Available online at www.sciencedirect.com
ScienceDirect
Pesticide Biochemistry and Physiology 86 (2006) 131–137
PESTICIDE
Biochemistry & Physiology
www.elsevier.com/locate/ypest

Toxic effects of acephate on *Paramecium caudatum* with special emphasis on morphology, behaviour, and generation time

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Received 4 January 2006; accepted 9 February 2006
Available online 23 March 2006

Abstract

The continuous increase in the number of new chemicals as well as the discharges of solid and liquid wastes triggered the need for simple and inexpensive bioassays for routine testing. In recent years, there has been increasing development of methods (particularly rapid tests) for testing environmental samples. This paper describes the quick toxic evaluation of an organophosphorus insecticide, acephate (*O*,*S*-dimethyl acetylphosphoramidothioate) on *Paramecium caudatum* for acute and sub-acute toxicity studies with reference to morphology, behaviour, and its generation time. The lethal concentrations for 10 min and 2 h were determined by probit method, as 500 mg L^{-1} and 300 mg L^{-1}, respectively. Higher concentrations of 10 min exposure caused cell lysis with disintegration of cell membrane and precipitation of protoplasm. Combination of conventional light microscopy and computerized video tracking systems were used to study the locomotor behaviour of paramecia. The test organism was under stress and exhibited an initial increase and subsequent decrease in the swimming speed when exposed to 1/4, 1/2, 3/4, and LC_{50} concentrations for 10 min (125, 250, 375, and 500 mg L^{-1}, respectively). Similar changes were also noticed when paramecia were exposed to LC_{50} for 2 h. In a separate set of experiments, the number of generations and generation time in 24 h was evaluated with respect to the different sub-lethal concentrations (30, 60, 120, and 240 mg L^{-1}). The number of generations decreased and generation time extended significantly in a concentration dependent manner. The results indicate that the Paramecium toxicity assay could be used as a complimentary system to rapidly elucidate the cytotoxic potential of insecticides. The major advantages associated with these tests are: they are inexpensive, simple, user-friendly, space saving, and seem to be attractive alternatives to conventional bioassays.

Keywords: *Paramecium caudatum*; Acephate; Cell lysis; Cytotoxic; Morphology; Behaviour; Generation number and time

1. Introduction

The extensive use of organophosphorus insecticides, during the past decades has led to a number of negative effects on terrestrial and aquatic organisms. Insecticides are being used in agriculture and they are found to be more hazardous than herbicides and fungicides. Acephate (*O*,*S*-dimethyl acetylphosphoramidothioate) a water soluble organophosphorus insecticide registered to control certain insect pests on a variety of field, fruit, and vegetable crops, in food handling establishments, on ornamental plants both in greenhouses and outdoors with residual systemic activity. Acephate and its primary metabolite, methamidophos, are toxic to various species. A number of studies were conducted on the toxicity of acephate on different organisms and indicated as a potent neurotoxicant [1]. It is also found to be mutagenic [2], carcinogenic [3], and cytotoxic [4]. Monitoring of aquatic ecosystem pollution represents one of the major activities involved in measures aimed at environmental protection.

Usage of non-targeted organisms in environmental toxicology is needed to understand the wide range of toxic effects caused by the pesticides on different organisms [5]. Fish and other aquatic biota that were commonly used as bioindicators of persistent organic pollutants [6] have been

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doi:10.1016/j.pestbp.2006.02.005

replaced in recent years successfully by ciliates [7]. Protozoan cells are often used as bioindicators of chemical pollution, especially in aqueous environment. Among protozoans, *Paramecium caudatum* is one of the ciliate models, most commonly used for laboratory research [8]. This unicellular ciliate facilitates the study of physiological processes and effects of pollutants on locomotory behaviour. It has been widely used to evaluate the toxic effects of several food dyes, carcinogens, synthetic chemicals, carbamate pesticides, and pollutants [7,9–11].

Mortality is obviously not the only endpoint to consider and there is a growing interest in the development of behavioural markers to assess the sub-lethal affects of toxicant. Behaviour is considered as a promising tool in ecotoxicology [12,13] and these studies are becoming prominent in toxicity assessments in unicellular organisms [14]. Locomotion has been found to be a consistently sensitive measure of toxic stress for a wide range of environmental contamination [15]. Diverse methods have already been developed and used to measure the locomotor activity of exposed organisms. Time-lapse video techniques have been successfully used to facilitate the documentation of behaviour of normal and stressed organisms [16]. With the recent development of computer-assisted electronics, video-camera tracking systems have been greatly improved (Ethovision, Noldus, The Netherlands) and used extensively in quantification of locomotor behaviour with a high degree of precision [17,18].

Further, multidisciplinary progress in research is very much needed to increase the significance and usefulness of behavioural markers for aquatic toxicology, and aim to highlight the specific areas for consideration. The application of unicellular organisms to study the toxic effects of pesticides from contaminated wastewater is relatively new throughout the world. The toxicity of acephate to primary consumers in aquatic food chain is generally uncharacterized [19]. *P. caudatum* test is more sensitive to investigate the direct toxicity of compounds [20,21]. Hence, in the present paper, we have studied the toxic impacts of acephate on *P. caudatum* with special emphasis on locomotor behaviour, proliferation rate, and morphological abnormalities.

2. Materials and methods

Stock cultures of *P. caudatum* were maintained in rice straw medium. Fresh cultures were initiated by seeding 100 ml of the rice straw medium with 1 ml of a stationary phase paramecium culture containing 1500–2000 organisms per ml. The cultures were maintained at room temperature (25 ± 2 °C); with a photo period of 14 h light and 10 h dark, pH 7.5–8.0 with loose fitting covers.

2.1. Determination of median lethal concentration (LC_{50})

The acute LC_{50} value of acephate was determined by static method. The technical grade of acephate was initially dissolved in 1 ml rice straw water and then diluted according to the test concentrations. The test concentrations were chosen based on the initial experiments to determine the lethal concentration (LC_{50}) for 10 min and 2 h.

The required concentrations of 460, 480, 500, 520, and 540 mg L^{-1} for 10 min, and 200, 250, 300, and 350 mg L^{-1} for 2 h exposure were maintained in 1 ml of rice straw water in 12-well microplates for definitive tests. Twenty numbers of active paramecia were released into each well and were exposed to selected concentrations with five replicates each. Simultaneously, control experiments were also performed without toxicant. The mortality record of the paramecia was maintained (10 min and 2 h of exposure) in each concentration of the toxicant and the mortality data was observed using inverted microscope (Nikon TMS) to estimate the median-lethal concentration (LC_{50}) using probit analysis [22]. Morphological abnormalities caused by different concentrations of acephate were observed on a glass slide and recorded as digital photographs using 'Easy Grab' software. The magnifications of snaps were calibrated with the aid of ocular and stage micrometers (ERMA, Tokyo, Japan).

2.2. Measurement of locomotor behavioural response of paramecium

In a separate set of experiments, locomotor behavioural response of paramecium was monitored by using a singlewell test apparatus. The apparatus is a combination of a microscopic slide and a silicon sheet (0.5 mm thickness) that possess centrally located well (5 mm diameter), where the glass slide served as the bottom of the apparatus [23]. Ten microliters of different stock concentrations were added individually to the test well containing 15 μl of rice straw water along with a healthy paramecium. The final concentrations of the toxicant were maintained individually to obtain 1/4, 1/2, 3/4, and LC_{50} concentrations for 10 min (125, 250, 375, and 500 mg L^{-1}, respectively) in the well. The test apparatus was placed under a compound microscope (Polyvar, Reichert-Jung light microscope) attached to a CCD camera (Sony CCD IRIS, Model No: SSC-M370CE) for continuous monitoring the locomotor behaviour of test organism for 6 min with five replicates each. Speed and distance travelled parameters of individual test organism were observed using the analysis module of Ethovision-2.3 package software (Noldus Information Technology, The Netherlands).

In addition, paramecia were exposed to LC_{50} concentration for 2 h (300 mg L^{-1}) in 100 ml of rice straw water. A paramecium with 25 μl of test solution was placed in the well to determine the altered locomotor behaviour (distance travelled in mm and velocity in mm s^{-1}) at regular intervals of 15 min. A minimum of five paramecia at each interval were used to evaluate their locomotor behaviour individually.

2.3. Population growth impairment and generation time determination

In a separate set of experiments, the culture was maintained in 12-well microplates for 24 h with sub-lethal