Evaluation of interactive toxic effects of chemicals in water using a *Tetrahymena pyriformis* toxicity screening test

JL Slabbert* and JP Maree
National Institute for Water Research, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, South Africa

Abstract

A *Tetrahymena pyriformis* oxygen uptake assay developed for rapid screening purposes was used to investigate toxic effects of combinations of chemicals commonly found in various water environments. Respiratory response was measured over a 5 min period at constant temperature by means of a biological oxygen monitoring system connected to a potentiometric recorder. Mixtures of chemicals produced antagonistic, synergistic, additive or neutral toxic effects, depending on the chemicals used in combination and individual concentrations. Irrespective of the type of combined effect when testing high chemical concentrations, mixtures of low concentrations of these chemicals produced additive effects. Enhancement of the toxic effect when chemicals were mixed indicated that water quality standards based on individual toxicity data do not sufficiently protect the aquatic environment. Biological monitoring can, however, detect such effects and is therefore a very important detection system.

Introduction

The effects of individual toxic chemicals on the aquatic environment have been studied extensively, using various test organisms. When pollution occurs in practice, however, several chemicals are usually present simultaneously. These may interact additively, synergistically or antagonistically, or the observed effect may be due to the toxicity of the dominant constituent.

Much of the work on toxic interaction of aquatic pollutants has been carried out using fish, and involved the development of various models for assessing and predicting combined toxicity (Bliss, 1939; Sprague, 1970; Muska and Weber, 1977). Very few authors have examined the effects of mixtures of toxic chemicals on micro-organisms. Dutka and Kwan (1982) compared four bacterial screening procedures for assessment of the effects of mixtures of toxic chemicals. Combined effects varied from antagonistic or neutral to additive and synergistic. The authors found that each system has its own toxicity sensitivity pattern. There are areas of general concurrence as well as areas of wide divergence in sensitivity. Because of the variety of toxicant concentrations to which biological species react the battery approach using various testing systems is recommended. Interaction of certain metal combinations on algae was examined by Wong and Beaver (1981) and Rai et al. (1981). In certain cases the metals acted antagonistically while in others the same metals had markedly synergistic effects. This was also observed by Gray (1974) and Parker (1979) while studying the effect of metal combinations on the growth of ciliate populations.

During recent years a number of rapid microbiological screening tests have been developed, one of which was the *Tetrahymena pyriformis* oxygen uptake bioassay (Slabbert and Morgan, 1982), which provides results within 10 min. Based on physiological functions, these microbiological tests detect low levels of toxicants. In this study the *T. pyriformis* toxicity bioassay was applied to test mixtures of chemicals commonly found in water. The objective of the research was to obtain a better understanding of the modes of action of sub-lethal concentrations of toxicants in mixtures and to evaluate the sensitivity of the system to combined toxicity. Results were used to establish whether water quality standards based upon individual toxicity data adequately protect the microbiota.

Materials and methods

Chemicals

The following chemicals were tested: Hg$^{2+}$ (HgCl$_2$), Cu$^{2+}$ (CuSO$_4$), Zn$^{2+}$ (ZnSO$_4$.7H$_2$O), Pb$^{2+}$ (PbCl$_2$), Cr$^{6+}$ [Cr(NO$_3$)$_3$], CN$^-$ (NaCN), F$^-$ (KF), As$^{3+}$ (As$_2$O$_3$), BO$^{3-}$ (H$_3$BO$_3$), NH$_3$ [(NH$_4$)$_2$SO$_4$] and C$_6$H$_5$OH. Deionized water was used for the preparation of test solutions and for control tests. Membrane filtered (0.45µm) activated sludge effluent from the Daspoort sewage works near Pretoria (Prinsloo et al., 1978) was used as solvent and control for one series of tests carried out on a mixture of ten chemicals added together at concentrations set as general standard for industrial effluents discharged into receiving waters in South Africa (1962). The chemical composition of the effluent has been reported elsewhere (National Institute for Water Research, 1982). After addition to the *T. pyriformis* cell suspension (20-fold dilution), the concentrations of some of the determinands were as follows: 1.6 mg/l COD (chemical oxygen demand), 0.59 mg/l DOC (dissolved organic carbon), 49 µg/l MBAS (methylene blue active substances), 2.1 µg/l phenol, 0.13 mg/l iN$H_3$, 31 µg/l BO$^3-$, 75.0 µg/l F$, 1.25$ µg/l Cr$^{3+}$, 1.25 µg/l Cu$^{2+}$, 4.25 µg/l Zn$^{2+}$, 1.25 µg/l Pb$^{2+}$ and 2.5 µg/lCN$^-$. For purposes of comparison this study also included tests on the individual chemicals making up the various mixtures. Part of the individual toxicity data have already been published in a methodological paper by Slabbert and Morgan (1982).

Test organism

*T. pyriformis* strain W was cultured axenically at 27°C in a 10 g/l protease peptone medium (Slabbert and Morgan, 1982). Logarithmic growth phase cells (24 h old) were suspended in Osterhout salt solution (Taylor and Strickland, 1935) by means of gravity filtration before use in toxicity tests. Cells were diluted to a concentration yielding an oxygen uptake rate of approximately 8 units(%)/min.

Toxicity test

The *T. pyriformis* oxygen uptake bioassay was carried out accord-