Toxicity testing of linear alkylbenzene cable oil using *Caenorhabditis elegans* in soil

Stephen Johnson*, Magali Castan[†], Lorna Proudfoot[+] and Nick Christofi

Pollution Research Unit and [+]Biomedical Research Group, Napier University, Edinburgh, EH5 10DT, UK.
*now at University of Greenwich at Medway, Central Avenue, Chatham Maritime, Kent, ME4 4AW, UK.
[†]Ecole Polytechnique Universitaire de Lille, Departement IAA, Boulevard Langevin – F, 59655 Villeneuve D'ascq Cedex, France

Abstract

Linear alkylbenzene (LAB) is an LNAPL used as a precursor in the manufacture of linear alkylbenzene sulphonate detergents, from where it makes its way into the aquatic environment. LAB is classified N; R50 Very toxic to aquatic organisms. In addition, LAB is used as an insulating oil in underground electricity transmission cables. Failure of a cable may result in contamination of soil with cable oil. To date, no data have been published on the toxicity of LAB to soil organisms. Nematodes occupy a central position in soil ecosystems, and since they live in interstitial water they are exposed to dissolved contaminants. They are therefore good candidates for ecotoxicity studies and have been used in the assessment of environmental contaminants. Caenorhabditis elegans is a non-parasitic soil nematode that is already well known because of its use in developmental studies. Its small size, hermaphroditism, short life cycle (Figure 1) and the occurrence of a stress-tolerant "dauer" larval stage means that it is easy to raise large numbers of genetically similar, age-synchronised adults in the laboratory. A 24-h acute exposure to heavy metals has been shown to be equivalent to a 14-d earthworm assay and an ASTM method (E2172-01) for acute toxicity of substances in soil has been published. Populations of C. elegans were exposed to several concentrations of LAB in natural soil. Nematodes were recovered using differential density centrifugation in colloidal silica. Mortality was determined after 24 h and the LC$_{50}$ was calculated by several methods. Trimmed Spearman-Karber analysis yielded the most conservative estimate at 0.52% w/w dry soil, as well as the narrowest 95% confidence interval, with upper and lower confidence limits of 0.405% and 0.68%, respectively (n = 360). This figure is higher than published toxicity to aquatic organisms but LAB adsorbs to clay and humic material in soil so the availability to soil organisms will be rather lower than the absolute amount.
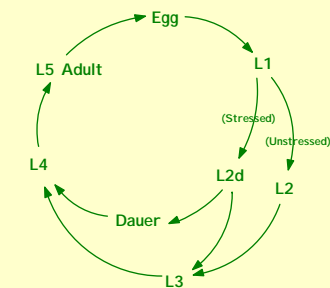
Figure 1 *C. elegans'* life cycle takes 5.5 d at 15°C, 3.5 d at 20°C, and 2.5 d at 25°C. Above 25°C, the worms are sterile.
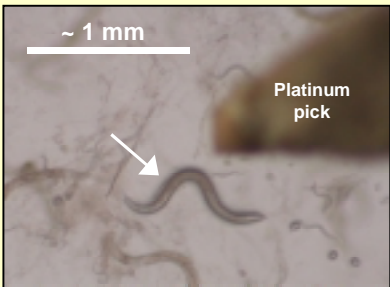

Plate 1 Handling of individual *C. elegans* (arrowed) using a platinum pick. Image shows an adult worm as well as larvae and eggs

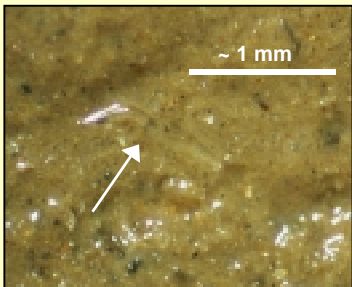Plate 2 Adult *C. elegans* (arrowed) on soil prior to burrowing below surface

Methods

The methods described in ASTM Method E2172-01 were followed throughout.

Test Organisms *C. elegans* strain N2 were cultured at 20ºC on a lawn of *Escherichia coli* OP50 (a uracil deficient strain) on nutrient agar. To produce a population of age-synchronised adults larvae were recovered from a 9 d-old culture, placed on *E. coli* OP50 plates and incubated at 20°C for 48 h. The adults were collected in M9 buffer. They were washed twice with dH$_2$O and treated with alkaline hypochlorite solution for 15 min. This killed the gravid adults and lysed the body wall to release the eggs contained inside. Vortexing briefly every 2 min during this treatment ensured that the embryos did not die of oxygen deprivation. Eggs were harvested by centrifuging and washing the pellet twice in 10 ml M9 buffer. The final pellet was resuspended in no more than 0.5 ml M9 buffer and the eggs transferred onto NA plates inoculated with *E. coli* OP50. These were incubated at 20°C for 4 d.

Test Method A measured quantity of LAB was added to natural soil. This was homogenised and the efficiency of mixing was confirmed by hexane extraction and GC-MS. Concentration was varied by mixing with appropriate amounts of uncontaminated soil. Soil was hydrated to 40% and 2.33 g (dry weight) was placed into 35 mm Petri dishes sufficient to allow three replicates of each concentration to be tested. These were stored at 20ºC to equilibrate for seven days. On the day of the test, 10 worms were added to each dish using a flamed platinum wire (Plates 1 and 2). After 24 hours, nematodes were recovered by shaking the soil in a colloidal silica suspension (1:1 Ludox HS-40 in dH$_2$O adjusted to pH 7 with HCl) and centrifuging at 2000 rpm for 2 min. After 15 min settling time the supernatant containing the worms was poured into a 90-mm Petri dish. The worms were counted and scored live/dead under a dissecting microscope.
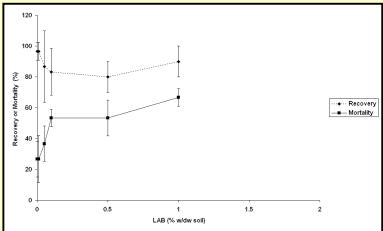
Figure 2 Experiment 3 - Plot of number of worms recovered and mortality against LAB % dry weight soil. Error bars = 1 SD, n = 30
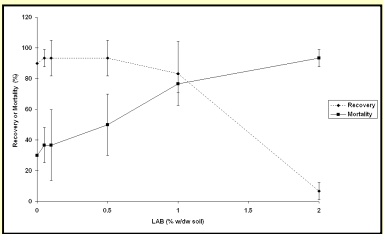

Figure 3 Experiment 6 - Plot of number of worms recovered and mortality against LAB % dry weight soil. Error bars = 1 SD, n = 30
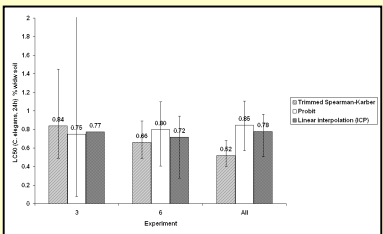

Figure 4 Comparison of LC50 (24h, *C. elegans* in soil) calculated by different methods. Data from experiments 3, 6 and pooled data. Error bars = 95% confidence intervals, n = 180 (for individual experiments) or 360 (pooled data).

Results and Discussion

As an endpoint for a toxicity assay, death is of limited usefulness. It is relatively easy to identify but it does not give any information about the long-term effects of sublethal concentrations on the soil community. Nevertheless, LC50 remains the most commonly used measure of environmental toxicity. Mortality data (Figures 2 and 3) were used to calculate LC50 values by a variety of methods using MS-DOS software downloaded from the US Environmental Protection Agency website at http://www.epa.gov/nerleerd/stat2.htm.

Figure 4 shows the concentration of LAB in soil required to kill 50% of a population of *C. elegans*, as calculated by a variety of commonly used methods. There were no significant differences between the different methods or between experiments. The upper 95% confidence interval for the Probit analysis was rather high (61%) and is not shown in full in order to keep the y-axis at a meaningful scale. The linear interpolation program was unable to generate 95% confidence intervals for experiment 3.

C. elegans provides an increasingly popular model for toxicology in soil. Its small size and hence large surface area to volume ratio means that it is sensitive to contaminants over a much shorter timescale than, for instance, earthworms. However, this small size does have implications for handling the animals and this was evident in the high mortality seen in control populations, especially in early experiments. It is likely that further practice and method development would reduce this to a more appropriate level.

Since there was no significant difference between the LC50 calculated from the two successful experiments, it was felt that it would be appropriate to pool the data to obtain a more accurate estimate.

Conclusions

The most conservative estimate, as well as the narrowest confidence interval, is given by pooling all the available data and applying the trimmed Spearman-Karber method.

LC50 for LAB in soil is calculated to be 0.52% dw of soil (5200 mg kg^{-1}), with 95% confidence that the actual value lies between 0.41% and 0.68% (4100-6800 mg kg^{-1}).