergence rate on day 22 was 83.33, 79.17, 81.25, 75.00 and 75.00 %, respectively.

On day 8, no individuals with presence of uneaten food were observed. At the end of the test, in the final assessment of the emergence on day 22, no emerged bees were recorded as being affected (i.e. malformation).

Conclusions

The Endpoints of the study are summarised in the following table.

Endpoints at Emergence on day 22 (D22)

Endpoints	Concentration		Dose	
	mg t.i./kg diet	mg a.s./kg diet	µg t.i./larva	µg a.s./larva
NOEC / NOED	≥ 3544.69	≥ 1246.76	≥ 545.88	≥ 192.00
LOEC / LOED	> 3544.69	> 1246.76	> 545.88	> 192.00
EC_{10} / ED_{10}	n.d.	n.d.	n.d.	n.d.
EC_{20} / ED_{20}	n.d.	n.d.	n.d.	n.d.
EC_{50} / ED_{50}	> 3544.69	> 1246.76	> 545.88	> 192.00

t.i.: test item (SALAMAN 510)
a.s.: active substance (Potassium phosphonate)
Test item equivalences were determined based on the content and density values of the Certificate of Analysis and MSDS
n.d.: not determined

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

Comments of zRMS:

The study was evaluated and accepted.

Salaman 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid) was applied in two application occasions, at a target rate corresponding to 8.75 L formulated product/ha each, one 25 days before full flowering and the second one at full-flowering (BBCH 64-65) in Rapeseed (*Brasica napus L.*) during daily honey bee foraging activity.
The effects on honey bee colonies under confined (semi-field) conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and brood cell development were evaluated.
The study consisted of three treatment groups: the test item group T Salaman 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid), a toxic reference item group R (Insegar containing a fenoxycarb) and a water-treated control C. The test item (T) was applied in two occasion, one 25 days before full-flowering and the second at full-flowering of Brassica napus (BBCH 63-64) during foraging activity of the honeybees while the toxic reference item (R) and tap water (c) were applied after one occasion at the same time of the second application of the test item at full flowering.
The test item was applied at a target rate of 8.75 L formulated product./ha (corresponding to 4.6113 kg a.i./ha).
Test item solutions were prepared shortly before the applications. The applicates were carried out with calibrated portable boom sprayer simulating a commercial application.
The following conditions during applications were met:

- crop at full flowering during application 2 (A2) (BBCH 64-65);
- bees were actively foraging during the applications in C, T and R (≥ 10 bees/m2 per treatment group shortly before application 2 (A2));
- wind speed did not exceed 1.9 m/s during all applications;
- air temperature was higher than 15 ºC and did not exceed 21.5 ºC;
- the accepted spray tolerance of ±10 % per treatment group was met in all treatment groups;
- there was no rainfall within at least four hours after the application.

The mortality was noted following observation scheme:

Activity Code	Time of the test	Timing	Evaluations of number of dead honey bees
EV	Pre-exposure period (inside tunnels)	At least 4DBA2 to 1DBA2	Once at the same time of day in the morning up to noon (dead bee traps)
	Day of application during bee-flight (C, T and R)	0DBA2	Once shortly before start of application in C, T and R
		0DAA2	1 h after application 2 h after application 4 h after application 6 h after application In the evening after daily flight activity of the bees (linen sheets and dead bee traps)
	Exposure period (inside tunnels)	1DAA2 to 7DAA2	Once a day at the same time in the morning up to noon (linen sheets and dead bee traps)
	Post-exposure period (outside tunnels)	8DAA2 to 28±2DAA2	Once a day at the same time in the morning up to noon (dead bee traps)

EV: evaluation; DBA/DAA: Days before/after the application

The flight intensity was noted following observation scheme:

Activity Code	Time of the test	Timing	Evaluations of number of dead honey bees
EV	Pre-exposure period (inside tunnels)	At least 4DBA2 to 1DBA2	Once a day during flight activity of the bees
	Day of application during bee-flight (C, T and R)	0DBA2	Once shortly before start of application
		0DAA2	1 h after application 2 h after application 4 h after application 6 h after application
	Exposure period (inside tunnels)	1DAA2	Three times during flight activity of the bees (preferably in the morning, mid-day and afternoon)
		2DAA2 to 7DAA2	Once a day during flight activity of the bees

EV: evaluation; DBA/DAA: Days before/after the application

The bee brood development in single cells was noted following observation scheme:

Activity Code	Timing	Determined/expected brood stage in marked cells
EV	2(-1)DBA2 (=BFD)	Egg
	BFD+5(±1)	Young to old larvae
	BFD+10(±1)*	Capped cells
	BFD+16(±1)*	Capped cells shortly before hatch
	BFD+22(±1)*	Empty cells or cells containing eggs, young larvae, nectar or pollen

EV: evaluation; BFD: brood area fixing day; DBA/DAA: days before/after application
* Assessments were performed at the monitoring site

The condition of colonies was assessed based on following parameters:

- Colony strength (number of bees, estimation adapted from IMDORF & GERIG, 1999, and IMDORF et al., 1987);
- Presence of a healthy queen (e.g. presence of eggs)
- Pollen storage area and area with nectar or honey (estimation adapted from IMDORF & GERIG, 1999, and IMDORF et al., 1987)
- Area containing cells with eggs, larvae and capped cells (estimation adapted from IMDORF & GERIG, 1999, and IMDORF et al., 1987)

The meteorological data during the study were provided.

The statistics for study results was presented for:

- mortality of worker honey bees, larvae and pupae;
- flight intensity for each treatment group per day.

The observation of honey bees behaviour, development of honey bee in individual cells, condition of the colonies were provided.

No test item (T) related adverse effects on adult honey bee mortality compared to the control group (C) resulted in the mean mortality during the confinement period from 0DAA2 to 7DAA2. On the other hand, T showed significantly differences on adult honey bee mean mortality compared to the control group (C) during the postapplication
period from 0DAA2 to 28DAA2. However, mean mortality data in this period was under the natural colony loss and did not impact on the others parameters evaluated.
No test item related adverse effects on mortality of larvae and pupae were observed in

	T. No reduction in foraging activity was seen in T throughout the study. No unusual behavior was observed in T. The quantitative assessment of brood development in individually marked cells performed in this study revealed that Salaman 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid) at a target rate of 8.75 L formulated product/ha and after two applications did not cause any treatment-related adverse effect on honey bee brood development at the end of the observation period (BFD22). No test item related adverse effects on colony strength or on the development of the brood and food storage area were observed in T.

Report:	KCP 10.3.1.6/01
Authors (year)	Gimeno, I. (2021)
Title:	SALAMAN 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid): A Semi-Field Study to Evaluate Side Effects on the Brood of Honey Bees (*Apis mellifera* L.) in Rapeseed (Brassica napus L.) in Spain in 2021.
Document No:	TrialCamp S.L.U., Report No.: S21-00857
Guidelines:	OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (PISTORIUS *et al*., 2012) OEPP/EPPO Guideline No. 170(4) (2010)
GLP:	Yes
Deviations	Specimens (Cs and Ts) of pollen loads from the Forager Bees sampled on sampling 3 (S3) could not be prepared and shipped to the Test Site for analytical verification. Reason: Forager Bees sampled on S3 did not have any enough pollen loads in both S and R subsamples to achieve an acceptable amount for specimen preparation. Impact on study: None.

Study Objective

This study was designed to determine the potential effects of SALAMAN 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid) on the honey bee (*Apis mellifera* L.) in one test item treatment group (T) after two applications under semi-field conditions in rapeseed in Spain, following the OECD guidance document No. 75, (2007) and partial integration of recommendations by EFSA (2013).

The evaluation of the treatment effects focused on mortality, flight intensity, behaviour, condition of the colonies and development of the bee brood assessed in individually marked cells within a time period of approximately four weeks. Residue loads in pollen and nectar from collected Forager Bees and flowers were determined.

Materials and methods

Test item

"SALAMAN 510" (Potassium phosphonate 510 g/L. as Phosphorous acid), batch 02515, purity for Phosphorous acid 51.0% w/v (nominal) / 52.7% w/v (analytical), density 1.47 g/mL, expiry May 2022.

Reference item

"Insegar" (fenoxycarb 25% w/w), batch SSP9F010.

Test organisms

Honey bee (*Apis mellifera* L.); adult honey bee and bee brood of all stages.

Source***:*** Commercial beehives, adequately fed, healthy and as far as possible disease-free and queen-right. Colonies used were not previously exposed to any chemical treatments within four weeks of test initiation.

Preparation of test organisms

The following criteria for each colony were met:

- At least 2 – 4 brood combs containing eggs, larvae and capped cells.
- At least 2 honey and pollen combs.
- Colonies were visibly free of *Nosema* and *Varroa* disease symptoms and foul brood and other bee diseases.
- All brood stages were present at the start of the test.
- Before start of the test, dead bee traps were fixed in front of each hive of the replicates intended for the biological assessment in order to record the number of dead honey bees per day.

Test design

Control (C):	C: control group (tap-water).
Test Item (T):	8.75 L formulated product/ha (equivalent to 4.6113 kg a.i./ha).
Reference item (R):	1200.00 g formulated product/ha (equivalent 300.00 g a.i./ha).

Treatments included four replicates (tunnels) each. Furthermore, two tunnels (one control and one test item treatment) were used for sampling for analytical verification purposes.

Two applications of the test item treatment were made, one 25 days before full-flowering and the second one at full flowering (BBCH 64-65). Control and reference item treatment groups were applied once at full-flowering (BBCH 64-65) under the same conditions as the application of the test item treatment group.

Dates of work: 25 March 2021 to 17 May 2021

Sampling

Samples of the Forager Bees for preparation of nectar and pollen as well as flowers were taken in three sampling occasions (S1 to S3). Nectar and pollen were prepared as soon as Forager Bees specimens were sampled. All samples were placed in the freezer at < -18 ºC until shipment and kept in dry ice during shipment to the Test Facility/Site.

Analytical verification

An analytical study was performed as a phase of this multisite study. Test item residues were determined to verify the content of test item in the samples taken.

Statistics

Statistical calculations were made with MS Excel 2016 and the statistical program ToxRatPro® Version 3.3.0.

Data from all treatment groups were tested for normality using the Shapiro-Wilk Test ($p > 0.01$) and for homoscedasticity using the Levene Test ($p > 0.01$). Data were statistically compared using t-Test pooled (one side, $p \leq 0.05$). During the exposure phase, right-sided tests were used for mortality of T and R compared to C. When homoscedasticity assumptions were not met Welch-t Test for inhomogeneous variances was used. Furthemore, when normality assumptions were not met Mann-Whitney U Test was used.

Mortality of worker bees was evaluated separately from mortality of larvae/pupae.

The data for brood indices, compensation indices and termination rates were compared to the control using the same pattern as de data of mortality and flight intensity, with left-sided tests for brood and compensation indices and right-sided tests for the termination rates after the application.

Results

Validity of the study

The application procedure resulted in precise test item application rates and a uniform distribution of the treatments over the tunnels. Colonies and crop were in good condition, adequate for the purposes of this study as can be seen by the termination rates of the control colonies and acceptable background mortality. The mean control brood termination rate was 26.90 % on BFD22 and therefore met the validity criteria.

Foraging intensity was sufficiently to ensure sufficient exposure to treated flowers. The reference item caused statistically significant effects on brood development.

The residue analysis of the specimens showed exposure of the colonies to the test item since residue levels of Phosphorous Acid were detected shortly after application 2 and at the end of the exposure period 7DAA2.

Therefore, it can be concluded that bees were exposed to the treated crop since the treated flowers were contaminated and pollen and nectar brought to the hives by the Forager Bees had quantifiable levels of residues.

These findings showed that the test set-up and the statistical analysis of the results were adequate to detect the effects on these parameters in case these occurred.

Mortality

Findings are summarised in the table below.

Mortality of adult worker bees and larvae/pupae per colony

Treatment group		Control (C)	Test item (T)	Reference item (R)
Daily mean mortality (dead worker bees/colony) ± SD	4DBA2 to 0DBA2	62.0 ± 11.2	52.0 ± 12.8	52.6 ± 17.1
	0DAA2	14.0 ± 4.4	38.5* ± 8.2	35.3* ± 8.4
	0DAA2 to 7DAA2	17.2 ± 3.2	28.3 ± 10.9	20.4 ± 2.8
	0DAA2 to 28DAA2	8.0 ± 0.9	13.6* ± 3.9	10.6* ± 2.5
	8DAA2 to 28DAA2	4.5 ± 0.4	8.1* ± 2.6	6.9 ± 2.5
Daily mean mortality (dead larvae + pupae/colony) ± SD	4DBA2 to 0DBA2	2.1 ± 0.4	1.9 ± 0.6	2.3 ± 1.5
	0DAA2	1.5 ± 2.4	2.8 ± 4.2	1.0 ± 0.8
	0DAA2 to 7DAA2	6.1 ± 4.7	3.8 ± 1.5	4.9 ± 3.2
	0DAA2 to 28DAA2	1.9 ± 1.3	1.1 ± 0.5	3.0 ± 0.7
	8DAA2 to 28DAA2	0.3 ± 0.3	0.1 ± 0.2	2.3* ± 1.0

DAA: days after application; DBA: days before application; SD: standard deviation
* Statistically significantly higher than control group

Throughout the period before exposure, mortality of adult bees across all treatments was similar indicating that background mortality was comparable before the start of the exposure.

On the day of the second application (DAA2) adult mortality was statistically significant (t-Test pooled, one side, $\alpha = 0.05$) in both test and reference item treatments groups in comparison to the control group.

During exposure phase from 0 until 7 DAA2, mean mortality of adult bees across all treatments was not statistically significant (t-Test pooled, one side, $\alpha = 0.05$) in comparison to the control during this period. However, statistically significant differences (t-Test pooled, one side, $\alpha = 0.05$) resulted in the daily assessment of 2DAA2 when compared the test item group to control group.

Throughout the post-application period from 0DAA2 to 28DAA2 the mean number of dead adult honey bees in the test item group differed statistically with the control treatment group (t-Test pooled, one side, $\alpha = 0.05$). Data was also analysed for the monitoring period from 8DAA2 to 28DAA2, statistically significant effects were also detected (Welch-t Test, one side, $\alpha = 0.05$). Statistically significant effect also resulted in the mean daily assessments on 12, 18, 19, 20, 21 and 23 DAA2 on the monitoring site period.

The mean number of dead adult bees in the R group show statistically significant differences with the control group over the post-application period from 0DAA2 to 28DAA2 (t-Test pooled, one side, $\alpha = 0.05$).

No statistically significant effects resulted in the monitoring period from 8DAA2 to 28DAA2 (t-Test pooled, one side, $\alpha = 0.05$). Statistically significant effect also resulted in the mean daily assessments on 9, 12 and 21 DAA2 on the monitoring site period.

The mean number of observed dead pupae and larvae before exposure was similar in all treatments groups.

The mean value of the pupae and larvae mortality in the test item treatment was not statistically significant over the post-application periods of 0DAA2 to 7DAA2 (Welch-t Test pooled, one side, $\alpha = 0.05$), 0DAA2 to 28DAA2 (Welch-t Test pooled, one side, $\alpha = 0.05$) and 8 to 28DBAA2 (t-Test pooled, one side, $\alpha = 0.05$) compared to control.

In the monitoring site period on 10, 23, 25 and 26DAA2 the value of the pupae and larvae mortality in the reference item treatment was statistically significant (t-Test, one side, $\alpha = 0.05$; and Mann-Whitney U-test, one side, $\alpha = 0.05$, for 25DAA2 evaluation). The mean value over the post-application period from 0DAA2 to 7DAA2 was not statistically significant (t-Test, one side, $\alpha = 0.05$) while in the monitoring period from 8DAA2 to 28DAA2 a statistically significant effect of the reference item group resulted in comparison to control group (t-Test, one side, $\alpha = 0.05$).

No abnormal behaviour such as locomotion problems, crampling, trembling, inactive bees, etc., were observed throughout the duration of the study in any treatment.

Flight Intensity

Findings are summarised in the table below.

Flight Intensity

Treatment group		Control (C)	Test item (T)	Reference item (R)
Daily mean flight intensity (bees/m²) ± SD	4DBA2 to 0DBA2**	17.0 ± 1.3	21.0 ± 3.0	19.8 ± 1.8
	0DAA2	24.0 ± 1.8	24.1 ± 3.5	18.5* ± 3.9
	1DAA2	15.9 ± 1.0	14.9 ± 2.0	16.7 ± 0.5
	0DAA2 to 7DAA2**	9.5 ± 1.5	10.2 ± 0.5	11.0 ± 1.0

DAA: days after application; DBA: days before application; SD: standard deviation
* Statistically significantly higher than control group
** Assessments on day 4 before application 2 and on day 4 after application 2 were excluded from evaluation due to poor weather conditions (rain) on these days

Mean foraging rates were similar between test item treatment and reference item treatment groups before exposure from 4DBA2 to 0DBA2 and both of them higher than value recorded in control group.

On the application day mean foraging rates were similar between control and test item treatment groups so no test item related effects were observed on that day. On the other hand, mean foraging rate in the reference item group was statistically significant lower (Welch-t Test pooled, $\alpha = 0.05$) in comparison to the control group.

On the day after application 1DAA2 mean foraging rates were similar between treatments groups so no test item related effects were observed (t-Test pooled, $\alpha = 0.05$) in comparison to the control group.

In the post-application period from 0DAA2 to 7DAA2 no statistically significant reduction (t-Test pooled, $\alpha = 0.05$) reduction was observed in any assessment day in the test item treatment group in comparison to the control group. Moreover, mean foraging activity in this period was similar and no statistically significant (t-Test pooled, $\alpha = 0.05$) reduction, in any of the treatments occurred in comparison to the control.

Development of Honey Bee Brood in Individual Cells

Findings are summarised in the table below.

Summary of the brood and compensation indices and termination rates

Treatment	Brood index / Compensation index at x days after brood area fixing day (BFD)				Termination rate (BFD+22)
	+5	+10	+16	+22	[%]
Control	**2.21 / 2.28**	**2.94 / 3.36**	**2.93 / 3.79**	**3.66 / 4.44**	**26.90**
SD	0.63 / 0.55	0.89 / 0.46	0.89 / 0.06	1.12 / 0.19	22.45
Test item T	**0.96* / 1.12***	**1.38 / 2.02**	**1.34 / 2.77**	**1.67 / 3.42**	**66.59**
SD	1.01 / 0.99	1.41 / 1.32	1.38 / 1.24	1.73 / 1.20	34.56
Reference item R	**0.33* / 0.51***	**0.30* / 1.02***	**0.14* / 1.92***	**0.16* / 2.38***	**96.73***
SD	0.38 / 0.50	0.35 / 0.72	0.23 / 1.29	0.26 / 1.11	5.15

BFD: brood area fixing day; SD: standard deviation
* Statistically significantly lower (brood and compensation indices) or higher (termination rate) compared to the control

In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of brood. The mean termination rate at the end of the observation period (BFD22) was at 26.90 %.

In the test item treatment group T, the mean termination rate at the end of the observation period (BFD22) was at 66.59 %. There was found a statistically significant difference (t-Test, one side, $\alpha = 0.05$) compared to the control group in both brood / compensation indices as well as brood termination rate for the first BFD assessment after application 2 (BFD5). Afterwards, there were no further statistically significant differences (t-Test, one side, $\alpha = 0.05$) recorded in comparison to the control group during the whole test until the end of the observation period on BFD22. Furthemore, it can be observed that the mean compensation index, which s an indicator of the recovery of the colonies, significantly increased on BFD10 until BFD22, resulting in a similar status of the colonies in comparison to those of the control group.

In the reference item treatment group R, the post treatment mean values of the brood and compensation indices were clearly lower than those observed in the control, indicating a strong adverse effect. The mean brood and compensation indices as well as the mean termination rates in R were statistically significantly different from the respective values in the control for all post treatment assessments (t-Test, one-sided, $\alpha = 0.05$). The mean termination rate at the end of the observation period (BFD22) was 96.73 %, indicating that the most of the initially marked eggs had not completed its development.

Strength of the Colonies

The overall development of colony strength (mean number of bees per colony) of all treatment groups showed fluctuations in a typical and normal range. The colony strength values of the test item treatment group T were on approximately the same level or even higher during the entire study than the corresponding values of the control group.

No test item related adverse effects on colony strength were observed.

Development of the Brood Area

The mean amount of brood in the colonies (sum of cells containing eggs, larvae, and pupae) was assessed.

Overall, honey bee brood development in the test item treatment group T was not affected when compared to the control group.

Development of the Food Storage Area

The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed.

The majority of the colonies were well provided during the course of the study. Thus, no test item related adverse effects on the development of the food storage area were observed.

Residue Analysis

Samples of pollen, flowers and nectar were analysed for residues of Phosphorous Acid. Honey bees were sampled in two additional replicates (Cs and Ts) before and after the exposure to honey bees started. Pollen loads and honey stomachs were extracted from the honey bees in the Test Facility and sent to the analytical laboratory. Flowers from each Cs – Ts replicates were kept stored in the Test Facility until shipment to the analytical laboratory.

No residues of Phosphorous Acid were detected at or above the LOD in any of the untreated samples.

Findings on the residues on pollen, nectar and flowers are summarised in the table below.

Sample Code	Activity Code / Timing	Commodity	Residue of Phosphorous Acid [mg/kg]	LOQ [mg/kg]
L21-00857-01-Cs-S1-FL-A	S1 / 1DBA2	Flowers	< LOQ	4.89
L21-00857-01-Cs-S3-FL-A	S3 / 7DAA2	Flowers	< LOQ	
L21-00857-01-Ts-S2-FL-A	S2 / 0DAA2	Flowers	260.56	
L21-00857-01-Ts-S3-FL-A	S3 / 7DAA2	Flowers	9.74	
L21-00857-01-Cs-S1-NFB-A	S1 / 1DBA2	NFB	< LOQ	1.96
L21-00857-01-Cs-S3-NFB-A	S3 / 7DAA2	NFB	< LOQ	
L21-00857-01-Ts-S2-NFB-A	S2 / 0DAA2	NFB	255.95	
L21-00857-01-Ts-S3-NFB-A	S3 / 7DAA2	NFB	< LOQ	
L21-00857-01-Cs-S1-PFB-A	S1 / 1DBA2	PFB	< LOQ	1.96
L21-00857-01-Ts-S2-PFB-A	S2 / 0DAA2	PFB	2932.24	

NFB: nectar from Forager Bees (after preparation);
PFB: pollen from Forager Bees (after preparation);
LOQ: Limit of Quantification.

Conclusion

No test item (T) related adverse effects on adult honey bee mortality compared to the control group (C) resulted in the mean mortality during the confinement period from 0DAA2 to 7DAA2. On the other hand, T showed significantly differences on adult honey bee mean mortality compared to the control group (C) during the post-application period from 0DAA2 to 28DAA2. However, mean mortality data in this period was under the natural colony loss and did not impact on the other parameters evaluated.

No test item related adverse effects on mortality of larvae and pupae were observed in test item (T).

No reduction in foraging activity was seen in test item (T) throughout the study.

No unusual behaviour was observed in test item (T).

The quantitative assessment of brood development in individually marked cells performed in this study revealed that Salaman 510 (Potassium Phosphonate 510 g/L, as Phosphorous Acid) at a target rate of 8.75 L formulated product/ha and after two applications did not cause any treatment-related adverse effect on honey bee brood development at the end of the observation period (BFD22).

On the other hand, brood development in individually marked cells of the reference item group showed a statistically significant differences for both brood and compensation indices as well as the brood termination rate compared to the control group in all of the assessment performed. Moreover, statistically significant effect in the mean number of dead larvae and pupae resulted when compared the reference item to the control group in the monitoring period from 8DAA2 to 28DAA2. Therefore, sensitivity of the test was proven.

No test item related adverse effects on colony strength or on the development of the brood and food storage area were observed in T.

Analytical verification confirmed that the honey bees were adequately exposed to the test item.

A 2.3.2 KCP 10.3.2 Effects on arthropods (other than bees)

Comments of zRMS:	The study was accepted. The study was conducted according to method based on guidelines proposed by Vogt, et al., 2000 and in accordance with the recommendations of the ESCORT workshops for higher tier testing. The validity criteria were met. Two rates of the test item, 5.7500 and 20.1250 L/ha of formulated product /ha were tested. The following observation were noted: • No lethal effects have been found with the rate 5.75 L FP/ha (6.7% mortality); • Juvenile mortality was 40% for 20.1250 L/ha; • No effect on fecundity and fertility was observed for the test substance at a rate of 5.75 L/FP/ha; • A reduction on fecundity 7.33 eggs per female was observed at a rate of 20.1250 L FP/ha.

The studies summarized in this point has been already submitted for the authorization of the product.

Report:	KCP 10.3.2/01
Authors (year)	Luna (2013)a
Title:	Side-effects of the product "SALAMAN 510" (Potassium phosphite 510 g/L, as Phosphorous acid) on *Chrysoperla carnea* (Neuroptera: Chrysopidae) under laboratory conditions**.**
Document No:	TRIALCAMP Study report TRC12-286BA
Guidelines:	Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC. WPRS/SROP. Edit: M.P Candolfi *et al.* Gent: 121-144
GLP:	Yes
Deviations	--

A study was carried out to determine the effects of fresh and dry residues of the formulation "SALAMAN 510" (Potassium phosphate 510 g/L) on the green lacewing (*Chrysoperla carnea*).

The study was carried out under GLP conditions and according to OECD Good Laboratory Practice Standards, the study plan TRC11-286BA and the appropriate SOPs. The study was conducted in accordance with the recommendations of the ESCORT workshops for higher tier testing (Barrett *et al.*, 1994 and Candolfi *et al*., 2000). The method is based on guidelines proposed by Vogt, *et al*., 2000.

Two rates of the test substance, 5.7500 and 20.1250 L/ha of formulated product (FP) /ha were assayed in a program of one application. After drying the residue during approximately 1-hour and 30 minutes on the application day, larvae 2-3 days old of *C. carnea* were exposed on the treated glass plates. Juvenile mortality of the larvae exposed to residues as well as sub-lethal effects on the reproductive performance of the emerging adults, were evaluated.

Application was performed on glass plates with a laboratory track sprayer with a CO2regulator, working at 300 kPa of pressure. Glass plates were sprayed at an application volume of 200 L/ha with the test substance and a water control and the reference substance (Dimethoate 400 g/l EC at 0.1%). The equipment was calibrated before each treatment application with five glass plates to verify that the fluid was 2.0 mg/cm2 ±10% (200 L/ha). To check the correct application of treatments, the extra glass plates (five) were placed among the glass plates in each treatment. The applications were considered acceptable.

First instar larvae of *Chrysoperla carnea* (2-3 days old) were isolated and exposed to the residues on glass. The larvae were continuously exposed to the residue on the glass until pupation.

Exposure was performed at 0 days after application. Thirty glass plates per treatment were placed in the exposure chambers.

Exposure to the residues and reproductive performance were carried out under controlled conditions; an environmental controlled room at 25 ± 2ºC, 60-90% RH, light regime of 16 hours light and 8 hours dark and a light intensity above 1000 lux.

Total juvenile mortality (larvae and pupae mortality) was significantly different (Fisher's exact test, 1-sided) when compared to the control for the assayed rate 20.1250 L FP/Ha; juvenile mortality was 40% for this treatment and 3.57% for the control. No lethal effects have been found with the assayed rate 5.75 L FP/ha (6.7% mortality). Mortality in the reference substance treatment was 100%.

No effect on fecundity and fertility was observed for the test substance at a rate of 5.75 L FP/Ha, since fecundity (mean number of eggs per female and day) was above 15 (24.88) and fertility (mean hatching rate) was above 70% (96.52%). These values were similar to those at the control treatment (23.05 eggs per female and 96.17% as hatching rate).

A reduction on fecundity was observed when the test product was applied at 20.1250 L FP/ha; 7.33 eggs per female, below the trigger value of 15 eggs per female. No effects were observed on fertility with 100% as hatching rate.

TRT	Test item	Rate L Test item/ha	Mortality (%)	Corrected mortality (%)	Fecundity Mean eggs per female	Fertility Mean eggs viability (%)
C	Control	--	3.6	--	23.05	96.17
T1	Salaman	5.75	6.7	3	24.88	96.52
T2	Salaman	20.125	40.0*	38	7.33**	100
R	Dimethoate 40% EC	0.2 (0.1%)	100	100	--	--

*Significantly different compared to the control (Fisher's Exact Test, 1 sided).
**Fecundity rate below the trigger value of 15 eggs / female / day.

* * * * *

Comments of zRMS:	The study was accepted. The study was conducted according to IOBC (Blümel *et al.* 2000), modified for the exposure on natural substrate (extended laboratory test). This study fulfils criteria of 2D study (extended laboratory study with formulation Salaman 510 and bean leaf discs). The validity criteria were met: • maximum acceptable cumulative mortality (dead larvae and pupae, adults dying during emergence or not successfully moulted): ≤20% (7.41%); • fecundity (mean number of eggs per female and day): ≥15 (40.75; • fertility (mean hatching rate): ≥70% (99.67%); • mortality in the reference substance treatment was higher than 50% (100%). The following endpoint for *Typhlodromus pyri* was derived: • LR_{50} > 24.0 L product/ha in 200 L water/ha;

Report:	KCP 10.3.2/02
Authors (year)	Luna (2013)c
Title:	An extended laboratory test to determine the LR_{50} of the product "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae)
Document No:	TRIALCAMP Study report TRC12-015BA
Guidelines:	Guidelines to evaluate side-effects of plant protection products to non-target arthropods.

	IOBC. WPRS/SROP. Edit: M.P Candolfi *et al*. Gent: 121-144
GLP:	Yes
Deviations	--

The aim of the study is to determine the LR_{50} with one application of the fungicide "SALAMAN 510" (Potassium phosphite 510 g/L as Phosphorous acid) on the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) under extended laboratory conditions.

The study was carried out under GLP conditions and according to OECD Good Laboratory Practice Standards. The trial was codified as TRC12-015BA.

The proposed rates were obtained according results from a preliminary non GLP range-finder test. A range of five rates of the test substance from 1.5 to 24.0 L/ha of formulated product was sprayed with a laboratory sprayer (Potter Tower).

Bean leaves (*Phaseolus vulgaris*) were used as a substrate for the tests according to Pia Ternes *et al*. (2001). Leaves were cut in fragments of 1.5 x 5 cm. The fragments were placed with the adaxial surface upward on moist filter paper in Petri dishes. The fragments were sprayed per treatment by means of the Potter Tower. The droplets covered uniformly paper surface. and the deposit was 2 ± 0.2 mg fluid/cm2. equivalent to a spray volume of 200 L/ha. Test was validated with a control treated with water and a reference substance (Dimethoate 400 g/L EC at 0.2 L/ha).

The treated fragments were maintained at ambient temperature in the laboratory during approximately 1 hour and 30 minutes until spray residue was dry. When dry. leaves were placed in the exposure units and mites were introduced. Five replicates per treatment were evaluated; each replicate consisted of 20 protonymphs less than 24 hours old that were exposed to the fresh dry residues.

The test units were placed into an environmental chamber at 25 ± 2 ºC. 60-90% RH. with a 16:8h L:D. These conditions were achieved throughout the study.

Mortality was evaluated after 1. 3. 7 days of exposure. Living. dead and escaped mites were counted in each of the five plates per treatment. The average of mortality has been expressed as a percentage of the number of dead and escaped individuals at the end of the exposure. Seven days after application. sex ratio of the surviving mites was determined. and fecundity was evaluated over a 7 days period.

Results and Conclusions

The estimated LR_{50} for the test product "SALAMAN 510" on the predatory mite *Typhlodromus pyri* was 24 L of formulated product /ha (equivalent to 13538.4 g potassium phosphate /ha according the certificate of analysis). A significant difference was only observed on lethal effects with the rate of 24.0 L FP /ha (13538.4 g potassium phosphate /ha) with a 23.81% of corrected mortality. below the trigger value of 50%.

The treatments of the test substance showed mortalities less than 50% so all them were used in the reproduction test (T1 to T5; 1.5 to 24.0 L /ha of formulated product). The effect on the reproductive capacity with the assayed rates was below the ESCORT 2 trigger value of 50%. A significant reduction on fecundity was only observed at the end of the study period with the rate 24.0 L /ha of formulated product (28.98% reduction with respect to the control).

Toxicity test	**LR_{50} – 7 days**
Test product	**LR_{50}> 24.0 L SALAMAN/Ha**
Active ingredient (H_3PO_3)	**LR_{50}> 13538.4 g H_3PO_3/Ha**

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Comments of zRMS:	The study was accepted. The study was conducted according to IOBC (Mead-Briggs *et al.* 2000). This study fulfils criteria of 3D study (extended laboratory study with formulation Salaman 510 and potted barley plants). The validity criteria were met.

	The LR_{50} for the test product "SALAMAN 510" on the parasitic wasp *Aphidius rhopalosiphi* was estimated greater than the maximum assayed rate 24.0 L formulated product/ha (equivalent to 13538.4 g potassium phosphate /ha according the certificate of analysis). No lethal effects were found with the assayed rates from 1.5 to 24.0 L formulated product/ha. The following endpoints for *Aphidius rhopalosiphi* were derived: • LR_{50} > 24 L product/ha for 200 L water/ha;

Report:	KCP 10.3.2/03
Authors (year)	Luna (2013)b
Title:	An extended laboratory test to determine the LR50 of the product "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)
Document No:	TRIALCAMP Study report TRC12-014BA
Guidelines:	Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC. WPRS/SROP. Edit: M.P Candolfi *et al.* Gent: 13-25
GLP:	Yes
Deviations	--

Methods:

An extended laboratory study was carried out to generate data on dose-response toxicity of the formulation "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) to *Aphidius rhopalosiphi* DeStephani Perez (Hymenoptera: Braconidae) under extended laboratory conditions in order to calculate the LR_{50}-values (application dose killing 50 % of the exposed organisms). The application of test substance and exposition of parasitoids were over bean leaves as foliar substrate.

The study was carried out under GLP conditions and according to OECD Good Laboratory Practice Standards. The trial was codified as TRC12-014BA.

The protocol was employed using appropriate SOP's and conducted under the guideline: "*A laboratory test for evaluating the effects of plant protection products on the parasitic wasp. Aphidius rhopalosiphi DeStephani-Perez (Hymenoptera: Braconidae)*" (Mead-Briggs M.A. *et al*. 2000) and the modifications described in Grimm *et al.* (2002) for the methodology using the excised leaf test method.

The endpoints were the following:

(1) To study the mortality at 48 hours after application (lethal effect)

(2) To study the fecundity of the survival females during one day in presence of their hosts.

The proposed rates were obtained according results from a preliminary non GLP range-finder test.

They were selected according to a geometrical progression (factor 2). A range of five rates of the test substance from 1.5 to 24.0 L/ha of formulated product was sprayed with a laboratory sprayer (Potter Tower). The Potter Tower was calibrated to deliver a target of 2 ± 0.2 mg spray solution/cm^2 corresponding to 200 L/ha and it was tested for the target rate of the test substance. the water control and a toxic standard. Dimethoate 40% EC. at 15 mL/ha. The leaves were air-dried in the laboratory for approximately 1 hour 30 minutes prior to the introduction of the wasps.

For mortality test. the exposure test unit (arenas) consisted of two treated bean leaf disks (3.8 cm diameter) fixed to plastic Petri dish (4 cm diameter) by means of a thin layer of Agar. Four replicates for each treatment were used and 10 adult wasps were placed in each arena. Once the wasps were introduced in the arenas. the test units were placed into an environmental chamber at 20 ± 2 ºC. 60-90% RH. with a 16:8h L:D photoperiod. Mortality assessments were carried out after 24 and 48 hours of exposure.

To assess any effects on the relative fecundity of the surviving insects. 15 surviving females per treatment were taken after to study mortality. These females were individually confined inside pots of aphid-infested cereal plants enclosed in plastic ventilated cylinders. After a period of 24h. females were removed again, and the survivors were disposed of by means of freezing.

The parasitized aphids within the fecundity arenas were left to develop in situ and the number of aphid mummies that develop was recorded 10 days after parasitization. Fecundity was expressed as number of mummies per female.

Results:

No effects on mortality and acceptable reproductive capacity were observed during the 48-hour exposure period in the control group. The toxic reference substance produced 90% mortality and confirmed the sensitivity of the test species and the test conditions.

Conclusion:

The LR50 for the test product "SALAMAN 510" on the parasitic wasp *Aphidius rhopalosiphi* was estimated greater than the maximum assayed rate 24.0 l formulated product/ha (equivalent to 13538.4 g potassium phosphate /ha according the certificate of analysis). No lethal effects were found with the assayed rates from 1.5 to 24.0 L formulated product/ha.

No significant adverse effects on the reproductive capacity of the parasitic wasp. *Aphidius rhopalosiphi*. were found for the assayed rates 1.5. 3. 6. 12 and 24 L of formulated product /ha. Reduction on fecundity with the assayed rates was always less than the ESCORT 2 trigger value of 50%.

Toxicity test	**LR_{50} – 7 days**
Test product	**LR_{50}> 24.0 L SALAMAN/Ha**
Active ingredient (H_3PO_3)	**LR_{50}> 13538.4 g H_3PO_3/Ha**

*** * * * ***

Comments of zRMS:	The study was accepted. The field study was conducted according to IOBC (Blumel *et al*. 2000). The immediate reduction, the persistence and the recovery potential of the predatory mites has been studied in a citrus orchard in commercial production. An untreated control and a reference substance were used to validate the results. The predatory mite population was assessed before the applications and at different intervals until 27 days after 3rd application. The distribution and density of predatory mites was studied before the first application of the test and reference substances, and plots were arranged according to a random design (EPPO guideline number PP 1/152(2)). The plot size was 60 m2 (3 trees). No significant difference between treatments was found at the beginning of the study (before the application) with the number of phytoseiids per leaf or number of leaves with presence of phytoseiids. The meteorological data (weather conditions) were collected. The statistical analysis was provided. For each treatment, a statistical analysis of the homogeneity and normality of replicate results was made using Levene' and Kolmogoroff-Smirnov's test procedures with results of the mean number of predators/leaf per plot, and the percentage of leaves with presence of mobile forms per plot. The three applications of the product "SALAMAN 510" at the rate of 8.75 L FP /ha did not cause significant reduction in the *E. stipulatus* population at any of the assessment occasions. Population levels in the treatment of the test substance were similar to the

	control treatment during the test period. *E. stipulatus* was increasing until after the 3rd application (C) in both treatments, control and "SALAMAN 510", and then, the population decreased. These changes in the populations of *E. stipulatus* correspond to the population dynamics of this species.

Report:	KCP 10.3.2/04
Authors (year)	Luna (2013)e
Title:	Side-effects of the formulated product "SALAMAN 510" (Potassium phosphite 510 g/L. SL) on the predatory mite. *Euseius stipulatus* (Athias-Henriot) (Acari: Phytoseiidae) in citrus under field conditions.
Document No:	TRIALCAMP Study report TRC12-283BA
Guidelines:	Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC. WPRS/SROP. Edit: M.P Candolfi *et al.* Gent: 121-144
GLP:	Yes
Deviations	--

A field study was conducted to assess the effect of the product "SALAMAN 510" (Potassium phosphite 510 g/L. SL) on population dynamics of the predatory mite *Euseius stipulatus* (Athias-Henriot) following three applications on citrus. The objective was to evaluate the immediate reduction. the persistence and the recovery potential of the phytoseiid mite populations.

The study was conducted in the framework of a protocol based on "Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards" (Blümel *et al*. 2000) and in accordance with GLP standards.

A field trial was conducted in Alfafar (Valencia. Spain). The trial site chosen was representative area of the test system (citrus) in Spain and had a stable predatory mite population. The crop was grown in a way typical of the producing region. At the beginning of the study the predatory mite population level in the different plots was uniform.

The test was performed in a random design with 3 treatments; five replicates (plots) for the control and test substance treatments. and 3 replicates for the reference substance treatment. Three trees per plot (60 m2) were used. At least one unsprayed buffer tree surrounded each of the plots.

The test product was tested at one rate: 8.75 L per hectare (4.9359 kg of active substance /ha according the certificate of analysis). A reference product (Mancozeb 80% WP at 0. 4%) and an untreated control were concurrently tested.

The test product "SALAMAN 510" (Potassium phosphite 510 g/L. SL) was applied three times at 20 days' interval (treatments T). The reference product (treatment R) was applied at the same time of the test substance. The control plots (Treatment U) were sprayed three times with water too. Plots were applied starting with control plots. thereafter the test substance. and finally. the toxic reference treatment.

The applications were performed according to the Good Agricultural Practices with a phonological development of citrus plants (BBCH-scale) of 76-79-81 (applications A. B and C respectively). The application equipment consisted of a motorized Maruyama backpack sprayer at 1800 kPa of pressure equipped with a handheld with one hollow cone nozzle (Albuz ATR Hollow Cone 1.2 ceramic Nozzle) simulating a conventional application in field (volume 2000 L/ha).

The equipment was calibrated before each application. Calibrations were performed at the Trial Site by using the volume/time method for liquid applications. Calibration runs (three independent runs) verified that the system was operating consistently. uniformly and as expected.

The application volume was fixed at 2000 L/ha according the local training system for the crop development and the size of canopy.

Spray mixture volume remaining after application was measured and the volume applied to the plots was calculated to determine actual delivery rates. It was verified that the volume applied did not differ by more than 10% of the delivery rate. So, applications were considered acceptable.

Assessments of population were performed before each application event and at defined intervals after applications. Leaves were observed immediately after being picked out with caution of predators did not fall.

30 leaves per plot were collected at random at each assessment; leaves were collected from at least four different orientations and from the inside canopy. The numbers of mobile stages of phytoseiid mites were counted in-situ with the help of magnifying glasses. The main specie found as phytoseiid mite was *Euseius stipulatus* (Athias-Henriot).

Mobile forms of *E. Stipulates* per plot for each treatment and sampling date. the mean number of predators/leaf per plot. and the percentage of leaves with presence of mobile forms per plot have been summarized and presented in the raw data.

The effect has been expressed as a percentage of reduction of the phytoseiid mite populations in test and reference product treatments compared to the control. calculated according to Abbott's formula. Data were analysed with a Dunnett's test at 95% when normality and homogeneity of variance were obtained. otherwise the non-parametric test. Mann-Whitney U was used.

Throughout the study, mean numbers of mites in control plots (30 leaves per plot) were acceptable and are considered to be representative of populations found in citrus orchards in Spain. The different population level in the performed assessments is considered in accordance to the population dynamic of the phytoseiid mite *Euseius stipulatus.*

The reference substance "Mancozeb 80% WP" had an effect of more than 50% compared with the controls from 18 days after the 1st application "A" (assessment 3 days before the 2nd application "B") and thus confirms the suitability of the test system and the study site.

The test substance, "SALAMAN 510". applied at a field rate of 8.75 L/ha of formulated product (FP) had no detrimental effect in populations of phytoseiid mites when compared to the control after one. two or three applications. The mean number of predators/leaf per plot and the percentage of leaves with presence of mobile forms per plot were similar to the control treatment.

It can therefore be concluded that after three applications of "SALAMAN 510" (Potassium phosphite 510 g/L. SL) applied at the rate of 8.75 L/ha with an interval of 20 days. are not be expected a reduction in phytoseiid mite populations.

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Comments of zRMS:	The study was accepted. The study was conducted according to method based on guidelines proposed by Vogt, *et al*., 2000 and in accordance with the recommendations of the ESCORT workshops for higher tier testing. This study fulfils criteria of extended laboratory study with formulation Salaman 510 sprayed on bean leaflets. Two rates of the test item, 5.7500 and 20.1250 L/ha of formulated product /ha were tested. The validity criteria were met. • maximum acceptable cumulative mortality (dead larvae and pupae, adults dying during emergence or not successfully moulted): ≤20% (7.41%); • fecundity (mean number of eggs per female and day): ≥15 (40.75); • fertility (mean hatching rate): ≥70% (99.67%); • mortality in the reference substance treatment was higher than 50% (100%).

	The following observation were noted: • no dose-related lethal effects were observed with the test product at the assayed rate of 5.75 L of formulated product/ha. • no significant difference compared to control was found when the results were statistical analyzed with the Fisher's exact Test (α=0.05). • mortality for this rate was below 20% (6.90%). • pre-adult mortality with the test substance at 20.1250 L /ha was less than 50% (42.4% corrected mortality); • a reduction respect to the control on fecundity was observed with the assayed rates of the test substance of 5.75 and 20.1250 L /ha, but the mean number of eggs per female and day was always above the trigger value of 15 (33.56 and 22.06 respectively); • fertility (mean hatching rate) was always above 70% with all assayed rates of the test substance and control treatments, so no effects on fertility were observed (99.81% and 98.70% egg viability for the rates 5.75 and 20.1250 L /ha respectively). LR_{50} >20.125 L formulated product/ha.

Report:	KCP 10.3.2/05
Authors (year)	Luna (2013)d
Title:	Side-effects of the product "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) on *Chrysoperla carnea* (Neuroptera: Chrysopidae) under extended laboratory conditions.
Document No:	TRIALCAMP Study report TRC12-156BA
Guidelines:	Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC. WPRS/SROP. Edit: M.P Candolfi *et al.* Gent: 121-144
GLP:	Yes
Deviations	--

The aim of the study was to determine under extended laboratory conditions the effects of fresh residues of the formulation "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) applied to bean leaflet. on the green lacewing *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae).

The study was carried out under GLP conditions and according to OECD Good Laboratory Practice Standards. the study plan TRC13-156BA and the appropriate SOPs. The study was conducted in accordance with the recommendations of the ESCORT workshops for higher tier testing (Barrett *et al.* 1994 and Candolfi *et al.* 2000). The method was based on guidelines proposed by Vogt. *et al.* 2000.

The test substance was sprayed at 2 different rates provided by the sponsors: 5.75 and 20.1250 L of formulated product /ha. A reference substance treatment (Dimethoate 400 g/L EC at 0.200 L/ha) and a water control was included in the test design:

TRT	Test item	Rate L Test item/ha	g/ha(1) H_3PO_3	Description
C	Water	--	--	control
T1	SALAMAN	5.75	3243.58	FIELD RATE; Grapevine. 3 appl. at rate 2.50 L FP/ha (MAF = 2.3)*
T2	SALAMAN	20.125	11352.51	FIELD RATE; Citrus. 3 appl. at rate 8.75 L FP/ha (MAF = 2.3)*
R	Dimethoate 40% EC	0.200	82.8	Toxic reference substance

The test substance rates have been recorded as L of formulated product (FP) per ha and the corresponding rate of active substance has been calculated according the analysis certificate. 564.1 g/L (1). For Reference substance. Dimethoate. 414 g/L in the analysis certificate and 400 g/L. as nominal value.
* MAF = multiple application factor in case that the product is applied two or more times.

Application was performed on bean leaflets (*Phaseolus vulgaris*) with a laboratory track sprayer with a CO2 regulator. working at 300 kPa of pressure. The equipment was calibrated before the application with four glass plates to verify that the fluid was 2 mg/cm2 ± 10% (200 L/ha). To check the correct application of treatments. the glass plates were placed among leaves in each treatment. The applications were considered acceptable.

First instar larvae of *Chrysoperla carnea* (2-3 days old) were isolated and exposed to the fresh and dry residues on the leaves. The larvae were continuously exposed to the residue on the leaves until pupation. Thirty larvae per treatment were individually confined within test units.

Exposure to residues was performed in an environmental controlled room at 25 ± 2ºC. 60- 91% RH. light regime of 16 hours light and 8 hours dark and a light intensity above 1000 lux. Juvenile mortality when larvae are exposed to residues on the leaf until pupation as well as sub-lethal effects on the reproductive performance (fecundity and fertility) of the emerging adults was evaluated.

Under the conditions of the present study (extended laboratory). it can be concluded that:

1. Pre-adult mortality (larvae and pupae) in the control treatment was less than 20% (7.41%). Mortality in the reference substance treatment was 100%.
2. No dose related lethal effects of the test substance "SALAMAN 510" on *Chrysoperla carnea* at the assayed rate of 5.75 L /ha was observed (corrected juvenile mortality was -0.6%); no significant difference was found with statistical analysis (Fisher's exact Test. α=0.05).
3. Pre-adult mortality with the test substance at 20.1250 L /ha was less than 50% (42.4% corrected mortality). Significant difference compared to the control with statistical analysis (Fisher's exact Test. α=0.05) was found.
4. A reduction respect to the control on fecundity was observed with the assayed rates of the test substance of 5.75 and 20.1250 L /ha. but the mean number of eggs per female and day was always above 15 (33.56 and 22.06 respectively).
5. Fertility (mean hatching rate) was always above 70% with all assayed rates of the test substance and control treatments. so no effects on fertility were observed (99.81% and 98.70% viability for the rates 5.75 and 20.1250 L /ha respectively).

It can therefore be concluded. that the product "SALAMAN 510". applied at rates 5.75 and 20.1250 L of formulated product /ha. is not expected to cause reductions in *Chrysoperla carnea* populations. according the results with these extended laboratory conditions.

TRT	Test item	Rate L Test item/ha	Mortality (%)	Corrected mortality (%)	Fecundity Mean eggs per female	Fertility Mean eggs viability(%)
C	Control	--	7.41	--	40.75	99.67
T1	SALAMAN	5.75	6.90	-0.6	35.56	99.81
T2	SALAMAN	20.125	46.67*	42.4	22.06	98.70
R	Dimethoate 40% EC	0.200	100	100	--	

(*): Significantly different compared to the control (Fisher's Exact Test).

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was accepted. The study was conducted according to OECD 222 guideline and ISO 11268 – 2: 1998.

	The validity criteria were met: • the mean rate of juveniles' production was of 327.75 ± 31.68 individuals per control container (mean ± SE), required at least 30 per control container; • the coefficient of variation of reproduction in the control was 19.33%, required not to exceed 30%; • the percent mortality of the adults observed in the control was 0.00%, required – less than 10. The NOEC for mortality and reproduction was determined to be 315.20 mg test item/kg soil dry weight equivalent to 119.78 mg Potassium phosphite/kg s dw.

Report:	KCP 10.4.1.1/01
Authors (year)	Ansaloni (2012)
Title:	A laboratory test to determine the chronic (sub-lethal) effects of "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) to the earthworm *Eisenia foetida* (Oligochaeta: Lumbricidae)
Document No:	TRIALCAMP Study report TRC11-295BA
Guidelines:	ISO 11268-2:1998 and OECD 222:2004
GLP:	Yes
Deviations	--

Methods

A study was carried out under laboratory conditions with the objective to determine the effects of the formulated product of formulated product "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) on the reproduction. growth and percentage mortality of the earthworm *Eisenia foetida* (Oligochaeta: Lumbricidae) after 28 days exposure to an artificial substrate treated with five test item concentrations.

The study followed OECD guideline 222 and the ISO 11268 – 2: 1998 and was conducted under study code TRC11-295BA.

Potassium phosphite 510 g/L. as Phosphorous acid. (SE12057/02). batch 1132015. purity 38.0% w/w (564.1g/L). density 1.4846g/ml. expiry 4 August 2013.

Adult earthworms of the species *Eisenia foetida* between two months and one year old. with clitellum and a wet mass between 250 mg and 600 mg. The test individuals were selected from a synchronised population and did not differ in age by more than 4 weeks. The earthworms were maintained under conditions identical to the experimental conditions for 2 days before the start of the study.

***Test product concentrations*:** Adult earthworms were exposed in an artificial soil (OECD. 10% peat) treated with "SALAMAN 510" at sub-lethal concentrations of 19.70. 39.40. 78.80. 157.60 and 315.20mg formulated product/kg dry soil and evaluated on day 28 after exposure for mortality and weight variation and on day 56 for reproduction.

The concentrations of the test product were selected with a spacing factor of 2. In addition. the fourth concentration (T4) of the selected range corresponds to approximately 5-fold the maximum Predicted Environmental Concentration (PEC) of the formulated product that can occur as residue in a 5cm soil layer.

***Experimental units*:** The experimental units consisted of glass containers, with capacity of approximately 1.5 liters, filled with 500g (dry weight) of either the treated or untreated (control) artificial soil mixed with 60% of its water holding capacity of de-ionized water. Four replicates consisting of 10 worms were evaluated for each treatment.

***Assessments*:** Assessment on adults' mortality and weight variation was carried out on day 28 after the application. and assessment on offspring production was carried out on day 56 after the application.

***Toxic reference*:** The toxic standard Carbendazim (OECD 222: 2004) is regularly tested; the last test was performed between February and April 2012.

Results

Mortality and weight variation: No mortality was observed for the control group and any of the concentrations of "SALAMAN 510" tested. Average weight variation at 28 days after the application with respect to the initial weight was positive (body mass gain) for the control group and all treatments with the test product. No significant difference in weight variation was observed for any of the treatments with the test product as compared to the control group.

Reproduction: Mean offspring production of the control individuals was of 327.75 ± 31.68 individuals (mean ± SE). Mean offspring production of the treated *E. foetida* adults ranged from 235.75 ± 34.25 individuals at the second highest rate tested (157.60mg f.p./kg dry soil) to 293.25 ± 17.35 individuals at the second lowest rate tested (39.40mg f.p./kg dry soil). Since no significant effect at the highest rate tested of 315.20mg f.p./kg dry soil was observed. significant effect in offspring production observed at a rate of 157.60mg f.p./kg dry soil is considered to be due to biological variability and not dose related (Dunnett's test. _ = 0.05).

Conclusion

Mortality. weight variation or offspring production of *Eisenia foetida* were not negatively affected by the assayed concentrations of "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) as compared to the control group. Therefore. it was determined that the 'No Observed Effect Concentration' (NOEC) under the conditions of this study for the test product on the earthworm *Eisenia foetida* corresponded to the highest rate tested of 315.20mg formulated product/kg dry soil. equivalent to 119.78mg Potassium phosphite. as Phosphorous acid/kg dry soil (analytical content).

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The study was accepted. The study was conducted according to OECD guideline 232. The validity criteria were met. The insignificant deviation was noted: The assessment of mortality and reproduction was carried out at 29 days after the application (DAA) instead 28 DAA as the study plan indicates. This fact had no negative effects on the study, as the OECD guideline 232 and the SOP of Trialcamp allows performing the assessment at 28 ± 1 DAA. In a 28-day *Folsomia candida* reproduction study, the LC_{50} > 1000 mg test item/kg soil dry weight, equivalent to 384.36 mg phosphorous /kg dry soil. the highest tested concentration. The NOEC for mortality was determined to be 185.64 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight, equivalent to 384.36 mg phosphorous /kg dry soil. The LOEC (lowest observed effect concentration) for the reproduction capacity was estimated to be greater than 1000 mg test item /kg dry soil, equivalent to 384.36 mg

	phosphorous /kg dry soil.

Report:	KCP 10.4.2.1/01
Authors (year)	Luna. F. (2015)
Title:	Effects of the formulation "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) on the non-target soil arthropod. *Folsomia candida* (Collembola. Isotomidae)
Document No:	TRIALCAMO Study report TRC13-297BA
Guidelines:	OECD/OCDE nº 232 (September. 2009)
GLP:	Yes
Deviations	--

Introduction

The fungicide "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) has been tested under laboratory conditions to assess the effects of a range of concentrations on the springtail *Folsomia candida* (Willem) (Collembola. Isotomidae).

The study was carried out at Trialcamp facilities under GLP conditions and according to OECD Good Laboratory Practice Standards and the OECD Guideline for testing chemicals "232": "Collembolan Reproduction Test in Soil"). The trial was codified as TRC13-297BA.

Materials and methods

Test Product

"SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid); Batch: 1132015; Purity: Phosphorous co tent 57.1% w/v (570.7 g/L)

Test design

The formulation "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) was evaluated with eight concentrations from 16.33 to 1000 mg of formulated product (FP)/kg dry mass of substrate (geometrical series at a factor 1.8). The objectives of the test were to estimate the LOEC/NOEC and to calculate the ECx (e.g. EC10 – EC50). where possible. The test was validated with a water control. The toxic standard "Boric Acid" (OECD 232: 2009) is regularly tested; the last test was performed between September and October 2013(See summary report in annex X). Ten juvenile *F. candida* (9-10 days old). obtained from a cohort reared at Trialcamp facilities. were introduced into each replicate. (four replicates per treatment and eight per control). Each replicate contained approximately 30 g fresh treated soil. Dry granulated yeast was provided as a food source. After 29 days. the number of the original springtails still surviving, and the number of offspring produced was recorded. One additional container per treatment was prepared for checking pH of the soil for the control and each treatment at the beginning and the end of the test.

Results

Residues of the product "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) resulted in corrected mortality of less than 50% at concentrations up to and including 1000 mg FP /kg of dry mass of substrate (dry soil). The **LC50** was estimated to be greater than 1000 mg FP/ dry soil. equivalent to 384.36 mg phosphorous /kg dry soil.

When mortalities were compared to control. significant difference was only found with the concentration of 1000 mg FP /kg dry soil (Jonckheere Tersptra Test. Monte Carlo sig. 99%. 1-tailed). Therefore. the NOEC (no-observed-effect concentration) based on lethal effects was 555.56 mg FP /kg dray soil (equivalent to 213.53 mg phosphorous /kg dry soil) and LOEC (lowest observed effect concentration) was 1000 mg FP /kg dray soil (equivalent to 384.36 mg phosphorous /kg dry soil).

Reproductive output of the introduced Collembola was not affected following the exposure to "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) at concentrations up to and

including 1000 mg FP/kg dry mass of substrate (dry soil). Reproduction rates with the test product were similar or although higher than in the control treatment; reduction on reproduction was always less than 5%.

Therefore. the NOEC (no observed effect concentration) was 1000 mg FP /kg dry soil (equivalent to 384.36 mg phosphorous /kg dry soil). The LOEC (lowest observed effect concentration) for the reproduction capacity was estimated to be greater than 1000 mg FP /kg dry soil.

ECx values (concentration that causes x% reduction on fecundity) were estimated according the percentage of reduction respective to the control (Table 1) as no effect on reproduction was observed with the tested concentrations. **EC_{50}, EC_{10}** and **EC_{20}** were not possible to be calculated and they were estimated to be greater than 1000 mg FP /kg of dry soil.

Test product	Parameter	LOEC	NOEC	LC_x / EC_x mg formulated product/kg dry soil	LC_x / EC_x mg phosphorous /kg dry soil
"SALAMAN 510" (Potassium phosphite 510 g/L, as Phosphorous acid)	Mortality	1000	555.56	$LC_{50} > 1000$	$LC_{50} > 384.36$
	Reproduction	> 1000	1000	$EC_{10}, EC_{20}, EC_{50} > 1000$	$EC_{10}, EC_{20}, EC_{50} > 384.36$

	Treatment	Concentration(1) mg fp /kg dry soil	% mortality(2)	% Corrected mortality(3)	Mean of juveniles	% reduction(4) (relative to control)
C	CONTROL (deionized water)	0	10.00	--	268.25	--
T 1	"SALAMAN 510" (Potassium phosphite 510 g/L, as Phosphorous acid)	16.33	7.50	-2.78	271.25	-1.12
T 2		29.40	0.00	-11.11	264.75	1.30
T 3		52.92	10.00	0.00	261.75	2.42
T 4		95.26	0.00	-11.11	290.75	-8.39
T 5		171.47	7.50	-2.78	272.50	-1.58
T 6		308.64	12.50	2.78	297.00	-10.72
T 7		555.56	22.50	13.89	283.25	-5.59
T 8		1000	27.50 SD	19.44	282.00	-5.13

(1): mg of the formulated product (fp) per kilogram dry mass of the soil.
(2): SD: Significant difference compared to control (Jonckheere-Terpstra test, α=0.05).
(3): Negative values indicate a decrease compared to the control.
(4): Negative values indicate an increase compared to the control.

* * * * * *

Comments of zRMS:	The study was accepted. The study was conducted according to OECD guideline 226. The validity criteria were met. The following endpoints for *Hypoaspis aculeifer* were derived: NOEC for mortality and reproduction was calculated to be 1000 mg test item/kg soil dry weight, equivalent to 384.36 mg phosphorous /kg dry soil.

Report:	KCP 10.4.2.1/02
Authors (year)	Ansaloni. T. (2016)
Title:	Side-Effects of "SALAMAN 510" (POTASSIUM PHOSPHITE 510 G/L. AS PHOSPHOROUS ACID) on the predatory mite. *Hypoaspis* (*Geolaelaps*) aculeifer Canestrini (Acari: Laelapidae) under laboratory conditions.

Document No:	TRIALCAMP Study report TRC13-298BA
Guidelines:	OECD/OCDE nº 226 (October. 2008)
GLP:	Yes
Deviations	--

Introduction

A dose response study was carried out under laboratory conditions with the objective to determine the effects on reproduction and percentage mortality of the mite *Hypoaspis* (*Geolaelaps*) *aculeifer* Canestrini (Acari: Laelapidae) exposed to a single limit rate of the formulated product "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) in artificial substrate. The study followed OECD guideline 226 and was conducted under study code TRC13-298BA.

Materials and methods

Test product

"SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid). batch 1132015. purity for Potassium Phosphite (as phosphorous acid) 57.1% w/v (570.7g/L). expiry April 2016.

Test species

Adult female mites of the species *Hypoaspis aculeifer* obtained from a synchronized cohort (7-14 days old adult females. equivalent to 28-35-day old individuals after eggs' laying).

Test design

Test product concentrations**:** Adult mites were maintained in an artificial substrate treated with "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) at a concentration of 1000.00 mg formulated product/kg dry substrate and removed on day 14 and evaluated on day 19 after exposure for mortality and reproduction.

A toxic reference treatment (Dimethoate 40% EC) at 7.75 mg of formulated product per kilogram dry mass of substrate and a negative (untreated) control were concurrently tested.

Experimental units**:** The experimental units consisted of glass containers. 45 x 70mm (diameter x height). covered with a glass cover lid. which is designed to reduce water evaporation and allowing gas exchange between the soil and the atmosphere. For each glass container. the quantity of substrate (artificial soil) was equivalent to 20g (dry mass). The substrate was moistened with distilled water to reach 45% of its total water holding capacity. Each experimental unit was aerated at least twice a week to guarantee gaseous exchange.

Eight replicates of the water control and of the treatments with the test and reference product consisting of 10 mites each were evaluated.

***Assessments*:** Mites' extraction started 14 days after the application and assessment of adult mortality and offspring production was carried out on day 19 after the application.

Results

Mortality and offspring production: Mortality was 5.00% ± 1.89% (mean ± SE) for the control group and it was 3.75% ± 1.83% (mean ± SE) in the treatment with the test product. No statistical analysis was performed on these data. Mean mortality in the reference product was of 81.25 ± 2.95% (mean ± SE).

Reproduction: Mean offspring production was of 130.88 ± 6.96 individuals (mean ± SE) in the control and it was of 130.00 ± 9.81 individuals (mean ± SE) in the treatment with the test product. No statistical analysis was performed on these data. Mean offspring production in the reference product was of 10.13 ± 1.83 individuals (mean ± SE).

Conclusion

Survivorship and reproduction of *Hypoaspis* (*Geolaelaps*) *aculeifer* adults were not negatively affected by the assayed concentration of "SALAMAN 510" (Potassium phosphite 510 g/L. as Phosphorous acid) as compared to the control group.

Therefore. it was determined that the 'No Observed Effect Concentration' (NOEC). under the conditions of this study. for the test product corresponded to the tested rate of 1000.00 mg f.p./kg dry substrate. equivalent to 384.36 mg Potassium Phosphite (as phosphorous acid)/kg dry substrate (CoA analytical content).

	Formulated product	Potassium phosphite, as Phosphorous acid
NOEC (mg/kg dry substrate)	1000.00	384.36

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was accepted. The study was conducted according to OECD guideline 216 (nitrogen transformation) and 217 (carbon transformation). The validity criteria were met. The formulation caused no adverse effect on on respiration activity, soil nitrate content and soil nitrate formation rate of soil microflora when applied up to 142.11 mg SALAMAN 510 (510 g/l Phosphorus acid)/kg soil dry weight.

Report:	KCP 10.5/01
Authors (year)	Hammesfahr. U. (2013)
Title:	Effects of SALAMAN 510 (510 g/l Phosphorus acid) on the Activity of the Soil Microflora in the Laboratory
Document No:	IBACON Study report No. 65677080
Guidelines:	OECD guideline 216 and 217 (2000)
GLP:	Yes
Deviations	--

Methods

***Test Item*:** SALAMAN 510 (510 g/l Phosphorus acid); Batch No. 1132015; Purity: 38.0% w/w (564.1 g/l in phosphorous acid)

***Test System*:** Biologically active agricultural soil: Mid loamy sand

***Test Design*:** Determination of carbon transformation in soil after addition of glucose.

Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. A BSB-Sensomat System® was used to determine the CO_2-production over a period of up to 24 hours at different sampling intervals.

Determination of nitrogen-transformation (ammonium-, nitrite- and nitrate-nitrogen levels) in soil enriched with Lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil.

3 replicates per treatment and concentration. NH_4-. NO_2- and NO_3- nitrogen formed from the nitrification process were determined by means of a Dionex ion chromatography system (DX-120 / ICS 1000, AS/AS 50 autosampler, VWD ICS UV photometer).

Sampling scheme: 0. 7. 14 and 28 days after treatment (soil carbon transformation test) and 0. 7. 14 and 28 after treatment (soil nitrogen transformation test). subsamples were withdrawn from the soil bulk batches and subjected to the analysis.

***Test Rates*:** Control.

Lower test concentration: 14.21 mg SALAMAN 510 (510 g/l Phosphorus acid)/kg soil dry weight (corresponding to the maximum field application rate). Higher test concentration: 142.11 mg SALAMAN 510 (510 g/l Phosphorus acid)/kg soil dry weight (corresponding to 10 times the maximum field application rate)

***Endpoints*:** Effects on O_2-consumption after 28 days of exposure (soil carbon transformation).

Effects on NO_3-nitrogen production after 28 days' exposure (soil nitrogen transformation).

***Reference Item*:** Sodium chloride was applied at a rate of 16 g/kg dry soil in a separate study (study code: 30696080) within one year of the start of the experimental phase of this study

***Test Conditions*:** Soil moisture: 48% to 53% of its maximum water holding capacity. Soil samples were incubated at 20 °C ± 2 °C while stored in plastic boxes covered by perforated lids.

Results

***Soil Respiration Rates*:** The soil respiration rates were clearly within the trigger value of ±25% set by OECD guideline 217 throughout the experiment. On day 28 the values differed by -3.39% and -4.01% from the control for the low and high dose rate. respectively. There were no statistically significant differences between control and test item treated groups within the experiment except the higher dose rate at day 0 (Student t-test). A summary of the results is shown in Table 10.5-1.

***Soil Nitrate Content*:** No adverse effects of SALAMAN 510 (510 g/l Phosphorus acid) on nitrogen transformation in soil could be observed in both test item concentrations (14.21 mg SALAMAN 510 (510 g/l Phosphorus acid)/kg dry soil and 142.11 mg SALAMAN 510 (510 g/l Phosphorus acid)/kg dry soil) after 28 days. Only slight deviations from the control of 1.08% (application rate 14.21 mg/kg dry soil) and -5.94% (application rate 142.11 mg/kg dry soil) were measured at the end of the 28-day incubation period. The statistical evaluation resulted in significant differences for day 28 of the higher treatment group (Student t-test. $\alpha = 0.05$). The results are summarized in Table 10.5-1.

***Nitrate Formation Rate*:** The soil nitrate formation rates were calculated on a cumulative basis (i.e. between day 0 and the sampling dates). The difference in the soil nitrate formation rate between the control and the both test concentrations was lower than 25% throughout the experiment. In the last interval between days 0 and 28. the deviations from control were 2.41% and -6.63% for the lower and higher test concentration of SALAMAN 510 (510 g/l Phosphorus acid).

Thus. the difference in the soil nitrate formation rates between the control and both test item treatments was clearly below the OECD guideline 216 trigger value of 25 % at the 0 to 28-day interval. All deviations were not statistically significant different. except for the higher dose rate at day 0- 28 interval (Student t-test. $\alpha = 0.05$). A summary of the results is shown in the following tables.

Soil Respiration (mg CO_2 / kg soil dry weight / h) Mean Values		
	Control	**Potassium phosphonate**

			14.21 mg SALAMAN 510 (510 g/L Phosphorus acid)/kg soil dw		142.1 mg SALAMAN 510 (510 g/L Phosphorus acid)/kg soil dw	
Sampling	**Respiration rate**	**Replicate variation[1]**	**Respiration rate**	**Deviation[2]**	**Respiration rate**	**Deviation[2]**
Day 0	11.161	1.53	11.389	2.04	10.438*	-6.48
Day 7	10.065	1.73	9.843	-2.21	9.680	-3.83
Day 14	9.363	3.12	10.109	4.91	9.278	-3.72
Day 28	9.716	0.77	9.387	-3.39	9.326	-4.01
NO_3 - Nitrogen (mg / kg soil dry weight) Mean Values						
	Control		**Potassium phosphonate**			
			14.21 mg SALAMAN 510 (510 g/L Phosphorus acid)/kg soil dw		**142.1 mg SALAMAN 510 (510 g/L Phosphorus acid)/kg soil dw**	
Sampling	**Nitrate-N content**	**Replicate variation[1]**	**Nitrate-N content**	**Deviation[2]**	**Nitrate-N content**	**Deviation[2]**
Day 0	13.417	2.32	13.049	-2.74	12.920	-3.70
Day 7	21.261	0.99	20.223	-4.88	21.415	0.72
Day 14	45.446	7.65	42.453	-6.59	44.042	-3.09
Day 28	59.918	1.81	60.566	1.08	56.361*	-5.94
NO_3 - Nitrogen Formation Rate (mg / kg soil dry weight per day)[3]						
	Control		**Potassium phosphonate**			
			14.21 mg SALAMAN 510 (510 g/L Phosphorus acid)/kg soil dw		**142.1 mg SALAMAN 510 (510 g/L Phosphorus acid)/kg soil dw**	
Interval	**Nitrate-N Formation**		**Nitrate-N Formation**	**Deviation[2]**	**Nitrate-N Formation**	**Deviation[2]**
Day 0-7	1.12		1.02	-8.93	1.21	8.04
Day 0-14	2.29		2.10	-8.30	2.22	-3.06
Day 0-28	1.66		1.70	2.41	1.55*	-6.63

Mineral Nitrogen[4] (mg / kg soil dry weight) Mean Values						
	Control		**Potassium phosphonate**			
			14.21 mg SALAMAN 510 (510 g/L Phosphorus acid)/kg soil dw		**142.1 mg SALAMAN 510 (510 g/L Phosphorus acid)/kg soil dw**	
Sampling	**Mineral N-content**	**Replicate variation[1]**	**Mineral N-content**	**Deviation[2]**	**Mineral N-content**	**Deviation[2]**
Day 0	15.595	3.21	16.461*	5.55	16.103	3.26
Day 7	22.160	0.95	21.122	-4.68	22.314	0.69
Day 14	44.670	6.06	43.352	-2.95	44.941	0.61
Day 28	61.022	1.83	61.641	1.01	57.335*	-6.04

1 = % variation within control replicates (coefficient of variation. calculated as standard deviation / mean value * 100)
2 = % deviation to control
3 = samplings related to test start (cumulative)
4 = mineral nitrogen = sum of nitrite- nitrate- and ammonium-nitrogen
positive values = stimulatory effect; negative values = inhibitory effect; dw = dry weight
* statistically significant different from control (Student t-test; $\alpha = 0.05$)

Soil Mineral Nitrogen

Content: The differences between the mineral nitrogen content of SALAMAN 510 (510 g/l Phosphorus acid) treated soil at both test concentrations and the control soil were also clearly below the 25 % trigger value at day 28 (required only by the EPPO and SETAC guidelines). At day 28 the differences were 1.01% and -6.04%. respectively.

***Validity Criteria*:** The validity criterion of the 15% limit for the deviation between control replicates is in force for the respiration rates and the soil nitrate content. The variation between the replicate control samples for both parameters was clearly within the validity criterion of 15% for both the carbon and nitrogen transformation tests (OECD test guidelines 216/217) throughout the study.

The validity of the test system was further confirmed by the sensitivity established in separate positive control experiments using sodium chloride at a concentration of 16 g/kg soil dry weight.

Conclusions

Based on the results of this study. it is concluded that SALAMAN 510 (510 g/l Phosphorus acid) had no impact on respiration activity. soil nitrate content and soil nitrate formation rate of soil microflora when applied up to 142.11 mg SALAMAN 510 (510 g/l Phosphorus acid)/kg soil dry weight.

It can be concluded that SALAMAN 510 (510 g/l Phosphorus acid) will not have any long-term influence on soil micro-organisms.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study was accepted. The study was conducted according to OECD guideline 227. The validity criteria were met. The ER_{50} vegetative vigour >23.63 L/ha. The study results are suitable for the risk assessment.

Report:	KCP 10.6.2/01
Authors (year)	Gimeno (2013)a
Title:	Effects of the formulated product "SALAMAN 510" (Potassium phosphite 510 g/L SL) on Vegetative Vigour of terrestrial non-target plants
Document No:	TRIALCAMP Study report TRC12-012BP
Guidelines:	OECD 227. 2006: Terrestrial Plant Test: Vegetative vigour test
GLP:	Yes
Deviations	--

A Laboratory study was carried out to determine the effects of the formulated product SALAMAN 510 on vegetative vigour of higher plants following exposure to the test product under controlled conditions.

The species selected are non-target crop species and are included in the list of suitable species in OECD 227. Monocotyledonous plants were represented by four species from two families; dicotyledonous species were represented by six species from six families.

The study was conducted in a controlled environment chamber at a temperature of 25 ± 3 ºC during the day and 20 ± 3 ºC during the night. relative humidity of 70 ± 5 % during light periods and 90 ± 5 % during dark periods. a 16:8 light/dark photoperiod and a light intensity of 292-306 microE/m^2/s at the top of the canopy.

The test product SALAMAN 510 (Potassium phosphite 510 g/L SL). batch 1132015. was tested in a range of ten rates in a geometric series with a factor of 2.1. The highest rate (T1) of the selected range corresponds to the equivalent to three applications at the maximum application rate (4.463 kg a.s./ha). An untreated control group per species was concurrently tested.

The test substance was sprayed onto the plants simulating realistic exposure conditions. At the time of application plants were at the 2-4 leaf stage.

Applications were performed according to the Good Agricultural Practices in a target volume of 400 L/ha. The application equipment used was a laboratory track sprayer equipped with a Hardi ISO F-110 orange flat fan nozzle at 300 kPa. The equipment was calibrated before the application; three independent runs verified that the system was operating consistently. uniformly and as expected.

Five replicates were evaluated per treatment group and for each control. Each replicate consisted of 6-8 plants (30-40 plants/treatment).

Effects were evaluated 7. 14 and 21 days after application. Endpoints measured were visual mortality. growth rate. assessment of visible detrimental effects on different parts of the plants. shoot height and fresh shoot weight of the shoots at the end of the study.

The study was considered valid for all species; emergence recorded was > 70% and mean survival of the control plants was > 90%. moreover. no phytotoxic effects were detected in the control plants.

Effects on mortality were not observed for any of the plant species. Visual phytotoxicity symptoms were not observed for any of the assayed species.

Effects on final weight were observed at one to several treatment rates for all the plant species with the exception of *Pisum sativum* (pea) and *Allium cepa* (onion).

The species *Lolium perenne* (perennial ryegrass) showed a reduction in final weight at several treatment rates but reduction did not reach 25%.

The species *Solanum lycopersicon* (tomato), *Cucumis sativus* (cucumber), *Lactuca sativa* (lettuce), *Brassica oleracea* (cabbage), *Brassica napus* (oilseed rape), *Daucus carota* (carrot) and *Zea mays* (corn) showed a reduction in final weight at one to several treatment rates but reduction did not reach 50%. Effective rates (25% effect) were estimated using regression curves. Rates at which mean plant weight was reduced by 25% with respect to the control group ranged from 11.1070 L test product/ha for *Daucus carota* (carrot) to 20.1945 L test product/ha for *Cucumis sativus* (cucumber).

Effects on final height were observed at one to several treatment rates for all the plant species with the exception of *Cucumis sativus* (cucumber), *Lactuca sativa* (lettuce), *Pisum sativum* (pea) and *Allium cepa* (onion).

The species *Solanum lycopersicon* (tomato). *Brassica oleracea* (cabbage). *Brassica napus* (oilseed rape). *Lolium perenne* (perennial ryegrass) and *Zea mays* (Corn) showed a reduction in final height at one to several treatment rates. but reduction in height did not reach 25%.

The species *Daucus carota* (carrot) showed a reduction in final height at several treatment rates but reduction did not reach 50%. Effective rate (25% effect) was estimated using regression curves. Rates at which mean plant weight was reduced by 25% with respect to the control group was 21.8461 L test product/ha.

Results are detailed in the following tables.

Effect on mortality (based on nominal rates)

Species	Common Name	Family	SALAMAN 510 (L/ha)		
			ER_{50}	**LOER**	**NOER**
Solanum lycopersicon	Tomato	Solanaceae	>23.6277	--	23.6277
Cucumis sativus	Cucumber	Cucurbitaceae	>23.6277	--	23.6277
Lactuca sativa	Lettuce	Compositae	>23.6277	--	23.6277
Pisum sativum	Pea	Leguminosae	>23.6277	--	23.6277
Brassica oleracea	Cabbage	Cruciferae	>23.6277	--	23.6277
Daucus carota	Carrot	Umbelliferae	>23.6277	--	23.6277
Brassica napus	Oilseed rape	Cruciferae	>23.6277	--	23.6277
Lolium perenne	Perennial ryegrass	Gramineae	>23.6277	--	23.6277
Zea mays	Corn	Gramineae	>23.6277	--	23.6277
Allium cepa	Onion	Amaryllidaceae	>23.6277	--	23.6277

Effect on final weight (based on nominal rates)

Species	Common Name	Family	SALAMAN 510 (L/ha)			
			ER_{25}	**ER_{50}**	**LOER**	**NOER**
Solanum lycopersicon	Tomato[(1)]	Solanaceae	18.4981	>23.6277	23.6277	11.2513
Cucumis sativus	Cucumber[(1)]	Cucurbitaceae	20.1945	>23.6277	23.6277	11.2513
Lactuca sativa	Lettuce[(1)]	Compositae	17.8542	>23.6277	11.2513	5.3578

Pisum sativum	Pea	Leguminosae	>23.6277	>23.6277	--	23.6277
Brassica oleracea	Cabbage(1)	Cruciferae	16.3699	>23.6277	23.6277	11.2513
Daucus carota	Carrot(1)	Umbelliferae	11.1070	>23.6277	5.3578	2.5513
Brassica napus	Oilseed rape(1)	Cruciferae	17.9214	>23.6277	11.2513	5.3578
Lolium perenne	Perennial ryegrass(2)	Gramineae	>23.6277	>23.6277	5.3578	**2.5513**
Zea mays	Corn(2)	Gramineae	18.6241	>23.6277	23.6277	11.2513
Allium cepa	Onion	Amaryllidaceae	>23.6277	>23.6277	--	23.6277

(1) Weight reduction at the highest rate tested was < 50%.
(2) Weight reduction at the highest rate tested was < 25%.

Effect on final height (based on nominal rates)

Species	Common Name	Family	SALAMAN 510 (L/ha)			
			ER_{25}	ER_{50}	LOER	NOER
Solanum lycopersicon	Tomato	Solanaceae	>23.6277	>23.6277	23.6277	11.2513
Cucumis sativus	Cucumber	Cucurbitaceae	>23.6277	>23.6277	--	23.6277
Lactuca sativa	Lettuce	Compositae	>23.6277	>23.6277	--	23.6277
Pisum sativum	Pea	Leguminosae	>23.6277	>23.6277	--	23.6277
Brassica oleracea	Cabbage(2)	Cruciferae	>23.6277	>23.6277	23.6277	11.2513
Daucus carota	Carrot(1)	Umbelliferae	21.8461	>23.6277	5.3578	2.5513
Brassica napus	Oilseed rape(2)	Cruciferae	>23.6277	>23.6277	11.2513	5.3578
Lolium perenne	Perennial ryegrass(2)	Gramineae	>23.6277	>23.6277	11.2513	5.3578
Zea mays	Corn(2)	Gramineae	>23.6277	>23.6277	11.2513	5.3578
Allium cepa	Onion	Amaryllidaceae	>23.6277	>23.6277	--	23.6277

(1) Height reduction at the highest rate tested was < 50%.
(2) Height reduction at the highest rate tested was < 25%.

It can be concluded that SALAMAN 510 had no effects on mortality (all the species tested). The test product had significant effects on shoot length (all the species with the exception of cucumber, lettuce, pea and onion) and on shoot biomass (all the species with the exception of pea and onion) at one or more treatment rates but reduction never reached 50%.

The overall lowest NOER was estimated to be 2.5513 L product/ha based on nominal treatment levels for *Daucus carota* (carrot. weight and height; perennial ryegrass, height).

*** * * * * ***

Comments of zRMS:	The study was accepted. The study was conducted according to OECD guideline 208. The validity criteria were met. The ER_{50} emergence: >23.63 L/ha. The study results are suitable for the risk assessment.

Report:	KCP 10.6.2/02
Authors (year)	Gimeno (2013)b
Title:	Effects of the formulated product "SALAMAN 510" (Potassium phosphite 510 g/L SL) on Seedling Emergence and Seedling growth
Document No:	TRIALCAMP Study report TRC12-011BP
Guidelines:	OECD 208. 2006: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test.
GLP:	Yes
Deviations	--

A Laboratory study was carried out to determine the effects of the formulated product SALAMAN 510 (Potassium phosphite 510 g/L SL) on the emergence and early growth of 10 species of terrestrial non-target plants.

The species selected are non-target crop species and are included in the list of suitable species in OECD 208. Monocotyledonous plants were represented by four species from two families; dicotyledonous species were represented by six species from six families.

The study was conducted in a controlled environment chamber at a temperature of 25 ± 3 ºC during the day and 20 ± 3 ºC during the night. relative humidity of 70 ± 5 % during light periods and 90 ± 5 % during dark periods. a 16:8 light/dark photoperiod and a light intensity of 286-306 microE/m2/s at the top of the canopy.

The test product SALAMAN 510 (Potassium phosphite 510 g/L SL). batch 1132015. was tested at a limit rate (23.6277 L/ha) corresponding to the equivalent to three applications at the maximum field application rate (4.463 kg a.s./ha). Additionally. the rate corresponding to the maximum field application rate was tested.

The test substance was applied to the soil surface. which represents the main route of exposure to the chemical from the proposed uses. Soil application was made to potted soil in which the seeds were already planted.

Applications were performed according to the Good Agricultural Practices in a target volume of 400 L/ha. The application equipment used was a laboratory track sprayer equipped with a Hardi ISO F-110 orange flat fan nozzle at 300 kPa. The equipment was calibrated before the application; three independent runs verified that the system was operating consistently. uniformly and as expected.

Eight replicates were evaluated per treatment group and for each control. Each replicate consisted of a minimum of 6 seeds (48 seeds/treatment).

Effects were evaluated in the period of 7 to 21 days after 50 % emergence of the seedlings in the control group. Endpoints measured were visual assessment of seedling emergence. mortality. fresh shoot weight. shoot height and assessment of visible detrimental effects on different parts of the plants.

The study was considered valid for all species; the emergence recorded in the control group was > 70% and mean survival of the seedlings was > 90%. moreover. no phytotoxic effects were detected in the control plants.

None of the tested rates of the test product “SALAMAN 510” (Potassium phosphite 510 g/L SL) significantly affected the emergence and the post emergence survivorship of any of the tested species.

Effects on final weight were not observed at any of the treatment rates for the species *Solanum lycoperssicon* (tomato), *Cucumis sativus* (cucumber), *Lactuca sativa* (lettuce), *Pisum sativum* (pea), *Brassica olearacea* (cabbage), *Daucus carota* (carrot) *and Lolium perenne* (Perennial ryegrass).

The species and *Brassica napus* (oilseed rape), *Zea mays* (corn) and *Allium cepa* (onion) showed a reduction in final weight at the highest treatment rate. but reduction in weight did not reach 25%.

Effects on final, *Cucumis sativus* (cucumber), *Lactuca sativa* (lettuce), *Pisum sativum* (pea), *Brassica olearacea* (cabbage), *Daucus carota* (carrot). *Lolium perenne* (Perennial ryegrass) *and Allium cepa* (onion)

The species and *Brassica napus* (oilseed rape) and Zea mays (corn) showed a reduction in final height at the highest treatment rate. but reduction in height did not reach 25%.

Results are detailed in the following tables.

Effect on emergence (based on nominal rates)

Species	Common Name	Family	SALAMAN 510 (L/ha)		
			ER_{50}	**LOER**	**NOER**
Solanum lycopersicon	Tomato	Solanaceae	>23.6277	--	23.6277
Cucumis sativus	Cucumber	Cucurbitaceae	>23.6277	--	23.6277
Lactuca sativa	Lettuce	Compositae	>23.6277	--	23.6277

Pisum sativum	Pea	Leguminosae	>23.6277	--	23.6277
Brassica oleracea	Cabbage	Cruciferae	>23.6277	--	23.6277
Daucus carota	Carrot	Umbelliferae	>23.6277	--	23.6277
Brassica napus	Oilseed rape	Cruciferae	>23.6277	--	23.6277
Lolium perenne	Perennial ryegrass	Gramineae	>23.6277	--	23.6277
Zea mays	Corn	Gramineae	>23.6277	--	23.6277
Allium cepa	Onion	Amaryllidaceae	>23.6277	--	23.6277

Effect on survivorship (based on nominal rates)

Species	Common Name	Family	SALAMAN 510 (L/ha)		
			ER_{50}	LOER	NOER
Solanum lycopersicon	Tomato	Solanaceae	>23.6277	--	23.6277
Cucumis sativus	Cucumber	Cucurbitaceae	>23.6277	--	23.6277
Lactuca sativa	Lettuce	Compositae	>23.6277	--	23.6277
Pisum sativum	Pea	Leguminosae	>23.6277	--	23.6277
Brassica oleracea	Cabbage	Cruciferae	>23.6277	--	23.6277
Daucus carota	Carrot	Umbelliferae	>23.6277	--	23.6277
Brassica napus	Oilseed rape	Cruciferae	>23.6277	--	23.6277
Lolium perenne	Perennial ryegrass	Gramineae	>23.6277	--	23.6277
Zea mays	Corn	Gramineae	>23.6277	--	23.6277
Allium cepa	Onion	Amaryllidaceae	>23.6277	--	23.6277

Effect on final weight (based on nominal rates)

Species	Common Name	Family	SALAMAN 510 (L/ha)			
			ER_{25}	ER_{50}	LOER	NOER
Solanum lycopersicon	Tomato	Solanaceae	>23.6277	>23.6277	--	23.6277
Cucumis sativus	Cucumber	Cucurbitaceae	>23.6277	>23.6277	--	23.6277
Lactuca sativa	Lettuce	Compositae	>23.6277	>23.6277	--	23.6277
Pisum sativum	Pea	Leguminosae	>23.6277	>23.6277	--	23.6277
Brassica oleracea	Cabbage	Cruciferae	>23.6277	>23.6277	--	23.6277
Daucus carota	Carrot	Umbelliferae	>23.6277	>23.6277	--	23.6277
Brassica napus	Oilseed rape	Cruciferae	>23.6277	>23.6277	23.6277	8.75
Lolium perenne	Perennial ryegrass	Gramineae	>23.6277	>23.6277	--	23.6277
Zea mays	Corn	Gramineae	>23.6277	>23.6277	23.6277	8.75
Allium cepa	Onion	Amaryllidaceae	>23.6277	>23.6277	23.6277	8.75

Effect on final height (based on nominal rates)

Species	Common Name	Family	SALAMAN 510 (L/ha)			
			ER_{25}	ER_{50}	LOER	NOER
Solanum lycopersicon	Tomato	Solanaceae	>23.6277	>23.6277	--	23.6277
Cucumis sativus	Cucumber	Cucurbitaceae	>23.6277	>23.6277	--	23.6277
Lactuca sativa	Lettuce	Compositae	>23.6277	>23.6277	--	23.6277
Pisum sativum	Pea	Leguminosae	>23.6277	>23.6277	--	23.6277
Brassica oleracea	Cabbage	Cruciferae	>23.6277	>23.6277	--	23.6277
Daucus carota	Carrot	Umbelliferae	>23.6277	>23.6277	--	23.6277
Brassica napus	Oilseed rape	Cruciferae	>23.6277	>23.6277	23.6277	8.75
Lolium perenne	Perennial ryegrass	Gramineae	>23.6277	>23.6277	--	23.6277
Zea mays	Corn	Gramineae	>23.6277	>23.6277	23.6277	8.75
Allium cepa	Onion	Amaryllidaceae	>23.6277	>23.6277	--	23.6277

It can be concluded that the test product SALAMAN 510% SL (Potassium phosphite 510 g/L SL) had no significant effects on emergence (all the species) on post emergence survivorship (all the species). The test product had significant effects at the highest treatment rate on shoot biomass for the species *Brassica napus* (oilseed rape), *Zea mays* (corn) and *Allium cepa* (onion) and on shoot length for the species *Brassica napus* (oilseed rape) and *Zea mays* (corn). The ER_{25} was >23.6277 L test product/ha.

The overall lowest NOER was estimated to be 8.7500 test product/ha.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data