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# Toxic Effects of Malathion and Endosulfan on Chick Embryo

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## ABSTRACT

**Toxic effects of malathion and endosulfan to fertile chicken eggs were determined. Injection of 1.25, 2.5, 5.0 and 10.0 mg/egg of malathion into yolk sac of fertile eggs prior to incubation caused mortality of 10.0, 30.0, 83.33 and 93.33% respectively. Similar trend was observed with injection of endosulfan doses. Malathion and endosulfan at 1.25 mg/egg caused no pronounced $LD_{50}$ increase in mortality as compared to control groups. The $LD_{50}$ values for these insecticides proved malathion to be more toxic than endosulfan. The hypothesis that the dose response of viable embryo fitted a linear regression line was statistically acceptable. Combination of malathion and endosulfan increased the mortality rate as compared with either one alone. With either compound, an increased dose generally resulted in a decrease in embryonic body weight.**


**Keywords:** Fertile chicken egg; Insecticides; Lethal effect.

## INTRODUCTION

Several insecticides are proven to be toxic in experimental animals [6, 7, 8, 20]. At the same time it is well known that insecticides for which suspicion of teratogenic, carcinogenic and/or mutagenic effects in man exist, are still in use [1, 14, 18]. Among insecticides used in Agriculture, malathion and endosulfan have been used in large scale for many years [2, 4].

In determining lethal effects, test material, time, route of administration, and dose level are of vital considerations [5, 17]. The prime requisite for any toxicological study is the availability of large numbers of uniform good quality and low cost test materials. The importance of uniform test materials as components of a reliable and sound bioassay system has been fully appreciated [5, 17]. Among the test materials, avian embryo particularly fertile chicken eggs posses a number of characteristics suited to toxicological studies. For example, chick embryo test for detecting lethal and teratogenic effects through injection of the toxicants into the yolk sac is an easy, reliable, rapid and inexpensive procedure that has been utilised [10, 12, 14, 15, 20, 22]. Despite the fact that several factors can affect the results, the chick embryo test allows for studies on toxicity and morphological disturbances during development [6, 7, 8, 9, 14, 20]. Embryonic abnormalities have been induced by organochlorine insecticides when injected into the chick egg during the embryonic development [14, 18, 20, 23]. Interest in the possible teratogenic potential of organophosphorous insecticides in mammals was induced by reports that this important class of insecticides caused nervous system lesions as well as skeletal abnormalities in various species of birds [14, 20]. Although malathion possesses advantages, in an environmental sense, however there is increasing concern over its possible deleterious side effects in man [1, 21].

Insecticide residues in commercial feed ingredients has been suggested as a possible factor in affecting growth, and hatchability of avian embryos [4, 11]. The acute toxicity levels of some insecticides have been reported on a number of avian species [6, 7,

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20, 22, 23] but as yet, no detailed work has been published on the toxicity of mixture of these insecticides. The present investigations were undertaken to determine the lethal effects of malathion and endosulfan individually and in combination to fertile chicken eggs.

## MATERIALS AND METHODS

Fertile eggs from in crossbred white Leghorn hens were obtained from a commercial supplier. During the 3 day pre- experimental period, eggs were stored small end down in a forced draft incubator at 37 ± 0.5$^0$C, 60% relative humidity being turned three times daily. After three days, eggs were candled and embryos of uniform size were selected randomly to be used in all experiments.

Two insecticides belonging to two major groups, Organophosphorous and Organochlorine, were selected for these studies. These were malathion (95.5% American Cyanamid Co., Hampshire, U.K.), and endosulfan (99.5% Hoechst, Norfolk, U.K.), respectively. Technical quality samples of each chemical were dissolved or suspended in pure corn oil. The test concentrations were obtained by a serial dilution of a fresh stock solution with corn oil. Each concentration was one half of the previous one. After initial dose fixing experiments, four concentrations for each insecticide ranging from 1.25 to 10 mg/egg were used. Each egg was injected with 0.05 ml of the insecticide in corn oil carrier. The solutions were applied in ascending order of concentration. A control group of 10 eggs was included in each of bioassays, 0.05 ml of corn oil being injected into the yolk sac of each egg. Ten eggs were injected with each dose and three replications, each on a different day, were made. A total of 30 eggs per treatment were used.

In order to avoid contamination, the injections were carried out in a room with a sterile atmosphere using formaldehyde vapor. Before injection, each egg was shaken with a quick twist of the wrist and eggs were placed on their sides to allow the embryos to rise to the top. The large end of the egg was wiped with a sterile cotton wool pad moistened with a 70% ethanol solution and a small hole 5 millimeter in diameter was drilled above the air cell through the shell. All particles of egg shell were removed with an aspirator. The needle (1 inch long no. 27) was inserted horizontally through the air cell into the yolk sac. Immediately after the injection, the hole in the shell was sealed with adhesive tape. The injected eggs were kept in incubator with large ends up. The incubator was rotated normally and was maintained at an optimum temperature of 37 ± 0.5$^0$C and relative humidity of 60%. Eggs were candled daily, and dead embryos being removed and autopsied. Experiments were terminated after 20 days of total incubation time by cracking the eggs open. Statistical evaluation of group differences in mean embryonic body weight was carried out using Student's t-test. The dose- mortality data were analyzed by probit analysis using SPSS [19].

## RESULTS

The cumulative mortality rates of embryos for compound injected groups were predominantly higher than control groups (Table 1). These data indicated that the mortality was correlated with the concentration of insecticide doses. For example injection of 1.25 mg of malathion or endosulfan into the yolk sac of fertile eggs produced no pronounced increase in mortality but when a higher dose of each insecticide was used, more mortality was observed. These data indicated that the highest level of malathion and endosulfan greatly affected hatchability of the fertile eggs. When malathion and endosulfan were injected together at a dose of 1.25 mg/egg, more mortalities were observed.

**Table 1.** Embryo mortalities resulted from injection of malathion and endosulfan into yolk sac of incubated chicken eggs.

| Treatment | Dose mg egg $^{-1}$ | Number of eggs injected | Number of dead embryos | Percent mortality |
|---|---|---|---|---|
| Control | 0.00 | 30 | 3 | 10.00 |
| Malathion | 1.25 | 30 | 3 | 10.00 |
| | 2.50 | 30 | 9 | 30.00 |
| | 5.00 | 30 | 25 | 83.33 |
| | 10.00 | 30 | 28 | 93.33 |
| Endosulfan | 1.25 | 30 | 2 | 6.66 |
| | 2.50 | 30 | 9 | 30.00 |
| | 5.00 | 30 | 19 | 63.33 |
| | 10.00 | 30 | 21 | 70.00 |
| Malathion +endosulfan[a] | | 30 | 5 | 16.66 |

[a] Each at a level of 1.25 mg/egg.

Mortality data for insecticide injected groups are presented in Table 2 as regression formula. The estimated $LD_{5O}$,slope, variance of slope, heterogeneity and R-squared for embryo mortality are set out in this Table. These data indicate that embryos are more susceptible to toxic effect of malathion as compared to endosulfan. The relationship between dose and mortality of malathion and endosulfan resulted R-squared values ,93.51 and 94.81% respectively. The R-squared values revealed a close fit of the model to the data.

Mean body weight and standard deviation for viable 20 day old embryos are given in Table 3. There was a trend towards lower body weight in embryos from eggs injected with higher doses. Viable embryos from the malathion injected eggs had lower mean body weights. Significant difference in mean body weight of viable embryos existed between 5 mg malathion injected groups and control.

## DISCUSSION

The incubation period from injection to occurrence of embryo death was inversely related to the dosage of insecticides. This is not surprising, because in assessing the susceptibility of any organism to a toxicant, the amount of toxin per body unit is a crucial factor [5, 17].

Abnormalities such as weight loss were observed more in embryo from insecticide treated eggs. The validity of these results has also been supported in a number of other

**Table 2**. Regression lines of chicken embryo mortality from treatments with malathion and endosulfan

| | Regression lines | $LD_{50}$ | Variance | Heterogeneity | | |
|---|---|---|---|---|---|---|
| | | Mg egg$^{-1}$ | of slope | $X^2$ | P | $R^2$ |
| Malathion | Y= 2.254+4.052X | 3.59 | 0.604 | 3.82 | 0.148[a] | 93.51 |
| Endosulfan | Y= 1.712+2.351X | 5.35 | 0.299 | 4.75 | 0.093[a] | 94.81 |

Results of three replicates per treatment pooled.

[a] No heterogeneity.

**Table 3.** Toxicity of injection of malathion and endosulfan into yolk sac of chicken eggs.

| Treatment | Dose mg egg$^{-1}$ | Number of viable embryo | | Body weight of viable embryo (g)[a] |
|---|---|---|---|---|
| | | Healthy looking | Malformed | |
| Control | 0.00 | 26 | 1 | 22.60±2.36[d] |
| Malathion | 1.25 | 24 | 3 | 21.68±2.23[d] |
| | 2.50 | 16 | 5 | 19.82±2.19[d] |
| | 5.00 | 5 | 0 | 18.39±2.11[e] |
| | 10.00 | 1 | 1 | 18.12[b] |
| Endosulfan | 1.25 | 27 | 1 | 22.23±2.48[d] |
| | 2.50 | 19 | 2 | 20.85±2.37[d] |
| | 5.00 | 9 | 2 | 19.91±2.30[d] |
| | 10.00 | 6 | 3 | 19.35±2.53[d] |
| Malathion+endosulfan[c] | | 18 | 7 | 21.47±2.45[d] |

[a] Mean ± SD of weight of five normal looking viable embryos except.

[b] Weight of one normal looking viable embryo.

[c] Each at a level of 1.25 mg/egg.

*d* and *e* Means followed by similar letters within a column are not significantly different from each other at 5% level.

cases [14, 20].

In studies with various insecticide mixtures Walker found that hatchability of eggs is depressed to a great extent by mixture of insecticides than by those injected individually [22]. Similarly Ioannis *et al.*,[10] working with scopolamine and hyosciamine found that a mixture of these toxicants has more lethal effects when injected into the yolk sac of developing chick embryos. Based on cumulative mortality data only an additive effect occurred in the present study. This discrepancy could be attributable to either the difference in toxicants tested, the technique used, or both. The results of this investigation demonstrated that the combined action of malathion and endosulfan in fertile chick embryo causes increased mortality and reduced embryonic body weight in comparison with action of each individual compound alone. Since malathion and endosulfan have been used extensively in many countries and for a long time [1, 11, 16], therefore due to environmental contamination people are exposed to these chemicals to varying degrees [1, 2, 3, 11, 13, 16, 21].

Although the results of mortality on chicken egg under laboratory conditions can not be directly extrapolated to the mammals, but it can be useful in predicting potential damage. These conclusions are meant only to serve as preliminary information for further research.

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## بررسی اثرات سمی مالاتیون و اندوسولفان در جنین تخم مرغ

## چکیده

دراین بررسی اثرات سمی مالاتیون و اندوسولفان در روی جنین تخم مرغ های نطفه دار تعیین گردید. تزریق ۱/۲۵، ۲/۵، ۵ و ۱۰ میلی گرم مالاتیون به زرده هر تخم مرغ قبل از شروع جوجه کشی به ترتیب سبب ۱۰، ۳۰ ، ۸۳/۳۳ و ۹۳/۳۳ درصدتلفات شد. از نظر تلفات جنینی روند مشابهی در اثر تزریق مقادیر اندوسولفان مشاهده گردید. تزریق مالاتیون و اندوسولفان به مقدار ۱/۲۵ میلی گرم به هر تخم مرغ، افزایش تلفات قابل توجهی را نسبت به تیمار شاهد ایجاد نکرد. براساس مقادیر $LD_{50}$ ثابت شد که مالاتیون سمی تر از اندوسولفان می باشد. این فرضیه که بین دز مصرفی و تلفات جنینی یک خط رگرسیون برازش داده می شود از نظر آماری قابل قبول می باشد. تزریق توام

مالاتیون و اندوسولفان به تخم مرغ ها در مقایسه با کاربرد انفرادی این سموم باعث افزایش تلفات جنینی تخم مرغ ها گردید. افزایش دز مصرفی برای هر کدام از دو ترکیب مالاتیون و اندوسولفان سبب کاهش وزن جنین شد.