Aluminium silicate (Kaolin)

DOCUMENT M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Annex to EU Regulation 283/2013

Ownership Statement

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

~~This document contains only summaries of studies, which were not available at the time of the first Annex I inclusion of aluminium silicate (kaolin) and were therefore not evaluated during the first EU review. To facilitate discrimination between new and original information, the original information is 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.~~
The previous EFSA Conclusion for aluminium silicate (kaolin) is published in the EFSA Journal 2012; 10(2): 2517 (subsequently referred to as EFSA (2012)).

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.

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 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 worldwide 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 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, kaolin is 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.

Kaolin's chemical composition is similar to common clay. 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, kaolin is insoluble in water and present as clay particles that sink to the bottom and become an integral part of the sediment and are undistinguishable from clay minerals naturally present. It is not translocated in plants or bioavailable and therefore it cannot be readily transported through the gut wall of animals.

For the above reasons, a waiver from conducting specific toxicity studies on birds, wild mammals, aquatic organisms, soil micro and macro organisms and non-target terrestrial plants has been requested as was previously done during the initial Annex I review and accepted due to the conclusion that aluminium silicate (kaolin) is of low risk to these organisms.

CA 8.1 Effects on Birds and Other Terrestrial Vertebrates

CA 8.1.1 Effect on birds

No new avian toxicity data are available or required for the renewal of aluminium silicate (kaolin). As discussed in the original DAR (Section B.9.1), 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, 2012).

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.

Table 8.1.1-1: 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

~~Waivers were requested and accepted during the initial EFSA review (EFSA 2012) for avian toxicity studies 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. Aluminium silicate (kaolin) is a natural component of the environment and birds have been 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.~~

~~Many birds are known to take clay dust baths to help reduce dermal parasites (Martin and Mullens, 2012[1]), but some birds like the macaw have even been observed to eat kaolin for the purpose of aiding their digestive systems (Grange J, 2003)[2]. Also, birds that eat earthworms and other soil dwelling invertebrates routinely consume soil (hence clay) adhering to the prey and present in their digestive tracts.~~

~~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 testing is considered necessary as supported during the initial Annex I approval and that no avian data were specified as required in EFSA (2012).~~

CA 8.1.1.1 Acute oral toxicity to birds

~~Please refer to above discussion under point 8.1.1.~~
No acute oral toxicity study with birds is being submitted.

[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)

A waiver is requested for an acute oral bird toxicity study with the active substance 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[3]).

- 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)[4]. The consumption of soil by the parrot has been studied extensively for decades (Brightsmith *et al*., 2010[5]) 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[6]), reduce bacterial contamination of the guts and reduce the detrimental effects of mycotoxin contaminated diets (Tauqir and Nawaz, 2001[7]). Kaolin also protects the intestinal mucosa, by adhering to pathogen and selectively promotes their excretion (Droy-lefain *et al*., 1985[8]).

- 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.

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[3] 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.

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

[5] 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

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

[7] 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

[8] 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.

- 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.
    - Friedland clay[9] 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 active substance 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).

CA 8.1.1.2 Short-term dietary toxicity to birds

~~Please refer to above discussion under point 8.1.1.~~
No data are available. Short-term dietary toxicity data to birds are not required according to Commission Regulation EU 283/2013.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Please refer to above discussion under point 8.1.1.

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 reproductive avian toxicity testing with the active substance 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). Nevertheless, data have been found in the open literature and have been summarised in support of this submission.

Reference:	KCA 8.1.1.3/01, Owen, O.J., Nodu, M.B., Dike, U.A., Ideozu, H.M., 2012
Title:	The effects of dietary kaolin (clay) as feed additive on the growth performance of broiler chickens
Report No.:	Published in: Greener Journal of Agricultural Sciences Vol. 2(6): 233-236
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study, efficacy trial

Executive summary

Investigation was carried out to elucidate the effects of dietary kaolin (clay) as feed additive on the growth parameters of broiler chickens. One hundred and twenty day-old Hubbard broiler chickens were used for 56 days. The birds were randomly assigned to 4 treatments consisting of 30 birds/treatment and 10

[9] 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.

birds/replicate. The 4 treatments were: basal diet only (control), basal diet + 10 g kaolin/kg, basal diet + 20 g kaolin/kg and basal diet + 30 g/kg kaolin. Results obtained revealed that incorporating kaolin into broiler diets significantly ($p<0.05$) improved feed intake and feed efficiency. It was concluded that dietary kaolin in the diet of broiler birds had beneficial effects on the growth performance.

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

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Kaolin
	Batch number:	Not reported.
	Content of a.s.:	Not reported
	Appearance:	Not reported.
2.	**Vehicle:**	Commercial broiler starter and finisher diets
3.	**Test organism**	
	Species:	Hubbard strain broiler chickens
	Age at test initiation:	120 days old
	Weight:	60 g (mean initial weight)
	Source:	Not reported. Experiment carried out in the poultry section of the Rivers State University of Science and Technology Teaching and Research Farm, South-South of Nigeria
	Acclimation:	Not reported
	Food:	Top feed with protein contents of 22% for starter and 18% for finisher. They contained 2800 ME/Kcal/kg and 2900ME/Kcal/kg, respectively
4.	**Treatment:**	10, 20 and 30 g a.s./kg feed (ppm)

5. Environmental conditions: Not reported

B. STUDY DESIGN AND METHODS

1. Test method:
A total of 120 Hubbard strain broilers of mixed sexes with an initial mean body weight of 60 g were used in the experiment that lasted for 56 days. The birds were housed in a deep litter with wood shavings as bedding material. The birds were divided into 4 groups of 30 birds each. Each treatment group was further sub-divided into 3 replicates of 10 birds in a completely randomized design (CRD). Feed and water were offered *ad-libitum.* Feed intake was measured on daily basis while body weight was observed weekly. At the expiration of the experiment, all the birds were weighed and 3 birds were selected from each treatment for histological assay.

4. Statistics:
All the data collected were subjected to Analysis of Variance and the differences in treatments, where it existed was separated using Duncan's New Multiple Range Test (DNMRT).

II. RESULTS AND DISCUSSION

1. Findings:
The performance of the birds fed the different levels of kaolin is shown in the following table.

Table 8.1.1.3/01-1: Performance characteristics of broiler chickens fed with kaolin diets

Parameter	Treatment			
	Control	10 g kaolin/kg	20 g kaolin/kg	30 g kaolin/kg
Mean initial weight (g)	60.00	60.00	60.00	60.00
Mean final weight (kg)	2.25	2.18	2.10	2.23
Mean total weight gain (kg)	2.20	2.12	2.04	2.23
Mean daily weight gain (g)	39.30	37.85	36.43	38.75
Mean total feed intake (kg)	5.10 a	4.70 b	4.90 b	4.90 b
Mean daily feed intake (g)	91.07 a	83.93 c	87.50 b	85.71 c
Feed conversion ratio (feed/gain)	2.32 b	2.23 a	2.40 b	2.21 a

a, b, c: means within the same rows with different letters are significantly different ($p < 0.05$)

The data for daily feed intake indicated that the birds in the control treatment showed significant difference among the experimental diets. The mean daily feed consumption was highest in the control (91.07 g), and ranged between 83.93 g - 87.50 g in the treated groups of birds. Significant differences also existed in the total feed intake. The control treatment recorded the highest total feed intake of 5.10 kg. The data showed that birds on 10 g, 20 g and 30 g dietary levels of kaolin had 4.70 kg, 4.90 kg and 4.80 kg of total feed intake, respectively. Although there was no significant differences in the mean weight gain, the control birds recorded the highest numerical body weight gain than the other groups.

There was significant difference in feed conversion ratio among the birds in the different dietary treatments. Birds fed 10 g kaolin and 30 g kaolin recorded the best feed: gain ratio of 2.23 and 2.21 respectively, which was significantly better than the control (0 g) and those fed 20 g kaolin.

The results on organ weight measurements indicated there were significant differences among the birds in the liver and gizzard. The 0 g (control) group recorded the highest values in these parameters. However, the result also indicated that significant differences did not exist in the remaining organs examined *vis-à-vis* kidney, heart, spleen and gall bladder among the groups.

III. CONCLUSION

There is indication from this study that kaolin as feed additive in broiler production improved feed efficiency at all levels of inclusion, when compared to the control. It is therefore concluded that dietary kaolin had beneficial effects on the growth performance of broiler chickens.

CA 8.1.2 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 (Section B.9.3), 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, 2012).

An acute mammalian toxicity study with aluminium silicate has been submitted previously. Full details of the study are provided in the previous EU DAR (2008) and related documents. The toxicity endpoint concluded in EFSA (2012) remains valid. No additional mammalian data were specified as required in EFSA (2012).

Table 8.1-2: Toxicity endpoint for mammals

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

CA 8.1.2.1 Acute oral toxicity to mammals

No new acute mammalian data are available or required for the renewal of aluminium silicate. Aluminium silicate (kaolin) is of low oral acute toxicity with an LD_{50} value of >5000 mg a.s./kg in rats (combined ♀ and ♂) (Wnorowski, 1997a; Report No.: 4903). Please refer to Document MCA, Section 5, Point 5.2.1 for details on the acute oral study on rats with the active substance agreed in EFSA (2012).

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

No new long-term and reproductive toxicity data for mammals are available or required for the renewal of aluminium silicate (kaolin). As discussed in the original DAR (2008) (Point B.9.3), 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, 2012).

~~Waivers were requested and granted during the initial EFSA review (EFSA (2012)) for long-term and reproductive toxicity studies for mammals based on the following information:~~

A waiver is requested for a long-term / reproductive mammalian toxicity study with the active substance 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 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 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[10]. For example, women in Budongo, Uganda consume forest clay mixed with

[10] 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.

water for stomach problems and during pregnancy (Reynolds *et al*., 2015)[11]. 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)[12], 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[13] 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[14] 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.

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 active

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[11] 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

[12] 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.

[13] 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).

[14] 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.

substance 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.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

In accordance with Commission Regulation (EU) No 284/2013, an assessment of the potential risk posed by bioconcentration in the prey of birds and mammals shall be provided for substances with a log Pow >3. Aluminium silicate (kaolin) is not lipophilic and is not soluble in water. In addition, as aluminium silicate is inorganic, partition coefficient information is not considered relevant (see Document M-CA, Section 2). Therefore, it can be classified as not bio-accumulative, hence an assessment for bioconcentration in prey for birds and mammals is not necessary.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

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 in 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.

CA 8.1.5 Endocrine disrupting properties

The available studies and literature search for terrestrial animals do not indicate that aluminium silicate (kaolin) would have endocrine disrupting properties. The mammalian section Document MCA Section 5, Point 5.8.1 notes that the available data do not suggest that aluminium silicate (kaolin) would be considered as an endocrine disrupting chemical, *i.e.* it does not cause adverse developmental, reproductive, neurological, and/or immune effects in animals.

CA 8.2 Effects on Aquatic Organisms

No new data are available for aquatic organism toxicity since the first approval of aluminium silicate (kaolin) (EFSA, 2012). A summary of the existing data is presented the following table.

Details of these studies are provided in the previous EU DAR and related documents and in the relevant sections below. The toxicity endpoints remain valid as agreed by EFSA (2012).

Table 8.2-1: 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) KCA 8.2.4.2/01 (EFSA Conclusion, 2012)
Daphnia magna	Surround WP	48 h	EC_{50} >600	Goodband (2006)

Species	Substance	Exposure System	Results	Reference
		(static)	mg product/L (nom) (>570 mg a.s./L)	KCP 10.2.1/01 (Report no.: 2120/0004)
Algae				
Scenedesmus subspicatus	Surround WP	72 h (static)	EC_{50} >600 mg product/L (nom) (>570 mg a.s./L)	Vryenhoef (2006) KCP 10.2.1/02 (Report no.: 2120/0003)

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 and algae are not available for the active substance and waivers are provided in the relevant sections below. Aluminium silicate (kaolin) is extremely common in the environment, including aquatic systems. The proposed use should not lead to surface water levels outside the normal range and clay particles would rapidly settle, thus additional chronic aquatic organism testing is not required. Furthermore, data with the formulated product is available for acute *Daphnia magna* and for green algae (chronic) and can be extrapolated for the active substance (see Point 8.2.4 and 8.2.6 below). Aluminium silicate (kaolin) is shown to have a very low ~~acute~~ toxicity to aquatic invertebrates, including *Daphnia magna*, and algae, and is non-soluble in water and has no potential for bioaccumulation in aquatic organisms. Therefore, additional ~~chronic~~ aquatic toxicity testing data with the active substance is not considered to be necessary.

CA 8.2.1 Acute toxicity to fish

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

Reference:	KCA 8.2.1/01 Goldes, S.A., Ferguson, H.W., Moccia, R.D. and Daoust, P.Y. 1988 (previously evaluated in DAR B9, IIA 8.2.2.1)
Title:	Histological effects of the inert suspended clay kaolin on the gills of juvenile rainbow trout, *Salmo gairdneri*
Report No.:	Richardson, Journal of Fish Diseases (1988), volume 11 pages 23-33
Guideline(s):	Not reported
Deviation(s):	None
GLP:	No, but study considered scientifically valid

Executive Summary

Juvenile rainbow trout were exposed to 56, 280, 1400 and 7000 mg/L of suspended kaolin clay for 64 days. The monitoring of water turbidity and suspended solids levels revealed that the actual average levels of kaolin concentration were 36, 171, 1017 and 4887 mg/L. Quantitative and qualitative histological analyses were carried out on their gills.

Intracellular kaolin was visible within filament and lamellar epithelium in gills exposed to 171, 1017, 4887 mg/L kaolin at 4, 8, 16, 32, and 64 days. However, there was no adverse branchial pathology observed at all sample times in fish exposed to 36, 171 and 1017 mg/L kaolin. In gills exposed to 4887 mg/L kaolin, lesions were found at 16 and 32 days, probably caused by the proliferation of a protozoan identified as *Ichtyobodo necator*. However, gills regained normal architecture by 64 days despite continuous exposure to kaolin.

In this experiment, the highest dose of kaolin might have been indirectly responsible for creating a favourable environment for protozoan colonization that caused branchial lesions. However, these lesions were reversible, and the fish recovered thanks to increased mucus secretion, immune response and turnover of branchial epithelium.

According to analysis by light microscopy, the exposure to such levels of kaolin clay probably has little direct effect on overall gill structure. The LC_{50} for kaolin against rainbow trout must be >7,000 mg a.s./L (nominal).

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolin
Description:	White powder
Lot/Batch #:	hydrite flat D
Purity:	Not specified
Stability of test component:	Stable
Particle Size:	Median 5 µm, range 0.3 to 30 µm
2. Test animals	Juvenile rainbow trout *Salmo gairdneri* Richardson 8 month old, 5.64±0.58 cm long
3. Testing Facility	Fish Pathology Laboratory, Department of Pathology, Ontario Veterinary College, University of Guelph, Ontario, Canada.

B. STUDY DESIGN AND METHODS

1. Experimental Design:

Three untreated controls: unexposed fed control, unexposed reduced-feed control, and unexposed optimally reared control. Four kaolin concentrations: 56, 280, 1400 and 7000 mg/L. The highest concentration of kaolin was set at 70% of the dose causing 28% mortality in pilot studies.

Treatments were assigned randomly to twelve 200 L fibreglass tanks. Within each tank 27 fish were placed in each of 3 plastic 8-L perforated buckets that were suspended in the water column, giving a total 81 fish per tank in a semi-static system. The kaolin was kept in suspension during the study by a submersible pump located on the bottom of each tank.

2. Observations:

During the experiment, the following water parameters were monitored: temperature, dissolved oxygen, pH, un-ionized ammonia and turbidity.

Fish were sampled at 0, 4, 8, 16, 32, and 64 days. At each sampling, 6 fish per tank were arbitrarily chosen, removed, killed, and four gill arches from their right side were examined under light microscopy. The gills were checked for histological alteration and the thickness of their lamellae was measured.

3. Statistical analysis:

Differences in lamellar thicknesses and water quality were evaluated using analysis of variance, t-tests and Duncan's multiple range tests.

II. RESULTS AND DISCUSSIONS

The monitoring of water turbidity and suspended solids levels revealed that the actual average levels of kaolin concentrations were 36, 171, 1017 and 4887 mg/L.

Qualitative histology: All gills samples were found normal, except for those taken at 16 and 32 days in fish exposed to 4887 mg/L kaolin, which showed lesions and the proliferation of a protozoan, *Ichtyobodo necator*. However, the morphology and parasite numbers returned back to normal at 64 days.

Quantitative study: Statistically significant increases in lamellar thickness only occurred at the base of lamellae in fish exposed to 4887 mg/L kaolin. However, there is no evidence that this loss of surface area had any significant effect on branchial function.

Mortalities were 1.7% in reduced-feed control and 8% in optimally reared control. Average percentage mortality per treatment ranged from 2.5% (36 mg/L) to 13.6% (4887 mg/L).

Analytics:
All kaolin concentrations were, on average, within 23% of desired levels for each treatment at time 0 of the 12 hours experimental cycle. On average, turbidity and suspended solids levels dropped 22 and 24%, respectively, by the end of the 12-h period.

III. CONCLUSIONS

The highest dose was selected at 70% of the dose where 28% had been observed in pilot toxicity tests. The results of mortality during the test period demonstrate that the LC_{50} value for kaolin against rainbow trout must be >7,000 mg a.s./L.

In terms of chronic effects, the absence of qualitative and quantitative branchial changes at 36, 171, and 1017 mg/L indicates that environmental concentrations of kaolin are unlikely to cause histological alteration to gill structure, and that kaolin alone at these levels probably does not lead to lesions that might facilitate secondary infection. Since there is no reliable data on the histological effects of kaolin, it is impossible to determine whether the lesions in fish exposed to 4887 mg/L at 16 and 32 days were characteristic of the effects of kaolin. It is probable that the branchial proliferation was indeed a consequence of protozoan colonization, and not damage by kaolin. The NOEC value after 64 days was 1017 mg/L (mean measured) based on histological alteration to gill structure.

Reference:	KCA 8.2.1/02 McFarland, V.A., and Peddicord, R.K. 1980 (previously evaluated in DAR B9, IIA 8.2.1/01)
Title:	Lethality of a suspended clay to a diverse selection of marine and estuarine macrofauna
Report No.:	Archives of Environmental Contamination and Toxicology (1980), volume 9, pages 733-741
Guideline(s):	Not reported
Deviation(s):	Not applicable, published paper
GLP:	No, but study considered scientifically valid

Executive Summary

Sixteen species of fish and aquatic invertebrates from marine and estuarine environments were exposed to different concentrations of suspended kaolin for different durations. All other water-quality parameters in the test aquaria were maintained close to natural levels. The concentrations of kaolin were varied in order to estimate lethal concentrations for 10, 20 and 50% of the population plus time-concentration mortality response curves for each species.

Naturally occurring marine and estuarine fish and invertebrates: Sea urchin (*Strongylocentrotus purpuratus*), Japanese clam (*Tapes japonica*), hermit crab (*Pagurush hirsutiusculus*), isopod (*Sphaeroma pentodon*), mud snail (*Nassarius obsoletus*), blue mussel (*Mytilus edulis*), tunicate (*Molgula manhattensis*), tunicate (*Styela montereyensis*), coast mussel (*Mytilus californianus*), spot tailed sand shrimp (*Crangon nigromaculata*), grass shrimp (*Palaemon macrodactylus*), Dungeness crab (*Cancer magister*), polychaete (*Neanthes succinea*), tunicate (*Ascidia ceratodes*), amphipod (*Anisogammarus confervicolus*), shiner perch (*Cymatogaster aggregate*).

A wide range of sensitivities to suspended kaolin was observed among the 16 species studied. For half of the species, no true Lethal Concentrations (LC) estimates could be made. There seemed to be some correlation between sensitivity and natural habitat. Organisms restricted to muddy bottoms were very insensitive, whilst species shown to be highly sensitive to high concentrations of suspended solids were either invertebrates occurring predominantly on sandy bottoms or in fouling communities, or open water fish. However, some tolerant species were also identified from this group.

The following table summarises the data obtained for the more sensitive species where acute toxic effects were sufficient to determine an LC_{50} value after 200 h (8.3 d) exposure to suspended kaolin.

Species name	Common name	LC_{50} (g/L)
Mytilus californicus	Coast mussel	96
Crangon nigromaculata	Spot tailed sand shrimp	50
Palaemon macrodactylus	Grass shrimp	>77*
Cancer magister	Dungeness crab	32
Neanthes succinea	Polychaete worm	48
Ascidia ceratodes	Tunicate	38
Anisagammarus confervicolus	Amphipod	50
Parophrys vetulus	English sole	70 - 117
Cymatogaster aggregata	Shiner perch	3

* 50% mortality was not reached, 77 g/L = LC_{20}

The fish, *Cymatogaster aggregata* (shiner perch), was found to be by far the most sensitive species with LC_{50} of 3000 mg/L suspended kaolin.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Kaolin
   - Description: White powder
   - Lot/Batch #: Hydrite flat D, obtained in a single batch
   - Purity: Not specified
   - Stability of test component: Stable

2. Test animals:
   Naturally occurring marine and estuarine fish and invertebrates: Sea urchin (*Strongylocentrotus purpuratus*), Japanese clam (*Tapes japonica*), hermit crab (*Pagurush hirsutiusculus*), isopod (*Sphaeroma pentodon*), mud snail (*Nassarius obsoletus*), blue mussel (*Mytilus edulis*), tunicate (*Molgula manhattensis*), tunicate (*Styela montereyensis*), coast mussel (*Mytilus californianus*), spot tailed sand shrimp (*Crangon nigromaculata*), grass shrimp (*Palaemon macrodactylus*), Dungeness crab (*Cancer magister*), polychaete (*Neanthes succinea*), tunicate (*Ascidia ceratodes*), amphipod (*Anisogammarus confervicolus*), shiner perch (*Cymatogaster aggregate*).

3. Testing Facility: US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

B. STUDY DESIGN AND METHODS

1. Experimental design:

The laboratory system was a flow-through system of 16 hemispherical 75-L aquaria supplied with suspended kaolin and a complementary volume of dilution water at estuarine or oceanic salinity. The major water-quality parameters (pH, D.O., salinity, temperature and suspended solids) were kept stable during the experiments.

For the mud snail and the blue mussel, the 16 aquaria were arranged into 4 replicates, each consisting of a control and 3 kaolin concentrations (the highest concentration tested was 117g/L). For all the other species, the 16 aquaria were arranged into 2 replicate sets of 8, each consisting of 2 controls and 6 concentrations of kaolin.

2. Observations:

Counts were made of living animals at approximately 8-hour intervals, and suspended solids concentrations were determined.

3. Statistical analysis:

Adjustments according to the mortality in the control aquaria were made by the method of Bliss (1935). The lethal concentration values (LC_{50}, LC_{20}, LC_{10}) were calculated with the logit method of Berkson (1953), and then regressed on exposure time to estimate the time-concentration mortality response.

II. RESULTS AND DISCUSSIONS

The following species had less than 10% mortality in the length of their exposure time, so that no lethal concentration estimates could be made:

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Table 1. Observed mortalities of species relatively insensitive to suspended kaolin

Species [a]	Exposure time in days	% Mortality at 100 g/L
Strongylocentrotus purpuratus (sea urchin)	9	0
Tapes japonica (Japanese clam)	10	0
Pagurus hirsutiusculus (hermit crab)	12	0
Sphaeroma pentodon (isopod)	12	0
Nassarius obsoletus (mud snail)	5	0
Mytilus edulis (blue mussel) (2.5 cm)	5	10
Mytilus edulis (blue mussel) (10 cm)[b]	11	10
Molgula manhattensis (tunicate)	12	9
Styela montereyensis (tunicate)	12	10

[a] Species grouped together were tested simultaneously in the same aquaria
[b] Tested simultaneously in the same aquaria with *Mytilus californianus* (Table 2)

However, the other species showed different sensitivities to suspended kaolin.

The following table summarises the data obtained for the more sensitive species where acute toxic effects were sufficient to determine an LC_{50} value after 200 h (8.3 d) exposure to suspended kaolin.

Species name	Common name	LC_{50} (g/L)
Mytilus californicus	Coast mussel	96
Crangon nigromaculata	Spot tailed sand shrimp	50
Palaemon macrodactylus	Grass shrimp	>77[15]
Cancer magister	Dungeness crab	32
Neanthes succinea	Polychaete worm	48
Ascidia ceratodes	Tunicate	38
Anisagammarus confervicolus	Amphipod	50
Parophrys vetulus	English sole	70 - 117
Cymatogaster aggregata	Shiner perch	3

The mortality response curves obtained following exposure to different concentrations and lengths of exposure are shown below:

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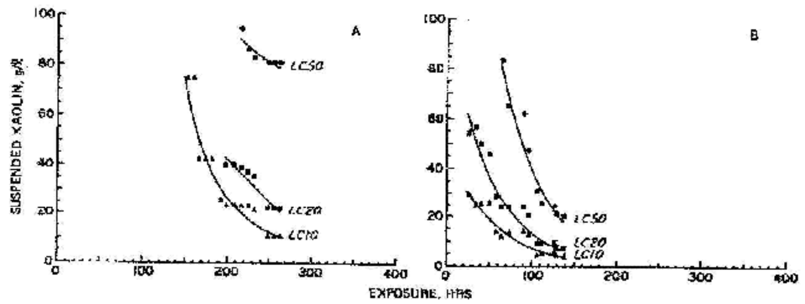

Fig. 1. Suspended kaolin time-concentration mortality curves: A. *M. californianus* (coast mussel), 31 ‰ salinity, 12°C B. *A. ceratodes* (tunicate), 33 ‰ salinity, 9°C.

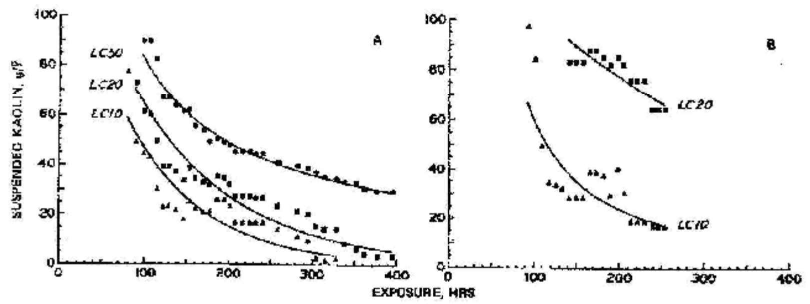

Fig. 2. Suspended kaolin time-concentration mortality curves: A. *C. nigromaculata* (spot-tailed sand shrimp), 31 ‰ salinity, 10°C B. *P. macrodactylus* (grass shrimp), 13 ‰ salinity, 11°C

[15] 50% mortality was not reached, 77 g/L = LC_{20}

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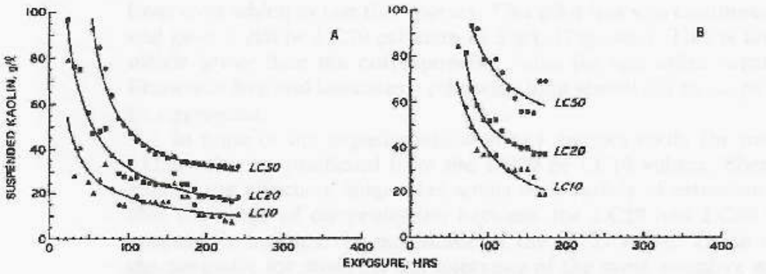

Fig. 3. Suspended kaolin time-concentration mortality curves: A. *C. magister* (dungeness crab), 32 ‰ salinity, 10°C B. *A. confervicolus* (amphipod), 13 ‰ salinity, 11°C

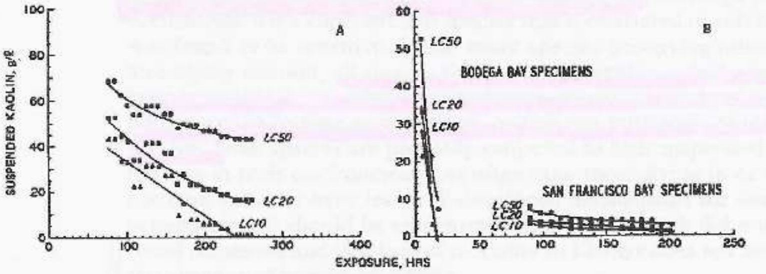

Fig. 4. Suspended kaolin time-concentration mortality curves: A. *N. succinea* (polychaete), 13 ‰ salinity, 11°C B. *C. aggregata* (shiner perch), Bodega Bay specimens: 14 to 89 g/L at 31 ‰ salinity and 9°C. San Francisco Bay specimens: 2 to 16 g/L at 16 ‰ salinity and 13°C

From the above results, it can be seen that the fish, shiner perch, was by far the most sensitive species, with a 200 h LC_{50} value of 3 g suspended kaolin/L whilst the Dungeness crab was marginally the most sensitive invertebrate, with a 200 h LC_{50} value of 32 g/L.

Analytics:
Suspended solids concentrations remained close to the desired levels in each aquarium with standard deviation of about 10% of the mean values, therefore LC_{50} values were based on nominal exposure concentrations. Positive control was not available.

III. CONCLUSIONS

Eight species, those whose natural habitat are soft muddy bottoms, were found to be relatively insensitive to suspended kaolin. Among the species that showed a mortality response, a wide range of sensitivities were found. The fish from pristine open water environments like the shiner perch (*Cymatogaster aggregate)* presented by far the highest sensitivity with an LC_{50} of 3 g/L.

Reference:	KCA 8.2.1/03 Isono, R.S., Kita, J., and Setoguma, T. 1998 (previously evaluated in DAR B9, IIA 8.2.1/02)
Title:	Acute effects of kaolinite suspension on eggs and larvae of some marine teleosts
Report No.:	Comparative Biochemistry and Physiology Part C (1998) volume 120, pages 449-455
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid

Executive Summary

In order to test the acute effects of kaolinite suspensions on marine fish early life stages, eggs of three pelagic species - red seabream, black seabream, striped beak perch - were exposed to concentrations of suspended kaolinite ranging from 0 mg/l to 10,000 mg/L for 24 hours. Their hatching success was then determined, as well as the development stage in terms of somite formation.

Previously unexposed larvae of red seabream, striped beak perch and threeline grunt were also exposed to 8 different concentrations of suspended kaolinite (again ranging from 0 to 10,000 mg/L) for durations up to 12 hours. The number of surviving larvae was counted.

The results show that the hatching success was high and stable, without appreciable decrease with increasing kaolinite concentration. Likewise, for all species, exposed eggs showed no marked delay in development. The results on larvae showed a general decrease of the survival rate with increase in both concentration and duration of exposure.

The LC_{50} values based on larvae survival for *O. fasciatus* were estimated at 710 mg/L (12h) and for *P. trilineatum* at 6200 mg/L (1h), 1500 mg/L (3h), 170 mg/L (12h). Over 50% mortality also occurred in larvae of *P. major* when exposed to 1000 mg/L for 12h. Kaolinite particles readily adhered to the body of *P. major* larvae at or above concentrations of 320 mg/L. However, even when the larvae were completely covered with kaolinite most still survived and responded to external stimuli. In contrast, larvae of the other two species showed little adherence of kaolinite particles although they were more sensitive overall.
In conclusion, the study shows that even very high concentrations of suspended kaolinite do not affect the early development and hatching of marine fish eggs from these marine species. In contrast, newly hatched fish larvae were more sensitive to suspended kaolinte. It is postulated that larvae have a larger and more delicate body surface relative to the body mass than do spherical eggs. This would make them more vulnerable to any abrasive effects of kaolinite particles. In addition, the highly agitated particle motion needed in the experiment may also have increased any abrasive effects and therefore be partially responsible for the mortality of kaolinite-exposed larvae.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolinite
Description:	Powder
Lot/Batch #:	117-100025, Wako Pure Chem, Japan
Purity:	Not specified
Stability of test component:	Stable
Particle size:	Mean diameter 4µm. Range 0.63 -12.7 µm

2. Test animals –	Pelagic eggs of **red seabream** (*Pagrus major*), **black seabream** (*Acanthopagrus schegeli*), **striped beakperch** (*Oplegnathus fasciatus*), and larvae of **red seabream** (*Pagrus major*), **striped beakperch** (*Oplegnathus fasciatus*) and **threeline grunt** (*Parapristipoma trilineatum*).
3. Testing Facility –	Central Laboratory, Marine Ecology Research Institute, Onjuku-machi, Chiba 299-51, Japan

B. STUDY DESIGN AND METHODS

1. Experimental design:

For tests on both eggs and larvae, the apparatus consisted of six exposure bottles containing 35 mL, rotating around a horizontal axis once every 10 seconds in order to maintain the kaolinite in suspension.

The highest kaolinite concentration tested (10000 mg/L) is one experienced only during man-made environmental perturbations.

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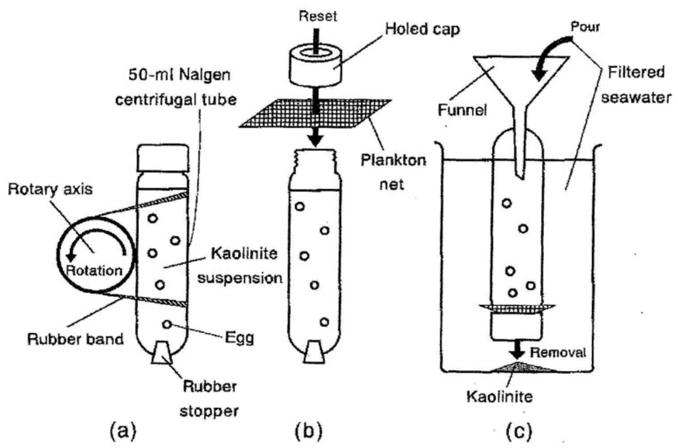

Fig. 1. Schematic of the exposure method and technique for kaolinite removal. (a) A set of exposure bottles, containing a kaolinite suspension with eggs or larvae, were fixed on the rotary axis of a roller pump; (b) after exposure under rotation, the cap of bottle was replaced by one with a central hole, to be tightened sandwiching a piece of 150-μm mesh plankton net; (c) then, the bottle was placed upside down in a 5-l aquarium, and filtered seawater was poured into the bottle to remove the kaolinite particles.

Groups of 20 eggs at 2-32 cell stages were exposed to 0, 320, 1000, 3200, 10000 mg/L suspended kaolinite concentrations for 24 hours. This test was run in triplicate.

Groups of 10 previously unexposed larvae were exposed to 0, 32, 100, 320, 560, 1000, 3200, 5600 and 10000 mg/L suspended kaolinite concentrations for durations up to 12 hours.

All data obtained were processed through analysis of variance and regression analysis.

II. RESULTS AND DISCUSSIONS

The results for hatching success and effects on numbers of embryonic somites showed that no tested concentration of suspended kaolin caused any delay in egg development. However, during exposure it was clear that the eggs became white and opaque and some showed a loss of buoyancy with more eggs settling at the bottom of the test vessel.

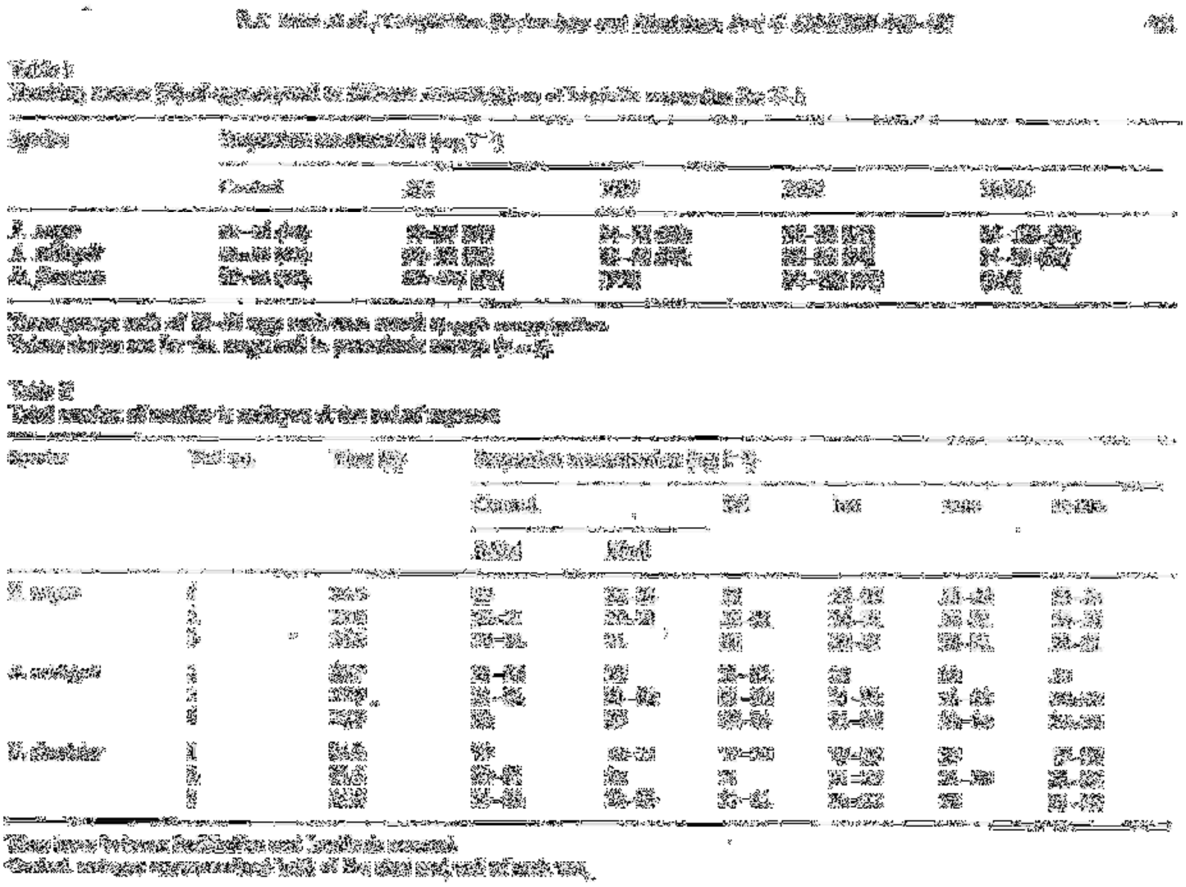

Table 1

[illegible]

Species	Suspension concentration (mg L−1)				
	Control	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

Table 2

[illegible]

Species	[illegible]	[illegible]	Suspension concentration (mg L−1)					
			Control		[illegible]	[illegible]	[illegible]	[illegible]
			[illegible]	[illegible]				
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

In contrast, larvae showed elevated mortalities with kaolin exposure, implying that they are more sensitive to suspended kaolinite than eggs. The LC_{50} values for *O. fasciatus* were estimated at 710 mg/L (12h) and for *P. trilineatum* at 6200 mg/L (1h), 1500 mg/L (3h), 170 mg/L (12h). Over 50% mortality also occurred in larvae of *P. major* when exposed to 1000 mg/L for 12h. Kaolinite particles readily adhered to the body of P. major larvae at or above concentrations of 320 mg/L. However, even when the larvae were completely covered with kaolinite most still survived and responded to external stimuli. In contrast, larvae of the other two species showed little adherence of kaolinite particles although they were more sensitive overall.

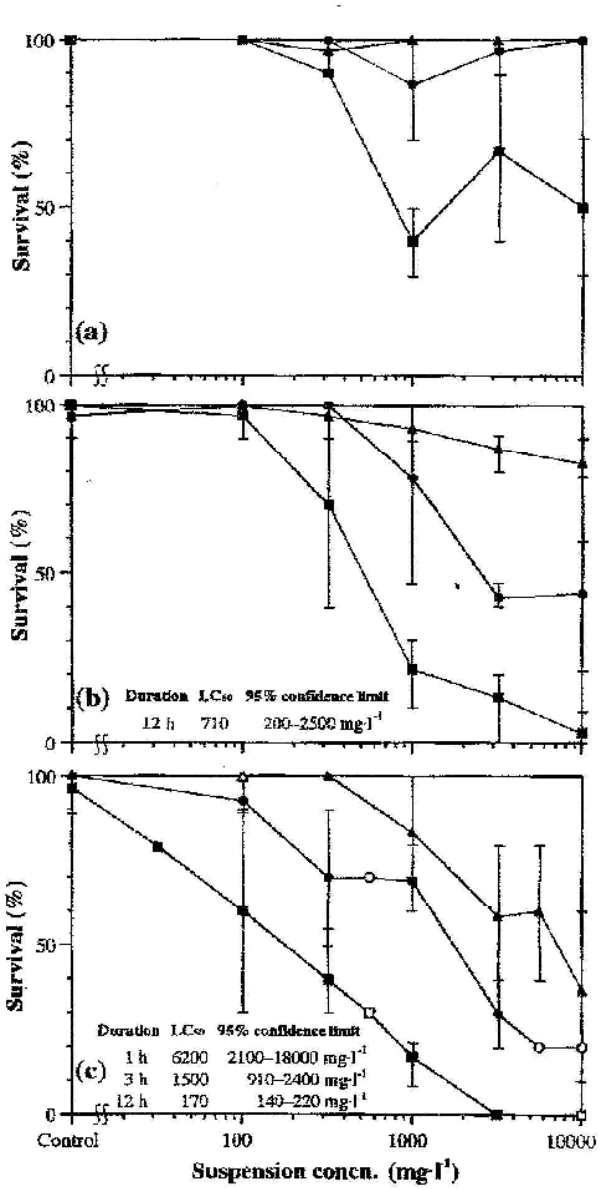

Fig. 2. Percentage survival of larvae exposed to different concentrations of kaolinite suspension for 1 h (▲), 3 h (●) and 12 h (■). (a) *P. major*; (b) *O. fasciatus*; (c) *P. trilineatum*. One to three groups each of 8–11 larvae were tested at each concentration. Values are given as the average and range for solid symbols. Open symbols show data with no replication. LC_{50} values are given with 95% confidence limit.

Analytics:
Not available

III. CONCLUSIONS

The study shows that even very high concentrations of suspended kaolinite do not affect the early development and hatching of marine fish eggs from these marine species. In contrast, newly hatched fish larvae were more sensitive to suspended kaolinite. It is postulated that larvae have a larger and more delicate body surface relative to the body mass than do spherical eggs. This would make them more vulnerable to any abrasive effects of kaolinite particles. In addition, the highly agitated particle motion needed in the experiment may also have increased any abrasive effects and therefore be partially responsible for the mortality of kaolinite-exposed larvae.

Reference:	KCA 8.2.1/04 Sherk J.A. Jr., O'Connor J.M., Neumann D.A., Prince R.D., and Wood K.V., 1973 (previously evaluated in DAR B9, IIA 8.2.1/03)
Title:	Effects of Suspended and Deposited Sediments on Estuarine Organisms, PHASE II
Report No.:	U.S. Department of Commerce, National Technical Information Service
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid

Executive Summary

This study looked at various aspects of the effects of suspended solids on estuarine fishes. Chapter I: Lethal effects of suspended solids on estuarine fishes covers toxicity tests conducted with kaolinite and Fullers Earth.

Species tested were: Menhaden (*Brevoortia tyrannus*), Striped killifish (*Fundulus majalis*), Mummichlog (*F. Heteroclitus*), Atlantic silverside (*Menidia menidia*), Striped bass (*Morone saxatilis*), White perch (*M. Americana*), Spot (*Leiostomus xanthurus*), Croaker (*Micropogon undulatus*), Weakfish (*Cynoscion regalis*) Hogchoker (*Trinectes maculatus*), Oyster toadfish (*Opsanus tau*).

After a 24 and 48 hour exposure to concentrations up to 140 g/L of kaolin, there was no mortality attributed to kaolin in any of the 11 species of fish tested. In comparison, significant mortality occurred in 11 species exposed to Fuller's earth suspensions between 96 and 140 g/L for 24 hours. Because of the lack of toxicity of kaolin, the rest of the study focused on experiments with Fuller's earth.

Concentrations up to 140 g/L of suspended kaolinite had no lethal effect on 11 species of estuarine fish, whereas fuller's earth showed higher toxicity to at least 11 of the 14 species tested.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolinite
Description:	White powder
Lot/Batch #:	Hydrite-10, Georgia kaolin Co.
Purity:	Not specified
Stability of test component:	Stable

2. Test animals:

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

1. [illegible]
2. [illegible]
3. [illegible]

3. Testing Facility: University of Maryland, Solomon's Natural Resources Institute.

B. STUDY DESIGN AND METHODS

1. Experimental design:

Eleven of the fourteen species were exposed to suspensions of kaolinite, because insufficient numbers of bluefish, cusk eel and bay anchovy were collected.

Tests were carried out in 27 L polyethylene tanks. Fish were starved for 2 to 5 days before being dispatched between one control group and 4 test concentrations groups. Concentrations of particles varied depending upon the species being tested, and whether the duration of the test was to be 12, 18, 20, 24, or 48 hours.

Sediment was maintained in suspension during tests by continuous submersible pumping and aeration. Concentrations in the experimental tanks were determined by weight. Replicate 5 mL samples were drawn from test and control tanks and dried.
Temperature was monitored and maintained.

2. Observations

Mortality, temperature, pH, dissolved oxygen were measured.

3. Statistical analysis:

Particles were analysed for size, organic content, and acid-extractable cations. LC_{10}, LC_{50}, LC_{90} were determined by normit analysis (Berkson, 1953).

II. RESULTS AND DISCUSSIONS

Kaolinite:
All the fishes exposed to kaolinite survived 24 hour exposures in concentrations up to 140 g/L. Several species (white perch, spot, toadfish, mummichog, hogchoker, menhaden) were exposed to 140 g/L kaolinite for 48 hours with the same result: no deaths directly attributed to the mineral solid.

Fuller's earth:
Three species showed no mortality (toadfish, cusk eel and hogchoker). Among the eleven species killed, six had mortality results consistent enough to calculate LC_{50} values, ranging between 2.40 g/L to 39.00 g/L.

Analytics:
Not available

III. CONCLUSIONS

Concentrations up to 140 g/L of suspended kaolinite had no lethal effect on 11 species of estuarine fish, whereas Fuller's earth showed higher toxicity to at least 11 of the 14 species tested.

CA 8.2.2 Long-term and chronic toxicity to fish

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

Reference:	KCA 8.2.2/01 Goldes, S.A., Ferguson, H.W., Moccia, R.D. and Daoust, P.Y. 1988 (previously evaluated in DAR B9, IIA 8.2.2.1)
Title:	Histological effects of the inert suspended clay kaolin on the gills of juvenile rainbow trout, *Salmo gairdneri*
Report No.:	Richardson, Journal of Fish Diseases (1988), volume 11 pages 23-33
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid

Executive Summary

Juvenile rainbow trout were exposed to 56, 280, 1400 and 7000 mg/L of suspended kaolin clay for 64 days. The monitoring of water turbidity and suspended solids levels revealed that the actual average levels of kaolin concentration were 36, 171, 1017 and 4887 mg/L. Quantitative and qualitative histological analyses were carried out on their gills.

Intracellular kaolin was visible within filament and lamellar epithelium in gills exposed to 171, 1017, 4887 mg/L kaolin at 4, 8, 16, 32, and 64 days. However, there was no adverse branchial pathology observed at all sample times in fish exposed to 36, 171 and 1017 mg/L kaolin. In gills exposed to 4887 mg/L kaolin, lesions were found at 16 and 32 days, probably caused by the proliferation of a protozoan identified as *Ichtyobodo necator*. However, gills regained normal architecture by 64 days despite continuous exposure to kaolin.

In this experiment, the highest dose of kaolin might have been indirectly responsible for creating a favourable environment for protozoan colonization that caused branchial lesions. However, these lesions were reversible, and the fish recovered thanks to increased mucus secretion, immune response and turnover of branchial epithelium.

According to analysis by light microscopy, the exposure to such levels of kaolin clay probably has little direct effect on overall gill structure. The LC_{50} for kaolin against rainbow trout must be >7,000 mg a.s./L (nominal).

In terms of chronic effects, the absence of qualitative and quantitative branchial changes at 36, 171, and 1017 mg/L indicates that environmental concentrations of kaolin are unlikely to cause histological alteration to gill structure, and that kaolin alone at these levels probably does not lead to lesions that might facilitate secondary infection. Since there is no reliable data on the histological effects of kaolin, it is impossible to determine whether the lesions in fish exposed to 4887 mg/L at 16 and 32 days were characteristic of the effects of kaolin. It is probable that the branchial proliferation was indeed a consequence of protozoan colonization, and not damage by kaolin. The NOEC value after 64 days was 1017 mg/L (mean measured) based on histological alteration to gill structure.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolin
Description:	White powder
Lot/Batch #:	hydrite flat D
Purity:	Not specified
Stability of test component:	Stable
Particle Size:	Median 5 µm, range 0.3 to 30 µm
2. Test animals:	Juvenile rainbow trout *Salmo gairdneri* Richardson 8 months old, 5.64±0.58 cm long
3. Testing Facility:	Fish Pathology Laboratory, Department of Pathology, Ontario Veterinary College, University of Guelph, Ontario, Canada.

B. STUDY DESIGN AND METHODS

1. Experimental Design:

Three untreated controls: unexposed fed control, unexposed reduced-feed control, and unexposed optimally reared control. Four kaolin concentrations: 56, 280, 1400 and 7000 mg/L. The highest concentration of kaolin was set at 70% of the dose causing 28% mortality in pilot studies.

Treatments were assigned randomly to twelve 200 L fibreglass tanks. Within each tank 27 fish were placed in each of 3 plastic 8-L perforated buckets that were suspended in the water column, giving a total 81 fish per tank in a semi-static system. The kaolin was kept in suspension during the study by a submersible pump located on the bottom of each tank.

2. Observations:

During the experiment, the following water parameters were monitored: temperature, dissolved oxygen, pH, un-ionized ammonia and turbidity.

Fish were sampled at 0, 4, 8, 16, 32, and 64 days. At each sampling, 6 fish per tank were arbitrarily chosen, removed, killed, and four gill arches from their right side were examined under light microscopy. The gills were checked for histological alteration and the thickness of their lamellae was measured.

3. Statistical analysis:

Differences in lamellar thicknesses and water quality were evaluated using analysis of variance, t-tests and Duncan's multiple range tests.

II. RESULTS AND DISCUSSIONS

The monitoring of water turbidity and suspended solids levels revealed that the actual average levels of kaolin concentrations were 36, 171, 1017 and 4887mg/L.

Qualitative histology: All gills samples were found normal, except for those taken at 16 and 32 days in fish exposed to 4887 mg/L kaolin, which showed lesions and the proliferation of a protozoan, *Ichtyobodo necator*. However, the morphology and parasite numbers returned back to normal at 64 days.

Quantitative study: Statistically significant increases in lamellar thickness only occurred at the base of lamellae in fish exposed to 4887 mg/L kaolin. However, there is no evidence that this loss of surface area had any significant effect on branchial function.

Mortalities were 1.7% in reduced-feed control and 8% in optimally reared control. Average percentage mortality per treatment ranged from 2.5% (36 mg/L) to 13.6% (4887 mg/L).

Analytics:
All kaolin concentrations were, on average, within 23% of desired levels for each treatment at time 0 of the 12-h experimental cycle. On average, turbidity and suspended solids levels dropped 22 and 24%, respectively, by the end of the 12-h period.

III. CONCLUSIONS

The highest dose was selected at 70% of the dose where 28% had been observed in pilot toxicity tests. The results of mortality during the test period demonstrate that the LC_{50} value for kaolin against rainbow trout must be >7,000 mg a.s./L.

In terms of chronic effects, he absence of qualitative and quantitative branchial changes at 36, 171, and 1017 mg/L indicates that environmental concentrations of kaolin are unlikely to cause histological alteration to gill structure, and that kaolin alone at these levels probably does not lead to lesions that might facilitate secondary infection. Since there is no reliable data on the histological effects of kaolin, it is impossible to determine whether the lesions in fish exposed to 4887 mg/L at 16 and 32 days were characteristic of the effects of kaolin. It is probable that the branchial proliferation was indeed a consequence of protozoan colonization, and not damage by kaolin. The NOEC value after 64 days was 1017 mg/L (mean measured) based on histological alteration to gill structure.

Reference:	KCA 8.2.2/02 Redding, J.M., Schreck, C.B., and Everest, F., 1987 (previously evaluated in DAR B9, IIA 8.2.1/04)
Title:	Physiological Effects on Coho Salmon and Steelhead of Exposure to Suspended Solids
Report No.:	Transactions of the American Fisheries Society (1987), volume 116, pages: 737- 744
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid

Executive Summary

Yearling coho salmon, *Oncorhynchus kisutch* and steelhead, *Salmo gairdneri* were exposed to high (2-3 g/L) or low (0.4-0.6 g/L) concentrations of three kinds of suspended solids (topsoil, kaolin clay, and volcanic ash) for up to 7-8 days.

Most of the study was conducted with suspended topsoil and will therefore not be summarised here in detail. However, one part of the study did compare the sublethal physiological effects of the three suspended solids, including kaolin clay. In this test plasma cortisol and sodium concentrations were measured and blood hematocrit levels were determined.

No lethal effects were observed in either species at the highest concentration, however both high and low concentrations of suspended solids did cause elevations in plasma cortisol but only high concentrations caused significant elevation in blood heamatocrit levels. These physiological changes are characteristic of sublethal stress. Neither concentration of suspended solids caused a significant change in blood sodium levels.

Chronic exposure to relatively high concentrations of suspended kaolin (2-4 g/L) caused some physiological changes that are characteristic of sub-lethal stress. It is suggested that chronic exposure to suspended solids at concentrations up to 4 g/L may be stressful to the fish initially but that physiological compensation probably occurs within a few days.

Elevated hematocrits were found in fish exposed to suspended solids, which may indicate a compensatory mechanism for impaired respiratory performance. However, no damage to gill epithelia was observed.

Overall stress was not severe but may have been able to reduce the fishes’ capacity to obtain food and resist disease.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Kaolin
   Description: White powder

Lot/Batch #:	Not stated. Origin: Anglo-American Clays, Atlanta, Georgia, USA
Purity:	Not specified
Stability of test component:	Stable
Particle Size:	Not specified

2. Test animals: *Salmo gairdneri.* Source**:** The Alsea or Marion Forks hatcheries. *Oncorhynchus kisutch.* Source: Fall Creek or Sandy hatcheries, Oregon Dept Fish & Wildlife

3. Testing Facility: Oregon State University Agricultural Experiment Station.

B. STUDY DESIGN AND METHODS

1. Experimental Design:

Comparison of topsoil, kaolin clay and volcanic ash effects on *Salmo gairdneri*:

Solids were sieved and dried before entering the flow-through system, consisting of a hopper, a vibrating tray, a 100-L chamber and a 20-L dilution chamber. The fish were tested in 100-L-tanks with a water inflow rate of 2 L/min. Solids were kept in suspension in the test tanks with water recirculation pumps and aerators.

During 48 hours, *Salmo gairdneri* were exposed to either high (2-3 g/L) or low (0.4- 0.6 g/L) concentrations of topsoil, kaolin clay, or volcanic ash. The concentration of suspended solids was monitored, as well as temperature, dissolved oxygen, conductivity and alkalinity of the water. Six fish were sampled from each replicate group before treatment and 3, 9, 24 and 48 hours after the initial exposure.

2. Observations

Plasma cortisol, plasma sodium concentration, and blood hematocrit levels were measured and gill histology was examined.

3. Statistical analysis

Data were subject to analysis of variance and the means for treatment and control groups were compared by Student's *t*-test where appropriate.

II. RESULTS AND DISCUSSIONS

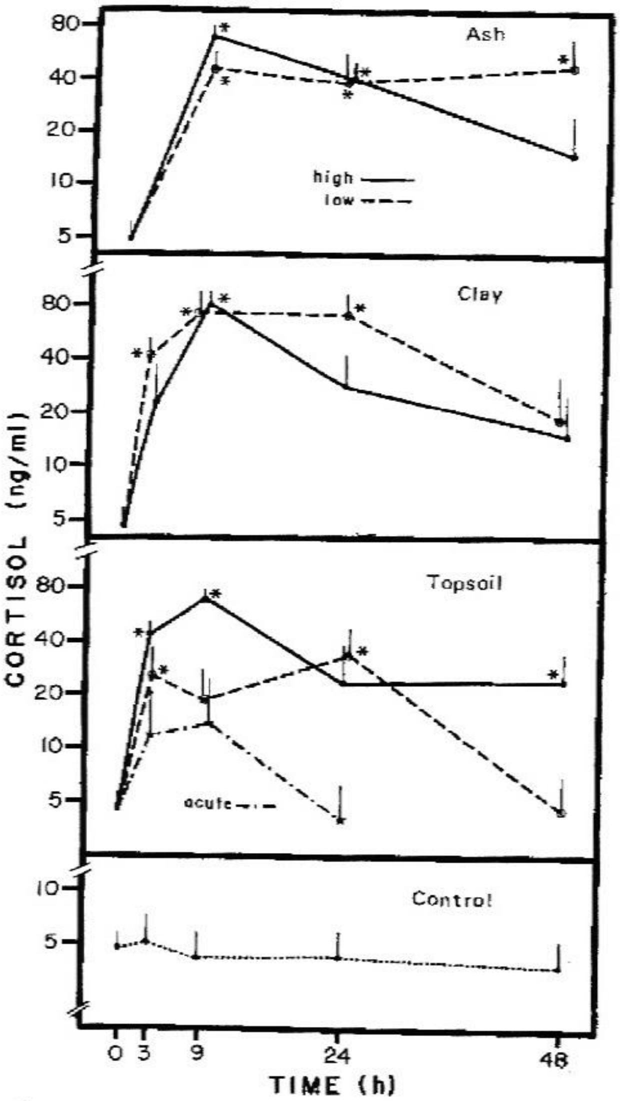

FIGURE 1.—Plasma cortisol concentrations (mean + SE) in yearling steelhead during continuous exposures to high (2-4 g/L) or low (0.4–0.6 g/L) concentrations of suspended volcanic ash, kaolin clay, or topsoil. Results from fish exposed acutely to 3 g/L suspended topsoil also are shown. Data are shown as antilogs of transformed ($\log_{10}X$) data, but the log scale is maintained. Asterisks indicate significant differences ($P < 0.05$) between treatment ($N = 11–13$) and pooled control groups ($N = 24–29$). Data from all groups were pooled for the hour-0 sample ($N = 60$).

There was no mortality in any of the three treatments. Plasma cortico steroid concentration increased significantly in fish exposed to both concentrations of the three types of suspended solids (Figure 1).

Hematocrits were consistently higher than in the control in fish exposed to high concentrations of the three types of suspended solids at 9 and 24 h after onset of the treatment (Table 2).

Plasma sodium concentrations were statistically similar in all treatment and control groups.

TABLE 2.—Blood hematocrits as percent packed red cells in yearling steelhead continuously exposed to high (2–4 g/L) or low (0.4–0.6 g/L) concentrations of suspended topsoil, kaolin clay, or volcanic ash. Data are presented also for fish acutely exposed to 3 g/L suspended topsoil. Data are means ± SE; sample sizes are in parentheses. Asterisks denote significant differences from control values at $P < 0.05$. Data at hour 0 were pooled for all groups within an experiment.

Exposure (h)	Topsoil				Clay			Ash		
	Control	Low	High	Acute	Control	Low	High	Control	Low	High
0	47±1 (17)				32±2 (12)			37±2 (17)		
3	48±2 (10)	47±2 (12)	47±1 (9)	47±1 (11)	44±2 (9)	45±2 (9)	44±2 (12)			
9	43±2 (8)	50±2* (8)	53±2* (9)	49±2 (6)	38±2 (11)	49±1* (12)	46±2* (11)	37±3 (6)	40±1 (9)	47±2 (11)
24	44±1 (12)	47±4 (5)	55±3* (7)	46±3 (11)	40±2 (6)	50±2* (10)	46±2* (11)	42±4 (6)	46±2 (11)	46±2 (7)
48	47±2 (11)	50±4 (7)	52±2 (9)		37±2 (6)	45±2* (11)	39±3 (9)	37±4 (5)	43±1 (11)	45±2 (11)

For *Salmo gairdneri* exposed for 2 days to either top soil, kaolin clay, or volcanic ash, gill tissue was examined histologically at magnifications of 10 x and 100 x. The appearance of gill tissue from treated fish was similar to that of control fish in all cases for both species.

Analytics:
Not available

III. CONCLUSIONS

Chronic exposure to relatively high concentrations of suspended kaolin (2-4 g/L) caused some physiological changes that are characteristic of sub-lethal stress. It is suggested that chronic exposure to suspended solids at concentrations up to 4 g/L may be stressful to the fish initially but that physiological compensation probably occurs within a few days.

Elevated hematocrits were found in fish exposed to suspended solids, which may indicate a compensatory mechanism for impaired respiratory performance. However, no damage to gill epithelia was observed.

Overall stress was not severe but may have been able to reduce the fish capacity to obtain food and resist disease.

The 48 h LC_{50} values for Coho salmon (*Oncorhynchus kisutch*) and Rainbow trout (*Oncorhnchus mykiss*) were estimated at >4 g/L, based on nominal exposure concentrations.

CA 8.2.2.1 Fish early life stage toxicity test

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

Reference:	KCA 8.2.2.1/01 Hashimoto, Y., Yamaguchi, M., Itō, T., and Tōi, J. 1986 (previously evaluated in DAR B9, IIA 8.2.2.2)
Title:	Effects of Kaolin on Hatching, Growth and Feeding Behaviour of Rainbow Trout, *Salmo gairdneri*
Report No.:	Bull. Tokai Reg. Fish. Res. Lab., (1986), volume 120 pages: 39 - 42
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid

Executive Summary

The effects of suspended kaolin on hatching (10 day exposure), growth and feeding behaviour of rainbow trout (*Salmo gairdneri*) were studied at 300, 100 and 30 ppm.

Turbidity had no impact on hatching rate, although slight retardation was noted at the highest concentration (300 ppm). Turbidity had no impact on growth; however, it reduced survival at the highest concentration tested. Feeding behaviour was most disturbed by turbidity, with effects seen at 30 ppm concentration.

In conclusion, high turbidity levels (300 ppm kaolin) has no or marginal impact on hatching and growth, but reduces feeding speed significantly, and reduces survival of the studied species.

The NOEC value is concluded to be 100 mg/L, based on the survival and growth of rainbow trout.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolin
Description:	White powder
Lot/Batch #:	Not stated. Origin: not stated
Purity:	Not specified
Stability of test component:	Stable
Particle Size:	Not specified
2. Test animals:	*Salmo gairdneri*. Source: not stated
3. Testing Facility:	Not stated.

B. STUDY DESIGN AND METHODS

1. Experimental Design:

Hatching: During 10 days, *Salmo gairdneri* eggs were exposed to 300, 100, 30 or 0 ppm suspended kaolin clay in water. A total of two replicates of 100 eggs per test concentration were observed. Concentration of suspended solids and temperature were most likely monitored. The number of hatched sacs was monitored every day from day 7 through to day 10.

Survival and growth: Juvenile *Salmo gairdneri* (30 per replicate per test concentration) were exposed to 300, 100, 30 or 0 ppm suspended kaolin clay in water. Concentration of suspended solids and temperature were most likely monitored.

Feeding: *Salmo gairdneri* individuals were exposed to following kaolin concentrations: 100, 30, 10 and 0 ppm and offered a fixed number of preys to consume.

2. Observations:

Hatching: Cumulative number of hatched eggs.

Survival and growth: Number of surviving individuals, final body length and final weight.

Feeding: Time taken to eat all offered preys. Time limit 30 minutes.

3. Statistical analysis

Not stated in the summary.

II. RESULTS AND DISCUSSIONS

Turbidity had no impact on hatching. A slight initial delay was observed at 300 ppm kaolin concentration (Table 1).

Table 1. The cummulative numbers of sac fry hatched from the eggs exposed to kaolin suspensions.

Concentration (ppm)	Replication	Days after the exposure started			
		7	8	9	10
300	A	5	82	94	100
	B	—*	—	97	100
100	A	55	88	100	100
	B	—	—	98	98
30	A	55	84	99	99
	B	58	84	100	100
0	A	24	84	99	99
	B	65	83	99	99

*No observation was made.

Turbidity had no impact on growth, although a slight impact on survival was noted at the highest test concentration of 300 ppm (Table 2).

Table 2. The effects of kaolin on the survival and growth of rainbow trout.

Concentration (ppm)	Survival	Average body length (cm)	Average weight (g)
300	23	3. 13	0. 56
100	28	3. 14	0. 55
30	28	3. 16	0. 59
0	30	3. 20	0. 57

Turbidity had a marked impact on feeding as time taken to consume all offered preys was significantly longer at 30 and 100 ppm. However, no impact was seen at 10 ppm exposure (Table 3)

Table 3. The effects of kaolin on the time required for eating all the given prey.

Concentration (ppm)	100	30	10	0
Time repuired for eating 100 daphnid (min.)	9	3	1	1
	13	1	·1	1
Time repuired for eating 20 carp fry (min.)	>30	>30	6	7
	>30	>30	7	7
	25	13	6	11
Time repuired for eating 100 carp fry (min.)	>30	22	9	8
•	>30	4	1	1
	>30	28	1	2
	>30	14	2	1

Analytics:
Not available

III. CONCLUSIONS

Although the effects of turbidity on hatching and growth are negligible, high levels of turbidity (300 ppm or 300 mg/L kaolin) have an impact on fish survival. Feeding times on some preys (carp fry) are dramatically increased at kaolin concentrations as low as 30 ppm (30 mg/L kaolin).

However, it may be noted that since growth itself in terms of weight and length is not reduced by the highest kaolin concentration tested, reduced survival could be attributed to the increased time and energy spent feeding.

In conclusion, high turbidity levels (300 ppm kaolin) have no or marginal impact on hatching and growth, but reduce feeding speed significantly, and reduce survival of the studied species.

The NOEC value is concluded to be 100 mg/L, based on the survival and growth of rainbow trout.

CA 8.2.2.2 Fish full life cycle test

Not required.

CA 8.2.2.3 Bioconcentration in fish

Aluminium silicate (kaolin) is not soluble in water and as a result has a very limited potential to bioaccumulate in fish, aquatic invertebrates, algae and aquatic plants. Hence no study on the potential bioconcentration of aluminium silicate has been performed.

Data point CA 2.8.1 requires the reporting of a constant for the equilibrium partition of the active substance in a water/n-octanol system. We request that this requirement be waived for the active substance in Surround WP which is calcined kaolin. The justifications for such a waiver are listed below:

- Calcined kaolin is practically insoluble in water or organic solvents[16]; the testing to determine the partition of the mineral in either phase is therefore of negligible value.

[16] "Kaolin (Kaolinum)", The International Pharmacopoeia – ed. 8th (2018), http://apps.who.int/phint/pdf/b/6.1.203.Kaolin-(Kaolinum).pdf

- Whereas the solubility of calcined kaolin in water has been reported at approximately 1.15 mg/L[17], such solubility is the result of the hydrolysis of lightly bound aluminium and silicon hydroxide moieties [18] and does not describe true solubilization in water. Natural deposits of surface kaolin are extremely stable and are not affected by rainfall. Kaolin exists stably in nature for millions of years.
- Analytical methodology for calcined kaolin is lacking due its inert nature and its unique semi-crystalline structure. The crystal structure of kaolin is disrupted during the calcination process therefore the typical analytical method for minerals, X-Ray Diffraction, provides no information on the identity of calcined kaolin. Atomic Absorption or Emission spectrometry focus on elemental analysis therefore cannot measure if water or octanol have any suspended particles of semi-crystalline calcined kaolin, and furthermore if there are trace solute amounts of aluminium and silicon hydroxide moieties these analytical methods cannot differentiate between clay types.

For the aforementioned reasons, we requests that the Log Pow data gap for calcined kaolin be waived.

CA 8.2.3 Endocrine disrupting properties

The available studies and literature search for aquatic animals do not indicate that aluminium silicate (kaolin) would have endocrine disrupting properties.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

No additional data are available for the acute toxicity to *Daphnia magna* since the approval of aluminium silicate (kaolin) (EFSA (2012)).

A waiver is requested for acute toxicity data with *Daphnia magna* for the active substance for the following reasons:

- A study with the formulated product is available. The findings from this single active substance formulation study with SURROUND® WP CROP PROTECTANT can be extrapolated and referred to since the formulated product consists of approximately 95% aluminium silicate (kaolin) and non-toxic inerts (please refer to Part C for details on formulation composition). The study with the formulated product, SURROUND® WP CROP PROTECTANT, is presented in Document MCP, Section 10 and showed low toxicity to *Daphnia magna* (EC_{50} >570 mg a.s./L); KCP 10.2.1/01; Goodband (2006)).

- In addition, Gordon and Palmer (2015)[19] collected aquatic organism responses to kaolin clay particle exposure from the open literature, including *Daphnia magna,* as they aimed to develop realistic water quality guidelines. A summary of the LC_{50} values collected from the open literature are summarised in the following table, demonstrating the high tolerance of aquatic invertebrates to suspended kaolin. *Daphnia magna* was the most sensitive overall, with stress induced through

[17] "Kaolin, calcined" from ECHA database, https://echa.europa.eu/registration-dossier/-/registered-dossier/13356/4/9
[18] Carroll, D., et al., Reactivity of Clay Minerals with Acids and Alkalies, Clays and Clay Minerals, 1971, Vol. 19, pp. 321-333. http://www.clays.org/journal/archive/volume%2019/19-5-321.pdf
[19] Gordon, A.K., and Palmer, C.G. (2015). Defining an exposure-response relationship for suspended kaolin clay particulates and aquatic organisms: work toward defining a water quality guideline for suspended solids. In: Environmental Toxicology and Chemistry, vol 34(4): 907-912

kaolin particles blocking the gut and ultimately leading to death. However, exposure was continuous for 7 days (not according to OECD 202), not allowing for settling or temporary displacement of the organism.

Table 8.2.4.1-1: Lethal point estimate data (lethal concentration affecting 50% of exposed organisms (LC_{50})) available for aquatic organisms exposed to suspended kaolin clay particles (from Gordon and Palmer, 2015)

Species name	Freshwater (FW) or saltwater (SW)	Exposure duration (d)	LC_{50} (mg/L)	Original reference
Mytilus californianus [a]	SW	8.3	96,000	McFarland & Peddicord (1980) Published ref (KCA 8.2.4.2/01)
Anisogammarus confervicolus [b]	SW	4.2	78,000	
Crangon nigromaculata [b]	SW	8.3	50,000	
Neanthes succinea [c]	SW	8.3	48,000	
Ascidia ceratodes [d]	SW	4.2	38,000	
Cancer magister [b]	SW	8.3	32,000	
Daphnia magna [b]	FW	7	75	Robinson *et al* (2010) Published ref (KCA 8.2.4.1/01)

[a] Bivalve. [b] Crustacean. [c] Annelida. [d] Tunicate.
*As the toxicity endpoints not generated under GLP conditions and are not based on OECD guidelines (*Daphnia magna* were exposed for 7 days), the values presented are to demonstrate the low toxicity of kaolin to aquatic invertebrates and not considered for quantifying the potential risk.

- Aluminium silicate will naturally settle provided water currents are slow enough to permit deposition (refer to Sutherland (2015), KCA 7.2.01 and discussion). Once settled (refer to Sutherland (2015), KCA 7.2.01 and discussion), aluminium silicate will be completely undistinguishable from naturally-present clay particles and become part of the sediment. Since aluminium silicate is not soluble in water, it is not unexpected that 100% of the product entering waterways will transfer to the sediment. This also explains the difficulty in maintaining kaolin-test solution concentrations within the aqueous phase when conducting a GLP laboratory study.

In light of these considerations, no additional acute toxicity testing with *Daphnia magna* is considered to be necessary with the active substance and the acute risk to aquatic invertebrates is concluded to be low.

Additional tests have not been conducted due to the low toxicity of aluminium silicate (kaolin) to *Daphnia*. However, a literature reference on the toxicity of *Daphnia magna* is available and summarised below, further supporting the low toxicity of kaolin to aquatic invertebrates.

Reference:	KCA 8.2.4.1/01 Robinson, S.E., Capper, N.A., and Klaine, S.J., 2009
Title:	The effects of continuous and pulsed exposures of suspended clay on the survival, growth and reproduction of *Daphnia magna*
Report No.:	Published in: Environmental Toxicology and Chemistry Vol 29 (1): 168-175
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid
Klimish score:	3, not known to be GLP, not guideline study design

Executive Summary

The objective of the present study was to characterize the effects of continuous and pulsed exposures of natural and defined clays on survival, growth, and reproduction of *Daphnia magna*. Two defined clays, montmorillonite and kaolinite, as well as clay isolated from the Piedmont region of South Carolina, USA, were used. Continuous exposures of clays elicited a dose dependent decrease in survival. Toxicity varied depending on clay source with montmorillonite > natural clay > kaolinite. Pulsed exposures caused a decrease in survival in a 24 h exposure of 734 mg/L kaolinite. Exposure to 73.9 mg/L also caused an increase in the time to gravidity, although there was not a corresponding decrease in neonate production over 21 days. No significant effects resulted from 12 h exposures even at 730 mg/L, almost 10 times the 24 h reproductive effects concentration. This suggests that exposure duration impacted toxicity more than exposure concentration in these pulsed exposures.

Overall, the mechanism of clay toxicity in these filter-feeding organisms is clogging of the gut tract, resulting in decreased food uptake and assimilation. When placed in clean water, daphnids can purge clay from their gut and recover.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 3, not guideline study
Reliability = 2, not known to be GLP

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolinite clay powder (KN)
Description:	Clay powder
Lot/Batch #:	Not reported. Obtained from VWR International
Purity:	Not specified
2. Reference item:	Montmorillonite clay (MN) and clay isolated (LC) from the Piedmont region of South Carolina, USA
3. Dilution water:	Reconstituted soft water (14.4 g $NaHCO_3$, 9.0 g $CaSO_4$, 9.0 g $MgSO_4$, 0.62 g KCl in 300 L of Milli Q water (Millipore) with a hardness of 45 ± 5 mg/L as $CaCO_3$ and alkalinity of 35 ± 5 mg/L as $CaCO_3$. Water with low hardness and alkalinity were used to minimize interaction between clay and dissolved ions, thus decreasing clay aggregates in test medium
4. Test organism	
Species:	*Daphnia magna*
Age at test initiation:	72 hours old
Source:	Obtained from cultures maintained at CU-ENTOX
Acclimation:	Not reported
5. Treatment groups:	<u>7-day test:</u> 0, 25, 50, 100, and 200 mg/L for KN, 0, 5, 15, 30, 50 75 mg/L for MN, and 0, 25, 50, 75, 100, and 150 mg/L for LC. <u>21 day test:</u> The 24 h exposure: 50, 100, 200, and 400 mg/L KN 5, 10, 25, and 50 mg/L MN, and 25, 50, 100, and 200 mg/L LC clay. The 12-h exposure: 100, 200, 400, and 800 mg/L KN The double pulse exposures were for 12 h: 800 mg/L KN for the first 12 h of the test period, then after a recovery period of 0, 48, 96, 192, or 384 h, were exposed to a second 12 h pulse of 800 mg/L.
6. Environmental conditions:	
Test vessels:	4 L plastic beakers on a stir plate. Organisms were held in the center of the beaker in glass test chambers within a modified test tube rack. Each test chamber consisted of 40 mm of 25 mm outer diameter, fused quartz glass

	tubing capped on both ends with 500 mm Teflon mesh. Mesh was secured to the bottom of the tube with aquarium safe silicon, and to the top with a 10-mm piece of 26 mm inner diameter fused quartz glass tubing.
Temperature:	Not reported
pH:	Not reported
Dissolved oxygen:	Not reported
Photoperiod:	16 hours light : 8 hours dark

B. STUDY DESIGN AND METHODS

1. Animal assignment and treatment:

Eight test chambers containing one *D. magna* each were placed in each test tube rack. Two replicate test beakers, each containing one test tube rack holding eight organisms, were used for each treatment for all tests. Test organisms were fed *Selenastrum capricornutum* every day at a final concentration of 150,000 cells/mL.

Daily renewal of test medium was conducted for the 7 day test.

In the 21-day test, episodic exposure bioassays were conducted to examine the effect of sediment pulses on mortality, growth, and reproduction for the majority of the daphnid life span. Organisms underwent a 24 h or less, single or double exposure, and were kept in clean medium for the remainder of the 21 d. For the single exposures, organisms were exposed for 24 h to each of the three types of clay or for 12 h to KN only.

During all episodic exposures, test beakers were only stirred on magnetic stir plates during the exposure. Medium was renewed daily, as with the 7-d tests.

2. Dose preparation:
Stock suspensions of the clays were prepared by mixing clay powder in deionized water on a stir plate overnight, followed by sonication. For 7-d tests, medium was prepared from stock suspensions to produce a concentration of 50 mg/L of each clay type.

3. Measurements/observations:
The 7 day test was conducted to determine mortality, growth, and reproduction endpoints. At the time of test medium renewal, the length of each organism was measured from its eye to the base of its tail using an ocular micrometer on a dissecting microscope, and the presence of eggs in the organisms' brood chamber was also recorded daily. Organisms that survived to the end of the test but had yet to become gravid were assumed to become gravid the following day and were assigned a value of 11 for days to gravidity.

In the 7 day test, test medium was renewed every day, with pH, dissolved oxygen and temperature measured before and after each renewal. Also, total suspended solids were measured at initiation, before and after each renewal, and at test termination. Due to the adhering of clay to surfaces, the concentration decreased over time; therefore, the average concentration was used in the analysis. During renewals, the test tube rack containing the test chambers was placed in a dish of clean medium while the test medium was replaced.

In the 21-day exposure test, on each day organism length was measured, the presence of the first brood of eggs in the brood chamber was noted, and neonates were counted to obtain the average number of neonates produced by each organism over the period of the test. Due to some mortality during the test, the average number of neonates per live organism for the extent of 21 d was calculated by taking the number of neonates on a particular day and dividing this by the number of live organisms in that beaker for that day. This value was then summed to obtain the average number of neonates produced per organism during the 21-d test period. During the second exposure of the double pulse, neonates could not be counted due to poor visibility in the media. For this 12-h period of time, the number of neonates was estimated by photographing each organism before and after the exposure and counting the neonates in the brood chamber that should have been released during the exposure.

Total suspended solids samples were analyzed using Standard Method 2540 D and Pall Metrigard glass fiber filters, with retention of 0.5 mm, were used as the filter medium.

4. Statistics:
Seven-day median lethal concentration (LC_{50}) values were calculated using Trimmed-Spearman-Karber with ToxStat.

Differences in days to gravidity, and the number of neonates produced per organism over 21 d, were analyzed using a one-way analysis of variance (ANOVA) and Tukey's multiple comparison test, using the statistical analysis software SAS. Growth rates were compared by defining a nonlinear growth curve model for each treatment, combining them into one model and comparing growth coefficients using a pairwise comparison. Also, the length of organisms recorded each day were compared using one-way ANOVA, and using sequential Bonferroni to adjust for type I and type II error.

II. RESULTS AND DISCUSSION

Concentrations of suspended clay decreased over the 24 h period between renewals, due to adhesion onto surfaces of the test chambers. Consequently, concentrations used to develop exposure response relationships were the average of the measured initial and final concentrations for each renewal.

A. 7-day test:
Daphnia magna exhibited dose-dependent mortality for all three clay types in the 7-d bioassays. The 7-d LC_{50} values of 5.17 (95% confidence interval 2.81, 9.54), 51.02 (31.25, 83.31), and 74.51 (65.08, 85.3) mg/L for MN, LC, and KN, respectively.

All three clay types decreased growth after 7 d ($p < 0.05$) (refer to figure below).

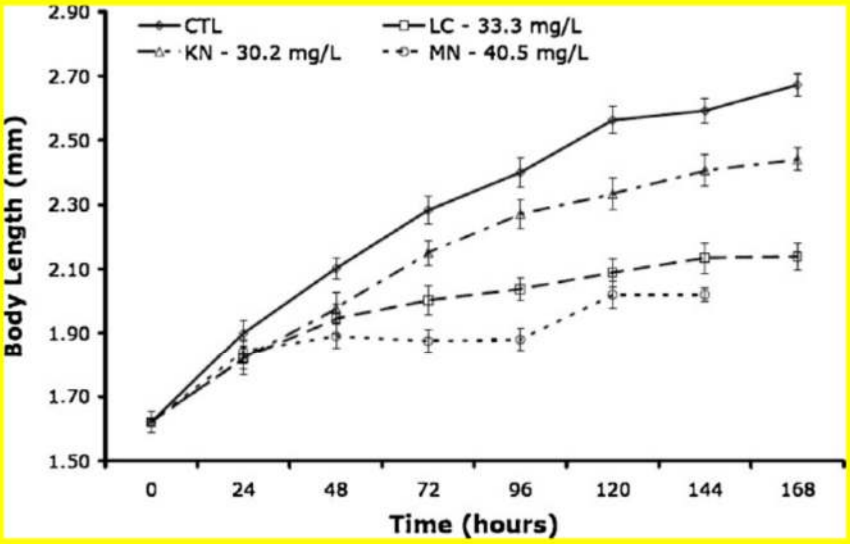

Fig. 2. Body length of *Daphnia magna* over time (mean ± standard error) for the control (CTL), Lost Creek (LC), kaolinite (KN), and montmorillonite (MN) treatments. Actual exposure concentrations are shown in the legend. Exposures were statistically different from controls at all time points > 24 h.

Fecundity was characterized by noting the day organisms became gravid. Kaolinite-exposed organisms did not show a difference in mean number of days to gavidity (mean number of days = 5.4) compared to the control (mean number of days = 5.4). While MN-treated organisms died before they became gravid and LC exposed daphnids experienced a significant increase in the number of days to gravidity (mean = 8.6).

B. Pulse exposure:

Organisms exposed to a single pulse of KN for 12 or 24 h, or MN or LC for 24 h, did not show any dose-dependent response for survival over 21 days. There were no exposure concentrations that led to a significant decrease in survival from the controls ($p > 0.05$). For the double pulse consisting of two 12 h KN exposures of 800 mg/L (measured concentration 734.2 mg/L), the organisms with a 0-h recovery time (or 24-h exposure) showed a significant decrease in survival from both the control and organisms with a recovery time greater than or equal to 48 h ($p<0.05$).

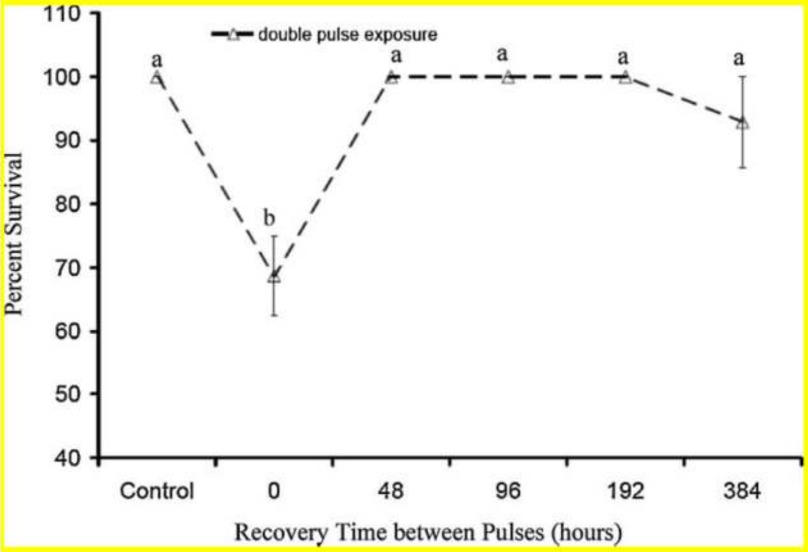

Fig. 3. Percent survival of *Daphnia magna* for double pulse exposures of varying recovery time between pulses (mean ± standard error). Only 0-h recovery time elicited a significant decrease in survival. Points with the same lowercase letter were not significantly different ($p > 0.05$).

Organisms exposed for 24 h to concentrations of 73.9, 152.5, and 312.0 mg/L KN; 9.0, 21.7, 48.9 mg/L MN; and 193.1 mg/L LC showed a significant increase in days to gravidity compared with control ($p < 0.05$). Days to gravidity for organisms exposed for 24 h at lower concentrations and all 12-h KN exposed organisms were not significantly different from the control ($p > 0.05$). Organisms exposed to all single pulse treatments showed no significant reduction in number of neonates produced over 21 d ($p>0.05$).

Organisms exposed to two 12-h KN exposures with a 0-h recovery time showed a significant increase in time to gravidity compared with control and organisms with a recovery time of 96 h or more ($p < 0.05$). Days to gravidity for the 0-h recovery was not significantly greater than organisms with the 48-h recovery ($p > 0.05$). There was no significant difference in the number of neonates produced for the double pulse exposures compared with controls ($p>0.05$; data not shown).

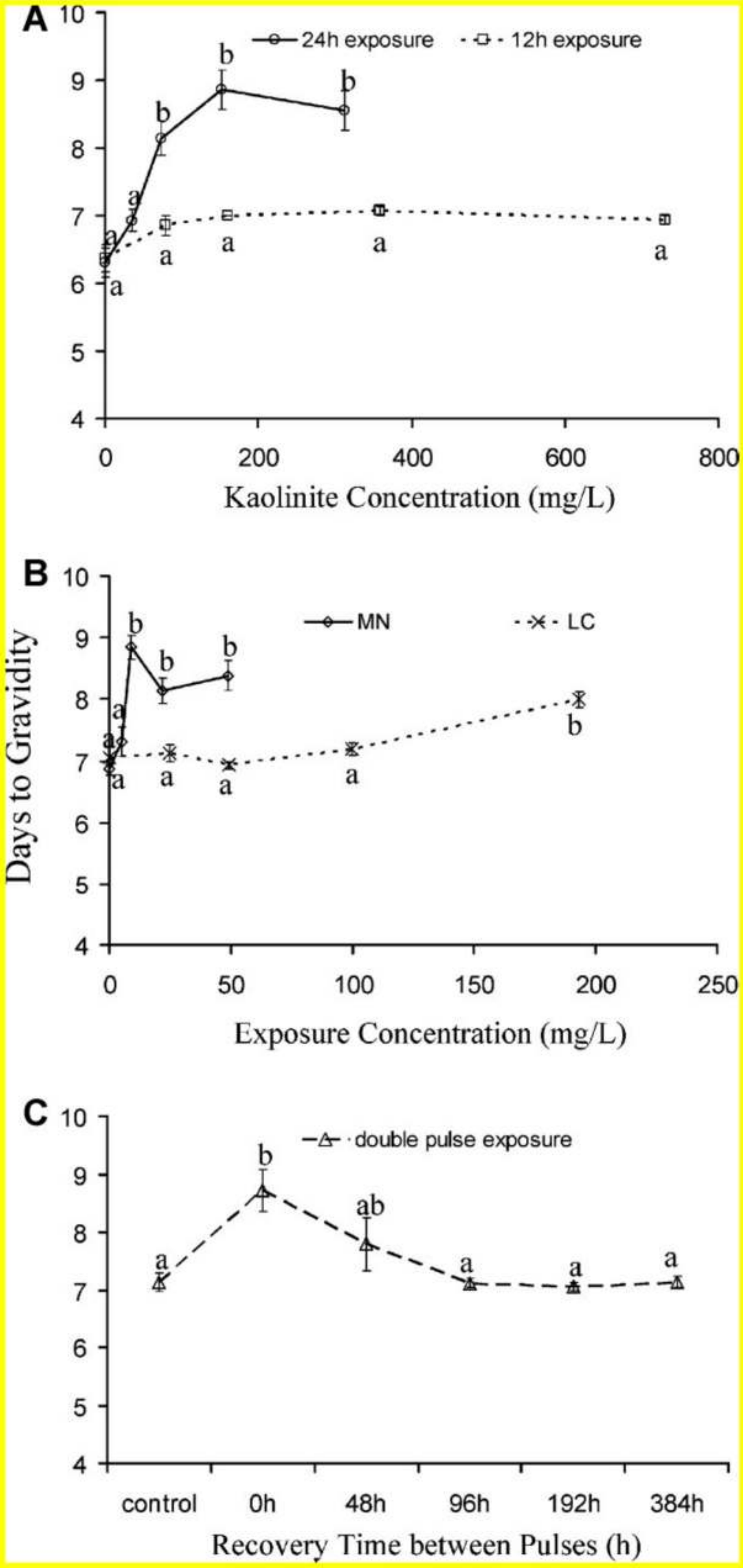

Fig. 4. Number of days from birth for organisms to become gravid, with first brood, for (**A**) single pulse kaolinite (KN), (**B**) montmorillonite (MN) and natural clay-sized particles (LC), and (**C**) double pulse kaolinite (KN) exposures (mean ± standard error). Note that experiments were started on day three of the organisms' lives. Points with the same lowercase letter were not significantly different ($p > 0.05$).

A significant difference in growth rate was observed over the 21-d bioassay For the 24-h exposures of all three clay types, there was a significant decrease in length, compared with the control, after the exposure. For day 1 to 14 (KN) and day 1 to 9 (MN and LC), there was at least one concentration that had significant difference in length ($p < 0.0056$, 0.0031, and 0.033, respectively). For the 12-h KN exposure, there was no significant difference after the exposure, but by day 16 the controls were significantly smaller than exposed organisms for all concentrations ($p < 0.05$). In the double pulse exposure only, organisms with a 0- or 48 h recovery time were significantly smaller than controls on days 1 through 13, and 5 through 19, respectively ($p < 0.05$).

III. CONCLUSION

The toxicity of the clays varied greatly among types of clay. Montmorillonite was the most toxic (7-d LC_{50} = 5.17 mg/L), KN the least toxic (7-d LC_{50} = 74.51 mg/L), and LC fell between these two (7-d LC_{50} = 51.02 mg/L).

These clays also reduced growth and reproduction in *D. magna*. These effects can also occur as a result of decreased food quality or quantity. It has been observed that surviving *D. magna* can purge their clay-clogged gut tract within approximately 30 min after transfer to clean medium. Organisms exposed to a single 12- or 24 h pulse of clay exhibited no significant mortality. Given that it required approximately 75 mg/L KN over 7 d for 50% of the organisms to die, it is expected that much higher concentrations would be required for significant mortality to be experienced with exposures of only 12 or 24 h. During the double-pulse exposures with a 0-h recovery time before the second pulse (essentially a 24-h exposure), there was a significant decrease in survival. This double-pulse exposure was at a concentration two times that used for highest 24-h exposures. Recovery times of 48 h or greater allowed organisms enough time to feed normally before being exposed again and having their feeding reduced again.

Single 12 h pulsed exposures did not significantly increase the number of days to gravidity; however, there was a significant increase in days to gravidity for single 24 h exposure at the three highest KN concentrations (73.9, 152.5, and 312 mg/L KN). There was also a significant increase in days to gravidity in the double pulse exposure when the recovery time was 0 h, which is essentially a 24 h exposure. The exposures that elicited a significant increase in days to gravidity (0 h recovery double pulse and the 24-h exposures of 312, 152.5, and 73.9 mg/L) were approximately equal, one-half, one-quarter, and one-tenth, respectively, of the highest 12 h KN exposure which showed no effect. This is one indication that duration of exposure is more important than concentration.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Additional tests have not been conducted due to the low toxicity of aluminium silicate (kaolin) to *Daphnia*. However, literature references on the toxicity of aquatic invertebrates ~~was previously submitted in support of this submission and the data approved (EFSA, 2012)~~ are available and summarised below, further supporting the low toxicity of kaolin to aquatic invertebrates.

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

Reference:	KCA 8.2.4.2/01 McFarland, V.A., and Peddicord, R.K. 1980 (previously evaluated in DAR B9, IIA 8.2.1/01)
Title:	Lethality of a suspended clay to a diverse selection of marine and estuarine macrofauna
Report No.:	Archives of Environmental Contamination and Toxicology (1980), volume 9, pages 733-741
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid

Executive Summary

Sixteen species of fish and aquatic invertebrates from marine and estuarine environments were exposed to different concentrations of suspended kaolin for different durations. All other water-quality parameters in the test aquaria were maintained close to natural levels. The concentrations of kaolin were varied in order to estimate lethal concentrations for 10, 20 and 50% of the population plus time-concentration mortality response curves for each species.

Naturally occurring marine and estuarine fish and invertebrates: Sea urchin (*Strongylocentrotus purpuratus*), Japanese clam (*Tapes japonica*), hermit crab (*Pagurush hirsutiusculus*), isopod (*Sphaeroma pentodon*), mud snail (*Nassarius obsoletus*), blue mussel (*Mytilus edulis*), tunicate (*Molgula manhattensis*), tunicate (*Styela montereyensis*), coast mussel (*Mytilus californianus*), spot tailed sand shrimp (*Crangon nigromaculata*), grass shrimp (*Palaemon macrodactylus*), Dungeness crab (*Cancer magister*), polychaete (*Neanthes succinea*), tunicate (*Ascidia ceratodes*), amphipod (*Anisogammarus confervicolus*), shiner perch (*Cymatogaster aggregate*).

A wide range of sensitivities to suspended kaolin was observed among the 16 species studied. For half of the species, no true Lethal Concentrations (LC) estimates could be made. There seemed to be some correlation between sensitivity and natural habitat. Organisms restricted to muddy bottoms were very insensitive, whilst species shown to be highly sensitive to high concentrations of suspended solids were either invertebrates occurring predominantly on sandy bottoms or in fouling communities, or open water fish. However, some tolerant species were also identified from this group.

The following table summarises the data obtained for the more sensitive species where acute toxic effects were sufficient to determine an LC_{50} value after 200 h (8.3 d) exposure to suspended kaolin.

Species name	Common name	LC_{50} (g/L)
Mytilus californicus	Coast mussel	96
Crangon nigromaculata	Spot tailed sand shrimp	50
Palaemon macrodactylus	Grass shrimp	>77*
Cancer magister	Dungeness crab	32
Neanthes succinea	Polychaete worm	48
Ascidia ceratodes	Tunicate	38
Anisagammarus confervicolus	Amphipod	50
Parophrys vetulus	English sole	70 - 117
Cymatogaster aggregata	Shiner perch	3

* 50% mortality was not reached, 77 g/L = LC_{20}

Eight species, those whose natural habitat are soft muddy bottoms, were found to be relatively insensitive to suspended kaolin. Among the species that showed a mortality response, a wide range of sensitivities was found. The fish from pristine open water environments like the shiner perch (*Cymatogaster aggregate*) presented by far the highest sensitivity. The most sensitive invertebrates were the Dungeness crab and the Tunicate with LC_{50} values of 32 and 38 g/L respectively.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolin
Description:	White powder
Lot/Batch #:	Hydrite flat D, obtained in a single batch
Purity:	Not specified
Stability of test component:	Stable

2. Test animals:

Naturally occurring marine and estuarine fish and invertebrates: Sea urchin (*Strongylocentrotus purpuratus*), Japanese clam (*Tapes japonica*), hermit crab (*Pagurus hirsutiusculus*), isopod (*Sphaeroma pentodon*), mud snail (*Nassarius obsoletus*), blue mussel (*Mytilus edulis*), tunicate (*Molgula manhattensis*), tunicate (*Styela montereyensis*), coast mussel (*Mytilus californianus*), spot tailed sand shrimp (*Crangon nigromaculata*), grass shrimp (*Palaemon macrodactylus*), Dungeness crab (*Cancer magister*), polychaete (*Neanthes succinea*), tunicate (*Ascidia ceratodes*), amphipod (*Anisogammarus confervicolus*), shiner perch (*Cymatogaster aggregate*).

3. Testing Facility: US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

B. STUDY DESIGN AND METHODS

1. Experimental design:

The laboratory system was a flow-through system of 16 hemispherical 75-L aquaria supplied with suspended kaolin and a complementary volume of dilution water at estuarine or oceanic salinity. The major water-quality parameters (pH, D.O., salinity, temperature and suspended solids) were kept stable during the experiments.

For the mud snail and the blue mussel, the 16 aquaria were arranged into 4 replicates, each consisting of a control and 3 kaolin concentrations (the highest concentration tested was 117 g/L). For all the other species, the 16 aquaria were arranged into 2 replicate sets of 8, each consisting of 2 controls and 6 concentrations of kaolin.

2. Observations:

Counts were made of living animals at approximately 8-hour intervals, and suspended solids concentrations were determined.

3. Statistical analysis:

Adjustments according to the mortality in the control aquaria were made by the method of Bliss (1935). The lethal concentration values (LC_{50}, LC_{20}, LC_{10}) were calculated with the logit method of Berkson (1953), and then regressed on exposure time to estimate the time-concentration mortality response.

II. RESULTS AND DISCUSSIONS

The following species had less than 10% mortality in the length of their exposure time, so that no lethal concentration estimates could be made:

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Table 1. Observed mortalities of species relatively insensitive to suspended kaolin

Species [a]	Exposure time in days	% Mortality at 100 g/L
Strongylocentrotus purpuratus (sea urchin)	9	0
Tapes japonica (Japanese clam)	10	0
Pagurus hirsutiusculus (hermit crab)	12	0
Sphaeroma pentodon (isopod)	12	0
Nassarius obsoletus (mud snail)	5	0
Mytilus edulis (blue mussel) (2.5 cm)	5	10
Mytilus edulis (blue mussel) (10 cm) [b]	11	10
Molgula manhattensis (tunicate)	12	9
Styela montereyensis (tunicate)	12	10

[a] Species grouped together were tested simultaneously in the same aquaria
[b] Tested simultaneously in the same aquaria with *Mytilus californianus* (Table 2)

However, the other species showed different sensitivities to suspended kaolin.

The following table summarizes the data obtained for the more sensitive species where acute toxic effects were sufficient to determine an LC_{50} value after 200 h (8.3 d) exposure to suspended kaolin.

Table 8.2.4.2/01-1: Summary of data obtained for the more sensitive species where acute toxic effects were sufficient to determine an LC_{50} value

Species name	**Common name**	**LC_{50} (g/L)**
Mytilus californicus	Coast mussel	96
Crangon nigromaculata	Spot tailed sand shrimp	50
Palaemon macrodactylus	Grass shrimp	>77[20]
Cancer magister	Dungeness crab	32
Neanthes succinea	Polychaete worm	48
Ascidia ceratodes	Tunicate	38
Anisagammarus confervicolus	Amphipod	50
Parophrys vetulus	English sole	70 - 117
Cymatogaster aggregata	Shiner perch	6

The mortality response curves obtained following exposure to different concentrations and lengths of exposure are shown below:

[20] 50% mortality was not reached, 77 g/L = LC_{20}

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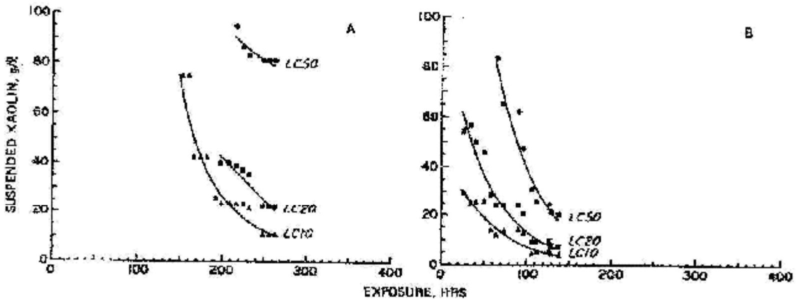

Fig. 1. Suspended kaolin time-concentration mortality curves: A. *M. californianus* (coast mussel), 31 ‰ salinity, 12°C B. *A. ceratodes* (tunicate), 33 ‰ salinity, 9°C.

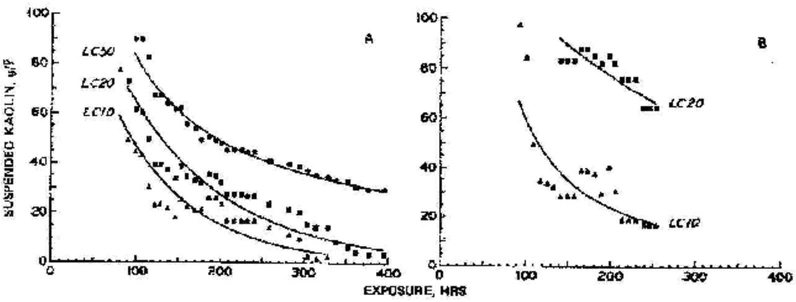

Fig. 2. Suspended kaolin time-concentration mortality curves: A. *C. nigromaculata* (spot-tailed sand shrimp), 31 ‰ salinity, 10°C B. *P. macrodactylus* (grass shrimp), 13 ‰ salinity, 11°C

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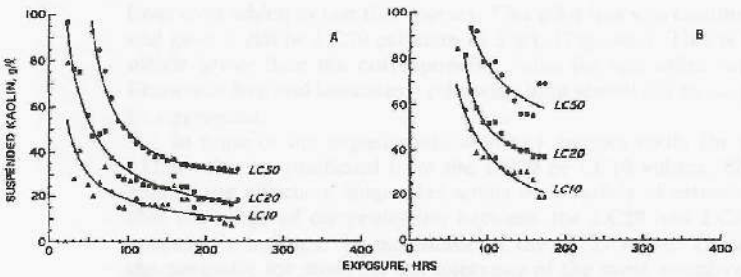

Fig. 3. Suspended kaolin time-concentration mortality curves: A. *C. magister* (dungeness crab), 32 ‰ salinity, 10°C B. *A. confervicolus* (amphipod), 13 ‰ salinity, 11°C

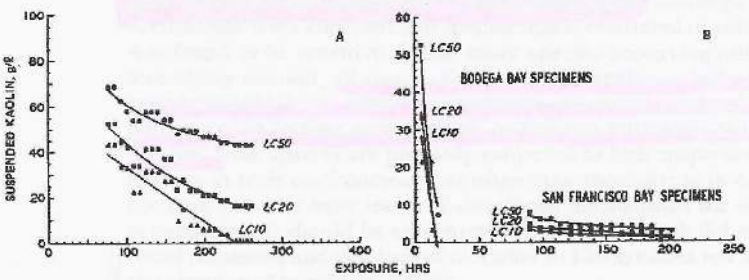

Fig. 4. Suspended kaolin time-concentration mortality curves: A. *N. succinea* (polychaete), 13 ‰ salinity, 11°C B. *C. aggregata* (shiner perch), Bodega Bay specimens: 14 to 89 g/L at 31 ‰ salinity and 9°C. San Francisco Bay specimens: 2 to 16 g/L at 16 ‰ salinity and 13°C

From the above results, it can be seen that the fish, shiner perch, was by far the most sensitive species, whilst the Dungeness crab was marginally the most sensitive invertebrate.

Analytics:
Suspended solids concentrations remained close to the desired levels in each aquarium with standard deviation of about 10% of the mean values, therefore LC_{50} values were based on nominal exposure concentrations. Positive control was not available.

III. CONCLUSIONS

Eight species, those whose natural habitat are soft muddy bottoms, were found to be relatively insensitive to suspended kaolin. Among the species that showed a mortality response, a wide range of sensitivities was found. The fish from pristine open water environments like the shiner perch (*Cymatogaster aggregate)* presented by far the highest sensitivity (with a 200 h LC_{50} value of 3 g suspended kaolin/L). The most sensitive invertebrates were the Dungeness crab and the Tunicate with 200 h LC_{50} values of 32 and 38 g/L, respectively.

Reference:	KCA 8.2.4.2/02 Gordon, A.K., Niedballa, J., and Palmer, G.C., 2013
Title:	Sediment as a physical water quality stressor on macro-invertebrates: A contribution to the development of a water quality guideline for suspended solids
Report No.:	WRC Report No. 2040/1/13
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid
Klimish score:	3, not known to be GLP, not guideline study design, toxicity endpoints could not be calculated, high variability of test concentrations

Executive Summary

The responses of three indigenous South African macroinvertebrates to suspended kaolin clay particles are reported. This particulate was chosen as commercial brands have a defined particle size range (< 4 µm), known particle shape (laminar), and are chemically inert with a low toxicity to aquatic species (WHO 2005).

All organisms exposed appeared to be very tolerant of the suspended kaolin, especially in terms of mortality which limited the generation of statistical point-estimates of effect. The most useful biological response measured was that of reproduction in shrimp *Caridina nilotica*. At a population endpoint level, however, the results were contradictory. Although there were fewer gravid females at 680 mg/L, the number of juveniles produced from this treatment were not statistically different from any other treatment. Furthermore, growth was not found to be a useful biological endpoint in *C. nilotica* as these organisms appeared to exhibit density dependent growth, growing larger at higher stress exposures as more individuals in the exposure vessel succumbed to mortality. An attempt was made to link mayfly kaolin exposure to a physiological response by linking damage to gills (either from abrasion or clogging) to two hypoxia biomarkers (lipid peroxidation and catalase activity). Unfortunately, a method could not be developed for viewing the mayfly gills under scanning electron microscopy (the gills were always damaged during the critical point drying process), and the biochemical responses exhibited no statistical exposure-response relationship. An important finding of these experiments was, however, that the biological consequences of settled particulates are much more pronounced than those of suspended solids.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 3 (not reliable), not known to be GLP, not guideline study design, toxicity endpoints could not be calculated, high variability of test concentrations

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Purified kaolin clay
Description:	Clay powder
Lot/Batch #:	Not reported. Obtained from Sigma Aldrich Chemicals
Purity:	Not specified
2. Test animals	Two mayfly nymph taxa (*Tricorythus discolor* and an Oligoneuridae sp.) and the fresh water shrimp *Caridina nilotica*
Source:	Mayfly nymphs were collected from the Balfour River in the Eastern Cape Province of South Africa. The shrimp were obtained from laboratory cultures maintained at the Institute for Water Research, Rhodes University
3. Test concentration:	
Trial 1:	60 ± 26, 229 ± 89, 325 ± 189, 751 ± 117 mg/L (measured)
Trial 2:	55 ± 3, 174 ± 12, 383 ± 26, 801 ± 57 mg/L (measured)
Trial 3:	Phase 1: 135 ± 43, 293 ± 81, 586 ± 161, 680 ± 166 mg/L (measured)
	Phase 2: 500, 1000. 1500, 2000 mg/L (nominal)
4. Environmental conditions:	
Trial 1:	Range: temperature: 17.7-18.0 °C, pH: 7.9-8.1, electrical conductivity: 90-118 mS/m, dissolved oxygen: 9.1 mg/L
Trial 2:	Range: temperature: 17.4-17.5 °C, pH: 8.3-8.4, electrical conductivity: 34.0-34.4 mS/m (initial) to 59.1-60.9 mS/m (final), dissolved oxygen: 9.1 mg/L
Trial 3:	Range: Phase 1: temperature: 25.1-26.9 °C, pH: 8.1-8.4, electrical conductivity: 40.3-40.7 mS/m (initial) to 59.1-65.2 mS/m (final), dissolved oxygen: 7.0-7.2 mg/L. Phase 2: temperature: 25.1-25.9 °C, pH: 7.8-8.3, electrical conductivity: 43.6-47.4 mS/m, dissolved oxygen: 6.9-7.2 mg/L.

B. STUDY DESIGN AND METHODS

The kaolin clay powder was added to a diluent of ‘aged’ dechlorinated tap water to constitute the exposure medium.

1. Exposure vessels:

The mayfly nymphs were exposed in laboratory-based recirculating artificial streams. Streams consisted of 1 m polyvinylchloride (PVC) channels, each receiving exposure medium *via* 15 mm tubing from a submersible pump. The flow rate down the channel averaged 0.36 m/sec. The experiment was conducted in a controlled environmental room. Two trials with mayfly nymphs were conducted, the first using three stones placed along the length of the channel for substrate, while the second trial used three square ceramic tiles instead.

As shrimp require lower flow rates compared to mayfly nymphs, 15 L rectangular glass tanks were used as the experimental vessels. Ten litres of experimental medium was used in each tank. Two air stones connected to an aeration pump were placed in opposite corners of each tank to provide oxygen for the organisms and to keep the suspended particulate exposure solutions in circulation. A stone was placed at the centre of each tank to act as additional substrate. An aquarium heater was placed in each tank to maintain the temperature between 24 °C-26 °C.

2. Measurements:

The water quality variables pH, electrical conductivity, water temperature and dissolved oxygen were measured daily (in the case of the mayfly trials and weekly during the longer shrimp experiment).

Measurements of the actual exposure concentrations of suspended kaolin particles were undertaken using two methods: the more rapid surrogate measure of turbidity (nephelometric turbidity units – NTU); and the more laborious measure of total suspended solids (TSS in mg/L). To determine TSS, 250 mL of exposure medium from each channel was filtered through pre-weighed filter paper, which was then dried and re-weighed to determine the sediment concentration per litre of water. A relationship between TSS concentration and turbidity was then quantified.

An analysis of the size particle distribution of kaolin clay used in the exposure-response tests was undertaken using a SediGraph 5100.

Trial 1

Ten *T. discolor* were placed in each of the 20 experimental channels and allocated into five treatments, including a control (i.e. four replicates per treatment). The experiment was conducted over a period of 10 days in an environmentally controlled room with air temperature set at 18 °C and a light:dark cycle of 12:12 hours. Organisms were not fed for the duration of the trial.

A wide range of kaolin exposure concentrations were used for the exposure treatments. However, these nominal suspended kaolin concentrations were seldom achieved in reality because of particle settling during the exposure trial. Consequently, only measured concentrations of suspended kaolin particles are reported. The biological endpoint measured was mortality.

Trial 2

Two mayfly taxa, *T. discolor* and an Oligoneuridae sp., were exposed together in the artificial streams. There were five treatments (including a control) with four replicates each, and 10 individuals of each taxon in each channel. The experiment was conducted over 13 days and from day 5 onwards an attempt was made to feed the mayflies every second day using very finely ground Tetramin® fish flakes.

Only measured concentrations of suspended kaolin exposures were reported. In addition to mortality, the measurement of three sublethal biological endpoints was attempted: the physiological response of gill damage from sediment particle abrasion; and two biomarkers of hypoxia – lipid peroxidation and catalase activity.

An attempt was made to photograph the gills of mayflies using the scanning electron microscope. In addition, on completion of the exposure trial, surviving *T. discolor* and Oligoneuridae sp. individuals were collected from each replicate stream and a catalase assay was performed and the lipid peroxidation assay was conducted. Lipid peroxidation is malondialdehyde (MDA) in terms of protein concentration rather than in relation to whole body mass was measured

Trial 3

This experiment was divided into two phases:

Day 0-17: Five females and five males shrimp were placed in each of the 15 glass tanks and allocated into five treatments (including a control) with three replicates each. After 17 days, the number of gravid females was counted and the adult mortality determined for each exposure treatment. Turbidity of the exposure medium prevented daily observations of mortality or determination of when females became gravid.

Day 17-71: Male shrimp were then removed from the tanks and the experiment was continued with the eggs from the gravid females being allowed to hatch. After 42 days of exposure, tanks were emptied and restarted with fresh exposure medium, while concurrently an initial count of the number of juveniles produced in each tank was conducted. After 71 days of exposure the final count of juveniles was conducted and the carapace length of juveniles measured. Once again, the turbidity of the exposure medium prevented more frequent observations of the numbers of juvenile shrimp produced over the course of the trial. Furthermore, as juvenile shrimp are very sensitive to handling it was decided not to capture and remove the shrimp from the tanks more frequently.

During both phases of the trial shrimp were fed every second day with crushed Tetramin® flakes. The growth of the shrimp was determined by measuring carapace length.

3. Statistical analysis:

The following procedure was followed when testing for significant differences in mortality, reproduction, growth and sublethal responses between exposure treatments and the control treatment. The data were tested for normality and equality of variances using the Shapiro- Wilk test and Levene's test respectively. The students t-test was applied to parametric data with equal variance, while the Mann-Whitney U test was applied to non-parametric data or data with unequal variance. All the above analyses (including correlation analysis) were undertaken using Statistica (version 9), with significance set at $P < 0.05$.

The calculation of LC_{50} values for mortality data was attempted using the Probit method. In the event that the data were not suited to this parametric model, the non-parametric Trimmed Spearman-Karber (TSK) method was attempted.

II. RESULTS AND DISCUSSIONS

Eighty percent of the size particle distribution indicated that kaolin particles are smaller than 1 µm, with the largest particles in the 3-4 µm range.

Trial 1:

Settling of kaolin particles in some replicates resulted in high variability of exposure concentrations within some treatments as indicated by the high standard deviations.

Exposure to suspended kaolin particles for 4 days resulted in low percentage mortality in all exposure treatments and no mortality in controls. At 4 days, the percentage mortality in exposure treatments was not determined to be significantly different ($p < 0.05$) from that of the control. However, between days 8-9 of the trial the majority of exposed mayflies died and mortality began occurring in the control treatment. By day 10 all treatments were significantly different to the control with >95% mortality across all kaolin concentrations and 23% in the control.

Trial 2:

After 4 days of exposure, mortality levels in *T. discolor* were below 10% for all treatments. Mortality rate in *T. discolor* began to accelerate after approximately 9 days of exposure, however by the end of the trial the highest treatment mortality was only 50%, compared to the 100% measured in trial 1. There was no dose dependent response and no significant difference in mortality between control and exposed treatments. An LC_{50} could not be determined.

The Oligoneuridae sp appeared to be more sensitive to kaolin exposure with between 10- 20% mortality after 4 days. In addition, morality rate began increasing from approximately day 4, earlier than for *T. discolor*, although once again by the end of the trial the highest treatment mortality was only approximately 50%. Unfortunately, the control mortality at the end of the trial was 25%. It is possible that the increased earlier mortality rate and higher control mortality may be related to the requirement of this mayfly species for fast water flows. Although an LC_{50} could not be determined, the two highest kaolin concentrations were found to cause significantly higher mortality than the control, giving a no observed effect concentration

(NOEC) of 174 mg/L.

Assay measurements:
An attempt was made to determine the effect of kaolin on the gills in both mayfly species, but this was not possible using SEM process.

Lipid peroxidation in *T. discolor* appeared to increase at the lower exposure concentrations and then decrease at the higher kaolin concentrations after both 4 and 13 days of the trial, although this is not statistically significant. Lipid peroxidation appeared to increase in this mayfly species over the course of the experiment with some individuals by day 13 experiencing high levels of cell damage in the exposed treatments. There appeared to be a small dose dependent increase in lipid peroxidation in Oligoneuridae sp. after both 4 and 13 days of exposure, although this was again not statistically significant. Unlike *T. discolour*, Oligoneuridae sp. showed no increase in lipid peroxidation between day 4 and 13.

In terms of catalase activity, *T. discolor* exhibited no dose dependent response during the trial. After both 4 and 13 days of exposure, average catalase activities in Oligoneuridae sp. indicate a possible dose response, although this was not statistically significant.

Trial 3:
Day 0-17: Mortality of *C. nilotica* over the 17 days of exposure was very low, with no mortality in the control treatment and less than 20% occurring at the highest kaolin concentration. The average mortality at 680 mg/L was statistically higher than in the control. The average number of gravid females occurring in each treatment decreased with increasing exposure concentration, being statistically lower at the highest concentration of 680 mg/L. Estimated NOEC is 586 mg/L.

Day 17-71: Excessive settling was significant, as in those replicates where it occurred, far fewer juveniles shrimp survived compared to other replicates within the same treatment. The removal of replicates with excessive settling from the dataset indicated that suspended kaolin particles at high concentrations did not negatively affect the number of juvenile shrimp produced, while settled particles did. Growth of juveniles, measured as final carapace length, was also not statistically affected by increasing concentrations of suspended kaolin particles, or excessive particles settling. However, an inverse relationship between the number of juveniles in each tank or exposure replicate and carapace length appears evident, suggesting density dependent growth for these organisms.

Table 8.2.4.2/02-1: Actual measured suspended kaolin concentrations (average ± SD), with associated juveniles produced, per replicate over the exposure period

Nominal concentration (mg kaolin/L)	Measured concentration (mg kaolin/L)	No. of juveniles/replicate
0	2 ± 3	215
	3 ± 3	133
	2 ± 3	174
500	296 ± 75	149
	281 ± 68	257
	271 ± 92	151
1000	628 ±225	266
	461 ± 246	97*
	595 ± 268	127
1500	814 ± 335	168
	923 ± 261	133
	466 ± 174	11*
2000	995 ± 419	209
	1265 ± 523	164
	576 ± 261	0*

*Replicates with excessive kaolin settling

III. CONCLUSIONS

There were technical challenges encountered with suspended kaolin: 1) maintaining particulates in suspension, and keeping the concentrations of those suspensions constant across replicates; and 2) the difficulty of observing responses of organisms in turbid treatments limited the type of biological endpoint that could be measured and how often this measurement could take place.

All organisms exposed appeared to be very tolerant of the suspended kaolin, especially in terms of mortality which limited the generation of statistical point estimates of effect. The most useful biological response measured was that of reproduction in shrimp *C. nilotica*. However, from a population point level of view the results were contradictory. Although there were fewer gravid females at 680 mg/L, the number of juveniles produce from this treatment were not statistically different from any other treatment. Growth was not found to be a useful biological endpoint in *C. nilotica* as these organisms appeared to exhibit density dependent growth, growing larger at higher stress exposures as more individuals in the exposure vessel succumb to mortality. An attempt to link mayfly kaolin exposure to a physiological mode of action by linking damage to gills (either from abrasion or clogging) to two hypoxia biomarkers (lipid peroxidation and catalase activity). Unfortunately, a method could not be developed for viewing the mayfly gills under scanning electron microscopy (the gills were always damaged during the critical point drying process), and the biochemical responses exhibited no statistical exposure-response relationship.

Lastly, the experiments undertaken during this study suggest that the biological consequences of settled particulates are much more pronounced than suspended solids.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

No new data are available or required for long-term and chronic toxicity to *Daphnia magna* since the approval of aluminium silicate (kaolin) (EFSA (2012)). As discussed in the original DAR, data available from the open literature confirm that no classification for toxicity of aluminium silicate (kaolin) to aquatic organisms was necessary; it was considered that the risk to non-target organisms from the representative use of aluminium silicate (kaolin) will be low.

A waiver is requested ~~and accepted during the initial EFSA review (EFSA (2012))~~ for long-term and reproductive toxicity studies for daphnids based on the following information:

- As detailed in the original DAR (Section B.9.2.5), 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.

- 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. As detailed in the original DAR (Section B.9.2.5), 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.

- This is further supported by the rational that under OECD ecotoxicity testing in water-sediment systems (i.e., OECD Technical Guidance No. 218[21], 225[22] and 239[23]) the standardized formulated sediment is prepared with 20% (dry weight) kaolin clay (kaolinite content preferably above 30%). As a comparison, overspray of a still pond 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 sediment density of 1.3 g/cm^3, and sediment layer thickness of 5 cm, this deposition of kaolin following application of SURROUND® WP CROP PROTECTANT represents less than 0.01% of the sediment weight (i.e. far lower than the 20% kaolin used in standard ecotoxicity tests).

- Aluminium silicate will naturally settle (refer to Sutherland (2015), KCA 7.2.01 and discussion) provided water currents are slow enough to permit deposition. Once settled, aluminium silicate will be completely undistinguishable from naturally-present clay particles and become part of the sediment. Since aluminium silicate is not soluble in water, it is not unexpected that 100% of the product entering waterways will transfer to the sediment. This also explains the difficulty in maintaining kaolin-test solution concentrations within the aqueous phase when conducting a GLP laboratory study.

- Chronic toxicity testing with *Daphnia magna* has not been conducted due to the low acute toxicity of aluminium silicate (kaolin) to aquatic invertebrates as demonstrated in the available acute toxicity studies.

- From the open literature, a summary of the NOEC values for aquatic organism responses to kaolin clay particle exposure, including *Daphnia magna,* are summarised in the following table that demonstrate aquatic invertebrate tolerance to kaolin.

Table 8.2.5.1/1: Sublethal no observed effect concentration (NOEC) data for freshwater organisms exposed to suspended kaolin clay particles**

Species name	Exposure duration (d)	NOEC (mg/L)	Biological response	Reference
Caridina nilotica [a]	17	586	Reproduction	Gordon *et al* (2013) Published ref (KCA 8.2.4.2/02)
Daphnia magna [b]	1	50*	Reproduction	Robinson *et al* (2010) Published ref (KCA 8.2.4.1/01)

[a] Crustacean, [b] Cladoceran
*Actual NOEC could be between 50-100 mg/L
**As the toxicity endpoints are not generated under GLP conditions and are not based on OECD guidelines (*Daphnia magna* were exposed for 1 day), the values presented are to demonstrate the low toxicity of kaolin to aquatic invertebrates and not considered for quantifying the potential risk.

- Furthermore, fish were demonstrated to be more sensitive to acute exposure from kaolin (LC_{50} = 494 mg a.s./L), compared to aquatic invertebrates (EC_{50} >570 mg a.s./L, reported in MCP, Section 10). As an ELS study is available for fish and considered in the risk assessment, the chronic risk to aquatic invertebrates is also considered to be addressed.

- In addition, a chronic algae study is available and also demonstrated a low toxicity from exposure to kaolin.

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[21] OECD TG 218 (2004), "Sediment-Water Chironomid Toxicity Using Spiked Sediment". Organisation for Economic Co-operation and Development, Paris, 2004
[22] OECD TG 225 (2007), "Sediment-Water *Lumbriculus* Toxicity Test Using Spiked Sediment". Organisation for Economic Co-operation and Development: Paris, 2007.
[23] OECD TG 239 (2014b), "Water-Sediment *Myriophyllum Spicatum* Toxicity Test". Organisation for Economic Co-operation and Development, Paris, 2014.

In light of these considerations, no long-term and chronic toxicity testing with *Daphnia magna* is considered to be necessary with the active substance for the purposes of renewal and the chronic risk to aquatic invertebrates is concluded to be low.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

No new data are available or required for long-term and chronic toxicity to an additional aquatic invertebrate species since the original approval of aluminium silicate (kaolin) (EFSA (2012)). As discussed in the original DAR, data available from the open literature confirm that no classification for toxicity of aluminium silicate (kaolin) to aquatic organisms was necessary. Furthermore, as kaolin is not an insect growth regulator, addition aquatic invertebrate testing is not required according to the Commission Regulation EU 283/2013.

As discussed above in Section CA 8.2.5.1, in light of the weight-of-evidence, no long-term and chronic toxicity testing with aquatic invertebrates is considered to be necessary and the chronic risk to aquatic invertebrates is concluded to be low.

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

No new data are available or required for *Chironomus riparius* since the original approval of aluminium silicate (kaolin) (EFSA (2012)). As discussed in the original DAR, data available from the open literature confirm that no classification for toxicity of aluminium silicate (kaolin) to aquatic organisms was necessary; it was considered that the risk to non-target organisms from the representative use of aluminium silicate will be low.

A waiver is requested for *Chironomus riparius* toxicity studies based on the following information:

- As detailed in the original DAR (Section B.9.2.7), aluminium silicate (kaolin) is present in sediments and suspended solids of natural water bodies (see Document MCP, Section 9 for natural background levels).
- It is also not soluble in water and thus not bioavailable to sediment dwelling organisms.
- Following settling as sediment, aluminium silicate (kaolin) will become indistinguishable of existing sediment and will have no impact on sediment dwelling organisms.
- According to OECD guidelines for ecotoxicity tests that require the use of sediment, i.e., OECD 218 or 233, the standardized formulated sediment is prepared with 20% (dry weight) kaolin clay (kaolinite content preferably above 30%). As a comparison, overspray of a still pond 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 sediment density of 1.3 g/cm^3, and sediment layer thickness of 5 cm, this deposition of kaolin following application of SURROUND® WP CROP PROTECTANT represents less than 0.01% of the sediment weight (i.e. far lower than the 20% kaolin used in standard ecotoxicity tests).

In light of these considerations, no toxicity testing with *Chironomus riparius* is considered to be necessary with the active substance for the purposes of renewal and the chronic risk to sediment dwellers is concluded to be low.

CA 8.2.5.4 Sediment dwelling organisms

No new data are available or required for sediment dwelling organism toxicity since the original approval of aluminium silicate (kaolin) (EFSA (2012)). As discussed in the original DAR, data available from the open literature confirm that no classification for toxicity of aluminium silicate (kaolin) to aquatic organisms was necessary; it was considered that the risk to non-target organisms from the representative use of aluminium silicate (kaolin) will be low.

A waiver is requested for toxicity studies on sediment-dwelling organisms based on the following information:

- As detailed in the original DAR (Section B.9.2.7), aluminium silicate (kaolin) is present in sediments and suspended solids of natural water bodies (see Document MCP, Section 9 for natural background levels).

- It is also not soluble in water and thus not bioavailable to sediment dwelling organisms.

- Following settling as sediment, aluminium silicate (kaolin) will become indistinguishable of existing sediment and will have no impact on sediment dwelling organisms.

- According to OECD guidelines for ecotoxicity tests that require the use of sediment, i.e., OECD 218 or 233, the standardized formulated sediment is prepared with 20% (dry weight) kaolin clay (kaolinite content preferably above 30%). As a comparison, overspray of a still pond 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 sediment density of 1.3 g/cm^3, and sediment layer thickness of 5 cm, this deposition of kaolin following application of SURROUND® WP CROP PROTECTANT represents less than 0.01% of the sediment weight (i.e. far lower than the 20% kaolin used in standard ecotoxicity tests).

In light of these considerations, no testing to sediment dwelling organisms is considered to be necessary with the active substance for the purposes of renewal and the chronic risk to sediment dwellers is concluded to be low.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

No new data are available for effects on algae since the original approval of aluminium silicate (kaolin) (EFSA (2012)).

A waiver is requested for an active substance study with algae based on the following information:

- Findings from the study with SURROUND® WP CROP PROTECTANT can be extrapolated and referred to instead since the formulated product consists of a single active substance with approximately 95% aluminium silicate and non-toxic inerts (please refer to Part C for detail on formulation composition). The study with the formulated product, SURROUND® WP CROP PROTECTANT, presented in Document MCP, Section 10 showed low toxicity to *Scenedesmus subspicatus* (EC_{50} >570 mg a.s./L); KCP 10.2.1/02; Vryenhoef (2006)).

- Aluminium silicate (kaolin) is present in sediments and suspended solids of natural water bodies. Aluminium silicate will naturally settle (refer to Sutherland (2015), KCA 7.2.01 and discussion) provided water currents are slow enough to permit deposition. Once settled, aluminium silicate will be completely undistinguishable from naturally-present clay particles and become part of the sediment. Since aluminium silicate is not soluble in water, it is not unexpected that 100% of the product entering waterways will transfer to the sediment. This also explains the difficulty in maintaining kaolin-test solution concentrations within the aqueous phase when conducting a GLP laboratory study.

- Kaolin is also not soluble in water and thus not bioavailable to algae or aquatic plants.

- It is common practice in the US state of Georgia, where most of the high quality kaolin is mined, that exhausted deposits are converted into fresh water lakes or ponds where algae rapidly colonise these newly available sites. Being a natural component of sediment, exposure to kaolin at the dose rates used in agriculture is very unlikely to have effects on algal growth or growth rate.

- However, algae are known to be sensitive to very high concentrations of some suspended clays (0.1 to 4.0 g/L), including kaolin, that have been used to control harmful algal blooms. A recent review of the information on using clays to control harmful algal blooms has been published by Sengo and Anderson (2004)[24]. Most of the work on this subject has been conducted with phosphatic montmorilinite clays, which seem much more effective than kaolins. The key points relating to kaolin have been extracted from the published review and are summarised below:
    - Clay flocculation has proved an efficient and safe medium to control Harmful Algal Blooms.
    - Clay minerals are small (< 2 µm) and dense, and they act to ballast the organisms, and to promote cell sinking, despite the organisms' natural motility and buoyancy.
    - The high removal efficiency, rapidity, cost effectiveness and potentially low environmental impacts of clay dispersal have made it one of the most promising control methods under investigation
    - In Japan, suspensions of pure kaolinite were sprayed onto the surface of a "red tide" algal bloom, *Cochlodinium sp.,* at 200 g/m^2 near fish enclosures. Shortly after treatment, the number of algal cells was greatly reduced at the surface, water transparency increased, and there was a marked recovery in the reared opaleye and yellowtail fishes.
    - In a 1996 report, workers in South Korea dispersed approximately 60,000 tons of dry yellow loess (a kaolinite-bearing sediment) by barges over 260 km^2 at a loading rate of 400 g/m^2. Removal rates of the "red-tide" alga, *Cochlodinium polykrikoides,* were calculated at 90% to 99 % up to 2 m depth, with virtually no reported mortality in the caged fish due to clay treatment. Water transparency improved to a depth of 4 m within hours of dispersal. Fisheries losses were reduced from $100 million the previous year, to $1 million during this first year of implementation.
    - Typically, montmorillonites and montmorillonite-containing sediments, such as phosphatic clay, have much higher removal abilities than kaolinites and zeolites. Most of the work in the USA has therefore focused on these clays.
    - Typical rates of using clays (bentonites, phosphatic clays, kaolins) for harmful algal control in the USA, Australia and Sweden are between 0.1 to 4.0 g/L.

It is clear from the above that very high levels of kaolin clays can have a detrimental effect on algae by causing flocculation of cells and sedimentation. However, the total seasonal input from the proposed use of SURROUND® WP CROP PROTECTANT is only 0.0032 g a.s./L (single maximum application would result in 0.0008 g a.s./L) (refer to Document MCP, Section 9, Point 9.2.5), hence no adverse effects on algae are expected.

[24] Sengo, M.R., and Anderson, D.M. (2004). Controlling Harmful Algal Blooms Through Clay Flocculation. J. Eukaryot. Microbiol. vol 51, no. 2: 169-172.

In light of these considerations, no chronic testing to algae is considered to be necessary with the active substance for the purposes of renewal and the chronic risk to algal organisms is concluded to be low.

CA 8.2.6.2 Effects on growth of an additional algal species

Not required.

CA 8.2.7 Effects on aquatic macrophytes

No new data are available or required for aquatic macrophytes since the approval of aluminium silicate (kaolin) (EFSA (2012)). As discussed in the original DAR, data available from the open literature confirms that no classification for toxicity of aluminium silicate (kaolin) to aquatic organisms was necessary; it was considered that the risk to non-target organisms from the representative use of aluminium silicate (kaolin) will be low.

A waiver is requested ~~and accepted during the initial EFSA review (EFSA (2012))~~ for aquatic macrophytes (i.e., *Lemna*) toxicity studies based on the following information:

- Aluminium silicate (kaolin) is present in sediments and suspended solids of natural water bodies.
- It is also not soluble in water and thus not bioavailable to algae or aquatic plants.
- It is common practice in the US state of Georgia, where most of the high quality kaolin is mined, that exhausted deposits are converted into fresh water lakes or ponds where plants rapidly colonise these newly available sites.
- Aluminium silicate will naturally settle (refer to Sutherland (2015), KCA 7.2.01 and discussion) provided water currents are slow enough to permit deposition. Once settled, aluminium silicate will be completely undistinguishable from naturally-present clay particles and become part of the sediment. Since aluminium silicate is not soluble in water, it is not unexpected that 100% of the product entering waterways will transfer to the sediment. This also explains the difficulty in maintaining kaolin-test solution concentrations within the aqueous phase when conducting a GLP laboratory study.
- Being a natural component of sediment, exposure to aluminium silicate (kaolin) at the dose rates used in agriculture is very unlikely to have effects on aquatic plant health.
- In a robust field mesocosm study (Parkhill and Gulliver, 2002 (KCA 8.2.7/01) examining the response of a mixed periphyton and macrophyte community to extended periods of suspended kaolin exposure (63 days), the actual NOEC based on whole stream respiratory rate, periphyton biomass and percentage macrophyte cover was estimated to be between 50 and 100 mg/L.

In light of these considerations, no testing to aquatic macrophytes is considered to be necessary with the active substance for the purposes of renewal and the risk to aquatic macrophytes is concluded to be low.

Tests have not been conducted due to the low toxicity of aluminium silicate (kaolin) to aquatic organisms. However, a literature reference on the toxicity of mixed periphyton and macrophyte community to extended periods of suspended kaolin exposure (63 days) is available and summarised below, further supporting the low toxicity of kaolin to aquatic macrophytes.

Reference:	KCA 8.2.7/01 Parkhill, K.L., and Gulliver, J.S, 2002
Title:	Effect of inorganic sediment on whole-stream productivity
Report No.:	Published in: Hydrobiologia 472: 5-17
Guideline(s):	Not reported
Deviation(s):	Not relevant, published paper
GLP:	No, but study considered scientifically valid
Klimish score:	2, not known to be GLP, not guideline study design

Executive Summary

To study the effect of inorganic sediment on plant and animal communities in stream ecosystems, kaolinite clay was tested using. The sediment loading rates were 300, 200, 100, and 50 mg/L. First dosing period (mid-August to November 1994) began at the start of a fall bloom in autotroph productivity, and the second (May to August 1995) began before the summer communities were established.

During both treatment seasons, the addition of clay significantly increased turbidity and sedimentation, and decreased light penetration in treated streams. In general, the macrophyte and periphyton communities responded quickly after only a few weeks exposure to the sediment additions. Wholes tream respiration was significantly lower in treated streams, decreasing as the amount of sediment added increased. Periphyton biomass on tiles and percent cover of macrophytes was significantly lower in treatment streams than in controls. Total whole-stream productivity was not significantly lower in streams receiving sediment loads than in control streams.

Based on the response of a mixed periphyton and macrophyte community to extended periods of suspended kaolin exposure (63 days), the actual NOEC based on whole stream respiratory rate, periphyton biomass and percentage macrophyte cover was estimated to be between 50 and 100 mg/L.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study
Reliability = 2, not known to be GLP

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Kaolinite clay
Description: Fine grained
Lot/Batch #: Not reported. Obtained from local mineral supplier
Purity: Not specified, all clay mined from one location

2. Test organism
Species: Mixed periphyton and macrophyte community
Age at test initiation: Varied

3. Treatment groups: Sediment loading rates were 300, 200, 100, and 50 mg/L

4. Environmental conditions:
Test vessels: Outdoor experimental streams 520 m long and 3.5 m wide at the Monticello Ecological Research Station (MERS), with water from the Mississippi River.
Temperature: Not reported
pH: Not reported

Dissolved oxygen: Not reported
Photoperiod: Natural

B. STUDY DESIGN AND METHODS

1. Stream:

The pools were roughly parabolic with a surface width of 3.5 m and a maximum depth of ~0.8 m, and the riffles were roughly trapezodial with a surface width between 1–2 m and a depth of ~0.2 m.

Different habitats and biotic communities existed in the pools and riffles. Pools were good macrophyte habitat, with slow water velocities (~1 cm/s), mud substrate, and shallow depth (60 cm). Higher water velocities (8–12 cm/s) and shallow rocky substrate in the riffles provided good epilithic habitat. Longitudinal O_2 profiles indicated that both macrophytes and periphytic algae contributed significantly to daily whole-stream productivity

2. Treatment:

The rates of clay addition corresponded to suspended sediment concentrations of roughly 200, 100, and 50 mg/L in the high, medium, and low dose streams respectively during the 1994 field season, and 300 and 100 mg/L in the high and medium dose streams during the 1995 field season. Two streams received each sediment treatment in both 1994 and 1995, and the remaining streams were untreated controls (two controls in 1994 and four in 1995).

Turbidity measurements were collected regularly (two to five collections/week) and suspended sediment measurements were collected on a weekly basis along the length of all the streams to characterize the sediment treatments.

Given the magnitude of the original treatment objectives, i.e., concurrent, continuous, and steady loadings of about 35 tons of fine-clay into six different experimental streams for nearly three months, the sediment delivery system performed very successfully.

3. Measurements/observations:

Downwelling total solar radiation measurements were collected on three sunny days in 1994 and two in 1995 using a SUD 400 sensor with cosine corrected filter (International Light) to determine the effect of sediment treatments on the underwater light environment.

Biomass and percent cover measurements were used to document the effect of clay on specific plant communities in the streams. Visual surveys were conducted to estimate changes in percent cover of macrophytes during the clay additions. The mean growth rate of a macrophyte, *Elodea*, was measured twice, once in 1994 for growth between September 30 and October 20, and again in 1995 for growth between June 30 and July 18. Additional measurements were collected to quantify periphytic algal production on a variety of different substrates, including ceramic tiles and plastic sheets in a variety of orientations.

Two-station diel oxygen O_2 surveys were used to estimate whole-stream productivity.

4. Statistics:

Not reported

II. RESULTS AND DISCUSSION

The clay added to the streams had a geometric mean diameter of 20.8 µm.

A. Community biomass

In 1994, there appeared to be a decreasing trend in growth with sediment dose, but the controls do not follow the trend, and there is minimal difference between the controls and the treated streams. In 1995, there was again a decreasing trend between the medium and high-dose streams, and there also was significantly less growth for *Elodea* in high-dose streams than in the controls. The dry weights for treated streams were always higher than controls, but this difference was due to larger ash weights, presumably from more particulate material attached to the plant surface. The *Elodea* growth rate observations are also consistent with macrophyte cover estimates from visual surveys. Percent cover of all macrophytes decreased significantly with increasing sediment loads ($p = 0.003$). Unlike Elodea growth rate, periphyton productivity measurements are inconsistent among the substrates, and often periphyton productivity appeared to increase with clay additions. Thus, the response of individual plant communities to clay additions was not uniform.

B. Whole stream metabolism

Whole-stream metabolism was significantly affected by clay additions in both 1994 and 1995, but not through the expected reductions in total daily photosynthetic productivity. Night-time measurements of whole-stream respiratory rates were significantly lower in high and medium-dose streams. Surprisingly, however, whole-stream daily photosynthetic productivity did not show consistent trends with respect to sediment treatments.

Whole-stream photosynthesis in the streams showed no consistent behavior either within treatments or relative to the other streams in response to the clay additions. Respiration rate coefficients in the high-dose streams were significantly lower than the control streams only days after dosing began in both 1994 and 1995 (LSD, $\alpha = 0.05$). Thus, sediment loadings appear to have immediate effects on whole-stream respiratory rates in these streams. The magnitude of clay impacts on whole-stream metabolism did not appear to increase during the chronic exposure for over 2 months.

III. CONCLUSION

Total daily photosynthetic production in the experimental streams treated with clay was not significantly different from the control streams. The efficiency of photosynthesis appeared to increase in experimental streams dosed with clay, so that the total daily photosynthetic productivity of the treated streams was not significantly different from the controls. Observed reductions in dark respiratory rate coefficients, however, in treated streams reflect measurable impact of clay additions to plant and animal communities. Dark respiration is a metabolic process that is often related to biomass.

Thus, whole-stream metabolism measurements in the MERS streams confirm the expectation that even small sediment loads can reduce the overall amount of biological activity in streams. These reductions, however, will not necessarily affect all communities in the lotic ecosystem, and specifically, autotroph productivity in the MERS streams was not diminished by sediment additions. Reduced primary productivity is one of the most often cited impacts of sediment pollution and increased turbidity. These findings suggest, however, that primary producers are among the most resistant members of the lotic ecosystem to sediment deposition and increased turbidity.

Based on the response of a mixed periphyton and macrophyte community to extended periods of suspended kaolin exposure (63 days), the actual NOEC based on whole stream respiratory rate, periphyton biomass and percentage macrophyte cover was estimated to be between 50 and 100 mg/L.

CA 8.2.8 Further testing on aquatic organisms

Not required.

CA 8.3 Effects on Arthropods

CA 8.3.1 Effects on bees

No new data are available for bee toxicity since the approval of aluminium silicate (kaolin) as a plant protection product (EFSA (2012)). A summary of the existing data is presented in the following table.

Details of these studies are provided in the previous EU DAR and related documents and summarised for completeness in the relevant sections below. The toxicity endpoints remain valid as agreed by EFSA (2012).

Table 8.3.1-1: Endpoints relevant for the risk assessment for honey bees – aluminium silicate (kaolin)

Species	Substance	Exposure	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)

*98.8% active kaolin

In accordance with Commission Regulation EU 283/2013, chronic data on adult bees and larval life stages are required. These data have been generated for the formulated product. The findings from these single active substance formulation studies with SURROUND® WP CROP PROTECTANT can be extrapolated and referred to, since the formulated product consists of approximately 95% aluminium silicate and non-toxic inerts (please refer to Part C for details on formulation composition). Please refer to Document MCP, Section 10, Points 10.3.1.2 and 10.3.1.3 for chronic honey bee adult and chronic larval study summaries, respectively.

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

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

Reference:	KCA 8.3.1.1.1/01 Hoxter, K.A., Palmer, S.J., and Krueger, H.O. 1997 (previously evaluated in DAR B9, IIA 8.3.1.1/01)
Title:	M-96-018 Kaolin: An Acute Dietary Toxicity Study with the Honey Bee
Report No.:	469-102
Guideline(s):	FIFRA subdivision L, Section 141-1 and EPPO Guideline 170
Deviation(s):	None
GLP:	Yes

Executive Summary

In an acute dietary toxicity study to honey bee (*Apis mellifera*), M-096-018 as manufactured, 98.8% kaolin, was administered orally as a mix with honey. Sixty bees per treatment group (3 replicates of 20) were exposed to 62.5, 125, 250, 500 or 1000 ppm M-96-018 per bee, with honey control, untreated blank control and toxic reference treatment (3 test concentrations of dimethoate). Observation took place 1 hour and 15 minutes, 2 hours and 45 minutes, 24 hours and 48 hours after treatment. The LC_{50} of M-96-018 Kaolin is >1000 ppm/bee and the NOEC is 1000 ppm/bee. M-96-018 Kaolin is not harmful to bees by the oral route. On the basis of this study, kaolin is not harmful to bees when administered as part of the diet.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	M-096-018
Description:	White powder
Lot/Batch #:	08145
Purity:	98.8% kaolin
Stability of test component:	Stable
2. Vehicle and/or positive control:	Methanol
3. Test animals:	
Species:	Honey bee (*Apis mellifera*)
Age:	One to seven days old, workers
Source:	Wildlife International Ltd, Maryland, USA.
Acclimation:	7 days
Diet:	Test material mixed with honey
Housing:	Environmental chamber
4. Environmental conditions:	
Temperature:	27.4-29.1 ºC
Humidity:	70-76%
Photoperiod:	darkness except during dosing and observation

B. STUDY DESIGN AND METHODS

1. In life dates:	18 – 20 June 1997

2. Animal assignment and treatment:

Newly hatched bees were kept for 7 days in an environmental chamber at 33ºC with unlimited honey and pollen as food sources.

Sixty bees per treatment group (3 replicates of 20) were exposed to 62.5, 125, 250, 500 or 1000 ppm M-96-018 per bee, with honey control, no treatment blank control and toxic reference treatment (dimethoate, 5, 16 and 45 ppm per bee).

3. Statistics:

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSIONS

A. MORTALITY

Details are provided in the following table.

Table 8.3.1.1.1/01-1. Mortality of honey bees fed with kaolin.

Dose (ppm)	Mortality
62.5	2/60
125	0/60
250	1/60
500	2/60
1000	0/60
Negative control	0/60
Positive control 0.05	51/60
Positive control 0.10	56/60
Positive control 0.20	60/60

LD_{50} > 1000 ppm/bee

B. CLINICAL OBSERVATIONS

The numbers of mortalities observed in the treatment groups were low and did not occur in a dose-responsive manner. Therefore, the mortalities and clinical signs of toxicity were not considered to be related to treatment with the test substance.

C. DEFICIENCIES

None.

III. CONCLUSIONS

The LD_{50} of M-96-018 Kaolin is >1000 ppm/bee and the NOEC is 1000 ppm/bee. M-96-018 Kaolin is not harmful to bees by the oral route. On the basis of this study, test material does not warrant classification as harmful or toxic to bees when administered as part of the diet.

CA 8.3.1.1.2 Acute contact toxicity

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

Reference:	KCA 8.3.1.1.2/01 Hoxter K.A., Palmer, S.J.and Krueger, H.O. 1997 (previously evaluated in DAR B9, IIA 8.3.1.1/02)
Title:	M-96-018 Kaolin: An Acute Contact Toxicity Study with the Honey Bee
Report No.:	469-101
Guideline(s):	FIFRA subdivision L, Section 141-1 and EPPO Guideline 170
Deviation(s):	None
GLP:	Yes

Executive Summary

In an acute contact toxicity study to honey bee (*Apis mellifera*), M-096-018, as manufactured 98.8% kaolin, was administered topically as a mix with methanol. Sixty bees per treatment group (3 replicates of 20) were exposed to 6.25, 12.5, 25.0, 50.0 or 100.0 µg M-96-018 per bee, with methanol solvent control, untreated blank control and toxic reference treatment (3 test concentrations of dimethoate). Observation took place at 1 hour, 1 hour and 30 minutes, 24 hours and 48 hours post dosing. The LD_{50} of M-96-018 Kaolin is >100.0 µg/bee and the NOEC is 100.0 µg/bee. M-96-018 Kaolin is not harmful to bees by contact. On the basis of this study, test material does not warrant classification as harmful or toxic to bees when administered topically.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	M-096-018
Description:	White powder
Lot/Batch #:	08145
Purity:	98.8%
Stability of test component:	Stable
2. Vehicle and/or positive control:	Methanol
3. Test animals:	
Species:	Honey bee (*Apis mellifera*)
Age:	One to seven days old, workers
Source:	Wildlife International Ltd, Maryland, USA.
Acclimation:	7 days
Diet:	50% sucrose
Housing:	Environmental chamber
4. Environmental conditions:	
Temperature:	28.6-28.8 ºC
Humidity:	64-76%
Air changes:	Not specified
Photoperiod:	darkness except during dosing and observation

B. STUDY DESIGN AND METHODS

1. In life dates:	18 – 20 June 1997

2. Animal assignment and treatment:

Newly hatched bees were kept for 7 days in an environmental chamber at 33ºC with unlimited honey and pollen as food sources.

Sixty bees per treatment group (3 replicates of 20) were exposed to 6.25, 12.5, 25.0, 50.0 or 100.0 µg M-96-018 per bee, with methanol solvent control, no treatment blank control and toxic reference treatment (dimethoate, 0.05, 0.10, 0.20 µg per bee).

3. Statistics:

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSIONS

A. MORTALITY

Details are provided in table below. One death occurred at 50 µg dose.

Contact LD_{50} > 100 µg/bee

B. CLINICAL OBSERVATIONS

With the exception of one bee that was immobile on day 0 in the negative control group, all bees appeared active and healthy throughout the study.

Table 8.3.1.1.2/01 -1. Doses, mortality / animals treated

Dose (µg)	Mortality
6.25	0/60
12.5	0/60
25	0/60
50	1/60
100	0/60
Negative control	1/60
Solvent control	0/60
Positive control 0.05	3/60
Positive control 0.10	24/60
Positive control 0.20	60/60

C. DEFICIENCIES

None.

III. CONCLUSIONS

The LD_{50} of M-96-018 Kaolin is >100.0 µg/bee and the NOEC is 100.0 µg/bee. M-96-018 Kaolin is not harmful to bees by the contact route. On the basis of this study, test material does not warrant classification as harmful or toxic to bees when administered topically.

CA 8.3.1.2 Chronic toxicity to bees

No new data are available or required with the active substance for chronic toxicity to bees since the original approval of aluminium silicate (kaolin) (EFSA (2012)). As discussed in the original DAR, results of the available field studies demonstrate a low risk to honey bees.

A waiver is requested for a chronic active substance study with bees based on the following information:

- Findings from the study with SURROUND® WP CROP PROTECTANT can be extrapolated and referred to instead, since the formulated product consists of a single active substance, aluminium silicate (kaolin), representing 95% of the formulation composition, and non-toxic inerts (please refer to Part C for formulation composition). The study with the formulated product, SURROUND® WP CROP PROTECTANT, is presented in Document MCP, Section 10, Point 10.3.1.2 and shows a low toxicity to honey bees (10 d LDD_{50} 1390~~.14~~ µg a.s./bee; KCP 10.3.1.2/01; Ansaloni (2019)).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

No new data are available or required with the active substance for effects on honey bee development and other honey bee life stages since the original approval of aluminium silicate (kaolin) (EFSA (2012)). As discussed in the original DAR, results of the available field studies demonstrate a low risk to honey bees.

A waiver is requested for a chronic honey bee larval toxicity study with the active substance based on the following information:

- Findings from the study with SURROUND® WP CROP PROTECTANT can be extrapolated and referred to instead. The formulated product consists of a single active substance of approximately 95% aluminium silicate and non-toxic inerts (please refer to Part C for formulation composition). ~~The study with the formulated product, SURROUND® WP CROP PROTECTANT, is presented in Document MCP, Section 10, Point 10.3.1.3. Based on range-finding results as the definitive study is yet to be concluded; the NOED is 150 µg kaolin/larva (based on the highest test dose); KCP 10.3.1.3/01; Ansaloni (2019)).~~
  The study with the formulated product, SURROUND® WP CROP PROTECTANT, is presented in Document MCP, Section 10, Point 10.3.1.3, and shows a low toxicity to honey bee larvae from repeated exposure; the NOED is 405 µg kaolin/larva; KCP 10.3.1.3/01; Ansaloni (2019)).

CA 8.3.1.4 Sub-lethal effects

Not relevant.

CA 8.3.2 Effects on non-target arthropods other than bees

No new data are available or required for effects on non-target arthropods since the approval of aluminium silicate (kaolin) (EFSA (2012)).

As discussed in the original DAR, several field studies for non-target terrestrial arthropods were available where the WP formulation was applied to orchards (multiple applications) up to a rate of 56 kg/ha. The results demonstrated that aluminium silicate (kaolin) had no adverse effects on the populations of beneficial arthropods that were investigated in these trials. However, in some trials a reduction in the populations of predatory mites and *Anthocoris* predators was noted. It was considered that this reduction might be attributed to the repellent effect of aluminium silicate and the limited availability of prey animals on the treated plants.

A waiver is requested ~~and accepted during the initial EFSA review (EFSA (2012))~~ for standardized laboratory tests on non-target terrestrial arthropods based on the following information:

- As detailed in the original DAR (Section 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 aluminium silicate (kaolin) (as the formulated product SURROUND® WP CROP PROTECTANT) should be evaluated under field conditions, which are more appropriate for this type of product. Please refer to Document MCP, Section 10.3.2 for non-target terrestrial arthropod study summaries with the formulated product.

In addition, data from public literature demonstrate that kaolin is of low toxicity, as confirmed by the information summarised below for several non-target arthropods. A study by Gharbi and Abdallah (2016, KCA 8.3.2/02) showed that kaolin applied to olive leaves at 5 kg a.s./hL (equivalent to 50 kg/ha) caused minimal adverse effects to *Anthocoris nemoralis* and *Chrysoperla carnea*. In general, the study showed that kaolin suspension (50 kg/ha) did not affect egg mortality and last instar larvae development of *C. carnea* and *A. nemoralis*. This suspension had practically no impact on mortality and longevity of the two predatory adults. However, the number of eggs laid by *A. nemoralis* females on leaves treated with kaolin was significantly reduced compared to the control. In contrast, *C. carnea* females showed a significant increase of oviposition on treated leaves. Moreover, the egg hatching rate was not influenced by kaolin treatment for both predators. While there were minor effects, kaolin was concluded by the authors to be a substance to be considered as a good alternative to pesticides in organic olive groves.

Bestete *et al*., (2018, KCA 8.3.2/03) demonstrated that direct spray to *C. externa* and *E. connexa* was not lethal with LR_{50} values > 100 g a.s./L. Overall, survival rates and development durations for *E. connexa* larvae topically treated with kaolin at different ages were similar to those for untreated larvae. However, larvae of *C. externa* experienced reduced survival and delayed development when treated with kaolin concentrations greater than the recommended field rate (≥ 80 g/L; equivalent to 80 kg/ha at 1000 L/ha spray volume). Otherwise, kaolin treatments did not affect prey consumption by larvae or adults of either predator species.

A summary of the data found in the public literature is presented in the following table. Details of these studies are provided below.

Table 8.3.2-1: Endpoints and effect values for non-target arthropods from the public literature

Species	Substance	Exposure System	Results	Reference
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	Bestet *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	Bestet *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)
Field or semi-field tests				
Seven weekly applications of kaolin (M-96-018) as a 3% solution in water + methanol had no significant effects on populations of ladybirds (Coccinellidae), lacewings (Chrysopidae) or spiders (Araneae). (KCP 8.3.2/01)				

Earlier work conducted on kaolin-based products was carried out at the USDA fruit research station in Kearneysville, West Virginia, USA. A field study conducted in 1997 on unformulated kaolin is given below to evaluate effects of the active substance.

The study was submitted and evaluated for the first inclusion on Annex I:

Reference:	KCA 8.3.2/01 Puterka, G.J. 1997 (previously evaluated in DAR B9, IIA 8.3.2/01)
Title:	Report on the Effect of M-96-018 Kaolin on Insect Predators
Report No.:	-
Guideline(s):	Not reported
Deviation(s):	None
GLP:	No, but study scientifically valid

Executive Summary

M-096-018 (98.8% Kaolin) was applied weekly as a 3% kaolin solution (+ 4% methanol) in apples. Predators (different life stages of naturally occurring insect predators: Ladybirds (Coccinellidae); Lacewings (Chrysopidae); Spiders (Araneae) were observed every two weeks throughout the season from June to August in treated orchards. Tree terminals (first 8 inches) were inspected for all stages (egg, nymph, adult) of each predator species with 20-25 terminals inspected per tree per treatment replicate in each fruit orchard

There are no definite trends indicating that M-96-018 Kaolin has a major impact on predator populations. Data indicate that predator populations were present at numbers near that of the untreated control most times in the season.

Seven weekly applications of kaolin (M-96-018) as a 3% solution in water + methanol had no significant effects on populations of ladybirds (Coccinellidae), lacewings (Chrysopidae) and spiders (Araneae). On the basis of this test, kaolin is not harmful to these insect predators when exposed under field conditions.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material: M-096-018
   - Description: White powder
   - Lot/Batch #: Not specified
   - Purity: Not specified but known to contain 98.8% kaolin
   - Stability of test component: Stable

2. Test animals: Different life stages of naturally occurring insect predators: Ladybirds (Coccinelids); Lacewings (Chrysoperlids); Spiders (Araneae)

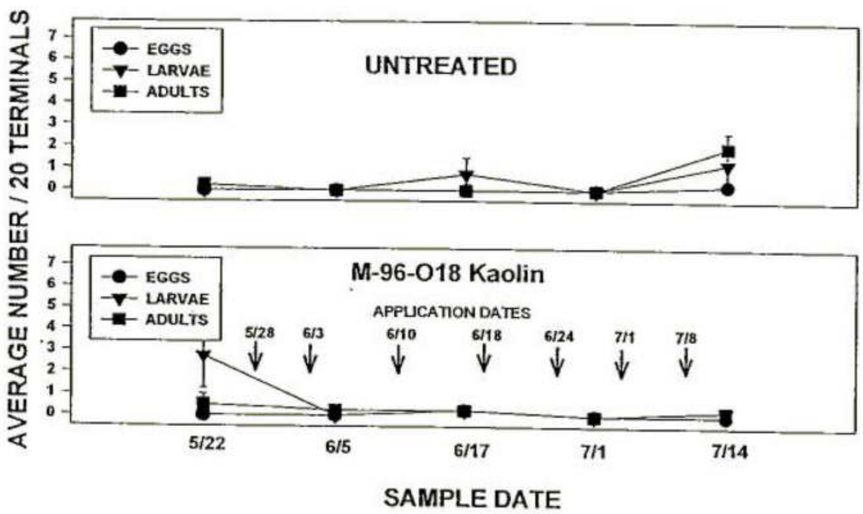
3. Testing Facility and trial location: USDA, Appalachian Fruit Research Station, Kearneysville, West Virginia, USA

B. STUDY DESIGN AND METHODS

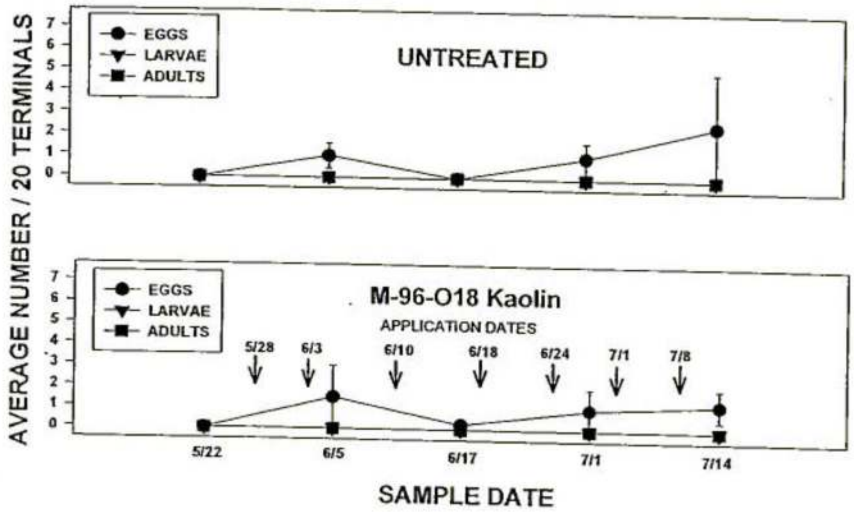
The trials consisted of 8 plots of apples, 4 were treated with the test substance, M-096-018, and 4 were left untreated. Seven applications of M-096-018, as a 3% solution (+ 4% methanol), were applied at weekly intervals from 28 May 1997 to 8 July 1997. Numbers of predators were observed every two weeks throughout the season from June to August in both treated and untreated plots. Tree terminals (first 20 cm) were inspected for all stages (egg, nymph, adult) of each predator species. In total, 20-25 terminals were inspected per treatment replicate. Differences between the kaolin treated plots and the untreated were assessed using ANOVA.

II. RESULTS AND DISCUSSIONS

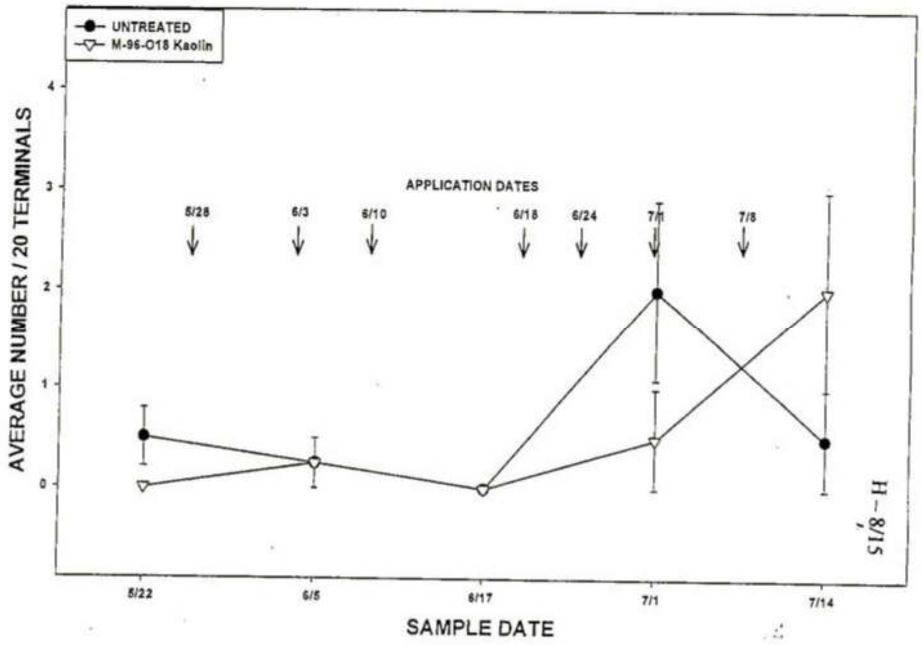
Ladybird beetles: Numbers were generally low throughout the trial. No significant differences were found post-treatment between M-96-018 Kaolin and the untreated control.



Green Lacewing: No significant differences were found between M-96-018 Kaolin and the untreated control.



Spiders: Significantly less spiders in M-96-018 Kaolin treated fields early in season (1 July), but significantly more spiders in M-96-018 treated fields from 14 July onwards



III. CONCLUSIONS

Seven weekly applications of kaolin (M-96-018) as a 3% solution in water + methanol had no significant effects on populations of ladybirds (coccinellids), lacewings (chrysoperlids) and spiders (Araneae). On the basis of this test, kaolin is not harmful to these insect predators when exposed under field conditions.

Reference:	KCA 8.3.2/02, Gharbi, N., and Abdallah, S.B. 2016
Title:	Laboratory evaluation of side-effects of kaolin on two predator species found on olive groves
Report No.:	Published in: Tunisian Journal of Plant Protection vol 11, no. 1: 83-90
Guideline(s):	Not reported
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 aim of the laboratory trials was to evaluate the side effects of kaolin-based treatments on the biology and the behavior of two predators frequently found in Tunisian olive groves; *Anthocoris nemoralis* and *Chrysoperla carnea*. Kaolin suspension applied at 5 kg test item/hL did not affect egg mortality and last instar larvae development of *C. carnea* and *A. nemoralis*. Kaolin had practically no impact on mortality and longevity of the two predatory adults. However, the number of eggs laid by *A. nemoralis* females on leaves treated with kaolin was significantly reduced compared to the control. In contrast, *C. carnea* females showed a significant increase of oviposition on treated leaves. Moreover, the egg hatching rate was not influenced by kaolin treatment for both predators. Based on the findings, the LR_{50} value based on survival for both *A. nemoralis* and *C. carnea* is >5 kg/hL (50 kg/ha). Effects on fecundity is concluded to be < 5 kg/hL for *A. nemoralis* and >5 kg/hL for *C. carnea*.

While some minor effects were observed, the authors concluded that kaolin could be considered as a good alternative to pesticides in organic olive groves.

According to the Klimisch score, the literature reference is concluded to be:
Relevance = 2, not guideline study and no toxicity endpoint reported (can only be estimated), however sufficient data well described
Reliability = 2 (reliable with restrictions), not known to be GLP

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Kaolin
	Batch number:	Not reported.
	Content of a.s.:	Not reported
	Appearance:	White fine-grained aluminosilicate mineral
2.	**Vehicle:**	Distilled water
3.	**Reference:**	None reported
4.	**Test organism**	
	Species:	*Anthocoris nemoralis* *Chrysoperla carnea*
	Age at test initiation:	See individual experiments in method section
	Source:	Collected from Tunisian olive groves in spring 2013. The adults and larvae of predatory bug and the larvae of green lacewing were reared in the laboratory (Olive Tree Institute, Tunis Station, Tunisia) on irradiated eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). The adults of green lacewing were kept in boxes with an ovipositing surface and provided with honey and water.
	Acclimation:	Under test conditions
5.	**Treatment:**	5 kg test item/hL
6.	**Environmental conditions**	
	Temperature:	25 ± 1 ºC
	Humidity:	50-60%
	Photoperiod:	16 h light: 8 h dark

B. STUDY DESIGN AND METHODS

Kaolin was applied to young olive trees using a hand sprayer (1 L) at an approximate distance of 20 cm until the liquid run-off from the leaves. The leaves were then left to dry and were then cut to be provided to the insects.

1. Egg mortality trials:
Kaolin was tested on *A. nemoralis* and *C. carnea* eggs to determine its effects on egg viability and larval hatching suppression. Untreated olive leaves were provided to 50 mated females of *A. nemoralis* to ensure oviposition within 12 hours. Then leaves were checked under a stereomicroscope to count the number of laid eggs. After 12 hours, 100 eggs were collected, of which 50 were sprayed with kaolin suspension and 50 treated with distilled water.

For *C. carnea* females, two bands of green paper (20 - 10 cm) used as ovipositing surfaces, were stacked upside down to the rectangular removable lid of the rearing box (36 × 22 × 20 cm) containing approximately 70 males and 70 females. After 12 hours, 50 eggs were selected on each band by eliminating the remaining ones. One band was sprayed with kaolin suspension and the other with distilled water.

After drying, the eggs of both species were kept in a rearing chamber under rearing conditions. After the incubation period, three days for *C. carnea* and six days for *A. nemoralis*, *E. kuehniella* eggs were sprinkled over the predator eggs in order to feed neonate larvae to prevent intraspecific predation.

For all trials, eggs were checked daily until the fifteenth day to ensure that hatching had ceased. Also, data regarding viable and nonviable eggs were recorded using a stereomicroscope. The number of live larvae hatched from the eggs was scored.

2. Last instar larval mortality trials:
Newly molted larvae (last instar) were chosen for this experiment. 100 recently molted larvae (less than 12 hours) of *A. nemorlis* and 100 *C. carnea* were selected from the rearing stock. Each specimen was transferred to Petri dishes containing a filter paper in order to prevent the accumulation of large droplets during the spraying process, half of them were sprayed with about 1 L of kaolin suspension and the other half with the same volume of distilled water. After spraying, individuals were subjected to a one-hour drying period after which the filter paper was removed and the larvae were fed and kept under rearing conditions. Tested individuals were checked daily for survival until adult emergence and larval mortality was recorded.

3. Adult mortality trials:
Fifty adults of both species were individually exposed to treated leaves in single Petri dishes for 72 hours and mortality was recorded. After 72 hours of incubation, the surviving individuals were transferred to clean Petri dishes to evaluate the effects of kaolin treatment on adult longevity.

4. Impact of kaolin-treated surface on the behaviour of insects:
Two young olive trees (height = 30 cm), covered with a ball-shaped net, were used for behavioral tests. One tree was treated with approximately 100 mL/hL of kaolin suspension and the other with the same volume of distilled water. These plants were used as an ovipositing arena for ten couples of each predator.

Freshly emerged adults (less than 12 hours) were coupled and transferred to small rearing boxes (0.9 L), kept under rearing environmental conditions and provided with adult food and water. After five days, ten couples of each predator were transferred to the ovipositing arena to lay eggs within a period of 48 hours. The eggs deposited on each plant (with or without kaolin treatment) were recorded. The same process was repeated five times.

4. Statistics:
All statistical analyses were performed using SPSS 20 for Windows.

The mean values of mortality were corrected using Abbott's formula: M(%)=(NDL-NDLC)×100/(TNL-NDLC), where M = Rate of mortality (%); NDL = Number of dead larvae; NDLC = Number of dead larvae in the control; TNL = Total number of larvae

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

For the oviposition and behavioral experiments, treatments were compared using a Fisher's least significant difference (LSD) test.

II. RESULTS AND DISCUSSION

1. Egg mortality:

For both predator species *C. carnea* and *A. nemoralis*, a slight decrease in their hatching rate was observed after kaolin treatment compared to control. However, this reduction is about 4.24% for *C. carnea* and approximately 11.81% for *A. nemoralis*. No statistical differences were found.

For the two predator species, no intra-specific cannibalistic behaviour was observed because the recently hatched larvae fed on the supplied *E. kuehniella* eggs.

Table 8.3.2/02-1: Egg hatching (in %) of *Chrysoperla carnea* and *Anthocoris nemoralis* sprayed either with kaolin or distilled water

Treatment	*C. carnea*	*A. nemoralis*
Kaolin	88.1 ± 7.8	76.8 ± 6.2
Control	92.2 ± 5.5	88.7 ± 4.6

Incubation duration is 3 to 4 days for *C. carnea* and 6 to 7 days for *A. nemoralis*.

2. Larval mortality of the last instar:

One hundred larvae (3rd instar for *C. carnea* and 5th instar for *A. nemoralis*) of both predator species were checked daily for survival until the adult emergence and larval mortality was recorded. The obtained results are illustrated in the following table. No difference in mortality was observed between kaolin-based treatment and control. The kaolin covering the larvae body did not affect normal larval development and adult emergence of both test species.

Table 8.3.2/02-2: Mean mortality (in %) in last instar larvae of the two predator species sprayed either with kaolin or distilled water

Treatment	*C. carnea*	*A. nemoralis*
Kaolin	10.4 ± 4.8	12.8 ± 6.2
Control	8.8 ± 3.5	11.7 ± 4.6

3. Adult mortality and life span:

After 72 hours, kaolin treatment caused a slightly higher adult mortality, but statistically insignificant, in both predators compared to the control. Moreover, kaolin did not produce any deleterious effects on longevity for the two predators.

Table 8.3.2/02-3: Mortality rate (after 72 h) and longevity of *Chrysoperla carnea* and *Anthocoris nemoralis* adults with or without kaolin treatment

Treatment	*C. carnea*		*A. nemoralis*	
	Mortality (%)	Longevity (%)	Mortality (%)	Longevity (%)
Kaolin	7.2 ± 1.9	31.7 ± 7.6	5.3 ± 2.2	25.8 ± 10.5
Control	6.4 ± 2.1	33.1 ± 5.8	4.5 ± 1.4	26.7 ± 12.8

4. Effects on reproduction and adult oviposition behaviour

After a period of 48 hours, kaolin treatment had significantly reduced the number of eggs laid by *A. nemoralis* females by about 69.91% on treated leaves compared to those treated only with distilled water. However, during adult longevity, the reduction of fecundity was about 60.97% on kaolin-treated olive leaves. When *A. nemoralis* females were able to choose, they preferred leaves treated with distilled water than those sprayed with kaolin and they laid significantly more eggs on control than on treated leaves. The egg hatching percentage was not significantly different for both treatments; it seems that kaolin had no deleterious effect on egg hatching.

The females of *C. carnea* laid almost all their eggs on the lower surface of leaves. Otherwise, the number of eggs laid by *C. carnea* females after 48 hours on kaolin-treated leaves had significantly increased compared to that recorded on control leaves treated with distilled water; this increase was about 32.71%. When females were able to choose, they preferred to lay their eggs on kaolin-treated leaves than on the control ones. Hence, the females laid more than twice the number of eggs on kaolin-treated leaves compared to leaves sprayed with distilled water only. As for *A. nemoralis*, it seems that kaolin had no deleterious effect on fertility of *C. carnea.*

Table 8.3.2/02-4: Mean number of eggs laid by females, fecundity and fertility of *Chrysoperla carnea* and *Anthocoris nemoralis* on olive leaves treated with kaolin or distilled water

Treatment	*C. carnea*			*A. nemoralis*		
	Mean number of eggs	Fecundity	Egg hatching (%)	Mean number of eggs	Fecundity	Egg hatching (%)
Kaolin	15.66 ± 7.25*	129.94 ± 17.14*	86.26 ± 15.44	2.60 ± 0.78*	36.28 ± 10.22*	82.85 ± 12.44
Control	11.80 ± 4.62	63.29 ± 10.85	87.39 ± 13.03	8.64 ± 3.58	92.96 ± 11.41	84.39 ± 14.57

* Statistically significantly different from control

III. CONCLUSION

The present study confirmed that direct kaolin applications did not affect larval mortality on the two tested predators, or cause deleterious effects on longevity of *C. carnea* and *A. nemoralis* females emerging from larvae (last instars) sprayed with kaolin. Moreover, kaolin-treated leaves led to reduced oviposition in *A. nemoralis*. Kaolin suspension had probably induced a structural modification in the treated leaf surfaces thus discouraging insect oviposition. Also, no effects were detected on hatching fertility of the laid eggs. A behavioural trial showed that *A. nemoralis* adults preferred non-kaolin-treated leaves when they were able to choose. However, kaolin did not result in negative effects on reproduction and longevity of *C. carnea* females. Otherwise, gravid females of *C. carnea* preferred ovipositing on kaolin-treated leaves, which were probably more suitable for anchoring the eggs.

Based on the findings, the LR_{50} value based on survival for both *A. nemoralis* and *C. carnea* is >5 kg/hL (50 kg/ha). Effects on fecundity is concluded to be < 5 kg/hL for *A. nemoralis* and >5 kg/hL for *C. carnea*.

The authors concluded that the potential effects of kaolin on natural enemies are lower than the impact caused by insecticides, such as dimethoate, commonly used in conventional Tunisian olive groves. Therefore, kaolin seems to be a promising compound for IPM and organic farming.

Reference:	KCA 8.3.2/03, Bestete, L.R., Torres, J.B., Pereira, F.F., 2018
Title:	Harmonious interaction of kaolin and two insect predator species in plant protection
Report No.:	Published in: International Journal of Pest Management, 64 (2): 166-172
Guideline(s):	Not reported
Deviation(s):	Not reported
GLP:	Not reported
Klimish score:	2, not guideline study but sufficient data well described

Executive summary

This work evaluated the development and survival of *Chrysoperla externa* and *Eriopis connexa* larvae of different ages treated with kaolin at a rate of 60, 80, and 100 g/L. Prey consumption when treated with kaolin at a field rate of 60 g/L was also evaluated. The prey used were the whitefly *Bemisia tabaci* and the aphid *Lipaphis erysimi*. Survival rates and development durations for *E. connexa* larvae topically treated with kaolin at different ages were similar to those for untreated larvae. However, larvae of *C. externa* reduced survival and delayed development when treated with kaolin concentrations greater than the recommended field rate (≥ 80 g/L). Otherwise, kaolin treatments did not affect prey consumption by larvae and adults of both predator species.

Based on the findings, the LR_{50} value based on survival for both *E. connexa* and *C. externa* is >100 g a.s./L. Effects on prey consumption is concluded to be > 60 g a.s./L for both species. Based on development, the NOEC value for *E. connexa* is > 100 g a.s./L and a NOEC value of 60 g a.s./L for *C. externa*.

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, but sufficient data well described.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Kaolin
	Batch number:	Not reported.
	Content of a.s.:	Not reported
	Appearance:	Not reported
2.	**Vehicle:**	Distilled water plus WillFix at 0.05% as a surfactant
	Reference item:	None reported
3.	**Test organism**	
	Species:	*Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae)
	Age at test initiation:	Larvae of different ages
	Source:	*Eriopis connexa* from in-house culture. A colony of *C. externa* was established from pupae provided by the Laboratory of Integrated Pest Management of the "Universidade Federal de Lavras (UFLA)," Lavras, Brazil
4.	**Treatment:**	Mortality test: 60, 80, and 100 g/L Prey consumption test: 60 g/L
5.	**Environmental conditions**	
	Temperature:	23.2-27.2 ºC
	Humidity:	44.5-85.3%
	Photoperiod:	14 h light: 10 h dark

B. STUDY DESIGN AND METHODS

1. Topical kaolin toxicity test:
Larvae of *C. externa* were exposed to kaolin at 2, 4, and 8 days old, and larvae of *E. connexa* were exposed at 3 and 8 days old. Larvae were subjected to topical kaolin treatment by spraying using the concentrations 0, 60, 80, and 100 g of kaolin/L. A random design was carried out with kaolin concentrations and predator age as treatments for each predator. Each treatment consisted of 50 larvae of *C. externa* and 30 of *E. connexa* individually reared after application and representing one replication for statistical purposes.

Kaolin dilutions were prepared using distilled water plus WillFix at 0.05% as a surfactant. The treatments were applied holding the larvae in a cylindrical plastic tray; 3.80 mL of kaolin dilutions was applied each time, corresponding to an average volume of field spraying of 300 L/ha. In the control treatments, larvae were sprayed only with distilled water plus surfactant.

After drying (1 h), treated-larvae were transferred to plastic Petri dishes and supplied with abundant *A. kuehniella* eggs as food. The larvae were reared individually and monitored daily to record mortality and the date of molting to pupa and the adult emergence.

2. Prey consumption test:
The experiment was carried out to measure the consumption of third-instar nymphs of the aphid *Lipaphis erysimi* (Kalt.) (Hemiptera: Aphididae) and the pupae of the whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae).

The experimental set consisted of caging predators with kaolin treated or untreated prey. For *C. externa*, larvae at 8 days old were used, and aphid nymphs and whitefly pupae offered as prey, with 39 and 27 replications per treatment using untreated and treated aphid nymphs and 15 and 13 replications per treatment using whitefly pupae, respectively. For *E. connexa*, larvae and adults at 8 days after hatching or molting were used and adult stage aphids were offered as prey. The experiment was set up using a random design with predator species and prey established separately due to the logistics of initial counting and surviving prey after caging.

Two treatments consisted of prey treated with kaolin at the field rate of 60 g/L (i.e., treated prey) and prey sprayed only with water plus surfactant; there were 15 and 17 replicates with untreated and treated aphids and 28 and 30 replications with untreated and treated whitefly pupae, respectively. Each replicate was composed of 100 prey to measure consumption by larvae and adult lady beetles. To isolate the mortality caused by the kaolin treatment, kaolin-treated prey were also evaluated without predator presence in five replicates. Thus, the mortality recorded in the kaolin treatment without a predator presence was used to correct the mortality obtained in the kaolin + predator treatments using Abbott's formula. For both predator and prey items, the number of replicates was uneven due to the availability of respective age individuals to be tested simultaneously.

Cotton or kale leaf discs were treated with kaolin as described in the previous experiment and allowed to dry under laboratory conditions. Next, the number of prey per leaf disc was set to 100 each. The leaf discs containing the known number of prey were transferred to clean plastic transparent dishes (9.0 cm diameter) lined with moistened filter paper. Larvae of *C. externa*, or larvae and adults of *E. connexa* were caged with prey (at rate of one individual per dish). To standardize the predator hunger level, they were held over-night without prey (ca. 12 h) prior to being used in the trials. The plastic dishes were covered and sealed with plastic film to avoid prey escaping. Then 24 h after caging, the predator was removed and the remaining number of prey alive was counted.

3. Statistics:
Based on the daily larvae mortality data, the viability of pupae at adult emergence was used to calculate the survival curves for each predator species, larval age, and kaolin concentration using the Kaplan-Meier method and compared using the Log-Rank test at a 0.05 significance level. To analyze the effect of kaolin concentrations (i.e., treatments) on the remaining time to pupation from spray date for each predator and its larval age, the data were subjected to normality (Kolgomorov-Smirnov's test) and homogeneity (Bartlett's test) analysis and transformed into the square root (x + 0.5) to run the analysis of variance (ANOVA). After the results were analyzed using ProcANOVA, means were compared using Tukey's HSD test at a 0.05 significance level. All analyses were performed using the statistical package SAS 9.0.

The number of prey consumed in the kaolin-predator treatment was adjusted using the natural prey death in the kaolin treatments without predators and adopted corrections by Abbott. Next, the corrected data were subjected to normality and homogeneity of variance tests (Kolgomorov-Smirnov's and Bartlett's tests, respectively) and to t-test for comparison between treated and untreated prey using Proc test with pooled method and equal variance in SAS.

II. RESULTS AND DISCUSSION

1. Toxicity test:

The direct spray of any of the kaolin concentrations on *E. connexa* larvae did not affect its survival. The larvae of *E. connexa* sprayed at 3 days old exhibited a pupation rate of 83.3%–93.3% and an adult emergence of 98%–100%. Thus, the survival average from larval spraying to adult emergence ranged from 83.1%–95.4%. For those larvae sprayed at 8 days old, 86.7%–96.7% molted to pupa, and 92.3%–100% of pupae emerged as adults, resulting in larvae survival average that ranged from 85.4%–94.9% from spray to adult emergence, irrespective of the kaolin concentration tested.

The duration that *E. connexa* larvae required to fulfill development did not differ as a function of kaolin spray, irrespective of larval age at spray date and kaolin concentrations. However, a longer pupal stage was observed for larvae treated at 3 days old with 80 and 100 g/L. For 8-day-old larvae, the duration of pupa was also variable but did not follow a pattern, with similar results between larvae treated with the highest kaolin concentration and the control treatment.

Table 8.3.2/03-1: Duration in days of larva and pupa of *E. connexa* after treatment

Treatment	Age of larvae at treatment date	
	3 days old	8 days old
Duration to complete larval stage		
Control	8.8	4.3
Kaolin 60 g/L	9.1	5.1
Kaolin 80 g/L	8.6	4.7
Kaolin 100 g/L	9.1	5.0
Duration of pupa		
Control	3.8 b	3.9 bc
Kaolin 60 g/L	3.6 b	3.5 c
Kaolin 80 g/L	4.4 a	4.4 a
Kaolin 100 g/L	4.3 a	4.0 ab

Means bearing different letters are significantly different in the column

The larvae of *C. externa* demonstrated reduced survival as a function of the kaolin concentration and age of the treated larvae. Two-day-old larvae sprayed with kaolin at the highest concentration had 27% reduction in survival compared to the control treatment. This result is due to the observation that 56% of the larvae molted to pupa, although from these pupae, 96.4% emerged as adults. Likewise, 8-day-old larvae under treatment with 80 g/L of kaolin also showed a reduction in survival. On the other hand, either 2 or 8-day-old larvae treated with kaolin at the remaining concentrations exhibited survival rates similar to that in the control treatment. Furthermore, 4-day-old larvae demonstrated similar survival rates across kaolin concentrations and control treatments.

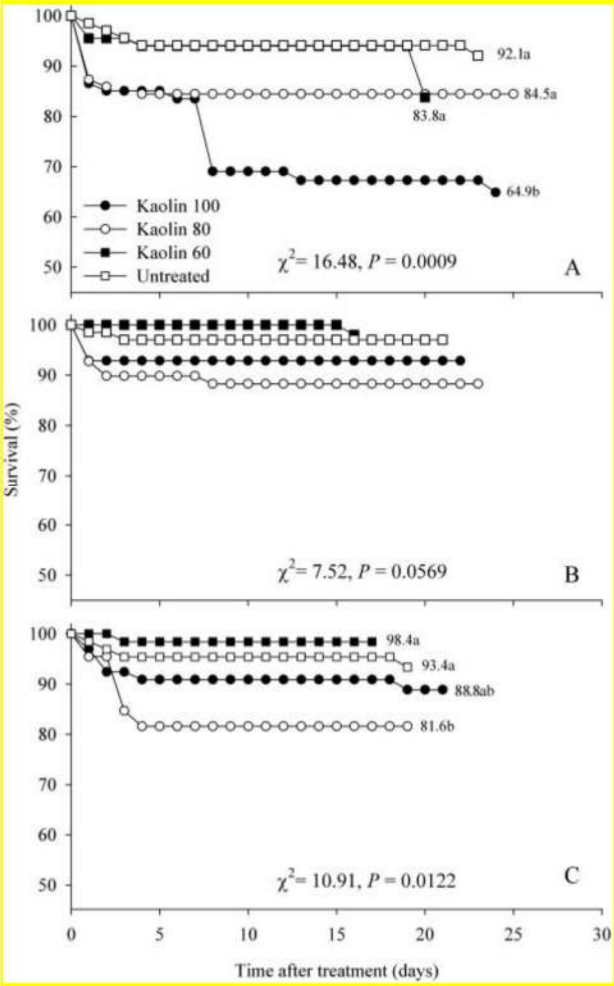


Figure 8.3.2/03-1: Age-specific survival (from spraying to adult emergence) for *C. carnea* larvae treated with kaolin at 2 (A), 4 (B) and 8 (C) days old

The duration to complete development of *C. externa* larvae exhibited a delay when sprayed at 2 and 4 days old with the two highest or only with the highest kaolin concentration, respectively; while at 8 days old, no effect was detected. Otherwise, the pupal development was similar across ages of treated larvae and kaolin concentrations.

Table 8.3.2/03-2: Duration in days of larva and pupa of *C. carnea* after treatment

Treatment	Age of larvae at treatment date		
	2 days old	**4 days old**	**8 days old**
Duration to complete larval stage			
Control	11.0 a	7.6 a	6.0 a
Kaolin 60 g/L	11.2 a	7.8 a	6.1 a
Kaolin 80 g/L	12.1 b	8.1 a	6.2 a
Kaolin 100 g/L	12.8 b	9.2 b	6.9 a
Duration of pupal stage			
Control	9.4 a	9.6 a	10.6 a
Kaolin 60 g/L	9.6 a	9.7 a	10.7 a
Kaolin 80 g/L	9.7 a	9.8 a	10.67 a
Kaolin 100 g/L	9.4 a	9.8 a	10.4 a

Means bearing different letters are significantly different in the column

2. Prey consumption:

The larvae of *C. externa* exhibited similar aphid consumption on kaolin-treated and untreated prey. The means for prey consumed were 31.5 ± 3.10 and 29.1 ± 3.63 for untreated and treated aphids, respectively. Likewise, the consumption of whitefly pupae treated and untreated with kaolin was similar. The means of whitefly consumed were 36.5 ± 2.17 and 34.6 ± 1.76 pupae for treated and untreated pupae, respectively.

The consumption of aphids by 8-day-old *E. connexa* larvae either treated or untreated with kaolin did not differ. The means of aphids consumed were 34.8 ± 3.50 and 36.9 ± 3.33 for treated and untreated aphids, respectively. Likewise, the adults of *E. connexa* consumed on average 59.7 ± 3.23 aphids treated with kaolin, which was statistically similar to the 53.3 ± 3.15 aphids consumed in untreated treatment.

III. CONCLUSION

This work evaluated the development and survival of *Chrysoperla externa* and *Eriopis connexa* larvae exposed to kaolin, as well as the consumption of kaolin-treated prey by *Chrysoperla externa* and *Eriopis connexa*. Survival rates and development durations for *E. connexa* larvae topically treated with kaolin at different ages were similar to those for untreated larvae. However, larvae of *C. externa* were observed to have reduced survival and delayed development when treated with kaolin concentrations > 80 g/L (equivalent to> 80 kg/ ha at 1000 L /ha spray volume). Otherwise, kaolin treatments did not affect prey consumption by larvae and adults of both predator species.

Based on the findings, the LR_{50} value based on survival for both *E. connexa* and *C. externa* is >100 g a.s./L. Effects on prey consumption is concluded to be > 60 g a.s./L for both species. Based on development, the NOEC value for *E. connexa* is > 100 g a.s./L and a NOEC value of 60 g a.s./L for *C. externa*.

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

Please refer to Section 8.3.2.

A waiver is requested for standardized laboratory tests on non-target terrestrial arthropods. Instead it is proposed that the potential effects of aluminium silicate (kaolin) (as the formulated product SURROUND® WP CROP PROTECTANT) have been evaluated under field conditions in existing studies. Please refer to Document MCP, Section 10.3.2 for non-target terrestrial arthropod study summaries with the formulated product.

CA 8.3.2.2 Effects on *Typhlodromus pyri*

Please refer to Section 8.3.2.

A waiver is requested for standardized laboratory tests on non-target terrestrial arthropods. Instead it is proposed that the potential effects of aluminium silicate (kaolin) (as the formulated product SURROUND® WP CROP PROTECTANT) have been evaluated under field conditions in existing studies. Please refer to Document MCP, Section 10.3.2 for non-target terrestrial arthropod study summaries with the formulated product.

CA 8.4 Effects on Non-Target Soil Meso- and Macrofauna

No new data are available or required for effects on non-target soil organisms since the approval of aluminium silicate (kaolin) (EFSA 2012). As discussed in the original DAR (Section B.9.6), a low risk can be concluded for soil organisms.

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)[25], it can be noted in the diagram below that a large area of Europe consists of 28 to 98% clay-based soil. 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.

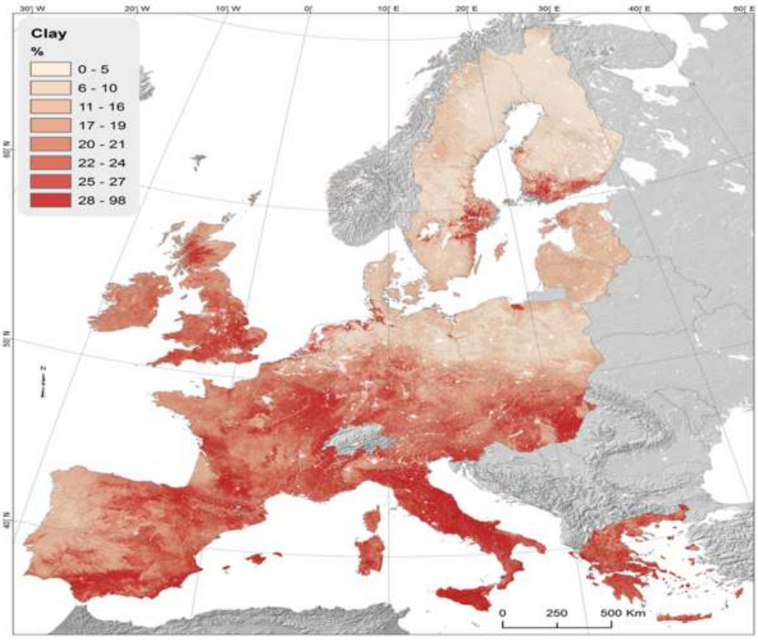


Figure 1: Clay concentration in European soils (JRC-ESDAC)

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. 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.

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. Agricultural soils typically contain between 5 and 50% clay and therefore, the quantity of kaolin added through the use of SURROUND® WP CROP PROTECTANT will not be

[25] https://esdac.jrc.ec.europa.eu/content/topsoil-physical-properties-europe-based-lucas-topsoil-data

enough (the added quantities represent mg/kg soil/year) to cause any measurable increase in the clay (aluminium silicate) content of agricultural soils.

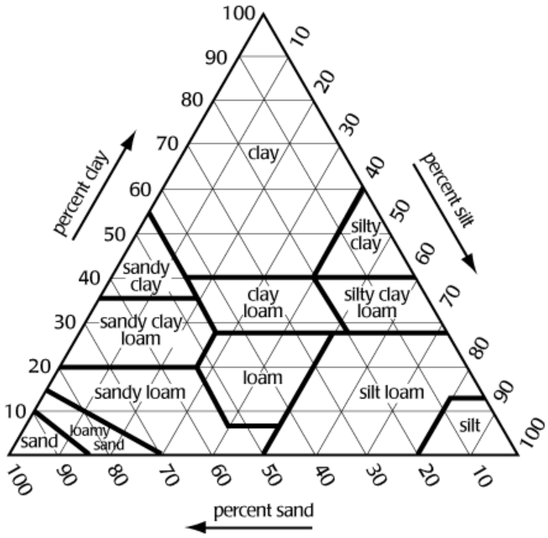


Figure 2: Soil texture triangle

CA 8.4.1 Earthworms – sub-lethal effects

A waiver is requested ~~and accepted during the initial EFSA review (EFSA (2012))~~ for a chronic earthworm toxicity study with the active substance based on the following information:

- 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 8.4 above). 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.

- Aluminium silicate is a natural component of most soils and is present at concentrations of 5 to 50% in agricultural soils (see Document MCP, Section 9).

- As detailed in the original DAR (Section B.9.6), it is estimated that earthworms contain about 30% soil (Hoerger & Kenaga, 1972[26]). Given that soils typically contain between 5-40% clay, 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.

- 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, demonstrating the absence of toxicity of kaolin towards earthworms as well as the need for kaolin in soil to support earthworm survival. As a comparison,

[26] Hoerger, F. and Kenaga, E.E. (1972). Pesticide residues on plants: correlation of representative data as a basis for estimation of their magnitude in the environment. *IN:* F. Coulston and F. Corte, eds., *Environmental Quality and Safety: Chemistry, Toxicology and Technology.* Vol 1. George Theime Publishers, Stuttgart, Germany. pp. 9-28.

application of 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 with the active substance is considered to be necessary for the purposes of renewal and the risk to soil organisms is concluded to be low.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

A waiver is requested ~~and accepted during the initial EFSA review (EFSA (2012))~~ for effects on non-target soil meso- and macrofauna toxicity studies with the active substance based on the following information:

- 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 8.4 above). The agricultural use of SURROUND® WP CROP PROTECTANT therefore is not expected to have any negative effects on soil organisms. 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 is a natural component of most soils and is present at concentrations of 5 to 50% in agricultural soils (see Document MCP, Section 9).

- As detailed in the original DAR (Section B.9.6), it is estimated that earthworms contain about 30% soil (Hoerger & Kenaga, 1972[27]). Given that soils typically contains between 5-40% clay, soil organisms 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.

- 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, demonstrating the absence of toxicity of kaolin towards soil organisms as well as the need for kaolin in soil to support soil organisms' survival.

- 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).

[27] Hoerger, F. and Kenaga, E.E. (1972). Pesticide residues on plants: correlation of representative data as a basis for estimation of their magnitude in the environment. *IN:* F. Coulston and F. Corte, eds., *Environmental Quality and Safety: Chemistry, Toxicology and Technology.* Vol 1. George Theime Publishers, Stuttgart, Germany. pp. 9-28.

In light of these considerations, no toxicity testing with other soil macro-organisms with the active substance is considered to be necessary for the purposes of renewal and the risk to soil organisms is concluded to be low.

CA 8.5 Effects on Nitrogen Transformation

No new data are available or required for effects on nitrogen transformation since the approval of aluminium silicate (kaolin) (EFSA 2012). As discussed in the original DAR (Section B.9.7), a low risk can be concluded for soil organisms.

A waiver is requested for studies on non-target micro-organisms based on the following information:

- 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 8.4 above). The agricultural use of SURROUND® WP CROP PROTECTANT therefore is not expected to have any negative effects on microbial activity. 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 is a natural component of most soils and is present at concentrations of 5 to 50% in agricultural soils (see Document MCP, Section 9).

- Given that soils typically contains between 5-40% clay, soil organisms 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.

In light of these considerations, no study on the effects on nitrogen transformation with the active substance is considered to be necessary for the purpose of renewal and the risk to soil microbial activity is concluded to be low.

CA 8.6 Effects on Terrestrial Non-Target Higher Plants

No new data are available or required for effects on non-target terrestrial plants since the approval of aluminium silicate (kaolin) (EFSA 2012). As discussed in the original DAR (Section B.9.8), a low risk can be concluded for non-target terrestrial plants.

CA 8.6.1 Summary of screening data

No data submitted.

CA 8.6.2 Testing on non-target plants

A waiver is requested ~~and accepted during the initial EFSA review (EFSA (2012)~~ for non-target terrestrial plant toxicity studies based on the following information:

- 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[28]).

- As detailed in MCA Section 7, clay makes a vital contribution to soil fertility. Loam soil that contains 15-25% clay provides an adequate surface for interaction with water and nutrients, and to have a friable structure beneficial for tillage and root growth.

- 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.

- 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 8.4 above). The agricultural use of SURROUND® WP CROP PROTECTANT therefore is not expected to have any negative effects on non-target terrestrial plants. On the contrary, the use of kaolin as a replacement for conventional pesticides could help to improve soil conditions through the elimination of potentially harmful residues of synthetic compounds within the soil.

- Aluminium silicate is a natural component of most soils and is present at concentrations of 5 to 50% in agricultural soils (see Document MCP, Section 9).

- In a root growth inhibition study by Wang *et al*. (2011[29]), 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).

In light of these considerations, no studies on non-target terrestrial plants with the active substance are considered necessary for the purposes of renewal and adverse effects on terrestrial vascular plants from the application of aluminium silicate (kaolin) are not expected.

CA 8.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

No additional data are available or required for the purposes of renewal. As detailed in the original DAR (Section B.9.8), aluminium silicate (kaolin) is a common component of the environment. It is inert and has no known toxic mode of action. Aluminium silicate (kaolin) added to the environment through agricultural uses (as with SURROUND® WP CROP PROTECTANT) contributes a negligible amount of aluminium silicate compared with that already present in clays from natural sources (please refer to Document MCP, Section 9 for natural background levels); it therefore has negligible effect upon organisms that might be exposed. Aluminium silicate (kaolin) has already been used for many years as an inert ingredient in numerous pesticide formulations (*e.g.,* WPs, DPs etc.).

[28] 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

[29] 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.

CA 8.8 Effects on Biological Methods for Sewage Treatment

No new data are available or required for effects on biological methods for sewage treatment (activated sludge study) since the approval of aluminium silicate (kaolin) (EFSA 2012).

A waiver is requested for effects on biological methods for sewage-studies based on the following information:

- As detailed in the original DAR (Section B.9.8), kaolin is a common component of the environment.

- As detailed in the original DAR (Section B.9.8), kaolin is inert and has no known toxic effects on any organisms. Kaolin has already been used for many years as an inert ingredient in numerous pesticide formulations (e.g. WPs, DPs etc.).

- As detailed in the original DAR (Section B.9.8), kaolin added to the environment through agricultural uses (as with SURROUND® WP CROP PROTECTANT) contributes a negligible amount of aluminium silicate compared with that already present in clays from natural sources. It will therefore have negligible effect upon organisms that might be exposed.

- Suspended clay particles routinely enter water and sewage treatment plants, which are equipped to deal with that type of particulate. If aluminium silicate (kaolin) from SURROUND® WP CROP PROTECTANT enters a sewage plant, it is inert and would not interfere with the microbial processes.

- As described above for aquatic organisms (Section 8.2) and soil organisms (Section 8.4), the use of SURROUND® WP CROP PROTECTANT will not significant increase clay concentrations compared to background levels.

In light of these considerations, no studies on biological methods for sewage treatment (activated sludge study) with the active substance are considered necessary for the purposes of renewal and adverse effects from the application of aluminium silicate (kaolin) are not expected. In addition, there are several useful publications that demonstrate aluminium silicate (kaolin) can be used as an absorbent to reduce the aquatic toxicity of certain industrial chemicals that might be found in sewage effluent. Summaries of these studies are given below.

The studies submitted and evaluated for the first inclusion on Annex I:

Reference:	KCA 8.8/01 Cary, G.A., McMahon, J.A., and Kuc W. J. 1987
Title:	The effect of suspended solids and naturally occurring dissolved organics in reducing the acute toxicities of cationic polyelectrolytes to aquatic organisms
Report No.:	Environmental Toxicology and Chemistry (1987), volume 6, pages 469-474
Guideline(s):	Not reported
Deviation(s):	None
GLP:	No, but study scientifically valid

Executive Summary

Cationic polyelectrolytes have high acute toxicities to aquatic organisms but react with suspended solids that flocculate them and may therefore be useful for water clarification. This study determines and compares the effects of suspended solids "SS" (bentonite, illite, kaolin and silica) and of dissolved organic carbon compounds "DOC" (humic, fulvic and tannic acids, lignin and lignosite) on the acute toxicities of four cationic water clarification aids to the fathead minnow (*Pimephales promelas*) and a cladoceran (*Daphnia magna*).

Compared with standard laboratory water test results, the addition of 50 mg/L of kaolin reduced the acute toxicities of the four cationic polyelectrolytes to *Daphnia magna* and to the fathead minnow by 0.87 to 11x (depending on the type of compound and the test species).

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolin
Description:	Powder
Lot/Batch #:	Not specified. Origin: J.T. Baker
Purity:	Not specified
Stability of test component:	Stable
Particle size:	Not specified

2. Test animals:	Fathead minnow (*Pimephales promelas*) Water flea (*Daphnia magna)*

3. Testing Facility:	Petrolite Corporation, Environmental Studies Group, St Louis, Missouri 63119

B. STUDY DESIGN AND METHODS

The fish toxicity tests were conducted in 5-gal glass aquaria containing 15 liters of water. Ten fish per concentration were used in the fathead minnow tests, with a geometric spacing of 1.75 between test concentrations. The test solutions for the daphnids tests were mixed in 500 ml of water and distributed among 250-ml beakers. Twenty daphnids were distributed among three replicates per concentration; a geometric spacing of 1.60 was used between concentrations.

The tests included a water control, polymer control (except for the *D. magna* tests), an SS/DOC control and 5 to 12 test concentrations of each polymer. Tests were initiated by adding the SS/DOC to the test water, followed by the addition of the polymer and finally the test organisms after 15 to 30 minutes. SS materials were added at 50 mg/L in all tests.

The temperature ranged from 19 to 21ºC and the photoperiod was set for 16 h light and 8 h dark. Temperature, pH and dissolved oxygen were determined. All tests were monitored at 24-hours intervals.

48-hour LC_{50} (*Daphnia*) and 96 hours, LC_{50} (Fathead minnow) were determined using the method of Stephan (1977).

II. RESULTS AND DISCUSSIONS

Acceptable survival rates were observed in all SS/DOC controls for both species.

Positive polymer controls in the fish studies resulted in 100% mortality within the 96-h test period.

In general, all Suspended Solids (SS)-including kaolin- and Dissolved Organic Carbon (DOC) reduced the acute toxicities of cationic polyelectrolytes to *D. magna* and to fathead minnows. However, kaolin was significantly less effective at reducing toxicity than ether bentonite or all of the DOCs.

Results for the different SS and DOCs are shown in the following tables.

Table 3. Acute toxicities (LC50, mg/L) of selected cationic polyelectrolytes to *Daphnia magna* and to fathead minnows in the presence of suspended solids and dissolved organics[a]

Substrate	Compound A		Compound B		Compound C		Compound D	
	Daphnids	Fatheads	Daphnids	Fatheads	Daphnids	Fatheads	Daphnids	Fatheads
Standard laboratory water	0.21	0.16	0.082	0.17	0.08	0.25	0.20	0.46
Bentonite[b]	20.1	7.3	>8.3	6.2	6.0	6.5	7.1	6.5
Illite[b]	1.0	1.1	3.6	0.27	0.95	0.95	1.2	0.55
Kaolin[b]	0.91	0.41	0.24	0.26	0.90	0.65	1.1	0.40
Silica[b]	0.26	0.35	0.37	0.28	0.12	0.42	0.14	0.39
Tannic acid[c]	17.4	4.6	>8.3	6.2	8.0	6.5	11.9	6.5
Lignin[c]	28.8	3.8	>8.3	3.2	4.0	3.5	>15.4	3.7
Humic acid[c]	10.5	6.4	7.7	6.2	5.0	4.0	7.4	6.5
Lignosite[c]	5.9	2.9	>8.3	3.5	4.7	3.8	7.9	3.7
Fulvic acid[c]	14.6	2.2	4.3	3.3	3.8	3.8	2.2	4.2

[a]See Table 1 for identification of compounds. 48-h LC50 calculated for *D. magna*; 96-h LC50 for fathead minnows.
[b]Test conducted in presence of 50 mg/L of substrate.
[c]Test conducted in presence of 10 mg/L of substrate.

Table 4. Reductions in acute toxicities, relative to standard laboratory water test results, of selected cationic polyelectrolytes[a] to *Daphnia magna* and to fathead minnows in the presence of suspended solids and dissolved organics

Substrate	Compound A		Compound B		Compound C		Compound D	
	Daphnids	Fatheads	Daphnids	Fatheads	Daphnids	Fatheads	Daphnids	Fatheads
Bentonite[b]	96×	46×	>101×	36×	75×	26×	36×	14×
Illite[b]	4.8×	6.9×	44×	1.6×	6.9×	3.8×	6.0×	1.2×
Kaolin[b]	4.3×	2.6×	2.9×	1.5×	11×	2.6×	5.5×	0.87×
Silica[b]	1.2×	2.2×	4.5×	1.6×	1.5×	1.7×	0.70×	0.85×
Tannic acid[c]	83×	29×	>101×	36×	100×	26×	59×	14×
Lignin[c]	137×	24×	>101×	19×	50×	14×	>77×	8×
Humic acid[c]	50×	40×	94×	36×	63×	16×	37×	14×
Lignosite[c]	28×	18×	>101×	21×	59×	15×	39×	8×
Fulvic acid[c]	70×	14×	52×	19×	48×	15×	11×	9×

[a]See Table 1 for identification of compounds.
[b]Test conducted in presence of 50 mg/L of substrate.
[c]Test conducted in presence of 10 mg/L of substrate.

III. CONCLUSIONS

Low amounts of kaolin (50 mg/L), can reduce the acute toxicity of some cationic polyelectrolytes to the fish and aquatic species by as much as 11 times.

Reference:	KCA 8.8/02 Maki, W., and Bishop, W.E. 1979
Title:	Acute Toxicity Studies of Surfactants to *Daphnia magna* and *Daphnia pulex*
Report No.:	Archives of Environmental Contamination and Toxicology (1979), volume 8, pages 599-612
Guideline(s):	Not reported
Deviation(s):	None
GLP:	No, but study scientifically valid

Executive Summary

This study determines the acute toxicity of linear alkyl benzene sulfonates (LAS) to daphnids. As part of the experiment, reductions in toxicity by adding 50 mg/L of suspended kaolin were examined.

Kaolin significantly reduced the toxicity of longer chain length LAS homologs but had no effect on non-ionic surfactant toxicity.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	Kaolin
Description:	White powder
Lot/Batch #:	Georgia origin, mean particle size 4 µ.
Purity:	Not specified
Stability of test component:	Stable
2. Test animals:	*Daphnia magna*
3. Testing Facility:	The Procter and Gamble Company USA, Ivorydale Technical Centre, Cincinnaty, Ohio, USA.

B. STUDY DESIGN AND METHODS

The methods used for culture procedures and acute toxicity tests followed the guidelines established by USEPA (EPA -600/3-75-009, 1975).

Details are included only for the part of the experiment that examined the effect of kaolin on surfactant acute toxicity:

1. Experimental design:
50 mg/L of kaolin clay was added to dilution water up to a total solution volume of 200 mL in 250 mL Pyrex beakers, for each surfactant concentration. All concentrations were tested in triplicate.

Five 24-hour-old *Daphnia* per beaker were tested. All Tests lasted 48 hours at a constant temperature of 21 ±1ºC under a 16-hour illumination period.

2. Observations
Mortality was recorded after 24 and 48 hours. Water hardness, dissolved oxygen, pH, as well as concentrations of nitrate, nitrite, copper, iron, lead, sodium and zinc were measured at the end of the 48-hour test.

3. Statistical analysis:
LC_{50} values were calculated using a computerized probit analysis program.

II. RESULTS AND DISCUSSIONS

Table 5. Effects of a 50 mg/L suspension of kaolin clay on the acute toxicity of a homologous series of anionic surfactants to *Daphnia magna*

		48 hr LC50 and Associated 95% Confidence Limits (ppm)	
Anionic Surfactants		Without Kaolin	With Kaolin
C_{11} LAS		19.3 (14.3 – 23.5)	13.0 (5.1 – 17.4)
C_{14} LAS		1.0 (0.94 – 1.1)	1.4 (1.2 – 1.5)
C_{18} LAS		0.09 (0.07 – 0.11)	0.18 (0.12 – 0.24)
Nonionic Surfactants			
$C_{10}AE_3$		1.9 (1.0 – 2.6)	1.7 (1.1 – 2.3)
$C_{14}AE_3$		0.12 (0.08 – 0.16)	0.12 (0.05 – 0.18)
$C_{18}AE_3$	young adults	5.0 – 20.0 >80.0	<5.0 >80.0

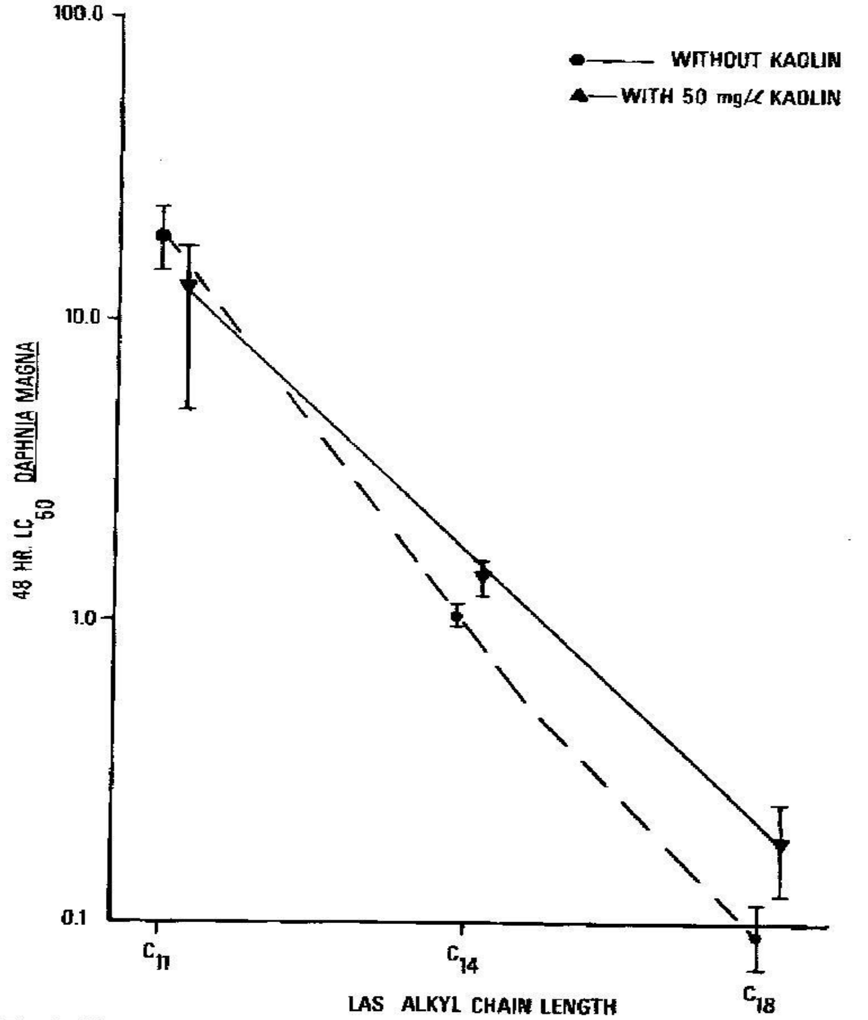


Fig. 3. The influence of suspensions of naturally-occurring Kaolin clay on the acute toxicity of linear alkyl benzene sulfonate to *Daphnia magna*.

The results of the tests with *Daphnia magna* demonstrate variable toxicity, with interactive effects between the 50 mg/L suspension of kaolin clay, the chemical class of surfactant tested, and individual alkyl chain lengths. For the anionic surfactants tested, the 48-hour LC_{50} values were observed at significantly higher concentrations of both the C_{14} and C_{18} homologs, but no significant difference was observed with C_{11} LAS (Figure 3).

III. CONCLUSIONS

These tests demonstrate that the presence of suspensions of purified, naturally occurring kaolin clay, significantly alters the observed acute toxicity of some long chain surfactants.

Reference:	KCA 8.8/03 Valenta, S., and Svobodova, Z. 1980
Title:	An Ichtyotoxicological Evaluation of Electrically Conductive Resins and Coating Mixtures used in the Paper Industry
Report No.:	Buletin VÚRH Vodňany (1980), volume 2
Guideline(s):	Not reported
Deviation(s):	None
GLP:	No, but study scientifically valid

The paper is in Czech language so only the English summary is presented.

Executive Summary

The electro-conductive resin Alcostat 576, a coating mixture with Alcostat 576, and a coating mixture with ECR 77 were tested for fish toxicity. Species used in the tests were rainbow trout (*Salmo gairdneri*), Guppy (*Poecilia reticulate*) and Common Carp (*Cyprinus carpio*). The test substances were included in a group of chemicals of known high toxicity to fish. After the use of potential deactivating substances (kaolin, sodium carbonate, calcium hypochlorite), the toxicity of these chemicals was reduced, allowing for the group of substances to be classified as just "toxic" to fish.

Out of the deactivating agents used for reducing the toxicity of the electro-conductive resins, kaolin is the most convenient because of its high flocculation effects. Kaolin is present in pulp and paper effluents and once combined with resins it can be removed with the sludge from the sewage-treatment facilities after sedimentation.

CA 8.9 Monitoring Data

No data available.