SURROUND® WP CROP PROTECTANT

DOCUMENT M-CP, Section 10

ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Annex to EU Regulation 284/2013

Ownership Statement

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CP 10 ECOTOXICOLOGICAL STUDIES ON PLANT PROTECTION PRODUCTS

This dossier refers to the product SURROUND® WP CROP PROTECTANT (the representative chemical product), containing calcined kaolin which was registered in 2008 under the term "Aluminium silicate". The regulatory term used throughout this dossier is therefore aluminium silicate, although in geological and mineralogical terms, the substance described therein is known as calcined kaolin.

SURROUND® WP CROP PROTECTANT is a foliar insect repellent for application to grapevines. It contains 95% w/w kaolin (aluminium silicate) as the active substance. The worst-case critical GAP is 30 kg product/ha (28.5 kg a.s./ha) applied 4 times a season with a minimum interval of 7 days to grapevines. Applications occur from BBCH 51 to 65.

SURROUND® WP CROP PROTECTANT was included into Annex I of Directive 91/414 (2008/127/EC). This product was the representative formulation.

Where study summaries were previously presented and summarised, or waivers accepted, the relevant review information or a reference to where such information can be found is within the previous EU review for aluminium silicate; the EFSA Conclusion for aluminium silicate (EFSA Scientific Report (2012) 10(2):2517).

This document contains summaries of all studies, including those that were available at the time of the first Annex I inclusion of aluminium silicate (kaolin) and were evaluated during the first EU review. To facilitate discrimination between new and original information, the executive summaries of the original information are shaded in grey.

~~Studies submitted by the notifier for the first Annex I inclusion are contained in the original dossier and are not summarised in this document. However, for ease of reference, executive summaries have been provided in the respective sections below. For all new studies, detailed summaries are provided in this supplementary dossier. In the case where final study reports were not available at time of submission, the draft results are shaded in blue and will be updated once the final report is available.~~

Introduction

The kaolin ores being mined today in the US were laid down over 25 million years ago. Kaolin is therefore extremely inert and will remain stable in any natural environment. Kaolin (aluminium silicate) is a common component of clay and is therefore ubiquitous throughout the world. When aluminium silicate (kaolin) is added to the environment through agricultural uses, the amount is so small that the impact on overall amounts of natural clays in soils, sediments or water will be negligible.

Heavy use of kaolin (28 million metric tonnes world-wide per year) over the years has not resulted in any reported incidences of toxicity or environmental impact. Numerous industries also continue to use the product on an extensive scale, including agriculture, where aluminium silicate (kaolin) has been used for decades as an inert filler in pesticide formulations.

Aluminium silicate (kaolin) also has an extremely low toxicity to all known organisms with no known mode of toxicity, and except for certain insects that are repelled and/or irritated by the particles, aluminium silicate (kaolin) is extremely unlikely to have any adverse effects. In addition, based on the Draft Working Document AIR IV Renewal Programme (SANTE-2016-10616-rev3, July 2016), kaolin is listed as a low-risk substance based on input from the EU low-risk expert group.

Aluminium silicate's chemical composition is similar to common clay. Aluminium silicate (kaolin) used in SURROUND® WP CROP PROTECTANT, is an ultra-pure, ultra-fine, calcined kaolin, a natural white clay mined in Georgia (USA) but present across the world. It is a natural mineral substance composed of silicon, aluminium and oxygen, just like a variety of other minerals. Once released into the environment, aluminium silicate (kaolin) is insoluble in water and present as clay particles that sink to the bottom and become an integral part of the sediment and is undistinguishable from clay minerals naturally present. It is therefore not translocated in plants or bioavailable and therefore it cannot be readily transported through the gut wall of animals.

Proposed use pattern

This section of the submission summarises the ecotoxicological effects of the formulation and evaluates the potential risk to various representatives of terrestrial, aquatic and soil organisms. The proposed use pattern is summarised in Table 10-1.

Table 10-1: Proposed use pattern of SURROUND® WP CROP PROTECTANT

Crop and/ or situation (crop destination / purpose of crop)	F G or I	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application			Application rate			Remarks: e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
			Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max	
Grapevines	F	*Frankliniella occidentalis.*	Broadcast spraying of entire vine	BBCH 51 - 65	a) 1-4 (7) b) 1-4 (7)	a) 30 kg/ha b) 120 kg/ha	a) 28.5 kg/ha b) 114 kg/ha	a) 500 – 1000 L/ha b) 2000 – 4000 L/ha	First spraying at emergence of overwintering females. Use sufficient spray volume, apply to near drip but avoid run-off. Re-apply each 7 to 21 days, depending on rainfall and crop development.

F = field use

Consideration of metabolites

Aluminium silicate is not metabolised and does not degrade or react. SURROUND® WP CROP PROTECTANT containing 95% aluminium silicate (kaolin) a stable compound, hence there is no formation of metabolites.

CP 10.1 Effects on Birds and Other Terrestrial Vertebrates

CP 10.1.1 Effects on birds

No new avian toxicity data are available or required for the renewal of aluminium silicate (kaolin). As discussed in the original DAR (2008), considering the nature of the active substance and that it is a widespread element of the environment to which wildlife will often be exposed; it has been concluded that the risk to non-target organisms from the representative use of aluminium silicate (kaolin) will be low (EFSA Scientific Report (2012) 10(2):2517).

However, data from the open literature are available for kaolin, showing minimal avian toxicity at high dose levels after intentional consumption *via* their diets. The reference is being submitted in support of aluminium silicate (kaolin). The findings are summarised in the following table and full details of the study are provided in the respective section of MCA-8.

The provision of further data on the representative formulation is not considered necessary as SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). Therefore, the formulated product is highly unlikely to be of higher toxicity compared to the active substance, kaolin.

~~Waivers were requested and accepted during the initial EFSA review (EFSA 2012) for avian toxicity studies based on the following information which still applies.~~

~~SURROUND® WP CROP PROTECTANT28.5 is composed of 95% aluminium silicate (kaolin) and 5.0% of well-known additives of no toxicological concern. Aluminium silicate (kaolin) is a natural component of the environment and birds have been routinely exposed to kaolin in the soil and through exposure from all the industrial uses of kaolin. In addition, no adverse effects have been observed upon birds in the areas where aluminium silicate (kaolin) has been routinely mined for decades.~~

~~Many birds are known to take clay dust baths to help reduce dermal parasites, but some birds like the Macaw have even been observed to eat kaolin for the purpose of aiding their digestive systems. Also, birds that eat earthworms and other soil dwelling invertebrates, routinely consume large quantities of soil (hence clay) adhering to the prey and present in their digestive tracts.~~

~~Moreover, aluminium silicate (kaolin) is inert and insoluble in aqueous and organic solvents. It does not become bioavailable when ingested. Experience has shown it is not absorbed through the gut wall.~~

~~Therefore, toxicity studies and conventional EU risk assessments are not considered necessary for a non-toxic, non-bioavailable, routinely ingested natural mineral such as kaolin clay as was reported in the EFSA Conclusion for aluminium silicate (2012).~~

Table 10.1.1-1: Toxicity endpoint for birds

Species	Substance	Exposure System	Results	Reference
Chicken	Kaolin	Dietary,56 d Subchronic	LD_{50} >30,000 mg a.s./kg diet (ppm) (>3000 mg/kg bw/d)*	Owen *et al.*, (2012) Published ref (KCA 8.1.1.3/01)

*Conversion factor of 0.1 according to EFSA/2009/1438, Section 2.3.1.1

Risk assessment for birds

Toxicity studies and conventional EU risk assessments are not considered necessary for a non-toxic, non-bioavailable, routinely ingested natural mineral such as kaolin clay. ~~Therefore,~~ Nevertheless, a chronic risk assessment has ~~not~~ been performed using the available literature data and the risk to birds is concluded to be low as agreed during the initial EFSA review (2012).

Acute data for birds exposed to kaolin are not available, nor considered to be required due to the weight-of-evidence waivers provided in Section CA 8.1.1.1 and CP 10.1.1.1. Sub-chronic data for chickens exposed to kaolin-enriched diet for 56 days from the open literature is available and has therefore been considered in the following risk assessment.

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).

Table 10.1.1-2: Screening and Tier 1: Chronic risk assessment for birds exposed to kaolin

Screening Assessment					
Intended use		Grapes			
Active substance		Kaolin			
Application rate (kg a.s./ha)		4 x 28.5 (7 day interval) (BBCH 51-65)			
Chronic toxicity (mg a.s./kg bw/d)		>3000			
TER criterion		5			
Crop scenario	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Vineyards	Small ominvorous bird	38.9	2.2 x 0.53	1292.7	**>2.3**
Tier 1 assessment (BBCH 51-65)					
Crop scenario	**Generic f0cal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Vineyards BBCH ≥20	Small insectivorous bird "redstart"	9.9	2.2 x 0.53	329.0	>9.12
Vineyards BBCH ≥40	Small granivorous bird "finch"	3.4	2.2 x 0.53	113.0	>26.55
Vineyards BBCH ≥40	Small omnivorous bird "lark"	3.3	2.2 x 0.53	109.7	>27.36

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. Values in bold indicate a potential risk

The chronic risk to birds feeding in kaolin-treated vineyards is demonstrated to be acceptable at the Tier 1 level. No further consideration to birds is required.

Drinking water risk assessment

Aluminium silicate (kaolin) is present in most natural water bodies and the use of SURROUND® WP CROP PROTECTANT in agriculture will not significantly alter the normal background levels (see Document MCP 9). Also, the aluminium silicate (kaolin) in SURROUND® WP CROP PROTECTANT is not expected to act any differently from natural clays with which it will be mixed and rapidly become part of the natural sediment. Hence, a drinking water risk assessment is not required and the risk to birds is concluded to be low.

Secondary poisoning

Aluminium silicate (kaolin) is not soluble in polar or non-polar solvents and thus has no octanol/water partition coefficient. It is considered that there is no potential for bioaccumulation of aluminium silicate (kaolin) in fatty tissues and therefore there is no potential for secondary poisoning. Moreover, aluminium silicate (kaolin) is naturally occurring compound and it is considered that additional exposure from the proposed use on vines will not lead to exposure levels above natural levels.

CP 10.1.1.1 Acute oral toxicity

~~Not relevant, please refer to point CP 10.1.1.~~

A waiver is requested for an acute oral bird toxicity study with the formulated product based on the following information:

- SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition).

- As detailed in the original DAR (Section B.9.1), aluminium silicate (kaolin) is a natural component of the environment and birds are routinely exposed to kaolin in the soil and through other sources. In addition, no adverse effects have been observed upon birds in the areas where kaolin has been routinely mined for decades.

- As detailed in the original DAR (Section B.9.1), many birds are known to take clay dust baths to help reduce dermal parasites (Martin and Mullens, 2012[1]).

- As detailed in the original DAR (Section B.9.1), some birds like the macaw, have even been observed to eat kaolin for the purpose of aiding their digestive systems (Grange, 2003)[2]. The consumption of soil by the parrot has been studied extensively for decades (Brightsmith *et al*., 2010[3]) and is noted to also be attributed to the animals' search for minerals, which are deficient in their diets, might also protect the birds from dietary toxins, treat ionic imbalance, stabalise gut pH, reduce intestinal parasitism, and reduce diarrhea. The parrots are also known to feed soil to their chicks.

- As detailed in the original DAR (Section B.9.1), birds that eat earthworms and other soil dwelling invertebrates routinely consume soil (hence clay) adhering to the prey and present in their digestive tracts.

- It has been reported that animal feed containing clay minerals such as kaolin promote weight gain and feed efficiency (Mumpton, 1999[4]), reduce bacterial contamination of the guts and reduce the detrimental effects of mycotoxin contaminated diets (Tauqir and Nawaz, 2001[5]). Kaolin also protects the intestinal mucosa, by adhering to pathogen and selectively promotes their excretion (Droy-lefain *et al*., 1985[6]).

- As detailed in the original DAR (Section B.9.1), aluminium silicate (kaolin) is inert and insoluble in aqueous and organic solvents. It does not become bioavailable when ingested. Experience has shown it is not absorbed through the gut wall.

- Similar products have been approved by EFSA when used as technological additive for animal species, such as Friedland clay (EFSA Journal 2014;12(11): 3904) and natural mixture of illite, montmorillonite and kaolinite (EFSA Journal 2016;14(1): 4342), to aid in fattening chickens.

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[1] Martin, C.D. and Mullens, B.A. (2012). Housing and dustbathing effects on northern fowl mites (*Ornithonyssus sylviarum*) and chicken body lice (*Menacanthus stramineus*) on hens. In: Medical and Veterinary Entomology. Vol 26 (3): 323-333.

[2] http://www.bbc.co.uk/nature/animals/features/318feature2.shtml (not available online anymore – please refer to original dossier)

[3] Brightsmith, D.J., McDonald, D, Matsafuji, D., Bailey, C.A. (2010). Nutritional content of the diets of free-living scarlet macaw chicks in Southeastern Peru. In: Journal of Avian Medicine and Surgery 24(1): 9-23

[4] Mumpton, F.A (1999). *La roca magica*: uses of natural zeolites in agriculture and industry. In: Proc. Nat. Acad Sci (USA): 3463 – 3470

[5] Tauqir, N.A. and H. Nawaz (2001). performance and economics of broiler chicks fed on rations supplemented with different levels of sodium bentonite. In: Int. J. Agric. Biol. 3: 137-149

[6] Droy –Lefain, M.T.; T. Drouet and B. Schatz (1985). Sodium glycodeoxycholate and spinability of gastrointestinal mucus: protective effect of smectitite. In: Gastroenter, 88(2): 1369.

- Friedland clay[7] is typically composed of the major constituents montmorillonite, illite, quartz and kaolin. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concludes that 20,000 mg Friedland clay/kg complete feed is safe for target species (chickens, sows, dairy cows and cattle)/categories and extends this conclusion to all animal species.
- A natural mixture of illite, montmorillonite and kaolinite with a minor amount of calcite and sanidine, is regarded as safe by FEEDAP as an additive in feeding stuffs for all animal species at a maximum concentration of 50,000 mg/kg.

In light of these considerations and for animal welfare reasons, unnecessary animal testing should be avoided for a non-toxic, non-bioavailable, routinely ingested natural mineral such as kaolin clay. Therefore, no additional acute oral avian toxicity testing with the representative formulation is considered necessary for the purposes of renewal, as supported during the initial Annex I approval and no avian data were specified as required in EFSA (2012).

CP 10.1.1.2 Higher tier data on birds

Not relevant, please refer to point CP 10.1.1.1. An acceptable risk to birds is concluded based on the available weight of evidence and first tier risk assessment.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

No new mammalian toxicity data are available or required for the renewal of aluminium silicate (kaolin).

As discussed in the original DAR (2008), considering the nature of the active substance and that it is a widespread element of the environment to which wildlife will often be exposed; it was considered that the risk to non-target organisms from the representative use of aluminium silicate (kaolin) will be low (EFSA Scientific Report (2012) 10(2):2517).

An acute mammalian toxicity study has been carried out with aluminium silicate (kaolin). Full details of the study are provided in the previous EU DAR (2008) and related documents. There is no available study for the formulated product since SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). The toxicity of SURROUND® WP CROP PROTECTANT can be determined by that of the active substance. The result of the study is summarized in the following table.

[7] Friedland clay originates from volcanic ash which sedimented in a marine milieu in the Scandinavian region during the Eocene epoch and, as a result of further geological activities, turned into clay. This clay is found in a very specific area of Germany, where it is produced. Friedland clay is typically composed of the major constituents montmorillonite, illite, quartz and kaolin.

Table 10.1.2-2: Toxicity endpoint for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Kaolin	Oral,1 d Acute	LD_{50} >5000 mg a.s./kg bw/d	Wnorowski (1997a) Report no.: 4903 KCA 5.2.1/01 (EFSA Conclusion, 2012)

No long-term toxicity data are available for mammals for the active substance (see waiver in MCA 8.1.2.2) and additional long-term toxicity data for mammals with the formulated product SURROUND® WP CROP PROTECTANT are not considered necessary.

~~A waiver from doing specific studies on terrestrial vertebrates is requested since aluminium silicate (kaolin) has no known modes of toxicity and is not bio-available, based on arguments presented and accepted during the previous dossier~~ review. Exposure to aluminium silicate from natural sources (e.g. clays), domestic and/or industrial uses are likely to be far higher than from any use of SURROUND® WP CROP PROTECTANT in agriculture.

A waiver is requested for a long-term / reproductive mammalian toxicity study with the formulated product based on the following information:

- SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 5.0% of well-known additives of no toxicological concern (Please refer to Part C for details on product consumption).

- Aluminium silicate (kaolin) has no known modes of toxicity and is not bio-available

- Exposure to aluminium silicate from natural sources (e.g. clays), domestic and/or industrial uses are likely to be far higher than from any use of SURROUND® WP CROP PROTECTANT in agriculture.

- As detailed in the original DAR (Section B.9.3), wild mammals are known to eat earthworms and other soil-dwelling invertebrates that contain a large amount of soil (including clay) and to take dirt or mud baths for either body cooling or parasite control reasons. Exposure to kaolin clays during these natural activities will be massively higher than any possible exposure resulting from the proposed use in agriculture.

- Geophagy, the deliberate consumption of earth materials, is common with animals, and can even occur with humans[8]. For example, women in Budongo, Uganda consume forest clay mixed with water for stomach problems and during pregnancy (Reynolds *et al*., 2015)[9]. The major hypothesis about its adaptive functions are the supplementation of essential elements and the protection against temporary and chronic gastrointestinal distress.
  - Ta *et al*., (2017)[10], tested this hypothesis to determine if there was a soil-type preference. Baboons preferred soil containing approximately 14% kaolinite and 0.8% sand over soil

[8] Dominy, N.J., Davoust E., Minekus M. (2004). Adaptive function of soil consumption: an *in vitro* study modeling the human stomach and small intestine. The Journal of Experimental Biology 207, 319-324. The Company of Biologists 2004.

[9] Reynolds, V., Llyod, A.W., English, C.J., Lyons, P., Dodd, H., Hobaiter, C., Newton-Fisher, N., Mullins, C., Lamon, N., Schel, A.M., Fallon, B. (2015). Mineral acquisition from clay by Budongo Forest Chimpanzees. PLOS one. July 28 2015

[10] Ta, C.A.K, Pebsworth, P.A., Liu, R, Hillier, S., Gray, N., Arnason, J.Y., and Young, S.L. (2017). Soil eaten by chacma baboons absorbs polar plant secondary metabolites representative of those found in their diet. Environ Geochem Health. Springer Netherlands.

containing 19% kaolinite and 3% sand. As a comparison, based on an expected crop yield of 7000 kg/ha[11] and a cumulative application rate of 120 kg/ha aluminium silicate per season, assuming no interception / degradation / dissipation, absolute worst-case residue on grapes can be estimated at [120 kg kaolin/ha / 7000 kg grape yield/ha] = 1.71%. It was reported that kaolin contributed to the absorption of toxins in the digestive system (Ta *et al.*, 2017).
    - Reynolds *et al.*, (2015) observed chimpanzees that drank regularly from clay-holes and rarely from surface water or free-flowing water (reduced clay in water sources). They also purposely ate clay (form of kaolinite) and termite mound soil. Again, it is reported that kaolinitie in particular is consumed to aid in digestion, counter over acidity and toxins, and aid in adsorption of nutrients in the gut.
    - White colobus monkeys, orangutans and gorillas are also noted to purposely seek out clay-licks, and/or soil-water (Reynolds *et al.*, 2015).

- Aluminium silicate (kaolin) is also a known substance internationally approved as a food additive, a pharmaceutical ingredient, an ingredient in cosmetics and toiletries and an industrial chemical, with no known long-term adverse effects (refer to Document MCA, Section 5, Point 5.6).

- Similar products have been approved by EFSA when used as technological additive for animal species, such as Friedland clay (EFSA Journal 2014;12(11): 3904) and natural mixture of illite, montmorillonite and kaolinite (EFSA Journal 2016;14(1): 4342), to aid in fattening sows, dairy cows and cattle.
    - Friedland clay[12] is typically composed of the major constituents montmorillonite, illite, quartz and kaolin. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concludes that 20,000 mg Friedland clay/kg complete feed is safe for target species (chickens, sows, dairy cows and cattle)/categories and extends this conclusion to all animal species.
    - A natural mixture of illite, montmorillonite and kaolinite with a minor amount of calcite and sanidine, is regarded as safe by FEEDAP as an additive in feeding stuffs for all animal species at a maximum concentration of 50,000 mg/kg.

- In a study summarised under Document MCA, Section 5, Point 5.6, Patterson and Staszak (1977) reported no observed teratogenic effects in rats fed a diet consisting of 20% kaolin prior to fertilization and during the gestation period.

- Moreover, aluminium silicate (kaolin) is inert, insoluble in aqueous and organic solvents; it does not become bioavailable when ingested. Experience has shown it is not absorbed through the gut wall.

Extensive contact with and use of aluminium silicate (kaolin) in day-to-day life, be it as a food additive (human and animal), a pharmaceutical ingredient, an ingredient in cosmetics and toiletry or as an industrial chemical, has never led to any reported cases of reproductive toxicity. Given the inert nature of this substance, its lack of oral absorption and therefore bioavailability, conducting toxicity studies is considered scientifically unjustified.

Overall, exposure to aluminium silicate (kaolin) during these natural activities will be massively higher than any possible exposure resulting from the use of SURROUND® WP CROP PROTECTANT in

[11] Source: Agreste Database, http://agreste.agriculture.gouv.fr/donnees-de-synthese/statistique-agricole-annuelle-saa/. The 7000 kg/ha standard yield is proposed by ANSES (FR).
[12] Friedland clay originates from volcanic ash which sedimented in a marine milieu in the Scandinavian region during the Eocene epoch and, as a result of further geological activities, turned into clay. This clay is found in a very specific area of Germany, where it is produced. Friedland clay is typically composed of the major constituents montmorillonite, illite, quartz and kaolin.

agriculture, therefore the risk to wild mammals is concluded to be low and the request for additional toxicity studies and conventional EU risk assessments are not considered necessary for a non-toxic, non-bioavailable, routinely ingested natural mineral such as kaolin clay as was reported in the EFSA Conclusion for aluminium silicate (2012).

In light of these considerations and for animal welfare reasons, unnecessary animal testing with vertebrates should be avoided. Therefore, no additional mammalian reproductive toxicity testing with the formuklarted product is considered to be necessary for the purposes of renewal (refer to Document MCA, Section 5, Toxicology for further details) and EFSA (2012) did not specify any mammalian long-term data in the list of data gaps.

Risk assessment for other terrestrial vertebrates

Toxicity studies and conventional EU risk assessments are not considered necessary for a non-toxic, non-bioavailable, routinely ingested natural mineral such as kaolin clay. Exposure to aluminium silicate from natural sources (e.g. clays), domestic and/or industrial uses are likely to be far higher than from any use of SURROUND® WP CROP PROTECTANT in agriculture. ~~Therefore~~, Nevertheless, an acute risk assessment has been performed with consideration of the available data and the risk to mammals is concluded to be low as agreed during the initial EFSA review (2012).

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).

Table 10.1.2-2: Screening and Tier 1: Acute risk assessment for mammals exposed to kaolin

Screening Assessment					
Intended use	Grapes				
Active substance	Kaolin				
Application rate (kg a.s./ha)	4 x 28.5 (7 day interval)				
Acute toxicity (mg a.s./kg bw)	>5000				
TER criterion	10				
Crop scenario	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Vineyards	Small herbivorous mammal	136.4	1.8	6997.3	**>0.7**
Tier 1 assessment (BBCH 51-65)					
Crop scenario	**Generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_m (mg/kg bw/d)**	**TER_a**
Vineyards BBCH ≥40	Large herbivorous mammal "lagomorph"	8.1	1.8	415.5	>12.0
Vineyards BBCH ≥20	Small insectivorous mammal "shrew"	5.4	1.8	277.0	>18.1
Vineyards BBCH ≥40, crop directed	Small herbivorous mammal "vole"	40.9	1.8	2098.2	>**2.4**
Vineyards BBCH ≥40, crop	Small omnivorous mammal "mouse"	5.2	1.8	266.8	>18.7

directed					

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER value in bold indicates an unacceptable risk

The acute risk to mammals feeding in kaolin treated vineyards is demonstrated to be acceptable at the Tier 1 level, except for the small herbivorous mammal "vole".

However, as discussed above, the risk to mammals from the consumption of kaolin in concluded to be low. Mammals are known to intentionally seek out clay to aid in digestion, counter over acidity and toxins, and aid in adsorption of nutrients in the gut. Kaolin has also been approved by EFSA for use as a feed additive, up to 50,000 mg/kg feed stuff (approximately 5000 mg/kg bw/d[13]). Furthermore, according to acute oral testing for rats (i.e., OECD 420), the limit dose in testing in rats is typically 2000 mg/kg or exceptionally 5000 mg/kg, hence a conservative acute TER of >2.4 for voles based on a greater than toxicity endpoint of 5000 mg a.s./kg does not equate to a potential risk. Based on the weight-of-evidence, as described below, the risk to mammals foraging in treated vineyards is concluded to be acceptable.

- SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product consumption).

- Kaolin is a natural component of the environment and mammals have been routinely exposed to kaolin in the soil and through other sources. In addition, no adverse effects have been observed upon mammals in the areas where kaolin has been routinely mined for decades.

- As detailed in the original DAR (Section B.9.3), wild mammals that eat earthworms and other soil-dwelling invertebrates, routinely consume soil (hence clay) adhering to the prey and present in their digestive tracts.

- Geophagy, the deliberate consumption of earth materials, is common with animals, and can even occur with humans[14]. For example, women in Budongo, Uganda consume forest clay mixed with water for stomach problems and during pregnancy (Reynolds *et al*., 2015)[15]. The major hypothesis about its adaptive functions are the supplementation of essential elements and the protection against temporary and chronic gastrointestinal distress.
    - Ta *et al*., (2017)[16], tested this hypothesis to determine if there was a soil-type preference. Baboons preferred soil containing approximately 14% kaolinite and 0.8% sand over soil containing 19% kaolinite and 3% sand. As a comparison, based on an expected crop yield of 7000 kg/ha[17] and a cumulative application rate of 120 kg/ha aluminium silicate per season, assuming no interception / degradation / dissipation, absolute worst-case residue on grapes can be estimated at [120 kg kaolin/ha / 7000 kg grape yield/ha] = 1.71%. It was reported that kaolin contributed to the absorption of toxins in the digestive system (Ta *et al*., 2017).

[13] Based on a conversion factor of 0.1 for sub-chronic exposure to rats as reported under EFSA/2009/1438, Section 2.3.1.1

[14] Dominy, N.J., Davoust E., Minekus M. (2004). Adaptive function of soil consumption: an *in vitro* study modeling the human stomach and small intestine. The Journal of Experimental Biology 207, 319-324. The Company of Biologists 2004.

[15] Reynolds, V., Llyod, A.W., English, C.J., Lyons, P., Dodd, H., Hobaiter, C., Newton-Fisher, N., Mullins, C., Lamon, N., Schel, A.M., Fallon, B. (2015). Mineral acquisition from clay by Budongo Forest Chimpanzees. PLOS one. July 28 2015

[16] Ta, C.A.K, Pebsworth, P.A., Liu, R, Hillier, S., Gray, N., Arnason, J.Y., and Young, S.L. (2017). Soil eaten by chacma baboons absorbs polar plant secondary metabolites representative of those found in their diet. Environ Geochem Health. Springer Netherlands.

[17] Source: Agreste Database, http://agreste.agriculture.gouv.fr/donnees-de-synthese/statistique-agricole-annuelle-saa/. The 7000 kg/ha standard yield is proposed by ANSES (FR).

  - Reynolds *et al*., (2015) observed chimpanzees that drank regularly from clay-holes and rarely from surface water or free-flowing water (reduced clay in water sources). They also purposely ate clay (form of kaolinite) and termite mound soil. Again, it is reported that kaolinitie in particular is consumed to aid in digestion, counter over acidity and toxins, and aid in adsorption of nutrients in the gut.
  - White colobus monkeys, orangutans and gorillas are also noted to purposely seek out clay-licks, and/or soil-water (Reynolds *et al*., 2015).

- Aluminium silicate (kaolin) is also a known substance internationally approved as a food additive, a pharmaceutical ingredient, an ingredient in cosmetics and toiletries and an industrial chemical, with no known long-term adverse effects (refer to Document MCA, Section 5, Point 5.6).

- Similar products have been approved by EFSA when used as technological additive for animal species, such as Friedland clay (EFSA Journal 2014;12(11): 3904) and natural mixture of illite, montmorillonite and kaolinite (EFSA Journal 2016;14(1): 4342), to aid in fattening sows, dairy cows and cattle.
  - Friedland clay[18] is typically composed of the major constituents montmorillonite, illite, quartz and kaolin. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concludes that 20,000 mg Friedland clay/kg complete feed is safe for target species (chickens, sows, dairy cows and cattle)/categories and extends this conclusion to all animal species.
  - A natural mixture of illite, montmorillonite and kaolinite with a minor amount of calcite and sanidine, is regarded as safe by FEEDAP as an additive in feeding stuffs for all animal species at a maximum concentration of 50,000 mg/kg.

- In a study summarised under Document MCA, Section 5, Point 5.6, Patterson and Staszak (1977) reported no observed teratogenic effects in rats fed a diet consisting of 20% kaolin prior to fertilization and during the gestation period.

Drinking water risk assessment

Aluminium silicate (kaolin) is present in most natural water bodies and the use of SURROUND® WP CROP PROTECTANT in agriculture will not significantly alter the normal background levels (see Document MCP 9). Also, the aluminium silicate (kaolin) in SURROUND® WP CROP PROTECTANT is not expected to act any differently from natural clays with which it will be mixed and rapidly become part of the natural sediment. Hence a drinking water risk assessment is not required and the risk to mammals is concluded to be low.

Secondary poisoning

Aluminium silicate (kaolin) is not soluble in polar or non-polar solvents and thus has no octanol/water partition coefficient. It is considered that there is no potential for bioaccumulation of aluminium silicate (kaolin) in fatty tissues and therefore there is no potential for secondary poisoning. Moreover, aluminium silicate (kaolin) is naturally occurring compound and it is considered that additional exposure from the proposed rate of use on vines will not lead to exposure levels above natural levels.

CP 10.1.2.1 Acute oral toxicity to mammals

[18] Friedland clay originates from volcanic ash which sedimented in a marine milieu in the Scandinavian region during the Eocene epoch and, as a result of further geological activities, turned into clay. This clay is found in a very specific area of Germany, where it is produced. Friedland clay is typically composed of the major constituents montmorillonite, illite, quartz and kaolin.

A waiver is requested for an acute oral mammalian toxicity study with the formulated product based on the following information:

- SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). Therefore, the formulated product is highly unlikely to be of higher toxicity compared to the active substance, kaolin. The toxicity of SURROUND® WP CROP PROTECTANT can be determined based on the available data for the active substance (please refer to Document MCP, Section 7, Point 7.1).

In light of this considerations and for animal welfare reasons, unnecessary animal testing with vertebrates should be avoided. Therefore, no additional mammalian acute toxicity testing with the formulated product is considered to be necessary for the purposes of renewal.

CP 10.1.2.2 Higher tier data on mammals

Not relevant, please refer to point CP 10.1.2. An acceptable risk to wild mammals is concluded based on the available weight of evidence and first tier risk assessment.

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

In accordance with Commission Regulation (EU) No 284/2013 setting out the data requirements for plant protection products the risk to vertebrate wildlife (including reptiles and amphibians) shall be addressed where effects cannot be predicted from the active substance. At the time of this submission there is no agreed guidance on this data requirement and no specific active substance data are available to address these points. However, vertebrate wildlife is repeatedly exposed to clay *via* the natural environment, and an inherent level of safety has been demonstrated based on the acceptable aquatic and terrestrial risk assessments that will cover the risk to these organisms.

Furthermore, as stated in the guidance document for aquatic organisms (EFSA Journal 2013;11(7):3290 Section 7.2.4.) the rainbow trout *Oncorhynchus mykiss* is a good surrogate test species for predicting the acute toxicity of plant protection products for larval stages of amphibian species living in the aquatic compartment of the environment. It is also noted in the aquatic guidance document that by using the same assessment factors as have been applied for fish, the same level of protection will be achieved for both groups. The toxicity to rainbow trout has been assessed for aluminium silicate (kaolin) and the risk assessment presented at point 10.2 of this document demonstrates a low risk to aquatic organisms. Therefore, the risk to amphibians arising from the proposed use of SURROUND® WP CROP PROTECTANT is considered to be covered by the risk assessment for the rainbow trout and no further data are required.

CP 10.2 Effects on Aquatic Organisms

Aquatic toxicity data from the open literature are available for aluminium silicate (kaolin) with fish and aquatic invertebrates and were submitted in support of aluminium silicate (kaolin) during the previous EU review. Full details of these studies are provided in the previous EU DAR (2008) and related documents (i.e., MCA, Section 8, Part 8.2).

During the initial EU evaluation, a data gap for algae was identified and data are now available with the formulated product and is being submitted to support the submission, along with an acute *Daphnia magna* study. Full details of the studies are provided and summarised in Section MCP 10.2.1, below. A summary of the available data is presented in the following tables.

Table 10.2-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – aluminium silicate (kaolin)

Species	Substance	Exposure System	Results	Reference
Acute fish				
Larvae of *Pagrus major, Oplegnathus fasciatus and Parapristipoma trilineatum*	Kaolin	12 hr (static)	LC_{50} = 494 mg a.s./L*, geometric mean (nom)	Isono *et al*. (1998) KCA 8.2.1/03 (EFSA Conclusion, 2012)
Oncorhynchus mykiss	Kaolin	-	LC_{50} >7000 mg a.s./L (nom)	Goldes *et al*. (1988) KCA 8.2.1/01 (2012 Dossier)
Cymatogaster aggregata	Kaolin	200 hr (flow-through)	LC_{50} = 3000 mg a.s./L (nom)	McFarland & Peddicord (1980) KCA 8.2.1/02 (2012 Dossier)
Parophrys vetulus	Kaolin	200 hr (flow-through)	LC_{50} = 70000 – 117000 mg a.s./L (nom)	McFarland & Peddicord (1980) KCA 8.2.1/02 (2012 Dossier)
Brevoortia tyrannus, *Fundulus majalis*, *F. heteroclitus, Menidia menidia*, *Morone saxatilis*, *M. americana*, *Leiostomus xanthurus*, *Micropogon undulates*, *Cynoscion regalis*, *Trinectes maculatus, Opsanus tau*	Kaolin	24-48 hr (static)	LC_{50} >140000 mg a.s./L (nom)	Sherk *et al*. (1973) KCA 8.2.1/04 (2012 Dossier)
Oncorhynchus kisutch & Oncorhynchus mykiss	Kaolin	48 hr (flow-through)	LC_{50} >4,000 (nom)	Redding, Schreck, & Everest (1987) KCA 8.2.2/02 (2012 Dossier)
Chronic fish				
Oncorhynchus mykiss	Kaolin	30 d (ELS) (static)	NOEC = 100 mg a.s./L (nom)	Hashimoto *et al.* (1986) KCA 8.2.2.1/01 (EFSA Conclusion, 2012)
Oncorhynchus mykiss	Kaolin	64 d	NOEC = 1400 mg a.s./L (nom)	Goldes *et al*. (1988) KCA 8.2.2/01 (2012 Dossier)
Oncorhynchus kisutch & Oncorhynchus mykiss	Kaolin	7-8 days	NOEC < 2000 mg a.s./L (nom)	Redding, Schreck, & Everest (1987) KCA 8.2.2/02 (2012 Dossier)
Acute aquatic invertebrate				
Cancer magister (Dungeness crab)	Kaolin	200 h (flow-through)	LC_{50} = 32000 mg a.s./L (nom)	McFarland & Peddicord (1980)

Species	Substance	Exposure System	Results	Reference
				KCA 8.2.4.2/01 (EFSA Conclusion, 2012)
Daphnia magna	Surround WP	48 h (static)	EC_{50} >600 mg product/L (nom) (>570 mg a.s./L)	Goodband (2006) Report no.: 2120/0004 KCP 10.2.1/01
Algae				
Scenedesmus subspicatus	Surround WP	72 h (static)	EC_{50} >600 mg product/L (nom) (>570 mg a.s./L)	Vryenhoef (2006) Report no.: 2120/0003 KCP 10.2.1/02
Higher-tier studies (micro- or mesocosm studies)				
Not required				

nom = nominal
*Study not relevant for risk assessment as explained in MCP Section 10, Part 10.2 as it is not a standard guideline study

Chronic data on aquatic invertebrates are not available for the active substance and a waiver has been requested in MCA 8.2.5.1. A waiver is also requested for chronic data on aquatic invertebrates with the formulated product since SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). Therefore, the formulated product is highly unlikely to be of higher toxicity compared to the active substance, kaolin.

However, fish were demonstrated to be more sensitive from acute exposure to kaolin (LC_{50} = 494 mg a.s./L), compared to aquatic invertebrates (EC_{50}>570 mg a.s./L). As an ELS study is available for fish and considered in the following risk assessment, the chronic risk to aquatic invertebrates is also concluded to be addressed. In addition, a chronic algae study with SURROUND® WP CROP PROTECTANT is available and demonstrated a low toxicity from the proposed application rate of kaolin. The proposed uses should not lead to surface water levels outside normal range and thus chronic testing is not required. Therefore, chronic toxicity data for aquatic organisms are not considered to be necessary and a waiver has been requested (under Section MCA 8.2.5).

Kaolin (as clay) is present in most natural water bodies and the use of SURROUND® WP CROP PROTECTANT in agriculture will not significantly alter the normal background levels. Also, the aluminium silicate (kaolin) in SURROUND® WP CROP PROTECTANT is only a select fraction of natural kaolin in which many of the impurities have been removed. It is not expected to act any differently from natural clays as it will become mixed with other natural components of sediments and suspended solids.

Aquatic organisms are also exposed to high levels of clay when floods, storms creating run-off or other natural phenomena, result in high turbidity in natural water bodies. Exposure to aluminium silicate (kaolin) from SURROUND® WP CROP PROTECTANT use in agriculture will always represent a minute fraction of the exposure to clay particles occurring during these natural events.

In addition, man also causes turbidity through dredging operations, boating activities or artificial impoundment. Studies have shown that long-term turbidity at high concentrations has a negative effect upon aquatic organisms (adverse effects mostly noted for freshwater organisms with potential in reduced feed and burial of eggs which leads to a reduction in hatching[19]). However, at the recommended rates and spray frequency for SURROUND® WP CROP PROTECTANT, natural streams or ponds (or larger water bodies) could never reach these turbidity levels through following label use recommendations. Also, often for studies on the influence of turbidity on the toxicity of chemicals to aquatic organisms, a standard turbidity laboratory water sample is prepared with kaolin (approximately 1 g kaolin/L of water). This level of kaolin is therefore well known not to be harmful to fish and aquatic invertebrates.

Finally, clay is a well-known water-tight liner used in the creation of artificial ponds and canals. It is also common practice in the US state of Georgia, where most of the high quality kaolin is mined, to convert exhausted deposits of aluminium silicate / kaolin into fresh water lakes or ponds where fish are stocked and go on to breed successfully, thus demonstrating that aquatic organism can tolerate massive exposures to aluminium silicate.

A thorough search of the public domain literature has found several publications on aluminium silicate (kaolin) and aquatic organisms. These are summarised below and confirm the very low toxicity of aluminium silicate (kaolin) to aquatic organisms. There are several useful publications that demonstrate kaolin can be used as an absorbent to reduce the aquatic toxicity of certain industrial chemicals that might be found in sewage effluent (Cary, *et al*. 1987; Maki, *et al.* 1979 and Valenta, *et al*. 1980; studies summarized in Document MCA, Section 8, Point 8.8, Talaat *et al*., 2011[20], Abu-Safa *et al*., 2012[21]).

Aluminium silicate (kaolin) is also not soluble in water and thus not bioavailable to fish, aquatic invertebrates, algae and aquatic plants.

Risk assessment for aquatic organisms

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).

~~Estimated worst case exposure (30 kg product/ha, 8.2% spray drift at 3 m) for aluminium silicate (kaolin) in surface water was calculated in MCP 9, Point 9.2.5 to be as follows:~~

~~PEC_{SW} = 0.802 mg a.s./L (max single spray)~~
~~PEC_{SW} = 3.208 mg a.s./L (total season)~~

Following the request of the co-RMS, the FOCUS STEPS 1-2 model was used to calculate PECsw values (please refer to Document M-CP S9 for details of input values and output).
All possible scenario combinations were modelled:

- North and South Europe
- Early application (minimal crop cover)
- Late application (full canopy)
- Treatment in October to February, March to May and June to September

Full results are presented in Document M-CP S9. Late application affords the highest PECsw value, which is identical in all time periods and for North and South scenario. Due to the inorganic nature of the active

[19] Kefford, B.J., Zalizniak, L., Dunlop, J.E., Nugegoda, D and Choy, S.C. (2010). How are macroinvertebrates of slow flowing lotic systems directly affected by suspended and deposited sediments? Environmental Pollution 158: 543-550.
[20] Talaat, H.A., et al. (2011). Evaluation of Heavy Metals Removal Using Some Egyptian Clay. 2011 2nd International Conference on Environmental Science and Technology. IPCBEE vol 6, Singapore.
[21] Abu-Safa, A., Abu-Safa, S., Mosa, M., and Gharaibeh, S. (2012). Low Cost Pre-Treatment of Pharmaceutical Wastewater. International Journal of Chemical and Biological Engineering vol 6.

substance, the model proposes higher surface water contamination for single application rather than multiple applications. Therefore, the higher single application value is used for worst-case risk assessment. The values are as follows:

- PEC_{SW} = 0.8028 mg/L (Single application)
- PEC_{SW} = 0.6656 mg/L (Multiple application)

This concentration is very low compared with the levels of aluminium silicate (kaolin) and clays typically found in natural water bodies.

In the following table, the Tier 1 ratios between predicted environmental concentrations in surface water bodies (PEC_{sw}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given. The lowest toxicity endpoints have been considered.

Table 10.2-2: Tier 1 Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aluminium silicate (kaolin) for each organism group based on worst-case PEC_{sw} for the use of SURROUND® WP CROP PROTECTANT in grapevines (4 x 28.5 kg a.s./ha)

Group	Fish acute	Chronic fish	Invertebrate acute	Algae
Test species	Fish	*Oncorhynchus mykiss*	*Daphnia magna*	*Scenedesmus subspicatus*
Endpoint	LC_{50}	LC_{50}	EC_{50}	EC_{50}
(mg a.s./L)	494	100	> 570	> 570
AF	100	10	100	10
RAC (mg/L)	4.94	10	>5.7	>57
PEC total season (mg/L)	~~3.208~~ 0.803			
PEC/RAC	~~0.649~~ 0.163	~~0.321~~ 0.0803	~~<0.563~~ < 0.1409	~~<0.056~~ < 0.0141

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

An acceptable risk to aquatic organisms from the proposed use of SURROUND® WP CROP PROTECTANT is demonstrated based on a first-tier risk assessment. No further consideration to aquatic organisms is necessary.

However, the notifier is not in agreement with the acute toxicity endpoint for fish. A revised risk assessment is presented below based on the following justification in support of a more appropriate acute fish toxicity endpoint in assessing the potential risk of the natural compound, kaolin.

Appropriate acute fish toxicity endpoint to be considered in the risk assessment:

According to current data requirements (Commission Regulations (EU) No 283/2013), the acute toxicity of the active substance to fish is obtained by typically testing rainbow trout according to OECD guideline 203. In the case of aluminium silicate (kaolin), no guideline studies are available. Information has been found in the public domain regarding the toxicity of aluminium silicate (kaolin) to aquatic organisms. These tests were not designed as regulatory studies, but for fundamental research. Therefore, the results obtained must be carefully considered for relevance in adhering to current guidelines and for their validity prior to being considered for risk assessment purposes. It is noted that additional data for vertebrates is to be avoided where possible.

Studies considered for risk assessment purposes should comply with OECD 203 (Fish, Acute Toxicity Test). When the rainbow trout is not used, the freshwater fish recommended for testing (including trout) range from 2.0 to 5.0 ± 1.0 cm in total length of test fish. This indicates the fish are juveniles (fingerling), free-feeding and fully developed. Therefore, the appropriateness of considering a study with eggs and larvae to derive the standard acute toxicity endpoint for fish is not deemed appropriate and rather overly conservative. Therefore, although the toxicity endpoint reported by Isono *et al*. (1998) is the lowest value, as it is based on the effects on marine larvae, it is not considered appropriate for use in the risk assessment for the following reasons.

Larvae are not free-feeding as they still carry a yolk-sac for their nutrients. When they have developed to the point where they are capable of feeding themselves, the fish are called fry. When, in addition, they have developed scales and working fins, the transition to a juvenile fish is complete and it is called a fingerling. Fingerlings are typically about the size of fingers. The juvenile stage lasts until the fish is fully grown, sexually mature and interacting with other adult fish.

The study by Isono *et al*. (1998) used newly hatched marine larvae (prior to the opening of the oral or gill chambers) which were exposed to kaolin for 12 hours in the toxicity study. Although an acute study, the design was related more to chronic OECD 236 (Fish Embryo Acute Toxicity (FET) Test) and parts of OECD 210 (Fish, Early-life Stage Toxicity Test) than satisfying the criteria of the selection of test species for OECD 203 (Fish, Acute Toxicity Test). Therefore, the results from larvae testing is not appropriate for use in a standard guideline acute fish risk assessment. Based on the available data, the lowest acute juvenile fish LC_{50} of 3000 mg/L is derived from the Shiner perch and is appropriate for risk assessment purposes, without conducting additional unnecessary vertebrate testing in the laboratory for aluminium silicate (kaolin).

However, as there are several available toxicity endpoints, further evaluation of the entire data set should be considered. The geomean and SSD (Species Sensitivity Distribution) were conducted based on the available acute fish data and are deemed appropriate compared to a larvae toxicity endpoint.

Geomean:
The use of the fish larva data only is not deemed appropriate as discussed above. The geometric mean has therefore been calculated using all tested species from the literature references (Table 10.2-3), including the larva data. In addition, the geometric mean has also been calculated for all fish excluding larval data (Table 10.2-4), and only those studies considered valid for risk assessment purposes (Table 10.2-5) during the initial Dossier review (including larval data).

Table 10.2-3: Acute fish geomean using all available fish and larval fish endpoints

Species	LC_{50} (mg a.s./L)	Valid (Y/N)	Reference
Red sunbeam (*Pagrus major*)	1000	N	Isono, *et al.* (1998)
Striped beakperch (*Oplegnathus fasciatus)*	710	N	
Threeline grunt (*Parapristipoma trilineatum)*	170	N	
English sole (*Parophrys vetulus*)	70000	Y	McFarland & Peddicord (1980)
Shiner perch (*Cymatogaster aggregat*e)	3000	Y	
Menhaden (*Brevoortia tyrannus*)	>140000	Y	Sherk, *et al* (1973)
Striped killifish (*Fundulus majalis*)	>140000	Y	
Mummichlog (*F. heteroclitus)*	>140000	Y	
Atlantic silverside (*Menidia menidia*)	>140000	Y	
Striped bass (*Morone saxatilis*)	>140000	Y	
White perch (*M. americana*)	>140000	Y	
Spot (*Leiostomus xanthurus*)	>140000	Y	
Croaker (*Micropogon undulatus*)	>140000	Y	
Weakfish (*Cynoscion regalis*)	>140000	Y	
Hogchoker (*Trinectes maculatus*)	>140000	Y	
Oyster toadfish (*Opsanus tau*)	>140000	Y	
Coho salmon (*Oncorhynchus kisutch*)	>4000	N	Redding, Schreck, & Everest (1987)
Rainbow trout (*Oncorhynchus mykiss*)	>4000	N	

Geomean:	**28,603**		

Table 10.2-4: Acute fish geomean estimate using fish data excluding larval data

Species	LC_{50} (mg a.s./L)	Valid (Y/N)	Reference
English sole (*Parophrys vetulus*)	70000	Y	McFarland & Peddicord (1980)
Shiner perch (*Cymatogaster aggregate*)	3000	Y	
Menhaden (*Brevoortia tyrannus*)	>140000	Y	Sherk, *et al* (1973)
Striped killifish (*Fundulus majalis*)	>140000	Y	
Mummichlog (*F. heteroclitus)*	>140000	Y	
Atlantic silverside (*Menidia menidia*)	>140000	Y	
Striped bass (*Morone saxatilis*)	>140000	Y	
White perch (*M. americana*)	>140000	Y	
Spot (*Leiostomus xanthurus*)	>140000	Y	
Croaker (*Micropogon undulatus*)	>140000	Y	
Weakfish (*Cynoscion regalis*)	>140000	Y	
Hogchoker (*Trinectes maculatus*)	>140000	Y	
Oyster toadfish (*Opsanus tau*)	>140000	Y	
Coho salmon (*Oncorhynchus kisutch*)	>4000	N	Redding, Schreck, & Everest (1987)
Rainbow trout (*Oncorhynchus mykiss*)	>4000	N	
Geomean:	**64,405**		

Table 10.2-5: Acute fish geomean estimate excluding studies that were not considered valid for risk assessment purposes during the initial EFSA review (2012)

Species	LC_{50} (mg a.s./L)	Reference
Red sunbeam (*Pagrus major*)	1000	Isono, *et al.* (1998)
Striped beakperch (*Oplegnathus fasciatus)*	710	
Threeline grunt (*Parapristipoma trilineatum)*	170	
English sole (*Parophrys vetulus*)	70000	McFarland & Peddicord (1980)
Shiner perch (*Cymatogaster aggregate*)	3000	
Menhaden (*Brevoortia tyrannus*)	>140000	Sherk, *et al* (1973)
Striped killifish (*Fundulus majalis*)	>140000	
Mummichlog (*F. heteroclitus)*	>140000	
Atlantic silverside (*Menidia menidia*)	>140000	
Striped bass (*Morone saxatilis*)	>140000	
White perch (*M. americana*)	>140000	
Spot (*Leiostomus xanthurus*)	>140000	
Croaker (*Micropogon undulatus*)	>140000	
Weakfish (*Cynoscion regalis*)	>140000	
Hogchoker (*Trinectes maculatus*)	>140000	
Oyster toadfish (*Opsanus tau*)	>140000	
Geomean:	**36,577**	

The geometric means were 28,603 mg/L when considering all the available data for acute fish exposed to kaolin, 64,405 mg/L when the larval data was excluded, yet using all the available fish data regardless of validity. However, in line with the 2012 EFSA review, the geometric mean considering all valid fish studies (including larval data) a geomean of **36,577 mg a.s./L** should be considered for risk assessment purposes. This value does not refute EFSA's view that kaolin can cause adverse effects to fish larvae, however, it does consider all valid data in assessing the acute risk to fish from 16 different species based on the geomean as supported in the current Aquatic guidance (2013). This value also remains conservative as it addresses the acute risk to fish at different growth stages and several of the endpoints are based on "greater" than values. Therefore, in theory, discussion of the relevance of the assessment factor (AF) of a 100 could also be considered as it is not standard to test larval stage fish and thus the AF of 100 can be considered to be very conservative.

Species Sensitivity Distribution (SSD):
As there are more than 8 test species, an SSD approach to address the wide variety in toxicity and reduce the assessment factor (AF) in the risk assessment was also considered. However, the majority of the endpoints are based on “greater” than values (unbound values). Such endpoints are not usually considered for SSD calculations, however, since there is a set of identical unbound toxicity values, these endpoints can be considered (without the > sign).

Using E_tX 2.1 programme[22], calculation of the HC_5 value was attempted. The program fits the log_{10} toxicity values to a normal distribution. The reliability in the statistical determination of the HC_5 value carries uncertainty since the Goodness-of-fit tests for normality are all rejected. Therefore, the SSD approach was not considered further as it is it not deemed appropriate for this data set.

Therefore, as there are multiple LC_{50} values for fish (16 valid species’ endpoints from the literature), consideration of the geomean and SSD was performed, as opposed to considering the geomean of larval fish toxicity data only. The geomean is considered instead of the SSD approach since the statistical analysis of the data set were all rejected and the majority on the toxicity endpoints were based on unbound values.

The most appropriate LC_{50} value for acute fish exposed to kaolin for use in the acute risk assessment is considered to be the geomean value of **36,577 mg a.s./L** based on data from all the valid acute toxicity studies and including the fish larval toxicity endpoints.

In the following table, the revised ratios between predicted environmental concentrations in surface water bodies (PEC_{sw}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given. The appropriate acute fish toxicity endpoints have been considered.

Table 10.2-6: Revised aquatic risk assessment: acceptability of risk (PEC/RAC < 1) for aluminium silicate (kaolin) for each organism group based on worst-case PEC_{sw} for the use of SURROUND® WP CROP PROTECTANT in grapevines (4 x 28.5 kg a.s./ha)

Group	Fish acute	Chronic fish	Invertebrate acute	Algae
Test species	Fish (geomean)	*Oncorhynchus mykiss*	*Daphnia magna*	*Scenedesmus subspicatus*
Endpoint (mg a.s./L)	LC_{50} 36,577	LC_{50} 100	EC_{50} > 570	EC_{50} > 570
AF	100	10	100	10
RAC (mg/L)	365.77	10	>5.7	>57
PEC total season (mg/L)	~~3.208~~ 0.803			
PEC/RAC	~~0.009~~ 0.002	~~0.32~~ 0.0803	~~<0.56~~ < 0.141	~~<0.056~~ < 0.014

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

[22] Van Vlaardingen PLA, Traas TP, Wintersen AM, Aldenberg T. (2004). ETX 2.1. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 601501028/2004, 68 pp.

An acceptable risk to aquatic organisms from the proposed use of SURROUND® WP CROP PROTECTANT is demonstrated based on a first-tier risk assessment with consideration of the more appropriate acute fish toxicity endpoint. No further consideration to aquatic organisms is necessary.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A waiver is requested for requiring an acute fish toxicity study with the formulated product based on the following:

- The acute toxicity of the formulation can be extrapolated from the active substance data since the SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). Therefore, the formulated product is highly unlikely to be of higher toxicity compared to the active substance, kaolin.

- The selection of endpoints for the risk assessment of fish is in line with the endpoint used for the previous EU review process.

- No new data for fish have been generated since the existing studies remain valid in support of aluminium silicate and for reasons of animal welfare.

- A low toxicity to fish has been demonstrated with a PEC/RAC ratio of 0.009, when considering the appropriate LC_{50} value of 36,577 mg a.s./L for fish.

In light of these considerations, no acute toxicity testing with fish exposed to SURROUND® WP CROP PROTECTANT is considered to be necessary for the purposes of renewal and the acute risk to fish is concluded to be low.

An acute study on *Daphnia magna* and a chronic green algae study with SURROUND® WP CROP PROTECTANT are available and the derived endpoints from the studies have been used in the risk assessment. The studies have been generated to satisfy the data requirements in accordance with Commission Regulation (EU) No 284/2013.

Reference:	KCP 10.2.1/01, Goodband, T.J. 2006
Title:	Surround WP Crop Protectant: Acute toxicity to *Daphnia magna*
Report No.:	2120/0004
Guideline(s):	OECD 202 (April, 2004)
Deviation(s):	None
GLP:	Yes

Executive Summary

The objective of this study was to determine the effects of Surround WP (nominally 95% kaolin) on *Daphnia magna* during a 48 hour exposure period under static test conditions. The study encompassed four treatment groups (2 dose rates of the test item, a control and positive control) each containing 20 individuals (10 daphnids per vessel in duplicate). The mobility of the daphnids was determined in a static 48 hour test by visual observation after 24 and 48 hours.

The toxic effect of the test item Surround WP to *Daphnia magna* was assessed in a static concentration-response test. The acute toxicity of the test material to the freshwater invertebrate *Daphnia magna* has been investigated and gave a 48 hours EC_{50} >600 mg test item/L (equivalent to >570 mg a.s./L). Correspondingly, the NOEC was 600 mg test item/L. Results were based on nominal test concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Surround WP
	Batch number:	AL060727
	Purity:	95% kaolin (analysed)
	Appearance:	Off-white powder
2.	**Reference item*:**	Potassium dichromate
	Test concentrations:	0.32, 0.56, 1.0, 1.8 and 3.2 mg/L

*A positive control study conducted in a separate study.

3.	**Dilution water:**	Reconstituted water
4.	**Test organism**	
	Species:	*Daphnia magna*
	Age at test initiation:	1st instar, < 24 hr old
	Source:	In-house culture (Safepharm Laboratories)
	Acclimation:	Not reported
5.	**Treatment groups:**	100 and 600 mg test item/L (nominal)
6.	**Environmental conditions:**	
	Test vessels:	250 mL glass jars, approximately 200 mL of test preparation were used.
	Temperature:	20.1 to 20.3 °C
	pH:	7.9 to 8.0
	Dissolved oxygen:	8.7 to 8.9 mg/L
	Photoperiod:	16 hours light : 8 hours dark

B. STUDY DESIGN AND METHODS

1. Animal assignment and treatment:
This study encompassed 4 treatment groups (2 dose rates of the test item plus control and positive control) in a static system. Twenty daphnids per control and test concentration, divided into 2 groups of 10 animals. Test organisms were exposed for 48 hours under static conditions.

2. Dose preparation:
An amount of test material (500 mL) was dispersed in reconstituted water and the volume adjusted to 500 mL to give a 1000 mg/L stock dispersion. Aliquots (50 and 300 mL) of this stock dispersion were each added separately to a final volume of 500 mL of reconstituted water to give the test concentrations of 100 and 600 mg/L, respectively.

The stock dispersions and each of the prepared concentrations were inverted several times to ensure adequate mixing and homogeneity.

3. Measurements/observations:
All test daphnids were observed for immobility and abnormal behavior or appearance, if any, at 24 and 48 h of exposure. Mobility of the daphnids was assessed by gently swirling the test container for 15 seconds and observing their swimming behaviour. Those daphnids unable to swim during the agitation of the test

container and organisms remaining settled on the water surface or settled at the bottom of the test vessel were recorded as immobile.

Temperature, dissolved oxygen and pH of the test media were measured at 0 and 48 h after the commencement of exposure.

The test concentrations in the test preparations were not determined by analysis at the request of the Sponsor.

4. Statistics:
The EC_{50} value and associated confidence limits at 24 hours and the slope of the response curve and its standard error were calculated by the maximum-likelihood probit method (Finney, 1971). The EC_{50} value and associated confidence limits at 48 hours a were calculated using the trimmed Spearman-Karber method.

II. RESULTS AND DISCUSSION

Based on the validity criteria, the study is concluded to be valid:
- Not more than 10% of the daphnids in the control are immobile (actual value = 0%)
- At study end, dissolved oxygen is ≥ 3 mg/L in control and test vessels (actual value > 8.7 mg/L)

The test media at 0 hours were observed to be cloudy pale grey/white homogeneous dispersions increasing turbidity with increasing concentrations. At 24 and 48 hours the test media were observed to be clear, colorless water columns with all the test material settled to the bottom of the vessels.

A. Immobilisation:
There was no immobilisation in 40 daphnids exposed to test concentrations of 100 and 600 mg/L or the control for a period of 48 hours. Resulting in a 48 hours EC_{50} > 600 mg test item/L (equivalent to >570 mg a.s./L). Correspondingly, the NOEC was 600 mg test item/L.

Table 10.2.1/01-1: Immobilisation of *Daphnia magna* exposed to Surround WP

Nominal test conc. (mg/L)	Number of larvae tested	Number of immobilised *Daphnia* after		% of immobilised *Daphnia* after	
		24 h	**48 h**	**24 h**	**48 h**
Control	20	0	0	0	0
100	20	0	0	0	0
600	20	0	0	0	0

Inspection of immobilisation data for the reference item at 3, 24 and 48 hours gave the following results:

Time (h)	EC_{50} (mg/L)	95% Confidence limits (mg/L)
3	> 3.2	-
24	0.88	0.75 – 1.0
48	0.60	0.53 – 0.68

The NOEC after 24 and 48 hours exposure was 0.32 mg potassium dichromate/L, EC_{50} was 0.82 mg/L.

III. CONCLUSION

The acute toxicity of the test material to the freshwater invertebrate *Daphnia magna* has been investigated and gave a 48 hours EC_{50} > 600 mg test item/L (equivalent to >570 mg a.s./L). Correspondingly, the NOEC was 600 mg test item/L. Results were based on nominal test concentrations.

Reference:	KCP 10.2.1/02, Vryenhoef, H. 2006
Title:	Surround WP Crop Protectant: Algal inhibition test
Report No.:	2120/0003
Guideline(s):	OECD 201 (1984)
Deviation(s):	None
GLP:	Yes

Execute summary

The aim of the study was to determine the inhibitory effect of the test item Surround WP on the growth of fresh water green algae *Scenedesmus subspicatus* in a 72 hours static test.

The study comprised two test item and a control. There were 3 replicates (= test units) for each treatment.

The effect of the test material on the growths of *Scenedesmus subspicatus* has been investigated and gave EC_{50} values greater than 600 mg test item/L (equivalent to >570 mg a.s./L). Correspondingly the NOEC was 600 mg test item/L. Results were based on nominal test concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Surround WP (95% kaolin)
Batch number: AL060727
Purity: 95% kaolin (analysed)
Appearance: Off white powder

2. Reference item: None

3. Vehicle: None

4. Dilution water: Reverse osmosis purified deionized water

5. Test organism
Species: Freshwater green algae, *Scenedesmus subspicatus* strain CCAP 276/20
Source: Culture Collection of Algae and Protozoa (CCAP), Scotland
Initial density: 10,000 cells/mL

6. Treatment groups: 100 and 600 mg test item/L

7. Environmental conditions:
Test vessels: 250 mL conical flasks each containing 100 mL of test solution. With continual shaking at 150 rpm
Temperature: 24 ± 1°C
pH: 7.3 -8.3
Photoperiod: Continuous lighting

B. STUDY DESIGN AND METHODS

1. Organism set up and treatment:
The test organisms, *Scenedesmus subspicatus*, were exposed to a range of 2 concentrations of the test item (100 and 600 mg/L in three replicate flasks per concentration) for 72 hours under constant illumination and shaking.

2. Dose preparation:
Amounts of test material (50 and 300 mg) were each separately dispersed in culture medium and the volume adjusted to 500 mL to give 100 and 600 mg/L stock dispersions. Each of these stock dispersions were inoculated with algal suspension (2.5 mL) to give the required test concentrations of 100 and 600 mg/L.

The stock dispersions and each of the prepared concentrations were inverted several times to ensure adequate mixing and homogeneity.

3. Measurements/observations:
Samples of the algal populations were removed daily (0. 24, 48 and 72 hours) and cell concentrations were determined for each control and treatment group, using a haemocytometer and light microscope.

The pH of each control and test flask was determined at initiation of the study and after 72 hours exposure. The temperature within the incubator was recorded daily.

The test concentrations in the test preparations were not determined by analysis at the request of the Sponsor.

4. Statistics:
The E_bC_{50} (72h) (biomass) was determined by inspection of the area under growth curve data after 72 hours. The E_RC_{50} (0 -72h) (growth rate) was determined by the inspection of the growth rates for the period 0 – 72 hours.

One way analysis of variance incorporating Bartlett's test for homogeneity of variance (Sockal and Rohlf 1981) and Dunnett's multiple comparison procedure for comparing several treatments with a control (Dunnett's 1955) was carried out on the 0-72 hour growth rate data for the control and the 100 and 600 mg/L test concentrations to determine any statistically significant differences between the test and the control groups.

II. RESULTS AND DISCUSSION

Based on the validity criteria, the study is concluded to be valid:
- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period (actual value = by a factor of 51)
- The mean coefficient of variation in the control cultures must not exceed 35%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*

At 0 hours the control cultures were clear colourless solutions, the 100 mg/L cultures were cloudy white dispersions and the 600 mg/L cultures were milky white dispersions After the 72 hour exposure period the control cultures were green dispersions, the 100 mg/L cultures were cloudy green dispersions and the 600 mg/L cultures were milky green dispersions. The green colouration at 72 hours was due to growth of algal cells.

A. Effect on cell density and growth rate:
Neither the growth nor the biomass of *Scenedesmus subspicatus* were significantly affected by the presence of the test item over the 72-hour exposure period.

Accordingly, the following results were determined:
E_bC_{50} (72h) > 600 mg test item/L
E_rC_{50} (0 -72h) > 600 mg test item/L

Statistical analysis of the growth rate data was carried out for the control and the 100 and 600 mg/L test item concentrations. There were no statistically significant differences (P≥ 0.05) between the control and the 100 and 600 mg/L test groups and therefore the NOEC was 600 mg/L.

All test and control cultures were inspected microscopically at 72 hours. In the 100 mg/L test culture clumping of the algal cells was observed whilst in the 600 mg/L test cultures clumping and enlarged cells were observed.

Table 10.2.1/02-1: Inhibition of growth rate and biomass

Nominal application rate (mg/L)	Area under curve at 72 hr	Inhibition (%)**	Growth rate (0-72hr)	Inhibition (%)**
Untreated control	9400000	NA	0.055	NA
100	8460000	10	0.054	2
600	8110000	14	0.053	4

NA not applicable.

III. CONCLUSION

The effect of the test material on the growth of *Scenedesmus subspicatus* has been investigated and gave EC_{50} values greater than 600 mg test item/L (equivalent to >570 mg a.s./L). Correspondingly the NOEC was 600 mg test item/L. Results were based on nominal test concentrations.

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A waiver is requested for additional long-term and chronic toxicity studies on aquatic organisms with SURROUND® WP CROP PROTECTANT which is composed of 95% aluminium silicate (kaolin) and 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (please refer to Part C for details on formulation composition). The toxicity of the formulated product can be extrapolated from the active substance.

CP 10.2.3 Further testing on aquatic organisms

An acceptable risk to aquatic organisms from the proposed use of SURROUND® WP CROP PROTECTANT is concluded, therefore no further studies are considered necessary.

CP 10.3 Effects on Arthropods

CP 10.3.1 Effects on bees

Study on the acute oral and contact toxicity to honey bees were carried out with kaolin and were submitted in support of aluminium silicate (kaolin) during the previous EU review. Full details of these studies are provided in the previous EU DAR (2008) and related documents and summarised in MCA 8.3.1.

Acute honey bee data are not required for the proposed formulated product SURROUND® WP CROP PROTECTANT. The acute effects on honey bees of SURROUND® WP CROP PROTECTANT can be predicted based on the active substance data since SURROUND® WP CROP PROTECTANT is composed of 95% aluminium silicate (kaolin) and 5.0% of well know additives of no toxicological concern (please refer to Part C for details on formulation comsposition). However, an acute oral study is available with the formulated product and is summarised in Table 10.3.1-1.

In accordance with Commission Regulation (EU) No 284/2013 setting out the data requirements for plant protection products, the chronic risk to bees including adult and larval life stages shall be addressed. Chronic data for adult honey bees and repeated larvae study are now available ~~(draft report)~~ for SURROUND® WP CROP PROTECTANT and the results are summarised below. ~~For the chronic larvae study, due to the limited optimal seasonal timing in conducting a successful, valid study in the laboratory, the definitive study could not be conducted in time of this submission. Range-finding results are presented in the table below and will be replaced once the definitive study in completed in 2018.~~

Table 10.3.1-1: Toxicity endpoints relevant for consideration for the honey bee risk assessment

Species	Substance	Exposure System	Results	Reference
Apis mellifera	M-96-018*	Acute oral	LC_{50} > 1000 ppm a.s./bee	Hoxter *et al*. (1997) Report no.: 469-102 KCA 8.3.1.1.1/01 (EFSA Conclusion, 2012)
Apis mellifera	M-96-018*	Acute contact	LC_{50} > 100 µg a.s./bee	Palmer *et al*. (1997) Report no.: 469-101 KCA 8.3.1.1.2/01 (EFSA Conclusion, 2012)
Apis mellifera	SURROUND® WP CROP PROTECTANT	Acute oral	LC_{50} > 2.0 mg product/bee (equivalent to >1900 µg a.s./bee)	Goodband (2006) Report no.: 2120/0005 KCP 10.3.1.1/01
Apis mellifera	SURROUND® WP CROP PROTECTANT	Oral, 10d Repeated exposure	LDD_{50} = 1390 µg a.s./bee/day LC_{50} = 56410 mg a.s./kg diet NOEDD = 660 µg a.s./bee/day NOEC = 29319 mg a.s./kg diet	Ansaloni (2019) Report no.: TRC17-208BA (KCP 10.3.1.2/01)
Apis mellifera	SURROUND® WP CROP PROTECTANT	22d Larvae toxicity Repeated exposure	NOED = 405 µg a.s./larva NOEC = 2.893 mg a.s./mL diet	Ansaloni (2019) Report no.: TRC17-184BA (KCP 10.3.1.3/01)
Higher-tier studies (tunnel test, field studies)				
Field studies in flowering pear and apple orchards in the USA demonstrated that the application of a kaolin preparation at 56 kg/ha did not have adverse effects on numbers of bees foraging and their behaviour. (Mayer, 199a (KCP 10.3.1.6/01) and Mayer 199b KCP 10.3.1.6/02)				

*98.8% active kaolin

Risk assessment for bees

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) has not yet been noted at the EU level. Nevertheless, the current SANCO guidance document does not address the risk to honey bee larvae and chronic adult honey bee toxicity. Endpoints for these are available according to EFSA Guidance[23], which will subsequently be referred to as EFSA/2013/3295.

In the absence of alternative approaches, it was agreed in a general ecotoxicology meeting, Pesticide Peer Review Meeting 133 which took place from 23 to 25 September 2015 that the risk assessment to honey bees should be performed (first tier) according to EFSA/2013/3295. On the basis of all the available information, a conclusion should be drawn with regard to the risk to honey bees. For bumblebees and solitary bees, it was agreed that if any data are submitted, they should be evaluated. However, currently it cannot be recommended to routinely perform a risk assessment for these organisms. No data for bumble bees or solitary bees have been submitted, and based on the recommendation, an assessment for bumble bees and solitary bees has not been conducted.

The evaluation of the risk for honey bees from exposure to SURROUND® WP CROP PROTECTANT was therefore performed in accordance with the recommendations to the first tier of EFSA/2013/3295.

There are no ecologically relevant metabolites to be considered in the risk assessment to honey bees.

Contact exposure

The acute contact risk to honey bees has been assessed for grapevines and the hazard quotient (HQ) is presented in the following table.

Table 10.3.1-2: Screening Step risk assessment for honey bees from contact exposure due to the use of SURROUND® WP CROP PROTECTANT in grapevines

Intended use		Grapevines			
Active substance		Aluminium silicate			
Application rate (kg a.s./ha)		4 × 28.5 (7 day interval)			
Exposure scenario		Acute contact (screening assessment)			
Test group	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**$HQ_{contact}$**	**Trigger**	**Acceptable risk?**
Honey bee (adult)	>100	28500	< 285	85	No

The acute risk to honey bees *via* contact exposure is not demonstrated to be acceptable based on the screening assessment. Whilst there was no evidence of any contact toxicity toward bees in the Tier 1 laboratory study the tested rate of 100 µg a.s./bee was not high enough to generate a hazard quotient below the trigger value of 85. A Tier I assessment has therefore been conducted to refine the risk to bees foraging on the treated crop, weeds in the treated field, and the field margin. Exposure from adjacent crops are covered by the assessment for field margin according to EFSA/2013/3295.

[23] EFSA (2013). 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, 268 pp.

Table 10.3.1-3: Tier I assessment of the risk to honey bees from contact exposure from foraging on the treated crop, weeds in the treated field and field margin/adjacent crops due to the use of SURROUND® WP CROP PROTECTANT in grapevines

Intended use		Grapevines				
Active substance		Aluminium silicate				
Application rate (kg a.s./ha)		4 × 28.5 (7 day interval) BBCH 51-65				
Exposure scenario		Acute contact (Tier I assessment)				
Test group	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**Fdep (default deposition)**	**$HQ_{contact}$**	**Trigger**	**Acceptable risk?**
Treated crop						
Honey bee (adult)	>100	28500	NA*	-	85	Yes
Weeds in treated field						
Honey bee (adult)	>100	28500	0.3**	< 85.5	42	No
Field margin / adjacent crop						
Honey bee (adult)	>100	28500	0.027 (early stage)	< 7.7	42	Yes
Honey bee (adult)	>100	28500	0.08 (late stage)	< 22.8	42	Yes

* Honey bees are only attracted to the grape pollen and not the nectar, hence Not Applicable as the pollen consumption of forager honey bees is negligible. **Application commences at BBCH principle growth stage 5.

The acute risk to honey bees *via* contact exposure is demonstrated to be acceptable based on the Tier I risk assessment, except when foraging on treated weeds in field.

Note that the toxicity value is based on a “greater than limit dose value” endpoint, and the conservative risk assessment assumes that weed coverage is 100% in the vineyards, and all weeds are at bloom during application. This is highly unlikely to be the situation as proper weed management is important for a healthy vineyard to minimise competition between grapevines and weeds for water, nutrients and light. There is typically a weed-free zone under the vines and the turf strips between vines are mowed or growth of weeds chemically controlled[24]. Therefore, the potential for blooming weeds to be present during application in grapevines is extremely limited and the acute risk from contact exposure to bees potentially foraging on weeds in treated field can be concluded as acceptable. This is further supported by the field studies (KCP 10.3.1.6/01 & /02) in flowering pear and apple orchards (which are far more attractive to bees for collection of pollen and nectar than grapevines) which demonstrated that applications of a kaolin preparation at 56 kg/ha did not have adverse effects on numbers of bees foraging or their behaviour.

Oral exposure

The acute and chronic oral risk to honey bees has been assessed and the exposure toxicity ratios (ETR) are presented in the following table.

[24] Dittmar, P.J., and Williamson, J.G. (2014). Weed Management in Grape. Horticultural Sciences Department. UF/IFAS Extension.

Table 10.3.1-5: Screening Step risk assessment of the risk for honey bees from oral exposure due to the use of SURROUND® WP CROP PROTECTANT in grapevines

Intended use	Grapevines				
Active substance	Aluminium silicate				
Application rate (kg a.s./ha)	4 × 28.5 (7 day interval)				
Exposure scenario	Oral (screening assessment)				
Test group	**Toxicity endpoint (µg a.s./bee)**	**SV (sideways spray)**	**ETR $_{oral}$**	**Trigger**	**Acceptable risk?**
Honey bee (adult) - acute	>1900	10.6	< 0.16	0.2	Yes
Honey bee (adult) - chronic	1390	10.6	0.22	0.03	No
Honey bee (larvae) - chronic	405	6.1	0.43	0.2	No

~~*Based on range finding results. To be revised when definitive study is completed.~~

The acute oral risk to adult honey bees is demonstrated to be acceptable based on the screening step assessment. The chronic oral risk to adult and larval honey bees was not demonstrated to be acceptable at the screening step. A Tier I assessment has therefore been conducted to refine the risk to bees foraging on the treated crop, an adjacent crop, weeds in the treated field, the field margin and foraging on a crop the following year.

Table 10.3.1-6: Tier 1 assessment of the risk to honey bees foraging on the treated crop from chronic oral exposure due to the use of SURROUND® WP CROP PROTECTANT in grapevines

Intended use			Grapevines				
Active substance			Aluminium silicate				
Application rate (kg a.s./ha)			4 × 28.5 (7 day interval)				
Exposure scenario			Oral (Tier 1 assessment)				
Test group	**Toxicity endpoint (µg a.s./bee)**	**Exposure factor (EF)**	**SV (sideward / upward spray)**	**TWA**	**ETR $_{oral}$**	**Trigger**	**Acceptable risk?**
Honey bee (adult) - chronic	1390	1	NA*	0.72	N/A*	0.03	Yes
Honey bee (larvae) - chronic	405	1	0.01	0.85	0.001	0.2	Yes

* Honey bees are only attracted to the grape pollen and not the nectar, hence Not Applicable as the pollen consumption of forager honey bees is negligible.

Table 10.3.1-7: Tier 1 chronic assessment of the risk to honey bees from oral exposure on adjacent crops, weeds in the treated field and the field margin due to the use of SURROUND® WP CROP PROTECTANT in grapevines after early application

Intended use		Grapevines					
Active substance		Aluminium silicate					
Application rate (kg a.s./ha)		4 × 28.5 (7 day interval)					
Exposure scenario		Oral (Tier 1 assessment, early application)					
Test group	**Toxicity endpoint (µg a.s./bee)**	**Exposure factor (EF)**	**SV (sideward / upward spray)**	**TWA**	**ETR $_{oral}$**	**Trigger**	**Acceptable risk?**
Adjacent crop							
Honey bee (adult) - chronic	1390	0.0047	5.8	0.72	0.0004	0.03	Yes
Honey bee (larvae) - chronic	405	0.0047	4.4	0.85	0.0012	0.2	Yes
Weeds in treated field							
Honey bee (adult) - chronic	1390	0.3*	2.9	0.72	0.013	0.03	Yes
Honey bee (larvae) - chronic	405	0.3*	2.2	0.85	0.039	0.2	Yes
Field margin							
Honey bee (adult) - chronic	1390	0.009	2.9	0.72	0.0004	0.03	Yes
Honey bee (larvae) - chronic	405	0.009	2.2	0.85	0.0012	0.2	Yes

*Application commences at BBCH growth stage 51.

Table 10.3.1-8: Tier 1 chronic assessment of the risk to honey bees from oral exposure on adjacent crops, weeds in the treated field and the field margin due to the use of SURROUND® WP CROP PROTECTANT in grapevines after a late application

Intended use		Grapevines					
Active substance		Aluminium silicate					
Application rate (kg a.s./ha)		4 × 28.5 (7 day interval)					
Exposure scenario		Oral (Tier 1 assessment, late application)					
Test group	**Toxicity endpoint (µg a.s./bee)**	**Exposure factor (EF)**	**SV (sideward / upward spray)**	**TWA**	**ETR oral**	**Trigger**	**Acceptable risk?**
Adjacent crop							
Honey bee (adult) - chronic	1390	0.0143	5.8	0.72	0.001	0.03	Yes
Honey bee (larvae) - chronic	405	0.0143	4.4	0.85	0.004	0.2	Yes
Weeds in treated field							
Honey bee (adult) - chronic	1390	0.3*	2.9	0.72	0.013	0.03	Yes
Honey bee (larvae) - chronic	405	0.3*	2.2	0.85	0.039	0.2	Yes
Field margin							
Honey bee (adult) - chronic	1390	0.027	2.9	0.72	0.001	0.03	Yes
Honey bee (larvae) - chronic	405	0.027	2.2	0.85	0.004	0.2	Yes

*Application commences as BBCH growth stage 51.

Table 10.3.1-9: Tier 1 assessment of the risk for honey bees from oral exposure due to the use of SURROUND® WP CROP PROTECTANT in grapevines –foraging in following year

Intended use		Grapevines					
Active substance		Aluminium silicate					
Application rate (kg a.s./ha)		4 × 28.5 (7 day interval)					
Exposure scenario		Oral (Tier 1 assessment – foraging in following year)					
Test group	**Toxicity endpoint (µg a.s./bee)**	**Exposure factor (EF)**	**SV (sideward / upward spray)**	**TWA**	**ETR oral**	**Trigger**	**Acceptable risk?**
Honey bee (adult) - chronic	1390	1	N/A*	0.72	N/A*	0.03	Yes
Honey bee (larvae) - chronic	405	1	0.002	0.85	0.0001	0.2	Yes

* Honey bees only attracted to the grape pollen not the nectar, hence Not Applicable as the pollen consumption of forager honey bees is negligible.

The chronic Tier 1 risk to adult and larvae honey bees is demonstrated to be acceptable for all scenarios. No further consideration is required. ~~However, note that the risk assessment for honey bee larvae will be revised once the definitive study is completed and valid results are available.~~

These findings are further supported by the low toxicity observed in the field trials. Field studies in flowering pear and apple orchards (which are far more attractive to bees for collection of pollen and nectar than grapevines) demonstrated that applications of a kaolin preparation at 56 kg/ha did not have adverse effects on numbers of bees foraging or their behaviour.

Drinking water assessment

Aluminium silicate (kaolin) is not soluble in water and will not dissipate but settle to the bottom of any water bodies. It is reasonable to assume that exposure from water sources would be minimal, if at all, hence the risk to bees drinking from surface water *via* water bodies and puddles present in the agricultural environment is concluded to be acceptable.

Based on the overall low risk to bees, exposure *via* guttation is considered to be minimal, especially in comparison to natural levels in the environment. Furthermore, aluminium silicate (kaolin) is not soluble in water and will settle.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Reference:	KCP 10.3.1.1.1/01 Goodband. T.J. 2006
Title:	Surround WP Crop Protectant: Acute toxicity to honeybees (*Apis mellifera*)
Report No.:	2120/0005
Guideline(s):	OECD Method 213
Deviation(s):	None
GLP:	Yes

Executive summary

The acute oral toxicity of SURROUND® WP CROP PROTECTANT to honey bees was tested in a 48-hour oral toxicity test. Toxicity of SURROUND® WP CROP PROTECTANT was assessed using young worker honey bees (*Apis mellifera* L.) derived from a healthy colony. The study was conducted as a Limit dose at the dose level of 2 mg SURROUND® WP CROP PROTECTANT /bee. A negative control (50% aqueous sucrose solution) and a reference item (dimethoate technical, 97% purity) were also tested.

The bees were initially fed with 200 µL of the treated diet. After approximately 6 hours, the feeding tubes were empty and the bees were fed 500 µL of a 50% w/v sucrose solution. A further 200 µL of a 50% w/v sucrose solution was given after 24 hours.

All the bees were observed for signs of mortality as well as any abnormal behavioural symptoms viz., regurgitation, disorientation, lethargy, distended abdomen, erratic movement, aggressiveness, trembling and tumbling at 4, 24 and 48 h post dosing.

There were no mortalities in 30 honey bees exposed to a test concentration of 2.0 mg/bee for a period of 48 hours. It is concluded that the median oral lethal dose (LD_{50}) of SURROUND® WP CROP PROTECTANT was greater than 2 mg test item/bee. Correspondingly, the No Observed Effect Concentration (NOEC) was 2.0 mg/bee.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	SURROUND® WP CROP PROTECTANT
	Description:	Off white powder
	Lot/Batch:	AL060727
	Purity:	95% w/w kaolin (calcined), analysed
2.	**Control:**	Untreated feed
	Vehicle:	None
3.	**Reference:**	Dimethoate technical, 97% purity

B. STUDY DESIGN AND METHODS

1.	**Test animals:**	Worker honey bee *(Apis mellifera* L.)
	Age/life stage:	>20 days old
	Source:	In-house culture (Safepharm Laboratories Limited)
2.	**Test units:**	Stainless steel wire mesh tubes (2-2.5 mm mesh size, 14 x 4 cm) with stoppers at both ends were used.
3.	**Test concentration:**	Limit dose, 2 mg test item/bee

4. Environmental conditions:

Temperature:	24.9 – 25.0 °C
Relative humidity:	59.8-60.3%
Photoperiod:	24 hrs darkness (except during observation)

5. Animal assignment and treatment:

The acute oral toxicity of SURROUND® WP CROP PROTECTANT to honeybee was tested in a 48-hour oral toxicity test. The study was conducted as a Limit test at 2 mg SURROUND® WP CROP PROTECTANT/bee. A negative control (50% aqueous sucrose solution) and a reference item (dimethoate technical, 97% purity) were also tested. The reference item was tested at the following rates: 0.05, 0.08, 0. 13, 0.21 and 0.33 µg dimethoate/bee. Three replicates per control, reference and test item were tested, each with 10 bees.

Groups of bees were the transferred impartially to the test cages. The bees were initially fed with 200 µL of the treated diet. After approximately 6 hours, the feeding tubes were empty and the bees were fed 500 µL of a 50% w/v sucrose solution. A further 200 µL of a 50% w/v sucrose solution was given after 24 hours.

6. Dose preparation:

The test material (1000 mg) was dispersed directly in 50% w/v sucrose solution and the volume adjusted to 10 mL to give 1000 mg/10 mL stock solution. An aliquot of 200 µL of the 1000 mg/10 mL stock dispersion was added to these feeding tubes to give the 2.0 µg/bee test concentration.

7. Measurements and observations:

All the bees were observed for signs of mortality as well as any abnormal behavioural symptoms viz., regurgitation, disorientation, lethargy, distended abdomen, erratic movement, aggressiveness, trembling and tumbling at 4, 24 and 48 h post dosing.

8. Statistics:

Not required. An estimate of the LD_{50} value was given by inspection of the mortality data for the test material. For the reference item, the LD_{50} values were calculated by the maximum-likelihood probit method (Finney 1971) using the ToxCalc computer software package. Probit analysis was used where two or more partial responses to exposure were shown.

II. RESULTS AND DISCUSSION

The study is considered valid. Average mortality was <10% in control after 48 hrs (actual value: 3%). The toxic reference LR_{50} was in the range of 0.10-0.35 µg/bee (actual value: 0.25 µg/bee) after 48 hours.

There were no mortalities in 30 honey bees exposed to a test concentration of 2.0 mg test item/bee for a period of 48 hours. It is concluded that the median oral lethal dose (LD_{50}) of SURROUND® WP CROP PROTECTANT was greater than 2 mg test item/bee. Correspondingly, the No Observed Effect Concentration (NOEC) was 2.0 mg test item/bee. No adverse effects of exposure were observed throughout the study.

Inspection of the mortality data for the reference item at 4 hours and analysis of the mortality data by the probit method at 24 and 48 hours based on the nominal test concentrations resulted in a 48 hr LD_{50} of 0.25 µg dimethoate/bee and a NOEC of 0.08 µg dimethoate/bee.

III. CONCLUSION

From the present study, it is concluded that the median oral lethal dose (LD_{50}) of SURROUND® WP CROP PROTECTANT was > 2 mg test item/bee. Correspondingly, the NOEC was 2.0 mg/bee.

CP 10.3.1.1.2 Acute contact toxicity to bees

No data are available or required for acute contact toxicity to bees since the authorisation of aluminium silicate (kaolin) as a plant protection product (EFSA (2012)). As discussed in the original DAR, it was considered that the acute contact risk to bees from the representative use of SURROUND® WP CROP PROTECTANT will be low.

A waiver is requested for acute contact honey bee toxicity data with the formulated product based on the following information:

- Testing with the formulated product is not required according to the data requirements set by the Commission Regulation EU 284/2013, since the toxicity of SURROUND® WP CROP PROTECTANT can be reliably predicted via the toxicity study with the active substance. The acute contact toxicity to bee with the active substance demonstrated a low risk with an LD_{50} > 100.0 µg/bee (maximum Limit test dose according to OECD 214: Honeybee, acute contact toxicity test). SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). Therefore, the formulated product is highly unlikely to be of higher toxicity compared to the active substance, kaolin (as noted with the acute oral toxicity data comparison between active substance and product testing).

In light of these considerations, no acute contact toxicity to bees with the formulated product is considered to be necessary for the purposes of renewal and the acute risk *via* contact exposure to honey bees is concluded to be low.

CP 10.3.1.2 Chronic toxicity to bees

Reference:	KCP 10.3.1.2/01, Ansaloni, T. 2019
Title:	Effects of Surround WP – Chronic oral toxicity to adult worker honey bees, *Apis mellifera* L. under laboratory conditions
Report No.:	TRC17-208BA
Guideline(s):	OECD No. 245
Deviation(s):	None
GLP:	Yes

Executive Summary

The objective of the study was to assess the effects of Surround WP (95% w/w kaolin) on adult worker honey bees (*Apis mellifera* L.) in a 10-day oral chronic toxicity test under laboratory conditions. Groups of 50 bees per treatment (5 replicates, each consisting of 10 bees in one cage) were orally fed with test item concentrations of 3957.98 to 79831.93 mg a.s./kg food; corresponding to 470.78 to 9500.00 µg a.s./bee/day.

The analytical results obtained by a digestion - ICP-OES analytical method demonstrated an overall recovery of 93% for both total aluminium and total silicon which fall under the SANCO/3029/99 rev.4 criteria of 70 to 110% mean recoveries.

The 10-day lethal daily dose $(LDD)_{50}$ value was determined to be 1390 µg a.s./bee/day and the LC_{50} value was determined to be 56410 mg a.s./kg diet. The 10 day no observed effect dietary dose (NOEDD) value was determined to be 660 µg a.s./bee/day based on the mean food consumption/bee/day corresponding to a NOEC of 29319 mg a.s./kg diet.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Surround WP (Kaolin Clay based partical film)
   Description: White powder
   Lot/Batch: AL170530
   Purity: 95% w/w kaolin

2. **Reference material:** BAS 152 11 I
   Description: Blue liquid
   Lot/Batch: FRE-001302
   Purity: 405.2 g/L dimethoate

3. **Control:** C (50 % (w/v) aqueous sucrose solution)
   Cs (50 % (w/v) aqueous sucrose solution + 0.1% Xanthan)
   Vehicle: 0.1% Xanthan (stabilizer to improve homogeneity of the treatment solutions)

B. STUDY DESIGN AND METHODS

1. **Test animals:** Honeybee *(Apis mellifera* L.)
   Age/life stage: 1-2 days old adult worker bees
   Source: Reliable commercial apiary
   Acclimation: 24 hours
   Diet: 50 % (w/v) aqueous sucrose solution, *ad libitum*

2. **Test units:** Stainless steel cages (approx.: base: 8.5 cm x 4.5 cm; height: 6.5 cm)

3. **Test concentration:** 3957.98, 6529.41, 10773.11, 17773.11, 29319.33, 48386.55 and 79831.93 mg a.s./kg food (equivalent to 470.78, 776.79, 281.70, 2114.81, 3489.44, 5757.58 and 9500.00 µg a.s./bee/day)

4. **Environmental conditions:**
Temperature: 30.66*– 34.03 °C (*short-term deviation less than 1 hr)
Relative humidity: 25.10* – 66.46 % (*short-term deviation less than 1 hr)
Photoperiod: 24 hrs darkness (except during observation)

5. **Animal assignment and treatment:**

The chronic feeding test was carried out as dose-response test with test duration of 10 days. The test comprised of two control treatment groups (C – untreated sucrose solution and Cs – aqueous sucrose solution + 0.1% Xanthan as a stabilizer), seven test item treatment groups and one reference item treatment group (0.107 µg dimethoate bee/day). Each treatment group consisted of 50 test organisms (divided into 5 replicates, containing 10 test organisms each).

Additionally, 10 test units without bees were placed in the climatic chamber for the evaluation of the evaporation: five with full food syringes containing pure 50 % (w/v) aqueous sucrose solution and five with full food syringes containing pure 50 % (w/v) aqueous sucrose solution + 0.1% Xanthan.

Freshly prepared feeding solutions replaced daily were offered to the test organisms of each test unit in feeders (plastic syringes, approx. 5 mL). The tip of each feeder was removed to give access to the feeding solution to the bees. A feeding volume of 1 mL/ replicate was offered to the bees *ad libitum*. The amount of feeding solutions consumed was determined by weighing the feeders before and after feeding using calibrated equipment. The syringes of 10 additional cages were filled with 1 mL of either pure 50 % (w/v) aqueous sucrose solution (5 cages) or 50 % (w/v) aqueous sucrose solution + 0.1% Xanthan (5 cages) and weighed daily for the determination of the evaporation.

6. **Dose preparation:**

The amounts of test item needed for the daily preparation of the test item solutions were measured using a calibrated balance. The test feeding solutions were freshly prepared every day by mixing the required (weighed) amounts of test item with 50 % (w/v) aqueous sucrose solution + 0.1% Xanthan. Xanthan gum was used as a stabilizer to improve homogeneity of the treatment solutions.

For the reference item treatment, a stock solution was prepared daily by dissolving a defined amount of the reference item in a defined amount of 50 % (w/v) aqueous sucrose solution as solvent. The definitive feeding solution was prepared fresh every day from the stock solution by dissolving a defined aliquot of the stock solution with 50 % (w/v) aqueous sucrose solution.

Samples of each freshly prepared control (C and Cs) and treated solution (T1 to T7) from D9 were taken directly after preparation and analysed by a digestion - ICP-OES analytical method for total aluminum and total silicon content.

7. **Measurements and observations:**

Mortality and sub-lethal effects were recorded daily at about the same time of the day (every 24 h ± 2 h), starting 24 ± 2 hours after start of the test period (initial feeding).

Sub lethal effects were quantitatively observed according to the following categories:

m: moribund
a: affected
c: cramps
ap: apathy

v: vomiting

Duplicate samples, one shipment and one retain sample (with a minimum amount of 2 mL) of the control groups and each treated solution from day 9 post study start (D9) were collected. Samples were stored in a freezer at ≤ - 18ºC until shipment and delivery to the analytical laboratory (CIP Chemisches Institut Pforzheim GmbH) for analytical determination of the actual concentration of the test chemical using acid digestion and ICP-OES.

8. Statistics:

The percentage of cumulative mortality was calculated for each treatment group and assessment from the number of dead individuals in relation to the number of introduced test organisms. The cumulative mortality of the test item treatments was corrected for the pooled controls mortality according to the formula ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The consumption of feeding solution per bee per day was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval. For each treatment group, the mean consumption of feeding solution per bee per day was calculated by averaging the replicate values.

Statistical calculations were made by using the statistical program ToxRat Professional®. In order to determine the LDD_{50} and the LC_{50} values a Trimmed Spearman-Karber procedure was used. A Multiple Sequentially-rejective U-test after Bonferroni-Holm (α= 0.05) was used to compare consumption data of the pooled controls and each test item treatment group and to determine if rejection of the test item solutions occurred. A Williams multiple sequential t-test procedure (one sided greater, α= 0.05) was used to compare mortality data of the pooled controls and each test item treatment group and to determine the NOEDD.

II. RESULTS AND DISCUSSION

The test was considered valid because the following criteria were satisfied:

- Average mortality for the control did not exceed 15% at the end of the test (actual value: 10% in C and 4% in Cs);
- Average mortality in the reference item treatment was ≥50% at the end of the test (actual value: 100.0%).

A. Feed consumption

The overall mean daily consumption of feeding solution over the entire test period was 21.71 µL/bee/day for the control group C (untreated 50 % (w/v) aqueous sucrose solution) and it was 20.43 µL/bee/day for the control group Cs (untreated 50 % (w/v) aqueous sucrose solution + 0.1% Xanthan). Since no statistical significant difference in mean daily consumption between the two controls was observed, statistical analysis for consumption data was performed with the pooled controls values.

The overall mean daily consumption of feeding solution at the test item concentrations of 3957.98, 6529.41, 10773.11, 17773.11, 29319.33, 48386.55 and 79831.93 mg a.s./kg food was 21.35, 21.63, 21.13, 20.30, 18.91, 19.16 and 22.56 µL a.s./bee/day, respectively. In the reference item treatment group, the overall mean daily consumption of feeding solution was 19.34 µL/bee/day. No significant differences in consumption of the sucrose solution was observed between any of the treatments with the test item and the pooled controls.

After 10 days of continuous exposure the accumulated mean uptake of kaolin at the treatment levels of 470.78, 776.79, 1281.70, 2114.81, 3489.44, 5757.58 and 9500.00 µg a.s./bee/day were 1005.25, 1680.39, 2708.46, 4293.57, 6597.45, 11028.96 and 21429.76 µg a.s./bee, respectively.

Table 10.3.1.2/01-1: Overall mean consumption of feeding solution

Treatment (µg a.s./bee)	**Concentration nominal (mg a.s./kg)**	**Overall mean consumption of feeding solution (µL/bee/day)**	**Dietary dose (µg a.s./bee/day)**	**Accumulated mean uptake (µg a.s./bee)**
Control	C (0.0)	21.70	-	-
Solvent control	Cs (0.0)	20.39	-	-
Pooled control	0.0	21.04	-	-
Reference item: dimethoate*	R (0.107)	19.34	0.021	0.186
Test item: Surround WP	T1 (470.78)	21.35	100.53	1005.25
	T2 (776.79)	21.63	168.04	1680.39
	T3 (1281.70)	21.13	270.85	2708.46
	T4 (2114.81)	20.30	429.36	4293.57
	T5 (3489.44)	18.91**	659.74	6597.45
	T6 (5757.58)	19.16	1102.90	11028.96
	T7 (9500.00)	22.56	2142.98	21429.76

* Cumulative over 9 days
** Statistically significantly lower than the pooled control (Multiple Sequentially-rejective U-test After Bonferroni-Holm, α = 0.05)

B. Mortality

On average, 10.0% mortality was observed in control C (untreated 50 % w/v aqueous sucrose solution) and 4.0% mortality in control Cs (untreated 50 % w/v aqueous sucrose solution + 0.1% Xanthan) was observed after 10 days of continuous feeding. Since no statistically significant difference in mortality between the two controls was observed, statistical analysis for mortality data was performed with the pooled controls values.

In the test item groups, at the concentrations of 3957.98, 6529.41, 10773.11, 17773.11, 29319.33, 48386.55 and 79831.93 mg a.s./kg food, mean cumulative mortalities of 14.0, 2.0, 2.0, 8.00, 10.0, 24.0 and 90.0 % were observed at the final evaluation after 10 days, respectively. Corrected mortality (with the pooled controls) was 7.53, -5.38, -5.38, 1.08, 3.23, 18.28 and 89.25 %, respectively.

The mean values for mortality are summarised in the following table.

Table 10.3.1.2/01-2: Mortality in the chronic 10-d oral honey bee test

Treatment (µg a.s./bee)	Concentration nominal (mg a.s./kg)	10d Cumulative mortality (%)	10d Corrected [1] mortality (%)
Control	C (0.0)	10.00	3.23
Solvent control	Cs (0.0)	4.00	-3.23
Pooled control	0.0	7.00	-
Reference item: dimethoate	R (0.107)	100.0	100.0
Test item: Surround WP	T1 (470.78)	14.00	7.53
	T2 (776.79)	2.00	-5.38
	T3 (1281.70)	2.00	-5.38
	T4 (2114.81)	8.00	1.08
	T5 (3489.44)	10.00	3.23
	T6 (5757.58)	24.00*	18.28
	T7 (9500.00)	90.00*	89.25

[1] Mortality corrected with the pooled controls mortality according to Schneider-Orelli, O. (1947)
* significantly different compared to the control (Williams multiple sequential t-test, one-sided greater, α = 0.05)

C. Behavioural

Symptoms of intoxication were observed sporadically for a few of the bees exposed to all of the test item concentrations starting on the second day of dosing. Symptoms observed throughout the study were mainly affected bees (lack of coordination). By the end of the study (day 10), the percentage of affected bees based on the surviving individuals ranged between 0.00 % at the three lowest concentrations (3957.98, 6529.41 and 10773.11 mg a.s./kg food) and 20.00 % at the highest concentration (79831.93 mg a.s./kg food).

Table 10.3.1.2/01-3: Behavioural abnormalities

TRT	Dose (µg as/bee/day)	D1					D2					D3					D4					D5				
		Al	A	Ap	C	Mb	Al	A	Ap	C	Mb	Al	A	Ap	C	Mb	Al	A	Ap	C	Mb	Al	A	Ap	C	Mb
C	--	49	0	0	0	0	48	0	0	0	0	47	0	0	0	0	47	0	0	0	0	47	0	0	0	0
Cs	--	49	0	0	0	0	49	0	0	0	0	49	0	0	0	0	49	0	0	0	0	49	0	0	0	0
T1	470.78	50	0	0	0	0	50	0	0	0	1	49	0	0	0	0	49	1	0	0	1	47	1	0	0	0
T2	776.79	50	0	0	0	0	50	0	0	0	0	50	0	0	0	0	50	1	0	0	0	50	0	0	0	0
T3	1281.70	50	0	0	0	0	50	0	0	0	0	50	0	0	0	0	50	0	0	0	0	50	1	0	0	0
T4	2114.81	50	1	0	0	0	50	1	0	0	0	49	2	0	0	0	49	0	0	0	0	49	3	0	0	0
T5	3489.44	50	0	0	0	0	50	0	0	0	0	50	2	0	0	0	49	2	0	0	0	48	1	0	0	0
T6	5757.58	48	0	0	0	0	48	0	0	0	0	48	1	0	0	0	47	2	0	0	0	47	3	0	0	0
T7	9500.00	49	0	0	0	0	47	0	0	0	0	45	3	0	0	0	42	1	0	0	0	39	1	0	0	0
TRT	Dose (µg as/bee/day)	D6					D7					D8					D9					D10				
		Al	A	Ap	C	Mb	Al	A	Ap	C	Mb	Al	A	Ap	C	Mb	Al	A	Ap	C	Mb	Al	A	Ap	C	Mb
C	--	47	0	0	0	0	47	0	0	0	0	46	0	0	0	0	46	3	0	0	1	45	0	0	0	0
Cs	--	48	0	0	0	0	48	0	0	0	0	48	0	0	0	0	48	0	0	0	0	48	0	0	0	0
T1	470.78	46	0	0	0	0	46	0	0	0	0	46	0	0	0	0	45	2	0	0	0	43	0	0	0	0
T2	776.79	50	0	0	0	0	50	1	0	0	0	50	0	0	0	0	50	1	0	0	0	49	0	0	0	0
T3	1281.70	50	0	0	0	0	49	0	0	0	0	49	0	0	0	0	49	2	0	0	0	49	0	0	0	0
T4	2114.81	49	1	0	0	0	49	0	0	0	0	49	1	0	0	0	48	3	0	0	0	46	1	0	0	0
T5	3489.44	48	1	0	0	0	48	0	0	0	0	48	0	0	0	0	46	3	0	0	0	45	1	0	0	0
T6	5757.58	47	1	0	0	1	46	0	0	0	0	44	3	0	0	0	43	1	0	0	1	38	2	0	0	0
T7	9500.00	36	1	0	0	0	31	8	0	0	0	17	2	0	0	0	9	4	0	0	0	5	1	0	0	0

Al = Alive; A = Affected; Ap = Apathy; C = Cramps; Mb = Moribund. No individuals with cramps or vomiting were observed throughout the test.

D. Toxicity endpoints

In summary, the toxicity endpoints for 10-d chronic feeding exposure to honey bee based on nominal test concentrations are as following:

Table 10.3.1.2/01-4: Honey bee toxicity endpoints for bees continuously exposed *via* the diet for 10-d to Surround WP under laboratory conditions

Treatment	Surround WP (Kaolin Clay based partical film)
LDD_{50} (95 % confidence limits) [1]	1389.55 µg kaolin/bee/day (1264.41 to 1527.08 µg kaolin/bee/day)
NOEDD [1]	659.74 µg kaolin/bee/day
LC_{50} (95 % confidence limits)	56409.64 kaolin/bee/day (51809.48 - 61418.25 mg kaolin/kg diet)
NOEC [1]	29319.33 mg kaolin/kg diet

[1] Based on actual consumption

E. Analytics

The analytical methods for determination of total aluminium and total silicon were successfully validated according to guideline SANCO/3029/99 rev.4 (2000) with regards to linearity, precision (repeatability), specificity and accuracy (recovery).

The specificity for the analysis of aluminium or silicon was demonstrated by using a highly specific method (ICP-OES, independent quantification and confirmation wavelengths). Mean recoveries for total aluminium were 92% and 94% respectively (overall: 93%). The mean recoveries for total silicon were 95% and 92% respectively (overall: 93%). Therefore, the SANCO requirement that mean recoveries for each level should be in the range 70% - 110% were fulfilled.

The limit of quantification (LOQ) was confirmed at 12 mg/L aluminium and silicon in final solution used for ICP analysis. Limit of detection (LOD) is defined as 30% of the LOQ. Therefore, the LOD was 3.6 mg/L aluminium and 3.6 mg/L silicon. The SANCO criteria for precision (repeatability) was fulfilled. The relative standard deviation for aluminium was 2.51% and 0.63%, respectively (overall 1.89%), and for silicon was 3.06% and 0.45% respectively (overall 2.59%). Linearity was also shown for both aluminium ($r^2 = 0.9996$) and silicon ($r^2 = 0.9997$).

III. CONCLUSION

The estimated LDD_{50}-value (Lethal Dietary Dose that kills 50% of the exposed individuals) for Surround WP corresponded to the mean consumed dose of 1390 µg kaolin/bee/day. The estimated NOEDD, based on the actual consumption for the respective feeding solutions, was determined to be 660 µg kaolin/bee/day.

Sub-lethal effects (i.e. affected bees) were observed throughout the exposure phase for some of the test item doses. By the end of the study (day 10), the percentage of affected bees based on the surviving individuals ranged between 0.00 % at the three lowest doses (470.78, 776.79 and 1281.70 µg kaolin/bee/day) and 20.00 % at the highest dose (9500.00 µg kaolin/bee/day).

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

~~A non-GLP range-finding test on the effects of Surround WP on honey bee larvae after a repeated exposure was conducted. As noted in the range-finder study, toxicity endpoints to be considered in the definitive larvae study are yet to be defined. Due to honey bee seasonality in conducting studies, repeating the range-finder has not allowed for an optimal testing window for honey bee larvae and the definitive test is yet to~~

be performed. The definitive study is scheduled to commence in 2018 when the bee colonies are healthy for the new season.

The non-GLP range-finding test on the effects of Surround WP on honey bee larvae after a repeated exposure is summarized below and will be replaced once a valid definitive study is completed.

Reference:	KCP 10.3.1.3/01, Ansaloni, T. 2019
Title:	Surround WP - Honey Bee Larval (*Apis mellifera* L.) Toxicity Test following Repeated Exposure under laboratory conditions
Report No.:	TRC17-184BA
Guideline(s):	OECD 239 (2016)
Deviation(s):	None
GLP:	Yes

Executive Summary
The objective of this study was to determine the effects of SURROUND WP (95% kaolin) on the honey bee (*Apis mellifera* L.) larvae, following a repeated feeding exposure under laboratory conditions. The study was carried out with 5 test item concentrations (0.107, 0.321, 0.964, 2.893 and 8.679 mg kaolin/mL diet, equivalent to cumulative doses of 15, 45, 135, 405 and 1215 µg kaolin/larva/developmental period), an untreated control and a reference item group (7.40 µg dimethoate/larva/developmental period). Each treatment group consisted of 48 larvae. Bee larvae were exposed for 22 days.

Analytical phase was performed to verify the concentration of the samples taken. For the analytical dose verification, aluminum and silicone residues were determined. Analysis of samples consistently provided recoveries over 80% and therefore nominal values were reported.

The 22 Day LC_{50} and LD_{50} values for honey bee larvae were empirically estimated to be > 1.071 mg a.s./mL diet and > 150 µg a.s./larva, respectively, i.e. the highest nominal diet concentration and dose tested.

In the repeated exposure 22-day larval toxicity test with SURROUND WP, the 22-day No Observed Effect Dose (NOED) value based on adult emergence was determined to be 405 µg kaolin/larva/developmental period. The No Observed Effect Concentration (NOEC) value based on adult emergence was determined to be 2.893 mg kaolin/mL diet. The 22-day adult emergence ED_x / EC_x values could not be estimated as no clear dose-response relationship was observed.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Surround WP
   Description: White powder
   Lot/Batch: AL170530
   Purity: 95% w/w kaolin (calcined), analysed

2. Control: Untreated feed
Vehicle: Deionised water

B. STUDY DESIGN AND METHODS

1. Test animals: Honey bee (*Apis mellifera* L.)
Age/life stage: 1st instar larvae
Source: In-house
Diet: The diet was prepared with deionized water using the following ingredients:

- Diet A (D1, volume administered: 20 µL/larva): 50.00 % weight of royal jelly + 37.00 % weight of deionized water + 1.00 % weight of yeast extract, 6.00 % weight of glucose and 6.00 % weight of fructose.
- Diet B (D3, volume administered: 20 µL/larva): 50.00 % weight of royal jelly + 33.50 % weight of deionized water + 1.50 % weight of yeast extract, 7.50% weight of glucose and 7.50 % weight of fructose.
- Diet C (from D4 to D6, volume administered: 30 µL/larva on D4, 40 µL/larva on D5 and 50 µL/larva on D6): 50.00 % weight of royal jelly + 30.00 % weight of deionized water + 2.00 % weight of yeast extract + 9.00 % weight of glucose and + 9.00 % weight of fructose.

For the preparation of the larval diet a commercial royal jelly was used.

2. Test units: Larvae were transferred into crystal polystyrene grafting cells (NICOTPLAST, Ø = 9 mm) sterilised by submerging for 30 min in ethanol 70 % (v/v), and then dried. Each cell was placed into a well of a sterile 48-well cellular culture plate (Greiner Bio-One), and the so prepared experimental units were placed under UV light for 15 minutes. The open plates of the control group and of all test product groups were individually placed into hermetically sealed Plexiglas desiccators, containing dishes filled with a saturated potassium sulphate (K_2SO_4) solution in order to keep a water saturated atmosphere from day 1 (D1) to day 8 (D8); on day 8 (D8), the well plates were transferred to another Plexiglas desiccator, containing a saturated sodium chloride (NaCl) solution in order to keep the established relative humidity until day 15 (D15). All desiccators were placed into the same incubator with forced air circulation. After the assessment on day 15 (D15), the test units were transferred to an emergence box (approx. 18 x 13 x 7 cm) with ventilated lids and placed inside the incubator. Each emergence box was supplied with 50 % (w/v) aqueous sucrose solution *ad libitum*.

3. Test concentration: 0.107, 0.321, 0.964, 2.893 and 8.679 mg kaolin/mL diet, equivalent to cumulative doses of 15, 45, 135, 405 and 1215 µg kaolin/larva /developmental period

4. Environmental conditions:
Temperature: 30.4– 35.3 °C
Relative humidity: 50.4-100.0%
Photoperiod: 24 hrs darkness (except during observation)

5. Animal assignment and treatment:
First instar larvae (L1) of the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae), were used as test organisms. The age-synchronized larvae originated from three different healthy, queen-right bee hives maintained by Trialcamp S.L.U.

The study was conducted as a dose response test with a duration of 22 days from grafting on day 1 (D1) to the final assessment on day 22 (D22). It comprised 1 control group; 5 test item groups with the cumulative doses of 15, 45, 135, 405 and 1215 µg kaolin/larva (equivalent to 0.107, 0.321, 0.964, 2.893 and 8.679 mg kaolin/mL diet), an untreated control and a reference item group with a cumulative dose of 7.40 µg dimethoate/larva. For each treatment group, 48 larvae from three different hives were tested over 22 days. Each hive equates to one replicate, 16 larvae from each replicate were used.

The larval Diet A was prepared freshly at D1 of the test; Diets B and C were prepared at the beginning of the test and then stored refrigerated (≤ 5 ºC) until each use. Each larva was fed once a day (except on day 2 (D2)) with a standardized amount of artificial diet until day 6 (D6).

6. Dose preparation:

For the preparation of the treated diets, a stock solution containing 22.8383 g test item with up to 250 mL deionized water was diluted into the series of four test solutions and aliquots of the stock solution and each dilution was mixed with the corresponding diet. The volume of the aliquots amounted for ≤ 10% of the final volume of the treated diet.

A unique stock solution (S5) was prepared on D3 and used for all dilutions (S1 to S4). All dilutions were prepared on the same day and stored in a refrigerator. Diet B was spiked on D3 for all application doses and, subsequently, stock solution S5 and associated dilutions were stored in the fridge. On D4, stock solution and dilutions were used to spike diet C for the applications of D4, D5 and D6. Spiked diet C was stored in the fridge between application days D4, D5 and D6.

A unique stock solution of the reference item was prepared on D3 with 0.0265 g dimethoate in up to 5 mL deionized water and was stored in the refrigerator to be used until D6 (last application).

Larvae of the control group (C) were fed with pure untreated diet from day 3 (D3) until day 6 (D6).

Daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period; the cumulative feeding volume from day 3 (D3) until day 6 (D6) of 140 µL diet per larva and the density of the diet (1.1 g/cm^3) were used to calculate the cumulative doses per larva. The treated diet was homogenized with a vortex mixer.

7. Measurements and observations:

Assessment of larval mortality was conducted before feeding on day 4 (D4), day 5 (D5), day 6 (D6), day 7 (D7) and day 8 (D8). On day 15 (D15) larvae that had not transformed into pupae were recorded as dead. Assessment of adult emergence was carried out on day 22 (D22). With the assistance of a stereo microscope, larvae were recorded as dead if no respiration (movement of spiracles) was observed. At each assessment time, dead larvae were removed for sanitary reasons. Other observations (larval appearance and size) were recorded. On day 8 (D8) during the assessment of mortality the presence of uneaten food was qualitatively recorded.

Analytical verification on samples of the stock solution S5 and its dilutions S1 to S4 was carried out by a digestion - ICP-OES analytical method. Since the test item is a natural mineral substance of undefined molecular mass, aluminium and silicone, which occur in a fairly constant ratio in kaolin, were quantified in the analytical verification.

8. Statistics:

A Chi² 2x2 Table Test with Bonferroni Correction was used to compare mortalities observed in the test item groups with mortalities of the pooled controls to determine the NOED / NOEC (No Observed Effect Dose / Concentration) on D22. The statistics program ToxRatPro Version 3.2.1® was used. Since no clear dose-response relationship was obtained, the ECx / EDx values could not be estimated.

II. RESULTS AND DISCUSSION

The test was considered valid because the following criteria were satisfied:

- The cumulative larval mortality in the control group from day 3 (D3) to day 8 (D8) was ≤ 15 % across all replicates (actual value: 6.25 %);
- On day 22 (D22) the adult emergence rate in the control group was ≥ 70 % across all replicates (actual value: 85.42 %);
- The cumulative larval mortality in the reference item groups was ≥ 50 % across all replicates on Day 8 (D8) (corrected mortality 97.56 %).

A. Mortality

In the control group (C), cumulative larval mortality on day 8 (D8) was 4.17%. On day 22 (D22), the adult emergence rate in the control group was 85.42% of the initial grafted larvae. Cumulative mortality (corrected with the control) in the reference item treatment group was 95.83% by D8. On day 8 (D8) in the test item doses of 15, 45, 135, 405 and 1215 µg kaolin/larva/developmental period, the cumulative mean mortality (corrected with the control) was -2.22, 4.44, 0.00, 4.44 and 4.44 %, respectively. On day 15 (D15) in the test item doses of 15, 45, 135, 405 and 1215 µg kaolin/larva/developmental period, the cumulative mean mortality (corrected with the control) was -2.33, 2.33, 0.00, 4.65 and 9.30 %, respectively. In the test item doses of 15, 45, 135, 405 and 1215 µg kaolin/larva/developmental period, the cumulative mean mortalities at 22 days (D22) after grafting were 14.58, 16.67, 12.50, 14.58 and 37.50 %, respectively (corrected mortalities with the control 0.00, 2.44, -2.44, 0.00 and 26.83 %, respectively). A summary of the mortality results over the test period is presented in the following tables.

Table 10.3.1.3/01-1: Cumulative mortality in the repeated larval test with Surround WP

Treatment Group	Dose		Cumulative Mortality (%)						
			D4	D5	D6	D7	D8	D15	D22
Control	---	---	4.17	6.25	6.25	6.25	6.25	10.42	14.58
Test item SURROUND WP	15	(µg a.i./larva/ developmental period) [a]	2.08	2.08	2.08	4.17	4.17	8.33	14.58
	45		8.33	10.42	10.42	10.42	10.42	12.50	16.67
	135		0.00	4.17	4.17	6.25	6.25	10.42	12.50
	405		0.00	4.17	6.25	10.42	10.42	14.58	14.58
	1215		6.25	8.33	10.42	10.42	10.42	18.75	37.50*
Reference Item (dimethoate)	7.40	(µg a.i./larva/ developmental period)	29.17	56.25	75.00	93.75	95.83	97.92	97.92

[a] Based on the analysed purity of active ingredient (kaolin)
* Significant difference compared to pooled control (Chi² 2x2 Table Test with Bonferroni Correction, α = 0.050; one-sided greater)

Table 10.3.1.3/01-2: Corrected mortality in the repeated larval test with Surround WP

Treatment Group	Dose		Corrected cumulative Mortality (%)						
			D4	D5	D6	D7	D8	D15	D22
Test item SURROUND WP	15	(µg a.i./larva/developmental period) [a]	-2.17	-4.44	-4.44	-2.22	-2.22	-2.33	0.00
	45		4.35	4.44	4.44	4.44	4.44	2.33	2.44
	135		-4.35	-2.22	-2.22	0.00	0.00	0.00	-2.44
	405		-4.35	-2.22	0.00	4.44	4.44	4.65	0.00
	1215		2.17	2.22	4.44	4.44	4.44	9.30	26.83
Reference Item (dimethoate)	7.40	(µg a.i./larva/developmental period)	26.09	53.33	73.33	93.33	95.56	97.67	97.56

[a] Based on the analysed purity of active ingredient (kaolin)

On day 8 (D8), no uneaten food was observed for any individual of the treatments with the test item and the control group.

The mean emergence rates were 85.42, 83.33, 87.50, 85.42 and 62.50 %, respectively. A significant difference in D22 mortality was observed between the control and the highest test item group (1215 µg kaolin/larva/developmental period), with no significant differences between the control and any of the treatment groups of ≤ 405 µg kaolin/larva/developmental period.

Table 10.3.1.3/01-3: Pupal mortality and mergence rate of honey bee larva exposed to Surround WP

Treatment Group	Dose		Mean Pupal Mortality % at D15[b]	Mean Pupal Mortality % at D22[c]	Emergence % D22[d]
Control	---	---	4.17	10.42	85.42
Test item SURROUND WP	15	(µg a.i./larva/developmental period) [a]	4.17	10.42	85.42
	45		2.08	14.58	83.33
	135		4.17	8.33	87.50
	405		4.17	10.42	85.42
	1215		8.33	29.17	62.50
Reference Item (dimethoate)	7.40	(µg a.i./larva/developmental period)	2.08	95.83	2.08

[a] Based on the analysed content of active ingredient (kaolin)
[b] Mean mortality at D15 with respect to the surviving larvae at D8
[c] Mean mortality at D22 with respect to the surviving larvae at D8
[d] With respect to the initial larvae at D3

B. Toxicity endpoints

In the repeated exposure 22-day larval toxicity test with SURROUND WP, the 22-day NOED value based on adult emergence was determined to be 405 µg kaolin/larva/developmental period. The NOEC value based on adult emergence was determined to be 2.893 mg kaolin/mL diet. The 22-day adult emergence ED_x / EC_x values could not be estimated as no clear dose-response relationship was observed. The calculated endpoints are presented in the following table.

Table 10.3.1.3/01-4: Toxicity endpoints for honey bee larvae repeatedly exposed for 22 days to Surround WP

Endpoint	µg kaolin/larva/developmental period
22-Day NOED	405
22-Day LOED	1215
22-Day ED_{10}	Not determined [a]
22-Day ED_{20}	Not determined [a]
22-Day ED_{50}	Not determined [a]
Endpoint	**mg kaolin/mL diet**
22-Day NOEC	2.893
22-Day LOEC	8.679
22-Day EC_{10}	Not determined [a]
22-Day EC_{20}	Not determined [a]
22-Day EC_{50}	Not determined [a]

a EDx/ECx values could not be determined as no clear dose-response relationship was observed.

C. Analytical verification:

The analytical methods for determination of total aluminium and total silicon were successfully validated according to guideline SANCO/3029/99 rev.4 (2000) with regards to linearity, precision (repeatability), specificity and accuracy (recovery).

The specificity for the analysis of aluminium or silicon was demonstrated by using a highly specific method (ICP-OES, independent quantification and confirmation wavelengths). Mean recoveries for total aluminium were 97.6% and 98.1% respectively (overall: 98%). The mean recoveries for total silicon were 99.5% and 101.9% respectively (overall: 101%). Therefore, the SANCO requirement that mean recoveries for each level should be in the range 70% - 110% were fulfilled (the 116% for silicon was detected in the lowest test concentration where undoubtedly contamination from glass vial (primarily composed of SiO_2), will be noticeable.

The limit of quantification (LOQ) was confirmed at 0.125 mg/L aluminium and silicon used for ICP analysis. Limit of detection (LOD) is defined as 30% of the LOQ. Therefore, the LOD was found at 0.038 mg/L aluminium and at 0.038 mg/L silicon for these solutions. The SANCO criteria for precision (repeatability) was fulfilled. The relative standard deviation for aluminium was found at 0.32% and 0.95%, respectively (overall 0.72%), and for silicon is was found at 0.36% and 0.16% respectively (overall 1.27%). Linearity was also shown for both aluminium (r^2 = 0.9999) and silicon (r^2 = 1.0000).

Results of the analytical verification are given in the following tables.

Table 10.3.1.3/01-5: Results of determination of total aluminium

Specimen ID	Total aluminium (mg/mL)	Total Kaolin, calculated from aluminium quantification (mg/mL)	Nominal content of kaolin in stock solution (mg/mL)	Recovery of kaolin in samples (%)
TRC17-184-S1-D4-AS	0.25	1.04	1.07	97
TRC17-184-S2-D4-AS	0.81	3.32	3.21	104
TRC17-184-S3-D4-AS	2.50	10.27	9.64	107

TRC17-184-S4-D4-AS	6.52	26.84	28.93	93
TRC17-184-S5-D4-AR1	18.75	77.19	86.79	89

Table 10.3.1.3/01-6: Results of determination of total silicon

Specimen ID	Total silicon [mg/mL]	Total Kaolin, calculated from silicon quantification (mg/mL)	Nominal content of kaolin in stock solution (mg/mL)	Recovery of kaolin in samples (%)
TRC17-184-S1-D4-AS	0.31	1.24	1.07	116*
TRC17-184-S2-D4-AS	0.87	3.45	3.21	108
TRC17-184-S3-D4-AS	2.62	10.37	9.64	108
TRC17-184-S4-D4-AS	7.42	29.33	28.93	101
TRC17-184-S5-D4-AR1	21.0	83.12	86.79	96

* Increased value is without much doubt traceable to a contamination from the glass vial (primarily composed of SiO_2), which will be only noticeable at lower silicon concentrations as contained in sample TRC17-184-S1-D4-AS.

III. CONCLUSION

In the repeated exposure 22-day larval toxicity test with SURROUND WP, the 22-day No Observed Effect Dose (NOED) value based on adult emergence was determined to be 405 µg kaolin/larva/developmental period. The No Observed Effect Concentration (NOEC) value based on adult emergence was determined to be 2.893 mg kaolin/mL diet. The 22-day adult emergence ED_x / EC_x values could not be estimated as no clear dose-response relationship was observed.

CP 10.3.1.4 Sub-lethal effects

Not relevant as field tests with honey bees have been conducted (see MCP 10.3.1.6).

CP 10.3.1.5 Cage and tunnel tests

Not applicable. Aluminium silicate has no direct toxic effects and small scale cage tests are not suitable to evaluate physical repellent barriers such as a kaolin particle film.

CP 10.3.1.6 Field tests with honeybees

Two non-GLP field tests were carried out to assess the impact of kaolin as an insect repellent on bees when applied during flowering in apple and pear orchards. These studies were submitted previously and have been reviewed as part of the EU assessment for the first approval of aluminium silicate.

Studies submitted and evaluated for the first inclusion on Annex I:

Reference:	KCP 10.3.1.6/01 Mayer, D.F. 1999a (previously evaluated in DAR B9, III.A 10.4.4/01)
Title:	Honey bee foraging in pear orchards treated with kaolin particle film
Report No.:	-

Guideline(s):	-
Deviation(s):	None
GLP:	No, but study scientifically valid

Executive summary

Kaolin Particle Film was applied at a rate of 56 kg/ha (50 lb/acre) in a mature pear orchard. Ten trees were randomly selected in the kaolin treated and untreated sections of the orchard and monitored for number of bees and their foraging behaviour, percent fruit set, fruit size, fruit weight and seed numbers.

There was no difference in number of bees or in their behaviour in the kaolin treated plot when compared with the untreated plot.

No differences were observed in percent fruit set and fruit diameter between the treated and untreated plots, although mean weight of fruits in the kaolin treated plot was significantly greater and the mean number of seeds was significantly lower than in the untreated plot.

Kaolin particle film applied at 56 kg/ha to flowering pear trees had no harmful effects on honey bee mortality or behaviour, fruit set and fruit diameter. However, pears from the treated trees were significantly heavier and contained fewer seeds than those from the untreated control.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Kaolin Particle Film
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: Not specified
   - Stability of test component: Stable

2. Vehicle and/or positive control: Water

3. Test animals –
   - Species: Honey Bee (*Apis mellifera*)
   - Age: Mixed – all life stages
   - Source: 24 colonies present on test site.

4. Trial location: Zillah, Washington State, USA

B. STUDY DESIGN AND METHODS

1. Test Method:

Twenty-four bee hives were placed around a 2.5 ha mature pear orchard. On 14 April 1999, 90% of the orchard was sprayed with Kaolin particle film at a dose rate of 56 kg/ha, using a commercial air blast sprayer delivering 935 L/ha spray volume. The remaining 10% of the orchard was left unsprayed to serve as an untreated control. At the time of application 50% of the flowers were open

On the day before treatment, 10 randomly selected trees in both the Surround® and untreated plots were marked for making assessments on honey bees and fruit quality and quantity.

2. Observations:

The following assessments were carried out on honey bees:

- Numbers foraging per tree per minute at 2, 4, 8 and 24h after application
- Foraging behaviour (nectar or pollen collectors) at 2h after application
- Time spent foraging individual flowers

Assessments were also made on the quantity and quality of fruit using the following methods:

- Flower clusters were counted on 4 branches on each of the 10 marked trees and the number of fruit was later counted on the same branches to determine % fruit set.
- On 6 August, 10 pears were collected from each of the 10 marked trees and taken to the laboratory where they were weighed, measured, cut open and the number of seeds counted.

3. Statistics:

Studentized Newman-Keuls range test.
Different letters denote significant differences at $P \leq 0.05$.

II. RESULTS AND DISCUSSIONS

There was no significant difference in number of foraging bees, foraging behaviour and time spent foraging between kaolin treated and untreated plots.

Table 10.3.1.6/01-1: Summary of Surround® and control honey bee results in pears

Treatment	Mean no. bees/tree/minute				% bee activity		Time (s) /flower	
	2h	4h	8h	24h	nectar	pollen	2h	4h
Kaolin	1.6a	3.2a	3.5a	3.7a	0a	100a	6.8a	6.4a
Control	2.7a	2.9a	3.8a	3.6a	0a	100a	6.4a	6.1a

There was no significant difference in percent fruit set and mean fruit diameter between treated and untreated trees. However, mean fruit weight was significantly greater in the treated plot compared to the untreated plot and fruits in the treated plot contained significantly less seeds than fruits in the untreated plot.

Table 10.3.1.6/01-02: Summary of pear fruit parameters in Surround® and control plots

Treatment	% fruit set	Mean weight	Mean diameter	Mean no. seeds
Kaolin	11.5a	125a	59a	2.8a
Control	11.2a	108b	57a	5.8b

III. CONCLUSIONS

Kaolin particle film applied at 56 kg/ha to flowering pear trees had no harmful effects on honey bee mortality or behaviour, fruit set and fruit diameter. However, pears from the treated trees were significantly heavier and contained fewer seeds than those from the untreated control.

Reference:	KCP 10.3.1.6/02 Mayer, D.F. 1999b (previously evaluated in DAR B9, III.A 10.4.4/02)
Title:	Honey bee foraging in apple orchards treated with kaolin particle film
Report No.:	-
Guideline(s):	-
Deviation(s):	None
GLP:	No, but study scientifically valid

Executive summary

Identical tests were carried out in two separate apple orchards. In each orchard, Kaolin Particle Film was applied at a rate of 56 kg/ha (50 lb/acre). Ten trees were randomly selected in the treated and untreated sections of the orchard and monitored for number of bees, honey bee foraging behaviour, percent fruit set, fruit size, fruit weight and seed production in fruit.

With the exception of observations made at 2 h after treatment in one test, there were no significant differences between kaolin treated plots and the untreated in the numbers of foraging honey bees, their behaviour and time spent visiting flowers. No differences were observed in fruit set fruit weight and size of apples, although there were more seeds in the Surround® treated apples in one test.

Application of kaolin particle film slightly reduced the number of foraging bees at 2 hours after application in one test in apples. However, numbers of foraging bees and their behaviour was then the same in both kaolin and untreated plots. No differences were observed between treated and untreated apples, except for a slightly higher number of seeds in fruit from the kaolin plots in one test.

On the basis of this study, kaolin has no negative effect on numbers of bees foraging, bee behaviour, fruit set, fruit weight and fruit size.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolin Particle Film
Description:	White powder
Lot/Batch #:	Not specified
Purity:	Not specified
Stability of test component:	Stable

2. Vehicle and/or positive control: Water

3. Test animals –	
Species:	Honey Bee (*Apis mellifera*)
Age:	Mixed – all life stages
Source:	16 colonies - test site 1
	12 colonies - test site 2
4. Trial location:	Naches, Washington State, USA

B. STUDY DESIGN AND METHODS

Two separate trials were conducted in different orchards in the same location.

Test 1:
A 1.6 ha apple orchard was split into 2 equal plots. The plot on the west of the orchard was treated with kaolin particle film on 4 May 2002 when 10% of the flowers were in bloom and again on 8 May when 80% of the flowers were in bloom.

Test 2:

A 2.4 ha apple orchard was split into 2 equal plots. The plot on the east of the orchard was treated with kaolin particle film on 11 May when 100% of the flowers were open.

1. **Methods common to both tests:**

Kaolin dose rate: 56 kg/ha
Spray volume: 935 L/ha
Sprayer: Commercial airblast

Ten randomly selected trees in both the Surround® and untreated plots were marked for making assessments on honey bees, fruit quality and quantity.

2. Observations:

The following assessments were carried out on honey bees:

- Numbers foraging per tree per minute at 2, 4, 8 and 24h after application
- Foraging behaviour (nectar or pollen collectors) at 2h after application
- Time spent foraging individual flowers

Assessments were also made on the quantity and quality of fruit using the following methods:

- Flower clusters were counted on 4 branches on each of the 10 marked trees and the number of fruit was later counted on the same branches to determine % fruit set.
- On 17 August, 10 pears were collected from each of the 10 marked trees and taken to the laboratory where they were weighed, measured, cut open and the number of seeds counted.

3. Statistics:

Studentized Newman-Keuls range test.
Different letters denote significant differences at $P \leq 0.05$.

II. RESULTS AND DISCUSSIONS

Test 1:

There were significantly fewer foraging bees in the treated plot as compared to the untreated plot at two hours after application both at 10% open bloom and 80% open bloom. However, no significant difference was observed at 4 and 24 hours post-application.

There was no significant difference in foraging behaviour and time spent foraging between treated and untreated plots.

Table 10.3.1.6/02-1: Summary of Test 1 honey bee results, apples 10% flowering

Treatment	Mean no. bees/tree/minute				% Foraging behaviour			Time (s) /flower*	
	2h	4h	8h	24h	top	side	pollen	2h	4h
Kaolin	1.0a	2.3a	1.6a	2.2a	3.6a	96.4a	16a	3.0a	2.8a
Control	2.2b	2.2a	1.4a	2.4a	3.8a	96.2a	18a	3.2a	3.2a

* observations for side feeders

Table 10.3.1.6/02-2: Summary of Test 1 honey bee results, apples 80% flowering

Treatment	Mean no. bees/tree/minute				% Foraging behaviour			Time(s)/flower*	
	2h	4h	8h	24h	top	side	pollen	2h	4h
Kaolin	5.6a	6.2a	8.6a	10.1a	5.1a	94.9a	17a	2.9a	3.3
Control	8.2b	6.2a	7.9a	11.4a	5.8a	94.2a	14a	3.0a	3.4

* observations for side feeders

There was no significant difference between treated and untreated trees in percent fruit set, fruit diameter or fruit weight. However, kaolin treated fruit had significantly more seeds than untreated apples.

Table 10.3.1.6/02-3: Summary of Test 1 fruit parameter assessments in apples

Treatment	% fruit set	Mean weight	Mean diameter	Mean no. seeds
Kaolin	11.4a	201a	7.7a	5.0a
Control	11.3a	191a	7.6a	4.2b

Test 2:

There was no significant difference in the number of foraging bees, foraging behaviour and time spent foraging between treated and untreated plots.

Table 10.3.1.6/02-4: Summary of Test 2 honey bee results in apples

Treatment	Mean no. bees/tree/minute				% Foraging behaviour			Time (s) /flower*	
	2h	4h	8h	24h	top	side	pollen	2h	4h
Kaolin	10.6a	12.8a	10.9a	7.9a	2.1a	98a	21a	3.0a	3.1a
Control	13.7a	13.1a	10.9a	7.7a	2.3a	98a	20a	3.0a	3.4a

* observations for side feeders

There was no significant difference in percent fruit set, fruit diameter, mean weight and number of seeds between treated and untreated trees.

Table 10.3.1.6/02-5: Summary of Test 2 fruit parameter assessments in apples

Treatment	% fruit set	Mean weight	Mean diameter	Mean no. seeds
Kaolin	8.6a	177a	6.6a	3.1a
Control	8.4a	172a	6.8a	3.0a

III. CONCLUSIONS

Application of kaolin particle film slightly reduced the number of foraging bees at 2 hours after application in one test in apples. However, numbers of foraging bees and their behaviour was then the same in both kaolin and untreated plots. No differences were observed between treated and untreated apples, except for a slightly higher number of seeds in fruit from the kaolin plots in one test.

On the basis of this study, Kaolin has no negative effect on numbers of bees foraging, bee behaviour, fruit set, fruit weight and fruit size.

CP 10.3.2 Effects on non-target arthropods other than bees

During the initial EU review (DAR 2008, B.9.5), a waiver from conducting standardised tests on non-target arthropods was requested and accepted because aluminium silicate (kaolin) does not have any direct toxic effects on arthropods. It was agreed by EFSA (2012) that the product is not suitable for use in standardised laboratory or semi-field tests where indirect effects (e.g., repellency and physical irritation) cannot be accurately evaluated. However, non-target arthropod data from the open literature are available for kaolin, including Tier I glass-plate studies with arthropod species commonly found in orchards showing minimal toxicity at high dose levels after direct spraying. These references are being submitted in support of aluminium silicate (kaolin).

The findings are summarised in the following table and full details of the studies are provided in the respective sections below.

Table 10.3.2-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Chrysoperla carnea (Neuroptera)	Surround WP	Direct spray (limit test)	LR_{50} > 50 g a.s./L (acute mortality, 3rd instar larvae or eggs) Signs of repellency	Porcel *et al.* (2011) Published ref (KCP 10.3.2.2/01)
Psyttalia concolor (Hymenoptera), *Chrysoperla carnea* (Neuroptera), *Chilocorus nigritus* (Coleoptera) and *Anthocoris nemoralis* (Hemiptera)	Surround 95 WP	Dried residue, glass plates (limit test)	*P. concolor*, *C. carnea* and *C. nigritus* 72 hr LR_{50} > 50 kg product/ha ER_{50} >50 kg product/ha *A. nemoralis* 72 hr LR_{50} > 50 kg product/ha ER_{50} < 50 kg product/ha (66.6% reduction on the number of eggs laid by female/day)	Bengochea *et al.* (2010) Published ref (KCP 10.3.2.2/02)
Chrysoperla externa	Kaolin	Direct spray and treated prey	Survival LR_{50} > 100 g a.s./L Development NOEC = 60 g a.s./L Effects on prey consumption > 60 g a.s./L	Bestete *et al* (2018) Published ref (KCA 8.3.2/03)
Eriopis connexa larvae	Kaolin	Direct spray and treated prey	Survival LR_{50} > 100 g a.s./L Development ER_{50} >100 g a.s./L Effects on prey consumption > 60 g a.s./L	Bestete *et al* (2018) Published ref (KCA 8.3.2/03)
Chrysoperla carnea	Kaolin	Extended laboratory study on olive leaves	72 hr L/EC_{50} > 5 kg/hL (50 kg/ha)	Gharbi & Abdallah (2016) Published ref (KCA 8.3.2/02)
Anthocoris nemoralis	Kaolin	Extended laboratory study on olive leaves	72 hr L/EC_{50} > 5 kg/hL (50 kg/ha) EC_{50} < 5 kg/hL (50 kg/ha)	Gharbi & Abdallah (2016) Published ref (KCA 8.3.2/02)
Chrysoperla carnea (Neuroptera)	Surround 95 WP	Glass-plates, Extended laboratory study on olive leaves & Semi-field (limit test)	72 hr LC_{50} > 47500 mg a.s./L EC_{50} > 47500 mg a.s./L Signs of repellency	Bengochea *et al.* (2014) Published ref (KCP 10.3.2.2/03)
Anthocoris nemoralis (Hemiptera), *Chelonus inanitus*, *Chelonus nigritus* and *Scutellysta cyanea* (all	Surround 95 WP	Extended laboratory study on olive leaves (limit test)	72 hr LR_{50} >50 kg product/ha ER_{50} >50 kg product/ha (*A. nemoralis*) Signs of repellency	Bengochea *et al.* (2013) Published ref (KCP 10.3.2.2/04)

Species	Substance	Exposure System	Results	Reference
Hymenoptera)				
Psyttalia concolor	Surround WP (95 WP)	Extended laboratory study on olive leaves & Semi-field (limit test)	L/ER_{50} > 5 kg product/hL (50 kg/ha) If given a choice, slight reduction in the percentage of parasitised hosts for kaolin	Bengochea *et al.* (2014) Published ref (KCP 10.3.2.2/05)
Trichogramma cacoeciae	Surround WP (95% a.s.)	Parasitism in *L. botrana* and *E. Kuehniella*	No significant effects on parasitism at the single rate tested (47.5 g a.s./L of water) (50 kg product/ha)	Pease *et al* (2016) Published ref (KCP 10.3.2.2/06)
Field or semi-field tests				
EFSA Conclusion (2012) (KCP 10.3.2.4/01 to /09): Nine field studies are available where the WP formulation of aluminium silicate (kaolin) was applied to orchards (multiple applications) up to the dose of 56 kg/ha. The results demonstrated that Surround was not harmful to many groups of non-target arthropods, including lacewings (Neuroptera: Chrysopidae), ladybirds (Coleoptera: Coccinellidae), hoverflies (Diptera; Syrphidae), some heteropteran bugs (e.g., Miridae), parasitic hymenopterans and spiders. However, in some trials a reduction in predatory mites (*Amblyseius spp.*) and anthocorid bugs was noted but this was attributed to the repellent effect of aluminium silicate (kaolin) and the limited availability to prey animals on the treated plants. Additional semi- and field studies have been submitted for the purposes of renewal where the WP formulation of aluminium silicate was applied to olive or apple orchards (multiple applications) up to the dose of 60 kg/ha. The results once again demonstrated that Surround is not harmful to lacewings (Neuroptera: Chrysopidae). For other taxa (bugs (Heteroptera), beetles (Coleoptera) and spiders (Araneae) and different species of *Orius* and Hymenoptera of the families Philodromidae, Scelionidae, Pteromalidae and Aphelinidae), a reduction in abundance and richness compared to control were noted. However, the applicant notes that in these studies the number of predatory bugs was generally very low in both control and treatment plots, so the statistical power of these studies was limited. Furthermore, the studies generally only sampled NTA numbers up to 3 months after the last application and hence the potential for recovery was not tested. The findings within the limited timeframes of these studies are not surprising as kaolin's mode of action is as a repellent/deterrent. In one trial (Markó *et al*. (2006) the commencement in the recovery of population abundance and diversity was noted in the 6 weeks post last application (following 12 x 45 kg product/ha (10 day interval) in apple orchards), and in another (Pascual *et al.* (2010a), abundance and diversity of arthropod communities in olive orchards treated 2 x 30 kg/ha for 3 years were approaching values comparable to controls within 2 months. Markó et al. (2006) (Published ref: KCP 10.3.2.4/12): Hydrophobic kaolin, M96-018, was applied at a rate of 45 kg/ha in a suspension of 30 g kaolin M96-018 and 40 mL methanol/L of water. The treatments were applied about every ten days, 12x between March 25 and August 5. In spite of the higher prey density, the numbers of the most important predators, *Forficula auricularia*, *Allothrombium fuliginosum* and *Exochomus quadripustulatus*, were significantly lower on the kaolin treated plots. This also was the case for the spiders. After the last treatment, the population density of *Allothrombium fuliginosum*, *Forficula auricularia* and *Exochomus quadripustulatus* did increase on the treated plots. However, a month after the last treatment, the population density of spiders was still lower on the treated plots than on the control plots. Iannotta *et al*. (2007) (Published ref: KCP 10.3.2.4/13): Surround WP applied at a rate of 2 x 5 kg/hL (50 kg/ha) in olive groves. Kaolin reduced the abundance of arthropods at canopy level (timimg/frequency of sampling not indicated), but it preserves a good Coenotic Balance among trophic guilds. On the canopy, only Lepidoptera were unaffected by the kaolin spraying, the other species were other Hymenoptera, Ichneumonoidea, Macrolepiotera, Neurptera, Mecoptera, Syrphidae, Coccinellidae, Aranease and Opiliones. The reduction in numbers in the canopy could be due to the interference between kaolin particle film and the feeding strategies utilised by pollinators, phytophagous and predators as kaolin had no impact on the soil arthropods communities (included: Araneae, Isopoda, Carabidae, Staphylinidae, other Coleoptera and Formicidae).				

Species	Substance	Exposure System	Results	Reference

Knight *et al.* (2001) (Published ref: KCP 10.3.2.4/14):
Population density of selected pests of apple and their natural enemy populations were measured after 7 or 10 applications of 56 kg M96-018/ha in the apple orchards in Washington State (USA) over a 2 year period. Beneficials analysed were spiders (Araneae), ants (Hymenoptera: Formicidae), ladybird beetle larvae and adults (Coleoptera: Coccinellidae) and earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae). The abundance of these species were lower in the treated crops compared to control (when sampled on the day to approximately 3 weeks post-application). The potential for recovery between last application in 1998 to first application in 1999 was not reported.

Pascual *et al.* (2010b) (Published ref: KCP 10.3.2.4/15):
Surround WP was applied at 2 x 3 kg/100 L (2 x 30 kg/ha) to an olive grove in Spain on 8 July and 16 September. Both PRC and two-way ANOVA identified the coccinellid *Scymnus mediterraneus* and the spider family Philodromidae as the taxa the most affected by kaolin. Numbers in the kaolin treated trees dropped after sprays were applied. Significant difference in kaolin treated plot versus untreated were on day of application and noted approximately 10 weeks post application. No further sampling occurred after 10 weeks, but the data indicates a trend of population numbers increasing.

Sánchez-Ramos *et al.* (2017) (Published ref: KCP 10.3.2.4/16):
The effects on the non-target arthropod fauna of the almond trees canopy in fields treated with 1 to 2 applications of Surround WP at 5 kg/100 L (50 kg/ha) over a 2 year treatment period reduced the abundance of natural enemies and other non-target arthropods. After treatment application (assumed 2-3 months monitoring post treatment), a significant reduction in the abundance of natural enemies in 2009 and 2010 and in the abundance of other arthropods in 2010 compared to the control plots. A significant reduction in the Shannon diversity index and in the number of species was observed in the kaolin plots compared with the control plots in both years except for the Shannon index in 2010 and no effect was observed for kaolin treated plots compared to the control in 2009, but a significant effect was observed in the kaolin-treated plots for the other non-target arthropod community in 2010, after treatment. No differences in the community composition of non-target arthropods were found among treated and control plots before treatment application either in 2009 and 2010. After treatment application, no effect was observed for kaolin treated plots compared to the control in 2009, but a significant effect was observed in the kaolin-treated plots for the other non-target arthropod community in 2010. (Specific timing of sampling only reported as "monthly, total of 5 and 6 sampling dates before and after treatment"). Arthopods evaluated were: phytophagous, spiders, parasitoids, other arthropods (Arachnida, Entognatha and Insecta, with Thysanoptera most abundant group). Poential for recovery was not addressed within the limited timeframe of this field study.

Sackett *et al.* (2007) (Published ref: KCP 10.3.2.4/17):
Surround WP, was applied in apple orchards to determine if applications affected the diversity of generalist arthropod predator assemblages in orchard foliage and the parasitism of the pest species *Choristoneura rosaceana*. In two orchards, kaolin was applied to orchard foliage once a week for 4 weeks, between mid-June and mid-July in 2004 and 2005 at a rate of 6 kg/100L and 1000 L/ha (total of 4 x 60 kg/ha at a 1 week interval). In the third orchard kaolin was applied to foliage twice over 2 weeks in June 2004 at a rate of 450 L/ha (total of 2 x 27 kg/ha at a 1 week interval). Kaolin altered the species composition of the generalist predator assemblages and reduced the relative abundances of certain generalist predators, most notably jumping and crab spiders (Salticidae and Philodromidae), assassin bugs (Reduviidae), ants (Formicidae) and coccinellids (Coccinellidae), after the fourth application of kaolin, but catch rates were no longer significantly lower 1 month after this final application in August. In contrast, the relative abundances of web-spinning spiders (Araneidae, Dictynidae, Theridiidae) were not affected. Kaolin did not affect the proportion of parasitized *C. rosaceana* larvae, which ranged from 24% to 47% in control and kaolin treatments, or the relative proportions of parasitoid taxa. The kaolin formulation did not affect the abundance of *C. rosaceana* larvae, but in one orchard, kaolin did reduce the abundance of the combined numbers of *C. rosaceana* and another tortricid pest, *Argyrotaenia velutiana*. Although kaolin does not affect parasitism of *C. rosaceana*, it significantly changes the composition of generalist predator assemblages in orchard foliage as noted from time of application.

Showler & Sétamou (2004) (Published ref: KCP 10.3.2.4/18):
Following a 2-year field trial in cotton fields treated with Surround at a rate of 42.3 L/ha applied weekly or biweekly from mid-April to the end of June (approximately 7 to 10 applications). Populations of *Aphis gossypii* increased in

Species	Substance	Exposure System	Results	Reference
kaolin treated cotton plots compared to control plots, but cicadellid populations were suppressed. Populations of dipterans, *Orius* spp., and wasps were reduced in the kaolin treatments only on 1 of 20 sampling dates over the two seasons. Foliar kaolin spray had no effect on other arthropod groups identified in this study (silverleaf whitefly, *Bemisia argentifoli* Bellows and Perring; herbivorous hemipterans and coleopterans; thrips; lepidopteran larvae; *Geocoris* spp.; *Nabis* spp.; reduviids; coccinellids; *Collops* spp.; neuropterans; and spiders). Scalericio *et al*. (2010) (Published ref: KCP 10.3.2.4/19): Two applications (6 weeks apart) of kaolin were applied to olive groves at a rate of 5 kg/hL (50 kg/ha). The selected arthropod taxa that were sampled include: *Arachnida*: *Araneae* and *Opiliones*; *Hymenoptera*: *Ichneumonidae*; other *Hymenoptera*; *Coleoptera*: *Coccinellidae*; *Lepidoptera*; *Neuroptera*; *Mecoptera* and *Diptera*: *Syrphidae*). Abbott's formula showed the negative effects of kaolin on *Araneae*, other *Hymenoptera* and *Lepidoptera* with 57.6, 39.0 and 7.2% reduction (based on sampling results up to 2 months after last application). Based on Coenotoc Balance, no significant differences were found between kaolin treated plots and untreated control. The other arthropods were not reduced by any treatments.				

Risk assessment for other non-target arthropods

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[25].

<u>**Exposure**</u>

In-field exposure

Non-target arthropods living in the crop can be exposed to residues from SURROUND® WP CROP PROTECTANT by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. The proposed GAP for SURROUND® WP CROP PROTECTANT is for an application rate of 28500 g a.s./ha to grapevines. SURROUND® WP CROP PROTECTANT is applied four times per season on grapevines and the foliar multiple application factor MAF is therefore 2.7 and 3.4 for soil for the use in vineyards (Appendix V, ESCORT 2).

The maximum in-field exposure (Predicted Environmental Rate, PER) to foliar-dwelling or soil-dwelling organisms, assuming the worst-case (contradiction) of 100% crop interception and 0% crop interception, is calculated below.

The in-field exposure (predicted environmental residue, PER) is calculated according to ESCORT 2 using the following equation:

$$PER_{in\text{-}field} = \text{Application rate (g a.s./ha) x MAF}$$

The maximum predicted in-field exposure rate used in the risk assessment is presented in Table 10.3.2-2.

Table 10.3.2-2: In-field exposure rate (PER) for SURROUND® WP CROP PROTECTANT

[25] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet MC, Lewis G, Oomen PA, Schmuck R and Vogt H (eds) (2001): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop. SETAC, Pensacola, 46 p.

Test item	Crop	Application rate	MAF	In-field exposure rate
SURROUND® WP CROP PROTECTANT	Grapevines	4 x 28500 g a.s./ha	2.7 (foliar)	76950 g a.s./ha
			3.4 (soil)	96900 g a.s./ha

Off-field exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to SURROUND® WP CROP PROTECTANT will be due mainly to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values as shown in the following equation:

$$PER_{off\text{-}field} = PER_{in\text{-}field} \times (\%drift/100) / \text{vegetation distribution factor}$$

Vegetation distribution factor: The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2.

For four applications in grapevines, the drift value at 3 m distance is 2.44% (early application) and 6.71% (late application) of the application rate (74th percentile drift). The foliar MAF value for four applications is 2.7. The resulting $PER_{off\text{-}field}$ values are shown in Table 10.3.2-3.

Table 10.3.2-3: Off-field foliar Predicted Environmental Rates (PER)

Study type	Application rate (g a.s/ha)	MAF	drift factor (% drift/100)	Vegetation distribution factor	Off-field foliar PER (g a.s./ha)
Early application					
2-D test system	28500	2.7	0.0244	10	187.76
3-D test system	28500	2.7	0.0244	1	1877.58*
Late application					
2-D test system	28500	2.7	0.0671	10	516.33
3-D test system	28500	2.7	0.0671	1	5163.35*

* PER value is corrected based on the vdf and CF for comparison with higher tier 3D effects data

Risk assessment

As agreed during the previous EFSA review (2012), laboratory toxicity studies are not suited for aluminium silicate (kaolin) with a reported mode of action as a repellent / deterrent. Hence, a conventional EU risk assessment is not considered necessary and a standard risk assessment has not been performed. Furthermore, hazard quotients (HQ) values have not been determined based on the data for non-standard laboratory arthropod species from the public literature as they have not been validated for consideration in a Tier 1 risk assessment.

The available data for non-target arthropods, as summarised in Table 10.3.1-1, suggests that adverse effects are generally noted at multiple doses greater than 50 kg product/ha (47.5 kg a.s./ha). Although the estimated theoretical worst-case in-field PER values are higher at 76.95 kg a.s./ha (foliar) and 96.9 kg a.s./ha (soil) than the maximum tested dose rate, the risk to non-target arthropods is concluded to be acceptable based on the findings reported from the 12 field studies and 1 semi-field study as concluded by EFSA (2012) during the evaluation for the original approval of aluminium silicate. An additional 7 orchard trials and one cotton field is being submitted to further support this conclusion.

Note that the risk to soil dwellers is observed to be lower than for canopy dwellers (Iannotta *et al* (2007, KCP 10.3.2.4/13). Any recorded reduction in arthropod numbers in the canopy could be due to the interference between kaolin particle film (repellency, disruption of movement, including grooming), the feeding strategies utilised by arthropods and the white coating on surfaces repel some insects by making the plant less visually recognizable[26], since kaolin has minimal impact on the soil arthropod communities. Especially since kaolin is undistinguishable compared to natural soils in which soil dwelling arthropods reside. Hence the risk to soil-dwelling non-target arthropods is negligible.

Off-field exposure (PER values = 0.188 to 5.16 kg a.s./ha) is significantly lower than tested rates reporting no unacceptable adverse effects greater than 50%, hence highly supporting the potential for recovery from off-field arthropod communities. In a 3-year study in olive orchards in Spain (Pascual *et al*. (2010a&b) KCP 10.3.2.4/10 & 15), arthropod community numbers and biodiversity were reported to be reaching levels of that of the control after 2 months following each last application per season; 2 x 30 kg/ha. In another trial (Marko *et al*. (2009 & 2006), KCP 10.3.2.4/11 & KCP 10.3.2.4/12, respectively), recovery in diversity and abundance on non-target arthropods was noted within the 6-week observation period following 12 x 45 kg product/ha (10 day interval) in apple orchards, except for spiders where numbers were still lower than in the treated plots one month after treatment compared to control plots.

A reduction in spiders was also observed in a study where four different routes of exposure were assessed using the orb-weaver spider[27]. Kaolin sprays (3% solution) were applied 10 times every week using a sprayer (100 mL) on (i) the surface, (ii) the prey (fly), (iii) the spider and (iv) both spider & prey; assays were maintained for 42 days. Results showed that sprays of kaolin significantly affected the survival of *Araniella curcubitina* when applications were made to the surface and on both spider and prey resulting in a reduction of 48% and 56%, respectively (treated prey alone was a reduction of 14% and spider treatment only was 2%). Differences observed were explained by the feeding behaviour of the species and on the consumption of the web by the spider and the ratio spider/fly for body size, according to the authors. However, in this study the daily application interval and laboratory exposure route represent a worse than realistic case.

In a 2-year apple orchard trial (at a high application rate of 4 x 60 kg/ha) (Sackett *et al.*, 2007 KCP 10.3.2.4/17), kaolin reduced the relative abundances of certain generalist predators, most notably jumping and crab spiders (Salticidae and Philodromidae), assassin bugs (Reduviidae), ants (Formicidae) and coccinellids (Coccinellidae). There was a significant decrease in the relative abundance of total spiders in the kaolin plots after the fourth application of kaolin, but catch rates were no longer significantly lower 1 month after this final application in August. In contrast, the relative abundances of web-spinning spiders (Araneidae, Dictynidae, Theridiidae) and sit-and-wait crab spiders were not affected. Scalericio *et al*. (2010, KCP 10.3.2.4/19), claim that kaolin at a rate of 2 x 50 kg/ha can have a negative impact on *Araneae* (57.6% reduction) and other *Hymenoptera* (39% reduction) (two groups which live in a close relationship with the substratum) since spiders are visual predators which have difficulty moving due to kaolin. *Hymenoptera*, similar to *Lepidoptera*, are mainly flower-visiting insects that have difficulties in finding and utilising alimentary sources within the kaolin-treated plot. Therefore, once kaolin is washed away, transient adverse effects on these species are reversible. Although effects on individual taxas may be noted, in the study by

[26] Showler, A.T, and Sétamou, M. (2004). Effects of kaolin particle film on selected arthropod populations in cotton in the lower Rio Grande Valley of Texas. Southwestern Entomologist, 29(2): 137-146
[27] Benhadi-Marin, J., Pereira, J.A., and Santos, S.A. (2016). Effects of kaolin particle films on the life span of an orb-weaver spider. Chemosphere 144: 918-24.

Scalericio *et al*. (2010), based on Coenotoc Balance there were no significant differences found between kaolin treated plots and untreated control.

However, multiple year studies in orchards repeatedly demonstrate the potential for recovery. Sánchez-Ramos *et al.* (2017, KCP 10.3.2.4/14) presented results for species abundance, presented in the Shannon diversity index and based on community composition, reporting that these parameters were comparable between the control and kaolin sprayed almond canopies treated with 1 to 2 applications of Surround WP at 5 kg/100 L (50 kg/ha) over a 2-year treatment period. The authors evaluated phytophagous, spiders, parasitoids, other arthropods (Arachnida, Entognatha and Insecta, with Thysanoptera most abundant group). Recovery was reported on the basis on there being no reported differencesa in arthropod community compodition between kaolin treated and control plots the following year (start on spray application in year 2).

In another 2-year field trial in cotton (Showler & Sétamou, 2004 KCP 10.3.2.4/19) treated with Surround at a rate of 42.3 L/ha (approximately 7 to 10 applications), no effect on arthropod groups were identified in this study (silverleaf whitefly, *Bemisia argentifoli* Bellows and Perring; herbivorous hemipterans and coleopterans; thrips; lepidopteran larvae; *Geocoris* spp.; *Nabis* spp.; reduviids; coccinellids; *Collops* spp.; neuropterans), including spiders. Only in a single sampling episode out of 20, was there a noted reduction in reported populations of dipterans, *Orius* spp., and wasps; being minimally impacted by the treatments. This could be explained how application timing is critical in potentially altering arthropod populations since kaolin is non-systemic and rates on treated foliage would rapidly decline (further supporting that lower rates are less harmful to arthropods). With aluminium silicate (kaolin), recovery would be expected to occur, especially after a rain event when the clay residue has been washed from the plant's surface.

The higher tier studies where the WP formulation of aluminium silicate (kaolin) was applied to orchards (multiple applications) up to the dose of 60 kg/ha demonstrated mixed results (Table 10.3.2-4). In some literature references, adverse effects on species abundance or arthropod fauna composition are reported. While in similar orchard trials at high rates do not report any significant changes in the treated plots compared to the controls. It is emphasized that kaolin is a repellent and could deter non-target arthropods from visiting the treated crops, and potentially create momentary obstacles in allowing egg attachment on treated leaves. Yet, laboratory studies repeatedly demonstrate a low toxicity to several different non-target arthropods at rates almost twice the recommended application rate of SURROUND® WP CROP PROTECTANT to vineyards at BBCH 51-65, early in the season when several sensitive species, such as spiders are not yet reproducing.

Based on the objective of kaolin being an alternative to synthetic chemicals as the plant protection option in treating grapevines from pests and avoiding fruit damage, an overall acceptable risk to non-target arthropod communities is concluded. The results demonstrated that SURROUND® WP CROP PROTECTANT is not harmful to many groups of non-target arthropods, including lacewings (Neuroptera: Chrysopidae), ladybirds (Coleoptera: Coccinellidae), hoverflies (Diptera: Syrphidae), some heteropteran bugs (e.g., Miridae), parasitic hymenopterans and some spiders. However, it has been reported in some trials a reduction in predatory mites (*Amblyseius*), orchard bugs (Heteroptera), beetles (Coleoptera), some spiders (Araneae) and different species of *Orius,* Hymeoptera of the families Philodromidae, Scelionidae, Pteromalidae, and Aphelinidae), and anthocorid bugs. However, these noted reductions may be attributed to the high doses tested (application rates evaluated in the reported studies are almost double or more of the proposed rate for SURROUND® WP CROP PROTECTANT in vineyards at 4 x 28.5 kg/ha), repellent effect of aluminium silicate (kaolin) and the limited availability to prey animals on the treated plants. Recovery from off-field canopy populations are also reported to occur.

The risk to in-field and off-field non-target arthropods is concluded to be acceptable without the need of additional laboratory data based on the repeated findings of acceptable risk observed in higher tier field trials, and the high potential for recovery.

Table 10.3.2-4: Summary of main findings in the field trial and potential for recovery (if reported in study)

Reference	Crop / duration	Application rate	Species tested	Effects reported compared to control	Sampling following last application
KCP 10.3.2.4/01 Lepine, 2004	Orchard (pear) 1 season	1 x 50 kg/ha 1 x 50 kg/ha + 3 x 25 kg/ha (~ 10 d interval)	Hymenoptera Mirid bugs	None	~ 1 to 2 month
KCP 10.3.2.4/02 Fraser, 2002a	Orchard (apple) 1 season	11 x 56 kg/ha (7-14 d interval)	Ladybirds (coccinellids) Hover flies (syrphids), Lacewings (chrysoperlids) Pirate bugs (*Orius*)	Not harmful to insect predators compared to non-toxic reference item.	Immediately after application
KCP 10.3.2.4/03 Fraser, 2002b	Orchard (apple) 1 season	8 x 56 kg/ha (7-14 interval)	Ladybirds (coccinellids), Lacewings (chrysoperlids) Mullein bugs (*Campylomma*)	Not harmful to insect predators compared to non-toxic reference item.	Immediately after application
Fraser, 2002c	Orchard (apple) 1 season	8 x 56 kg/ha (7-14 d interval)	Ladybirds (Coccinellids), Lacewings (Chrysoperlids), Pirate (*Orius)* Mullein bugs (*Campylomma*).	Slight effect on predatory mites compared to non-toxic reference item (statistical significance not reported)	Around time of application
KCP 10.3.2.4/05 Fraser, 2002d	Orchard (apple) 1 season	6 x 56 kg/ha (7-14 d interval)	Ladybirds (coccinellids), Lacewings (chrysoperlids), Hover flies (syrphids) Spiders	Slight effect on very low numbers of predatory mites compared to non-toxic reference item (statistical significance not reported)	~ 2 months
KCP 10.3.2.4/06 Fraser, 2002e	Orchard (apple)	15 x 56 kg/ha (7-14 d interval)	lacewings (chrysoperlids), *Orius*, general spider mite predators and spiders	Not harmful to insect predators compared to non-toxic reference item.	Around time of application
KCP 10.3.2.4/07 Peusens and Creemers, 2004a	Orchard (pear) 1 season	4 x 10 to 30 kg/ha (~ 14 d interval)	*Anthocoris*	25-80% reduction post Effects on *Anthocoris* are probably due to removal of prey and the general repellent effect of Surround®	1 to 3 months
KCP 10.3.2.4/08 Peusens and Creemers, 2004b	Orchard (pear) 1 season	3 x 300 or 500 L/ha (~ 10 d interval)	*Anthocoris*	Numbers significantly lower	2 days
KCP 10.3.2.4/09 Puterka, 2001	Orchard (pear and apple) 1 season	10 x 3 to 6 kg/hL (pear) 12 x 3 to 6 kg/hL (apple) (5 to 14 d interval)	Lacewing (Chrysoperlid) Ladybird (Coccinellid) Pirate bug (*Orius triscolor)*	Minimal impact	Soon after application

KCP 10.3.2.4/10, Pascual *et al*., 2010a	Orchard (olive) 3 yr trial	2 x 30 kg/ha (~2-3 month interval)	Numerous arthropods	Reduced abundance and diversity of arthropod community. Recovery of numbers over the winter reported and only reductions immediately after each application (increasing back to control levels within a couple of months). This indicates a high potential for recovery.	2 month
KCP 10.3.2.4/11, Markó *et al*., 2009	Orchard (apple) 1 season	10 x 45 kg/ha (10 d interval)	Heteropteran Beetle Spider	Reduced total abundance Signs of recovery reported by increasing numbers	6 weeks
KCP 10.3.2.4/12, Markó, *et al*., 2006	Orchard (apple) 1 season	12 x 45 kg/ha (~10 d interval)	*Forficula auricularia*, *Allothrombium fuliginosum* *Exochomus quadripustulatus*, Spiders	Reduction in numbers. Population density increase except for spiders which were still lower.	1 month
KCP 10.3.2.4/13, Iannotta *et al*., 2007	Orchard (olive) 1 season	2 x 50 kg/ha (5 week interval)	15 taxa	Reduced abundance of arthropods at canopy level (statistically not analysed), but good Coenotic Balance. No impact on the soil arthropods communities.	3 months
KCP 10.3.2.4/14, Knight, *et al*., 2001	Orchard (apple) 2 yr trial	7 to 10 x 56 kg/ha (~14 to 21 d interval)	Spiders (Araneae), Ants (Hymenoptera: Formicidae), Ladybird beetle (Coleoptera: Coccinellidae) Earwig (*Forficula auricularia* L.)	Reduced abundance Potential for recovery between last application in 1998 to first application in 1999 was not reported.	~ 2 months
KCP 10.3.2.4/15, Pascual, *et al*., 2010b	Orchard (olive) 1 yr trial	2 x 30 kg/ha (10 week interval)	Coccinellid *Scymnus mediterraneus* Spider family Philodromidae	Reduced abundance Data indicates a trend of population numbers increasing.	day of application and ~10 weeks
KCP 10.3.2.4/16, Sánchez-Ramos, *et al*., 2017	Orchard (almond) 2 yr trial	1 to 2 x 50 kg/ha (~8 to 10 week interval)	Spiders Parasitoids Other arthropods	Reduction in abundance and diversity Recovery noted as no difference in community composition between kaolin treated plot and non-treated the following year.	2-3 months
KCP 10.3.2.4/17, Sackett, *et al*., 2007	Orchard (apple) 2 yr trial	4 x 60 kg/ha (7 d interval) or 2 x 27 kg/ha (14 day interval)	Jumping and crab spiders (Salticidae and Philodromidae), Assassin bugs (Reduviidae), Ants (Formicidae) Coccinellids (Coccinellidae), Web-spinning spiders (Araneidae,	Altered the species composition. Recovery in abundances, following last application	1 month

			Dictynidae, Theridiidae)		
KCP 10.3.2.4/18, Showler and Sétamou, 2004	Cotton field 2 yr trial	7 to 10 x 42.3 L/ha (7 to 14 d interval)	Silverleaf whitefly Herbivorous hemipterans Coleopterans; Thrips; Lepidopteran larvae; *Geocoris* spp.; *Nabis* spp.; reduviids; coccinellids; *Collops* spp.; neuropterans; Spiders	No reduction in population	4 hours to 2 weeks or fortnightly for 3 months
KCP 10.3.2.4/19, Scalercio *et al.* 2010	Orchard (olive) 1 yr trial	2 x 50 kg/L (5 week interval)	*Arachnida*: *Araneae* and *Opiliones*; *Hymenoptera*: *Ichneumonidae*; other *Hymenoptera*; *Coleoptera*: *Coccinellidae*; *Lepidoptera*; *Neuroptera*; *Mecoptera* and *Diptera*: *Syrphidae*	Reduced abundance on *Araneae*, other *Hymenoptera* and *Lepidoptera* with 57.6, 39.0 and 7.2%, respectively Based on Coenotoc Balance, no significant differences were found The other arthropods were not reduced by any treatments.	2 months

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

The mode of action of aluminium silicate (physical repellency) is not suited to testing under small scale Tier 1 laboratory conditions as supported during the previous EFSA review (2012). As a consequence, only higher tier studies have been conducted.

A waiver is requested for standardized laboratory tests with the formulated product on non-target terrestrial arthropods based on the following information:

- As detailed in the original DAR (B.9.5), aluminium silicate (kaolin) does not have any direct toxic effects on arthropods, as observed effects are mostly in the form of physical irritation and behavior disruption (e.g. repellency). It is therefore not a suitable product for use in standardized laboratory or semi field tests, where indirect effects (e.g., repellency and physical irritation) cannot be accurately evaluated. It is therefore proposed that the effects of the formulated product SURROUND® WP CROP PROTECTANT should be evaluated under field conditions, which are more appropriate for this type of product (please see CP 10.3.2.4).

In addition, data from public literature demonstrate that kaolin is of low toxicity, as demonstrated by the information summarised in MCA 8.3.2 and below (MCP 10.3.2.2) for several non-target arthropods. The provision of further data on the representative formulation is not considered necessary as SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). Therefore, the formulated product is highly unlikely to be of higher toxicity compared to the active substance, kaolin.

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

The mode of action of aluminium silicate (physical repellency) is not suited to testing under small scale laboratory conditions. As a consequence, only field studies following typical 'worst-case' applications have been conducted. Nevertheless, data can be found in the open literature and have been summarised in support of this submission.

Reference:	KCP 10.3.2.2/01, Porcel, M., Cotes, B., and Campos, M., 2011
Title:	Biological and behavioural effects of kaolin particle film on larvae and adults of *Chrysoperla carnea* (Neuroptera: Chrysopidae)
Report No.:	Published in: Biological Control 59: 98-105
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

Several laboratory and field experiments were conducted to investigate the effect of kaolin particle film formulation Surround WP on the biology and behavior of the common generalist predator *Chrysoperla carnea*.

Kaolin 5% (w/v) suspension direct spray (50 g/L) did not affect third instar larvae development to adulthood. The hatchability of recently laid eggs, subjected to the same spraying process, was also unaltered. However, third instar larvae coated with particle film after kaolin spraying showed slightly hampered movement capacity. Parameters extracted from recorded larvae movement on a kaolin film surface showed similar decreased mobility results as well as preference for the clean control surface. Additionally, the larvae had difficulty grasping treated leaves. *C. carnea* adult females showed a predominant preference for treated leaves in oviposition choice tests.

In the field trial, no difference in *C. carnea* adult abundance was found between kaolin-treated and control olive trees. These results indicate that disruption of movement capacity and dislodgement from the plant surface may be the principal negative effects of particle film on *C. carnea* larvae. Despite the positive trend in oviposition towards kaolin treated surfaces, a particle film attraction effect on adults was not observed at field level.

It can be concluded that the LC_{50} for *C. carnea* larvae and eggs based on mortality is greater than 50 g kaolin/L. Although there were noted behavioural differences, these parameters did not appear to negatively impact the population in the field.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2 (reliable with restrictions), not known to be GLP, toxicity endpoints not calculated, hence not reliable for use in a qualitative risk assessment.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Surround WP (5% kaolin suspension)
   Batch number: Not reported. Product manufactured by NovaSource (Salt Lake, USA)
   Content of a.s.: Kaolin
   Appearance: Not reported.

2. **Vehicle:** Mineral water

3. Test organism

Species:	*Chrysoperla carnea* (Stephens)
Age at test initiation:	<12 hr old recently molted 3rd instar larvae
Source:	Koppert Spain
Acclimation:	Under test conditions
Food:	50% honey and 50% pollen

4. Treatment: 50 g kaolin/L

5. Environmental conditions

Temperature:	25 ± 1 ºC
Humidity:	50-60%
Photoperiod:	16 h light: 8 h dark

6. Dose application: Calibrated airbrush spray gun (Model 350) connected to an air compressor generating a cone spray pattern. The spray gun was fixed by means of an adjustable height laboratory arm at 33 cm over the table surface, preventing excessive air flow over the spraying spot position.

B. STUDY DESIGN AND METHODS

1. Acute mortality to larvae:

C carnea larvae were directly sprayed with the 5% kaolin suspension in order to assess any possible effects on mortality. Newly molted third instar larvae were chosen for the experiment given that previous observations had revealed that molting caused effective removal of the particle layer deposited on the larva's dorsal cuticle when spraying first and second instars.

Recently molted larvae (less than 12 h), homogeneous in age and size, were selected and set aside from the stock. Individuals were transferred by gently tapping the underside of the Petri dish or by using a camel paintbrush, to 4.5 cm ø Petri dishes containing a double filter paper layer in order to prevent the formation of large droplets during the spraying process. Larvae were chilled for 15 min, after which they were sprayed with either kaolin suspension or water. After spraying, the thoroughly wetted larvae typically presented the formation of one or more droplets on the insects' dorsum. The Petri dish was then covered, and the individuals were subjected to a 1 h drying period, after which, in the case of kaolin-sprayed larvae, the particle film became visually apparent. Finally, the filter paper was removed, and the larvae were fed and kept in culture conditions. The experiment used a randomised complete block design with replications consisting of 10 insects per treatment replicated five times over time. Tested individuals were checked daily for survival up to adult emergence and the developmental stage in which mortality occurred was recorded.

2. Acute mortality to eggs:

Kaolin film applications were tested on *C. carnea* eggs to determine their effects on egg viability and larval hatching suppression. Two bands (17 x 9 cm) of self-adhesive green velvety paper used as ovipositing surfaces in the *C. carnea* stock colony were attached upside-down to the rectangular removable lid (36 x 22 cm) of the adults' rearing box containing approximately 100 mixed male and female adults. After 12 h, 30 eggs were selected on each band by discarding the rest. One band was sprayed with the kaolin suspension and the other with the control water. Both treatments were delivered by handholding the airbrush at an approximate distance of 10 cm and by spraying the whole band area for 30 seconds.

The eggs were observed under the microscope to detect small droplets of kaolin suspension. After drying, the suspension droplets produced fragments of particle film adhering to the egg's surface. The lid containing the two treated sets of eggs was kept in a cabinet under culture conditions. After three days, *E. kuehniella* eggs were sprinkled over the bands in order to feed neonate larvae to prevent intraspecific egg predation. From the fourth day onwards, newly hatched *C. carnea* larvae were removed daily from the bands by means of a camel paintbrush, and the eggs were checked daily for 8 days to ensure that hatching had ceased. Data regarding viable and non-viable eggs was recorded under a microscope. The nonviable eggs were identified as those that desiccated as well as neonate larvae that failed to free themselves from the eggshell and died in the process. The experimental design used a random design block with up to 10 replications.

3. Effect on leaf-grasping ability

Larvae's capacity to grasp leaf surfaces covered by kaolin particle film as compared to untreated leaves was assessed. Olive leaves were attached side by side to a 100 cm^2 square glass, forming a continuous leaf surface platform. Several platforms were created as described, using either the upper or lower side of the leaves for each platform. Leaf-covered platforms were pressed by means of a weight and kept at cold temperatures to avoid moisture loss. Following standard spraying methodology, the platforms were firstly sprayed with water and left to dry for 2 h. Newly molted (less than 12 h) third instar individuals were used in this experiment. The larvae were transferred to the leaf surface and allowed to move for several seconds. When the larva was located in the central part of a leaf (sufficiently away from the edge) and still in motion, the platform was inverted for 30 seconds to determine whether the larvae could continue gripping the surface or falls off the platform. This procedure was repeated using 10 different larvae, after which the platform was sprayed with kaolin, left to dry and used to assay 10 new larvae on the particle film. The whole process was repeated using the underside of the leaf surface. The experiment was carried replicated five times. Two new leaf platforms (upper and lower surfaces) were constructed per replication.

4. Effect of particle film covering *C. carnea* larvae on mobility and behavior:

Directly sprayed kaolin-covered larvae were assessed for changes in behavioral and locomotor parameters. The same size and age of third instar larvae were selected and were sprayed with either kaolin or mineral water. Once sprayed, the larvae were starved for a period of 24 h prior to bioassay. Each individual was moved to a controlled environment, placed in a Petri dish and viewed with a Panasonic CCTV video camera (Mastsushita Electric Industrial Co., Ltd., Japan). Larval movement was recorded for a period of 15 min, and the track was transferred to a computer as part of the EthoVision XT integrated video tracking system. The EthoVision software automatically determines the location of the individual larva in the area and calculates several movement parameters derived from changes in position. The parameters chosen were (1) total distance moved (cm), (2) mean velocity (mm/s), (3) mean angular velocity (degrees/cm) and (4) movement (%). The movement variable was defined as the fraction of time the larvae spent in motion. One individual at a time was tested, and each larva was used only once. The experiment was conducted on the basis of a completely randomized design over five consecutive days, running 10 to 15 trials of both treatments every day up to a total number of 30 valid replicates per treatment (60 replicates).

5. Effect on particle film surface on larval mobility and selection:

In this experiment, it was determined whether kaolin-covered substrates interfered with larval mobility parameters and whether they showed a substrate preference between treated and control surfaces. Newly molted third instars were obtained from eggs. The larvae were subjected to a 24 h starvation period prior to study start. The Petri dish experimental arena was divided into two surface halves of equal size. One half was sprayed with kaolin and the other half with the control water. A semicircular waterproof plastic was used to cover treatment areas during the spraying process, ensuring that they never came in contact with the particle film, as the treatment was always delivered after the control area had been sprayed and dried for 2 h. Given that only the lower part of the Petri dish was sprayed, the experimental design was restricted to this area by coating the dish's circular edge with Fluon, thus preventing the larva from climbing up to the lid. The experimental setup and procedures were similar to those described in the previous experiment. The parameters extracted from the recorded tracks were (1) total distance moved (cm), (2) mean velocity (mm/s), (3) mean angular velocity (degrees/cm), (4) movement (%) and (5) time spent in each zone (kaolin and control). The parameters were calculated for each zone individually for the purpose of comparison. Unlike the previous experiment, each individual trial generated the complete set of variables for both treatments. The experiment was carried out over five consecutive days for a total of 50 replicates. Each block of replicates consisted of 10 trials conducted on the same day with the same experimental arena, and a new arena was therefore sprayed each day.

6. Adult oviposition preference:

C. carnea adults were placed in an oviposition arena and given the choice between kaolin film and control surfaces. The arena consisted of an 8.5 cm ø 2.5 cm high Petri dish with two ovipositing leaf surfaces, one upside down attached to the lid and the other attached to the bottom of the Petri dish. Before mounting the arena, equal semicircular zones were sprayed with kaolin and water. As a result, both leaf surfaces contained particle film and control zones of equal area.

Newly emerged (after less than 12 h) adults were coupled and transferred to small rearing boxes (0.9 L), kept in culture environmental conditions and provided with adult food and water. After seven days, individual couples were transferred to the ovipositing arena to lay eggs for a period of 48 hours. Water, supplied by a moistened piece of sponge, and food, supplied directly, were attached to the sides of the dish, while avoiding interference with the surfaces. The eggs deposited in each designated area (upper and lower surfaces, kaolin film and control zones) were recorded. Females that did not oviposit were excluded from the experiment. Ten couples were successively assayed in the same arena, and the process was repeated with a total of five different arenas (50 individual replicates).

7. Effect on adult abundance. Field study

A field experiment was conducted to determine whether a particle film sprayed on olive trees had an immediate effect on adult lacewing presence. The experiment was conducted in a 258 ha commercial non-irrigated olive orchard under integrated pest management (IPM) situated in the province of Granada, Spain. No insecticidal treatment had been applied during the year, and natural regeneration cover was present between trees from the beginning of spring until natural drying in June. A 50 g/kaolin solution (treatment) and water (control) were applied to 16 trees (grown 11 x 11 m apart) in a square plot by means of a tractor-drawn turbo atomizer (Sistromatic AL-TAR 2000N) at a rate of 95 L/ha, delivering approximately 0.58 kg of kaolin per tree. Treatment and control plots were situated at a minimum distance of 80 m apart. The experiment was replicated up to four times (total 8 plots), each plot separations of at least 150 m. Kaolin was applied twice: on 15th June and 24th September 2009. The chrysopids were sampled seven days after the applications on two consecutive days using an insect aspirator (Modified CDC Backpack Aspirator Model 1412) to sample all the trees in the plot. Inner and outer branches of each olive tree were suctioned up to a height of 2 m for a period of 2 min. The adult chrysopids collected were counted and identified at species level. Precipitation data obtained from a public agroclimatic station (IFAPA, Junta de Andalucía) revealed a single rainfall event (5.4 mm) that took place the afternoon after the kaolin treatment was carried out on 15th June and three rainfall events between 23rd September and 1st October, adding up to total rainfall of 10.2 mm.

4. Statistics:

All the statistical analysis was carried out using the SPSS Statistics package for Windows.

To analyze larvae and egg acute mortality and larvae leaf-grasping experiments, percentages were arcsin-transformed for normalization and compared using the Student's t-test ($\alpha = 0.05$) for paired comparisons and analysis of variance tests (ANOVA) for multiple comparisons. ANOVA analysis was followed by Tukey tests ($\alpha = 0.05$) to identify mean differences.

For larval mobility analysis, the parameters were log10 (x + 0.5) transformed for normalization and compared by means of a Student's t-test ($\alpha = 0.05$). In all cases, untransformed means were presented. Whenever the data distribution failed to satisfy parametric analysis assumptions, data was subjected to nonparametric Mann–Whitney U tests ($\alpha = 0.05$). Statistically significant differences at a confidence level of $\alpha = 0.10$ were shown.

In the oviposition preference experiment, as the data set violated parametric analysis assumptions, the treatments were compared using a Kruskal–Wallis test, followed by paired Mann–Whitney U tests for individual comparisons. The α value was adjusted by means of the Bonferroni–Holm correction ($\alpha \leq 0.05$).

C. carnea adult capture data from the field case study was analyzed using a generalized linear mixed model (GLMM) with a Poisson error distribution (R software). Treatment (kaolin and control) and date were used as explanatory variables and block as a random effect. No data over dispersion was detected.

II. RESULTS AND DISCUSSION

1. Acute mortality to larvae:

No difference in mortality was observed between the treatment and control individuals (Table 10.3.2.2/01-1) neither at the different developmental stages nor in the percentage of adult emergence. The clearly observable kaolin film particle covering the larvae dorsum did not interfere with *C. carnea*'s normal development from the third instar state.

Table 10.3.2.2/01-1: Mean mortality in different development stages and mean adult emergence of 3rd instar larvae sprayed either with water (control) or kaolin

Treatment	% Mean mortality			% Mean adult emergence
	3rd instar	Pre-pupa	Pupa	
Control	12.0	4.0	10.0	74.0
Kaolin	14.0	8.0	8.0	70.0

2. Acute mortality to eggs:

Egg hatching and early survival of newly emerged first instars were not affected by the kaolin treatment on eggs under the tested environmental conditions. Specifically, 80.0 ± 8.3% (mean ± SD) particle film sprayed eggs produced normally hatching individuals, while the hatching rate in water sprayed eggs was 84.3 ± 5.9%. Despite a lower hatching rate resulting from the kaolin treatment, no statistical differences were found, which can be considered of little biological significance. No cannibalistic egg predation was observed while recording the viable and non-viable eggs.

3. Effect on leaf-grasping ability

Kaolin particle film covering both sides of olive leaves clearly affected *C. carnea* larval ability to grasp the leaf surface. Their grasping capacity was altered by kaolin treatment on both the upper and lower side of the leaf and was especially notable in the case of the upper side, where the number of larvae that remained on the kaolin-treated surface decreased by 66%. With respect to the upper and lower parts of the leaf, no difference in grasping performance was observed when comparing water sprayed surfaces. However, the ability to grasp the treated lower surface was significantly greater than the capacity to grasp the treated upper surface.

4. Effect of particle film covering *C. carnea* larvae on mobility and behavior:

Observation of the recorded tracks did not reveal the existence of a clear behavioral change resulting from the particle film adhering to the insect's surface. Despite this, the numerical variables derived from the tracks provided by the EthoVision XT software showed small but significant differences in all of the measured parameters. Kaolin-treated larvae covered a shorter distance within the arena than water-treated individuals. The reduction in the distance moved can be interpreted as both an effect produced by a decreased mean velocity of kaolin-treated individuals and an increased frequency of pausing or spending a lower fraction of the total trial time in motion. Kaolin treatment also affected the shape of the path travelled by the larvae; kaolin-covered individuals showed a significantly higher turning rate per unit of time.

5. Effect on particle film surface on larval mobility and selection:

As above, direct observation did not reveal a distinct reaction caused by the presence of a kaolin film. The individuals showed a recurrent trend to attempt to climb the dish edge from kaolin and control surfaces alike. Analysis of track data showed differences in movement parameters induced by the kaolin film. The tested individuals covered a shorter distance at a lower velocity on the treated surface. The larvae spent significantly less time on the kaolin film surface and showed a higher stopping frequency as compared to the control surface. No differences were detected in the path shape given that the angular velocity exhibited was similar on both surfaces.

6. Adult oviposition preference:

C. carnea female adults laid 94.9 ± 6.4% (mean ± SD) of the total number of eggs on the kaolin-treated semi-circular zone compared with the control zone.

7. Effect on adult abundance. Field study

From a total of 256 samples, 111 adult chrysopids belonging to four different species were captured. Overall, no significant effect of kaolin treatments on *C. carnea* adult abundance was detected (Table 10.3.2.2/01-2).

Table 10.3.2.2/01-2: Number of Chrysopidae adults captured by suction sampling

Species	June sample		October sample	
	Control	Kaolin	Control	Kaolin
Chrysoperla carnea (Stephens)	8	3	36	55
Dichocrysa prasina (Burmeister)	0	0	1	0
Dichocrysa flavifrons (Brauer)	0	0	2	1
Chrysopa formosa (Brauer)	0	0	2	3
Total	**8**	**3**	**41**	**59**

III. CONCLUSION

Kaolin 5% (w/v) suspension direct spray (50 g/L) did not affect third instar larvae development to adulthood. The hatchability of recently laid eggs, subjected to the same spraying process, was also unaltered. However, third instar larvae coated with particle film after kaolin spraying showed slightly hampered movement capacity. Parameters extracted from recorded larvae movement on a kaolin film surface showed similar decreased mobility results as well as preference for the clean control surface. Additionally, the larvae had difficulty grasping treated leaves. *C. carnea* adult females showed a predominant preference for treated leaves in oviposition choice tests.

In the field trial, no difference in *C. carnea* adult abundance was found between kaolin-treated and control olive trees. These results indicate that disruption of movement capacity and dislodgement from the plant surface may be the principal negative effects of particle film on *C. carnea* larvae. Despite the positive trend in oviposition towards kaolin treated surfaces, a particle film attraction effect on adults was not observed at field level.

It can be concluded that the LC_{50} for *C. carnea* larvae and eggs based on mortality is greater than 50 g/L. Although there were noted behavioural differences, these parameters did not appear to negatively impact the population in the field.

Reference:	KCP 10.3.2.2/02, Bengochea, P., *et al*. 2010
Title:	Side effects of kaolin on natural enemies found on olive crops
Report No.:	Published in: Pesticides and Beneficial Organisms vol 55: 61-67
Guideline(s):	IOBC scheme for fecundity studies
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study and no toxicity endpoint reported (can only be estimated), however sufficient data well described

Executive summary

The main goal of this work was the evaluation of possible side effects of kaolin used against *B. oleae* on the auxiliary fauna. *Psyttalia concolor*, *Chrysoperla carnea*, *Chilocorus nigritus* and *Anthocoris nemoralis* were selected to be exposed to dried residues of kaolin sprayed on glass plates for 72 hours according to IOBC sequential scheme

Based on the results obtained in this study, kaolin seems to be not too much toxic to the natural enemies tested when they were in contact with kaolin-treated surfaces. According to IOBC categories, kaolin was classified as harmless (1) or slightly toxic (2), depending on the insect and the parameter studied. Based on the reference, the L/Er_{50} values for *P. concolor*, *C. carnea* and *C. nigritus* is greater than the tested dose of 5 kg test item/hL (50 kg product/ha). *A. nemoralis* resulted in a LR_{50} > 5 kg test item/hL (50 kg product/ha) (based on mortality), but a strong reduction (66.6%) on the number of eggs laid by female per day was noted.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2 (reliable with restrictions), not known to be GLP, toxicity endpoints not calculated, hence not reliable for use in a qualitative risk assessment.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Surround 95 WP
   Batch number: Not reported.
   Content of a.s.: Kaolin
   Appearance: Not reported.

2. **Vehicle:** Distilled water

3. **Reference:** Danadim Progress 40EC; a.s. Dimethoate

4. **Test organism**
   Species: *Psyttalia concolor* (Szèpligeti) (Hymenoptera: Braconidae)
   Chrysoperla carnea (Steph.) (Neuroptera: Chrysopidae)
   Chilocorus nigritus (F.) Coleoptera: Coccinellidae)
   Anthocoris nemoralis (F.) (Hemiptera: Anthocoridae)

   Age at test initiation: *P. concolor*: <24 hr
   C. carnea: <48 hr
   C. nigritus: adults
   A. nemoralis: adults

Source: *P. concolor*: reared in-house
C. carnea: Biobest Biological Systems, Spain.
C. nigritus: Entocare Biological Crop Protection, Netherlands
A. nemoralis: Biobest Biological Systems, Spain

Acclimation: Under test conditions
Food: *P. concolor*: 4:1 sugar:yeast
C. carnea: artificial diet
C. nigritus: *Ephestia kuehniella* eggs
A. nemoralis: *Ephestia kuehniella* eggs

5. **Treatment:** 5 kg test item/hL (50 kg product/ha)

6. **Test units:** Glass plates and a round metacrilate frame joined by two crossed rubber bands

7. **Environmental conditions**
Temperature: 25 ± 2 ºC
Humidity: 75 ± 5%
Photoperiod: 16 h light: 8 h dark

B. STUDY DESIGN AND METHODS

To evaluate residual contact activity, glass plates were treated under a Potter precision spray Tower with 1 mL of each test solution at a pressure of 55 kPa to obtain a homogenous deposit of 1.5-2 mg fluid per cm^2. A systemic insecticide, dimethoate at 150 mL/hL, was used as a commercial standard, and distilled water as control. As soon as the plates were dry, the corresponding group of insects per replicate was kept in glass dismountable cages.

P. concolor: 10 insects, 4 replicates
C. carnea: 6 insects, 4 replicates
C. nigritus: 12 insects, 5 replicates
A. nemoralis: 9 insects, 5 replicates

1. *P. concolor* trials:
After three days of exposure to a treated surface, 5 surviving females per replicate and control, were isolated for four days in plastic cages. Every day, 30 fully-grown *C. capitata* larvae were offered to each group of females for parasitisation. One hour later, *C. capitata* larvae exposed were placed into Petri dishes to let them pupate. Parasitism ability was measured as the percentage of attacked host (percentage of puparia without medfly emergence) and progeny size (percentage of parasitoids emerged from parasitized medfly puparia). Data of first day of parasitisation was not considered since females need to learn how to parasitize.

2. *C. carnea* trials:
Three days after exposure to the treated surfaces, survivors were moved to new cages and both fecundity (mean number of eggs per female laid in a 7-days period) and fertility (percentage of egg hatched) were assessed.

3. *A. nemoralis* trials:
Eggs laid in every piece of treated bean used as substrate for oviposition during the three days of exposure were counted. Then, they were transferred one by one to a plastic cages (9 x 3 cm) to count the emergence of neonates. Eggs laid per day and female and percentage of eggs hatched were recorded.

4. *C. nigritus* trials:

After being exposed to a contaminated substrate for three days, all survivors from the same replicate and insecticide were moved to ventilated plastic cages (11 x 5 cm) to evaluate life span. Mortality of insects was recorded every week until the death of the last insect. Life span was measured as the average of days that all the insects in each replicate.

4. Statistics:
Not reported.

II. RESULTS AND DISCUSSION

1. Findings:
Kaolin was classified as harmless (1) to adults of *C. nigritus*. No deleterious effect was detected. Neither mortality measured after 72 hours of exposure nor life span was modified due to the kaolin exposure. Nevertheless, possible negatives effects on fecundity and fertility remained unknown and need to be determined.

There were no effects on mortality on *C. carnea* and *P. concolor*, although *C. carnea* fecundity and *P. concolor progeny* were slightly reduced (2) compared to water control.

A. nemoralis was the most sensitive of the four insects tested. Forty per cent of mortality was recorded after 72 hours of exposure, when mortality was stabilised. The most important observed effect was the strong reduction (66.6%) on the numbers of eggs laid by female per day.

Table 10.3.2.2/02-1: IOBC toxicity rating after residual treatment in laboratory with kaolin

Test item	*P. concolor*			*C. carnea*			*A. nemoralis*			*C. nigritus*	
	M	AH	P	M	Fec	Fert	M	Fec	Fert	M	LS
Kaolin	1	1	2	1	2	1	2	2	1	1	1
Dimethoate	4	-	-	4	-	-	4	-	-	4	-

IOBC toxicity rating for laboratory: 1= harmless (<30%), 2 = slightly harmful (30-79%), moderately harmful (80-99%), 4= harmful (>99%).
M=Mortality after 72 h of exposure. AH=attacked host. P=Progeny. Fec = Fecundity. Fert. = Fertility. LS= Life span.

III. CONCLUSION

Based on the results obtained in this study, kaolin seems to be not toxic to the natural enemies tested when they were in contact with kaolin-treated surfaces. According to IOBC categories, kaolin was classified as harmless (1) or slightly toxic (2), depending on the insect and the parameter studied. Based on the reference, the L/Er_{50} values for *P. concolor*, *C. carnea* and *C. nigritus* is greater than the tested dose of 5 kg test item/hL. *A. nemoralis* resulted in a LR_{50} > 5 kg test item/hL (based on mortality), but a strong reduction (66.6%) on the number of eggs laid by female per day was noted.

Reference:	KCP 10.3.2.2/03, Bengochea, P., *et al*. 2014
Title:	Non-target effects of kaolin and copper applied on olive trees for the predatory lacewing *Chrysoperla carnea*
Report No.:	Published in: Biocontrol Science and Technology, vol 24, no 6: 625-640
Guideline(s):	IOBC scheme for fecundity studies
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

The effect of kaolin particle film formulation Surround WP (95 WP) on *Chrysoperla carnea* was evaluated in four separate extended laboratory studies to evaluate (1) the side effects of kaolin on all of the developmental stages of the predator *C. carnea* and (2) to evaluate the effects on *C. carnea* behaviour when larvae are able to choose (or not) between feeding on kaolin-contaminated and not contaminated food.

In the first study, the effects on *C. carnea* egg viability and larval hatching suppression by topical application was determined. In the second study, L3 larvae were exposed to the pesticide residues on treated olive tree leaves. Third, a series of three residual tests in adults were sequentially performed as follows: residues on glass surfaces, on olive tree leaves and on small olive trees. Finally, kaolin- and water-treated eggs of *Ephestia kuehniella* were offered to L3 larvae in both dual- and no-choice tests.

C. carnea egg hatching was reduced by the kaolin treatment compared to the control treatment; 24% after egg dipping and 29% from being sprayed. Residual treatments were harmless to larvae and adults, without any deleterious effects on reproduction; L/ER_{50} > 47500 mg a.s./L. L3 larvae consistently preferred to feed on water-treated *E. kuehniella* eggs compared to kaolin-treated eggs; consuming 87.2% and 85.7% of the water-tested eggs ad 67.3% and 56.9% of the kaolin-treated eggs in the no-choice and choice tests, respectively. The larvae from the no-choice test that had fed *ad libitum* until pupation on kaolin-treated *E. kuehniella* eggs, pupated and emerged as healthy adults in reduced proportions compared with those that fed on water treated eggs; only 56% reached pupal stage and 65.4% of formed pupae emerged as healthy adults.

In conclusion, the data suggest that kaolin products appeared to be largely harmless or only slightly harmful to the predator.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2 (reliable with restrictions), not known to be GLP, details of studies not reported

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Surround WP (95 WP)
   Batch number: Not reported. Product manufactured by BASF (Spain)
   Content of a.s.: Kaolin
   Appearance: Not reported.

2. **Vehicle:** Distilled water

3. **Reference:** Danadim Progress 40EC; a.s. Dimethoate

4. **Test organism**
   Species: *Chrysoperla carnea*

Age at test initiation: L1 larvae
Source: Biobest Biological Systems (Spain)
Acclimation: Under test conditions
Food: *Ephestia kuehniella* Zeller (Lep. Pyralidae) ad libitum and adults were provided with an artificial diet and distilled water as described by Vogt *et al.* (2000).

5. **Treatment:** 47500 mg a.s./L

6. **Environmental conditions**
Temperature: 25 ± 2 ºC
Humidity: 75 ± 5%
Photoperiod: 16 h light: 8 h dark

7. **Dose preparation:** Fresh solutions of the commercial product in distilled water were prepared prior to the assays according to the maximum field recommended concentration and the delivery rate was 1000 L water/ha.

B. STUDY DESIGN AND METHODS

1. Ovicidal activity:
Kaolin was tested on *C. carnea* eggs to determine their effects on egg viability or larval hatching suppression by the following two different treatments: dipping and spraying the eggs. Eggs were collected those attached to oviposition gauze. Each oviposition gauze, containing 40–80 eggs (less than 24-h-old), was considered as a replicate. Three (egg dipping) or four (egg spraying) gauzes per treatment were collected. After the treatments, eggs were allowed to dry for 2 h on a glass plate at room temperature. They were subsequently incubated for hatching in plastic containers (9 cm in diameter by 3 cm in height) to determine their viability. *E. kuehniella* eggs were supplied to avoid cannibalism among the newly emerged larvae. Afterwards, 24 neonates per treatment and control were randomly selected and were individually caged in the wells of a 24-well tissue-culture plate (3.5 mL) and fed on a diet of *E. kuehniella* eggs. The larval development was monitored until pupation. The percentages of pupation and adult emergence from formed pupae were scored.

Eggs dipped in each solution, or water, were immersed for 3 sec. Eggs were sprayed to the point of run-off with the different compounds. If the rates of egg hatching were not significantly different from those found in the former experiment, the development of the larvae was not subsequently monitored.

2. Laboratory extended test: larval exposure to pesticide residues on olive tree leaves (live substrate):
Pesticide-free olive tree leaves were collected from trees grown in a greenhouse in Madrid and taken to the laboratory. The leaves were sprayed using hand sprayers until the point of liquid run-off. Once dried, 10 leaves were carefully placed into each plastic experimental cage (9 cm in diameter by 3 cm in height, with a 5.5-cm diameter ventilation hole covered with mesh).

Newly emerged third instar larvae were maintained singly in each cage. A treatment replicate consisted of 10 cages. A total of four replicates per treatment were performed (40 larvae). *E. kuehniella* eggs were supplied as food on the surface of the treated leaves to encourage the larvae to walk on them. After 72 h of exposure, most of the insects had spun their cocoons, which were then transferred to larger plastic cages until the adults emerged.

Next, three couples (♀, ♂) of the same age that emerged from each treatment were kept in the oviposition cages previously described in the experiment on ovicidal activity. Four replicates per compound and control were performed. Eggs were daily collected during a one-week period after the beginning of oviposition period (approximately five days after the emergence of the females). All of the eggs attached to the gauze pads (less than 24 h) collected at day four after the beginning of oviposition were incubated for hatching as described above. Fecundity was measured as the mean number of eggs per female per day. Fertility was measured as the mean egg-hatching rate.

3. Sequential exposure scheme according to the International Organisation for Biological Control (IOBC) protocol for testing *C. carnea* adults:

Laboratory test:

To evaluate the residual contact activity of pesticide, glass plates (12 × 12 × 0.5 cm) were treated with the chemical under a Potter Precision Spray Tower. One milli-litre of each test solution was applied at a pressure of 55 kPa to obtain a homogenous deposit of 1.5–2 mg fluid per cm^{-2}.

Three couples per replicate were exposed to the dry residues of the insecticides after spraying on the glass surfaces. Each treatment consisted of four replicates. Cumulative mortality was recorded at 24, 48 and 72 h after treatment. Afterwards, the surviving adults were transferred to the described oviposition cages to evaluate the reproductive parameters, fecundity and fertility, as explained above.

Extended laboratory experiment:

Adults were exposed to pesticide residues on olive tree leaves following the same procedure as described in the extended laboratory test for larvae. In this case, 18 leaves (three small branches with six leaves per branch) were maintained in a plastic cage (12 cm in diameter by 5 cm in height and a 5.5-cm diameter ventilation hole covered with mesh on the top), and three pairs of adults were introduced into each cage. Each treatment had four replicates. Cumulative mortality, fecundity and fertility were measured as described above.

Semi-field experiment:

Small (55 cm) pesticide-free olive trees (cv. Picual), grown in a greenhouse located at the ETSI Agrónomos in Madrid, Spain, were used for the experiments. Environmental conditions in the greenhouse were recorded.

Trees were treated with hand sprayers until the liquid ran off the leaves. As soon as trees dried, they were covered with a cage and meshing to maintain the insects within. Water and artificial diet was provided. Each cage with a tree was considered a replicate, and three replicates per treatment and control were performed. Eight pairs of *C. carnea* (less than 48-h-old) were introduced per cage. The adults were exposed to the treated and control trees for three days, after which the survivors from the same treatment were recovered from the cages and moved to the laboratory cabinet. Next, the survivors were distributed into the oviposition cages described for the ovicidal test. Three couples of adults were introduced in each cage. Four replicates per treatment were done to evaluate fecundity and fertility as described above.

4. Choice and no-choice tests: feeding on kaolin-treated eggs:

The day before the experiment, newly moulted, third instar larvae were collected from the rearing cages and singly confined in small round plastic cages (4 x 2.5 cm, with a hole in the lid covered with mesh for aeration) without food. *E. kuehniella* eggs were placed on filter paper in a single layer and treated either with kaolin particle film or distilled water. For this experiment, *E. kuehniella* eggs were obtained from a small rearing culture of the laboratory [Crop Protection Unit, ETSIA, Technical University of Madrid (Madrid, Spain)] because commercial eggs are often mixed with eggs of other species and/or rice to absorb humidity or are not turgid enough. Once dried, only turgid eggs were selected and glued on a piece of black cardboard (3 × 1.5 cm) using a fine paintbrush. In both the choice and no-choice tests, 20 replicates of 40 eggs were used. Each replicate consisted of a plastic cage (7 x 3 cm, with a hole in the lid of 1 cm diameter, covered with a mesh to facilitate ventilation) containing a piece of cardboard with 40 eggs and one starved L3 larvae in the middle of each cardboard. In the no-choice tests, all of the eggs were treated with water or kaolin (20 replicates each). In the dual-choice test, the cardboard of each replicate was divided in two parts, one containing 20 kaolin treated eggs and the other with 20 water-treated eggs (20 replicates each). For both tests, the L3 larvae were fed for one and a half hour. Next, they were removed from the cages and the consumed eggs counted using a binocular stereomicroscope. Preliminary trials indicated that the experimental period was sufficient for the complete consumption of the eggs if the larvae were hungry. After that, eggs were checked under binocular lens. We considered the consumed eggs those without turgidity or with the holes caused by the penetration of the larval mandibles. The larvae from the no-choice experiments were transferred singly to clean cages to continue feeding *ad libitum* on treated and untreated eggs, depending on the former treatment, until pupation. The numbers of pupae formed and adult emergence were scored. The entire experiment was repeated twice. Once being sure that there were not significant differences amongst them, all the data were pooled.

4. Statistics:

The data were subjected to a one-way analysis of variance (ANOVA). Fisher's least significant difference (LSD) test was used to compare the responses at the maximum field concentrations. All of the statistical analyses were performed using Statgraphics® version 5.1. If necessary, the data were transformed using arcsin $\sqrt{(x/100)}$ for percentages or log (x + 1) for absolute values. If any of the assumptions of the ANOVA were violated after the appropriate transformations, the non-parametric Kruskal–Wallis test was applied. Median values were considered significantly different if the 95% confidence intervals of the medians did not overlap.

For egg consumption, the data distribution failed to satisfy parametric analysis assumptions, and thus, the data were subjected to the non-parametric Mann–Whitney U-test. The mean values of each parameter studied were corrected using the Schneider–Orelli formula for mortality or the Abbott formula for reproductive parameters. These corrected values were used to rank the products according to IOBC standards.

II. RESULTS AND DISCUSSION

1. Egg treatment:

Kaolin did not elicit any significant effect on the rate of egg hatching after dipping. The hatching percentage recorded for kaolin (57.7%) was slightly reduced compared to that of the control treatment (75.8%) and dimethoate (79.2%). There were no significant differences among the percentages of pupation or adult emergence in comparison to the values scored in the controls. The same pattern was observed when eggs were sprayed instead of dipped; thus, different application techniques produced similar results. The hatching percentage recorded for kaolin (47.7%) was also reduced in this assay compared to the control treatment (67.4%)

Table 10.3.2.2/03-1: Toxicity of kaolin on *C. carnea* egg hatching

Treatment	Concentration (mg a.s./L)	Mean egg hatching (%)	Reduction (%)[a]	IOBC[b]
Egg dipping[c]				
Control	0	75.8	-	-
Kaolin	47500	57.7	23.9	1
Reference	600	79.2	-	-
Egg spraying[d]				
Control	0	67.4	-	-
Kaolin	47500	47.7	29.2	1
Reference	600	62.6	7.1	1

[a] Percentages of egg hatching were corrected following the Abbott formula: P (%) = [1 – (Ptreated/Pcontrol)] × 100.
[b] IOBC toxicity rating: Laboratory: (1) harmless (<25%).
[c] Fifty to 80 eggs per replicate and compound. Three replicates per compound.
[d] Forty to 70 eggs per replicate and compound. Four replicates per compound.

2. Extended laboratory test on larvae:

No effects on mortality were detected when larvae walked on the treated substrate. No negative effects were scored on pupal spinning. Moreover, the reproductive parameters of *C. carnea* (fecundity and fertility) remained significantly unaffected by the treatments.

Table 10.3.2.2/03-2: Toxicity of kaolin on the reproductive parameters of *C. carnea*, when treated as third instar in an extended laboratory test

Treatment	Concentration (mg a.s./L)	Mean fecundity[a]	Reduction[c] (%) (IOBC)	Mean fertility[b]	Reduction[c] (%) (IOBC)
Control	0	40.4	-	83.7	-
Kaolin	47500	35.7	11.6 (1)	70.2	16.1 (1)
Reference	600	19.9	50.7 (2)	84.3	0 (1)

[a] Fecundity (eggs laid by female and day) of *C. carnea* females.
[b] Mean egg-hatching rate (percentage).
[c] Parameters corrected following the Abbott formula: P (%) = [1 – (Ptreated/Pcontrol)] × 100.
IOBC toxicity rating: Extended laboratory: (1) harmless (<25%) (2) slightly harmful (25-50%).

3. IOBC sequential exposure scheme for *C. carnea* adults:

No mortality was recorded after 72 h for *C. carnea* exposure to an inert residue of the test material. No significant differences were observed in terms of fecundity or fertility. Nevertheless, although no statistically significant reduction in fecundity was recorded when the females were exposed to kaolin (32.9%), they were previously classified as slightly toxic according to IOBC guidelines. In the extended laboratory experiment, similar results as in the previous experiment were recorded. The products did not cause any mortality of the adults by 72 h after the treatment with kaolin. No significant differences in reproductive parameters among treatments were found. In the semi-field trial, no negative effects on reproduction were found.

Table 10.3.2.2/03-3: Toxicity of kaolin on *C. carnea* adults after residual treatments

Treatment (rate)	Mean mortality 72 h (%)	Reduction[a] (%) (IOBC)	Mean fecundity	Reduction[b] (%) (IOBC)	Mean fertility	Reduction[c] (%) (IOBC)	Highest IOBC score
Laboratory test							
Control (0 mg a.s./L)	0.0	-	39.8	-	75.0	-	-
Kaolin (47500 mg a.s./L)	0.0	0 (1)	27.7	32.9 (2)	75.6	0 (1)	2
Reference (600 mg a.s./L)	100.0 *	100 (4)	-	-	-	-	4
Extended laboratory test							
Control (0 mg a.s./L)	0.0	-	36.5	-	72.6	-	-
Kaolin (47500 mg a.s./L)	0.0	0 (1)	38.1	0 (1)	73.2	0 (1)	1
Reference (600 mg a.s./L)	91.7 *	91.7 (4)	-	-	-	-	4
Semi-field test							
Control (0 mg a.s./L)	0.0	-	24.6	-	83.1	-	-
Kaolin (47500 mg a.s./L)	0.0	0 (1)	26.6	0 (1)	78.6	5.4 (1)	1
Reference	75.9*	75.9 (4)	-	-	-	-	4

(600 mg a.s./L)							

*Statistical significantly different (ANNOVA, LSD; P> 0.05)
[a] Mortality corrected following the Schneider–Orelli formula: M (%) = [(Mtreated – Mcontrol) / (100 – Mcontrol)] × 100.
[b] Fecundity and fertility corrected following the Abbott formula: P (%) = [1 – (Ptreated/Pcontrol)] × 100.
IOBC toxicity rating: Laboratory: (1) harmless (<30%); (2) slightly harmless (30-79%); (3) moderately harmful (8-=99%); (4) harmful (>99%). Lab extended and semi-field: (1) harmless (<25%); (2) slightly harmful (25-50%), (3) moderately toxic (51-75%), (4) toxic (>75%).

4. Dual-choice and no-choice tests: feeding on kaolin- or water-treated eggs:
Starved larvae of *C. carnea* consistently preferred to feed on water-treated *E. kuehniella* eggs compared to the kaolin-treated eggs. This behaviour was enhanced when they had the possibility of choice. They consumed 87.2% and 85.7% of the water-treated eggs and 67.3% and 56.9% of the kaolin-treated eggs in the no-choice and choice tests, respectively. As previously stated, larvae from the no choice experiments continued to feed *ad libitum* on the treated or non-treated *E. kuehniella* eggs, depending on the previous feeding option, until pupation. When fed on water-treated eggs, 100% of the larvae spun cocoons and 90% of the adults emerged, whereas after feeding on kaolin-treated eggs, only 65% reached the pupal stage and 65.4% of formed pupae emerged as healthy adults.

III. CONCLUSION

C. carnea egg hatching was reduced by the kaolin treatment compared to the control treatment; 24% after egg dipping and 29% from being sprayed. Residual treatments were harmless to larvae and adults, without any deleterious effects on reproduction; L/ER_{50} > 47500 mg a.s./L. L3 larvae consistently preferred to feed on water-treated *E. kuehniella* eggs compared to kaolin-treated eggs; consuming 87.2% and 85.7% of the water-tested eggs ad 67.3% and 56.9% of the kaolin-treated eggs in the no-choice and choice tests, respectively. The larvae from the no-choice test that had fed *ad libitum* until pupation on kaolin-treated *E. kuehniella* eggs, pupated and emerged as healthy adults in reduced proportions compared with those that fed on water treated eggs; only 56% reached pupal stage and 65.4% of formed pupae emerged as healthy adults.

In conclusion, the data suggest that kaolin products appeared to be largely harmless or only slightly harmful to the predator.

Reference:	KCP 10.3.2.2/04, Bengochea, P., *et al*. 2013
Title:	Kaolin and copper-based products applications: Ecotoxicology on four natural enemies
Report No.:	Published in: Chemosphere 91: 1189-1195
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

The effect of kaolin on *Anthocoris nemoralis*, *Chelonus inanitus*, *Chilocorus nigritus* and *Scutellysta cyanea* were investigated under extended laboratory conditions.

Exposure to kaolin for 72 hours did not increase the mortality of any of the insects, with the exception of *A. nemoralis*. The sublethal effects, however, differed depending on the parameter evaluated and the insect studied. Kaolin slightly, but significantly, reduced the life span of *C. inanitus* and *S. cyanea*. Number of eggs laid by *A. nemoralis* females were reduced, but not significantly compared to the controls. In the behavioural experiments, clear preference for remaining on kaolin-untreated surfaces when insects were able to choose was observed.

Based on the findings reported, the following toxicity endpoints can be estimated:

- The estimated LR_{50} based on mortality for all test species is greater than 5 kg/hL (50 kg/ha), the highest test dose.
- The estimated ER_{50} based on reproduction for *A. nemoralis* is greater than 5 kg/hL (50 kg/ha), the highest test dose.

Despite having some negative effects, the negative impact on natural enemies was lower than the impact caused by products commonly applied in olive groves. Therefore, kaolin can be considered as alternative products to be applied in olive groves if an effective resistance management programme is to be developed. Furthermore, kaolin is allowed in organic farming, in which the number of products that can be applied is more restricted.

According to the Klimisch score, the literature reference is concluded to be:

Relevance = 2, not guideline study

Reliability = 2 (reliable with restrictions), not known to be GLP, toxicity endpoints not calculated, hence not reliable for use in a qualitative risk assessment.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Surround WP (95 WP)
	Batch number:	Not reported. Product manufactured by BASF (Spain)
	Content of a.s.:	Kaolin
	Appearance:	Not reported.
2.	**Vehicle:**	Distilled water
3.	**Reference:**	Danadim Progress 40EC; a.s. Dimethoate
4.	**Test organism**	
	Species:	*Anthocoris nemoralis* (F.) (Hem. Anthocoridae), *Chelonus inanitus* (L.) (Hym. Braconidae), *Chilocorus nigritus* (F.) (Col. Coccinellidae) and *Scutellysta cyanea* Motschulsky (Hym. Pteromalidae)
	Age at test initiation:	Adults
	Source:	*A. nemoralis*: Biobest Biological Systems (Spain) *C. nigritus*: Entocare Biological Crop Protection (Netherlands) *S. cyanea*: Nijhof BGB (Netherlands) *C. inanitus*: Division of Developmental Biology, Bern University(CH)
	Acclimation:	Not reported
	Food:	See below
5.	**Treatment:**	5 kg/hL (50 kg/ha)
6.	**Environmental conditions**	
	Temperature:	25 ± 2 ºC
	Humidity:	75 ± 5%
	Photoperiod:	16 h light: 8 h dark
7.	**Dose preparation:**	Fresh solutions of the commercial product in distilled water were prepared prior to the assays according to the maximum field recommended concentration.

B. STUDY DESIGN AND METHODS

Olive tree leaves were collected from two-year-old trees (cv. Picual) grown in a greenhouse in Madrid (Spain) and taken to the laboratory. Test items were applied using hand sprayers until the liquid ran off the leaves. Once the leaves were dried, they were transferred into a plastic cage. Every single plastic cage was considered a replicate and each test item was considered a different treatment (Table 10.3.2.2/04-1).

All insects were exposed to treated leaves for at least 3 days. Mortality was always scored at 72 hours. Subsequently, the surviving individuals were moved to clean cages to evaluate the sublethal effects. Effects on life span were measured for *C. inanitus, C. nigritus* and *S. cyanea*. The effects on reproduction were evaluated for *A. nemoralis*. For this insect, one green bean per replicate was used instead of olive tree leaves because it is not possible to evaluate *A. nemoralis* reproductive parameters using olive tree leaves as oviposition substrata dry before nymphs' emergence. Green beans were checked under a stereoscopic microscope to count the number of eggs laid. Five to seven days later, the number of nymphs hatched from the eggs was scored.

For the behavioural experiments, vegetal material was treated with kaolin. For *A. nemoralis*, green beans were offered to adults, while for *C. nigritus* the vegetal material was butternuts.

Table 10.3.2.2/04-1: Materials and methodology used in the experiments

Insect species	Plastic cages[a]	N olive tree leaves per replicate	N replicates	Developmental stage	Adults per replicate	Parameters evaluated	Food and water[d]
Anthocoris nemoralis	A	1 green bean	5	Adult	6 males	Mortality	*E. kuehniella* eggs[e]
					6 females	Fecundity[b] Fertility[b]	No water
Chelonus inanitus	B	18	5	Adult	10 unsexed	Mortality Life span[c]	Semisolid diet[f] Water
Chilocorus nigritus	B	18	4	Adult	8 unsexed	Mortality Life span[c]	*E. kuehniella*[e] No water
Scutellista cyanea	S	5	4	Adult	5 unsexed	Mortality Life span[c]	Honey[g] No water

[a] A (25.5 × 9 × 7 cm^3); B (12 cm in diameter by 5 cm high); S (9 cm in diameter by 3 cm high). All the cages had a ventilation hole covered with mesh on the top for ventilation (13 × 3.5 cm^2, for A, and 5.5 cm in diameter for B and S).
[b] Fecundity was measured as the number of eggs laid per female and day and fertility as the percentage of nymphs hatching from the eggs.
[c] The life span of adults was measured as the average number of days that the insects survived per replicate.
[d] Food was supplied in small plastic stoppers (24 × 6 mm). Distilled water was offered *ad libitum* in glass vials (30 × 35 mm).
[e] *Ephestia kuehniella* Zeller (Lep. Pyralidae).
[f] 66.67 g of honey, 33.33 mL of distilled water and 0.5 g of agar.
[g] Small drops of honey (0.1 mm in diameter) placed on the olive tree leaves and on the mesh of the cages.

4. Statistics:
The data (mean values and standard errors (S.E.)) were subjected to a one-way analysis of variance (ANOVA). Fisher's least significant difference (LSD) test was used to compare the responses. All statistical analyses were performed using Statgraphics version 5.1 (STSC, 1987). If necessary, the data were transformed using arcsin for percentages or log (x + 1), otherwise. If any of the assumptions of the analysis of variance were violated after appropriate transformations, the non-parametric Kruskal–Wallis test was applied. Median values were considered significantly different if the 95% confidence intervals of the medians did not over-lap. The mean values of each parameter studied were corrected using the Schneider–Orelli or the Abbot formula for mortality and the reproductive parameters, respectively, and used to rank the products into four toxicity categories, according to the IOBC.

II. RESULTS AND DISCUSSION

1. Mortality:
No statistical differences were detected in the percentage of mortality of four out of the five insect species tested after 72 h of kaolin exposure. For *A. nemoralis*, however, the products caused a slightly higher mortality than that found for the controls (approximately 27%). In great contrast, dimethoate caused 100% mortality in all the species tested.

The estimated LR_{50} based on mortality for all test species is greater than 5 kg/hL, the highest test dose.

2. Life span:
The life span was reduced to less than 24 h for the three insects in the case of dimethoate. For *C. nigritus*, kaolin did not produce any deleterious effects on life span (7.1% reduction). In contrast, for *C. inanitus* kaolin produced a significant reduction (35.0% reduction). For *S. cyanea*, kaolin produced a statistically significant difference compared to the controls (15.8% reduction). Findings are summarized in Table 10.3.2.2/04-2.

3. Effects on reproduction:
Kaolin did not significantly diminish the fecundity and the fertility of *A. nemoralis*. However, there was a reduction on fecundity (32.0%) compared to the controls caused by kaolin. No data for dimethoate was obtained because the high mortality scored after exposure hindered the obtaining of eggs and nymphs in amounts enough to be statistically analyzed. Findings are summarized in Table 10.3.2.2/04-2.

The estimated ER_{50} based on reproduction for *A. nemoralis* is greater than 5 kg/hL, the highest test dose.

Table 10.3.2.2/04-2: Sublethal effects of kaolin

Treatments	*C. inanitus*		*C. nigritus*		*S. cyanea*	
	Life span[a]	Red.[b] (%)/IOBC[c]	Life span[a]	Red.[b] (%)/IOBC[c]	Life span[a]	Red.[b] (%)/IOBC[c]
Control	$25.9_{ab} \pm 2.6$	–	$117.8_{a} \pm 7.2$	–	$37.4_{a} \pm 3.0$	–
Kaolin	$16.9_{c} \pm 2.0$	35.0/2	$108.7_{a} \pm 11.2$	7.1/1	$31.4_{b} \pm 1.2$	15.8/1
Bordeaux mixture	$22.5_{bc} \pm 3.4$	13.1/1	$134.9_{a} \pm 11.3$	−14.6/1	$18.1_{c} \pm 0.7$	51.4/3
Copper oxychloride	$32.1_{a} \pm 3.8$	−23.6/1	$126.9_{a} \pm 26.8$	−7.7/1	$26.9_{b} \pm 1.2$	27.9/2
Dimethoate	$1.0_{d} \pm 0.0$	96.1/4	$1.1_{b} \pm 0.1$	99.1/4	$1.0_{d} \pm 0.0$	97.3/4
	$K^{d} = 18.03$ $P = 0.0012$		$F_{4,20} = 14.8$ $P < 0.0001$		$F_{4,15} = 79.48$ $P < 0.0001$	

Treatments	*A. nemoralis*			
	Fecundity[e]	Red.[f] (%)/IOBC[c]	Fertility[g]	Red.[f] (%)IOBC[c]
Control	$4.5_{a} \pm 0.5$	–	$46.4_{a} \pm 4.5$	–
Kaolin	$3.0_{a} \pm 0.7$	32.0/2	$67.8_{a} \pm 8.6$	−46.3/1
Bordeaux mixture	$3.3_{a} \pm 0.6$	26.2/2	$53.6_{a} \pm 6.8$	1.9/1
Copper oxychloride	$4.6_{a} \pm 0.6$	−2.5/1	$45.5_{a} \pm 4.8$	−15.7/1
Dimethoate	–	–	–	–
	$F_{3,16} = 1.47$ $P = 0.2603$		$F_{3,16} = 2.62$ $P = 0.0868$	

Data followed by the same letter are not significantly different (ANOVA, LSD; $P \geqslant 0.05$).

[a] Average of number of days that individuals survived after the treatments.

[b] Reduction (%): Life span corrected following the Abbot formula: $P(\%) = [1 - (P_{treated}/P_{control})] \times 100$.

[c] IOBC toxicity rating: Extended laboratory: 1, harmless (<25%); 2, slightly harmful (25–50%); 3, moderately harmful (51–75%); 4, harmful (>75%).

[d] Data analysed using Kruskal–Wallis test.

[e] Fecundity (number of eggs per female and day).

[f] Parameters corrected following the Abbot formula: $P(\%) = [1 - (P_{treated}/P_{control})] \times 100$.

[g] Mean egg-hatching rate (%).

4. Behavioural effects:
No statistically significant impact on toxicity and fertility of *A. nemoralis* resulted in the no-choice experiments. However, there was a reduction of 27.9% in the number of eggs laid in kaolin-treated beans. When females were able to choose, they laid significantly more eggs in the untreated beans. The deterrence index for fecundity was 0.54. Because the percentage of hatching was not significantly different for both, dual choice and no-choice experiment, it seems that kaolin had no deleterious effect on fertility.

In the dual choice test, 62% of *A. nemoralis* adults found on the beans were on the water-treated beans, whereas 38% were on kaolin-treated beans. In the no-choice test, in the control cages 83.1% and 16.9% of adults were found on and out of the green beans, respectively, while in the kaolin cages these percentages were 76.9% and 23.1%, respectively. Insect out of the beans were walking on the cage or close to the *E. kuehniella eggs*.

For *C. nigritus*, most of the adults were found on the cages, rather than on the butternuts, in all the observations done (in the no-choice experiment 85.5% of the adults were found out of the butternuts in the control replicates and 87.6% in the kaolin replicates; in the dual choice assay this percentage was 87.9%). In the no-choice experiment, statistical differences were found when the level of *A. nerii* infestation was compared. More adults were found on the infested butternuts than on the uninfested ones. In contrast, no differences were found when the treatment was compared, suggesting that *C. nigritus* adults are more likely to be on the infested butternuts, no matter whether they are treated or not. In the dual choice assay, statistical differences were found either when the treatment or the infestation level were compared. The results suggested that there was an interaction among the two considered parameters (i.e. infestation level and treatment) and adults preferred the untreated parts of the infested butternuts.

III. CONCLUSION

Exposure to kaolin for 72 hours did not increase the mortality of any of the insects, with the exception of *A. nemoralis*. The sublethal effects, however, differed depending on the parameter evaluated and the insect studied. Kaolin slightly, but significantly, reduced the life span of *C. inanitus* and *S. cyanea*. Number of eggs laid by *A. nemoralis* females were reduced, but not significantly compared to the controls. In the behavioural experiments, clear preference for remaining on kaolin-untreated surfaces when insects were able to choose was observed.

Based on the findings reported, the following toxicity endpoints can be estimated:
- The estimated LR_{50} based on mortality for all test species is greater than 5 kg/hL (50 kg/ha), the highest test dose.
- The estimated ER_{50} based on reproduction for *A. nemoralis* is greater than 5 kg/hL (50 kg/ha), the highest test dose.

Despite having some negative effects, the negative impact on natural enemies was lower than the impact caused by products commonly applied in olive groves. Therefore, kaolin can be considered as alternative products to be applied in olive groves if an effective resistance management programme is to be developed. Furthermore, kaolin is allowed in organic farming, in which the number of products that can be applied is more restricted.

Reference:	KCP 10.3.2.2/05, Bengochea, P., Budia, F., Viñuela, E., and Medina, P. 2014
Title:	Are kaolin and copper treatments safe to the olive fruit fly parasitoid *Psyttalia concolor*?
Report No.:	Published in: J Pest Sci 87: 351-359
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

The aims of this study were to evaluate direct mortality and sub-lethal effects on emergence of adults from treated pupae and on beneficial capacity of females to the parasitoid wasp, *Psyttalia concolor,* caused by Surround WP (95% kaolin) through four different experiments; three at laboratory level and one in semi-field conditions. Dual choice and no-choice experiments were also performed to test kaolin oviposition repellence.

The results indicated that kaolin was not harmful to the parasitoid, *P. concolor*, at a dose rate up to and including 5 kg Surround WP/hL (4.25 kg a.s./hL). No lethal toxicity or effects on beneficial capacity were recorded. The behavioural experiments, however, showed that when females could choose between parasitising through a kaolin-treated surface and a water-treated one, there was a slight reduction in the percentage of parasitised hosts for kaolin. No differences were detected when females were not given a choice.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2 (reliable with restrictions), not known to be GLP.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Surround WP (95 WP)
	Batch number:	Not reported. Product manufactured by Basf Española S.L. (Barcelona, Spain)
	Content of a.s.:	Not reported, but 95 WP formulation
	Appearance:	Not reported.
2.	**Vehicle:**	Distilled water
	Reference item:	Danadim Progress 40 EC (dimethoate)
3.	**Test organism**	
	Species:	*Psyttalia concolor* (Szèpligeti) (Hym. Braconidae), parasitoid wasp
	Age at test initiation:	See each test method below
	Source:	Reared culture on *Ceratitis capitate* (Wiedemann) in the laboratory of the Crop Protection Unit, ETSIA, Technical University of Madrid (Spain). Originally from El Encin (INIA, Madrid, Spain), strain from Spanish field.
	Acclimation:	See each test method below
	Food:	Distilled water in glass vials (30 x 35 mm^2) and diet (icing sugar: brewer's yeast 4:1, v:v) in plastic stoppers (24 x 6 mm^2) were offered *ad libitum* to adults during the assays.
4.	**Treatment:**	5 kg Surround WP/hL (4.25 kg a.s./hL)

5. Environmental conditions: See each test method below

B. STUDY DESIGN AND METHODS

Four experiments were performed to assess the effects of kaolin on the parasitoid wasp, *P. concolor*.

Fresh solutions of kaolin in distilled water were prepared prior to the assays for an application rate of 5 kg/hL for Surround WP and 150 mL/hL for dimethoate. Distilled water was used as a control.

1. Adult emergence from topical treatment of pupae:
The objective of this experiment was to test the possible side effects of the kaolin on the most protected stage of *P. concolor*, i.e. when parasitoids have pupated inside the host pupae. 150 pupae per treatment (five replicates per treatment and 30 pupae per replicate) were topically treated using hand sprayers until they were completely wet. Immediately after spraying them, they were transferred to a dry surface to avoid further penetration of the test item to the pupae. Once they were dried (1 h after application), they were transferred to fully enclosed plastic cages (12 x 5 x 5.5 cm) with ventilation. Five to six days after the treatment, and 1 to 2 days before adult emergence, the cages were provided with distilled water and diet. Emergence was evaluated daily for 10 days. Immediately after emergence, the adults were transferred to untreated cages. Beneficial capacity was evaluated as described below. Five replicates per treatment (five females per replicate) were carried out.

2. Female exposure to residues on olive tree leaves and parasitisation surface:
The objective of this experiment was to determine whether kaolin might modify the beneficial capacity of *P. concolor* females when they parasitised through a treated surface. The lethal and the sublethal effect assessments were tested at the same time. Pesticide-free olive tree leaves were collected from 2-year-old olive trees. Three small branches with six leaves were used per replicate (five replicates per treatment). Both the mesh of the bottom hole of the cages (parasitisation surface; see the parasitisation cages described below) and the olive tree leaves (contact surface) were treated using hand sprayers (until the liquid ran off the leaves and the mesh of the cages was totally covered). Five females (72-h-old) per replicate were used. The day before the experiment, the females were placed in untreated parasitisation cages (and without olive tree leaves), to allow them to acclimate in these cages. *Ceratitis capitata* larvae were offered to parasitise. The following day, the females were transferred to the treated cages, and the effects on beneficial capacity were measured. After the females were in the treated cages for 4 days, they were transferred again to untreated cages (without olive tree leaves), to record the potential recovery capacity of females after exposure. Beneficial capacity was measured 72 hours later over four more days. It was then possible to record the potential recovery capacity of females after the exposure to kaolin.

3. Female exposure under semi-field conditions
To evaluate the toxicity of kaolin under more realistic conditions, small (55 cm) pesticide-free olive trees grown in a greenhouse were used. The average environmental conditions (mean data ± SEM) in the greenhouse during the experiment were 14.06 ± 0.36 °C, 64.88 ± 0.81% RH. Parasitisation was always evaluated at noon. The average temperature at that time during the 5 days females were parasitising was 22.9 °C. Trees were treated with hand sprayers until the liquid ran off the leaves. As soon as trees dried, they were covered with a wooden cage. Each cage had a base dimension of 25 x 25 cm^2 and a height of 60 cm. At the base, there was a rectangular hole for the trunk of the tree. To contain insects in the cage, plasticine was used to seal the space between the trunk and the floor. The top and three sides of the cage were covered with mesh. A fourth side consisted of a methacrylate sheet to easily monitor the experiment. Each tree was considered a replicate, and three replicates per treatment were performed.

Each cage contained two small glass vials that supplied water and two plastic stoppers that supplied the food diet. A total of 30 *P. concolor* females (<24-h in age) were introduced per cage and exposed to the pesticides for a week. Mortality was recorded daily. The beneficial capacity was measured directly in the greenhouse. Since the mortality did not increase after 72 h of exposure to the compounds (with the exception of dimethoate), the measurement of the beneficial capacity was made using six times more *C. capitata* larvae. Therefore, 180 larvae were offered to the females of each replicate. The larvae were sandwiched between two mesh pieces held together with a wooden frame (15 cm in diameter). A sand bag was placed on the top of the frames to prevent the larvae from jumping. After 1 hour, the larvae were placed onto Petri dishes and transferred to a climate-control chamber in the laboratory to allow pupation.

4. Kaolin oviposition repellency: no-choice and dual choice tests
This experiment aimed to evaluate whether there are differences on *P. concolor* parasitisation when females find a treated or an untreated surface through which they parasitise. In a no-choice test, both the top and the bottom meshes of the parasitisation cages described below were sprayed with kaolin or water (control). Six replicates per treatment were used. In a dual choice test, one of the meshes was sprayed with kaolin and the other one with water. To prevent a potential effect of mesh position on choice, the experiment was varied between treating the upper and the bottom meshes, with six replicates per treatment position. The evaluation of the beneficial capacity was done as described below with a slight modification. Fifteen *C. capitata* larvae were offered on the top of the cages, and another 15 were offered on the bottom. The larvae placed on the top mesh were covered with a piece of parafilm and a small plastic stopper (4 cm in diameter) to prevent them from being mashed. The larvae placed on the bottom side were immobilised as explained below. The experimental setup (the cage, the plastic stopper and the glass pot) was also held together with two rubber bands.

Description of evaluating beneficial capacity

Mortality of *P. concolor* adults was daily scored until the end of the experiments described above. The sublethal effects of kaolin on the beneficial capacity of females were evaluated using parasitisation plastic cages (12 x 5 cm) with ventilation holes covered with mesh on the top and the bottom. Five mated females per cage were used. Before choosing the females for the different experiments, they were in contact with males for 2 to 3 hours. Thirty fully grown *C. capitata* larvae were offered for 1 hour to females during five consecutive days. The larvae were immobilised by sandwiching them between the mesh of the cage's floor and a piece of parafilm. It was placed on the floor of a glass pot turned upside down. The experimental setup (the cage and the glass pot) was held together with two rubber bands. The data of the first day of parasitisation were rejected, because previous assays had shown that naïve females (i.e. wasps with no prior oviposition experience) need 1 day to acclimate to the cage. The females' beneficial capacity was measured as the percentage of hosts attacked (percentage of puparia without medfly emergence) and progeny size (percentage of parasitoids emerged from parasitised puparia). To determine if the emergence failure was due to the parasitisation of *P. concolor*, a control with non-parasitised medfly was performed. Each single cage was considered as a treatment and a replicate, respectively. Five replicates were performed.

4. Statistics:
In the experiments to test the effects of kaolin, mortality rates and the percentage of attacked hosts and progeny size were analysed using one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. All statistical analyses were performed using Statgraphics version 5.1. When necessary, data were arcsin $\sqrt{(x/100)}$ transformed if the data were expressed as a percentage, or log (x + 1) transformed if the data were not expressed as percentage. If the transformations failed to meet the assumptions of ANOVA, the non-parametric Kruskal-Wallis test was applied instead. Median values were considered significantly different if 95% confidence intervals of medians did not overlap.

In the behavioural experiments, significant differences between treatment means were tested using the two sample t-tests. If any of the assumptions of the analysis were violated, the non-parametric Mann-Whitney U test was used instead.

II. RESULTS AND DISCUSSION

1. Adult emergence from topical treatment of pupae:

Significant differences in the percentage of adult emergence between reference item (dimethoate) and the rest of the treatments were found when *C. capitata* pupae (previously parasitised by *P. concolor*) were treated. A reduction of 32.9% emergence was scored for dimethoate compared to the controls, while no differences between the kaolin compared to the controls were observed (data summarised in table below). As almost 100% of medfly adults emerged from non-parasitized medfly pupae, failures of emergence are supposed to be due to female parasitisation and not due to failures of medfly's development.

Table 10.3.2.2/05-1: Percentages of emergence, attacked hosts and progeny size from exposure *via* topically applied kaolin to pupae (mean data ± SEM)

Treatment	Topical toxicity on pupae		
	% Emergence	Attacked hosts	% Progeny size
Control	55.7 ± 4.3	99.1 ± 0.5	31.6 ± 9.8
Kaolin	66.5 ± 6.6	96.4 ± 1.5	38.6 ± 8.3
Reference item (dimethoate)	37.4 ± 2.7 *	89.5 ± 7.1	29.6 ± 6.7

*Significantly different compared to control

2. Mortality of adult females:

Kaolin did not cause any deleterious effect on the percentage of mortality 72 hours after the treatments in the laboratory residual contact and the semi-field experiments compared to controls. In contrast, dimethoate killed 100% of insects within the first 24 hours of all the treatments, except in the semi-field experiment, in which almost 100% of *P. concolor* adults were killed 72 hours after the treatment (data summarised in the following table).

Table 10.3.2.2/05-2: Percentages of mortality, attacked hosts and progeny size from exposure to kaolin residue (mean data ± SEM)

Treatment	Exposure *via* residues on glass surfaces			
	% Mortality		Attacked hosts	% Progeny size
Control	0.0 ± 0.0		83.0 ± 6.6	54.8 ± 7.2
Kaolin	0.0 ± 0.0**		97.7 ± 1.3**	65.0 ± 3.9**
Reference item (dimethoate)	100.0 ± 0.0*		-	-
Treatment	Exposure *via* residues on olive tree leaves and parasitisation surface			
	% Attacked hosts		% Progeny size	
	Treated	After treatment	Treated	After treatment
Control	92.1 ± 4.7	94.5 ± 1.3	73.4 ± 3.5	76.0 ± 5.8
Kaolin	89.4 ± 2.2	94.2 ± 2.4	70.8 ± 1.4	71.2 ± 4.5
Treatment	Exposure under semi-field conditions			
	% Mortality		Attacked hosts	% Progeny size
Control	1.1 ± 1.1		92.7 ± 3.3	49.2 ± 4.8
Kaolin	1.1 ± 1.1		94.3 ± 2.7	45.2 ± 4.3
Reference item (dimethoate)	98.9 ± 1.1*		-	-

*Significantly different compared to control
**Data from Bengochea *et al*. (2010). Side effects of kaolin on natural enemies found on olive crops. IOBC/wprs Bull 55:61–67

3. Sublethal effects on beneficial capacity of females

The beneficial capacity of *P. concolor* females during or after the treatments remained unaffected in all experiments. The percentage of attacked hosts was higher than 85% in most of the cases. However, the percentage of progeny size values was more variable. Nevertheless, no significant differences were found in any of the experiments (see tables above). Thus, differences could be related to the specific characteristics and fitness of the females and the medfly larvae used for each experiment. The beneficial capacity of the females treated with dimethoate was not evaluated, because they did not survive long enough to oviposit, with the exception of the experiment in which pupae were treated. The reproductive parameters of females that emerged from dimethoate-treated pupae did not present any significant differences when compared to controls.

4. Sublethal effect due to kaolin oviposition repellency

No significant differences were found when comparing kaolin with controls in the no-choice experiment for the percentage of attacked hosts and for the percentage of progeny size. Slight significant differences were found, however, for the percentage of attacked hosts in the dual choice experiment but there were no differences in the percentage of progeny size. The reduction in the percentage of attacked hosts in the dual choice test using kaolin could be attributed to a behavioural effect, rather than a physiological disturbance. However, kaolin may affect host location strategies and larval habits of parasitoids and hence, affect parasitism rates.

Table 10.3.2.2/05-3: Percentages of attacked hosts and progeny size in the dual choice and the no-choice experiments when *P. concolor* females parasitise through a kaolin-treated surface (mean data ± SEM)

	% Attacked hosts	% Progeny size
No-choice		
Control	74.0 ± 3.2	43.8 ± 1.8
Kaolin	59.1 ± 6.2	48.7 ± 8.0
Dual choice		
Control	81.7 ± 5.7	48.3 ± 4.0
Kaolin	63.8 ± 8.0*	38.0 ± 5.0

*Significantly different compared to control

In the dual choice experiment, it was noted that females parasitised the bottom mesh more frequently than the upper one (daily observations every 15 min were done during parasitisation time). However, these differences could be explained by the position of the treated mesh than by either repellency or kaolin toxicity. Females found it harder to parasitise through the upper surface coated with kaolin, perhaps because they were not able to grip the surface easily, as it seems that *P. concolor* host searching and discrimination ability may be due to the perception of variations in the physiological parameters of the host

III. CONCLUSION

In summary, the results obtained in these experiments provide scientific evidences that kaolin particle films are harmless for the olive fruit fly parasitoid *P. concolor* at a dose rate up to and including 5 kg Surround WP/hL (4.25 g a.s./hL). Hence, kaolin should be considered together with other insecticides for effective resistance management programs, both for integrated pest management systems and organic oliviculture. Yet, special attention should be paid to the potential effects on insect behaviour, especially to sublethal effects of kaolin as oviposition repellent when females are able to choose between a treated and an untreated surface.

Reference:	KCP 10.3.2.2/06, Pease, C.E., López-Olguín, J. F., Pérez-Moreno, I., Marco-Mancebón, V., 2016
Title:	Effects of kaolin on *Lobesia botrana* (Lepidoptera: Tortricdae) and its compatibility with the natural enemy, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae)
Report No.:	Published in: Journal of Economic Entomology, 109(2): 740-745
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

The aim of the study was to evaluate the effect of kaolin on oviposition, egg hatch, and neonate mortality of the parasitoid wasp, *Trichogramma cacoeciae*. The efficacy on the pest *L. botrana* neonate larvae, oviposition, and egg hatch was also evaluated. The effects of kaolin on parasitism and emergence of *T. cacoeciae* from *L. botrana* and *Ephestia kuehniella* eggs were also evaluated. Application was made at a rate of 47.5 g a.s./L of water (50 kg product/ha).

Lobesia botrana egg hatch and oviposition rates were reduced, and neonate larvae mortality was significantly greater in kaolin-treated arenas and when included in synthetic neonate larvae diet. Kaolin had no effect on *T. cacoeciae* parasitism in both hosts. There was only a slight but statistically insignificant effect on *T. cacoeciae* progeny emergence from *L. botrana* eggs and no effect from *E. kuehniella*. The results involving reductions in *L. botrana* oviposition and egg hatch and increase in larval mortality with kaolin suggest this compound may contribute to reduction in pest population densities and can be considered in rational integrated pest management strategies for *L. botrana*.

Based on the laboratory results presented on parasitoid emergence, it appears kaolin can be compatible with the non-target arthropod *T. cacoeciae* in *L. botrana* pest management.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2 (reliable with restrictions), not known to be GLP.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Surround WP
Batch number: Not reported. Product manufactured by Engelhard Corporation, NJ, USA
Content of a.s.: 95% refined kaolin
Appearance: Not reported

2. Vehicle: Water
Reference item: None reported

3. Test organism
Species: *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae)
Trichogramma cacoeciae Marchal (Hymenoptera: Trichogrammatidae)
Age at test initiation: Varied, dependant on bioassay
Source: Laboratory culture. *Lobesia botrana* was originally collected in an organic vineyard in La Rioja, Spain. The *T. cacoeciae* individuals originated from an endemic population collected in La Rioja. The *E. kuehniella* eggs originated in the Polytechnic University of Madrid.

4. Treatment: 47.5 g a.s./L of water (50 kg product/ha)

5. Environmental conditions: During the experiments, not reported.
Colonies reared and maintained in a growth chamber at the constant, standard conditions of 24 ± 1 °C, 60±5% relative humidity, and a photoperiod of 16 hr light:8 hr dark.

B. STUDY DESIGN AND METHODS

1. Test method:
Two types of bioassays were carried out: choice and no choice.

In the choice bioassays, the individual had equal access to both kaolin treatment or control and could choose where to reside, lay its eggs, or, in the case of *T. cacoeciae*, parasitize. In the no-choice bioassays, the individuals were placed in arenas of only one condition (kaolin treatment or control). The parameters measured, such as oviposition, percentage of egg hatch, and neonate larvae mortality, also determined the kind of bioassay. Only behavioral and mechanical effects on oviposition and egg hatch were measured. Parasitism by *T. cacoeciae* in *E. kuehniella* and *L. botrana* eggs and survival of its progeny from each species was evaluated in accordance with each specific bioassay.

2. Application:
A quantity of 5.5 mL of solution was applied using a Potter Tower, which produced a deposit of 0.05±0.005 mL/cm^2 yielding an equivalent field coverage of 50 kg product/ha. All grape clusters used were prepared by immersion in a solution of kaolin or the water carrier for 5 s during continual mixing to avoid settling of the kaolin particles in the treatment solution. The semi-synthetic laboratory diet disks were treated by submersion for 5 s in the solution of kaolin with continual mixing or in the water carrier. All materials used were set to dry before their addition to the bioassay arenas.

1. Effect of kaolin on *L. botrana* (pest) oviposition and egg hatch on synthetic substrate
In all *L. botrana* oviposition assays, after a pre-oviposition period of 24 hours, one female and two males were placed in each replicate. The arenas consisted of upside down plastic cup with hexagonal base inside a Petri dish containing a Petri hydration dish filled with water-soaked cotton.

In the choice bioassay, three alternate sections of the chamber were treated with the kaolin solution. In the no-choice bioassay, the oviposition chambers in the kaolin condition were completely covered with kaolin. The adults were moved to new arenas every 48 hours for three consecutive time periods. The number of eggs laid was recorded after moving the adults to new oviposition chambers. The previous chambers were maintained in the experimental conditions until egg hatch. After the incubation period of 5 days, the number of eggs hatched in each condition was also recorded. Forty-eight replicates were evaluated in the choice bioassays, whereas thirty-one of each, kaolin-treated and control replicates, were evaluated in the no-choice bioassays.

2. Effect of kaolin on *L. botrana* (pest) oviposition and egg hatch on grape
One female and two male moths were used in each replicate. The experimental arenas consisted of one transparent plastic 9 x 21 cm-tall cylindrical box containing respiration holes in the lid, and one hydration container as described above. All plastic surfaces were covered with filter paper to avoid oviposition on the chamber. In all trials, grape clusters (Vitis vinifera var. Tempranillo) of 6 ± 1 g were treated.

In the no-choice bioassays, one kaolin or one control treated grape cluster was placed inside each chamber. In the choice trial, two clusters, kaolin and control, were added to each experimental arena. Oviposition was allowed during 48 hours on each set of grape clusters for three consecutive periods. The number of eggs laid on each grape cluster was recorded after removal of the cluster from the oviposition chamber. The grape clusters were thereafter maintained in standard conditions until egg hatch. The number of eggs hatched in each condition was recorded after their incubation period of 5 days. Twenty-five replicates were evaluated in the choice bioassays, whereas 20 and 13 replicates of control and kaolin treated, respectively, were evaluated in the no-choice bioassays.

3. Effects of kaolin on *L. botrana* (pest) neonate larvae mortality

Five neonate larvae, < 24 h old were placed in the centre of each replicate. The arenas consisted of one 9 x 3 cm-tall cylindrical plastic box containing two layers of filter paper lining the bottom. Semi-synthetic laboratory diet was added. Every container was topped with a lid. All experimental arenas were treated with the kaolin solution or water. The percentage of surviving individuals was recorded 72 hours after the beginning of the trial. Fifteen replicates of each, treatment and control, were evaluated in the statistical analysis

4. Effects of kaolin on the parasitism of *E. kuehniella* (pest) eggs and *T. cacoecias* (parasitoid) progeny emergence

One *T. cacoeciae* female < 48 h old was used in each replicate. The experimental arenas consisted of one 6.5 x 4.5 x 2.5 cm, rectangular, transparent plastic box with ventilation lids covered with filter paper. On the inner side of the lid two 5 mL drops of honey were placed at opposite edges of the ventilation hole to provide nutrients to the female. Groups of 20 sterlised *E. kuehniella* eggs were glued to yellow cards and added. The yellow cards with eggs were treated with kaolin in the Potter tower and set to dry before introduction into the experimental arena.

The choice bioassays used two cards (40 eggs offered), one of each condition, whereas the no-choice experiments contained one card with one of the two conditions, kaolin treated or control. Cards containing the host eggs were replaced every 24 hours over four consecutive periods. These egg groups were isolated at standard conditions after removing from the oviposition chambers until parasitoid emergence. At the end of the 10 days developmental period, parasitism (percentage of eggs parasitized in respect to those offered) and parasitoid emergence (percentage of adults emerged in respect to parasitized eggs) was evaluated and recorded. In the choice bioassays, 27 replications were evaluated. In the no-choice bioassays, 40 replications of each treatment were assessed. Due to the fact that emergence data are implicitly unpaired in these bioassays, parasitized replicates from both the choice and no-choice bioassays were used in combination to analyze the effect of kaolin on parasitoid offspring survival.

5. Effects of kaolin on the parasitism of *L. botrana* (pest) eggs and *T. cacoeciae* (parasitoid) progeny emergence

The number of females used and arena materials were the same as those in the evaluation of parasitism and parasitoid progeny survival from the *E. kuehniella* host, except the eggs of the *L. botrana* host had been previously laid on plastic substrate. Groups of approximately 20 *L. botrana* eggs were used on their respective sections of their oviposition chambers after sterilization. During four consecutive 24 hours periods, the *L. botrana* egg groups were replaced and those of the previous days isolated until parasitoid emergence. In the choice bioassay, two groups of *L. botrana* eggs with their respective treatments were added to the experimental arenas. The percentages of parasitism and parasitoid emergence were recorded after the 10 days parasitoid developmental period. Twenty replications of both conditions in both the choice and no-choice bioassays were carried out.

8. Statistics:

All statistical analyses were performed with Statgraphics. The paired-sample t-test (choice bioassays data) or two-sample t-test (no-choice bioassays data) were used at a significance level of alpha = 0.05. The normality assumption was met by Kolmogorov– Smirnov test applied to residuals, and equality of variances assumptions were met by the Levene test. Abbott's formula for the correction of neonate larvae mortality data was used.

II. RESULTS AND DISCUSSION

1. Effect of kaolin on *L. botrana* (pest) oviposition and egg hatch:

Females laid fewer eggs on all kaolin treated surfaces. Higher egg mortality was also observed in the kaolin treatment in all bioassays

Table 10.3.2.2/06-1: Mean number of *L. botrana* eggs laid and mean percentage egg hatch with or without kaolin

Choice (mean number)		% difference from control	No choice (mean number)		% difference from control
Control	Kaolin		Control	Kaolin	
Synthetic substrate					
16.5	13.1	11.6%	29.4	14.9	49.4%
Grape					
12.5	1.1	83.6%	9.7	0.6	93.8%
Synthetic substrate (% egg hatch)		**% difference from control**	**Grape (% egg hatch)**		**% difference from control**
Control	**Kaolin**		**Control**	**Kaolin**	
78.0%	56.3%	21.7%	87.2%	40.4%	37.1%

2. Effects of kaolin on *L. botrana* (pest) neonate larvae survival

The average percentage of neonate larvae mortality in the kaolin treatment was 78.7%, compared with 37.1% in the control. There was a significant difference between neonate larvae mortality rates with a corrected mortality percentage in the kaolin group of 66.1%.

3. Effects of kaolin on *T. cacoeciae* parasitism of *E. kuehniella* (pest) and *L. botrana* (pest) and parasitoid offspring emergence

No significant difference between treatments was found when the parasitism and emergence data sets were evaluated. This held true for both the choice and no-choice bioassays involving the two hosts, *E. kuehniella* and *L. botrana*.

Table 10.3.2.2/06-2: Mean percentage of eggs parasitized by *T. cacoeciae* and mean percentage emergence of adult parasitoids from kaolin treated *L. botrana* eggs in choice and no-choice assays

Parasitism choice		Parasitism no-choice		Parasitoid emergence	
Control	Kaolin	Control	Kaolin	Control	Kaolin
16.1%	14.5%	33.3%	34.4%	55.6%	47.2%

III. CONCLUSION

In the laboratory trials, exposure to 50 kg Surround WP/ha (equivalent to 47.5 kg kaolin/ha) had a number of effects on the pest *L. botrana* such as a reduction in oviposition, lower neonate emergence, and higher than normal neonate mortality. Kaolin had no significant effects on the non-target *T. cacoeciae*, one of the parasitoids most widely used in inundative biological control strategies for the management of Lepidopteran pest species. The authors concluded that with its effects on the pest *L. botrana*, its lack of detrimental impacts detected on the non-target parasitoid *T. cacoeciae* in laboratory bioassays, kaolin could lead to another rational, sustainable, and nontoxic management option to be included in integrated management of *L. botrana*.

CP 10.3.2.3 Semi-field studies with non-target arthropods

Not relevant as field studies have been conducted.

CP 10.3.2.4 Field studies with non-target arthropods

Studies submitted and evaluated for the first inclusion on Annex I:

Reference:	KCP 10.3.2.4/01 Lepine, J. 2004 (previously evaluated in DAR B9, III.A 10.5.2/01)
Title:	Evaluate the efficacy of Surround against *Cacopsylla pyri*, applied just after the end of the winter period
Report No.:	Report number FENG045059
Guideline(s):	CEB Method no.77
Deviation(s):	Not relevant
GEP:	Yes

Executive Summary

Four single un-replicated large plots (420 m^2) of pears were treated as follows:

- single spray of SURROUND® WP CROP PROTECTANT at 50 kg/ha
- SURROUND® WP CROP PROTECTANT at 50 kg/ha followed by 3 sprays at 25 kg/ha
- single spray of Decis at 17.5 kg a.s./ha
- untreated as the control.

Pear *Cacopsylla* eggs, nymphs & adults (a pest of fruit trees) were assessed on six occasions after treatment and beneficial arthropods were assessed on four occasions. SURROUND® WP CROP PROTECTANT applied at 50 kg/ha and then 25 kg/ha gave excellent control of first generation *Cacopsylla*. Single application of SURROUND® WP CROP PROTECTANT at 50 kg/ha gave moderate *Cacopsylla* control similar to the reference product Decis. Beneficial arthropods (sampling up to 1 to 2 months post application) were mainly parasitic Hymenoptera and Mirid bugs. Numbers were generally low but no major differences were observed between the treatments.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Surround® WP Crop Protectant
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: 95% kaolin
   - Stability of test component: Stable

2. Vehicle and/or positive control: Water – spray volume 500 L/ha

3. Test animals:

Different life stages of naturally occurring insect predators and parasites: Ladybirds (coccinelids), hemipterous bugs and hymenopterous parasites.

4. Testing Facility: Solevi, 26400 Crest, France

5. Trial Location: 37130 Lignières de Touraine, France

B. STUDY DESIGN AND METHODS

Experimental design: single un-replicated large (420m^2) plots

Spray equipment: Pneumatic Stihl mistblower

Spray volume: 500 L/ha

Treatments:
1) Untreated
2) Surround® WP @ 50 kg/ha (1 spray)
3) Surround® WP @ 50 kg/ha (1 spray) + 25 kg/ha (3 sprays)
4) Decis Protech 15EW @ 1.167L/ha (1 spray) 17.5 g ai/ha

Applications:
T1- 13 Feb' 2004: Treatments 2, 3 & 4
T2- 24 Feb' 2004: Treatment 3
T3- 16 March 2004: Treatment 3
T4- 30 March 2004: Treatment 3

Arthropod sampling: *Cacopsylla* adults were sampled by beating 30 branches per plot
Cacopsylla eggs, nymphs and beneficials were sampled by visual observations on 4 x 10 shoots per treatment
Sampling was carried out on the following dates:
- *Cacopsylla* – 05/04, 14/04, 27/04, 04/05, 18/05, 25/05
- Beneficials – 27/04, 04/05, 18/05, 25/05

Statistics: Data were transformed to log (x+1) analysed by ANOVA and if significant difference a Dunnets tests was used to compare treatments and control. Treatment means with different letters denote significant difference at $P \leq 0.05$.

I. RESULTS AND DISCUSSIONS

Surround® applied at 50 kg/ha followed by 25 kg/ha provided excellent control of the first generation of pear *Cacopsylla*. The single application of Surround® at 50 kg/ha was similar to the reference product Decis but significantly inferior. No control of the second generation was achieved by any of the treatment regimes.

Table 10.3.2.4/01-1: Numbers of *Cacopsylla pyri* pests in Surround® and reference plots

Treatments ↓	Sampling Dates								
	05/04		14/04	27/04	04/05	18/05	25/05		
	Numbers								
	eggs	nymphs	nymphs	adults	adults	adults	eggs	nymphs	adults
Untreated	43	85	69	1	29	23	488	310	18
Surround® 50	0	24	19	6	19	19	1052	240	37
Surround® 50+25	0	0	0	1	6	36	1341	136	62
Decis 17.5	0	36	15	13	15	29	571	283	33

The most important non-target arthrpods were parasitic Hymenoptera and Mirid heteropteran bugs (*Philophorus* & *Deraeocoris*). Other predators that were present only infrequently included, pirate bugs (*Anthocoris*), earwigs (Dermaptera), ladybirds (*Propylea, Stethorus*) and damsel bugs (*Nabis*).

Overall numbers of beneficial arthropods were rather low in all treatments. However, there were no treatment related effects noted in the numbers of the main two groups, Hymenoptera and Mirid bugs, during the trial.

Table 10.3.2.4/01-2: Beneficial arthropods in Surround® and reference plots

Treatments↓	Sampling Dates							
	27/04		04/05		18/05		25/05	
	Numbers: 4 x 10 shoots							
	Hym	Mir	Hym	Mir	Hym	Mir	Hym	Mir
Untreated	1	0	1	0	3	3	5	0
Surround® 50	3	0	1	1	0	10	4	0
Surround® 50+25	0	0	2	0	1	7	1	6
Decis 17.5	3	2	1	0	0	4	1	1

Hym = parasitic Hymenoptera, Mir = Mirid bugs (eg *Philophorus, Deraeocoris*)

III. CONCLUSIONS

Surround® applied at 50 kg/ha and then 25 kg/ha gave excellent control of first generation *Cacopsylla* pests. Single application of Surround® at 50kg/ha gave moderate *Cacopsylla* control similar to the reference product Decis. Beneficial arthropods were mainly parasitic Hymenoptera and Mirid bugs. Numbers were generally low but no major differences were observed between the treatments.

Reference:	KCP 10.3.2.4/02 Fraser, H. 2002a (previously evaluated in DAR B9, III.A 10.5.2/02)
Title:	Evaluation of a season long insect pest control programme with Surround WP in an Ontario apple orchard
Report No.:	Report number 2002-1
Guideline(s):	None
Deviation(s):	Not relevant
GLP:	No, but study scientifically valid

Executive Summary

SURROUND® WP CROP PROTECTANT was applied 11 times at 56 kg/ha to large replicated plots of apples at 7-14 day intervals from April to August 2002. Pest control in the SURROUND® WP CROP PROTECTANT treated plots was superior to the reference treatment that was based on an organic transition programme. Arthropod predators were assessed by beating 25 tree branches per plot in both late July and late August. Overall, the numbers of predators, (e.g. Syrphids, Chrysoperlids, *Orius*) found were generally similar for both treatments.

Season long SURROUND® WP CROP PROTECTANT applications appeared to have a minimal impact on insect predators like ladybirds, lacewings and *Orius*. On the basis of this test, SURROUND® WP CROP PROTECTANT is not harmful to insect predators in the field, even when sampled immediately following 11 applications at 56 kg/ha with 7-14 day intervals.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Surround® WP Crop Protectant
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: Not specified
   - Stability of test component: Stable

2. Test animals:
   - Species: Hoverflies (Syrphids)

	Lacewings (Chrysopids) Pirate bugs (*Orius*) Lady bird beetles (Coccinellids)
Age:	Not applicable
Source:	Naturally present on test site.
Trial Location:	Leamington, Ontario, Canada

3. Testing Facility: Ontario Ministry of Agriculture & Food, Vineland, ON, Canada

B. STUDY DESIGN AND METHODS

Surround® WP Crop Protectant was applied to 3 plots of apples of about 0.8 ha each using a commercial air blast sprayer delivering 56 kg/ha product in 935 L/ha. A total of 11 applications were applied at 7 to 14 day intervals between late April 2002 to end August. A grower standard of 3 x 0.8 ha plots was used as a reference. These plots were treated with mainly products suited for organic apple production. Pests present included plum curculio (*Conotrachelus nenuphar*), spring feeding lepidoptera complex and internal feeding Lepidoptera complex (e.g *Cydia pomonell, Grapholita molesta*). Pest damage was assessed by external and internal examination of the apples and leaves. Insect predators were collected by beating 25 branches in each plot. Both phytophagous (*Panonychus ulmi*) and predatory (e.g. *Amblyseius*) mites were sampled by examining 50 leaves per plot.

Statistics:
Kruskal Wallis ANOVA

II. RESULTS AND DISCUSSIONS

Surround® gave significantly better control of the main pests, plum curculio and internal Lepidoptera. Spider mites were more numerous in the Surround® treated plots but still were well below the treatment threshold. There were extremely few predatory mites in either treatment. In general, the numbers of predators were relatively low in both treatment regimes and consisted mainly of ladybirds (coccinellids) hover flies (syrphids), lacewings (chrysoperlids) and pirate bugs (*Orius*). Overall, the numbers of insect predators in the Surround® WP treated plots were very similar to the grower standard.

Table 10.3.2.4/02-1: Arthropods predators from beating 25 branches

Treatment	Date	Spider mite destroyers	Lacewings	Ladybirds	*Orius*	Mullein bugs	Other preds
Surround®	26/7/02	10	0	7	0	2	3
Grower	26/7/02	12	0	1	2	1	3
Surround®	23/8/02	5	35	40	4	3	0
Grower	23/8/02	14	9	9	6	1	3

III. CONCLUSIONS

Season long Surround® WP Crop Protectant applications appeared to have a minimal impact on insect predators like ladybirds, lacewings and *Orius*. On the basis of this test, Surround® WP Crop Protectant is not harmful to insect predators in the field, even when sampled immediately following 11 applications at 56 kg/ha with 7-14 day intervals.

Reference:	KCP 10.3.2.4/03 Fraser, H. 2002b (previously evaluated in DAR B9, III.A 10.5.2/03)
Title:	Evaluation of a season long insect pest control programme with Surround WP in an Ontario apple orchard
Report No.:	Report number 2002-2
Guideline(s):	None
Deviation(s):	Not relevant
GLP:	No, but study scientifically valid

Executive Summary

SURROUND® WP CROP PROTECTANT was applied 8 times at 56 kg/ha to large replicated plots of apples at 7-14 day intervals from end May to end August 2002. Pest control in the SURROUND® WP CROP PROTECTANT treated plots was superior to the reference treatment that was based on a growers IPM programme. Arthropod predators were assessed by beating 25 tree branches per plot in both late July and late August. Overall, the numbers of predators, (e.g. *Campylomma*, coccinellids, chrysoperlids) found in both treatments were generally similar, except for predatory mites, which appeared to be lower in the SURROUND® WP CROP PROTECTANT treated plots.

Season long SURROUND® WP CROP PROTECTANT applications appeared to have a minimal impact on insect predators like lacewings, ladybirds and Mullein bugs. Numbers of predatory mites were generally low but there were fewer in the SURROUND® WP CROP PROTECTANT treated plots. On the basis of this trial, SURROUND® WP CROP PROTECTANT is not harmful to insect predators in the field but has an apparent effect on predatory mites when applied 8 times at 56 kg/ha at 7-14 day intervals.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Surround® WP Crop Protectant
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: Not specified
   - Stability of test component: Stable

2. Test animals:
   - Species: Ladybirds (LBB – coccinellids); Lacewings (chrysoperlids); Mullein bugs (*Campylomma*); Predatory mites (*Amblyseius*)
   - Age: Not applicable
   - Source: Naturally present on test site.
   - Trial Location: Simcoe, Ontario, Canada

3. Testing Facility: Ontario Ministry of Agriculture & Food, Vineland, ON, Canada

B. STUDY DESIGN AND METHODS

Surround® WP Crop Protectant was applied to 3 plots of apples of about 0.8 ha each using a commercial air blast sprayer delivering 56 kg/ha product in 935 L/ha. A total of 8 applications were applied at 7 to 14 day intervals between late May 2002 to end August. A grower standard of 3 x 0.8ha plots was used as a reference. These plots were treated following an IPM programme. Major pests present included plum curculio (*Conotrachelus nenuphar*) and an internal feeding Lepidoptera complex (e.g *Cydia pomonella, Grapholita molesta*). Pest damage was assessed by external and internal examination of the apples and leaves. Insect predators were collected by beating 25 branches in each plot. Both phytophagous (*Panonychus ulmi*) and predatory (e.g. *Amblyseius*) mites were sampled by examining 50 leaves per plot.

Statistics:
Kruskal Wallis ANOVA

II. RESULTS AND DISCUSSIONS

Surround® gave significantly better control of the main pests, plum curculio and internal Lepidoptera. Spider mites were more numerous in the Surround® treated plots but still were well below the treatment threshold. There were few predatory mites (*Amblyseius*) in either treatment, although the numbers were generally lower in the Surround® treated plots. Predators consisted mainly of ladybirds (coccinellids), lacewings (chrysoperlids) and Mullein bugs (*Campylomma*). Overall, the numbers of insect predators in the Surround® WP treated plots were very similar to the grower IPM standard.

Table 10.3.2.4/03-1: Arthropods predators from beating 25 branches

Treatment	Date	Lacewings	Ladybirds	Mullein bugs	Other preds
Surround®	18/7/02	7	3	7	0
Grower	18/7/02	0	2	5	4
Surround®	15/8/02	6	4	0	1
Grower	15/8/02	0	1	0	4

III. CONCLUSIONS

Season long Surround® WP Crop Protectant applications appeared to have a minimal impact on insect predators like lacewings, ladybirds and Mullein bugs. Numbers of predatory mites were generally low but there were fewer in the Surround® treated plots. On the basis of this trial, Surround® WP is not harmful to insect predators in the field but has an apparent effect on predatory mites when applied 8 times at 56 kg/ha at 7-14 day intervals.

Reference:	KCP 10.3.2.4/04 Fraser, H. 2002c (previously evaluated in DAR B9, III.A 10.5.2/04)
Title:	Evaluation of a season long insect pest control programme with Surround WP in an Ontario apple orchard
Report No.:	Report number 2002-5
Guideline(s):	None
Deviation(s):	Not relevant
GLP:	No, but study scientifically valid

Executive Summary

SURROUND® WP CROP PROTECTANT was applied 8 times at 56 kg/ha to large replicated plots of apples at 7-14 day intervals from end May to mid-August 2002. Pest control in the SURROUND® WP CROP PROTECTANT treated plots was superior to the reference treatment that was based on a growers

IPM programme. Arthropod predators were assessed by beating 25 tree branches per plot in both late July and late August (i.e. within the application season). Overall, the numbers of predators, (e.g. *Campylomma, Coccinellids, Chrysoperlids* & *Orius*) found in both treatments were generally similar, except for predatory mites, which appeared to be very slightly lower in the SURROUND® WP CROP PROTECTANT treated plots.

Season long SURROUND® WP CROP PROTECTANT applications appeared to have a minimal impact on insect predators like Coccinellids, Chrysoperlids, Pirate and Mullein bugs. Numbers of predatory mites were generally low but there were fewer in the Surround® treated plots. On the basis of this trial, SURROUND® WP CROP PROTECTANT is not harmful to insect predators in the field but had an apparent slight effect on predatory mites when applied 8 times at 56 kg/ha.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Surround® WP Crop Protectant
Description:	White powder
Lot/Batch #:	Not specified
Purity:	Not specified
Stability of test component:	Stable
2. Test animals:	
Species:	Ladybirds (Coccinellids Lacewings (Chrysopids) Mullein bugs (*Campylomma*) Predatory mites (*Amblyseius*)
Age:	Not applicable
Source:	Naturally present on test site.
Trial Location:	Simcoe, Ontario, Canada
3. Testing Facility:	Ontario Ministry of Agriculture & Food, Vineland, ON, Canada

B. STUDY DESIGN AND METHODS

Surround® WP Crop Protectant was applied to 3 plots of apples of about 0.8 ha each using a commercial air blast sprayer delivering 56 kg/ha product in 935 L/ha. A total of 8 applications were applied at 7 to 14 day intervals between May 2002 to end August. A grower standard of 3 x 0.8 ha plots was used as a reference. These plots were treated following an IPM programme. Major pests present included an internal feeding Lepidoptera complex (e.g *Cydia pomonella, Grapholita molesta*), spring feeding Lepidoptera complex and various biting insects. Pest damage was assessed by external and internal examination of the apples and leaves. Insect predators were collected by beating 25 branches in each plot. Both phytophagous (*Panonychus ulmi*) and predatory (e.g. *Amblyseius, Zetzellia*) mites were sampled by examining 50 leaves per plot.

Statistics:
Kruskal Wallis ANOVA

II. RESULTS AND DISCUSSIONS

Surround® gave significantly better control of the main pests, especially internal Lepidoptera. Spider mite numbers were low and appeared similar in both treatment regimes. There were very few predatory mites (*Amblyseius*) in either treatment, although the numbers were generally lower in the Surround® treated plots. Predators consisted mainly of ladybirds (Coccinellids), lacewings (Chrysoperlids), Pirate (*Orius)* and

Mullein bugs (*Campylomma*). Overall, the numbers of insect predators in the Surround® WP treated plots were very similar to the grower IPM standard.

Table 10.3.2.4/04-1: Arthropods predators from beating 25 branches

Treatment	Date	Lacewings	Ladybirds	Mullein bug	Other preds
Surround®	18/7/02	7	2	4	1
Grower	18/7/02	0	2	2	0
Surround®	15/8/02	11	10	0	1
Grower	15/8/02	6	8	0	3

(statistical significance not reported)

III. CONCLUSIONS

Season long Surround® WP Crop Protectant applications appeared to have a minimal impact on insect predators like Coccinellids, Chrysoperlids, Pirate and Mullein bugs. Numbers of predatory mites were generally low but there were fewer in the Surround® treated plots. On the basis of this trial, Surround® WP is not harmful to insect predators in the field but had an apparent slight effect on predatory mites when applied 8 times at 56kg/ha.

Reference:	KCP 10.3.2.4/05 Fraser, H. 2002d (previously evaluated in DAR B9, IIIA.A 10.5.2/05)
Title:	Evaluation of a season long insect pest control programme with Surround WP in an Ontario apple orchard
Report No.:	Report number 2002-6
Guideline(s):	None
Deviation(s):	Not relevant
GLP:	No, but study scientifically valid

Executive Summary

SURROUND® WP CROP PROTECTANT was applied 6 times at 56 kg/ha to large replicated plots of apples at 7-14 day intervals from end May to end July 2002. Pest control in the SURROUND® WP CROP PROTECTANT treated plots was similar or superior to the reference treatment that was based on a growers IPM programme. Arthropod predators were assessed by beating 25 tree branches per plot. Overall, the numbers of predators (e.g. spiders, coccinellids, chrysoperlids & syrphids) were low but nevertheless were generally similar.

Season long SURROUND® WP CROP PROTECTANT applications appeared to have a minimal impact on insect predators like coccinellids, chrysoperlids, spiders and syrphids. Numbers of predatory mites were generally very low but there were fewer in the SURROUND® WP CROP PROTECTANT treated plots. On the basis of this trial, SURROUND® WP CROP PROTECTANT is not harmful to insect predators in the field but had an apparent slight effect on very low numbers of predatory mites when applied 6 times at 56 kg/ha.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Surround® WP Crop Protectant
   - Description: White powder
   - Lot/Batch #: Not specified

Purity: Not specified
Stability of test component: Stable

2. Test animals:
Species: Ladybirds (Coccinellids
Lacewings (Chrysopids)
Spiders (Araneae)
Predatory mites (*Amblyseius*)
Age: Not applicable
Source: Naturally present on test site.
Trial Location: Brantford, Ontario, Canada

3. Testing Facility: Ontario Ministry of Agriculture & Food, Vineland, ON, Canada

B. STUDY DESIGN AND METHODS

Surround® WP Crop Protectant was applied to 2 plots of apples of about 0.8 ha each using a commercial air blast sprayer delivering 56kg/ha product in 935 L/ha. A total of 6 applications were applied at 7 to 14 day intervals between May 2002 to end July. A grower standard of 3 x 0.8 ha plots was used as a reference. These plots were treated following an IPM programme. Major pests present included an internal feeding Lepidoptera complex (e.g *Cydia pomonella, Grapholita molesta*), spring feeding Lepidoptera complex, apple maggot (*Rhagoletis*) and leafrollers (*Choristoneura*). Pest damage was assessed by external and internal examination of the apples and leaves. Insect predators were collected by beating 25 branches in each plot. Both phytophagous (*Panonychus ulmi*) and predatory (e.g. *Amblyseius, Zetzellia*) mites were sampled by examining 50 leaves per plot.

Statistics:
Kruskal Wallis ANOVA

II. RESULTS AND DISCUSSIONS

Surround® gave significantly better control of apple maggots and leafrollers. Spider mite numbers were low but appeared slightly higher in the Surround® plots. There were very few predatory mites (*Amblyseius*) in either treatment, although the numbers were generally lower in the Surround® treated plots. Predators consisted mainly of ladybirds (coccinellids), lacewings (chrysoperlids), hover flies (syrphids) and spiders. Overall, the numbers of insect predators in the Surround® WP treated plots were very similar to the grower IPM standard.

Table 10.3.2.4/05-1: Arthropods predators from beating 25 branches

Treatment	Date	Ladybirds	Spiders	Other preds*
Surround®	18/7/02	2	3	1
Grower	18/7/02	2	6	2
Surround®	15/8/02	1	1	1
Grower	15/8/02	5	0	2

III. CONCLUSIONS

Season long Surround® WP Crop Protectant applications appeared to have a minimal impact on insect predators like coccinellids, chrysoperlids, spiders and syrphids. Numbers of predatory mites were generally very low but there were fewer in the Surround® treated plots. On the basis of this trial, Surround® WP is not harmful to insect predators in the field but had an apparent slight effect on very low numbers of predatory mites when applied 6 times at 56 kg/ha.

Reference:	KCP 10.3.2.4/06 Fraser, H. 2002e (previously evaluated in DAR, B9 III.A 10.5.2/06)
Title:	Evaluation of a season long insect pest control programme with Surround WP in an Ontario apple orchard
Report No.:	Report number 2002-7
Guideline(s):	None
Deviation(s):	Not relevant
GLP:	No, but study scientifically valid

Executive Summary

SURROUND® WP CROP PROTECTANT was applied 15 times at 56 kg/ha to large replicated plots of apples at 7-14 day intervals from end April to mid-August 2002. Pest control in the SURROUND® WP CROP PROTECTANT treated plots was similar or superior to the reference treatment that was based on a transitional organic programme. Arthropod predators were assessed by beating 25 tree branches per plot. Overall, the numbers of predators (e.g. spiders, *Orius*, chrysoperlids & general spider mite predators) were generally similar in the 2 treatment regimes.

Season long SURROUND® WP CROP PROTECTANT applications appeared to have a minimal impact on insect predators like ladybirds, lacewings, spiders and general spider mite predators. On the basis of this trial, SURROUND® WP CROP PROTECTANT is not harmful to insect predators in the field even when applied 15 times at 56 kg/ha.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Surround® WP Crop Protectant
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: Not specified
   - Stability of test component: Stable

2. Test animals:
   - Species: Pirate bugs (*Orius*)
Lacewings (Chrysopids)
Spiders (*Araneae*)
General spider mite predators
   - Age: Not applicable
   - Source: Naturally present on test site.
   - Trial Location: Wheatley, Ontario, Canada

3. Testing Facility: Ontario Ministry of Agriculture & Food, Vineland, ON, Canada

B. STUDY DESIGN AND METHODS

Surround® WP Crop Protectant was applied to 2 plots of apples of about 0.4 ha each using a commercial air blast sprayer delivering 56 kg/ha product in 935 L/ha. A total of 6 applications were applied at 7 to 14 day intervals between end April 2002 and mid-August. A grower standard of 2 x 0.4 ha plots was used as a reference. These plots were treated following a transitionary organic programme. Major pests present included plum curculio (*Conotrachelus nenuphar*) an internal feeding Lepidoptera complex (e.g *Cydia pomonella, Grapholita molesta*) and a general sucking pest complex. Pest damage was assessed by external and internal examination of the apples and leaves. Insect predators were collected by beating 25 branches in each plot.

Statistics:
Kruskal Wallis ANOVA

II. RESULTS AND DISCUSSIONS

Surround® gave significantly better control of plum curculio, internal Lepidoptera complex and general sucking pests. Predators consisted mainly of lacewings (chrysoperlids), *Orius*, general spider mite predators and spiders. Overall, the numbers of insect predators in the Surround® WP Crop Protectant treated plots were very similar to the grower IPM standard.

Table 10.3.2.4/06-1: Arthropod predators from beating 25 branches

Treatment	Date	Spider mite destroyer	Lacewings	Orius	Spiders
Surround®	26/7/02	1	0	0	4
Grower	26/7/02	8	1	0	0
Surround®	23/8/02	13	12	4	1
Grower	23/8/02	9	15	12	1

III. CONCLUSIONS

Season long Surround® WP Crop Protectant applications appeared to have a minimal impact on insect predators like ladybirds, lacewings, spiders and general spider mite predators. On the basis of this trial, Surround® WP Crop Protectant is not harmful to insect predators in the field even when applied 15 times at 56kg/ha.

Reference:	KCP 10.3.2.4/07 Peusens, G., and Creemers, P. 2004a (previously evaluated in DAR B9, III.A 10.5.2/07)
Title:	Biological efficacy evaluation of Surround WP against the pear sucker, *Cacopsylla pyri* L., on pear
Report No.:	20040617 412 BE 388 GEP
Guideline(s):	EPPO guideline "*Cacopsylla*". EPPO Standards (1997)
Deviation(s):	Not relevant
GEP:	Yes

Executive Summary

Observations were carried out on the predatory bug, *Anthocoris nemoralis*, in 3 out of 5 trials carried out with SURROUND® WP CROP PROTECTANT against the pest pear sucker *Cacopsylla* in Belgium. SURROUND® WP CROP PROTECTANT was applied at 10 to 30 kg/ha to large blocks of pears. Four applications were made from early February to late March at crop stages BBCH 52-56. Assessments were made on pest *Cacopsylla* adults, eggs and nymphs. The non-target predatory bug *Anthocoris* was assessed using a branch beating technique in mid-April and/or June (i.e 1-3 months after application).

SURROUND® WP CROP PROTECTANT treatments generally gave excellent control of *Cacopsylla* adults and nymphs, particularly at the 20 and 30 kg/ha dose rates. Numbers of *Anthocoris* were generally low even in the untreated controls. Nevertheless, they were somewhat reduced in the SURROUND® WP CROP PROTECTANT treated plots. This reduction was usually between 25-80% and therefore SURROUND® WP CROP PROTECTANT could be considered slightly to moderately harmful to *Anthocoris* predators. It is proposed that the effects on *Anthocoris* are probably due to removal of prey (*Cacopsylla* nymphs) and the general repellent effect of the Surround® particle film on the mobile adults present at the time of the SURROUND® WP CROP PROTECTANT applications.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: M99099
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: Not specified
   - Stability of test component: Stable
   - Reference substance: Deltamethrin (Decis 25EC)

2. Beneficial test animals:
   - Species: *Anthocoris nemoralis*
   - Age: Adults
   - Source: Naturally present on test site.

3, Testing facility: Royal Station for Fruit Growing, Gorsem, Belgium

B. STUDY DESIGN AND METHODS

Sprayers: 1) Stihl knapsack mistblower, delivering 1,000 L/ha
2) Munckhof axial fan sprayer, delivering 375 – 500 L/ha

Sampling: *Cacopsylla* adults - beating 3 branches on each of 10 trees per plot
Cacopsylla eggs - visual count of numbers on 10 branches (20cm) per plot
Cacopsylla nymphs - visual count of numbers on 20 clusters per plot
Anthocoris adults & nymphs - beating 3 branches on 10 trees per plot

Statistics:
Percent control was calculated using the Abbott formula.

Homogeneity of variance was assessed using Bartletts chi-square tests and raw numbers were transformed to their Log+1 values as appropriate. Significant differences between treatments were tested using ANOVA followed by with Duncan's multiple comparison test. Treatment means with different letter subscripts indicate significant differences at P≤ 0.05%.

Five separate field trials were conducted but assessments on *Anthocoris* were made in only 3.

II. RESULTS

1) 02/PSYLPY.05 (location: Velm)

Three single large (200 trees) un-replicated blocks of pear trees were used for the trial.
Treatments and applications dates were as follows:

- untreated water control
- Surround® WP, 30 kg/ha applied 4 times:
  i. 8 February, BBCH 52
  ii. 28 February, BBCH 53
  iii. 8 March, BBCH 54
  iv. 21 March, BBCH 56
- Decis EC, 0.6 L/h (15 g ai/ha) applied once.
  i. 8 February, BBCH 52

Biological assessments were carried out on the following dates:

- *Cacopsylla* adults – 11 March, 13 May, 21 May, 28 May
- *Cacopsylla* eggs – 25 March
- *Cacopsylla* nymphs – 3 May
- *Anthocoris* adults – 14 April, 28 May, 24 June
- *Anthocoris* nymphs – 28 May, 24 June

The results obtained for pear *Cacopsylla* and *Anthocoris* are summarised below.

Table 10.3.2.4/07-1: Summary of *Cacopsylla* (pest) results in trial 02/PSYLPY.05

	11/03	25/03	03/05	13/05	21/05	28/05
	adults	eggs	nymphs	adults	adults	adults
	Number					
untreated	0.8a	11.8a	32a	47.5a	36.8a	62a
	Mortality Abbott %					
Decis	66.7a	-10.6a	93.8b	42.6b	0a	0a
Surround®	100a	100b	98.4b	64.2c	25.2b	0a

A different letter indicates a statistical significance difference in the column

Surround® WP Crop Protectant gave excellent control of pear *Cacopsylla* eggs, nymphs and moderate control of second generation adults. In general, it was superior to the standard Decis 25EC which had a more limited impact on over-wintering adults, eggs and F2 adults.

Populations of the non-target predatory bug *Anthocoris* in the early spring period when Surround® was applied were low and it is difficult to see if there were any real effects of either treatment. However, in June (3 months after treatment), numbers (particularly nymphs) had increased significantly in the untreated control. At this time, there were fewer in both the reference and Surround® treated plots, although the differences were not statistically significant.

Table 10.3.2.4/07-2: Summary of non-target predatory bugs *Anthocoris* results in trial 02/PSYLPY

	14/04	28/05		24/06	
	adults	nymphs	adults	nymphs	adults
	Number				
untreated	2a	0.8a	1a	15	3
	Mortality Abbott %				
Decis	62.5a	66.7a	50a	73.3	33.3
Surround®	87.5a	33.3a	50a	26.7	66.7

A different letter indicates a statistical significance difference in the column

2) 02/PSYLPY.06 (location: Brustem)

Four single large (497 trees) un-replicated blocks of pear trees were used for the trial.
Treatments were as follows:

- untreated control
- Surround® WP, 10 kg/ha applied 4 times
- Surround® WP, 20 kg/ha applied 4 times
- Surround® WP, 30 kg/ha applied 4 times:

Application dates were as follows:

- 8 February
- 28 February
- 8 March
- 21 March

Biological assessments were carried out on the following dates:

- *Cacopsylla* eggs – 25 March
- *Cacopsylla* nymphs – 10 April
- *Anthocoris* adults – 25 June
- *Anthocoris* nymphs – 25 June

Cacopsylla was assessed on four plots in each block whereas *Anthocoris* was assessed on only 2 plots per treatment block. No statistical analysis was therefore carried out on the *Anthocoris* counts. The results obtained for pear *Cacopsylla* and *Anthocoris* are summarised below.

Surround® appeared to have a moderate but not significant effect on *Cacopsylla* eggs. However, all 3 dose rates of Surround® gave good control of *Cacopsylla* nymphs. Numbers of *Anthocoris* nymphs and adults were lower in Surround® treated plots, particularly the 20 and 30kg/ha dose rates. Nevertheless, *Anthocoris* was still present in reasonable numbers. Indeed, when compared with the numbers of *Cacopsylla* nymphs, the predator: prey ratio was considerably higher in the Surround® treated blocks than in the untreated.

Table 10.3.2.4/07-3: Summary of *Cacopsylla pyri* and *Anthocoris nemoralis* in trial 02/PSYLPY.06

	***Cacopsylla pyri* (pest)**		***Anthocoris nemoralis* (non-target predatory bug)**	
	25/03	10/04	25/06	
	Number			
	eggs	nymphs	nymphs	adults
Untreated	89a	16.3a	16	8
	Mortality (% Abbott)			
Surround® 10 kg	61.2a	96.9b	6.3	25
Surround® 20 kg	45.5a	93.8b	87.5	87.5
Surround® 30 kg	64.6a	98.5b	50*	62.5

**Anthocoris* counts of nymphs were made only on 2 replicate plots in each block and therefore no statistical analysis of this data was carried out.

3) 02/PSYLPY.08 (location: Sint Truiden)

Eight large (565 - 630 trees) blocks of pear trees were used for the trial.
There were 4 treatments, each replicated twice:

- untreated control
- Surround® WP, 10 kg/ha applied 4 times
- Surround® WP, 20 kg/ha applied 4 times
- Surround® WP, 30 kg/ha applied 4 times:

Application dates were as follows:

- 8 February, BBCH 52
- 28 February, BBCH 53
- 8 March, BBCH 54
- 21 March, BBCH 56

Biological assessments were carried out on the following dates:

- *Cacopsylla* eggs – 25 March
- *Cacopsylla* nymphs – 10 April
- *Anthocoris* adults – 18 April, 25 June
- *Anthocoris* nymphs – 25 June

All *Cacopsylla* counts and *Anthocoris* on 10 April were made on four plots in each replicated block. *Anthocoris* counts of nymphs on 24 June were made only on 2 replicate plots in each block and therefore no statistical analysis of this data was carried out.

The results obtained for pear *Cacopsylla* and *Anthocoris* are summarised below.

Surround® appeared to have no effect on *Cacopsylla* eggs, however, the 2 higher dose rates of Surround® gave good control of *Cacopsylla* nymphs. Numbers of non-target predatory bugs *Anthocoris* nymphs and adults were generally low even in the untreated control blocks. There were reduced numbers in Surround® treated plots, although generally only 25-50% lower than the untreated and statistical analysis was not performed. This suggests that Surround® was slightly harmful to *Anthocoris* bugs. Indeed, when compared with the numbers of *Cacopsylla* nymphs, the predator: prey ratio was again considerably higher in the Surround® treated blocks than in the untreated control.

Table 10.3.2.4/07-4: Summary of *Cacopsylla pyri* and *Anthocoris nemoralis* in trial 02/PSYLPY.08

	Cacopsylla pyri		***Anthocoris nemoralis***		
	25/03	10/04	10/04	25/06	
	Number				
	eggs	nymphs	adults	nymphs	adults
Untreated	115.5a	17.5a	1.8a	6	2
	Mortality (% Abbott)				
Surround® 10kg	0a	21.4a	0a	25	100
Surround® 20kg	0a	92.9c	100a	25	25
Surround® 30kg	0a	71.4b	100a	33.3	50

A different letter indicates a statistical significance difference in the column

4) 02/PSYLPY.17 (location: Velm)

No observations were carried out on *Anthocoris* in this trial.

5) 02/PSYLPY.18 (location: Velm)

No observations were carried out on *Anthocoris* in this trial.

III. CONCLUSIONS

In the 3 trials where observations were made on predatory bugs, Surround® gave good control of the pest pear sucker *Cacopsylla*, particularly the nymphal stages. Numbers of non-target predatory bugs *Anthocoris* were generally low even in the untreated control. However, they were reduced in numbers in the Surround® treated plots when sampled 3 months after application. This reduction was normally between 25-80%, but statistical analysis of the data was not possible because replication was too low. It is considered likely that this effect on *Anthocoris* is probably due to a combination of the removal of their food supply (eg

Cacopsylla nymphs) plus a general repellent effect of the Surround® particle film on the mobile adult forms of *Anthocoris* present at the early stage in the season.

Reference:	KCP 10.3.2.4/08 Peusens, G., & Creemers, P. 2004b (previously evaluated in DAR, B9, III.A 10.5.2/08)
Title:	Biological efficacy evaluation of Surround WP against the pear sucker, *Cacopsylla pyri* L., on pear
Report No.:	20040617 460 BE 421 GEP
Guideline(s):	EPPO guideline "*Cacopsylla*". EPPO Standards (1997)
Deviation(s):	Not relevant
GEP:	Yes

Executive Summary

SURROUND® WP CROP PROTECTANT was applied as a 2% concentration at 300 and 500 L/ha to large unreplicated blocks of pears. Three applications were made from mid-March to mid-April at crop stages BBCH 52-59. From just before the first application to end April assessments were made on the pest *Cacopsylla* adults, eggs and nymphs. Non-target predatory bug *Anthocoris* was sampled using a branch beating technique in mid-April (2 days after the last application). SURROUND® WP CROP PROTECTANT treatments gave excellent control of all pear *Cacopsylla* life stages. Numbers of *Anthocoris* were generally very low but they were significantly reduced in the SURROUND® WP CROP PROTECTANT treated plots. This is thought to be due to removal of prey (*Cacopsylla* nymphs) and general repellent effect of SURROUND® WP CROP PROTECTANT particle film on the mobile adults present at this time of year.

SURROUND® WP CROP PROTECTANT generally gave excellent control of all life stages of pear *Cacopsylla*. Numbers of *Anthocoris* were low but they were significantly lower in the SURROUND® WP CROP PROTECTANT treated plots when sampled 2 days after the last application. This reduction was probably due to a combination of the removal of their food supply (eg *Cacopsylla* nymphs) plus the general repellent effect of SURROUND® WP CROP PROTECTANT on the mobile adult forms of *Anthocoris* present at this early stage in the season.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: M99099
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: Not specified
   - Stability of test component: Stable

2. Test animals:
   - Species: *Anthocoris nemoralis*
   - Age: Adults
   - Source: Naturally present on test site.
   - Trial Location: Jeuk (Gingelom), Belgium

3.Testing facility: Royal Station for Fruit Growing, Gorsem, Belgium

B. STUDY DESIGN AND METHODS

Four single large un-replicated blocks of pear trees were used for the trial. One block was left untreated as the control and the other 3 were treated with Surround® WP as a 2% solution on 3 dates between 14 March and 14 April 2003 (crop stages BBCH 52-59). One of the Surround® blocks was treated using a spray volume of 500 L/ha whilst the other 2 were treated using 300 L/ha.

Within each treatment block, four separate plots were established for sampling. Over-wintering adult pear *Cacopsylla* were assessed by beating 3 branches on each of 10 trees per plot. *Cacopsylla* eggs and nymphs were assessed by counting numbers on 10 branches of about 20cm long per plot. Branches were tagged before treatments commenced so the same branches could be assessed in post-treatment assessments. *Anthocoris* populations were assessed by beating 3 branches on each of 10 trees per plot and catching the displaced insects on a tray positioned under the branch. Samples of *Anthocoris* were collected on 16 April, two days after the last application.

Statistics:
% control was calculated using the following formulae:
Henderson-Tilton – *Cacopsylla* ggs and adults corrected for pre-treatment differences.
Abbott – *Cacopsylla* nymphs and *Anthocoris* adults.

Homogeneity of variance was assessed using Bartletts chi-square tests and raw numbers were transformed to their Log+1 values as appropriate. Significant differences between treatments were tested using ANOVA followed by Duncan's multiple comparison tests.

II. RESULTS AND DISCUSSIONS

Surround® applied at 300 and 500 L/ha gave excellent control of *Cacopsylla* adults, eggs and nymphs on all assessment dates. *Anthocoris* was assessed only once on 16/04/03. Numbers in the untreated plots were still low (6 adults per 30 branches). However, in all Surround® treated plots there was a significant reduction in *Anthocoris* compared with the untreated.

Table 10.3.2.4/08-1: Percent control of *Cacopsylla* and effects on *Anthocoris*

	***Cacopsylla* pyri**								***Anthocoris***
	14/03/03		**3/04/03**		**16/04/03**			**30/04/03**	**16/04/04**
	Numbers								
	adults	eggs	adults	eggs	adults	eggs	nymphs	nymphs	**Total**
Untreated	84.5 a	70 a	26 a	56.7 a	10.0 a	15.0 a	4.5 a	11.5 a	6.0 a
	Number		Henderson-Tilton %				Abbott %		
Surround® 2% 500 L/ha	84.5 a	65.5 a	93.3 b	91.9 d	96.2b	89.4 a	100 b	98.2 b	100 b
Surround® 2% 300 L/ha	91.6 a	67.9 a	81.1 b	74.9 c	91.2 b	100 a	100 b	94.2 b	94 b
Surround® 2% 300 L/ha	98.3 a	73.4 a	90 b	53.1 b	94.6 b	80.4 a	100 b	94.2 b	100 b

Different letter subscripts denote significance difference between treatments at P≤ 0.05

III. CONCLUSIONS

Surround® generally gave excellent control of all life stages of pear *Cacopsylla*. Numbers of *Anthocoris* were low (only 6 adults per 30 branches in the untreated control) and found to be significantly lower in the Surround® treated plots 2 days after application. This reduction was probably due to a combination of the removal of their food supply (eg *Cacopsylla* nymphs) plus the general repellent effect of Surround® on the mobile adult forms of *Anthocoris* present at this early stage in the season.

Reference:	KCP 10.3.2.4/09 Puterka, G.J., 2001 (previously evaluated in DAR, B9, III.A 10.5.2/09)
Title:	Impact of Surround® WP Particle Film on Arthropod Predators in Tree Fruits
Report No.:	-
Guideline(s):	-
Deviation(s):	Not relevant
GLP:	No, but study scientifically valid

Executive Summary

SURROUND® WP CROP PROTECTANT was applied at 3 and 6% concentrations at a spray volume of 935 L/ha in small replicated plots of apples and pears. In total, 10 applications were made in pears and 12 in apples at 5 to 14 day intervals (May to August). Numbers of predators were assessed every 2 weeks throughout the season of application (June to August) by inspecting 15 terminals per tree per treatment replicate.

These season long applications of SURROUND® WP CROP PROTECTANT had a minimal impact on lacewing (Chrysoperlid) larvae and adults (egg laying), ladybird (Coccinellid) adults and pirate bug (*Orius triscolor)* adults, even when sampled immediately after application. The few significant differences that were observed were difficult to attribute to treatment related effects.

Season long applications of SURROUND® WP CROP PROTECTANT on pears and apples appeared to have a minimal impact on lacewing (Chrysoperlid) larvae and adults (egg laying), ladybird (Coccinellid) adults and pirate bugs (Orius triscolor) adults.

On the basis of this trial, SURROUND® WP CROP PROTECTANT can be considered as only slightly harmful to insect predators even when applied at extremely high rates and numbers of applications.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Surround® WP Crop Protectant
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: Not specified
   - Stability of test component: Stable

2. Vehicle and/or positive control: Water – spray volume 935 L/ha

3. Test animals: Different life stages of naturally occurring insect predators: Ladybirds (Coccinelids); Lacewings (Chrysoperlids); Pirate bugs (*Orius triscolor*)

4. Testing Facility: USDA, Appalachian Fruit Research Station, Kearneysville, West Virginia, USA

   No insecticide applications for 12 months.

B. STUDY DESIGN AND METHODS

Experimental design: 6 single tree replicates, complete randomised block design
Spray equipment: Commercial air blast sprayer

Spray volume: 935 L/ha (100 US gall/acre)

Treatments:
1) Surround® 3% (3 kg/hL)
2) Surround® 6% (6 kg/hL)
3) Untreated control

Applications:
Pears – 10 sprays at 5-14 d intervals between 11 May & 23 July
Apples – 12 sprays at 5 – 14 d intervals between 11 May & 21 August

Arthropod sampling: Twice weekly throughout the season from June to August. 15 twig terminals (first 20 cm) per tree were inspected for all stages (egg, nymph, adult) of each predator species. Numbers of leaves were also recorded to facilitate predator counts on a per leaf basis.

Statistics: ANOVA followed by LSD tests. Treatment means with different letters denote significant difference at $P \leq 0.05$.

II. RESULTS AND DISCUSSIONS

a) <u>Pear</u>

The primary predatory insects present in the pears were complexes of Chrysoperlids and Coccinellids.

Lacewing (chrysoperlids): Lacewing eggs were present in good numbers from mid-June onwards. No significant differences were found between untreated control and the two Surround® WP treatments until the last assessment on 23 July when 3% Surround® WP appeared to have significantly lower numbers than either 6% Surround® WP or the untreated control. Given this dose inversion it seems unlikely that this effect was treatment related. Numbers of lacewing larvae were quite low but present form mid-June onwards. Neither of the Surround® WP treatments had any negative effect on lacewing larvae.

Ladybirds (coccinellids): There were relatively low numbers in treated and control plots and therefore it was difficult to make conclusions. Nevertheless, in mid-season significantly fewer ladybirds were observed in plots sprayed with 6% Suround WP compared with either 3% Surround® WP or untreated control.

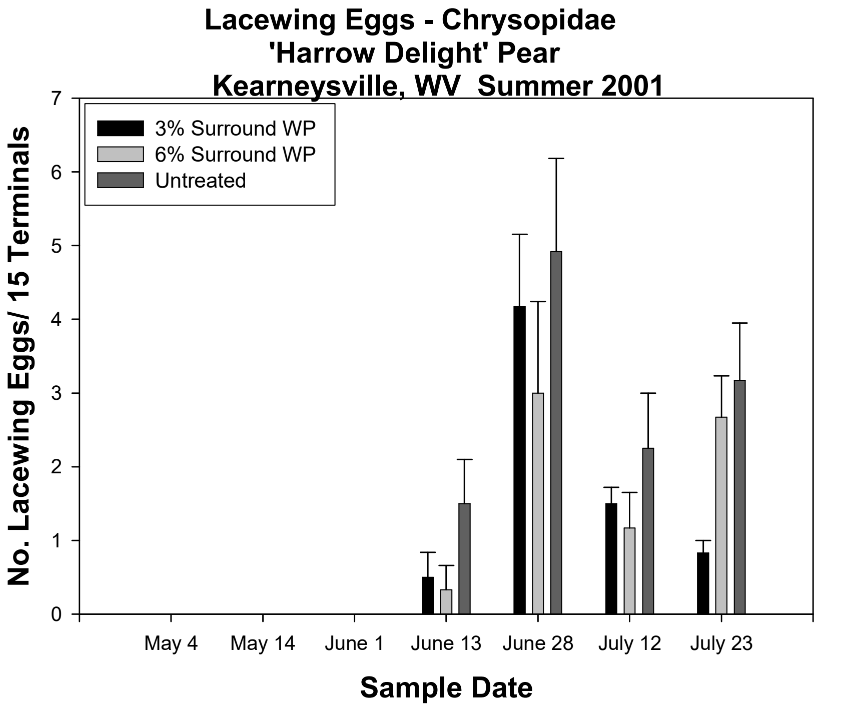

Figure 10.3.2.4/09-1: Effects of Surround® WP on lacewing eggs in pears

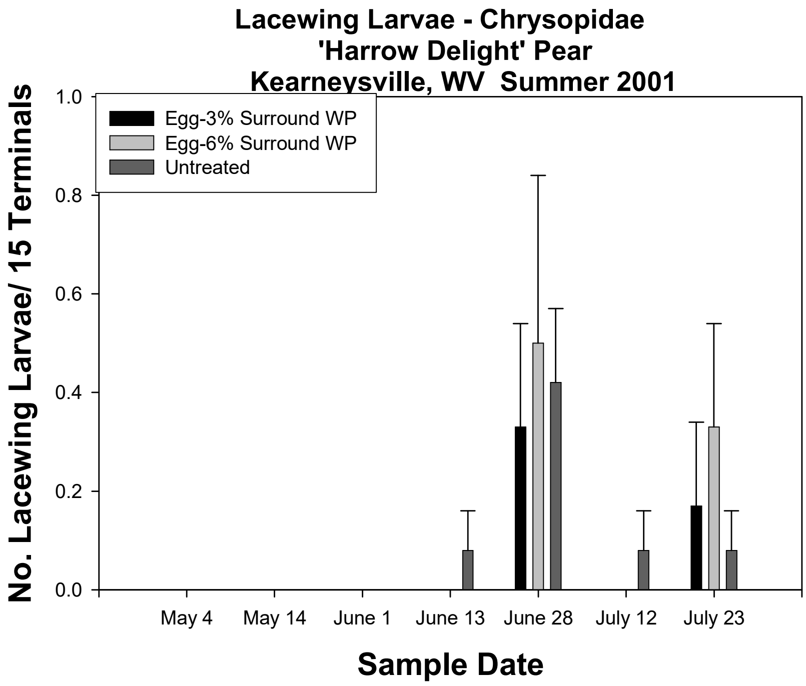

Figure 10.3.2.4/09-2: Effects of Surround® WP on lacewing larvae in pears

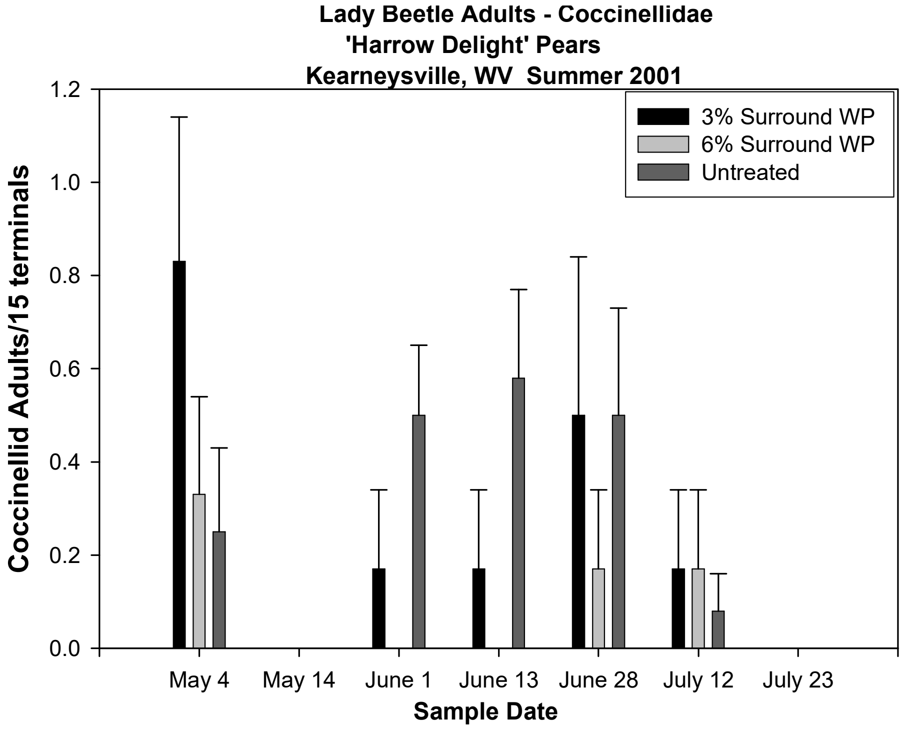

Figure 10.3.2.4/09-3: Effects of Surround® WP on ladybird adults in pears

b) <u>Apples:</u>

The primary beneficials were lacewing (chrysoperlids) eggs and larvae, ladybird (coccinellids) adults and pirate bug (*Orius tricolour*) adults.

Lacewing (chrysoperlids): Surround® WP treatments had no adverse effects on either lacewing egg laying or numbers of larvae. In fact, on two sampling dates (2 July and 14 August) there were significantly higher numbers of lacewing larvae recorded from both Surround® treatments than from the control. This was probably due to the higher numbers of aphids present in the plots on these dates.

Ladybirds (coccinellids): Ladybird adults were present throughout the trial period. No adverse effects on numbers were observed in either of the 2 Surround® treatments.

Pirate bug (*Orius tricolour*): Numbers of *Orius* were rather variable throughout the trial, although reasonable numbers were observed in July. During this time, significant differences were noted on several occasions, firstly in both Surround® dose rates, then with the 3% dose rate and finally with the 6% doses rate. Given the erratic nature of these fluctuations in numbers it is difficult to apportion them to any real treatment related effects.

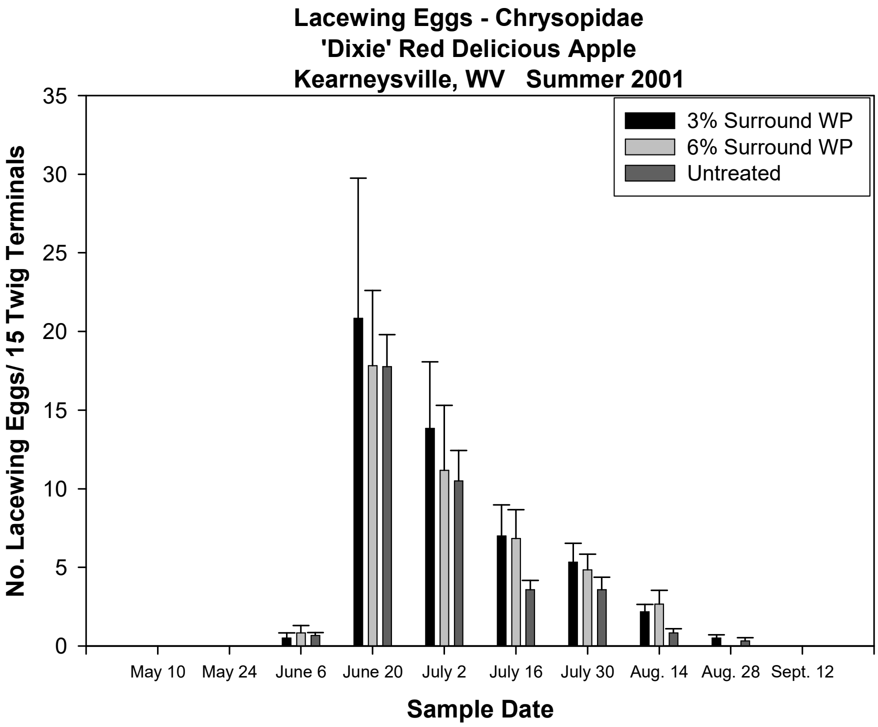

Figure 10.3.2.4/09-4: Effects of Surround® WP on lacewing eggs in apples

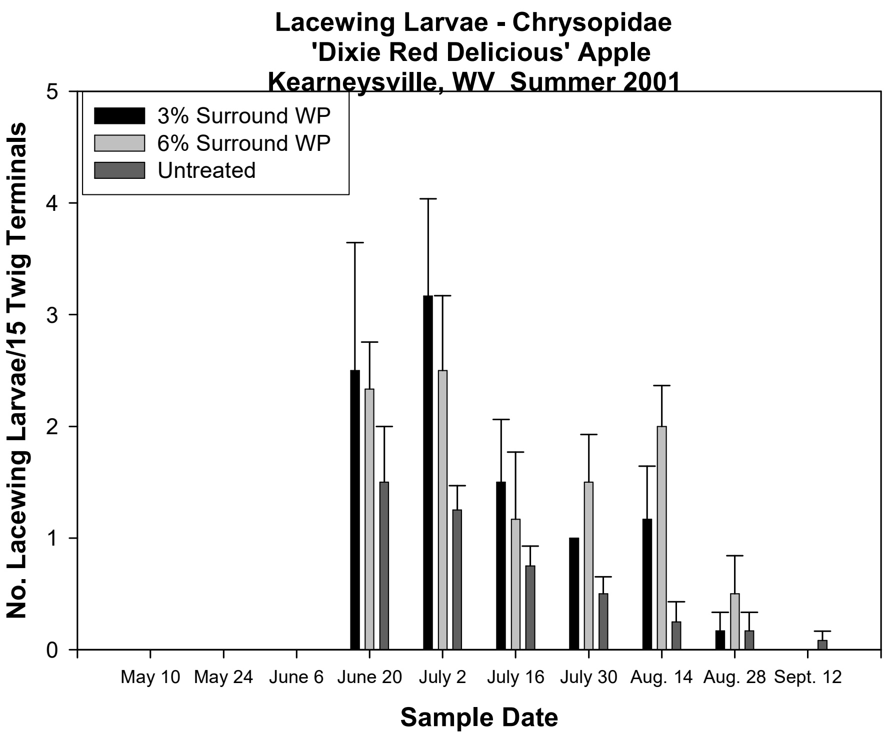

Figure 10.3.2.4/09-5: Effects of Surround® WP on lacewing larvae in apples

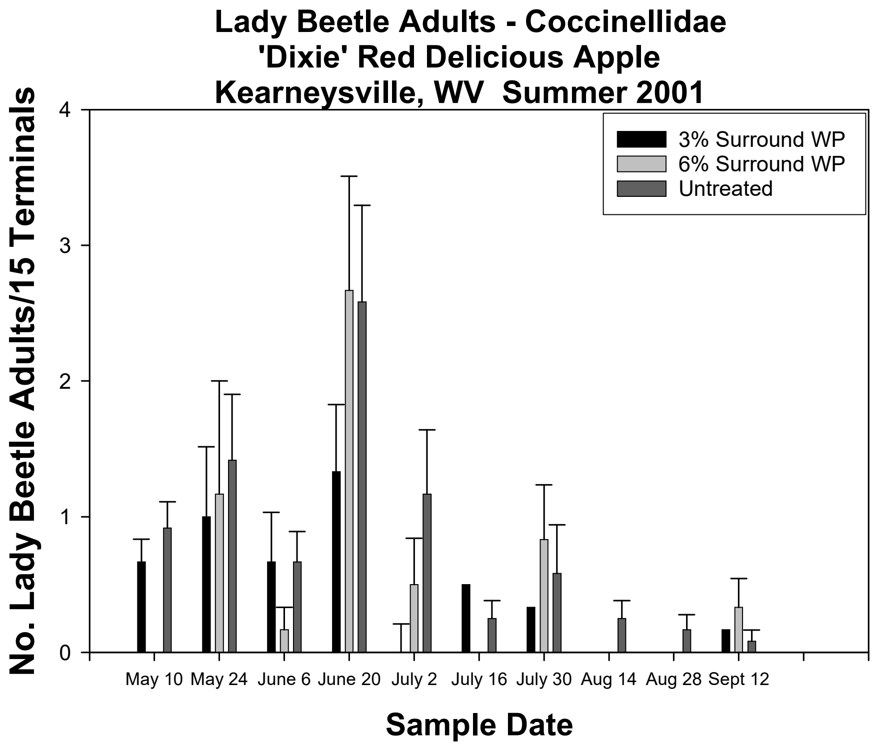

Figure 10.3.2.4/09-6: Effects of Surround® WP on ladybird adults in apples

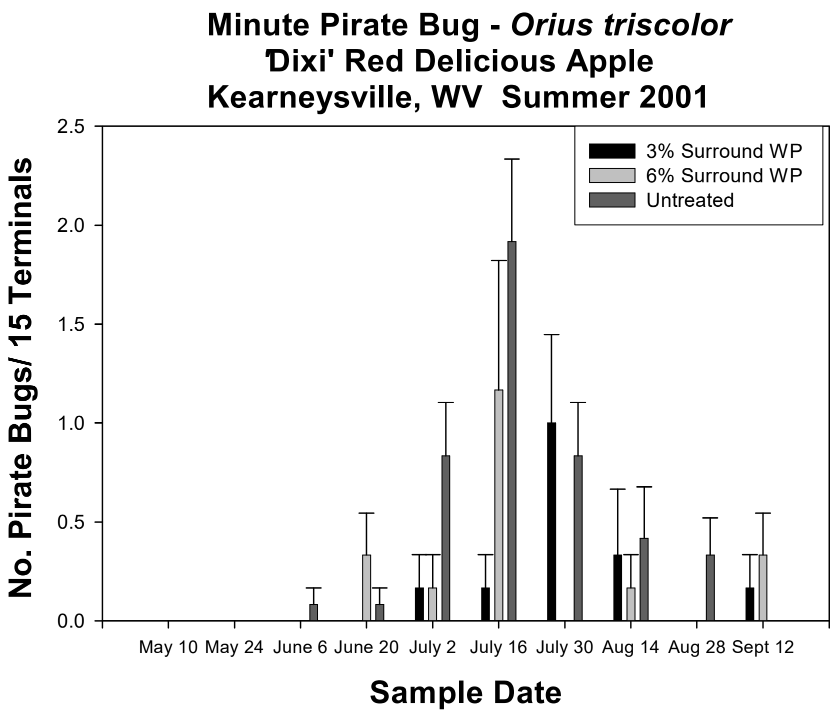

Figure 10.3.2.4/09-7: Effects of Surround® WP on *Orius* adults in apples

III. CONCLUSIONS

Season long applications of Surround® WP Crop Protectant on pears and apples appeared to have a minimal impact on lacewing (Chrysoperlid) larvae and adults (egg laying), ladybird (Coccinellid) adults and pirate bugs (*Orius triscolor*) adults, even when sampled immediately after application. The few significant differences that were observed were difficult to attribute to treatment related effects.

On the basis of this trial, Surround® WP Crop Protectant can be considered as only slightly harmful to insect predators even when applied at extremely high rates and numbers of applications.

Reference:	KCP 10.3.2.4/10, Pascual, S., Cobos, G., Seris, E., and Gonzalez-Nunez, M. 2010a
Title:	Effects of processed kaolin on pests and non-target arthropods in a Spanish olive grove
Report No.:	Published in: J Pest Sci 83:121-133
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

A 3-year field experiment was conducted from 2005 to 2007 at Villarejo de Salvane´s, Spain to assess the effects of Surround WP on the arthropod community of olive trees and on natural enemies. Both the abundance and the diversity of arthropods were reduced by Surround WP treatment following spray treatment, but appeared to be recoverying based on the data collected 2 months post treatnment. The principal response curve (PRC) analysis revealed a significant deleterious effect of Surround WP on the natural enemy arthropod community of the olive grove. The most affected taxa were the following: *Scymnus mediterraneus*, *Stethorus punctillum, Hyperaspis reppensis*, *Brachynotocoris ferreri* and different species of *Orius* and the families of Philodromidae, Scelionidae, Pteromalidae, and Aphelinidae, and Chrysopidae. However, the finding is not surprising as the mode of action of processed kaolin is basically a repellent/deterrent effect.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not known if guideline study
Reliability = 2 (reliable with restrictions), no known if GEP/GLP.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Surround WP
Batch number: Not reported. From Engelhard Corp, NJ, USA
Content of a.s.: Kaolin
Appearance: Not reported.

2. Reference: Dipagrex 80 (Trichlorfon (80%) bait spray)

3. Treated area: 4.0 ha olive grove in Villarejo de Salvane´s, south-eastern Madrid, Spain. The variety of olive trees was 'Manzanilla' and they were planted at a density of 100 trees per hectare.

4. Treatment: 3 kg/100 L (30 kg/ha)

B. STUDY DESIGN AND METHODS

To evaluate the effects on arthropod communities in olive groves after the use of Surround WP, two treatments were applied, the first one at the beginning of summer, when olive fruits are big enough for the attack of *B. oleae*, and the second one in autumn, to guarantee the coating until the end of the crop season, for three consecutive years.

Each treatment was applied to an area of about 0.8 ha. Experimental plots were randomly located in the olive grove. No additional pest control measures were used except for one treatment with Biobit XL applied at bloom to all test plots to control *Prays oleae* (Bernard).

1. Sampling:
The arthropod fauna from the canopy of olive trees was sampled using a beating method. In the laboratory, samples were kept in a freezer prior to analysis and after thawing and cleaning the specimens were classified into four groups: phytophagous on olive trees, predators, parasitoids and other. Biological diversity was assessed by calculating the Shannon index.

4. Statistics:
In order to investigate the changes in abundance of predators and parasitoids in the canopy of olive trees, a principal response curve (PRC) analysis was carried out using the program CANOCO 4.51 (Biometris, Plant Research International, Wageningen, The Netherlands).

In order to determine the effects of Surround WP on different taxa, 'Species Weights' and PRCs for Surround WP relative to the untreated control were calculated for each year.

II. RESULTS AND DISCUSSION

1. Effect of treatments on the abundance and diversity of arthropods:
When comparing Surround WP to the control plots, Surround WP treatment reduced the number of arthropods captured per tree for some of the sampling dates and always after the second Surround WP treatment of the season. Over all, Surround WP treatment resulted in a reduction in the number of species and also in the Shannon index. This reduction was observed at the end of the crop season in the 3 years of study. No consistent observation between trichlorfon and control plots were noted.

2. Effect of treatments on natural enemy communities:
The principal response curves (PRCs, figure below) indicated that there was a significant deleterious effect of the Surround WP treatment on the natural enemy arthropod community of the olive grove compared to the untreated control for the 3 years of study ($P = 0.022$). The analysis captured 34.8, 52.3 and 41.0% of the variance caused by the treatment effect in 2005, 2006 and 2007, respectively. On the other hand, trichlorfon-bait sprays did not have a significant effect in any of the years studied.

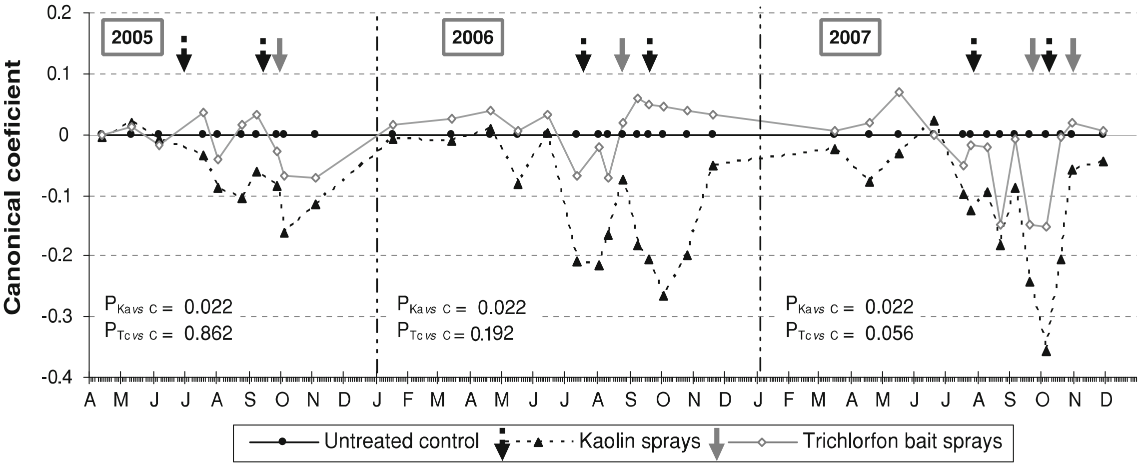

Figure 10.3.2.4/10-1: PRC diagram showing the effects of Surround WP on the beneficial community of arthropods over the growing season between 2005 and 2007 in an olive grove. Arrows indicate treatment applications.

The contribution of the different taxa to the overall community response in the Surround WP-treated plot is shown by the PRCs in the next figure, in which trichlorfon treatment was excluded from the analysis. Taxa indicated with a positive weight are expected to decrease in abundance relative to the control after treatment with Surround WP. *S. mediterraneus* had the highest positive weight in 2005 and 2006, and it also had a positive weight in 2007. Other species with positive weights in 2005 and 2006 were *S. punctillum*, *B. ferreri* and *Hyperaspis reppensis* (Herbst). In 2007, the taxon with the highest weight was the family Philodromidae, which also had a positive weight in 2006. Different species of Orius and the family Aphelinidae had high positive weights only in 2007. The families Scelionidae, Pteromalidae and Chrysopidae had positive weights for all 3 years. Taxa with negative weights in the PRCs are expected to increase after Surround WP treatment. Results were not conclusive in this respect as taxa with negative weights were different from one year to another, and the number of taxa with negative weights was very small.

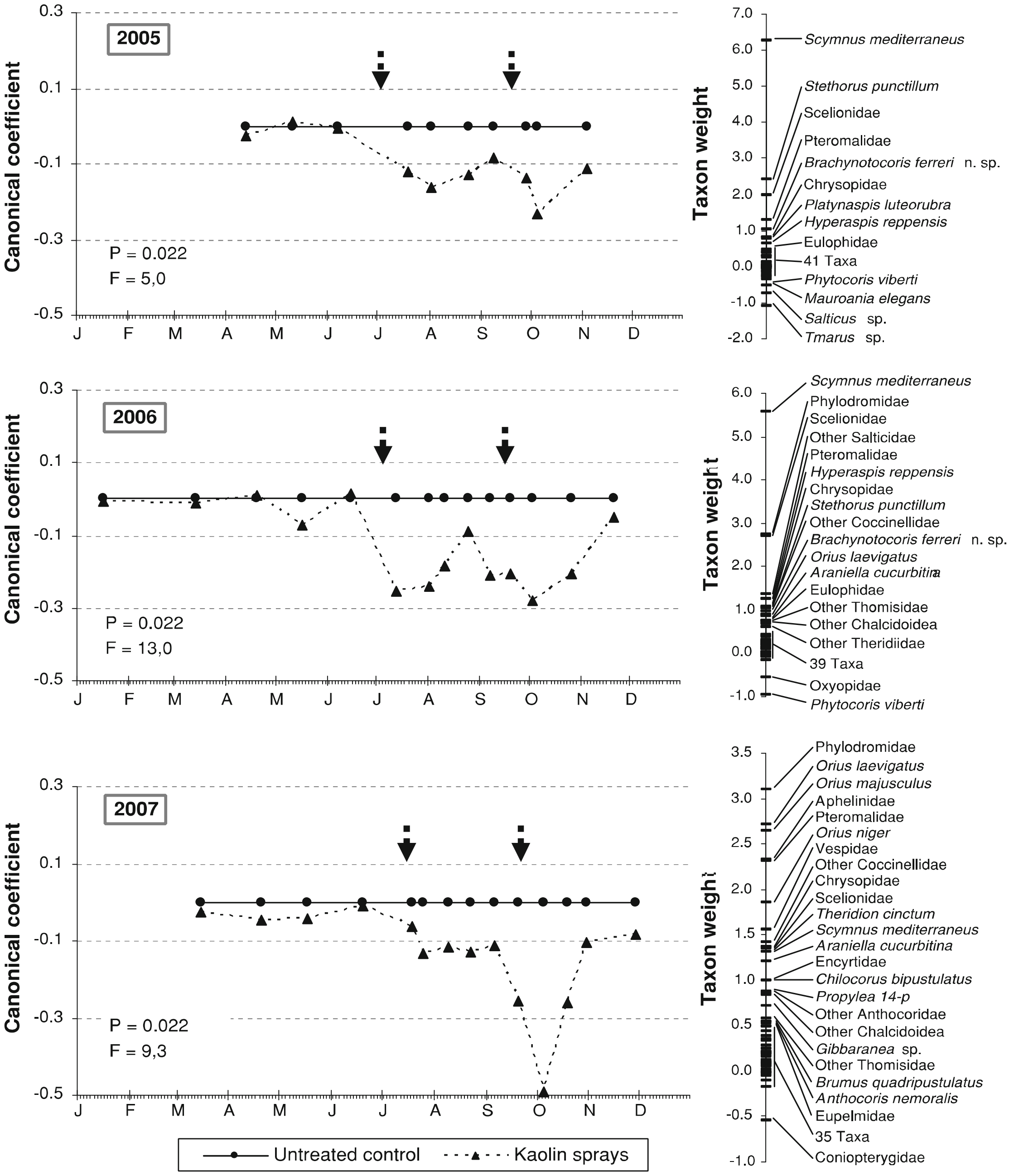

Figure 10.3.2.4/10-2: PRC diagrams and taxon weights for beneficial arthropods sampled in control and Surround WP plots. Arrows indicate treatment applications

III. CONCLUSION

This study shows that the processed kaolin Surround WP alters the arthropod community in olive trees. It affects insect pests and natural enemies. Also, it reduces the abundance and diversity of the arthropod community as a whole. This finding is not surprising as the mode of action of processed kaolin is basically a repellent/deterrent effect, and therefore, an a priori selectivity against phytophagous insects should not be expected. However, different responses to Surround WP treatment were found in different arthropod taxa. Differences in the effects of Surround WP were also noted in the 3 years of study. Environmental conditions were very different over the 3 years, and this affected the evolution of insect populations.

Furthermore, although not reported in the study, based on Figures 10.3.2.4/10-1 and 2 the differences between control and treated varied throughout the year, showing recovery of numbers over the winter and only reductions immediately after each application (increasing back to control levels within a couple of months). This indicates a high potential for recovery.

Reference:	KCP 10.3.2.4/11, Marko, V., Bogya, S., Kondorosy, E., and Blommers, L.H.M. 2009
Title:	Side effects of kaolin particle films on apple orchard bug, beetle and spider communities
Report No.:	Published in: International Journal of Pest Management vol 56: 189-199
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

The effects of multiple applications of hydrophobic kaolin particle film on apple orchard bug (Heteroptera), beetle (Coleoptera) and spider (Araneae) assemblages were studied in the Netherlands. Insecticide-free orchard plots served as a control. The kaolin applications significantly reduced the abundance and species richness of the communities and also altered their composition and diversity. The treatments disrupted many non-target groups notably mycophagous, predacious and tourist beetles, zoophagous bugs and spiders. Among spiders, wanderer spiders (Thomisidae, Philodromidae) were most affected, whereas web building spiders (Dictynidae) were least affected. After ceasing the applications in July, the between-treatment differences in composition of all communities and diversity of heteropterans and spiders diminished while the differences in abundance and species richness remained for a long time, until the end of September. Many predator species with good colonisation ability recovered slowly after the treatments, mainly due to the scarcity of prey. The trial ended approximately 8 weeks after last application.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not known if guideline study
Reliability = 2 (reliable with restrictions), not known to be GLP/GEP.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Hydrophobic kaolin M96
Batch number: Not reported. From Engelhard Corp, NJ, USA
Content of a.s.: Kaolin
Appearance: Not reported.

2. **Reference:** None

3. **Treated area:** 32 year old apple orchard De Schuilenburg in Kesteren, Netherlands. Had four blocks each of +0.23 ha (504–660 trees) consisting of cultivars James Grieve M7 (two rows), Golden Delicious M9 (two rows) and Cox's Orange Pippin M9 (two rows) at a planting distance of 4 x (1.5–2.25 m).

4. **Treatment:** 45 kg/ha

B. STUDY DESIGN AND METHODS

The treatments were carried out 12 times at average intervals of 10 days, between 24 March and 5 August in 1997. The applications were more frequent after rainstorms and less frequent during prolonged dry periods, and the treatments were interrupted during the apple blooming period between 16 April and 15 May. The treatments were completed earlier on the early (summer) cultivar James Grieve (on 15 July), so at that site only 10 applications of kaolin were made.

1. Sampling:

Beating-funnel samples were collected by jarring the entire canopy of two randomly chosen trees per plot (eight trees per treatment). Two additional, randomly chosen, trees were sampled in the fourth block, both in the kaolin-treated and control plots, but these samples were used only for calculating Renyi diversity and community composition. The heteropterans and the adults of beetles, and also most spiders collected, were identified to species level. The bug, beetle and spider communities were characterized in terms of abundance, species richness, composition and diversity.

Last application was 5 August, while last sampling was 28 September (i.e. approximately 8 weeks after the last application).

4. Statistics:

Percent reduction due to kaolin treatment was determined by Abbott's formula (Abbott 1925).

The numbers of individuals and species richness were compared by Welch's modified t-test with the post hoc Games–Howell test when the data were normally distributed. If the data did not conform to a normal distribution, the adjusted rank Welch test, followed by tests of pairwise stochastic equalities with Bonferroni correction, was applied. Because of small sample size, the comparison of guilds, families and species was performed only on data pooled across the growing season or across that part of the season when the kaolin was being periodically sprayed and after the applications had finished. All the statistical analyses were carried out by using the software package RopStat.

Renyi diversity ordering was chosen for calculating the diversity.

II. RESULTS AND DISCUSSION

1. Heteropteran community:

The kaolin treatments significantly decreased both the total annual numbers collected and the species richness of the Heteroptera community (Table 10.3.2.4/11-1). Most of the individuals belonged to the predatory guild. This group was most affected by kaolin treatments while the phytophagous guild decreased to a lesser extent.

The number of heteropterans was often lower in the kaolin-treated plots, although the difference was statistically significant only at two dates shortly following application. The mean species richness (number of species collected on two trees) alternated between 0.75 and 4.5 during the growing season. Generally, the number of species was higher in the control plots and on 28 September, 8 weeks after the last kaolin application, the species richness of bugs still was significantly higher in the control compared to kaolin plots.

The Renyi diversity was significantly greater in the control plots both during the part of the season when the kaolin was being periodically sprayed and during the rest of the season after kaolin spraying had ceased. The kaolin applications significantly increased the diversity at higher alpha values however, after finishing the treatments, the Renyi diversity profiles became identical.

Table 10.3.2.4/11-1: The mean total abundance (individuals/2 trees) of the heteropteran community, the main guilds and the mean species richness in the kaolin treated and control plots (based on annual data collection)

	Control	Kaolin	Reduction (%)
Abundance	52.8	21.0*	60
Species richness	10.8	7.8*	-
Carnivorous	47.5	15.5**	67
Omnivorous	3.5	1.5	57
Phytophagous	1.8	4.0	-122

*P<0.05, ** P<0.01, calculated according Abbott's formula.

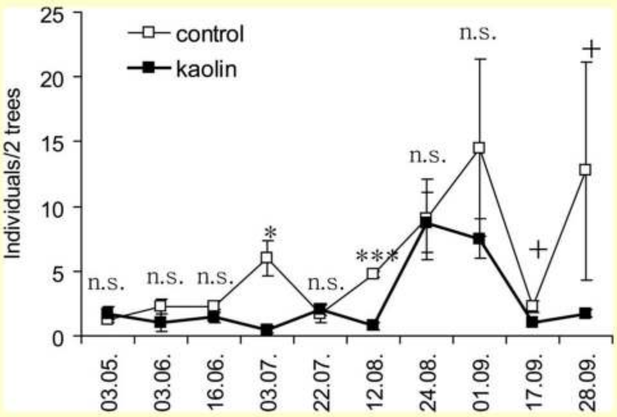

Figure 1. Seasonal abundance (individuals/2 trees ± SD) of heteropterans in the kaolin-treated and control plots. n.s., non significant, + $P < 0.1$, *$P < 0.05$, ***$P < 0.001$.

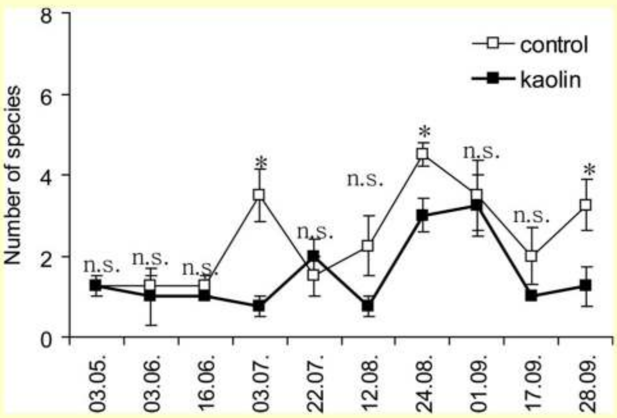

Figure 2. Species richness (number of species/2 trees ± SD) of heteropterans in the kaolin-treated and control plots during the season. n.s., non significant, *$P < 0.05$.

2. Beetle community:

The kaolin applications were disruptive to the beetle community: both the total annual abundance and the species richness were lower in the kaolin-treated plots (Table 10.3.2.4/11-2). However, there were major differences between the beetle guilds. The kaolin treatments mainly affected the mycophagous and xylophagous guilds, and had the lowest efficacy against the guild of apple-feeders.

Both abundance and species richness were usually significantly lower in the kaolin-treated plots during the whole season and the increase observed in control plots in late summer early autumn was not observed (abundance) or was so only moderately (species richness).

During the treatments, the Renyi diversity of the beetle communities was significantly higher in the kaolin treated plots and became identical at higher alpha values when considering the common species. After ceasing the treatments, the Renyi diversity values increased at alpha > 0 in the kaolin-treated and decreased in the control plots resulting in a clear difference between the treatments and control 6 weeks after the last application.

Table 10.3.2.4/11-2: The mean total abundance (individuals/2 trees) of the Coleoptera community, the main guilds and the mean species richness in the kaolin treated and control plots(based on annual data collection)

	Control	Kaolin	Reduction (%)
Abundance	240.8	57.0**	76
Species richness	22.5	12.5**	-
Mycophagous	109.5	12.0**	89
Xylophagous	5.5	1.3	76
Predators	29.3	8.3+	72
Tourists	12.3	3.5**	71
Apple feeders	87.5	40.0***	53

+P < 0.10, *P<0.05, ** P<0.01, calculated according Abbot's formula.

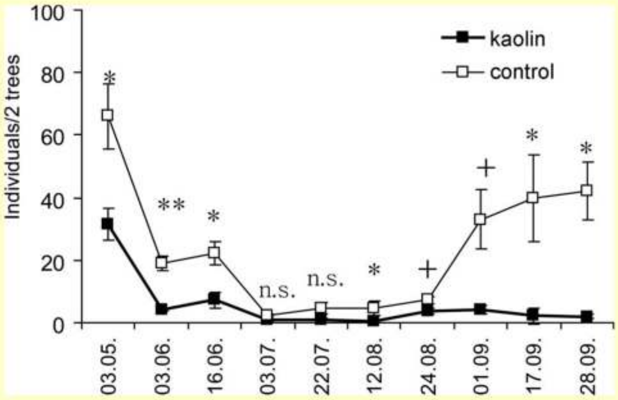

Figure 5. Seasonal abundance (individuals/2 trees ± SD) of beetles in the kaolin-treated and control plots. n.s., non significant, + $P < 0.1$, *$P < 0.05$, **$P < 0.01$.

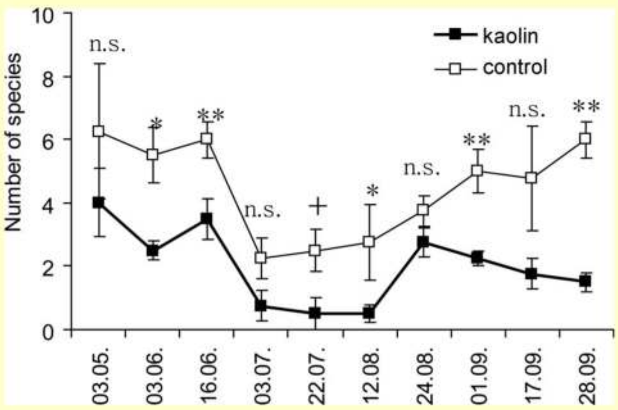

Figure 6. Species richness (number of species/2 trees ± SD) of beetles in the kaolin-treated and control plots during the season. n.s., non significant, + $P < 0.1$, *$P < 0.05$, **$P < 0.01$.

3. Spider community:

The kaolin formulation reduced both the annual abundance and species richness (Table 10.3.2.4/11-3). Generally, the spider guild 'wanderers' was more sensitive to kaolin applications than the guild 'web-builders' though there were major differences between the families belonging to the same guild. For example, one of the most sensitive families (Theridiidae) and the less sensitive Tetragnathidae both belong to the web-builders. Similarly, within wanderers the kaolin treatments strongly affected Philodromidae but to a much lesser extent Clubionidae.

Kaolin applications showed a very strong negative effect both on spider abundance and on the number of genera during not only the applications but also 6 weeks afterwards when the spider community consisted almost exclusively of juveniles. The increase of numbers after the first part of August was apparent in the control plots and negligible in the kaolin treatments.

The Renyi diversity was significantly higher not only during but also 6 weeks after kaolin applications. In the first part of the growing season, the kaolin applications reduced the diversity of spiders. Later in the season, 6 weeks after the last applciation, the diversity profile of the kaolin and non-kaolin treated plots became identical.

Table 10.3.2.4/11-3: The mean total abundance (individuals/2 trees) of the Araneae community, the main families and the mean species richness in the kaolin treated and control plots

	Control	Kaolin	Reduction (%)
Abundance	163.8	50.5***	69
Species richness	5.5	2.5*	-
Wanderers	59.0	14.3***	76
Web-builders	103.0	35.8***	65
Philodromidae	46.8	7.8***	83
Thomisidae	4.0	0.8*	81
Theridiidae	50.8	10.0***	80
Linyphiidae	12.8	3.3**	74
Araneidae	6.2	2.8	55
Dictynidae	31.5	18.5*	41
Clubionidae	7.5	5.0	33
Tetragnathidae	1.8	2.0	-11

*P<0.05, ** P<0.01, *** P<0.001, calculated according Abbot's formula.

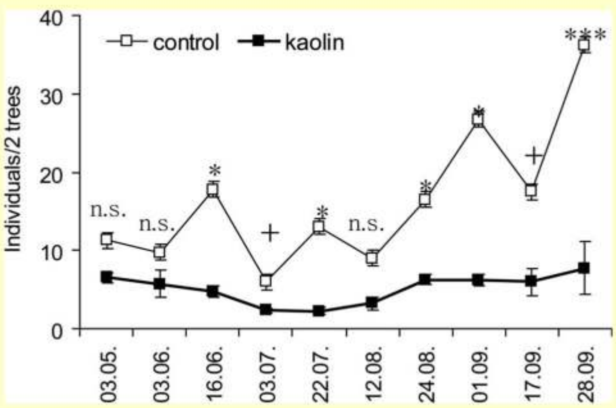

Figure 8. Seasonal abundance (individuals/2 trees ± SD) of spiders in the kaolin-treated and control plots. n.s., non significant, + $P < 0.1$, *$P < 0.05$, ***$P < 0.001$.

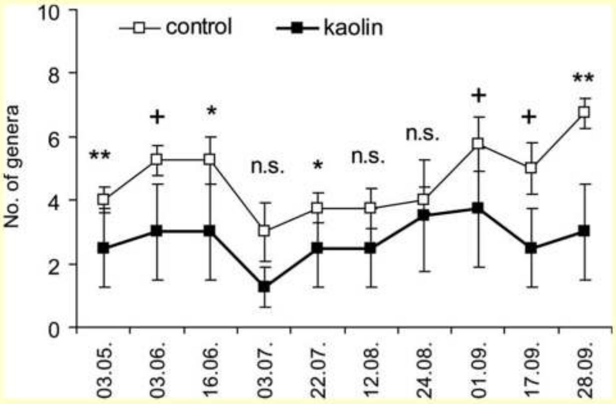

Figure 9. Mean number of spider genera (number of genera/2 trees ± SD) in the kaolin-treated and control plots during the season. n.s., non significant, + $P < 0.1$, *$P < 0.05$, **$P < 0.01$.

III. CONCLUSION

In the field trial, the application of kaolin particle film reduced the abundance and species richness of the apple orchard heteropteran, beetle and spider communities, the main guilds and the most common species during the growing/application season. However, the degree of reduction was different in many taxa, causing differences between the composition and diversity of the communities in the kaolin-treated and control plots.

Note that many predator species were reported during the 6 week observation period to be recovering after the treatments.

Reference:	KCP 10.3.2.4/12, Markó, V., Blommers, L.H.M., Bogya, S., Helsen, H., 2006
Title:	The effect of kaolin treatments on phytophagous and predatory arthropods in the canopies of apple trees
Report No.:	Published in: J Fruit Ornam Plant Res, 14 (suppl 3): 79-87
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described, also efficacy related information

Executive summary

The effect of a kaolin-based particle film formulation on apple pests and their natural enemies was investigated in an experimental apple orchard in the Netherlands. Each plot chosen for the experiment was planted with 260 trees of ‘Golden Delicious’, ‘James Grieve’ and ‘Cox’s O.P’. Four 0.2-hectare plots were divided into two parts. One part was treated with kaolin particle film, while the other part served as the control. Kaolin was applied in the form of hydrophobic kaolin M96-018, which was applied at a rate of 45 kg/ha in a suspension of 30 g kaolin M96-018 and 40 mL methanol/L of water. The treatments were applied about every ten days twelve times between March 25 and August 5. Beating-tray samples were collected nine times by tapping the entire canopies of ten trees per treatment. Data recorded included the number of aphid colonies, communal caterpillar webs, and leaf mines, and the population densities of *Hoplocampa testudinea* and *Eriosoma lanigerum*. All the samples were collected from the cultivar James Grieve.

The kaolin treatment reduced the population density of *Aphis pomi*, *Anthonomus pomorum*, and *Empoasca vitis*, and the number of communal caterpillar webs. The proportion of fruits infested with *H. testudinea* was 9.31% on the treated plots and 35.7% on the control plots. On the other hand, there were more mines formed by *Lyonetia clerkella*, *Phyllonorycter blancardella* and *Phytomyza heringiana* on the treated plots than on the control plots. Kaolin particle films also increased the population density of *Eriosoma lanigerum* so that, by the end of the season, the treated plots were severely infested. In spite of the higher prey density, the numbers of the most important predators, *Forficula auricularia*, *Allothrombium fuliginosum* and *Exochomus quadripustulatus*, were significantly lower on the kaolin treated plots. This also was the case for the spiders. After the last treatment on August 5, the population density of *Allothrombium fuliginosum*, *Forficula auricularia* and *Exochomus quadripustulatus* increased on the treated plots.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study, efficacy information
Reliability = 2 (reliable with restrictions), not known to be GLP.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Hydrophobic kaolin, M96-018
	Batch number:	Not reported. Manufactured by Engelhard Corporation, New Jersey, USA
	Content of a.s.:	Not reported
	Appearance:	Fine white particles
2.	**Vehicle:**	40 mL methanol/L of water
	Reference item:	Not reported
3.	**Test organism**	
	Species:	Aphid colonies, communal caterpillar webs, and leaf mines, and the population densities of *Hoplocampa testudinea* and *Eriosoma lanigerum*
	Age at test initiation:	Varied ages
	Source:	De Schuilenburg Experimental Apple Orchard in Kesteren, the Netherlands
4.	**Treatment:**	45 kg/ha in a suspension of 30 g kaolin M96-018 and 40 mL methanol/L of water

5. Environmental conditions: European maritime climate

B. STUDY DESIGN AND METHODS

1. Method:
The effect of a kaolin-based particle film formulation on apple pests and their natural enemies was investigated in an experimental apple orchard in the Netherlands. Each plot chosen for the experiment was planted with 260 trees of 'Golden Delicious', 'James Grieve' and 'Cox's O.P'. Four 0.2-hectare plots were divided into two parts. One part was treated with kaolin particle film, while the other part served as the control. Kaolin was applied in the form of hydrophobic kaolin M96-018, which was applied at a rate of 45 kg/ha in a suspension of 30 g kaolin M96-018 and 40 mL methanol/L of water. The treatments were applied about every ten days twelve times between March 25 and August 5.

2. Sampling:
Beating-tray samples were collected nine times by tapping the entire canopies of ten trees per treatment between May 3 and September 17. Data recorded included the number of aphid colonies, communal caterpillar webs, and leaf mines, and the population densities of *Hoplocampa testudinea* and *Eriosoma lanigerum*. All the samples were collected from the cultivar James Grieve.

3. Statistics:
Welch's modified t-test to determine significant differences between treated and control plots; $p < 0.01$.

II. RESULTS AND DISCUSSION

1. Effects of kaolin on pests:
Pest species were always present at higher numbers in the control plots at all sampling times. However, the difference between the treated and control plots was statistically significant only on June 16, when 3.5 times as many individuals were collected on the control plots that on the treated plots.

The mean amount of damage caused by the apple sawfly damage was 9.31% ±0.04% on the treated plots and 35.7% ±0.12% on the control plots, the difference was statistically significant.

Twelve times as many green apple aphids (*Aphis pomi*) were found on the control plots than on the treated plots, which was statistically significant.

There were no significant differences between the treated plots and the control plots for rosy apple aphids (*Dysaphis plantaginea*) and rosy leaf-curling aphids (*Dysaphis devecta*).

Significantly fewer caterpillar webs were found on the treated plots than on the control plots; most common was the vine leafhopper (*Empoasca vitis*), which was present at an average density of 81.00 ±23.30 individuals per tree on the control plots, and 23.50 ±22.56 individuals per tree on treated plots. The difference was statistically significant.

Mines of six species were found, mainly the spotted tentiform leafminer (*Phyllonorycter blancardella*), the apple leafminer (*Lyonetia clerkella*), and the agromyzid fly, *Phytomyza heringiana*. Significantly more mines were found on the treated plots than on the control plots.

Many more woolly apple aphids were found on the treated plots than on the control plots. On average, twenty of the twenty-five trees examined on the control plots received a score of 0, while fifteen of the twenty-five trees examined on the treated plots received a score of 8. On the treated plots, the level of infestation was severe and much higher than the economic threshold level.

2. Effects of kaolin on natural enemies:
The most common predators encountered were spiders, followed by the common earwig (*Forficula auricularia*). Throughout the growing season, they were less numerous on treated plots than on control plots. The population density of spiders was very low on treated plots. A month after the last treatment, the population density of spiders was still lower on the treated plots than on the control plots, but further later samples were not collected to measure any potential recovery.

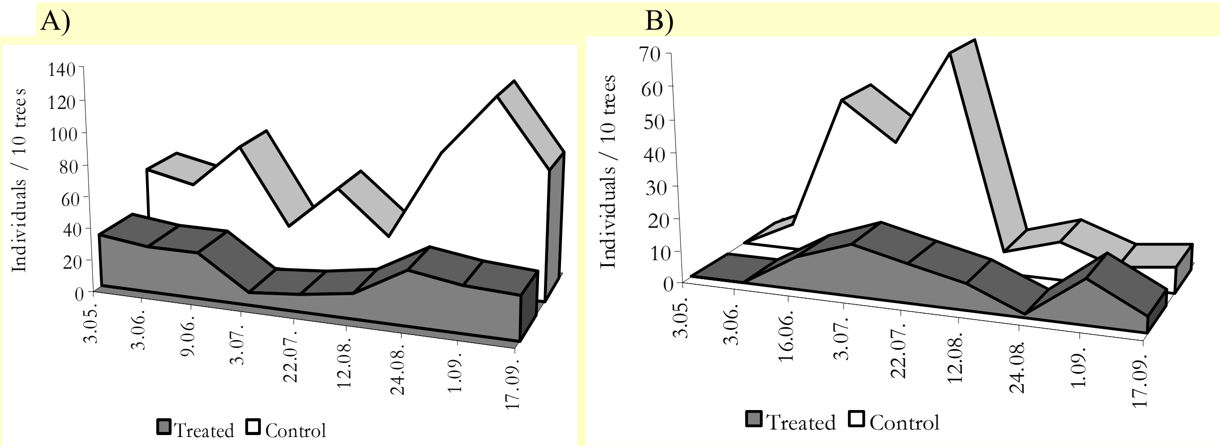

Figure 10.3.2.4/12-1: A) Number of spider and B) Number of common earwig collected during the season

Between May 5 and August 24, significantly fewer individuals of the predatory mite *Allothrombium fuliginosum* were found on the treated plots than on the control plots. An average of 0.30 ±0.45 individuals per tree were found on the treated plots, while an average of 3.30 ±1.52 individuals per tree were found on the control plots.

Between May 5 and August 24, significantly fewer individuals of the pine ladybird (*Exochomus quadripustulatus*) were found on the treated plots than on the control plots. An average of 1.30 ±0.75 individuals per tree were found on the treated plots, while an average of 4.90 ±2.16 individuals per tree were found on the control plots. After the last treatment on August 5, the population density of *Allothrombium fuliginosum*, *Forficula auricularia* and *Exochomus quadripustulatus* increased on the treated plots.

1. CONCLUSION

After the last treatment, the population density of *Allothrombium fuliginosum*, *Forficula auricularia* and *Exochomus quadripustulatus* did increase on the treated plots. However, a month after the last treatment, the population density of spiders was still lower on the treated plots than on the control plots.

Reference:	KCP 10.3.2.4/13, Iannotta, N., Belifiore, T., Noce, M.E., Scalercio, S., Vizzarri, V., 2007
Title:	The impact of some compounds utilized in organic olive groves on the non-target arthropod fauna: canopy and soil levels
Report No.:	Published in: Ecoliva 2007, VI Jornadas Internacionales de Olivar Ecologico, Puente de Génave (Jaén), España, 22-25 marzo 2007
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study, however sufficient data well described. Unfortunately, frequency and timing of sampling not reported, hence it is unknowm when adverse effects were noted or if recovery was observed

Executive summary

The aims of this research were (1) to evaluate the impact of compounds allowed in organic olive farming, (2) searching for more eco-compatible olive farming strategies, and (3) searching for bioindicators of olive ecosystem health among arthropods. The research was carried out in Southern Italy. Experimental olive groves were untilled, and the grass cover was periodically managed. Six orchards with 200 plants were randomly chosen and sprayed with different pesticides, including Surround WP Crop Protectant at a rate of 2 x 5 kg test item/hL. Arthropods were sampled at canopy and soil levels.

Dimethoate had negative effects on arthropod fauna of both the canopy and the soil. Kaolin reduced the abundance of arthropods at canopy level, but it preserves a good Coenotic Balance among trophic guilds and had no impact on the soil arthropods communities. On the canopy, only, Lepidoptera were unaffected by the kaolin spraying, and for soil no taxa seem to be significantly affected. This could be due to the interference between kaolin particle film and the feeding strategies utilised by pollinators, phytophagous and predators.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2 (reliable with restrictions), not guideline study, not known to be GLP

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Surround WP Crop Protectant
Batch number: Not reported. Product manufactured by Engelhard Corporation, NJ, USA
Content of a.s.: Kaolin, content not reported
Appearance: Not reported

2. Vehicle: Not reported

3. Reference: Rogor 40 (dimethoate)
(additional plots were treated with rotenone, and copper oxychloride with propolis)

4. Test organism
Species: At the canopy level: nine taxa (Arachnida: Araneae and Opiliones; Insecta: Hymenoptera Ichneuomonoidea, other Hymenoptera, Coleoptera Coccinellidae, Macrolepidoptera, Neuroptera, Mecoptera, Diptera Syrphidae)
At the soil level: six taxa (Arachnida: Araneae; Crustacea: Isopoda; Insecta: Coleoptera Carabidae, Coleoptera Staphylinidae, other Coleoptera, Hymenoptera Formicidae)
Age at test initiation: Varied
Source: Directly from within the experimental field of CRA - Experimental Institute for Olive Growing

5. Treatment: 5 kg Surround WP/hL

6. Test units: 15-18 year old olive trees

7. Environmental conditions: Mediterranean climate

B. STUDY DESIGN AND METHODS

The study area was located in Calabria, Southern Italy, within the experimental field of CRA - Experimental Institute for Olive Growing which consists of 15-18 year old olive plants. Experimental olive groves were untilled, and the grass cover was periodically managed.

Data were collected from late June to early December, i.e. from ripening to harvest. A plot of 200 plants were randomly chosen and treated on 21 August and 28 September with kaolin (5 kg/hL of Surround WP Crop Protectant). Two plots were treated from August to October with three applications of dimethoate (150 mL/hL of Rogor 40). An additional plot was treated twice with 300 mL/hL of rotenone and one plot treated twice with 250 g/hL cooper oxychloride and 150 mL/L propolis. One untreated plot was utilised as control. Arthropods were sampled at canopy and soil levels. Time and frequency of sampling was not reported.

1. Statistics:

Collected data were submitted to various analyses in order to detect the differences in community structure, the responses of sampled taxa to treatments, and the effects of compounds on the efficiency of trophic levels.

The ratio after/before treatments (*A/Bratio*) of the abundance of sampled taxa was used to provide information on the effect of treatments. This analysis was carried out at canopy and soil levels.

An index of Coenotic Balance (CB) was used in order to evaluate the efficiency of trophic levels. It is assumed that (1) in natural ecosystems antagonists are less abundant than indifferent insects which represent the major part of their preys, and that (2) the use of pesticides alters this ratio causing a relative higher decreasing of indifferent insects in the short time in respect to antagonist insects. The index of Coenotic Balance is coded as follows: $CB = n_I/n_A$, where n_I equals to the number of individuals belonging to indifferent insect taxa, and n_A equals to the number of individuals belonging to antagonist insect taxa. Higher values are determined by better Coenotic Balances. This analysis was carried out at canopy level only by grouping Araneae, Opiliones, Ichneumonoidea, Coccinellidae, Neuroptera and Syrphidae in the Antagonists category (*A*), including predators and parasitoids, and other Hymenoptera, Macrolepidoptera and Mecoptera in the Indifferent category (*I*), including saprofagous, phytophagous and pollinators. The superfamily Ichneumonoidea was chosen as representative of antagonist taxa because of relatively simple to identify. As consequence, the surrogate index of Coenotic Balance ($CB_{hym/ichn}$) is: $CB_{hym/ichn} = n_{hym}/n_{ichn}$, where n_{hym} equals to the number of individuals belonging to Hymenoptera, and n_{ichn} equals to the number of individuals belonging to Ichneumonoidea.

II. RESULTS AND DISCUSSION

1. Canopy level:

A total of 2,902 individuals belonging to selected taxa were collected. The most abundant taxon was other Hymenoptera (n = 1,003; 34.6%), followed by Ichneumonoidea (n = 884; 30.4%). The highest number of individuals was collected within the control plots, whilst the lowest one was collected within kaolin treated plots. Neuroptera, Macrolepidoptera and Syrphidae were more abundant in the plots treated with dimethoate.

Table 10.3.2.4/13-1: Abundance at the canopy level of sampled taxa

Taxa	Control	Kaolin	Dimethoate		Total*	%*
Other Hymenoptera	238	137	111	127	1003	34.6
Ichneumonoidea	230	104	125	162	884	30.4
Macrolepidoptera	39	37	76	44	259	8.9
Neuroptera	38	4	106	54	233	8.0
Mecoptera	83	29	4	4	163	5.6
Syrphidae	10	18	26	39	136	4.7
Coccinellidae	40	10	10	11	130	4.5
Araneae	18	17	15	5	92	3.2
Opiliones	0	2	0	0	2	0.007
Total	696	358	473	446	2902	-
%	24.0	12.3	16.3	15.4	-	-

*Total values also consider the findings for the other two treatments (rotenone and copper oxychloride plus propolis)

The ratio after/before treatments (A/B_{ratio}) showed that dimethoate and kaolin had the higher knock-down effect on the sampled arthropod community at the canopy level. Although Neuroptera were very abundant in plots treated with dimethoate, they showed a very high decrease due to treatments. Kaolin caused the greatest effects on the number of other Hymenoptera and Coccinellidae, from all treatments.

According to the Coenotic Balance (CB) computed utilising all the sampled taxa and hymenopteran taxa, plots treated with kaolin resulted in the higher CB value and, consequently, the best coenotic balance.

Table 10.3.2.4/13-2: The ratio after/before treatments (A/B_{ratio}) and results of Coenotic Balance (CB) at canopy level

Taxa	Control	Kaolin	Dimethoate	
Araneae	2.49	1.46	1.64	
Other Hymenoptera	0.90	0.56	1.17	
Ichneumonoidea	2.85	1.21	2.31	
Coccinellidae	0.53	0.34	1.30	
Macrolepidoptera	0.48	1.78	1.18	
Neuroptera	11.61	1.00	0.17	
Syrphidae	0.00	-	8.66	
Total	1.57	0.93	0.85	
CB	1.07	1.31	0.68	0.65
CB*ime/icne*	1.03	1.31	0.89	0.78

Plots were grouped and successively analysed as a unique sample.
No ratios are disposable for Opiliones and Mecoptera because no individuals were collected before the treatments.
Data for Syrphidae were not significant because of the late appearance of the adult stage.

2. Soil level:

A total of 23,393 individuals belonging to selected taxa were collected. The most abundant taxon was Formicidae (n =10,303; 44.0%), followed by Isopoda (n = 5,528; 23.6%). The least number of individuals were collected within the plot treated with dimethoate. For kaolin treated plot, no significant differences were noted compared to the control.

The ratio after/before treatments (A/B_{ratio}) wth dimethoate has the higher knock-down effect on the sampled arthropod community at the soil level. Carabidae and Staphylinidae were seriously affected by dimethoate. Araneae and other Coleoptera were the only taxa that were more abundant within the untreated plot than within the treated ones. Kaolin resulted in the lowest incidence on the arthropod populations at the soil level.

Table 10.3.2.4/13-3: Abundance at the soil level of sampled taxa

Taxa	Control	Kaolin	Dimethoate		Total*	%*
Formicidae	1464	2829	824	873	10303	44.0
Isopoda	1244	696	143	477	5528	23.6
Carabidae	640	504	287	355	2492	10.7
Arneae	589	584	83	133	2406	10.3
Other Coleoptera	475	344	140	151	2254	9.6
Staphylinidae	203	112	7	10	407	1.7
Opiliones	1	1	0	1	3	0.01
Total	4616	5070	1484	2000	23393	-
%	19.7	21.7	6.3	8.6	-	-

*Total values also consider the findings for the other two treatments (rotenone and copper oxychloride plus propolis)

Table 10.3.2.4/13-4: The ratio after/before treatments (*A/B*$_{ratio}$) and results pf Coenotic Balance (CB) at soil level

Taxa	Control	Kaolin	Dimethoate
Araneae	0.54	0.36	0.40
Isopoda	1.24	1.26	1.39
Carabidae	1.36	1.65	0.22
Staphylinidae	2.55	2.42	0.16
Other Coleoptera	0.59	0.20	0.42
Formicidae	0.65	0.58	0.66
Total	0.86	0.67	0.55

Plots were grouped and successively analysed as a unique sample.
No ratios are disposable for Opiliones because of any individuals were collected before the treatments.
Data about Staphylinidae were not significant because of the collection of very scarce populations.

III. CONCLUSION

The results obtained at canopy level were in some cases different from results obtained at soil level, showing different responses of arthropods communities to treatments according to both their behavioural features and the properties of their habitat.

Two applications (approximately 5 week interval) of 5 kg Surround WP Crop Protectant/hL reduced the abundance of arthropods at canopy level, but it preserved a good Coenotic Balance (CB) among trophic guilds and had no impact on the soil arthropod communities. On the canopy, only, Lepidoptera were unaffected by the kaolin spraying, and for the soil, no taxa seem to be significantly affected. This could be due to the interference between kaolin particle film and the feeding strategies utilised by pollinators, phytophagous and predators.

Reference:	KCP 10.3.2.4/14, Knight, A.L., Christianson, B.A., Unruh, T.A., 2001
Title:	Impacts of seasonal kaolin particle films on apple pest management
Report No.:	Published in: The Canadian Entomologist 133: 413-428
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	3, not guideline study, an efficacy study with random sampling and no aim in evaluating potential recovery

Executive summary

The impact of multiple applications (7 to 10 applications of 56 kg/ha) of the kaolin-based particle film, M96-018, on the population density of selected pests of apple, *Malus domestica*, and their natural enemy populations were measured in three Washington State orchards from 1997 to 1999.

Densities of the pest *Phyllonorycter elmaella* were significantly higher and percent parasitism was generally lower in treated plots than in untreated plots. The effect of M96-018 on green aphid pests *(Aphis* spp.) was variable between orchards; however, populations of rosy apple aphid, *Dysaphis plantaginea* were higher in treated plots than in untreated plots. M96-018 reduced the mean density of the pest *Typhlocyba pomaria* and the density of non-target spiders (Araneae) compared with untreated plots. Fruit injury by the pests *Cydia pornonella* and *Archips argyrospilus* was significantly reduced in the treated plots compared with the untreated plots. The effect of M96-018 on the non-target predatory bug *Campylomma verbasci* was variable between orchards and years. Stink bug density and related fruit injury were unaffected by M96-018. Fruit infestation by the pest *Quadraspidiotus perniciosus* was significantly higher in treated plots than in untreated plots. Discontinuing applications of M96-018 at mid-season reduced the number of aphid-infested leaves and increased fruit injury by leafroller compared with a full-season program. No differences in pest densities occurred in plots treated for one *versus* two consecutive years; however, the density of the apple orchard pest, phytophagous mites, was significantly higher the subsequent year after plots were treated with M96-018.

The seasonal use of M96-018 had a range of impacts on apple pest management in tested orchards during this 3-year study. Decline in non-target arthropods numbers were reported in the treated crops samples on the day to approximately 3 weeks post an application. Data was not presented to determine if recovery occurred since last application in 1998 to first application in 1999. As the objective of the study was to evaluate the efficacy of kaolin on orchard pests, it can not be concluded if there are irreversibkle adverse effects on beneficial.

According to the Klimisch score, the literature reference is concluded to be:

Relevance = 3 not guideline study, efficacy information, recovery of any tested arthropods not considered

Reliability = 3 (reliable with restrictions), not known to be GLP, rational for sampling periods unclear

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: M96-018

Batch number: Not reported. Product manufactured by Engelhard Corporation, NJ, USA

Content of a.s.: Kaolin-based particle film

Appearance: White fine-grained aluminosilicate mineral

2. Vehicle: 0.04 L methanol/L

3. Reference: None reported

4. Observed organism

Pests:

- *Phyllonorycter elmaella* (Doganlar and Mutuura) (Lzpidoptera: Gracillariidae), western tentiform leafminer
- *Aphis* spp. (Hemiptera: Aphididae), green aphids
- *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae), rosy apple aphid
- *Typhlocyba pomaria* (McAtee) (Hemiptera: Cicadellidae), white apple leafhopper
- *Cydia pornonella* (L.), codling moth
- *Archips argyrospilus* (Walker) (Lepidoptera: Tortricidae), fruittree leafroller
- *Campylomma verbasci* (Meyer) (Hemiptera: Miridae), mullein bug
- Stink bug (Hemiptera: Pentatomidae)
- *Quadraspidiotus perniciosus* (Comstock) (Hemiptera: Diaspididae), San Jose
- Phytophagous mites (Acari: Tetra*n*ychidae)
- European Red Mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae)
- Two-spotted spider mite
- *Typhlodromus occidentalis* (Nesbitt) (Acari: Phytoseiidae)

Non-target arthropods:

- Spiders (Araneae)
- Ants (Hymenoptera: Formicidae)
- Ladybird beetle larvae and adults (Coleoptera: Coccinellidae)
- Earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae)

Age at test initiation: Varied

Source: Washington State orchards from 1997 to 1999

5. Treatment: 56.0 kg product/ha

6. Test units: 1) 5 year old Fuji apple orchard (USDA orchard) on the US Department of Agriculture experimental farm,
2) 30 year old Red Delicious and Golden Delicious orchard (Garza orchard) near Moxee,
3) 8 year old Red Delicious orchard (Methow orchard) near Methow

7. Environmental conditions: Washington State, USA, climate

B. STUDY DESIGN AND METHODS

1. Application:
M96-018 was sprayed on apple trees at a rate of 0.03 kg plus 0.04 L methanol/L water. M96-018 was mixed separately with methanol and then added to water in the sprayer tank. Continuous agitation was required to keep the material in solution. Trees were sprayed to runoff and were visually inspected and resprayed to ensure complete coverage. Orchards were sprayed at a standard rate of 1866 L water/ha, which deposited 56.0 kg product/ha unless otherwise noted.

2. Effect of M96-018 rate on leafminer density:
Five replicates of three spray rates of M96-018 plus an untreated control were established with four-tree plots at the USDA orchard using a randomized complete block design. Spray rates used in this test were 0.015, 0.03, and 0.06 kg/L water and applied four times. Applications were made on 23 July, 6 and 22 August, and 5 September 1997.

3. Single-season study:
Ten replicated four-tree plots arranged in a completely randomized block design were established at the Garza Orchard using only 'Red Delicious' trees. M96-018 was applied at the standard rate on 10 dates during the season (applications made 3 March, 16 April, 19 May, 1, 10 and 23 June, 14 July, 1 and 17 August 1997).

4. Two-year study:
Twenty, four-tree plots spaced >6.0 m apart were established at the Methow Orchard. One half of these plots were randomly selected and treated with M96-018 and the other 10 plots were left untreated. M96-018 was applied to plots seven times. Following the last spray application, each of the four-tree plots treated with M96-018 were subdivided into paired, adjacent two-tree plots. One of each of the paired, two-tree subplots was randomly selected and sprayed with M96-018 three more times. The other pair was left unsprayed and was protected with a tarp on each of the spray dates.

The effects on apple pest management of a 2-year spray program of M96-018 and the potential effects in plots the year following the use of M96-018 were evaluated in the second year of the study. Five of the plots treated the first year were randomly selected to be sprayed with M96-018 during the following year and the other five were left untreated. In addition, five four-tree plots were randomly selected from among the 10 unsprayed plots previously monitored. These were treated with the standard M96-018 rate in the second year of the study. M96-018 was applied at the standard rate 10 times through the season. Applications made 26 March, 9 and 23 April, 6 and 20 May, 2 and 17 June 1998 the first year and 31 March, 17 April, 1, 18 and 31 May, 20 June, 10 July, 8 and 31 August, 12 September 1999 the second year. The five remaining plots were left untreated for a second year.

5. Arthropod sampling methodology:
Western tentiform leafminer, *Phyllonorycter elmaella*, was sampled in all studies by selecting four or five leaves per tree (third leaf down the shoot from the leaf visually selected) and counting the number of active tissue feeding mines. Percent parasitism of leafminers was recorded by inspecting 10-20 mines per replicate in the laboratory. Leafminers were sampled on 8 September 1997 in the USDA orchard (3 days after

application); 11 June (day post-application), 15 July (5 days post application), and 24 August (6 days post-application) 1998 in the Garza orchard; and 2 June (same day as application), 8 July (approximately 2 weeks post-application), and 26 August (approximately 5 weeks post-application) 1998 and 7 September (approximately 1 weeks post-application) 1999 in the Methow orchard.

Beating-tray samples (2025 cm^2) were conducted to assess populations of a number of arthropods. Data were summed across all trees within a replicate, and data are reported as the mean number per beating tray. Beating-tray samples were taken in the Garza orchard on 22 May (3 days post-applicsation) 1998 and in the Methow orchard on 6 and 20 May (day of application for both sampling periods), 22 July (approximately 3 weeks post-application), and 26 August (approximately 5 weeks post-application) 1998; and on 8 May (approximately 1 week post-application), 6 June (approximately 3 weeks post-application), 1 and 19 July (approximately 2 weeks post application for both sampling periods), 7 and 22 August (approximately 1 month and 2 weeks post-application, respectively), and 5 and 19 September (approximately 1-2 weeks post-application) 1999.

Fruittree leafroller, *Archips argyrospilus* populations were sampled near petal fall in the Methow orchard by visually inspecting four upright, vegetative shoots per tree for the presence of leafroller larvae. Sampling was conducted on 20 May 1998 and 17 May 1999

The population density of green aphids was assessed by counting the number of leaves infested. Five shoots per tree were cut from each plot. A leaf with one or more live aphids was considered infested. The population density of the rosy apple aphid, *Dysaphis plantaginea* was estimated by counting the number of infested shoots per tree in a 3-minute visual sample in the Garza orchard. Aphid populations were sampled in the Garza orchard on 7 June 1998; and in the Methow orchard on 2 and 17 June, 8 July, and 19 August 1998, and 30 May, 20 June, 15 July, 7 and 22 August, and 5 September 1999. The population density of the rosy apple aphid, *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae), was esti- mated by counting the number of infested shoots per tree in a 3-min visual sample on 14 July 1998 in the Garza orchard.

Egg, nymph, and adult densities of white apple leafhopper, *Typhlocyba pomaria* were estimated in the Methow orchard in both years. The number of overwintering eggs was counted from one 10 cm shoot per tree (four shoots per replicate) randomly collected from year-old wood on 26 March 1998 and 22 April 1999. The density of nymphs was sampled by visually inspecting both sides of 10 leaves per tree on 20 May 1998 and 30 May 1999. The combined nymph and adult density of the second generation was sampled with a beating tray on 26 August 1998 and 22 August 1999. Leafhopper populations were also sampled in the Garza orchard with a beating tray on 22 May 1998.

The population densities of the following orchard pests, two-spotted spider mite, European Red Mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae), and predatory mite, *Typhlodromus occidentalis* (Nesbitt) (Acari: Phytoseiidae), were assessed in the Methow orchard with a leaf-brushing machine. Ten leaves were collected randomly from each tree within each replicate and the 40 leaves per replicate were brushed together onto a glass plate covered with a film of detergent on 17 June and 28 August 1999. The number of immature and adult motile stages for *T. urticae* and *P. ulmi* were combined and reported as the number of tetranychid mites per leaf. Counts of predatory mites in the leaf-brushing samples were low across all treatments on both dates (<1 mite per 40-leaf sample), and these data were not analyzed.

6. Fruit injury assessment:
Fruit injury caused by a number of pests was assessed prior to harvest in both the Garza and Methow orchards. Fruit injury by codling moth was assessed in the Garza orchard on 5 September 1998 by visually inspecting 10 fruit high and low in the canopy from each tree (80 fruit per replicate). Fruit injury in the Methow orchard was assessed in both years by inspecting 100 fruit at random from boxes of fruit picked from each replicate. Fruit injury in the Methow orchard was characterized as feeding injury by leafroller, fruitworm, mullein bug, and stink bug. Fruits infested by San Jose scale, *Quadraspidiotus perniciosus* were also recorded. Fruit injury caused by the stink bugs *Euschistus conspersus* Uhler and *Acrosternum hilare* (Say) (Hemiptera: Pentatomidae) was similar in appearance to a physiological disorder, bitter pit. Fruits

were scored with stink bug injury when this type of injury occurred on the upper rim of the apple.

4. Statistics:
All count and proportion data were square root [$(x \pm 0.01)^{0.5}$] arcsine square root [$\arcin(x)^{0.5}$] transformed, respectively, before conducting unpaired *t* tests and ANOVA. Means were separated with the Neuman-Keuls multiple comparison test where significant differences occurred ($P < 0.05$). Data were analyzed with GraphPad Prism 3.0 (GraphPad Software, Inc 1999).

II. RESULTS AND DISCUSSION

1. Effect of M96-018 rate on leafminer density:
Plots treated with M96-018 had higher leafminer densities and lower parasitism of leafminers than the untreated plots in the Moxee orchard. Leafminer density per leaf was highest on trees treated with the lowest M96-018 rate and was significantly lower on trees treated with the highest *versus* the lowest rate, and significant, different from untreated plots. The number of leafminer per leaf were 0.71, 3.51, 2.50 ad 2.00 in the untreated, 0.015, 0.030 and 0.060 kg M96-018/L plots, respectively. No difference in the level of parasitism occurred among the three M96-018 treatments (74.3, 25.0, 18.0 and 13.7% larval parasitism in the untreated, 0.015, 0.030 and 0.060 kg M96-018/L plots, respectively).

2. Single-season study:
The population density of a number of apple pests and natural enemies assessed with beating-tray samples differed on trees treated with 10 sprays of M96-018 *versus* trees left untreated in the Garza orchard during. The mean density of leafhoppers and green aphids was significantly higher and the density of spiders was lower on trees treated with M96-018 than on untreated trees. No significant difference in the density of mullein bug was found between treatments. The mean number of green aphid infested leaves per shoot and the number of rosy apple aphid infested shoots per tree were significantly higher in plots treated with M96-018 than in unsprayed plots. Leafminer larval density was low during the first generation, and no difference between treatments was found on the June sampling date. The density of leafminers, however, was significantly higher for both second and third generations on trees treated with M96-018 than on untreated trees. Percent parasitism of leafminers was low in the plots treated with M96-018 during the second generation and was significantly higher in the untreated plots than in the plots treated with M96-018 during the third generation. Fruit injury by codling moth was significantly lower in plots treated with M96-018 than in untreated plots.

2. Two-year study:
Spiders (Araneae) were the most abundant and consistently present predator sampled with the beating-tray method in the Methow orchard during 1998 and 1999. Spider numbers increased in the untreated plots throughout the season but were lower in the plots treated with M96-018 both years. Counts of spiders in the beating-tray samples in plots treated with only seven (M96-018 x 7) applications were not different from those in plots treated with either 9 or 10 sprays. The density of spiders did not differ in plots treated with M96-018 for one or two consecutive seasons. Data was not presented to determine if recovdery occurred since last application in 1998 to first application in 1999.

Several other groups of generalist predators were present at moderate levels in beating-tray samples and their population densities were often lower in the plots treated with M96-018 than in the untreated plots. For example, the mean densities of ants (Hymenoptera: Formicidae) and ladybird beetle larvae and adults (Coleoptera: Coccinellidae), and earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae) were lower in trees treated with M96-018 than in untreated trees.

Note that sampling occured on the day of a spray treatement or approximately 3 weeks post a treatment.

The proportion of leafminer larvae parasitized during the first generation was significantly lower in the M96-018 treatment than in the untreated control during 1998. During the second and third generations, however, the mean proportion of mines parasitized among treatments differed by less than twofold. Larval parasitism during 1999 was significantly lower in the M96-018 (1999) **x** 7 plots than in either the M96-018 (1998) or untreated plots.

3. Pest population:
The density of first-generation (June) leafminers in 1998 was lower in the M96-018 treatment than in the untreated control. In contrast, leafminer densities were higher on trees treated with M96-018 than on untreated trees during both the second (July) and third (August) generations. Leafroller larval populations were lower in the plots treated with M96-018 than in the untreated plots.

The density of overwintering leafhopper eggs did not differ among treatments in either year, but M96-018 treatments significantly reduced the density of nymphs per leaf (May 1998 only) and nymphs and adults compared on the untreated trees. No difference in leafhopper density occurred in 1998 between trees treated with seven applications and those treated with 10 applications. During 1999, no difference in leafhopper population density was found between trees treated with either M96-018 treatment and those left untreated in both years.

There were no differences among treatments in the density of pentatomid bugs *E. conspersus* and *A. hilare* and (or) the mullein bug, *Campylomma verbasci*. Mullein bug populations, however, in 1999 were significantly lower in the plots treated with M96-018 than in the untreated plots. No difference in mullein bug densities was found in plots treated for one or two consecutive years of M96-018 treatment.

During 1998, significant differences in the number of green aphid were found among treatments at mid-season (July) and late season (August) sampling dates. Aphid infestations at mid-season were higher in the plots treated with M96-018 than in the untreated plots, and in August were significantly higher in the M96-018 x 10 plots than in either the untreated or the M96-018 x 7 plots. In contrast, during 1999 no differences in aphid densities were detected among treatments.

No difference in mite densities were found among treatments in June; however, tetranychid mite densities were significantly higher in the M96-018 (1998) treatment than in other treatments for samples collected in August.

4. Fruit injury:
Fruit injury by leafrollers, San Jose scale, and mullein bug varied among treatments in both years.

III. CONCLUSION

The seasonal use of M96-018 had a range of impacts on apple pest management in tested orchards during this 3-year study. Decline in non-target arthropods numbers were reported in the treated crops samples on the day to approximately 3 weeks post an application. Data was not presented to determine if recovery occurred since last application in 1998 to first application in 1999. As the objective of the study was to evaluate the efficacy of kaolin on orchard pests, it can not be concluded if there are irreversibkle adverse effects on beneficial.

Reference:	KCP 10.3.2.4/15, Pascual, S., Cobos, G., Medina, P., Budia, F., Viñuela, E., González-Núñez, M., 2010b
Title:	Field assessment of effects of control strategies against the olive fruit fly (*Bactrocera oleae* (Rossi)) on predatory arthropods: comparison of different methods of data analysis
Report No.:	Published in: Pesticides and Beneficial Organisms IOBC/wprs Bulletin vol 55: 11-18
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, aim of the study was to determine sensitivity of two analytical methods, not guideline study and no toxicity endpoint reported

Executive summary

Numbers of predators captured in a one-year field trial in a Spanish olive grove that received different treatments to control the olive fly (*Bactrocera oleae* (Rossi)) were analysed with two different statistical analysis methods. The treatments were kaolin (Surround WP, at 2 x 3 kg/100 L), trichlorfon bait spray as a positive control and unsprayed control. Two applications (10 week interval) of kaolin and one bait spray were done in July and September, and fourteen samplings were carried out using a beating method (5 samples before application between January and June; and 5 samples after the first application and 4 samples after the second application, with the last sample approximately 2 months after the last application in late November).

Two methods of analysis were used: Analysis of variance (ANOVA) and Principle Response Curve analysis (PRC). One-way ANOVA was carried out on total numbers of predators captured at each sampling date and two-way ANOVA was carried out on numbers of specimens of the most abundant taxa, with “treatment” and “sampling date” as factors. Changes in abundance of the different taxa of predators were also investigated using the multivariate method Principle Response Curves (PRC) analysis.

Results/conclusions based on one-way ANOVA and PRC analysis were similar, as both showed a significant decrease of the predatory arthropod community in the kaolin treated trees (on day of application and up to 10 weeks). PRC shows the effect of kaolin on the community of predators in an easier and quicker way, but only allows comparisons between two treatments. Also, PRC gives a global result for the whole season while by using ANOVA it is possible to pinpoint dates in which the effect of treatment is significant.

Both PRC and two-way ANOVA identified the coccinellid *Scymnus mediterraneus* Iablokoff-Khnzorian and the spider family Philodromidae as the taxa the most affected by kaolin. For other, less numerous predatory groups it seems that in general PRC analysis was able to detect effects of the kaolin treatment which were not revealed by two-way ANOVA analysis.

Sampling ended after 10 weeks post last application. According to the findinfs, population numbers were increasing but were still statistically different compared to the untreated control.

According to the Klimisch score, the literature reference is concluded to be:

Relevance = 2 method development study, not guideline study for data collection

Reliability = 2 (reliable with restrictions), not known to be GLP, analysis not completed (further analysis between kaolin and positive control needed), hence not reliable for use in a qualitative risk assessment.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Surround WP
   Batch number: Not reported.
   Content of a.s.: 95% Kaolin
   Appearance: Fine-grained clay

2. **Vehicle:** Water

3. **Reference:** Trichlorfon bait spray (Trichlorfon + protein hydrolysate (Nulure)

4. **Observed organism**
   Species: Predatory arthropods
   Age at test initiation: Varied
   Source: Olive grove in Madrid

5. **Treatment:** 3 kg/100 L (30 kg/ha)

6. **Test units:** Olive grove in Madrid, Spain

7. **Environmental conditions:** Mediterranean, climate

B. STUDY DESIGN AND METHODS

The aim of this work was to compare results and conclusions obtained from different types of analysis applied to the same dataset and to identify their advantages and disadvantages.

1. Application:
The field trial was conducted in a 4.0 ha olive grove in Madrid, Spain. Three different treatments were compared: kaolin, trichlorfon bait spray used as positive control and unsprayed control. Each treatment was applied to an area of about 0.8 ha and experimental plots were randomly located in the olive grove.

Two applications of Surround WP (kaolin 95%, Engelhard Corp., Iselin, New Jersey, USA) were done at 3 kg/100 L, covering the total foliage of the olive trees (on 8 July and 16 September). One treatment of trichlorfon bait was sprayed on 2-3m^2 spots on the south-eastern face of the tree, using two component mixture in water: Dipagrex 80 (trichlorfon 80%, DEQUISA, Paterna, Valencia, Spain) at 5g/L plus the protein hydrolysate Nu-Lure (Miller Chemical & Fertilizer Corp., Hanover, Pensylvania, USA) at 10g/L. In the untreated control plot, no measures against *B. oleae* were applied and only one treatment with *Bacillus thuringiensis* (Biobit XL) was applied to all test plots when the populations of *P. oleae* reached the treatment threshold.

2. Monitoring:
Predatory arthropod fauna of the canopy of olive trees was monitored periodically using a beating method. In every sampling, four olive trees were randomly chosen per plot and four branches per tree (one in each orientation) were beaten three times. A total of 14 samplings were done during the year of study, starting from mid July to December.

4. Statistics:
Numbers of predators captured were analysed by two different methods: Analysis of variance (ANOVA) and Principle Response Curve analysis (PRC).

One-way ANOVA ($P \leq 0.05$) was carried out on total numbers of predators captured at each sampling date and when differences were detected means were separated by a HSD Tukey test. Also, a two-way ANOVA was applied on numbers of specimens of the most abundant taxa of predators (accounting for at least 1.5% of the total predators captured), with "treatment" and "sampling date" as variable factors. ANOVA tests were performed using the software Statgraphics Centurion XV (StatPoint, 2005).

Changes in abundance of the different taxa of predators were also investigated using the multivariate method PRC analysis. This method summarizes the effect of treatments on the abundance of all sampled taxa on every sampling date and also generates "Taxon Weights" values that express how closely each taxon´s response fit the PRC and thus, which are the most affected taxa. PRC analysis was carried out using the program CANOCO 4.51 (Biometris, Plant Research International, Wageningen, The Netherlands) which detects significant differences between curves by means of an F-type permutation test (Monte Carlo simuation, 499 permutations in this study) and provides the parameters necessary to generate PRCs (canonical coefficients) and "Taxon Weights". Species were used for PRC analysis, but when identification was not possible, wider taxonomic groups were considered. PRCs for Surround WP-treated trees and for the positive control (trichlorfon bait spray) in relation to the untreated control were obtained for the year of sampling. To determine the effects of Surround WP on different taxa, "Taxon Weights" and PRCs for Surround WP relative to the untreated control were calculated. Values of taxon weight between 0.5 and –0.5 are likely to show either a weak response or a response that is unrelated to that shown in the PRC and therefore are not considered significant. Data on the number of captures of each taxon were transformed to ln(x+1) prior to analysis.

II. RESULTS AND DISCUSSION

A total of 2005 species were captured. From the total, 764 were from the control plot, 369 from kaolin treated plot and 873 from trichlorfon bait spray plot.

Results from the one way ANOVA and the PRC analysis applied to the total number of predators captured along the season both follow a similar pattern and show a deleterious effect of kaolin sprays on populations of predators. In the case of ANOVA, the analysis allows one to pinpoint at which sampling dates there are significant differences amongst treatments. Numbers of predators captured in the olive trees treated with kaolin were lower than those from the untreated control and trichlorfon plot in five dates and 7 dates compared to untreated control, only. Numbers in the treated trees (kaolin and trichlorfon) dropped after sprays were applied. As indicated in the graph below significant difference in kaolin treated plot versus untreated were on day of application and noted approximately 10 weeks post application.

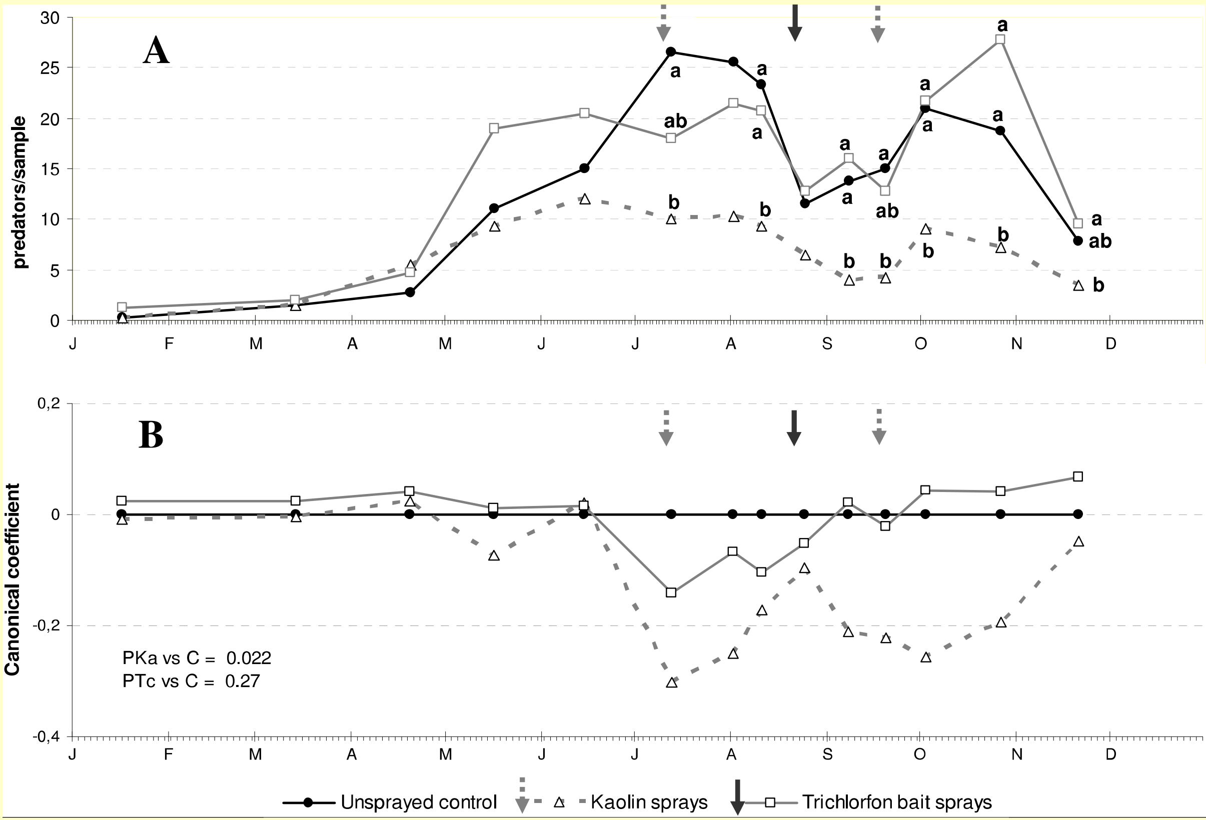

Figure 10.3.2.4/15-1: Assessment of predator populations from plots treated with trichlorfon, kaolin or untreated.
A) One-way Anova, mean numbers of predators captured in different treatment plots. Different letters in a sampling date indicate significant difference (P≤0.05, HSD Tukey).
B) PRC analysis showing the effect compared to an untreated control (y = 0 line). The P values denote the significance of each treatment relative to control based on an F-type permutation test (Monte Carlo simulation, 499 permutations)

The PRC method only gives information on significant differences between treatments compared to a single control, along the whole season. The results indicate that kaolin treatment caused a significant reduction in numbers of predators compared to the untreated control, while trichlorfon treatment did not affect these numbers. Further PRC analysis would be needed to know whether the effect of kaolin differs from the effect of trichlorfon, which is set in this field trial as a positive control.

PRC analysis uses the whole dataset and therefore is more robust than an ANOVA analysis carried out on data from a single sampling date. This could mean that for treatments with only a slight effect on predator populations, these effects would be undetected by one way ANOVA but not by PRC analysis. The usefulness of identifying the dates at which reductions are significant (one-way ANOVA) is to make sure that those reductions are not due to environmental factors but a consequence of treatment applications. It allows one to determine population recovery after treatments.

Based on taxon weights from the PRC analysis comparing kaolin versus untreated control plot, and the results from two-way ANOVA analysis on the most numerous groups of predators, both methods indicate that the main taxa of predators affected by kaolin treatment were the coccinellid, *S. mediterraneus* and the spiders belonging to the family Philodromidae. Other important groups of predators are shown by the PRC analysis as groups affected by the kaolin treatment because their taxon weights are positive and higher than 0.5, while the ANOVA analysis did not detect significant differences between control and kaolin treatment.

Some of these groups are for example chrysopids, *Stethorus punctillum* Weise, *Araniella cucurbitina* (Clerck) or *Brachynotocoris ferreri* n. sp. Baena (*in litteris*). Thus, for these groups it seems that PRC analysis is more sensitive than ANOVA.

III. CONCLUSION

Results from the one way ANOVA indicated that the numbers of predators captured in the olive trees treated with kaolin were lower than those from the untreated control and trichlorfon plot in five dates and 7 dates compared to untreated control, only. Numbers in the treated trees (kaolin and trichlorfon) dropped after sprays were applied. Significant difference in kaolin treated plot versus untreated were on day of application and noted approximately 10 weeks post application. No further sampling occurred after 10 weeks.

The PRC method only gives information on significant differences between treatments compared to a single control, along the whole season. The results indicate that kaolin treatment caused a significant reduction in numbers of predators compared to the untreated control, while trichlorfon treatment did not affect these numbers. Further PRC analysis would be needed to know whether the effect of kaolin differs from the effect of trichlorfon, which is set in this field trial as a positive control.

Reference:	KCP 10.3.2.4/16, Sánchez-Ramos, I., Marcotegui, A., Pascual, S., Fernández, C.E., Cobos, G, González-Núñez, M., 2017
Title:	Compatibility of organic farming treatments against *Monosteira unicostata* with non-target arthropod fauna of almond trees canopy
Report No.:	Published in: Spanish Journal of Agricultural Research 15(2), e1004
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study, comparison test for efficacy

Executive summary

The aim of this study was to evaluate the effects observed on the non-target arthropod fauna of the almond trees canopy in fields treated with 1 to 2 applications of kaolin at 5 kg/100 L. An untreated control was included in the trial, along with a second test item, potassium salts of fatty acids combined with thyme essential oil (PSTEO).

After treatment application (timeframe not specificed, presumable 2-3 months), a significant reduction in the abundance of natural enemies in 2009 and 2010 and in the abundance of other arthropods in 2010 compared to the control plots. A significant reduction in the Shannon diversity index and in the number of species was observed in the kaolin plots compared with the control plots in both years except for the Shannon index in 2010 and no effect was observed for kaolin treated plots compared to the control in 2009, but a significant effect was observed in the kaolin-treated plots for the other non-target arthropod community in 2010, after treatment. No differences in the community composition of non-target arthropods were found among treated and control plots before treatment application either in 2009 and 2010. After treatment application, no effect was observed for kaolin treated plots compared to the control in 2009, but a significant effect was observed in the kaolin-treated plots for the other non-target arthropod community in 2010. Prior to treatments, specifically in 2010, there was no difference between control and kaolin-treated plots. Arthopods evaluated were: phytophagous, spiders, parasitoids, other arthropods (Arachnida, Entognatha and Insecta, with Thysanoptera most abundant group).

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study, more of an efficacy trial
Reliability = 2 (reliable with restrictions), not known to be GLP.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Surround WP

Batch number: Not reported. Product manufactured by BASF Aktiengesellschaft, Germany)

Content of a.s.: Kaolin 95% w:w (wettable powder)

Appearance: Not reported.

2. Vehicle: Not reported

Reference item: Potassium salts of fatty acids combined with thyme essential oil (PSTEO): OleatBio-to (40% w:w soybean and sunflower fatty acids; 5% w:w potassium salts; 6% w:w thyme essential oil) (TRABE S.A., Murcia, Spain)

3. Test organism

Species: Arthropods found in almond orchard in Spain

Age at test initiation: Varied

Source: From commercial almond orchards in Murcia, Spain

4. Treatment: 5 kg/100 L

5. Environmental conditions: Mediterranean climate

B. STUDY DESIGN AND METHODS

1. Application:

Field trials were conducted in 2009 and 2010 in commercial almond orchards in Murcia, Spain. Surround WP was sprayed at a dose of 5 kg/100 L. Two applications were given each year: the first one in mid-spring and the second one in early summer, to guarantee coating until the end of the crop season. Potassium salts of fatty acids combined with thyme essential oil (PSTEO) was sprayed at a dose of 300 mL/100 L, but additional details of this treatment group are not summarised here as not relevant to the risk assessment of kaolin. Concurrently, an unsprayed control was included in the trial.

The experimental design consisted of randomised blocks with four (2009) or seven (2010) replications. Within each block, four contiguous trees of each cultivar were randomly assigned to each treatment.

2. Sampling:

The overall arthropod fauna from the canopy of almond trees was sampled using a beating method. Beating sampling was performed monthly in spring and summer, with a total of five and six sampling dates in 2009 and 2010, respectively. Samples were taken to the laboratory and the specimens were assigned to the following groups: 1) natural enemies, 2) phytophagous or 3) other arthropods. This work focuses on non-target arthropods, *i.e.* groups 1 and 3. Biodiversity was assessed by the number of morphospecies and the Shannon biodiversity index.

3. Statistics:

The effect of the factors considered on the number of individuals captured, the number of species and the Shannon biodiversity index was tested by linear mixed-effects models. Interactions among all fixed factors were considered in the models. The best covariance structure for the repeated-measures (date) factor was selected according to the lowest value of the Akaike and Schwarz's Bayesian information criteria fit statistics. The models were fitted using a restricted maximum likelihood estimation method. If convergence was not achieved or the final Hessian matrix was not positive definite, the random factor was removed from the model as it was identified as redundant variable. When necessary, data were previously transformed by ln(x+1) for normality. The significance level was always $p < 0.05$. Statistical tests were performed using

SPSS statistical program.

Analyses were performed for the periods before and after the first treatment application. In the first case, to verify the absence of significant differences among plots and in the second case, to examine the effect of the treatments on abundance and diversity of non-target arthropods. Differences in abundance and diversity of non-target arthropods among plots assigned to each treatment were evaluated separately against the untreated control in 2009 because of the different application schedule of kaolin and PSTEO. For 2010 data, differences with regard to the control were established by an LSD test when statistical significance was found.

To investigate changes in abundance and species composition of the non-target arthropod community in the canopy of almond trees, a principal response curve (PRC) analysis was performed using the program CANOCO 4.51. The significance of the deviations from the line representing the untreated control (y=0), because of each treatment, was tested using an F-type permutation test (Monte Carlo simulation) with 499 permutations. PRCs for kaolin and PSTEO plots in relation to the untreated controls were obtained before and after the first treatment applications for each year. Additionally, to determine treatment effects on different taxa, 'species weights' were also considered in those cases in which the PRCs were significant. Data on the number of captures of each taxon were transformed to $\ln(x+1)$ before analysis.

II. RESULTS AND DISCUSSION

1. Species diversity:

In the 2009 orchard, the great majority of arthropods captured were phytophagous potentially harmful on almond trees (87.5-90%), with the other groups appearing in very small proportions (predators (mostly spiders): 3.7-4.7%; parasitoids (immature Hymenopteran): 1.9-2.0%; other arthropods (Arachnida, Entognatha and Insecta, with Thysanoptera most abundant group): 4.4- 5.4%. In 2010, the situation was different, with phytophagous again as the dominant group (53.8-58.8%), but with the "other arthropods" group showing a much higher proportion (37.2-42.0%). Predators (3.1-4.0%) and parasitoids (0.6-1.0%) were again minor groups.

2. Effects of kaolin on non-target arthropods

Before the treatment applications in both years, no significant differences were found between kaolin and untreated control plots in abundance of non-target arthropods. After treatment application, it was found a significant reduction in the kaolin-treated plots in the abundance of natural enemies in 2009 and 2010 and in the abundance of other arthropods in 2010 compared to the control plots.

Concerning diversity and number of species of non-target arthropods, no significant differences were found between kaolin and the untreated control plots before treatment application both in 2009 and 2010. After treatment application, a significant reduction in the Shannon diversity index and in the number of species was observed in the kaolin plots compared with the control plots in both years except for the Shannon index in 2010.

No differences in the community composition of non-target arthropods were found among treated and control plots before treatment application either in 2009 and 2010. After treatment application, no effect was observed for kaolin treated plots compared to the control in 2009, but a significant effect was observed in the kaolin-treated plots for the other non-target arthropod community in 2010.

Table 10.3.2.4/16-1: Abundance, Shannon index and number of species per sample of non-target arthropods (natural enemies and other arthropods) captured by beating in almond trees before and after being sprayed with kaolin or potassium soap with thyme essential oil (PSTEO) and in the untreated trees

Plots	Natural enemies		Other arthropods		Shannon index		Number of species	
	Before	After	Before	After	Before	After	Before	After
2009								
Control	5.6	11.6	7.6	6.7	1.7	2.0	7.9	12.2
Kaolin	6.9	8.7*	7.4	5.0	1.7	1.8*	8.1	9.3*
2010								
Control	3.7	6.1	77.6	13.4	1.1	1.9	6.1	9.3
Kaolin	5.0	4.7*	92.3	3.7*	1.3	1.6	8.1	5.9*

*Significant differences compare with control

In those cases, where the PRC analysis was significant, the contribution of different taxa to non-target arthropod community response in the treated plots is revealed by the species scores obtained. Taxa with a positive weight over 0.5 are expected to decrease in abundance compared to the control after treatment application. For the effect of kaolin on other non-target arthropods in 2010, the affected taxa were (in decreasing order of effect) Melandryidae (3.3), Curculionidae (2.8), Formicidae (2.4), Psocoptera (2.0), Thysanoptera (1.4), Issidae (0.6), Phalacridae (0.6) and Anthicidae (0.5). On the other hand, taxa with negative weights below -0.5 in the PRCs are expected to increase after treatment application. According to that, Tettigoniidae (-0.5) abundance increased after the kaolin treatment in 2010.

Table 10.3.2.4/16-2: Significance of PRC analyses on the community

Year	Group	Period	Comparison			
			Global		Kaolin vs control	
			F ratio	*p* value	F ratio	*p* value
2009	Natural enemies	Before	-	-	1.140	0.7420
		After	-	-	1.438	0.5420
	Other arthropods	Before	-	-	1.189	0.5520
		After	-	-	1.277	0.7300
2010	Natural enemies	Before	1.987	0.3620	-	-
		After	3.691	0.0020*	2.070	0.0640
	Other arthropods	Before	3.129	0.1260	-	-
		After	6.393	0.0040*	5.309	0.0020*

*Significant differences compare with control

III. CONCLUSION

In this work, some adverse effects of kaolin on non-target arthropod fauna was observed (approximately 2-3 months post treatment, but timeframe not reported). Effects prior to trearment were comparable to control. Potential for recovdery could not be determined based on the limited timeline of the data collection.

Reference:	KCP 10.3.2.4/17, Sackett, T.E., Buddle, C.M., Vincent, C., 2007
Title:	Effects of kaolin on the composition of generalist predator assemblages and parasitism of *Choristoneura rosaceana* (Lep., Tortridae) in apple orchards
Report No.:	Published in: J. Appl. Entomol. 131(7): 478-485
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study, no toxicity value, quantitative information only

Executive summary

Kaolin clay particle film, Surround WP, was applied in three apple orchards to determine if applications affected the diversity of generalist arthropod predator assemblages in orchard foliage and the parasitism of the pest species *Choristoneura rosaceana*.

In two orchards, kaolin was applied to orchard foliage once a week for 4 weeks, between mid-June and mid-July in 2004 and 2005 at a rate of 6 kg/100L and 1000 L/ha (60 kg/ha). In the third orchard kaolin was applied to foliage twice over 2 weeks in June 2004 at a rate of 450 L/ha (27 kg/ha). The proportion of larvae *C. rosaceana* parasitized, larval populations, and the relative abundance and assemblage composition of generalist predators (spiders and insects) in the orchards was quantified.

Kaolin altered the species composition of the generalist predator assemblages and reduced the relative abundances of certain generalist predators, most notably jumping and crab spiders (Salticidae and Philodromidae), assassin bugs (Reduviidae), ants (Formicidae) and coccinellids (Coccinellidae). In contrast, the relative abundances of web-spinning spiders (Araneidae, Dictynidae, Theridiidae) were not affected. Kaolin did not affect the proportion of parasitized *C. rosaceana* larvae, which ranged from 24% to 47% in control and kaolin treatments, or the relative proportions of parasitoid taxa. The kaolin formulation did not affect the abundance of the pest *C. rosaceana* larvae, but in one orchard, kaolin did reduce the abundance of the combined numbers of *C. rosaceana* and another tortricid pest, *Argyrotaenia velutiana*. Although kaolin does not affect parasitism of *C. rosaceana*, it significantly changes the composition of generalist predator assemblages in orchard foliage up to 1 month post last application.

In conclusion, Surround WP affected the diversity of generalist arthropod predator assemblages in orchard foliage and the parasitism of the pest species *Choristoneura rosaceana*.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study, no toxicity value, quantitative information only
Reliability = 2 (reliable with restrictions), not known to be GLP.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Surround WP
	Batch number:	Not reported. Product manufactured by Engelhard Corporation, NJ, USA
	Content of a.s.:	Kaolin, content not reported
	Appearance:	Clay particle film
2.	**Vehicle:**	Not reported
	Reference item:	None reported
3.	**Test organism**	
	Species:	Arthropods found in apple orchards in Quebec, Canada. Specific focus on

spiders (Araneae).

Age at test initiation: Varied

Source: From two orchards at the Agriculture and Agri-Food Canada Experimental Farm in Frelighsburg, Quebec and one commercial orchard near Mount St Hilaire, Quebec

4. **Treatment:** In Frelighsburg: 6 kg/100 L and 1000 L/ha (equivalent to 60 kg/ha)
In St Hilaire: 450 L/ha (equivalent to 27 kg/ha)

5. **Environmental conditions:** Northern American climate

B. STUDY DESIGN AND METHODS

6. **Application:**

The kaolin trial was made in three 15-year-old orchards during 2004 and 2005. These included two orchards at the Agriculture and Agri-Food Canada Experimental Farm in Frelighsburg, Quebec (McIntosh, semi-dwarf root stock), and one commercial orchard near Mount St Hilaire, Quebec (Cortland and McIntosh, standard rootstock). Effects of kaolin on generalist predators were tested in two orchards in Frelighsburg in 2004 and 2005, one orchard per year. The effects of kaolin on the parasitism of the pest *C. rosaceana* were determined in 2004 in one orchard in Frelighsburg and one orchard in Mt St Hilaire.

A fixed block design was used. In Frelighsburg, Orchard F1 (2004) was 0.5 ha, and was divided in 12 blocks, each with 24 trees (3 rows x 8 trees). Orchard F2 (2005) was 0.8 ha in size, and was divided into 12 blocks, each with 54 trees (3 rows x 18 trees). In Mount St Hilaire, Orchard H3 (2004) was 2 ha in size, and divided into six blocks of 45 trees each (3 rows x 15 trees).

There were two treatments in each orchard, a control treatment (no spray), and a kaolin treatment. In Frelighsburg, a rate of 4 x 60 kg/ha at a 1 week interval was used. Kaolin was applied within 4 days of first male flight, and re-applied once per week until at least after peak egg hatch. In both years, this resulted in four applications. In orchard H3 in 2004, kaolin was applied within several days of first male flight and applied only once the following week, before peak egg hatch at a rate of 450 L/ha (2 x 27 kg/ha with a 1 week interval).

2. **Sampling:**

Densities of generalist predators were quantified in orchards F1 and F2 in 2004 and 2005 *via* random collection using the beating method and all spiders (Araneae) from three trees (five branches/tree/10 beats). There were five collection dates, one after each application of kaolin, and the final collection 1 month after the final kaolin application. In Orchard F2 in 2005, four trees per block were sampled using the same technique, but all generalist predators were counted, including spiders, harvestmen (Opiliones), beetles (Coleoptera), ants (Formicidae), stink bugs (Pentatomidae) and assassin bugs (Reduviidae). As very few spiders were found from the first three collections of 2004, in 2005 there were only two collections: immediately after the fourth and last kaolin application, and 1 month after this kaolin application. Insect predators were identified to family, and spiders to family and species when possible. Spiders were also divided into three age classes: spiderlings (newly hatched spiders, classified as such when <1.5 mm in length), juveniles (immature spiders but not newly hatched) and adults. As beating was the only collection method, the samples were biased towards diurnal species, as well as those found in foliage, rather than on tree bark.

3. **Effects of kaolin on the pest *C. rosaceana* parasitism**

In 2004, larval *C. rosaceana* (approximately fourth instar) were sampled by visually searching branches and fruit from ground height to 2 m. In orchard F1, four trees per block (six blocks/treatment), 10 min search per tree. In orchard H3 (n = 3), 6 trees per block, 15 min per tree. Larvae of pest species of tortricids, which included mainly *C. rosaceana* but also *Argyrotaenia velutinana* (Walker) were counted. *Choristoneura rosaceana* larvae were brought back to laboratory to be reared, species confirmed and emergent endoparasitoids were collected. Parasitoids were classified to family, and the rate of parasitism and proportion of each family were compared in kaolin and control treatments.

4. Statistics:
The relative abundances of generalist predators, *C. rosaceana* larvae, and the proportion of larvae parasitized, were compared between control and kaolin treatments using ANOVA (PROC GLM). Relative abundance of generalist predator taxa was analysed using raw data, unless the data for a particular group were non-normal or had heterogeneous variances, in which case they were square root trans-formed. To compare proportion of larvae parasitized, raw data were arcsine-square root transformed before analysis. SAS V8 (SAS Institute 2000) and Statview V5 (SAS Institute 1998) were used for these statistical tests. The proportions of each parasitoid order and family in kaolin and control treatments were compared using Fisher's exact tests. On-line applets with expanded contingency tables were used for Fisher's exact tests, allowing analysis of tables with expected cell frequencies below 5, for which chi-square analysis is inappropriate.

Non-metric multidimensional scaling (NMDS) was used to examine patterns of generalist predator community composition (based on family data) and to see whether kaolin affected this composition. Data from 2004 and 2005 were analysed separately, and samples from each treatment block and collection date were analysed to determine if either treatment or time affected the assemblages. Non-metric multi-dimensional scaling was chosen over other ordination methods because it does not require linear relationships among variables, it does not limit configurations based on a pre-determined model, and its distance measure can be specified. PC-ORD V4 to perform NMDS was used twice.

To determine the statistical significance of the differences in predator composition between the treatments, non-parametric multi-response permutation procedures (MRPP) was used, using a Sorenson distance measure to correspond with the NMDS metric. Samples were grouped by treatment block and collection date, as with the NMDS. The P-value of the MRPP test statistic as well as the agreement statistic, A, which describes the within-group homogeneity as compared with random expectations, and reflects effect size.

II. RESULTS AND DISCUSSION

1. Effects of kaolin on generalist predators:
In 2004, 654 spiders were collected over the five sampling dates. The relative abundances of spiders in both plots in the first three sampling dates were several times lower than the fourth and fifth samples. There was a significant decrease in the relative abundance of total spiders in the kaolin plots after the fourth application of kaolin, but catch rates were no longer significantly lower 1 month after this final application in August. The increase in collected spiders on the fourth sampling was mainly caused by an increase in the number of spiderlings and immature spiders, as many orchard spider species reproduce during July. On this sampling date, both spiderlings and older spiders (includes juveniles and matures) were significantly lower in kaolin plots than control plots.

Table 10.3.2.4/17-1: Relative abundances (n=6) and total number of generalist predators collected immediately after (July) and 1 month after (August) the final kaolin application in orchards F1 (2004) and F2 (2005)

Species	July 2004		August 2004		July 2005		August 2005	
	Control	Kaolin	Control	Kaolin	Control	Kaolin	Control	Kaolin
Saltcidae	11.7	5.5*	10	6.3*	13	6.8*	10.3	4.7*
Philodromidae	5.7	2.3*	3.8	3.2	3.3	1.2*	2.3	1.8
Clubionidae	0	0	0.2	0.2	0.3	0.3	0.3	0
Thomisidae	0.2	0.5	0.3	0.3	2.2	1.3	1.5	0.7
Araneidae	1.0	1.2	1.8	1.0	4.7	2.3	5.3	4.2
Dictynidae	2.2	1.2	2.3	2.2	0.8	1.3	2.7	1.5
Theridiidae	2.3	0.8	1.8	0.8	1.8	0.7	1.3	0.8
Opilionidae	nc[2]	nc	nc	nc	0.8	0.3	0.3	0.2
Reduviidae	nc	nc	nc	nc	6.3	0*	7.5	0.2*
Pentatomidae	nc	nc	nc	nc	1.5	0.5	0.3	0.3
Formicidae	nc	nc	nc	nc	13.2	4.2	7.8	2.7*
Coccinellidae	nc	nc	nc	nc	0.7	0*	0.5	0.2
Total spiders	128	87	146	75	192	115	149	85
Total other	nc	nc	nc	nc	135	30	95	21
Grand total	**128**	**87**	**146**	**75**	**327**	**145**	**244**	**106**

*Statistically significantly different compared to the control
[2] Only spiders were collected in 2004
nc = not collected

Spiders were the most common generalist predators in the orchards, in both control and kaolin blocks. In 2005, from the two sampling dates pooled, spiders accounted for 59% of the total predators in the control treatment, and 80% in the kaolin treatment. Ants were the second most common taxa in both treatments, accounting for 22 and 16% of individuals in control and kaolin treatments, respectively. Most other groups (harvest-men, beetles and lacewings (Neuroptera)) accounted for <1% each of collected arthropods, except the assassin bugs (mainly *Zelus luridus* Stal), which were 15% of the predators in the control treatment, but <1% in the kaolin treatment.

7. *C. rosaceana* parasitism and density

The proportion of parasitized *C. rosaceana* larvae was not affected by kaolin in either orchard F1 (four kaolin applications) or H3 (two kaolin applications). In F1, the percent parasitism was 47% (11/23 larvae) in control plots and 37% (6/16 larvae) in kaolin blocks, and there was no significant difference between the treatments. In orchard H3, the percent parasitism was 44% (25/61 larvae) in control blocks and 24% (17/71 larvae) in kaolin blocks, and was not significantly different.

There was no significant effect of kaolin on *C. rosaceana* populations in either orchard. However, when *Argyrotaenia velutiana* was included in the analysis for F1 there were significantly fewer larvae of these two species in kaolin blocks as compared with control blocks.

III. CONCLUSION

The kaolin treatment significantly altered the species composition of generalist predator assemblages in the Frelighsburg orchards in both years and reduced the relative abundance of the most common families of spiders as well as important insect predators, such as assassin bugs, ants and coccinellids. There was a significant decrease in the relative abundance of total spiders in the kaolin plots after the fourth application of kaolin, but catch rates were no longer significantly lower 1 month after this final application in August. Kaolin did not affect the overall percent parasitism, or the composition of parasitoids, of *C. rosaceana* larvae.

Reference:	KCP 10.3.2.4/18, Showler, A.T, and Sétamou, M., 2004
Title:	Effects of kaolin particle film on selected arthropod populations in cotton in the lower Rio Grande Valley of Texas
Report No.:	Published in: Southwestern Entomologist, 29(2): 137-146
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study

Executive summary

In the following 2-year field trial, kaolin was applied 7 to 10 times at a rate of 42.3 L/ha to cotton (from mid-April until end of June) (7-14 day interval). Leaf counts and dvac sampling indicated that pest cotton aphid, *Aphis gossypii* Glover, populations increased in kaolin treated cotton plots compared to control plots, but cicadellid pest populations were suppressed. Populations of dipterans, *Orius* spp., and wasps were reduced in the kaolin treatments only on 1 of 20 sampling dates over the two seasons. Foliar kaolin spray had no effect on other arthropod groups identified in this study (silverleaf whitefly, *Bemisia argentifoli* Bellows and Perring; herbivorous hemipterans and coleopterans; thrips; lepidopteran larvae; *Geocoris* spp.; *Nabis* spp.; reduviids; coccinellids; *Collops* spp.; neuropterans; and spiders).

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2 (reliable with restrictions), not known to be GLP.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Surround (wettable powder)
Batch number: Not reported. Product manufactured by Engelhard Corporation, NJ, USA
Content of a.s.: >85% kaolin
Appearance: Bright white colour

2. Vehicle: Not reported
Reference item: None

3. Test organism
Species: Herbivorous arthropods
Age at test initiation: Varied
Source: Cotton field at the USDA-ARA Kika de la Garza Subtropical Agricultural Research Center, Texas

4. Treatment: 42.3 L/ha

5. Environmental conditions: Subtropical

B. STUDY DESIGN AND METHODS

1. Application:
Twenty-four randomized design plots, each 8.1 m wide (8 rows) x 15.2 cm long with a 1 m bare ground buffer between plots were used. The herbicide, pendimethalin, was used to control weeds at 925 g a.s./ha after planting. Kaolin suspensions were applied with a tractor-mounted boom sprayer at 42.3 L/ha. Treatments were reapplied weekly to eight plots and fortnightly to 8 plots for a year, starting mid-April until end of June. Each application consisted of two passes by the tractor to maximize coverage. Three

weeks after the first application each year, larger application nozzles were used to increase coverage. The remaining 8 plots were not treated (control). No insecticides were applied to any of the plots. Treatment occurred for 2 years.

2. Measurements:

Kaolin retention on cotton leaves at 4 hour, 1 week and 2 weeks after the first application the first year was measured on randomly selected fully expanded leaves collected from the biweekly treated plots.

A randomly selected leaf from among the top 6 fully expanded leaves on 50 different cotton plants was examined in each plot for cotton aphids on 7 separate occasions throughout the study (April to June). Other arthropods and cotton aphids were sampled using a dvac on several cotton plants (n = 8) at fortnightly intervals in April and June (i.e. within the period of applications).

3. Statistics:

Repeated measures analysis was used to detect significant differences between treatments and sampling dates, and interactions. Insect numbers were log(x+1)-transformed before repeated measures analysis. However, transformed means are presented (Analytical Software).

II. RESULTS AND DISCUSSION

1. Kaolin retention:

Mean particle density on leaves 4 hours after the first application was 360.0 ± 18.7 µg kaolin/cm^2 leaf surface. After 1 and 2 weeks in the field (with biweekly applications), particle densities were 319.9 ± 20.8 and 201.0 ± 13.2 µg kaolin/cm^2, respectively.

2. Findings:

Kaolin effects on pest aphids were highest in the weekly kaolin treatment in late April of both years when populations were high.

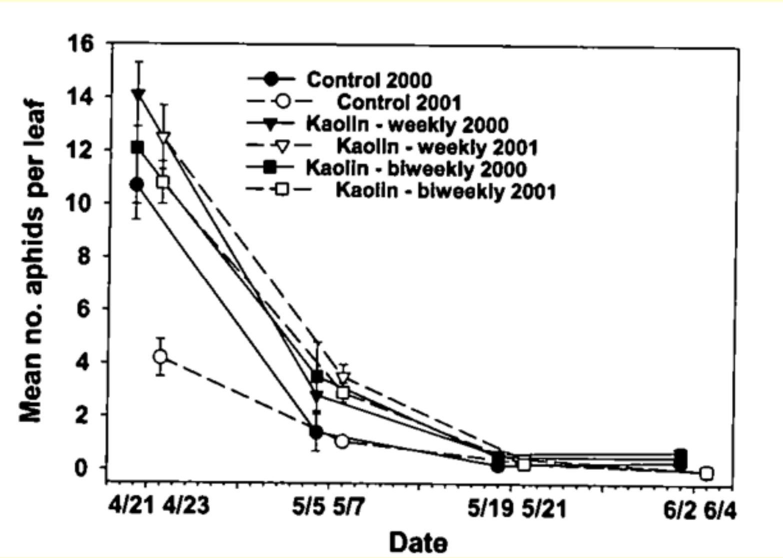

Figure 10.3.2.4/18-1: Mean number of pest cotton aphids per leaf (n=400)

Herbivorous arthropods counted in dvac samples included pests and non-target (beneficials), including aphids, silverleaf whitefly (*Bemisia argentifoli* Bellows and Perring), cicadellids, hemipterans (mostly mirids, coreids and pentatomids) and coleopterans (mostly elaterids and anthicids), dipterans (mostly drosophilids, cecidomyiids and muscids), thrips and lepidopteran larvae. Natural enemies were comprised of *Geocoris* spp., *Orius* spp., *Nabis* spp., reduviids; coccinellids, *Collops* spp., neuropterans, wasps and spiders.

Kaolin sprays significantly decreased mean numbers of cicadellids and dipterans the first year. In the second year, kaolin significantly increased aphids, but reduced the mean number of cicadellids, dipterans, *Orius* spp., and wasps. The effects of time were significant for 16 and 15 of the 18 arthropod groups in the first and second year, respectively. Only Collops bettles were not affected over time, but mean populations were always low. Interactions between treatment and time effects were detected for collected pest aphid populations in the first year, and for aphids, dipterans, *Orius* spp., and coccinellids in the second year.

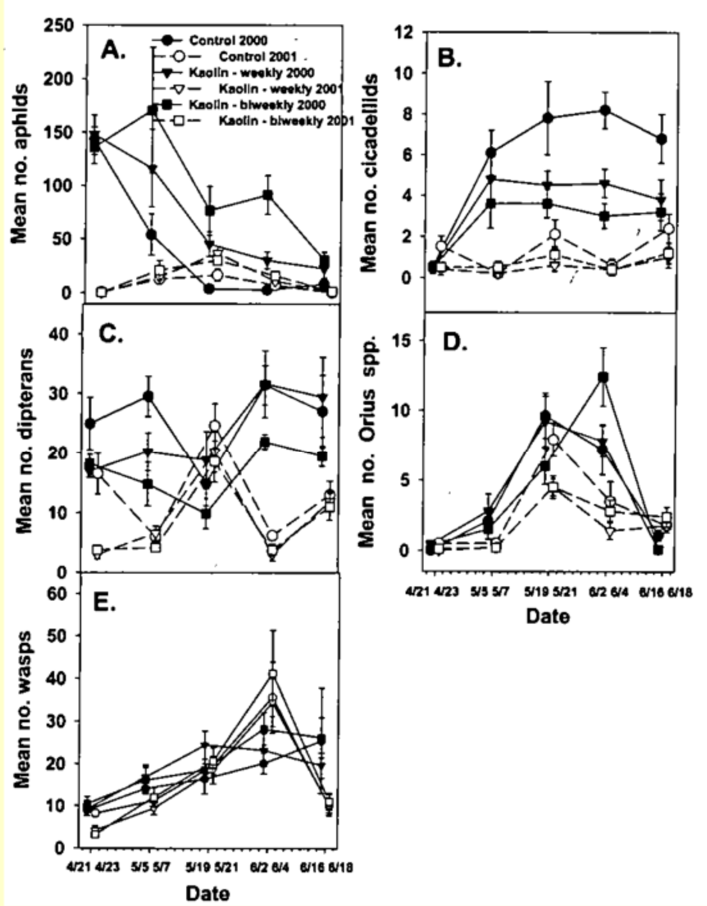

Figure 10.3.2.4/18-2: Mean numbers of selected arthropod groups collected from cotton plants

III. CONCLUSION

In the following 2-year field trial (applications and sampling between April and June only), leaf counts and dvac sampling indicated that pest cotton aphid, *Aphis gossypii* Glover, populations increased in kaolin treated cotton plots compared to control plots, but cicadellid populations were suppressed. Populations of dipterans, *Orius* spp., and wasps were reduced in the kaolin treatments only on 1 of 20 sampling dates over the two seasons (within the application period). Foliar kaolin spray had no effect on other arthropod groups identified in this study (silverleaf whitefly, *Bemisia argentifoli* Bellows and Perring; herbivorous hemipterans and coleopterans; thrips; lepidopteran larvae; *Geocoris* spp.; *Nabis* spp.; reduviids; coccinellids; *Collops* spp.; neuropterans; and spiders).

Reference:	KCP 10.3.2.4/19, Scalercio, S., Belfiore, T., Noce, M.E., Vizzarri, V., Iannotta, N., 2010
Title:	Impact of kaolin and *Beauveria bassiana* treatments against olive fly on the non-target arthropods of the olive ecosystem
Report No.:	Published in: Acta Horticulurae, 873: 329-336
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

The aim of this study was to evaluate the environmental impact of kaolin clay at a rate of 5 kg/hL in managing against the pest *Bactrocera oleae* in an olive grove in Southern Italy. Selected arthropod taxa (*Arachnida*: *Araneae* and *Opiliones*; *Hymenoptera*: *Ichneumonidae*; other *Hymenoptera*; *Coleoptera*: *Coccinellidae*; *Lepidoptera*; *Neuroptera*; *Mecoptera* and *Diptera*: *Syrphidae*) were utilised as bio-indicators.

The abundance of *Araneae, Hymenoptera* and *Lepidoptera* were reduced in the kaolin-treated plots. The ratio of pest: beneficial arthropods were unchanged after treatments. Results demonstrate that kaolin utilised against the pest *Bactrocera oleae* showed few negative effects on beneficial arthropod populations. The taxon *Araneae* was identified as the best bio-indicator, as these species were able to indicate well-preserved environmental conditions. More investigation on the efficacy of *B. bassiana* against the olive fly and on its impact on the above ground arthropods is required.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2 (reliable with restrictions), not known to be GLP.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Kaolin
Batch number: Not reported. Manufactured by Progetto Geovita, Italy
Content of a.s.: Not reported
Appearance: Bright white colour

2. Vehicle: Not reported
Reference item: Spores of the fungus *Beauveria bassiana* (150 mL/hL)

3. Test organism
Species: *Arachnida*: *Araneae* and *Opiliones*; *Hymenoptera*: *Ichneumonidae*; other *Hymenoptera*; *Coleoptera*: *Coccinellidae*; *Lepidoptera*; *Neuroptera*; *Mecoptera* and *Diptera*: *Syrphidae*
Age at test initiation: Varied ages
Source: 20 year old olive grove located in Cosenza, Italy

4. Treatment: 5 kg/hL

5. Environmental conditions: Mediterranean

B. STUDY DESIGN AND METHODS

1. Application:

The study was conducted in a 20 year old olive grove located in Cosenza, Italy. Three plots were chosen for the trials. Each plot was composed of 400 olive trees grown in alternate rows. Two applications of kaolin approximately 6 weeks apart starting August 2nd were sprayed in one plot (no replicate plots). One untreated plot was used as a control and to compare efficacy, the 3rd plot was treated once with a bioinsecticide based on the vital spores of the fungus *Beauveria bassiana* (150 mL/hL). The products were sprayed as liquid suspension by conventional spray equipment, in order to provide adequate and uniform canopy coverage.

2. Sampling:

Arthropods were collected using three yellow sticky traps per plot. Traps were placed in the center of each plot, 20 meters apart, to avoid border effects. Data were collected every 10 days from July to November (i.e. approx. one month before to 3 months after application). The abundance of *Araneae*, *Opiliones*, *Ichneumonidae*, other *Hymenoptera*, *Coccinellidae*, *Lepidoptera*, *Neuroptera*, *Mecoptera* and *Syrphidae* was recorded in detail.

3. Statistics:

Data of abundance were normalised using the Density of Activity (DAa), taking in account the number of traps and the number of days of trap exposure. As an abundance value, dominance (Do), expressed as *n*i/*N*, where *n*i equalled the number of individuals belonging to the taxon *i*, *N* equalled the number of individuals of all taxa registered within a given community was also used. Data were analysed using (a) the index of Coenotic Balance (CB), which measured the balance among functional units (feeding habits) of communities, and (b) Abbott's formula, which evaluated changes in insect populations in response to treatments.

II. RESULTS AND DISCUSSION

1. Arthropod abundance:

667 individuals belonging to the selected arthropod taxa (*Arachnida*: *Araneae* and *Opiliones*; *Hymenoptera*: *Ichneumonidae*; other *Hymenoptera*; *Coleoptera*: *Coccinellidae*; *Lepidoptera*; *Neuroptera*; *Mecoptera* and *Diptera*: *Syrphidae*) were sampled.

The selected taxa were found in all plots, with the exception of *Opiliones*, which was collected only from the untreated plot, but numbers were too low (n = 1) for further consideration. In the untreated and kaolin-treated plots the more abundant arthropods were *Hymenoptera*, followed by *Lepidoptera*. *Araneae* showed a two-fold value of density of activity in the untreated plot in comparison with treated ones, but no significant differences were found among plots for any arthropod group.

Abbott's formula showed the negative effects of kaolin on *Araneae*, other *Hymenoptera* and *Lepidoptera* with 57.6, 39.0 and 7.2% reduction based o the two sampling periods (last sample approximately 2 weeks post-application). The other arthropods were not reduced by any treatments.

2. Evaluation of Coenotic Balance (CB):

The CB computed using all considered groups attained the highest value in the untreated plot ($CB_{I/A}$ = 1.62) followed by kaolin treated plots ($CB_{I/A}$ = 1.57), but no significant differences were found. The CB computed using only *Hymenoptera* confirmed that the untreated plot ($CB_{hym/ichn}$ = 2.56) showed the highest balance value. $CB_{hym/ichn}$ =2.24 for kaolin.

III. CONCLUSION

Kaolin had a negative impact only on *Araneae* and other *Hymenoptera*, two groups which live in a close relationship with the substratum (based on sampling up to 2 months post treatrment). *Araneae* are visual predators which have difficulty moving due to kaolin. *Hymenoptera*, similar to *Lepidoptera*, are mainly flower-visiting insects that have difficulties in finding and utilising alimentary sources within the kaolin-treated plot. Based on the CB evaluation, no significant differences were found between kaolin treated plots and untreated control. The tested preparation had no evident negative effects on the arthropods of olive groves.

CP 10.3.3 Other routes of exposure for non-target arthropods

Not relevant.

CP 10.4 Effects on Non-Target Soil Meso- and Macrofauna

CP 10.4.1 Earthworms

~~Aluminium silicate's chemical composition is similar to common clay. Aluminium silicate (kaolin) used in SURROUND® WP CROP PROTECTANT, is an ultra-pure, ultra-fine, calcined kaolin, a natural white clay mined across the world. It is a natural mineral substance composed of silicon, aluminium and oxygen, just like a variety of other minerals. Once released into the environment, kaolin is insoluble in water and present as clay particles that sink to the bottom and become an integral part of the sediment and is undistinguishable from clay minerals naturally present. It is therefore not translocated in plants or bioavailable and therefore it cannot be readily transported through the gut wall of animals.~~

No data are available or required for effects on non-target soil organisms since the approval of aluminium silicate (kaolin) as a plant protection product (EFSA (2012)). As discussed in the original DAR, a low risk can be concluded for soil organisms.

A waiver is requested for studies on earthworms and other soil macro- and micro-organisms with the formulated product based on the following information:

- SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). Therefore, the formulated product is highly unlikely to be of higher toxicity compared to the active substance, kaolin.

- Aluminium silicate (kaolin) used in SURROUND® WP CROP PROTECTANT, is an ultra-pure, ultra-fine, calcined kaolin, a natural white clay mined across the world.

- Aluminium silicate (kaolin) is a natural mineral substance composed of silicon, aluminium and oxygen, just like a variety of other minerals.

- Aluminium silicate is essentially purified natural clay and is therefore not subject to adsorption on or desorption from soil particles, at it is part of said soil particles.

- Aluminium silicate's chemical composition is similar to common clay. From "topsoil physical properties for Europe" (based on LUCAS topsoil data): JOINT RESEARCH CENTRE European Soil Data Centre (ESDAC)[28], it can be noted in the diagram that a large area of Europe consists of

[28] https://esdac.jrc.ec.europa.eu/content/topsoil-physical-properties-europe-based-lucas-topsoil-data

28 to 98% clay based soil. Soil Data Centre (ESDAC)[29], it can be noted in the diagram that a large area of Europe consists of 28 to 98% clay based soil.

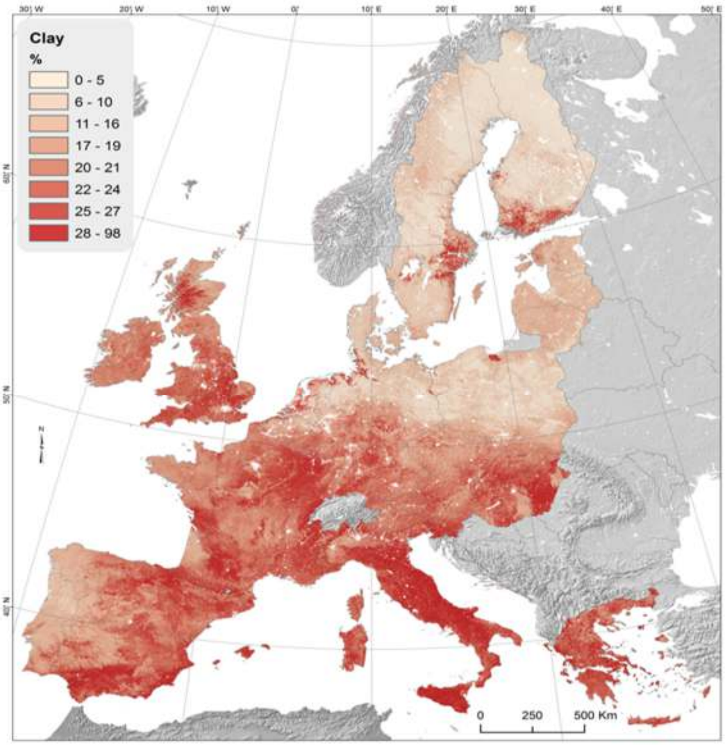

Figure 10.4.1-1: Clay concentration in European soils (JRC-ESDAC)

- When applied to soil, the aluminium silicate particles will readily mix with the other soil components. Some organic materials (for example fulvic acids) will adsorb onto the particle surfaces, similarly to the aluminium silicate already existing in the soil. Adsorption and desorption of aluminium silicate to soil contaminants is therefore well described in regulatory evaluation dossiers as all adsorption/desorption studies involving standard soils will involve aluminium silicate as a soil component.

[29] https://esdac.jrc.ec.europa.eu/content/topsoil-physical-properties-europe-based-lucas-topsoil-data

- The proportion of natural clay in soil varies from 0% in pure sand to 100% in pure clay soil as shown in the following soil diagram. However, agricultural soils normally contain between 5 and 50% clay and therefore, the quantity of kaolin added through the use of SURROUND® WP CROP PROTECTANT will not be enough (the added quantities represent mg/kg soil/year) to cause any measurable increase in the clay (aluminium silicate) content of agricultural soils.

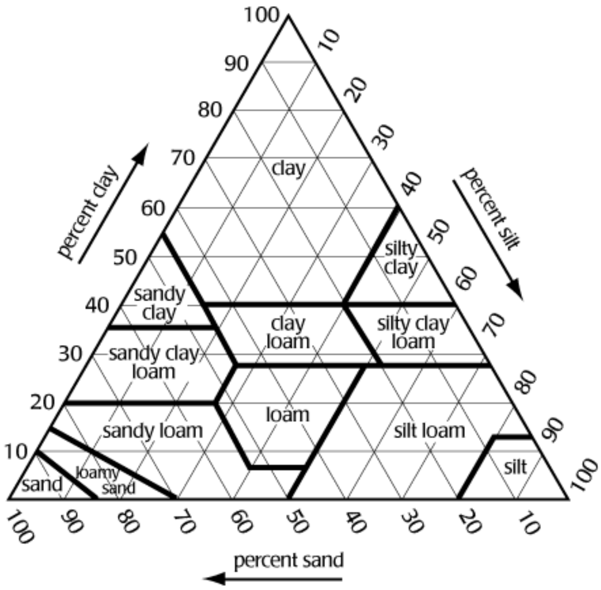

Figure 10.4.1-2: Soil texture triangle

- The use of SURROUND® WP CROP PROTECTANT in grapevines does not significantly increase the concentrations (PEC_{soil} = 0.024 g/kg per application or 0.096 g/kg total season, please refer to Document MCP, Section 9) of clays in the environment and it is not expected to have any negative effects on soil organisms.

- As detailed in the original DAR (Section B.9.6), it is estimated (Hoerger & Kenaga, 1972) that earthworms contain about 30% soil. Given that soils typically contains between 5-50% clay (see Document MCP, Section 9), earthworms are being continuously exposed to much higher concentrations of aluminium silicate (kaolin) than any that might arise from the use of SURROUND® WP CROP PROTECTANT.

- Kaolin does not translocate in plants, nor is it bioavailable, and therefore it cannot be readily transported through the gut wall of animals.

- Aluminium silicate (kaolin) occurs naturally in most soils and the quantity of kaolin added through the use of SURROUND® WP CROP PROTECTANT will not cause any measurable increase in the clay (aluminium silicate) content of agricultural soils. The agricultural use of SURROUND® WP CROP PROTECTANT therefore is not expected to have any negative effects on soil organisms, including earthworms. On the contrary, the use of kaolin as a replacement of conventional pesticides could help to improve soil conditions through the elimination of potentially harmful residues of synthetic compounds within the soil

Overall, exposure to aluminium silicate (kaolin) resulting from the use of SURROUND® WP CROP PROTECTANT in grapevines is minimal compared to its natural presence in the environment. Therefore, adverse effects to soil organisms is concluded to be low and the request for toxicity studies and conventional EU risk assessments are not considered necessary for a non-toxic, non-bioavailable, routinely ingested natural mineral such as kaolin clay as was reported in the EFSA Conclusion for aluminium silicate (2012).

In light of these considerations, no toxicity testing with macro or micro soil organisms with the formulated product is considered to be necessary for the purposes of renewal and the risk to soil organisms is concluded to be low.

Risk assessment for earthworms

Not relevant, please refer to discussion above.

CP 10.4.1.1 Acute oral toxicity

Not relevant, please refer to point CP 10.4.1. Furthermore, acute earthworm toxicity studies are no longer required in accordance with Commission Regulation EU 284/2013.

CP 10.4.1.2 Earthworms – sub-lethal effects

Not relevant, please refer to point CP 10.4.1.

A waiver is requested, as was accepted during the initial EFSA review (EFSA, 2012), for a stdy on the chronic toxicity to earthworms based on the information provided above, point CP 10.4.1, which still applies. Furthermore, according to OECD 207 or 222, for the preparation of the artificial soil test substrate used in the earthworm toxicity tests, 20% kaolin clay (kaolinite content preferably above 30%) is indicated as part of the dry constituents of the substrate.

As a comparison, overspray on bare soil with SURROUND® WP CROP PROTECTANT at a rate of 50 kg/ha would result in deposits of 5 g/m^2. Based on a standard soil density of 1.5 g/cm^3, and soil layer thickness of 5 cm, this deposition of kaolin following application of SURROUND® WP CROP PROTECTANT represents less than 0.01% of the soil weight (i.e. far lower than the 20% kaolin used in standard ecotoxicity tests).

In light of these considerations, no toxicity testing with earthworms on the formulated product is considered to be necessary and the risk to soil organisms is concluded to be low.

CP 10.4.1.3 Earthworms – field studies

Not relevant.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

~~Please refer to waiver request for earthworms above, point CP 10.4.1.~~

No studies are available for non-target soil meso- and macrofauna, nor are they required.

A waiver is requested for studies on other soil macro- and micro-organisms with the formulated product based on the information provided above for earthworms, point CP 10.4.1. Furthermore, according to OECD 232 (Collembolan Reproduction Test in Soil) and OECD 226 (Predatory mite (*Hypoaspis* (Geolaelaps) *aculeifer* reproduction test in soil), for the preparation of the artificial soil test substrate used in these reproductive toxicity tests, 20% kaolin clay (kaolinite content preferably above 30%) is indicated as part of the dry constituents of the substrate.

As a comparison, overspray on bare soil with SURROUND® WP CROP PROTECTANT at a rate of 50 kg/ha would result in deposits of 5 g/m^2. Based on a standard soil density of 1.5 g/cm^3, and soil layer thickness of 5 cm, this deposition of kaolin following application of SURROUND® WP CROP PROTECTANT represents less than 0.01% of the soil weight (i.e. far lower than the 20% kaolin used in standard ecotoxicity tests).

In light of these considerations, no toxicity testing with soil organisms on the formulated product is considered to be necessary and the risk is concluded to be low.

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

Not relevant, please refer to point CP 10.4.1.

CP 10.4.2.1 Species level testing

Not relevant.

CP 10.4.2.2 Higher tier testing

Not relevant.

CP 10.5 Effects on Soil Nitrogen Transformation

~~Please refer to waiver request for earthworms above, point CP 10.4.1.~~

No studies are available for effects on soil nitrogen transformation, nor are they required.

A waiver is requested, as was accepted during the initial EFSA review (EFSA, 2012), from studies on microbial activity based on the information provided above for earthworms, point CP 10.4.1, which still applies.

In light of these considerations, testing for effects on soil nitrogen transformation is considered to be necessary and the risk is concluded to be low.

Risk assessment for Soil Nitrogen Transformation

Not relevant, please refer to point CP 10.4.1.

CP 10.6 Effects on Terrestrial Non-Target Higher Plants

No studies have been conducted with SURROUND® WP CROP PROTECTANT on non-target terrestrial plants, nor are they required (please refer to waiver request below under CP 10.6.2).

Risk assessment for Terrestrial Non-Target Higher Plants

Not relevant, please refer to discussion below.

CP 10.6.1 Summary of screening data

No data has been submitted and is not required (please refer to waiver under CP 10.6.2).

CP 10.6.2 Testing on non-target plants

A waiver is requestedfor studies on non-target terrestrial plants with the formulated product based on the following information:

- SURROUND® WP CROP PROTECTANT is composed of 95% kaolin clay, 4.3% of food-grade additives and 0.7% of well-known additives of no toxicological concern (Please refer to Part C for details on product composition). Therefore, the formulated product is highly unlikely to be of higher toxicity compared to the active substance, kaolin.

- Aluminium silicate (kaolin) as SURROUND® WP CROP PROTECTANT is currently used outside Europe as an insect repellent and a protection against sunburn in fruit bearing vascular plants such as pears, apples, olives or peppers.

- Aluminium silicate is efficacious as an insect repellent and can improve fruit quality through heat protection. There have been no side effects to the use of aluminium silicate (kaolin) other than a slight maturation delay, without any reduction in the quality of the crop (Glenn and Puterka, 2005[30]).

- Aluminium silicate (kaolin) occurs naturally in most soils and the quantity of kaolin added through the use of SURROUND® WP CROP PROTECTANT will not cause any measurable increase in the clay (aluminium silicate) content of agricultural soils (refer to section 10.4.1 above). The agricultural use of SURROUND® WP CROP PROTECTANT therefore is not expected to have any negative effects on non-target terrestrisal plants. On the contrary, the use of kaolin as a replacement of conventional pesticides could help to improve soil conditions through the elimination of potentially harmful residues of synthetic compounds within the soil.

- Aluminium silicate (kaolin) is inert and will not be absorbed or metabolised by plants.

- Aluminium silicate has no known mode of toxicity, is insoluble in water and does not become bioavailable. Hence, it is not bioavailable to plants.

- In a root growth inhibition study by Wang *et al*. (2011[31]), seedlings of four different plants (tomato, cucumber, lettuce and carrot) were exposed to concentrations up to 2000 mg kaolin solution/L for 4 days. Results showed that kaolin suspension had no obvious phytotoxicity on all treated plants (no adverse effect of root length).

Overall, exposure to aluminium silicate (kaolin) resulting from the use of SURROUND® WP CROP PROTECTANT in grapevines is minimal compared to its natural presence in the environment. Therefore, adverse effects to non-target terrestrial plants is concluded to be low and the request for toxicity studies and conventional EU risk assessments are not considered necessary for a non-toxic and non-bioavailable natural mineral such as kaolin clay as was reported in the EFSA Conclusion for aluminium silicate (2012).

[30] Glenn, D.M., and Puterka, G.J., 2005. Particle Films, A New Technology for Agriculture. Horticultural Reviews. Vol 31. Edited by Janick K. John Wiley & Sons, Inc
[31] Wang, M., Chen, L, Chen, S. and Ma, Y. (2011). Alleviation of cadmium-induced root growth inhibition in crop seedlings. Y nanoparticles. Ecotoxicology and Environmental Safety 79 (2012): 48-54.

In light of these considerations, no toxicity testing with non-target terrestrial plants with the formulated product is considered to be necessary for the purposes of renewal and the risk is concluded to be low.

CP 10.6.3 Extended laboratory studies on non-target plants

Not relevant.

CP 10.6.4 Semi-field and field tests on non-target plants

Not relevant.

CP 10.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

Aluminium silicate (kaolin) is non-toxic, non-bioavailable and inert to mammals, fish, birds, arthropods and plants. SURROUND® WP CROP PROTECTANT's mode of action is one of repellency through the establishment of a particle film barrier. No additional testing on non-target organisms is required.

CP 10.8 Monitoring Data

No data available.