Bull. Environ. Contam. Toxicol. (1991) 47:628–633

Bulletin of Environmental Contamination and Toxicology

Hemopathological Changes Associated with Experimental Aldicarb Poisoning in Fish (*Puntius conchonius* Hamilton)

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Aldicarb, 2-methyl-2(methylthio) propionaldehyde-O-(methylcarbamoyl)-oxime, is an oxime carbamate insecticide used against a variety of insects and nematodes on crops such as cotton, sugar beets, sugarcane, citrus fruits, potatoes, sweet potatoes, and peanuts. Its half-life is 1 to 2 wk under laboratory conditions and could be shorter or longer in the soil depending upon field conditions. The acute toxicity of aldicarb, caused by rapid, reversible inhibition of acetylcholinesterase, is probably the highest among the commonly used insecticides (NAS 1977). Detection of aldicarb in ground water resources in Wisconsin and 15 other states nationwide (USEPA 1988) has prompted serious concern over the toxic effects of this carbamate in humans and animals (Olson et al. 1987; Risher et al. 1987; Thomas et al. 1987; Mirkin et al. 1990).

The immunomodulating effects of aldicarb have been examined in humans exposed to this carbamate at levels ranging from 1 to 61 μg/L in drinking water (Fiore et al. 1986). These authors found an association between aldicarb ingestion through drinking water and a significant increase in the IgG and the number of a particular subset of T lymphocytes, the CD8+ cells. Since B cells are the source of immunoglobulins (Terhorst and David 1987) and T cells are responsible for cell-mediated immune responses, alterations in the lymphocyte distribution due to aldicarb exposure may have clinical significance both in humans and experimental animals. The current study was undertaken to evaluate the adverse effects of aldicarb on a non-target organism, the rosy barb, *Puntius conchonius*, a teleost found in most countries. Circulating populations of leucocytes (small and large lymphocytes, neutrophils, monocytes, basophils, and thrombocytes) and erythrocytes and hemoglobin content were chosen as indicative parameters of experimental aldicarb poisoning over a 4 wk exposure period at sublethal concentration. In addition, the aldicarb-exposed fish were allowed recovery for a week in clean water to see if the blood changes were reversible.

MATERIALS AND METHODS

All the fish (rosy barb, *Puntius conchonius* Hamilton) used in this experiment were collected from Naini Tal Lake, India (altitude 1938 m). Before aldicarb exposure began, the fish were acclimatized to laboratory conditions in glass aquaria (50 L) filled with well aerated tap water (pH 7.3, dissolved oxygen 7.9 mg/L, EDTA hardness 398 mg/L, and temperature 17°C). During this acclimatization period, the fish were fed commercial fish food ad libitum and received natural photoperiod (13L/11D). The mean body weight was 5 g (4.5 to 5.5 g). Fish were randomly distributed into two 50-L capacity glass aquaria (n=40 individuals each). Group I was exposed to aldicarb (Temik granules, 99% purity,

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Union Carbide India Ltd., New Delhi) at an arbitrarily chosen concentration of 0.806 mg/L (1/3rd of the 96-hr median tolerance limit, TLm, Pant 1982) for 4 wk. Group II served as controls. For recovery tests, a third group of 10 fish was exposed to 0.806 mg/L aldicarb for 4 wk and thereafter moved to clean water (without added aldicarb) and allowed to recuperate for 1 wk. The medium in control and experimental tanks was continuously aerated and renewed every week. Fish were fed once a day to satiation.

Blood samples were collected immediately at sacrifice by decapitation at the end of 1, 2, 3, and 4 wk, and after recovery, from 6-8 individuals each from the control and experimental groups. Total erythrocyte counts were made on a Spencer's brightline hematocytometer in blood diluted 1:200, and the hemoglobin determined according to Sahli-Helliege method (Hesser 1960). Blood smears were air dried, fixed in methanol, and stained with 0.2% Leishman's stain. Different categories of leucocytes (small and large lymphocytes, neutrophils, basophils, monocytes, and thrombocytes) were scored on blood smears, 2 slides per animal, and averaged (n=6-8). Control and experimental values were compared by Student's t test for significance of differences at 5, 1, and 0.1% levels.

RESULTS AND DISCUSSION

Aldicarb at sublethal concentration caused statistically significant ($p<0.05$) polycythemia together with an increase in the hemoglobin content (Fig. 1). The fish previously exposed to aldicarb for 4 wk, when allowed recovery in clean water for 1 wk, showed reversal of effects noted during carbamate exposure. Insecticidal exposure influences blood parameters in fishes and the findings are so diverse that no consistent pattern can be drawn with regard to the clinical manifestations and the kind of chemical used. Another carbamate, sevin, and an OP, sumithion, lowered RBC counts, hemoglobin, and hematocrit in *Tilapia mossambica* (Koundiya and Ramamurthi 1979). Organochlorine (OC) insecticidal poisoning, on the other hand, induced an increase in erythrocytic parameters in *Labeo rohita* (Bansal et al. 1979). An erythropenia, leucopenia and decrease in hematocrit were described in the golden shiner, *Notemigonus crysoleucas*, exposed to parathion (Butler et al. 1969).

Physiologically, the erythrocytic responses observed in the rosy barb seem to be secondary to a typical stress situation caused by the presence of aldicarb in the medium. Possibly, the fish have respiratory difficulty when they confront a toxic environment and try to compensate for the reduced oxygen uptake at the gill surface by increasing the level of blood constituents concerned with oxygen uptake and delivery. However, a prolonged exposure to aldicarb exhausts the hematopoietic potential as revealed by the lowered RBC counts and hemoglobin noted in the fish previously exposed to aldicarb and allowed recovery for a week.

Enumeration of leucocyte subpopulations (lymphocytes, neutrophils, monocytes, basophils, and thrombocytes) is routinely used to assess physiological responses in fishes exposed to a variety of environmental situations, including stress due to chemical pollutants (Niimi and Lowe-Jinde 1984). In the present study, protracted exposure to sublethal concentration of aldicarb resulted in pronounced lymphocytosis caused by a statistically significant increase in large lymphocyte population ($p<0.01$ after1 wk, $p<0.001$ after 2, 3, and 4 wk) (Fig. 2). The small lymphocyte counts were also found to be raised with the peak occurring after 2 wk exposure ($p<0.01$). After recovery for a week, however, the lymphocyte counts returned to the baseline values. Changes in the lymphocyte counts observed in the rosy barb, *Puntius conchonius*, suggest that aldicarb-induced lymphocytosis in this fish is not due to transformation of small lymphocytes into large ones. Lymphocyte transformation has been described in mice and rats poisoned with cadmium chloride and cadmium oxide

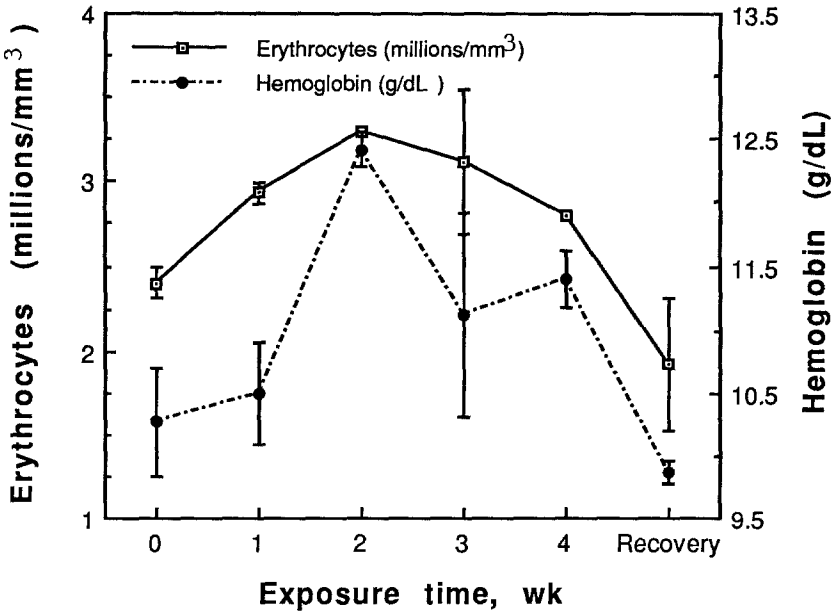

Figure 1. Changes in erythrocytes and hemoglobin in Puntnius conchonius chronically exposed to 0.806 mg/L aldicarb. Mean±SE (verticals bars) (n=6-8).

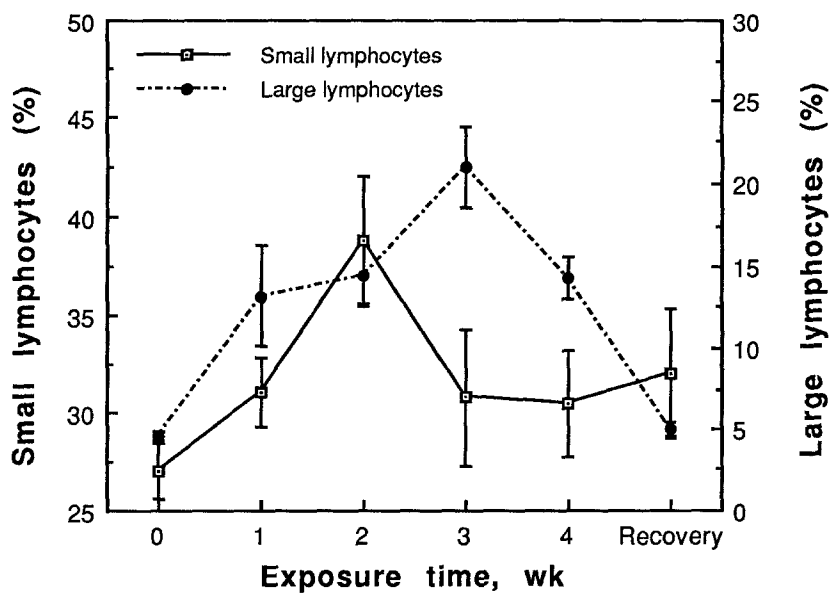

Figure 2. Changes in small and large lymphocytes in Puntius conchonius chronically exposed to 0.806 mg/L aldicarb. Mean±SE (vertical bars) (n=6-8).

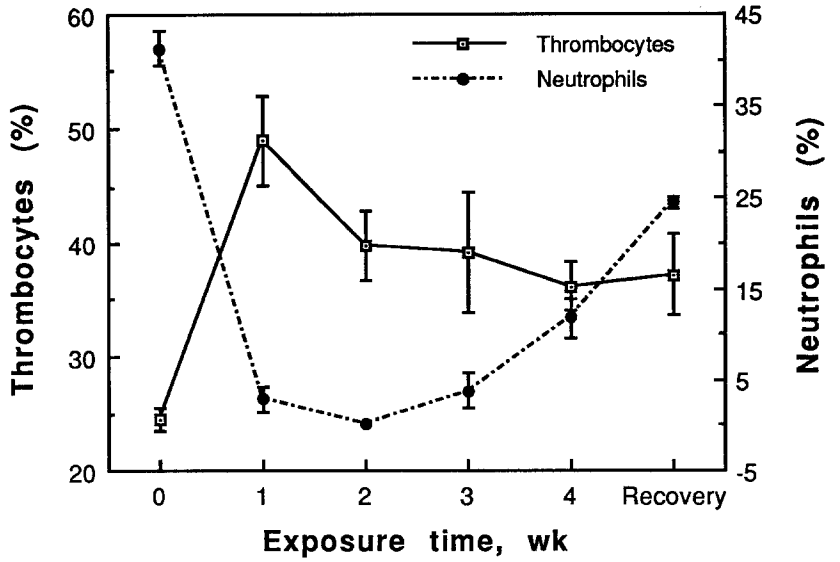

Figure 3. Changes in thrombocytes and neutrophils in Puntius conchonius chronically exposed to 0.806 mg/L aldicarb. Mean±SE (vertical bars) (n=6-8).

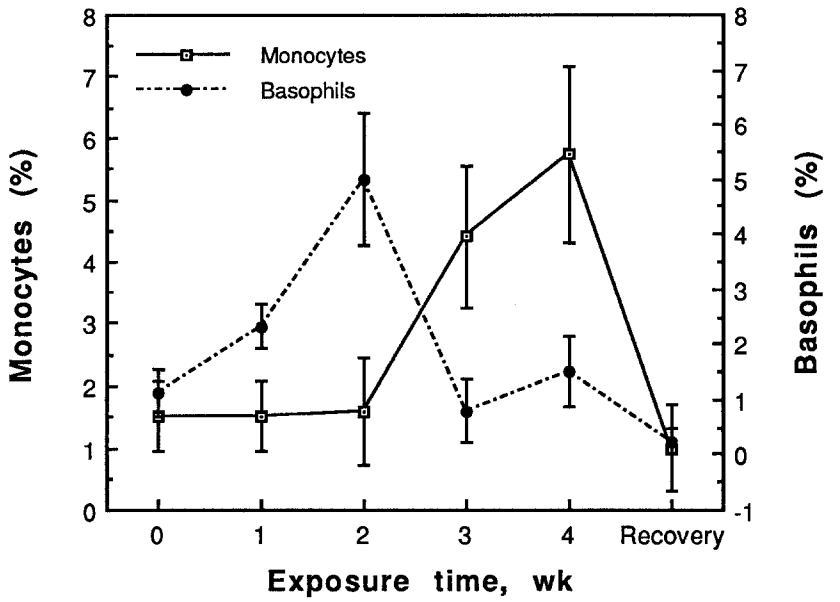

Figure 4. Changes in monocytes and basophils in Puntius conchonius chronically exposed to 0.806 mg/L aldicarb. Mean±SE (vertical bars) (n=6-8).

(Ohsawa and Kawai 1981). Since the small lymphocyte counts were also found to be raised in our study, a cytological shift can be ruled out. Perhaps, the exposed fish endeavor to counter the aldicarb poisoning by an accelerated production of lymphocytes and/or the carbamate triggers the release of already differentiated lymphocytes from hematopoietic loci into circulating blood. However, pesticide exposure is also known to cause lymphopenia, e.g., in *Cyprinus carpio* (Kawatsu 1977) and *Clarias batrachus* (Dalela et al. 1980).

Chronic aldicarb poisoning also elicited marked thrombocytosis ($p<0.001$ after 1, 2 and 4 wk, $p<0.01$ after 3 wk) which persisted even after recovery for a week in clean water (Fig. 3). An increase in thrombocyte numbers has been described in *Clarias batrachus* and *Cirrhina mrigala* exposed for 15 and 30 days to 1/2 , 1/4, and 1/8 fractions of the 96-hr LC50 of aldrin (Dalela et al. 1980). Lone and Javaid (1976) and Srivastava and Mishra (1983), however, found thrombocytopenia in different piscine species exposed to OP pesticides, and related the finding to capillary hemorrhage and/or insufficient thrombopoiesis.

Other leucocytic responses in the rosy barb included a consistently highly significant neutropenia ($p<0.001$ after 1, 2, 3, and 4 wk) (Fig. 3), in addition to a marked monocytosis ($p<0.05$ after 3 wk, $p<0.01$ after 4 wk) and basophilia ($p<0.01$ after 2 wk) (Fig. 4). At the end of recovery period, the percentage of these cell types was found to lie within the normal range. Metelyev and Grischenko (1970) showed an increase in monocytes and neutrophils of the fish treated with dimethoate. Although most reports describe neutrophilia in response to pesticide exposure (Kawatsu 1977; Mukhopadhyay and Dehadrai 1980), in some species at least, e.g., *Clarias batrachus* and *Cirrhina mrigala* (Dalela et al. 1980), neutropenia was found to manifest the exposed fish, resembling the situation in the rosy barb. A sustained stimulation of the granulopoietic precursors to meet increased demand for circulating neutrophils during aldicarb intoxication possibly leads to exhaustion and eventually a reduction in the circulating numbers of the cells in question. The monocytes which act as phagocytes to salvage debris from necrosed tissues, appear to constitute the phagocyte system in the fish as well (Ellis et al. 1978). Monocytosis observed in the present study could reasonably be associated with an increased phagocytic activity in order to remove debris from lesioned tissues, for pesticide poisoning in this species is known to cause tissue necrosis (Gill et al. 1988). Overall, the afflicted fish would suffer from immunosuppression causing decreased disease and parasite resistance, inability to secure and assimilate food for basal energy requirements, lack of physical strength to escape predators, and physiological impairments affecting vital processes.

Acknowledgments. This work was supported by funds received from the University Grants Commission, New Delhi, and the Council of Scientific and Industrial Research, New Delhi. We appreciate the excellent technical skills of Mrs.Manjit Gill and Mr.Rajendra Singh in preparation of this manuscript.

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Received July 27, 1990; accepted October 29, 1990.