Dominant Lethal Effects of Cyfluthrin and Malathion on Mice

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ABSTRACT

Male ICR strain mice were treated with three sublethal doses of malathion and cyfluthrin (Baythroid) through oral administration and intraperitoneal injection. One week after treatment, they were caged with two females and were allowed to mate. Seven days after the appearance of vaginal plugs, the females were dissected and observed for dominant lethality. Cyfluthrin came out as the more effective inducer of mutagenesis. The study found out that cyfluthrin was dose and route dependent but such results were not exhibited by malathion.

INTRODUCTION

Researches are becoming more inclined to anticipated problems posed by chemical pesticides such as insecticides, molluscides and herbicides not only in this country but worldwide. Scientists are aware that man is most likely to be affected due to direct exposure to these chemicals during manufacturing, formulation and application states or indirectly through the environment, such as the accumulation of residues in the food chain. Various studies have shown that most, if not all, pesticides carry potential hazards to man.

One of the pesticides that is commonly used in the Philippines is Baythroid, wherein the main component is synthetic pyrethroid called cyfluthrin (Behrenz et al., 1983) and malathion [0,0-dimethyl S-(1,2-dicarbethoxythyl)diphosphate].

They were found to be effective against insects that are of special importance as carriers of human and animal diseases. Although malathion is one of the least toxic of the commercial organophosphorus insecticides, it contains several impurities that potentiate its toxicity (Imamura and Talcott, 1985; Mason and Crozier, 1988). Baycarb, on the other hand, is found to have alkylating properties and can be correlated to mutagenesis or carcinogenesis (Aizawa, 1982; Wild, 1985). The dominant lethal test (DLT) is probably the most widely-used mutagenic procedure that employs animals, more particularly mice and rats (Anderson, 1984). The reliable indicator of dominant lethality is statistically significant in that it increases the number of early implantation deaths of females mated to the treated males. The genetic basis of dominant lethality is production of chromosomal breaks and translocation in sperm, which precludes development of a fertilized egg beyond the implantation stage (Mayer and Flamm, 1981).

Studies involving organisms like mice based on dominant lethal test have been a good indicator of genotoxic effects of gamma irradiation (Baev et al., 1977), x-ray (Cox and Lyon, 1975); chemicals, particularly pesticides (Anderson et al., 1978; Generoso et al., 1985; Suter and Generoso, 1976). Statistical analysis evaluating dominant lethal test was also done by Ryttman (1976) and Dean and Johnstone (1977). It has been established that DLT in death of embryo was caused by mutation inherited from one parent due to interference in the chromosome, like breaks which can be induced by mutagens (Anderson et al., 1978).

Dominant lethal mutation is lethal to an individual carrying the mutation in a single dose. Mutation can happen to a germ cell (egg or sperm) prior to and after fertilization. It is killed either in the fertilized egg or the subsequent blastula (Venitt and Parry, 1984). It is relevant that an abortion or miscarriage in humans can be a manifestation of dominant lethal mutation especially to those exposed to mutagens. One disadvantage of the DLT is that the conceptus dies as blastula or soon afterwards and, thus, not available for genetic analysis in succeeding generations.

OBJECTIVES

This study determined the dominant lethal activities of malathion and cyfluthrin; it also determined the dose dependence of these mice on different sublethal concentrations of these pesticides.

SIGNIFICANCE OF THE STUDY

Most pesticides are potent killers. Though not instantaneous in effects, sublethal doses may produce long term results that may even be worse than death. In cases when these pesticides are found to cause dominant lethal mutation, their relevance to man stems from the fact that this mutation induces chromosomal aberrations which may be associated with some congenital abnormalities and spontaneous abortion (Robert and Rand, 1978). Since prevention of the wide use of pesticides is almost impossible, the ones which have a lesser causal effect will be recommended to users with necessary precautions. This study intends to encourage other researchers to further formulate control measures that will not cause dominant lethal mutations and other health problems to users of these chemicals. Findings from this work will provide additional information and knowledge in the field of Genetics. The resulting genetic toxicity of pesticides to mammals which are usually non-target organisms is an additional factor which destabilizes the ecological balance, thus it provides another ground for controlling the use of these pesticides and the option to biological means.

METHODS

Test Animals

Breeding cages were placed in the laboratory located in the Natural Sciences Research Institute. Mice were fed with conditioner (mixed seeds and pellets) feeds and supplied with water ad libitum. Experimental cages were made of plastic trays (14" x 10") divided by plywood and covered with wire net (Fig. 1). Male and female (ICR) strain mice (Fig. 2) were purchased at the Veterinary Medicine Hospital, University of the Philippines, Diliman, Quezon City, and acclimatized in the laboratory for a week.

Preparations of Working Solution

The mice were weighed and the dose of working solutions was computed as: dose = weight of mice x dose mg/ml x 1000 (ul)

available g/1 of pesticides

The pesticides used were in liquid form. Malathion used was in liquid form. Malathion was available in 570 g/l and cyfluthrin (Baythroid) in 50 g/l.

LD50 Determination

The LD50 for the two pesticides: malathion and cyfluthrin (Baythroid) (Fig. 3) were determined by intraperitoneal injection (IP) and oral introduction. Only in cyfluthrin did the oral differed from intraperitoneal in the LD50. The sublethal doses were based on the 1/2, 1/6, and 1/3 of the LD50 of each pesticide to the ICR strain mice (Table 1).

Fertility Testing and Random Breeding for Dominant Lethal Test

Ten to 12 week-old healthy male mice which had successfully impregnated females were used in the experiment. They were dosed orally and intraperitoneally (Fig. 4), with 3 sublethal doses (mg/kg) of malathion (115, 230, and 460) and 3 sublethal doses oral administration in mg/kg of cyfluthrin oral LD50 (52, 103, and 206) and intraperitoneal (29.17, 58, and 116). Control mice were injected intraperitoneally with 0.25 ml. distilled water and those for oral administration were fed with the same amount of distilled water. Each treated mouse in the duplicated setup including the control were caged with two randomly picked virgin females. These matings were maintained until vaginal plugs and the swelling of the nipples were observed in the females. Females were sacrificed by cervical dislocation seven days after the appearance of vaginal plugs. Live and dead implants were counted and scored after dissection of the uteri.

Dominant Lethal Test

In the experimental setup on dominant lethal test (DLT) using malathion, four healthy mice, 10 to12 weeks old, weighing 15 to 21 g were used. Each male received sublethal doses through oral administration and intraperitoneal injection. Control mice received 0.25 ml of distilled water which was also the amount used in dissolving the pesticides. For the second trial, four male mice of the same age weighing 19.16 to 22.21 g were used. Each male mouse for both trials was caged with two virgin females for a period of 20 days. The same procedure was followed in the use of cyfluthrin; the mice used weighed 23 to 29 g and were 10 to 12 weeks old. The doses used for the pesticides were higher in the oral than those used for intraperitoneal administration (Table 1).

The mating period was prolonged over those prescribed in other reports in order to ensure that the females would get pregnant. The lights were kept on in the laboratory to prolong the photoperiod condition for 12 hours. Dissection was done seven days after the appearance of the vaginal plugs which is assumed at the start of pregnancy.

RESULTS AND DISCUSSION

The effects of the drug on the mating behavior can be ruled out as evidence of vaginal plugs. This is assumed as true in all pesticide treatments. The presence of vaginal plugs demonstrates the secretory functioning of the coagulating gland of the male reproductive tract (Meistrich et al., 1985).

There were live and dead fetuses observed in the uterus of the mother. The defective fetuses appear to be brown or black in color in comparison to the healthy pinkish color of the live implants (Fig. 5). This, according to Anderson (1984), persists in full term in rodents as a deciduum or mole which co-exists with the live embryos (Fig. 6). The deciduum is a growth of maternal tissue resulting from a stimulus from the implanting egg. The mole remains unaltered up to the 11th day of pregnancy except that the upper part of its necroses form the deciduum capsularis.

Table 2 shows the results of the number of live and dead implants obtained from four female ICR strain mice per sublethal dose of malathion. The percentage mutagenic index was determined by dividing the number of dead implants over the total number of live implants multiplied by 100. The higher the mutagenic index, the higher is the dose for both oral and intraperitoneal administration. When the mortality rate in the control group was compared with that of the other groups, it was found that the results in the control group was significantly different from the malathion-treated groups. However, no significant difference in the mortality rate was seen among the groups induced with 115, 236, and 460 mg/kg of malathion. That means the mortality rate among the treated groups is more or less the same. This observation is true for both types of treatment. Results for the groups of mice injected intraperitoneally did not differ significantly from those induced orally. The above findings reinforce the results that LD50 for oral and intraperitoneal treatments are the same. In general, no matter how much malathion was administered to the mice, either orally or intraperitoneally, the control group yielded lower mortality rates than the treated ones or none at all.

Table 3 shows the results of the experiment using cyfluthrin (Baythroid) on male mice weighing 23 to 29 g that were 10 to 12 weeks old. The higher the dose, the higher is the mutagenic index in both the oral and in the intraperitoneal administration. However, based on the statistical analysis, there is no significant difference in the results between the untreated and the 52 mg/kg treatment, but is significantly different from the results of the two higher treatments. The results in the two higher doses are either statistically significant or insignificant from each other, but the results of two lower-dose treatments are significantly different from the results of the highest dose-treatment which has the highest mortality. The results of the control in the intraperitoneal are significantly different from the treated. There is no significant difference between the 29 and 56 mg/kg treatments, but both results are significantly different from the results of the highest dose (117 mg/kg). The higher the LD50 in oral over that of intraperitoneal treatment indicates the cyfluthrin is more potent intraperitoneally than oral administration. This is further augmented by the results in the 3 sublethal dose levels in both administrations. Intraperitoneal treatment is more effective possibly because of the interference of the mucus and enzymes present in the buccal cavity of the mice. Dominant lethal mutations are nuclear alterations, which can affect the zygote even if present in a single dose. Such mutations prevent the development of the zygote to maturity, leading to zygotic and embryonic death from the break-fusions, bridge cycles, and chromosome imbalance brought about by chromosome breakage. It is associated with depression in mitotic rate leading to complete cessation of mitosis in the second or third cleavage divisions (Roberts and Rand, 1978).

CONCLUSION AND RECOMMENDATION

The results of this investigation indicate that the two test pesticides induce dominant lethality in mice. Cyfluthrin is a more effective inducer of abnormality, given intraperitoneally or orally. However, it shows dose dependence for both oral and intraperitoneal administration especially if doses are at wide intervals. However, dose dependence was not exhibited by the results for malathion. Intraperitoneal is more potent than the oral introduction in cyfluthrin but not in malathion. But despite the trend established, it is also recommended that determination of the genotoxic effect of cyfluthrin and malathion be done at the cellular, chromosomal, ultrastructural, and protein-enzyme level investigation with the use of a larger number of mice. While the two pesticides used affected the fertility of the male mice, as proven by the micronucleus and sperm mor-

phology test (Pagulayan and Baoanan, 1992), the long-term effects on the treated mice and their mutagenic effect on the live implants could be another aspect for further study.

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Table 1. Sublethal doses based on the 1/3, 1/6 & 1/12 of the LD50 to the ICR strain mice

	LD50	1/12 mg/kg	1/6 mg/kg	1/3 mg/kg
Malathion				
Oral	1380 gm/kg	115	230	460
IP				
Cyfluthrin				
Oral	620 mg/kg	52	103	207
IP	350 mg/kg	29	58	117

Table 2. Experimental results on the number of live and dead implants obtained from four female ICR strain mice per sublethal dose of malathion

Dose mg/kg)	Ave # of live implants	Ave. # of dead implants*	Ave. # of % Mutagenic Index
Oral Control	8.5	OA	O
115	8.5	2.25B	26.47
230	8.5	2.50B	31.35
460	6.75	3.50B	51.85
IP Control	9.50	OA	O
115	9.0	1.75B	19.44
230	7.75	2.75B	35.46
460	5.50	3.25B	65.00

* Values followed by the same letter are not significantly different at 0.01% level (Test for the difference of proportions.)

Table 3. Experimental results of the number of live and dead implants obtained from four female mice per sublethal dose of cyfluthrin (Baythroid)

Dose (mg/kg)	Ave. # of live implants	Ave. # of dead implants**	Ave. # of % Mutagenic Index
Oral Control	9.0	O A	O
52	9.5	1.5 A	17.65 A
103	7.25	2.25 B C	31.03
207	6.0	4.0 C	66.67
IP Control	8.75	O A	O
29	6.0	2.0 B	33.33
56	6.5	3.5 B	53.85
117	6.00	4.25 C	70.03

Table 4. Comparison of the dominant lethal activity of malathion and cyfluthrin

Dose	Average % Mutagenic	
	Malathion	Cyfluthrin
ORAL Control	0.0	0.0
1/12	26.47	17.65
1/6	31.25	31.03
1/3	51.85	66.67
INTRAPERITONEAL Control	0.0	0.0
1/12	19.44	33.33
1/6	35.48	53.85
1/3	65.00	70.83

** Values followed by the same letter are not significantly different at 0.01%

(Test for the difference of proportions.)

Figure 1. Experimental set-up in the animal house. Plastic trays were divided into two to conserve space. Mice were supplied daily with food and water.

Figure 2. Ventral view of the ICR strain mice showing the male (right) and female (left) genitalia.

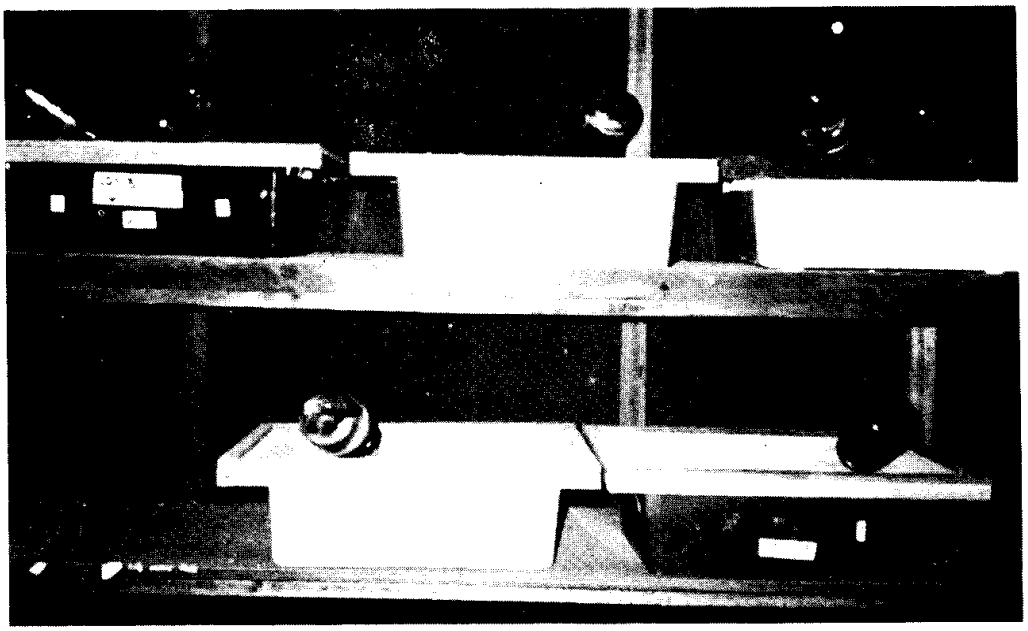


Figure 3. Experimental set-up in the determination of the LD90. The mice used were all male. They were monitored every hour for 12 hours and every 2 hours until the 24th hour of treatment.

Figure 4. Intraperitoneal injection of the ICR strain mice. The skin is lifted so that the needle would only reach the buccal cavity/peritoneum and not the internal organ.

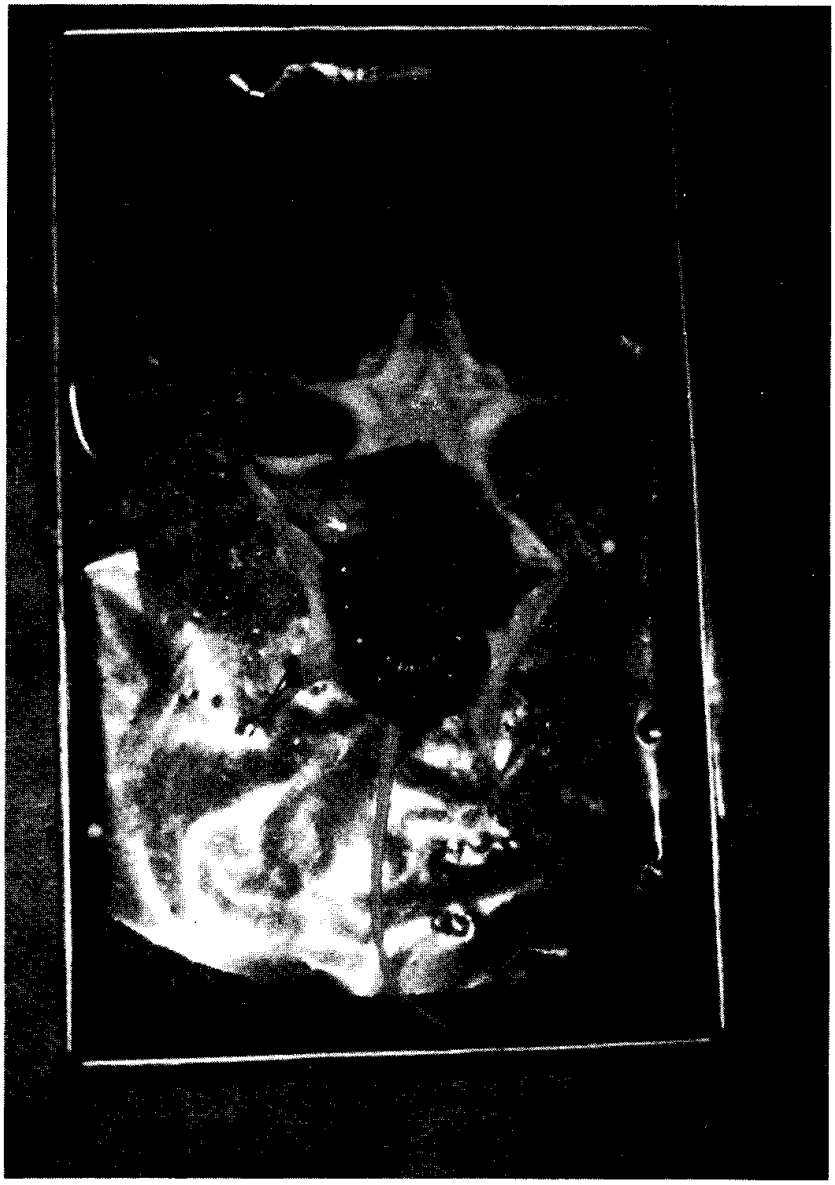


Figure 5. Dissected pregnant female mated with control male. Shown here are the live fetuses.

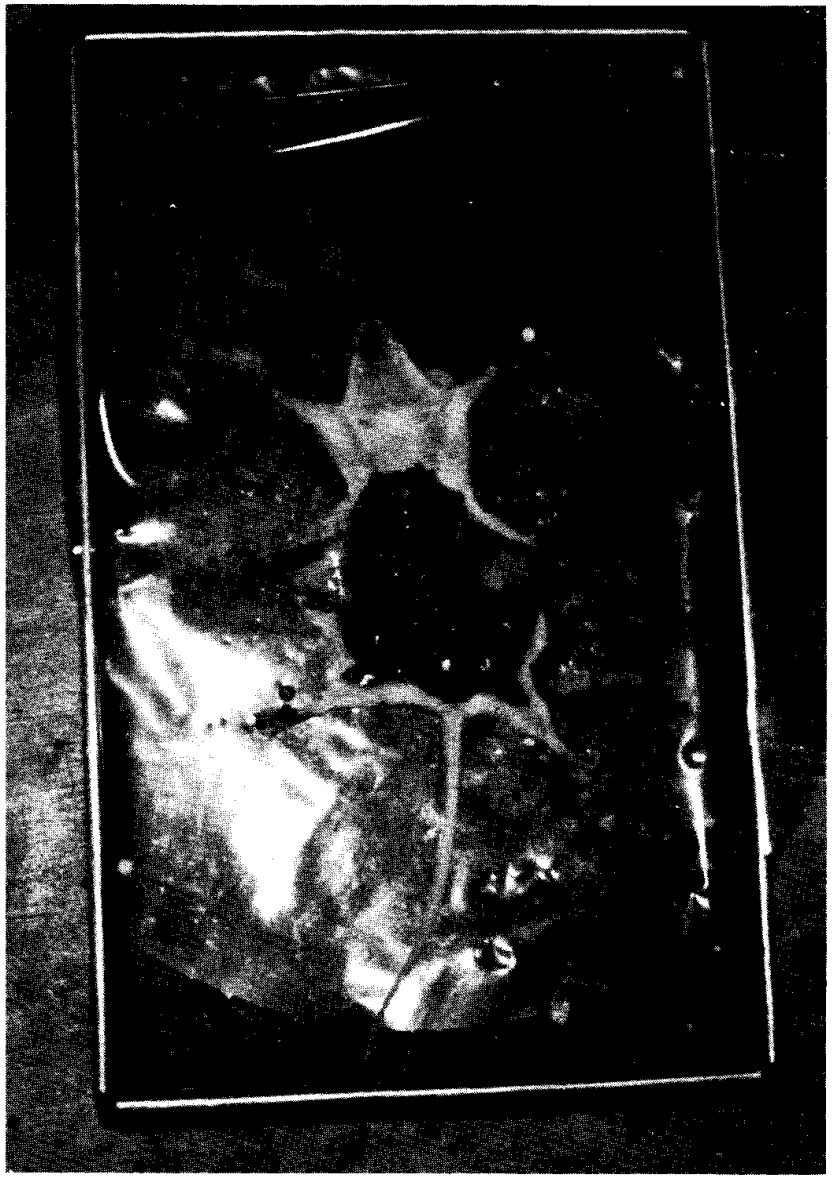
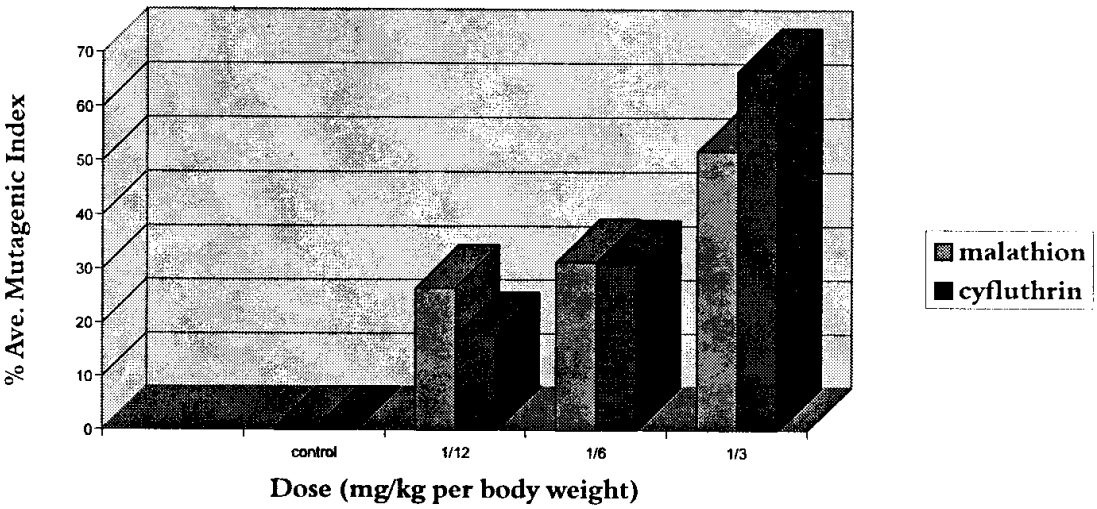


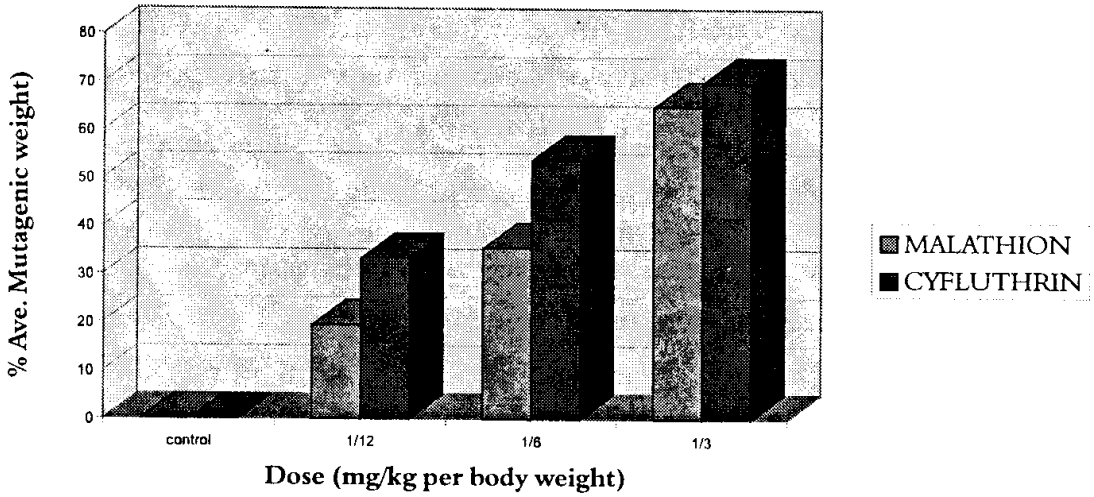
Figure 6. Dissected pregnant female mated with cyfluthrin (Baythroid) - treated male. Scoring of live and dead fetuses was done.

Figure 7. Comparison of Dominant Lethal Activity **(Oral)**



DOSE (Mg/kg)	MALATHION	CYFLUTHRIN
control	0	0
1/12	26.47	17.65
1/6	31.35	31.03
1/3	51.85	66.67

Figure 8. Comparison of Dominant Lethal Activity **(IP)**



DOSE (mg/kg)	MALATHION	CYFLUTHRIN
control	0	0
1/12	19.44	33.33
1/6	35.46	53.85
1/3	65.00	70.03