Table 1. Slide agglutination reactions in various titres of antigen (NPV) and antiserum

NPV (Polyhedra/ml)	Dilution of antiserum					
	1:10	1:100	1:1000	1.2000	1.5000	1.10,000
10^9	V/M	V/M	V/M	V/M	M	M[b]
10^8	V/M	V/M	V/M	V/M	V[a]/M	M[b]
10^7	M	M	M	M	M	M[b]
10^6	M	M	M	M[b]	—	—
$10–10^5$	—	—	—	—	—	—
PBS	—	—	—	—	—	—

Note: The dilutions were carried out in PBS.

[a] Detected with difficulty.
[b] Low agglutination.
V, Agglutination detected visually.
M, Agglutination detected microscopically
— No reaction.

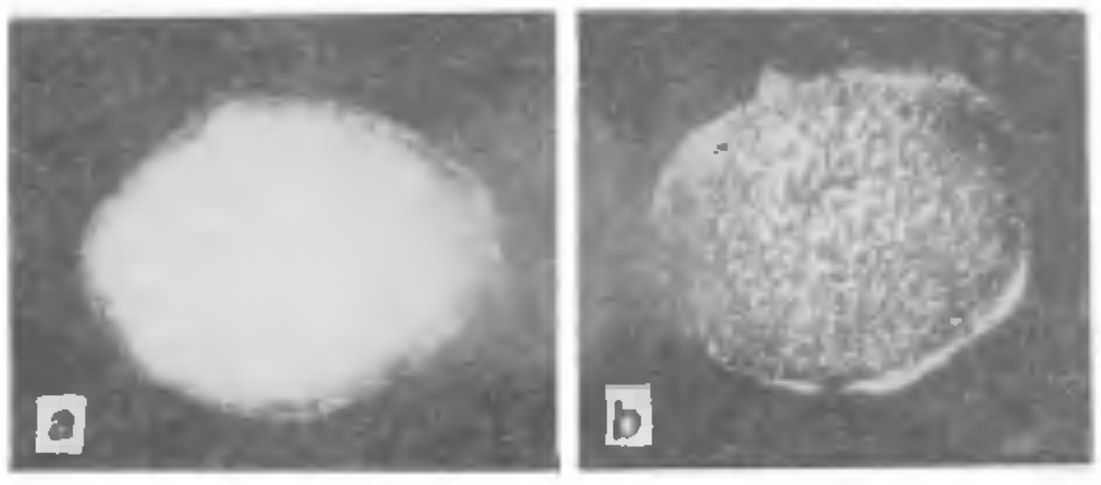

Figure 1. Direct visualization of agglutination reactions. *a*, NPV mixed with PBS solution (negative reaction, control); *b*, NPV mixed with antiserum (positive reaction).

accumulation of PIB in individual larva should be $2.757 \pm 0.092 \times 10^8$/g body weight or $2.946 \pm 0.122 \times 10^8$/ml body volume. The technique which is sensitive up to 10^8 polyhedra/ml enables the detection of infection in larva well ahead of the manifestation of disease symptoms. If the concentration of polyhedra in a single larva is lower, escaping detection, the infection can still be detected in the population from a sample of five to ten larvae.

An early and low level of infection in one lot, prior to manifestation of disease symptoms, could be detected using this simple method. This shows that the method is suitable for even the unskilled farmers.

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ACKNOWLEDGEMENT. We thank Dr K. Sengupta, Director, CSR&TI, Berhampore, for providing necessary guidance and help during this investigation.

Received 17 December 1992, revised accepted 31 May 1993

Effect of Endosulfan on the integrated density of the erythrocytes of *Channa punctatus* (Bloch)

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Amongst the various organochlorine pesticides used to augment agricultural production in India, Endosulfan is a commonly used pesticide and its toxicity is primarily due to its sulphur content. This paper reports that the integrated density (area in $\mu m^2 \times$density) of nucleus, cytoplasm and cell membrane of the erythrocytes of *Channa punctatus* decreased when exposed to sublethal concentrations of Endosulfan for different durations.

HAEMATOLOGY plays an important role in diagnosing the diseases in fishes and assessing the toxic effects of pollution on them. A normogram to determine different haematological parameters is needed to be developed for different species of fishes on the same pattern as that of human beings[1,2]. Fishes being poikilothermic aquatic vertebrates exhibit changes in their physiology at different stages in their life history, hence complete formation of normogram is very difficult. To understand the deviations from the normal condition, the maximum number of haematological parameters is required to be studied.

The most commonly studied haematological parameters are erythrocyte counts, haemoglobin concentration, haematocrit value, erythrocyte sedimentation rate and blood pH[3,4]. In the present study a freshwater fish *C. punctatus* was exposed to different concentrations of an organochlorine pesticide, Endosulfan, and the effects on the integrated density of nucleus, cytoplasm and cell membrane of erythrocytes were recorded.

Adults of *C. punctatus* were procured from a local pond and transported to the laboratory. The fish were acclimatized to laboratory conditions for 7 days at 25 ± 3°C (SD = 1.81). They were fed with zooplanktons once a day. The LC_{50} value (96 h)[5] of Endosulfan for *C. punctatus* was found to be 0.0070 mg/l (technical grade, i.e. 35% w/w of Thiodan 35 EC).

Endosulfan stock solution of 1 g/l of water was prepared. Aliquots of this stock solution were added to each experimental tank to bring the Endosulfan concentration to 0.0022 mg/l and 0.0035 mg/l (sublethal concentrations), representing 32% and 50% of the 96 h LC_{50}, respectively. A parallel control group was maintained in toxicant-free tap water (pH 6.8; total hardness 86 mg/l and dissolved oxygen 8.91 mg/l). A batch of ten fishes was released in each tank.

After 5 and 15 days of exposure, fish were bathed in clean water and blood smears (2 slides per individual) were prepared and fixed in methanol and stained

Table 1. Alteration in the integrated density of cell membrane, cytoplasm and nucleus in erythrocytes of control *Channa punctatus* (Bloch) and those exposed to 0.0022 mg/l and 0 0035 mg/l of Endosulfan

		Cell membrane	Cytoplasm	Nucleus
Concentration	Exposure time	Mean, SD, S E M.	Mean, SD, S.E M.	Mean, SD, S E M
Control	0 day	1.120, 0 086, 0 027	1 309, 0 141, 0.044	1 520, 0 143, 0 045
Control	5 days	0.880, 0 107, 0 033	0 880, 0 097, 0 030	1.090, 0 310, 0 098
0 0022 mg/l	5 days	0 980, 0 146, 0 046	1 090, 0 053, 0 016	1.320, 0.105, 0.033
0 0035 mg l	5 days	0 920, 0 120, 0 037	1 090, 0 085, 0 027	1 270, 0.060, 0.019
Control	15 days	0 890, 0.078, 0 024	1 012, 0.119, 0.037	1 140, 0 139, 0.044
0 0022 mg l	15 days	0 870, 0 080, 0.025	0.930, 0 078, 0.024	0.950, 0.058, 0.018
0.0035 mg l	15 days	0.770, 0 140, 0 044	0 850, 0.154, 0 048	0 920, 0.064, 0 020

SD = standard deviation; S E M. = standard error of mean.

uniformly with Giemsa's stain for 15 min. Erythrocytes were studied using Vickers scanning microdensitometer[6].

The integrated density of the cell membrane, cytoplasm and nucleus was determined with a spot size of 0.5 μm at a wavelength of 540 nm. The results (Table 1) revealed that the integrated density of these three regions of erythrocytes decreased with increase in Endosulfan concentration and exposure period, compared to the control fish.

To determine significant differences of the integrated density of different cell regions, Student's t test[7] was applied. It has been estimated that there was significant difference between cytoplasm and nucleus ($t^{12}_{005} = 3.95$), and cell membrane and nucleus ($t^{12}_{005} = 4\ 99$), whereas no significant difference was observed between cell membrane and cytoplasm ($t^{12}_{005} = 1.44$). This indicates that nuclear material gets affected more easily by Endosulfan than cell membrane and cytoplasm. The reduction in the nuclear density on exposure to Endosulfan indicates that nuclear material, i.e. DNA, RNA and nucleoproteins, is decreased per unit volume. This may be due to the disruption of DNA replication and transcription, leading further to decreased protein synthesis.

Visual changes like chromatin condensation, vacuolation and mild swelling of erythrocytes have been reported when *Puntius conchonius* was exposed to different concentrations of cadmium and chromium[8,9]. But in *C. punctatus* because of the hardy nature of the fish and the presence of air-breathing organs, such changes were not observed under light microscope after exposure to Endosulfan. Hence, the technique of determination of integrated density of erythrocytes was applied and significant alterations observed.

This technique can be successfully applied for determining the effects of different toxicants on fishes.

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ACKNOWLEDGEMENT. We thank the University Grants Commission, New Delhi, for financial assistance.

Received 5 March 1993, revised accepted 7 May 1993