RESEARCH NOTE

Effect of Bayluscide WP 70® on the Kinetic Behaviour of *Biomphalaria straminea* in Laboratory Conditions

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Kinesis is defined as a phenomenon of general stimulation of organisms, caused by external agents, which may result in an increase or decrease in locomotion speed. Since the ability of responding to adverse stimuli of the environment simply by increasing the locomotion speed may result in moving away from unfavourable places, kinetic behaviour has special adaptive importance (GS Fraenkel & DL Gunn 1961 *The Orientation of Animals: Kineses, Taxes and Compass Reactions*, Dover Publications, New York, 376 pp.).

There is evidence suggesting the ocurrence of kinetic behaviour in the snail hosts of schistosomiasis. Thus, the snails tend to avoid areas of high light intensity by increasing their locomotory activity (VT Schall et al. 1985 *Mem Inst Oswaldo Cruz 80*: 101-111). They are also able to detect extremely low concentrations of copper sulfate and sodium pentachlorofenate, and avoid moving towards lethal doses of these molluscicides (FJ Etges 1963 *Am J Trop Med Hyg 12*: 701-704). However, little is known about the kinetic behaviour of snail hosts in response to niclosamide, the only molluscicide used in control programmes. This is probably because the snails are immobilised and killed at very low levels of this product (FJ Etges & DE Gilbertson 1966 *Am J Trop Med Hyg 15*: 618-624).

In the present work, a computerised video system was used to evaluate the effect of Bayluscide® (ethanolamine salt of niclosamide) on the speed and duration of locomotory activity of *B. straminea* snails originated from transmission sites in a highly endemic area of schistosomiasis in Northeast Brazil.

The batch of Bayluscide WP 70® used in the tests, as well as the preparation of the stock solution and dilution were described in a previous paper (O Sarquis et al. 1997 *Mem Inst Oswaldo Cruz 92*: 619-623). The chosen dose of 0.01 mg/l corresponds to 1/10 of the medium lethal dose (DL_{50}) obtained by those authors in toxicity tests with *B. straminea* snails.

The test snails were from laboratory colonies founded by *B. straminea* collected from transmission sites in São Lourenço da Mata, sugar-cane zone of Pernambuco, Brazil. The colonies were maintained in flow-through aquariums as previously described (RDA Dannemann & OS Pieri 1992 *Mem Inst Oswaldo Cruz 87*: 87-90). One year after the colonies were initiated, 80 snails with 5-6 mm in shell diameter were randomly selected and placed, in batches of 10 snails, in 1-l glass beakers containing 900 ml of dechlorinated filtered tap water and fresh lettuce. These snails were kept up to 24 hr for acclimatisation in environmental chambers (FANEM, model 346 CGD) set at 26 ± 1°C and 12 hr light, 12 hr dark.

The kinetic behaviour of the snails was monitored through VIDEOMEX-V, a system developed by Columbus Instruments, Ohio, USA, for recording and analysing the behaviour of small animals by video image. The equipment includes a digital-analogical microprocessor, a camera, a video monitor and a printer. This system has different programs for recording and quantifying several behavioural parameters, including locomotion speed and duration. The image is automatically recorded, analysed and digitalised. The digitalised image may be observed on the monitor during all the test, and printed at preset intervals together with the behavioural parameters.

The snails were tested in a chamber previously used for behavioural recording of snails (OS Pieri et al. 1980 *Mem Inst Oswaldo Cruz 75*: 57-63), adapted to have a video camera on the top. Light was provided by two fluorescent lamps of 15 W each, "daylight" type, located on the ceiling. A double sheet of greaseproof paper was placed beneath the lamps to diffuse the light. The temperature inside the chamber was maintained constant (26 ± 1°C). Of the 80 snails, 40 were chosen randomly for exposure to the 0.01-mg/l dose of niclosamide (experimental group) and the others were tested in dechlorinated filtered tap water (control group). Each snail was placed at the centre of a clear polystyrene culture dish (140 x 20 mm), filled with the liquid (250 ml). Since only four

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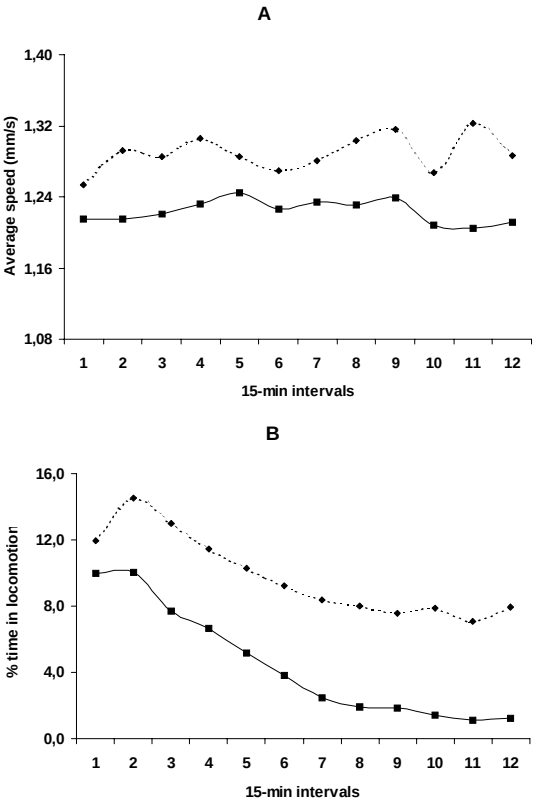
dishes could fit in the chamber, two snails from the experimental group and two from the control group were tested at a time. Test snails were monitored only during the first 3 hr of exposure to the moluscicide, as preliminary tests had showed that most snails of the experimental group would contract inside the shell or remain still after this time. After the 3-hr monitoring period, the snails were individually transferred, along with the liquid, to 250-ml glass containers. These containers were covered with a nylon mesh to prevent snails from escaping, and placed in the environmental chamber used for acclimatisation. The snails stayed up to 24 hr (exposure period) at 26 ± 1°C and 12 hr light, 12 hr dark. After this, those snails showing evident signs of decomposition were removed. The remaining snails were rinsed and placed individually in cleaned glass containers with dechlorinated filtered tap water and fresh lettuce, and kept for more 24 hr (recovery period). After the 48 hr of experiment, the dead and surviving snails were identified and counted.

Quantitative recordings were obtained through the program MODT (Multiple Object Distance Travelled) supplied with the VIDEOMEX-V. This program gave printed information on the distance travelled, time in locomotion, time inactive and average speed for each snail at 15-min intervals. The relevant parameters obtained from the experimental and control groups were compared statistically through the Wilcoxon test, matching the 12 pairs of data obtained from the 15-min intervals.

The Fig. shows the average speed and the percentage of time in locomotion of *B. straminea* during the first 3 hr of contact with the 0.01 mg/l dose of niclosamide (experimental group), in comparison with the control group. The average locomotion speed was significantly lower in the experimental group than in the control (T=0; N=12; $p<0.05$), varying from 1.20 mm/s to 1.24 mm/s in the former and from 1.25 mm/s to 1.32 mm/s in the latter. The percentage of time in locomotion was also significantly lower in the experimental group than in the control (T=0; N=12; $p<0.05$). For both groups this percentage decreased progressively during the 3-hr monitoring period, from 10.0% to 1.1% in the experimental group and from 14.4% to 7.0% in the control group. At the end of the monitoring period, 30% of the snails in the control group (12 out of 40) and 60% in the experimental group (24 out of 40) were inactive, either motionless or contracted inside the shell. From the total of 80 snails tested, only 11 (13.8%) died during the experiment, 4 in the control group and 7 in the experimental group.

These results show that even a sublethal dose of niclosamide is able to affect significantly the



Average locomotion speed (A) and percent time in locomotion (B) of *Biomphalaria straminea* at 15-min intervals in the first 3 hr of exposure to 0.01 mg/l of niclosamide (continuous line) compared with the control group (dotted line).

kinetic behaviour of *B. straminea*, reducing not only the locomotion speed but also the time spent in this activity. Since the snails were unable to escape from the moluscicide by moving faster and for a longer period, they remained in the area of moluscicide action, being continuously exposed to their adverse effects. It may be inferred that doses higher than 0.01 ppm, even if sublethal, would further reduce the locomotion activity of *B. straminea*, and increase their immobility or contraction inside the shell.

The inhibition, in this species, of kinetic behaviour in response to low doses of niclosamide in the environment confirms its superiority as molluscicide (F McCullough 1992 *Who/Schisto 107*: 1-34). It is interesting to note that *B. alexandrina* shows a severe reduction on the oxygen consumption and, consequently, on the locomotory activity, when exposed to 0.01 mg/l of Bayluscide WP 70® for 3 hr or more (MM Ishak & AM Mohamed 1975 *Hydrobiologia 47*: 499-512). However, the adverse effects of sublethal doses of niclosamide on the target snails are not permanent, tending to

decline as the action of the product ceases (P Andrews et al. 1983 *Pharmac ther 19*: 245-295). For this reason, application of niclosamide should last sufficiently long to guarantee a satisfactory toxic effect.

Inhibition of the kinetic behaviour of *B. straminea* in the presence of a dose of niclosamide 10 times lower than the medium lethal dose (DL_{50}) is of special relevance for the chemical control of this species. As lethal concentrations of a moluscicide may not reach all areas of the habitat, those products capable of reducing the kinetic behaviour of the snails will be of advantage over others that do not have this quality. Therefore, it is of interest that new promising molluscicides, such as *Euphorbia splendens* latex (VT Schall et al. 1998 *Am J Trop Med Hyg 58*: 7-10), should be comparatively tested as regards their behavioural effects on the host snails.

The automatic recording of host snail behaviour may be of help in behavioural assays, as protective modes of behaviour in response to different products, such as retraction into the shell and emigration from the water, may be easily quantified and accurately compared (Pieri et al. *loc. cit.*). Computerised video systems are particularly useful by allowing the digital processing of images and the automatic analysis of data through built-in programs. According to DC Miller et al. (1982 p. 206-220 in JG Pearson, RB Foster, WE Bishop (eds), *Aquatic Toxicology and Hazard Assessment: Fifth Conference, ASTM STP 766*, American Society for Testing and Materials), video recording followed by computerised analysis of the behaviour of aquatic animals in response to chemical agents has great applicability in toxicological studies, and should be incorporated to the routine of laboratory testing.