RESPONSE OF *DAPHNIA MAGNA* TO EPISODIC EXPOSURES OF SEVERAL TYPES OF SUSPENDED CLAY

Sarah E Robinson and Stephen J Klaine

AUTHORS: Sarah E Robinson and Stephen J Klaine PhD, 509 Westinghouse Rd, Pendleton, SC 29625
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Suspended solids are a natural component of aquatic systems and consist of both organic matter and inorganic sediments and clays. The amount of suspended solids in lakes and streams can be significantly increased by anthropogenic activity, when soils are exposed for development and agriculture, especially during rain events. It has been shown that constant exposure to suspended clays and silts effects the growth and reproduction of *Daphnia*, leading to a decrease in population growth rate (McCabe and Obrien, 1983; Kirk and Gilbert, 1990; Kirk, 1992). The major mechanism of suspended clay toxicity to *Daphnia* is a decrease in food uptake rate and assimilation efficiency (Arruda et al., 1983; McCabe and Obrien, 1983; Kirk, 1991; Levine et al., 2005). Previous work exposing D. magna continuously to different types of clay for seven days has also demonstrated a difference in toxicity between clay types. Montmorillonite was the most toxic, followed by natural clay sized particles (Lost Creek clay) extracted form sediment from a local creek, and kaolinite was the least toxic (Capper, 2006). In the environment suspended clay exposures tend to be episodic in nature due to stormwater runoff. Daphnia can purge clay from the gut when they are placed in clean water. The objective of this research was to first look at the response of survival, reproduction and growth of *Daphnia magna* when exposed episodically to clay particles, comparing duration of exposure and concentration of the exposure, and secondly, to compare the response between episodic exposures to three clay types: kaolinite, montmorillonite, and natural clay particles (Lost Creek clay).

For these experiments 72 hour old *Daphnia magna* were placed in 4 cm high, 2.5 cm diameter glass tubes that were capped on the top and bottom with 500 µm Teflon mesh. Eight of these test chambers were placed in a modified test tube rack and suspended in a 4 L plastic beaker. Each beaker contained a two inch stir bar and was placed on a stir plate to keep clay particles in suspension. During experiments organisms were exposed to a single pulse of suspended clay and then monitored for a total of 21 days, noting mortality, the day each organism became gravid, measuring the length of each organism, and counting the number of neonates produced daily. For the first objective organism were exposed to concentrations of kaolinite varying from 50 to 400 mg/L for the first 24 hours or concentrations from 100 to 800 mg/L for the first 12 hours of the test period. They were then moved to clean water and monitored for the remainder of 21 days. Differences in growth, days to gravidity, and number of neonates per organism were analyzed statistically using a one way ANOVA. Exposure duration and concentration were then compared. For the second objective organisms were exposed to montmorillonite at concentrations ranging from 5 to 50 mg/L or to natural clay sized particles extracted from sediment collected from Lost Creek (located in Saluda River Valley of South Carolina) at concentrations ranging from 25 to 200 mg/L for the first 24 hours of the test period and then moved to clean water for the remainder of 21 days. The concentrations used for these experiments were lower than those used for kaolinite because they were adjusted due to the lower 7d LC50. The effects of 24h exposures of all three clay types were compared. Exposure concentrations were divided by the 7d LC50 of the corresponding clay to convert the exposure to toxic units, and the effects were compared.

When *Daphnia* were exposed to kaolinite there was no significant decrease in survival for those exposed for 12 or 24 hours. Organisms did however exhibit a significant increase in the number of days to become gravid for those exposed to 100, 200 and 400 mg/L for 24 hours. Days to gravidity for organisms exposed for only 12 hours exhibited no significant difference from the control, even though the exposure concentrations were up to 10 times greater than the lowest 24 hour exposure that showed a significant effect. This is one indication that the exposure duration is more import than exposure concentration. Although there was a significant increase in the days it took organisms to become gravid this was not reflected in the number of neonates produced over the test period. *Daphnia* reproduction was able to recover; there was no significant difference in the number of neonates produced per organism over 21 days. The growth of the *Daphnia* over 21 days was similar to the days to gravidity data with respect to comparing exposure duration to exposure concentration. The organisms that were exposed for 24 hours were significantly smaller than the controls in the days following the exposure though by day 16 there was no difference in length. For those exposed for only 12 hours there was no difference in length in the days following the exposure until at least day 9 when the exposed organisms were significantly larger than the control. This may be a result of organisms funneling energy into growth after being exposed allowing the 24 hour exposed organisms, that were inhibited initially, to catch up with the control, where the 12 hour exposed organisms, that were not inhibited, actually exceeded that of the control.

The second experiment was a study to see if the difference in toxicity between clay types observed for continuous exposures of previous work also existed for episodic exposures, and if so was there a relationship between the 7d LC50 and the toxic response. Data for the 24 hour kaolinite exposure was used in addition to data for 24 hour exposures to both montmorillonite and natural clay sized particles extracted from sediment (Lost Creek clay). The data for both montmorillonite and Lost Creek clay was similar to that from kaolinite. There was no significant decrease in survival or the number of neonates produced over 21 days. There was a significant increase in days to gravidity for the 10, 25, 50 and 100 mg/L montmorillonite treatment and for the 200 mg/L Lost Creek treatment. Organisms were also significantly smaller in the days following the exposure but no significant difference from the control in any of the treatments after day 10 and 15 for montmorillonite and lost creek clay, respectively.

To compare the response from the three clay types the concentrations used were converted to toxic units by dividing the concentration by the 7d LC50 for the corresponding clay. This allowed the response of the different clays to be graphed together on the same scale to compare the response of days to gravidity. Organisms exposed to kaolinite and montmorillonite exhibited a very similar response when adjusted based on the 7d LC50. This means that when relative toxicity is taken into account *D. magna* reproduction responds the same to both types of pure clays. The response of days to gravidity when exposed to Lost Creek clay was reduced compared to the other clays. Organisms exposed to Lost Creek clay only exhibited a significant increase in days to gravidity, compared to controls, for the highest concentration, 3.78 toxic units. At around 2 toxic units organisms exposed to Lost Creek clay were not significantly different from the control, but those exposed to kaolinite and montmorillonite exhibited a significant increase in days to gravidity. It is possible that the natural clay particles are not as toxic when Daphnia are only exposed to it for short duration. It could contain organic matter that might be used as a food source at lower concentrations.

Many of the results observed can be explained by considering the major mechanism of suspended clay toxicity, which is a decrease in feeding efficiency. Exposure duration is more important than exposure concentration because for a range of concentrations the gut tract is filled with clay after some time being exposed and feeding is inhibited until they are placed in clean water. If the duration of exposure is increased then the amount of time feeding is reduced is increased. This increase in time of decreased feeding, affects the *Daphnia* more than the amount of clay present. The mechanism of toxicity can also help explain why for 24 hour exposure, days to gravidity is not affected as much by natural clay sized particles than it is by pure kaolinite and montmorillonite. The pure minerals contain no organic matter and therefore should not provide any possible food source. The natural clay particles could contain some organic matter which may provide some food for the short duration of exposure, even though when exposed for seven days this clay was more toxic than kaolinite.

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