A laboratory investigation into the potential use of BACSAN as a control method for adult zebra mussels.

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On behalf of:

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Client:

Water Development Program Ltd

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1. Summary

This report is the first of six reports in which the product BACSAN is investigated as a potential control agent for zebra mussels. In this first study, we treated adult zebra mussels with BACSAN and a regenerating agent (Potassium MonoPersulphate) in a controlled laboratory setting. Substantial mussel mortality was found at most doses, with a 7-day BACSAN LC_{50} of 14.03ppm (approx 0.5mg/l Copper), a 15-day BACSAN LC_{50} of 5.31ppm (approx 0.2mg/l Copper), and a 28-day BACSAN LC_{50} of 1.26ppm (approx 0.05mg/l Copper). There were only slight differences in the sizes of mussels killed at different times during the experiment.

2. Methods.

Adult zebra mussels were collected from the Rapid Gravity Filter inlets at Coppermills Water Treatment Facility and immediately returned to the laboratory for use in toxicity tests. In the laboratory, ten zebra mussels of over 20mm in shell length were placed into each of thirty 500ml plastic bowls containing aged tap water. The bowls were transferred into a temperature-controlled room on a 12h-light/12h-dark cycle, set at a temperature of 19-20ºC. Each bowl was placed on a mini-stirrer set at 60rpm (Stem mini-stirrer, Fisher Scientific UK), and aerated by an air pump (Hiblow HP100, Hiblow USA Inc., 1300 Tefft Ct. Suite 8, Saline, MI, USA), as shown in Figure 1. The mussels were allowed to acclimatise to the test conditions for 24 hours before the initiation of experiments. Prior to testing, each bowl was checked for zebra mussel mortality, and on the rare occasion that dead zebra mussels were found, these were replaced with mussels from a stock kept in the temperature-controlled room.

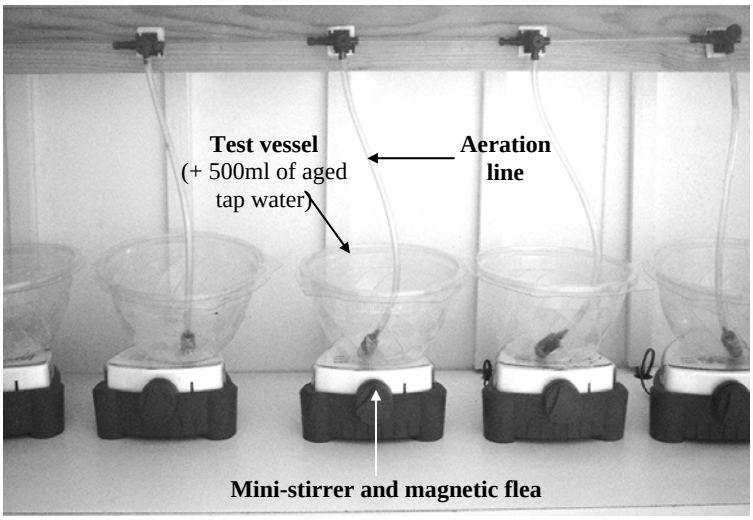


Figure 1. The tests vessels used in laboratory mortality trials with zebra mussels (10 mussels placed into each vessel).

Static toxicity tests were conducted using nine different pair-wise combinations of BACSAN and a regenerator (Potassium MonoPersulphate). Each combination of toxins was applied to 3 separate test vessels. A control experiment was also conducted, which involved no addition of toxin to 3 vessels. Zebra mussel mortality was recorded every day for the next 11 days, and then at approximately 3-day intervals for the following 17 days. After every mortality assessment, the concentrations of copper ions and active oxygen were measured in a sub-sample of the test vessels (using a supplied test kit). The vessels were then re-injected with more BACSAN and/or potassium MonoPersulphate to restore the desired concentrations of active ingredients.

Mortality was defined as a failure of zebra mussels to close in response to a prod by a blunt probe. For high potassium ion concentrations, mortality assessment was complicated by potassium ions inhibiting contraction of the posterior adductor muscle, causing living mussels to remain open even with tactile stimulus. However, it was readily apparent that living, non-responsive mussels were extremely turgid in comparison to dead mussels. In the rare event of ambiguity, the mussel was removed from the container for 5 minutes, after which time living mussels started to close. During each test, any dead mussels were immediately removed from the test vessels and measured in length.

$LC(toxin)_{50}$ values and 95% confidence were calculated using the Probit methods of the Spearman-Karber Method (Hamilton *et al.,* 1977; Waller *et al.,* 1993). Data analysis was conducted using the Trimmed Spearman-Karber Program Version.1.5 (Ecological Monitoring Research Division, USEPA, Cincinnati, Ohio, 1999), downloaded under licence from: www.epa.gov/ceampubl/fchain/lc50/. LC_{50}s are considered to be significantly different when confidence intervals do not overlap.

3. Results.

There was no mortality in any of the control vessels at any of the measurement times. Figure 2 shows the mortality of zebra mussels with a fixed dose of regenerator (10ppm Potassium MonoPersulphate), but with varying concentrations of BACSAN. A dose rate of 13.33ppm BACSAN (0.5mg/l Copper) and 10ppm Potassium MonoPersulphate gave a 50% mortality of zebra mussels in around 6-7 days and a 100% mortality after 11 days. There is clear evidence that as the concentration of BACSAN

increased, so too did the rapidity of mortality. However, there was mortality with even low concentrations of BACSAN. For instance, with a dosage of 5ppm, 97% mortality was achieved after 28 days. The results of individual dosage combinations, along with their associated standard errors can be found in Appendix 1.

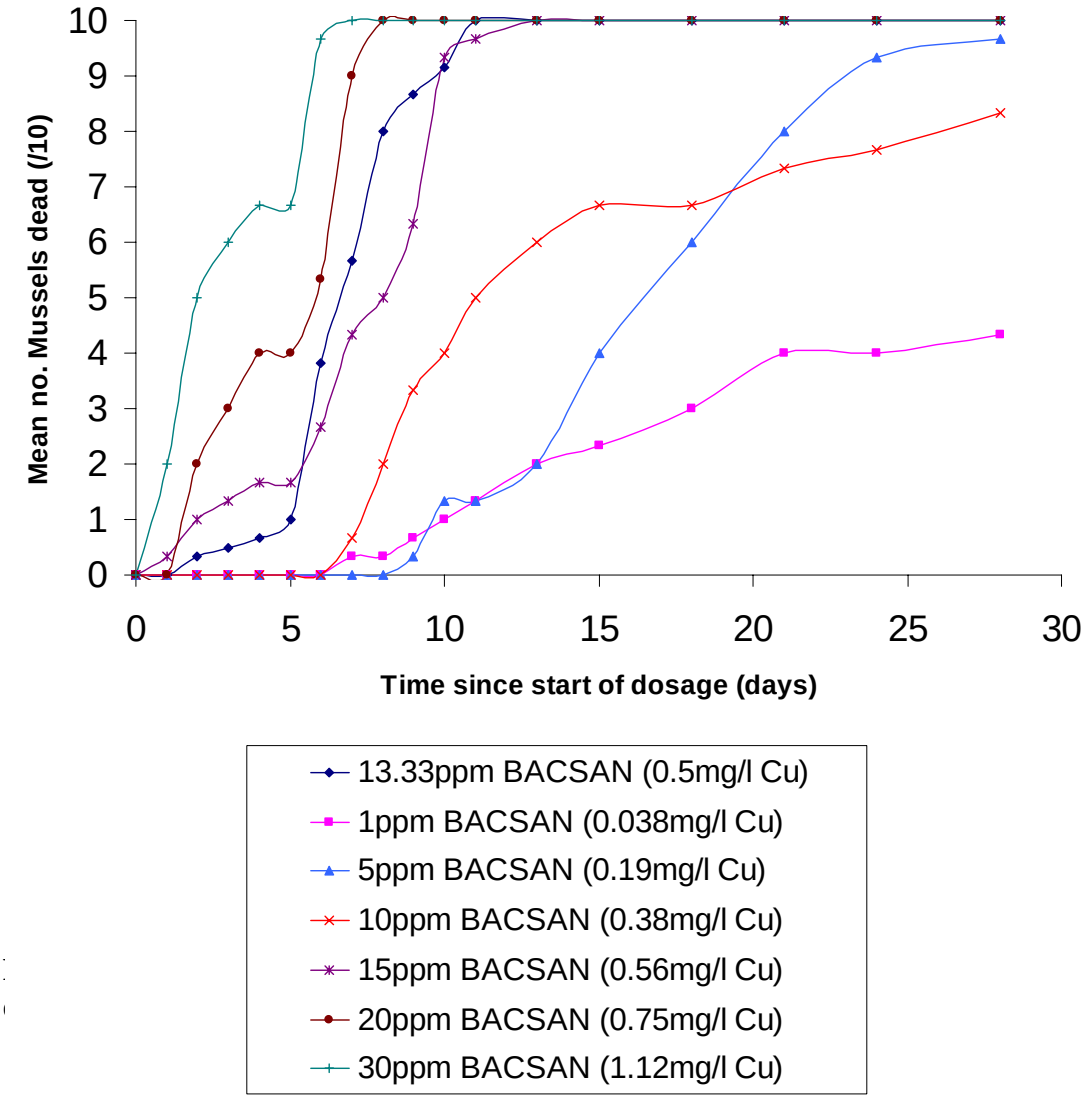


Figure 2. Zebra mussel mortalities following dosages of BACSAN (in varying concentrations) and Potassium MonoPersulphate (10ppm).

From these results, the LC_{50}s of BACSAN (when added with 10ppm MonoPersulphate) were calculated (See Table 1, Figure 3). The LC50's vary from 30ppm BACSAN (approximately 1.125mg/l Copper) after 1 day, down to 1.26ppm (approximately 0.05mg/l Copper) after 28 days.

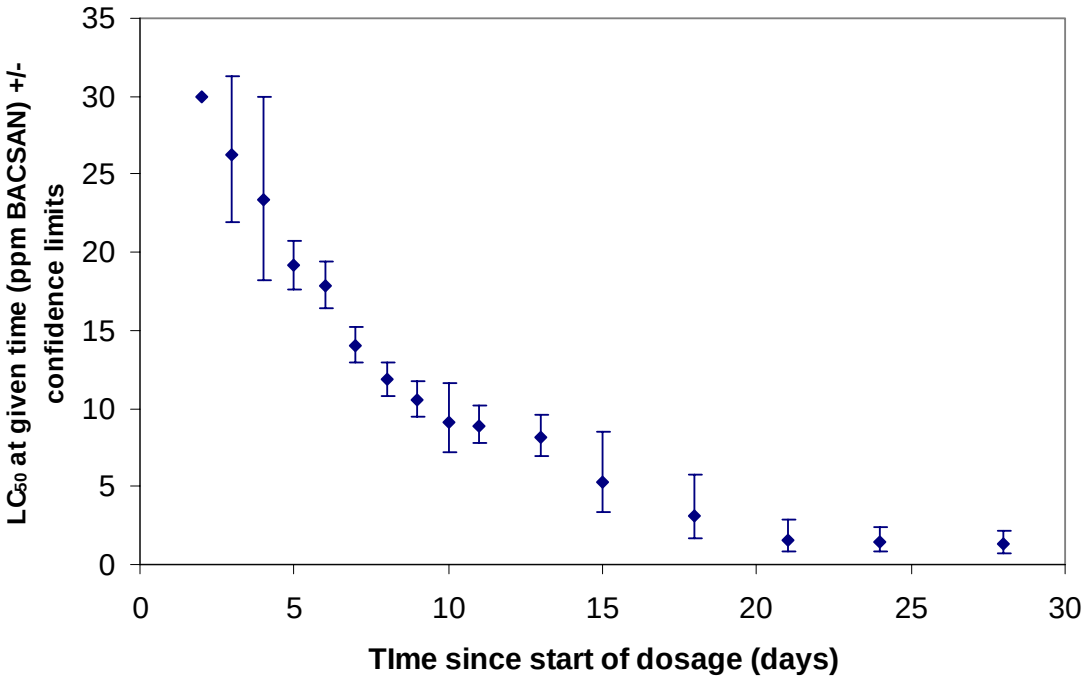


Figure 3. Calculated LC_{50}s for BACSAN after different durations of dosing. (10ppm of Potassium Monopersulphate was also present in all treatments)

Table 1. Calculated LC_{50}s for BACSAN after different durations of dosing. (10ppm of Potassium Monopersulphate was also present in all treatments)

Time (Days)	2	3	4	5	6	7	8	9
LC_{50} (mg/l)	30	26.21	23.33	19.13	17.85	14.03	11.82	10.57
Lower Conf.Lim		21.94	18.2	17.67	16.43	12.98	10.83	9.51
Upper Conf.Lim		31.31	29.91	20.7	19.4	15.17	12.9	11.76

Time (Days)	10	11	13	15	18	21	24	28
LC_{50} (mg/l)	9.13	8.87	8.13	5.31	3.07	1.55	1.43	1.26
Lower Conf.Lim	7.2	7.77	6.93	3.31	1.63	0.85	0.86	0.74
Upper Conf.Lim	11.57	10.14	9.54	8.5	5.8	2.83	2.38	2.15

When the dosage of Potassium MonoPersulphate was altered and the dosage of BACSAN was kept constant at 13.33ppm, there was less of an impact on mortality (Figure 4, Appendix 1). The 5ppm dose of Potassium MonoPersulphate gave a marginally lower mortality, but this was barely significant.

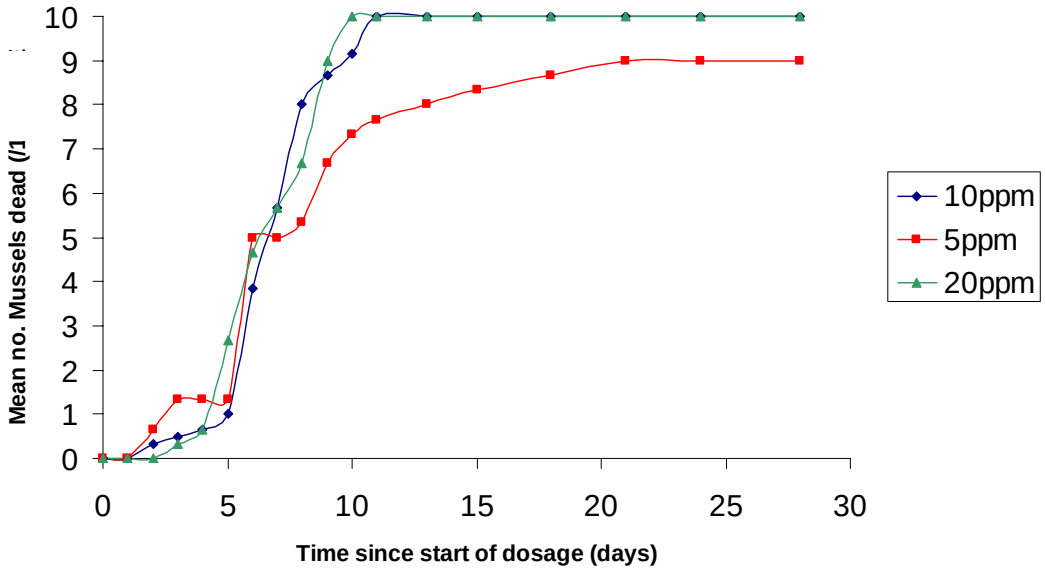


Figure 4. Zebra mussel mortalities following dosages of BACSAN (13.33ppm) and Potassium MonoPersulphate (varying concentrations).

The sizes of the dead mussels removed at the various measurement times did show a slight change across the duration of the experiment (1-way ANOVA, d.f=16, p= 0.03 Figure 5), with some slightly larger mussels being removed during the later stages. However, this effect was relatively minor.

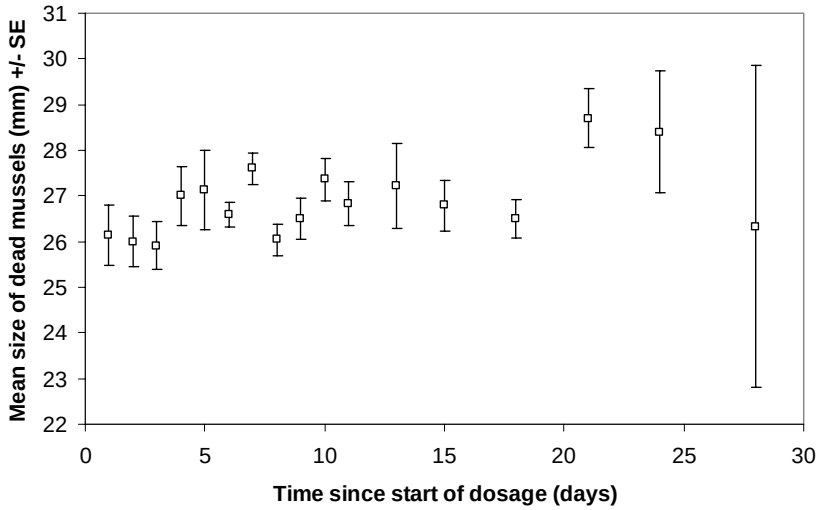


Figure 5. The sizes of dead mussels retrieved at different periods after the start of dosing.

4. Suggestions for Future Studies

- The next phase of experimentation, already under way, is the treatment of larval zebra mussels with BACSAN and Potassium Monopersulphate. Doses of between 0.01 and 5ppm BACSAN will be used, with varying doses of Potassium Monopersulphate.

- In July, a set of Large-scale trials of BACSAN are proposed. These will allow testing of BACSAN toxicity to zebra mussels under natural conditions of pH, temperature and riverine water quality. These tests could be conducted in a Yorkshire water-treatment facility, but we already have a set of flow-through flumes at a London water treatment facility which could be used for this purpose. The flume apparatus consists of thirty, 4m-long flumes, each stocked with zebra mussels from a nearby filter bed (Figure 6). Each flume is fed with untreated reservoir water from a $4m^3$ header tank, and flows can be manipulated to reach up to $300ml.s^{-1}$. For chemical dosing, groups of five adjacent flumes can each be equipped with a set of dosing apparatus (Figures 6 and 7), allowing different concentrations of chemical to be applied in replicate. At present, the dosing apparatus for each set of five flumes consists of a set of water-butts containing a 500-litre supply of control chemical solution (Figure 7). This water is fed by gravity at $50ml.s^{-1}$ into a 40-litre mixing tank positioned above the flumes and out into the flumes at a rate of 10ml.s-1 (by gravity). The treated water then flows out of the flumes and into a wastewater area of the facility, preventing any environmental discharge. This technique has been appropriate for short treatment regimes in the past, but for longer doses, more advanced dosage equipment will be necessary.

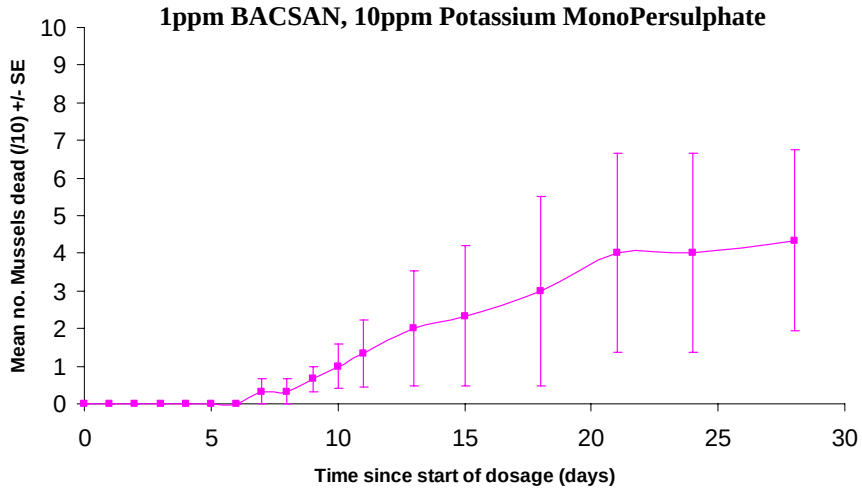
  If the Water Development Program wish to proceed with these large-scale experiments, they will take a total of 3 consultancy days in the first week of dosing (with measurements taken every day), together with an additional two consultancy days for every additional week of dosing/measurement.

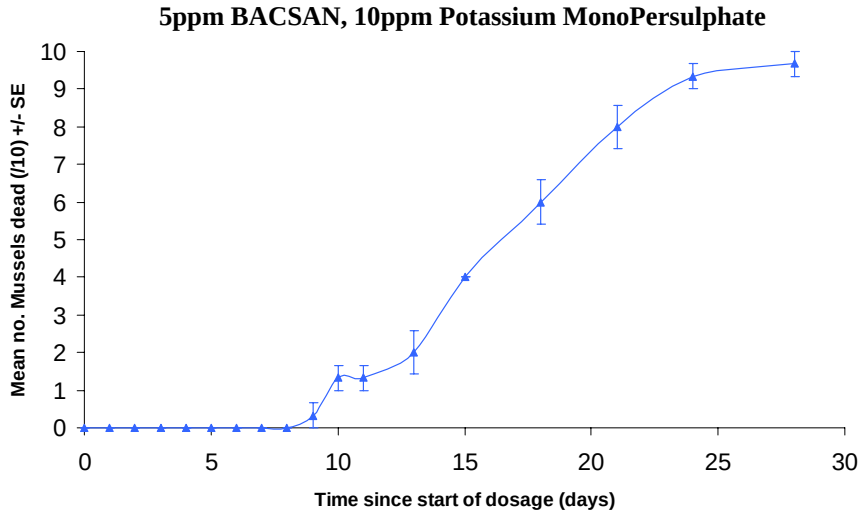
Figure 6. View of the experimental flumes at a London WTW which could be used in chemical dosing trials of BACSAN.

Figure 7. View of the dosing apparatus which could be used in large-scale field trials of BACSAN

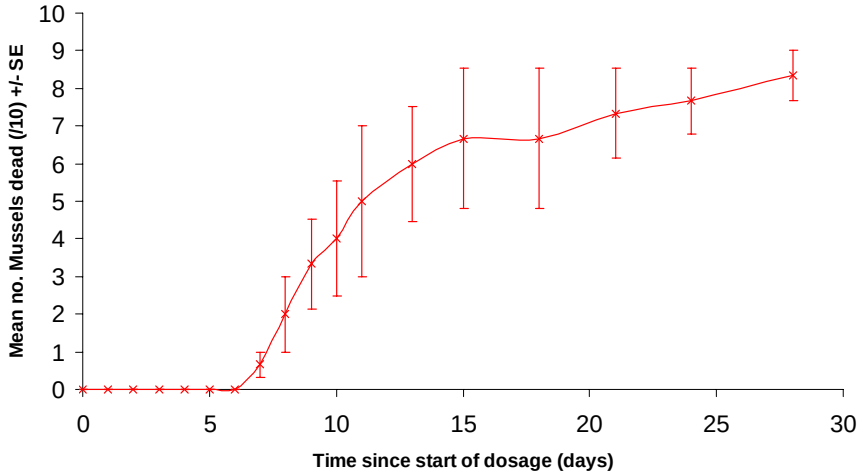
Appendix 1.

The following graphs show the zebra mussel mortalities +/- standard errors following treatment with different combinations of BACSAN and Potassium MonoPersulphate.

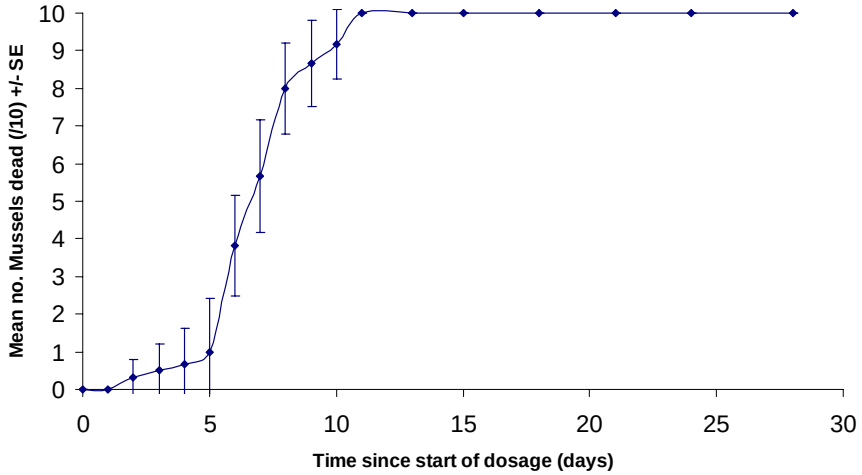




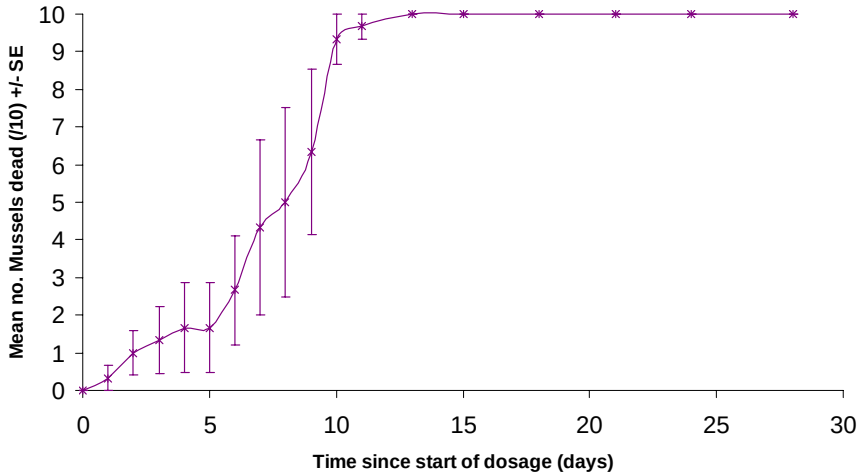
10ppm BACSAN, 10ppm Potassium MonoPersulphate



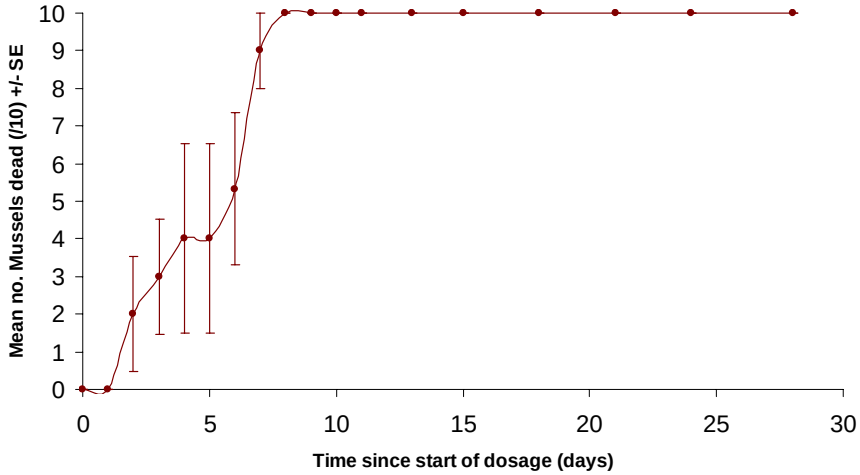
13.33ppm BACSAN, 10ppm Potassium MonoPersulphate



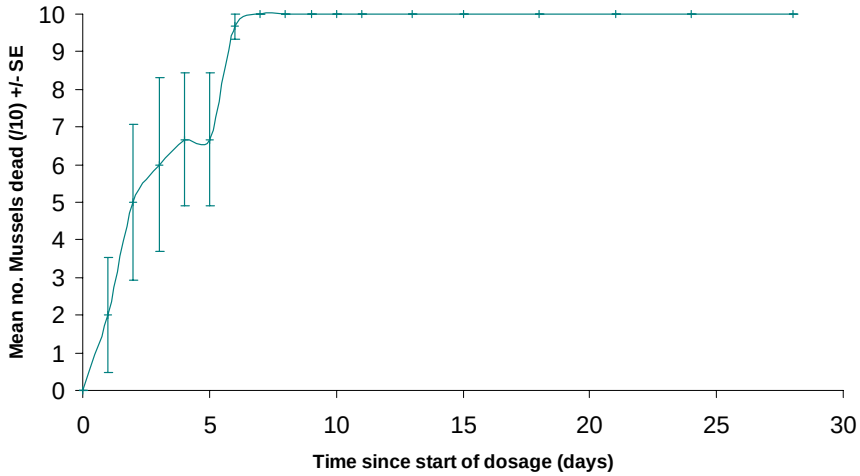
15ppm BACSAN, 10ppm Potassium MonoPersulphate



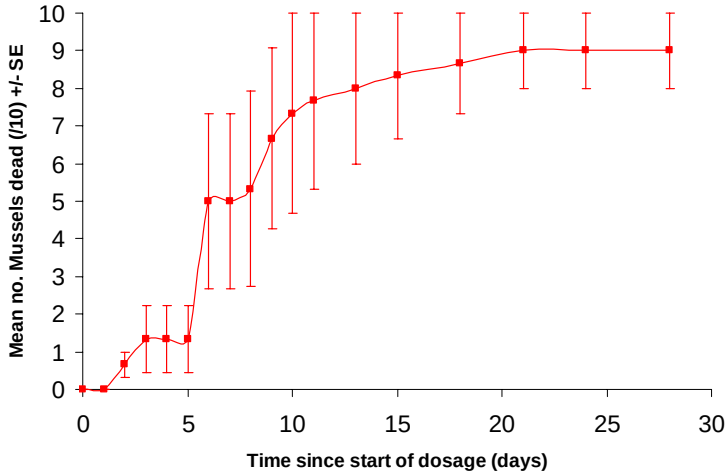
20ppm BACSAN, 10ppm Potassium MonoPersulphate



30ppm BACSAN, 10ppm Potassium MonoPersulphate



13.33ppm BACSAN, 5ppm Potassium MonoPersulphate



13.33ppm BACSAN, 20ppm Potassium MonoPersulphate