EXECUTIVE SUMMARY

BACKGROUND

In recent years, increased public awareness and interest in environmental issues have highlighted the problem and the effects of the high levels of accumulated persistent pesticides and other toxins in the environment. The increased concern and attention around this issue has led to an increased need for effective methods of detection of these substances in potentially contaminated areas and systems (Iwata *et al.*, 1994). Current methods can offer highly sensitive and effective detection of toxicants; however, they also have their limitations. These methods include chromatography (gas chromatography, high performance liquid chromatography) (Muir and Sverko, 2006) and spectroscopy (UV spectroscopy, mass spectroscopy, fluorescence spectroscopy) (Pogačnik and Franko, 2001). The disadvantages of these methods are the requirement for expensive equipment and materials which makes it costly, as well as the need for highly skilled operators (Mazzei *et al.*, 2004; Swart and Pool, 2007). Further limitations include the lack of portability of this equipment to the contaminated site, and the fact that these methods can be time consuming, making it unsuitable for rapid, on-site detection (Mazzei *et al.*, 2004).

Enzymatic methods of detection offer a potentially simpler, more rapid and cost effective alternative for the detection of water contaminants. For the detection of pesticides, the inhibition of enzymes affected by specific pesticides can be monitored using enzyme assays, the degree of inhibition being proportional to and thus giving an indication of the concentration of pesticides in the water (Breuer, 1982). Such enzymes include cholinesterases (inhibited by organophosphorus and carbamate pesticides) and alkaline phosphatases (inhibited by heavy metal ions, as well as organophoshorus and organochlorine pesticides) (Garcia Sanchez *et al.*, 2003; Chouteau *et al.*, 2004; Gokcimen *et al.*, 2006). However, only a limited number of contaminants have been tested based on these enzymatic detection methods and there is therefore a need to determine the response of a wide range of contaminants on the activity of key enzymes.

It has been reported that significant levels of various pesticides have been detected in water and sediments in South Africa, including pyrethroids, organochlorines (DDT and DDE), organophosphates and carbamates (Slabbert *et al*., 1998; Sereda and Meinhardt, 2003; Burger and Nel, 2008; Slabbert *et al*., 2004); as well as polychlorinated dibenzo-ρ-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Vosloo and Bouwman, 2005). To ensure the ongoing identification and management of water pollution by pesticides, it is necessary to investigate the development of rapid and simple detection systems such as proposed in this study.

AIMS

The ultimate aim of this work was to develop a rapid enzymatic assay for the detection of organochlorine pesticides (OCPs). This was accomplished by addressing the following specific aims, which were:

a) To develop and establish a rapid enzyme assay for the detection of OCPs,
b) To test the reactivity of these OCPs, present either alone or in combination (i.e. in a mixture), in surface water,
c) To establish proof of concept for a subsequent, more detailed study (3-5 years) using environmental samples from around the Eastern Cape.

METHODOLOGY

A standard alkaline phosphatase (ALP) assay that made use of calf intestine ALP, with *p*-nitrophenyl (pNPP) where, in the reaction, the ALP cleaves a phosphate off the pNPP, yielding a yellow product, was used. The method was modified from that originally used by Gasser and Kirschner (Gasser and Kirschner, 1987). The effect of several OCPs (2, 4'-DDE (DDE), heptachlor (HEP), 2, 4'-DDT (DDT), toxaphene (TOX), dieldrin (DIE), aldrin (ALD), mirex (MIR), endosulfan (END), α-chlordan) on the ALP assay was investigated. Each OCP was detected and quantified separately using suitable inhibition kinetics. Once the effect of each OCP on the ALP assay had been established, the OCPs will be mixed in various combinations and the effect of these combinations on the ALP activity was evaluated. The effect of organophosphorus pesticides (OPPs), carbamate pesticides (CPs) and heavy metals on the ALP assay was also investigated, as reports in literature have hinted to the fact that these compounds may interfere with the assay.

RESULTS

This study showed that ALP was not an effective target enzyme for the design of a suitable bioprobe or biosensor product. ALP was not significantly inhibited by any of the OCPs investigated in this study, and in many cases a slight enhancement of the enzyme activity was observed. Slight enhancement of ALP was also observed in the presence of OPPs and CPs. No synergistic or even additive effects were noted when mixtures or combinations of the OCPs were assayed. The effect of various metal ions such as Cadmium, Mercury, Nickel and Zinc on ALP activity was also investigated. From the data obtained during the course of this study it was found that all these metal ions had a dramatic effect on the ALP enzyme, increasing the relative activity of the ALP by 200% even at very low concentrations of metals of 0.001 mg/l. This, perhaps, is the most significant result of our study. ALP activity was enhanced in the presence of several environmental samples (mainly rivers from around the Eastern Cape), indicating the potential presence of several metal ions in these samples.

CONCLUSIONS

This study, however, showed that ALP was not an effective target enzyme for the design of a suitable bioprobe or biosensor product. ALP was not significantly inhibited by any of the

OCPs investigated in this study, and in many cases a slight enhancement of the enzyme was observed. No synergistic or even additive effects were noted when mixtures or combinations of the OCPs were assayed. ALP is probably more suitable as an enzyme target for the detection and monitoring of heavy metal compounds in water. In conclusion, we believe that the cholinesterases, such acetylcholinesterase and butyrylcholinesterase, present better target enzymes for the detection, especially for the newer generation pesticides (OPPs and CPs) classes.

RECOMMENDATIONS FOR FUTURE RESEARCH

Based on the results obtained during the course of this study, we would like to make the following recommendations:

a) ALP is probably more suitable as an enzyme target for the detection and monitoring of heavy metal compounds in water, and a follow-up study focussing on the use of ALP for heavy metal ion detection should be investigated.

b) The cholinesterases present better target enzymes for the detection of the newer generation pesticides (OPPs and CPs). These pesticides also have the added benefit of being more soluble and are more easily degraded in the environment. Currently, we are busy with a follow-on grant based on this approach (WRC 1902), extending the potential detection not only to the pesticides, but also to their degradation products in the water environment.