Egypt. J. Aquat. Biol. & Fish., Vol. 17, No. 1: 13-19 (2013) ISSN 1110 –1131

Effect of certain pesticides on the fragility of erythrocyte stabilization and lysis (In Vitro).

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ABSTRACT

The effect of four pesticides on erythrocyte membrane was investigated herein. This was attempted by studying their lytic or antilytic effects on erythrocytes (Red Blood Cells) of human and fish blood in critical hypotonic saline media. The insecticides used are representatives of four major groups; organochlorines (Lindane); Pyrethroids (Decamethrin); Carbamates (methomyl) and Organophosphorus (Malathion). These insecticides were tested at concentration range of (10^{-12}–10^{-4} M).

The first two compounds exert antilytic effect on both types of erythrocyte (RBCs) by preventing disruption of the membrane and increased its integrity in hypotonic solution. The order of effect was Decamethrin > Lindane. On the other hand, cell lysis was observed with the other two compounds with the order of effect, Malathion > methomyl. The latter effect was attributed to the disruption of the cell membrane by such insecticides.

Furthermore, the antilytic as well as lytic effects depend to large extent on the molar concentration of the insecticide.

Keywords: Lindane, decamethrin, methemyl and malathion.

INTRODUCTION

Pesticides occupy rather unique position among many chemicals that man encounters daily, in that they are deliberately added to the environment for the purpose of pest control in all agricultural programs. In fact, most of such chemicals are not highly selective and generally toxic to many non-target species including man and other animals (Murphy, 1975).

The most important widespread used insecticides, are four classes: Organochlorines, carbamates, organophosphates and pyrethroids (Doull *et al*., 1985).

Most insecticides have low water solubility and thus are incorporated in lipid-rich cellular structures. Therefore, biomembranes are good candidates as targets of insecticides action. Blood may serve as carrier of insecticides. The lipid moiety of erythrocyte membrane may be also considered as a site of interaction. Moreover, Red Blood Cells (RBCs) are excellent as biomembrane model for the study of the interaction of drugs and other compounds which are biologically active. In their studies, Antunes and Madeira (1979) showed that most pesticides increase the permeability of artificial lipid membrane to non-electrolytes and ionophore complexes.

In another study by the same authors they indicated that these effects may be mediated by disordering action on the bilayer membrane (Antunes-Madeira *et al*., 1980).

The mode of action of the carbamates, like organophosphates and pyrethroids is inhibition of acetylcholinesterase (AchE) (O'Brien, 1967; Abdel Raheem *et al*., 1988 and El-Elaimy *et al*., 1994)-and the signs of symptoms of poisoning are typically cholinergic with lacrimation, severe convulsions and death (Hutson and Roberts, 1985).

As a class, the organochlorine insecticides are also considered to be less acutely toxic, but of greater potentiality for chronic toxicity than organophosphates and carbamate insecticides. However, their mechanism of action is not the same as that of the organophosphates and still unknown for most of them (Hayes, 1971).

The neurotoxicity due to organochlorines is initiatied by altering specific properties of the axon membrane responsible or delayed repolarization of the action potential (Narahashi and Haas, 1968 and Brooks, 1974). Pyrethroids, however, induced similar neurotoxicity by affecting sodium channel gating kinetics (Hutson and Roberts, 1985).

In the present study, four insecticides (representing the four major insecticide groups) known to be neurotoxic to both mammalian and fish species (Hutson and Roberts, 1985), were used. The osmotic fragility of erythrocytes (for man and fish) was used as model for studying the effects of these pesticides on the barrier properties of biomembrane.

MATERIAL AND METHODS

a- Preparation of Biological Materials:

Fresh blood samples were taken from a human volunteer (28 years old, healthy male) and from live adult male fish (*Oblada melanura* (Kahla). To these samples sodium heparin (1000 U/ml) was added so that the final concentration was 1 unit to 1 ml blood.

Aliquots of 1 ml of the heparinized blood were then centrifuged at 1500 g for 5 min. The plasma and buffy coat were removed by suction. To each portion, 12.5 ml of sodium chloride solution (154 mM NaCl in 10 mM phosphate buffer, pH 7) was added.

The erythrocytes were resuspended by cooling and stored at 4^oC then the samples were analyzed within 24 hours

2- Construction of normal hemolysis curves:

To determine the concentration of sodium chloride necessary to produce 50% hemolysis for human and fishes blood, fractional concentration of 154 mM sodium chloride in buffer were made.

The erythrocyte suspension (0.1 ml) was added to 1.5 ml of each concentration and samples were mixed several times briefly and allowed to stand at room temperature (28 ± 1^oC) for 10 min. The suspension was then centrifuged at 1500 g for 1 min. The optical density of the clear supernatant was read at 540 nm. in spectrophotometric device (Bousch and Lomb). The point at which 50% hemolysis was read from hemolysis curve (Fig. 1).

The concentration of NaCl in phosphate buffer produced 50% hemolysis were 90 mM (human blood) and 100 mM (Fish blood).

Stock solution of the two concentrations were prepared and used throughout experimentation.

3- Estimation of hemolytic effect of pesticides:

The effects of the four pesticides on erythrocytes were studied using modified method described by Seeman and Weinstein 1966).

Different pesticide concentration ($10^{-12} – 10^{-2}$ M) were dissolved in acetone, portion of 10-100 ul of each compound was added to 1.5 ml of the stock solution to which 0.1 ml of erythrocyte suspension was added.

The above procedure for obtaining the hemolysis curve was followed.
Blanks with corresponding amounts of acetone were run currently.

4- Pesticides used:

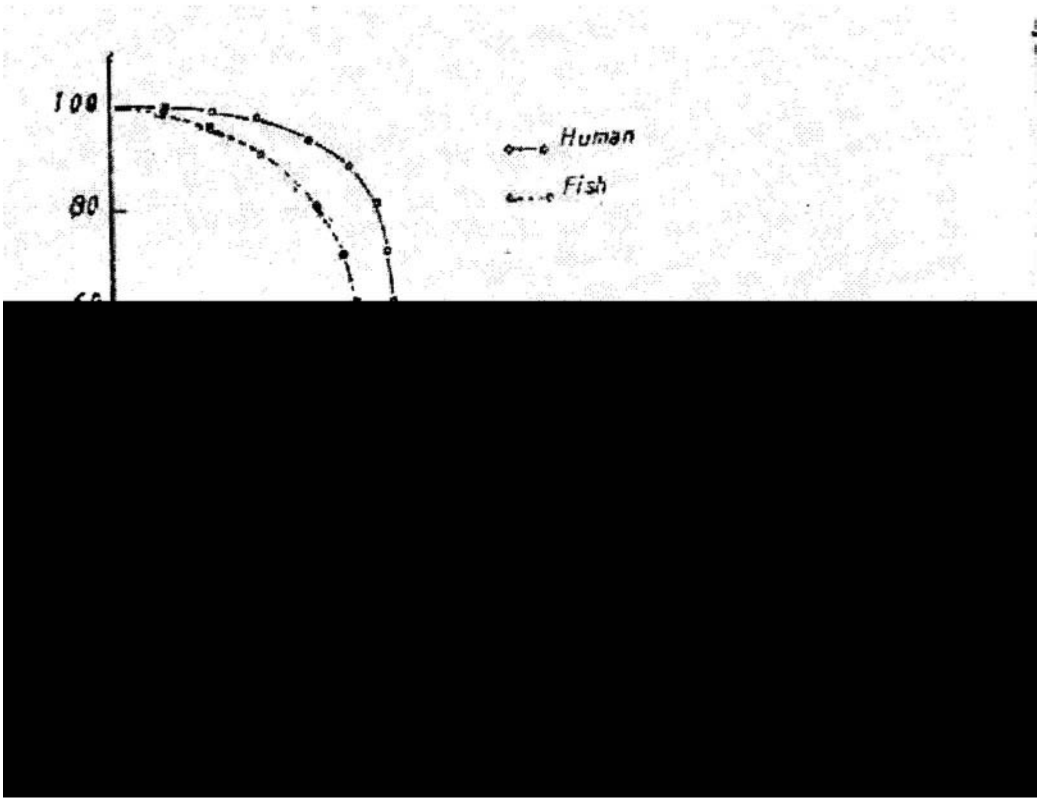
Four pesticides representing the main insecticide groups were tested, these are malathion, methomyl, lindane and decamethrin. They were obtained from Ministry of Agriculture, Tripoly-Libya.

RESULTS

The present study was attempted to study the erythrocyte hemolysis under the influence of different insectidies. Therefore, it was necessary to construct the hemolysis curves of both human and fish blood.

Hemolysis curves (Fig. I) show the variation of erythroytic lysis with different concentrations of sodium chloride. This curve was prepared for each type of blood, i.e., for human volunteer as well as blood from a marine fish *(Oblada melanura* (Kahla). Using such curves it was easy to deduce the concentration of sodium chloride that produces 50% hemolysis for each type of blood.

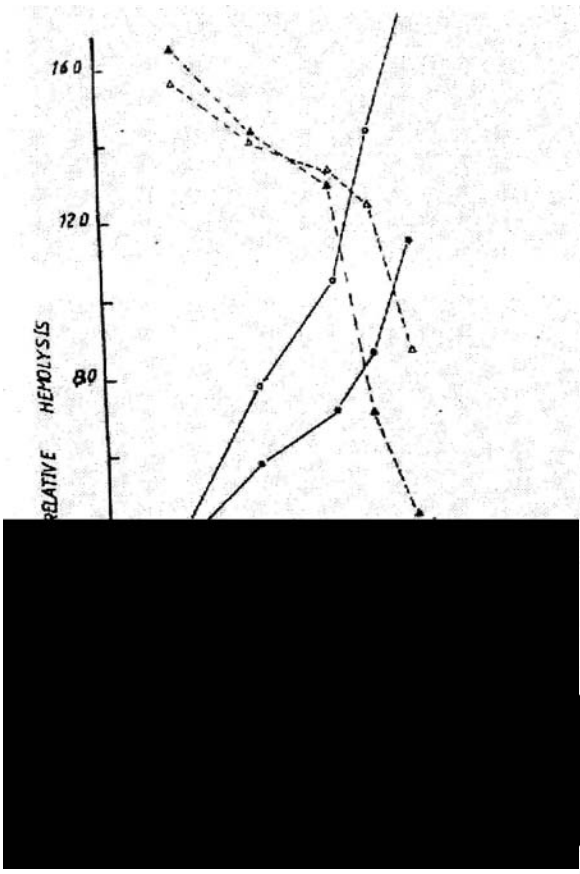
The values obtained were 80 mM and 95mM NaCl on both fish and human blood respectively.



These concentrations were then employed with various concentrations of the four pesticides used where the relative hemolysis was calculated. The data recorded expressed as relative hemolysis from controls (NaCl produce 50% hemolysis).

The pesticides, malathion, methomyl, lindane and decamethrin were tested at concentration ranges from (10^{-12}–10^{-4} M). Evidently, the constructed concentration hemolysis curves (Figs. 2,3) indicated that, at lower concentration 10^{-12} M, the relative hemolysis shows the highest values with Lindane and Decamethrin. The percentage values were 114%; 115% (Fish blood); 156%; 164% (human blood) for the two compounds respectively.

These values were sharply declined as the concentrations of the pesticides increase, indicating protection against erythrocytes fragility at higher concentration of both lindane and decamethrin. On the other hand, the other two pesticide, malathion and methamyl increased lysis of erythrocytes in both types of blood. The percentage increases in relative hemolysis (Figs. 2, 3) depended on the concentration of the pesticides. The highest values recorded at 10^{-4}m M were 95, 98% (fish blood) and 164%; 115% (human blood) for Malathion and Methamyl respectively.



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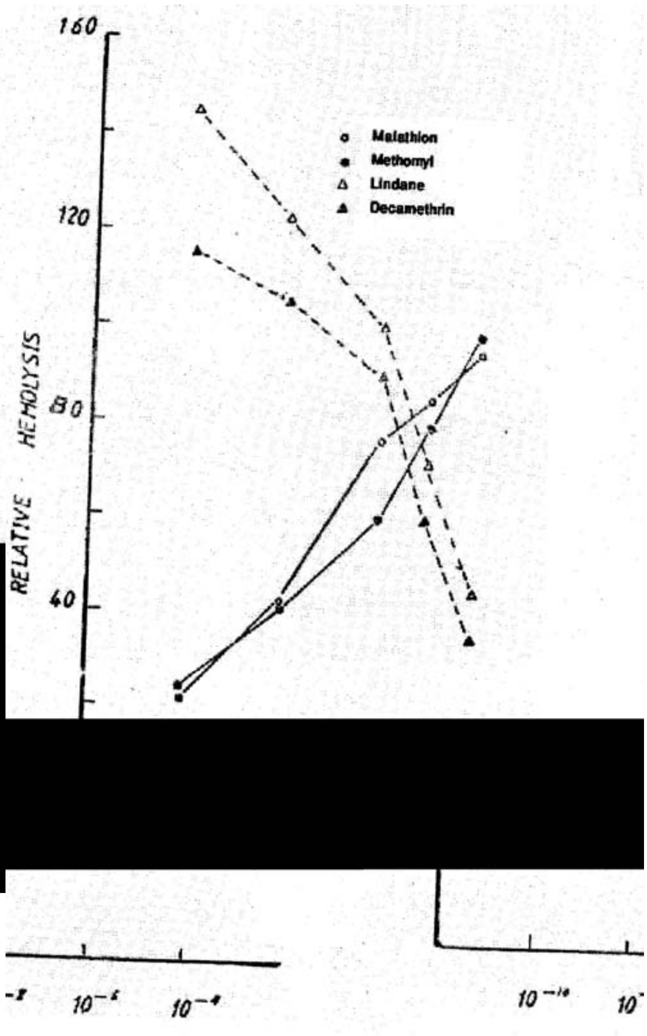

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DISCUSSION

It is clear from the present results that the four pesticides studied, protect red cells against osmotic hemolysis. The effectiveness of their protection follows the Order Decamathrin > Lindane. The potency of these compounds was much higher with increasing concentrations.

Essentially, these observations run in harmony with those previously described for DDT and its analogs (O'Brien and Hilton, 1978); Aldrin; DDT and other pesticides (Antunes-Madeira *et al*., 1981). The antihemolytic behaviour of the above pesticides was somewhat surprising, since these compounds are expected to increase the permeability of lipid-ionophore complexes (Antunes and Madeira, 1979).

Organochlorine, like DDT appears to associate preferentially with lipid rich biological structures (Murakami and Fukami, 1979) as a consequence of the very high partition coefficients of the compounds in a polar phase (O'Brien and Hilton, 1978) and (Murakami and Fukami, 1979). Therefore, it was suggested that these pesticides combine preferentially with the lipid moiety of the membrane leading to separation of protein and lipid-rich regions. Clustering of protein particles would favour their mutual interactions. Moreover, withdrawal of lipids from their interactions with proteins would produce extended regions of bilayer (Antunes nad Madeira, 1981).

Another hypothesis that antihemolytic pesticides may potentiate the binding capacity of divalent cations was reported to have condensing effects on membranes, reducing physical disorder (Champman *et al*., 1974 and Van Dijck, 1975).

The forementioned hypothesis is further supported by finding of other investigators that Ca^{++} and other divalent cations acting in the intercellular spaces, induce alterations and shrinkage of red cells (Johnson and Robinson, 1976). Moreover, addition of divalent cations during hypotonic hemolysis prevents extensive disruption of erythrocyte membrane (Imre *et al*., 1978).

The ability of pesticides to potentiate the binding capacity to cell membrane of divalent cations remained to be elucidated and need more investigation.

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ARABIC SUMMARY

تأثير مبيدات حشرية معينة على هشاشة أغلفة كريات الدم الحمراء للانسان وسمكة الكحلة

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تم خلال هذه الدراسة دراسة تأثير أربعة أنواع من المبيدات الحشرية العضوية كثيرة الاستخدام في الأغراض الزراعية في ليبيا على غشاء كرات الدم الحمراء في كلا دم الإنسان ودم أسماك الكحلة المنتشرة في البحر المتوسط والساحل الليبي. وتم ملاحظة التأثيرات المدمرة والتأثير الوقائي على عينات دم الإنسان وكذلك دم الأسماك محل الدراسة.

استخدمت لذلك مجموعة تمثل المجموعات الأربعة الرئيسية من المبيدات الحشرية الفسفورية العضوية (الملاثيون) والمبيدات العضوية الكلورينية (الليندين) والكرباميت (ميثوميل) والبيروثريدات (الديكامثيرين) وذلك بتركيزات تصل من 10^{-12} – 10^{-4} مول جزئ.

وقد لوحظ من النتائج المتحصل عليها الآتي:

١. إن مبيد الليندين أظهر تأثير مضاد لتكسير كرات الدم الحمراء في كلا عينات دم الرجال (سن ٢٨ سنة) وعينات دم سمكة الكحلة المستخدمين في التجربة، وكذلك مبيد (الديكامثرين) أظهر نفس النتائج من كلا عينات دم الإنسان والأسماك. وذلك بسبب زيادة صلابة غشاء كرات الدم الحمراء على تحمل التركيزات المنخفضة من المحاليل الفسيولوجية والمبيدات.
وكان تأثير مبيد الديكاميثرين أعلى من مبيد الليندين.

٢. وقد أظهر المبيدان الآخران تأثير معاكسا وكان تأثير الملاثيون أعلى من تأثير مبيد الميثوميل وذلك بسبب تحلل الغشاء الخلوى للكرات الدموية الحمراء تحت تأثير هذين المبيدين.

ولوحظ من الدراسة أن التأثيرات على غشاء الخلية الدموية الحمراء تعتمد على التركيزات الجزئية لكل من المبيدات الأربعة محل الدراسة. وقد نوقشت آلية عمل هذه المبيدات في إحداث التأثيرات الخاصة بكل منها.