

TERATOGENIC EFFECT OF
ORGANOPHOSPHATE PESTICIDE DIMETHOATE
30 EC IN RATS

THESIS SUBMITTED FOR
UNIVERSITY OF BOMBAY

THE DEGREE OF
MASTER OF PHARMACY
IN
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SUBMITTED BY
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UNDER THE GUIDANCE OF
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BY

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"Honour and shame from no conditions arise
Act well your part there all the honour lies"

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C O N T E N T S

			<u>Page No.</u>
1.	INTRODUCTION		1 - 4
2.	REVIEW OF LITERATURE..		5 - 17
3.	CHOICE OF ANIMAL MODEL		18 - 20
4.	MATERIALS AND METHODS		21 - 26
5.	RESULTS		27 - 47
6.	DISCUSSION		48 - 64
7.	CONCLUSION		65 - 66
8.	REFERENCES		67 - 74
9.	APPENDIX (1 to 18)...		75 - 127
10.	SYNOPSIS		1 - xv
	STATEMENT		

* * * * *

1. I N T R O D U C T I O N

INTRODUCTION

Past couple of decades have seen widespread use of pesticides for improving agricultural practices, in health programmes and for household purposes. The fear of adverse effects of pesticides in man, domestic animals and wild life have become a matter of public concern. Toxicity may be the result of access to treated areas, stored or discarded pesticide containers, contaminated food, air and water.

Pesticides have made a great impact on human life by its widespread use. In agricultural practices, pesticides are used for preservation and protection of crops, fibres, fruits, grains and cereals from spoilage by microbial diseases and pest attack. Pesticides are also used for the control of vectors transmitting human and animal diseases, and for the control of household pests.

The widespread application of pesticides for agricultural purposes and in industrial and residential premises, exposes large group of population of different ages, diseased individuals and sex to hazards. The hazards due to pesticides occur at different stages, such as occupational during manufacture, operational hazards during

application of pesticides, persistence of pesticides and contamination of food, air and water. Apart from these, hazards may result due to accidental or intentional poisoning.

Organophosphate pesticides are used all over the world today. The first organophosphorous compound, Triethyl pyrophosphate (TEPP) was developed in Germany during the World War II¹. Since then many new pesticides have been introduced. Organophosphate pesticides are esters, amides or thiol derivatives of phosphoric, phosphonic phosphorothioic and phosphonothioic acids.²

In a study conducted by the Indian Agricultural Research Institute, the estimated requirement of organophosphate pesticides for the year 1983-1984 was about 10,900 tonnes. Amongst the pesticides, Dimethoate is one of the largest pesticides used in India (900 tonnes during 1983-1984)³. Dimethoate $\text{[O,O-dimethyl S-(N-methyl, carbamoyl methyl)phosphorodithioate]}$ is marketed under the commercial names of Rogor, Cygon, Daphene, De-Fend, Trimethion, Dimeton, Ferkethion, Fortion, Fosfamid, Fosfotox, Lurgo, Perfektion, Rebelate, and Roxion.

Dimethoate is used as a residual spray in farm buildings for the control of house flies and wide range of

insects and mites on food grains, cash crops, vegetables, fruits and ornamental plants.⁴ The retail use of Dimethoate is in the form of emulsifiable concentrates (EC).

During their actual usage, pesticides are generally used as their formulations. Formulations are composed of different concentrations of technical grade material along with various solvents, emulsifiers, stabilizers and other additives. Because of variation in percentage of technical grade material in different EC formulations, the impurity levels vary and the possible toxicity. Impurities may also be found in formulated pesticides because of degradation which may arise during synthesis and storage. The levels of impurity may differ according to the route of synthesis chosen, the formulating ingredients added or the storage conditions.

The percentage of varying impurities may be present in the form of solvents, emulsifiers, stabilizers and other additives. These ingredients may exert their own toxicity or interfere with the metabolism/kinetics of technical grade material in the formulation. For example, the solvents used in formulation increase the chance of contact of the pesticides and the target and non-target organisms. The presence of impurities in formulations may result in various degrees of toxicity to

the biological system on exposure to these pollutants.

Various toxicity studies are required by State and Federal regulations to assess and prevent the possible hazards of pesticide chemicals. Different toxicity studies such as acute, sub-chronic, chronic, neurotoxicity, mutagenicity, carcinogenicity and teratogenicity are conducted in different species of animals to assess the potential toxicity of chemicals so as to extrapolate the data obtained in animal studies to human beings.

Amongst the specialized toxicity studies, teratogenicity is one of the most important study as it directly deals with the fetus or birth defects. The study is concerned with the generation, cause and manifestation of structural and functional alterations in fetal development. The data regarding the potential hazards of formulations to pregnant females is inadequate. Therefore, the present study was undertaken to evaluate the teratogenic effects in rats of a widely used, indigenously manufactured formulation Dimethoate 30 EC and compare its effects with Aspirin, a known teratogen (positive control).⁵

2. REVIEW OF LITERATURE

REVIEW OF LITERATURE

The term teratology was first coined by Etienne and Isidore Geoffrey Saint-Hilare in between 1822-1832 during their investigations of malformations or monsters (teras - plural-terata, from Greek monsters) mostly in the chick embryos.^{6,7} However, mankind's intense interest in humans born with deficiencies and deformities is known since centuries. The first recorded account of malformations dates back to as early as five thousand years ago in Egyptian wall paintings.⁸ Till the 19th century it was felt that the fetus is inviolate by the environment. The first evidence of the role of environment in the production of abnormal offspring was brought about in the 20th century.

The credit for experimental mammalian teratology goes to Hale who in his experiments between 1933-1937 reported that pregnant sows fed with vitamin A deficient diet gave birth to piglets without eye balls. Some piglets also had facial clefts, accessory ears, misplaced kidneys and occasionally malformed hind legs. By mating blind male to the blind dam (blind sister) normal pigs were born, which proved that these birth defects were not hereditary.⁹ Studies with various teratogens soon followed and during the last 50 years, scientific attempts

underlying the basis for such birth defects were made. Greg in 1941 reported a German measles epidemic in Australia which resulted in offspring with cataracts, deafness and congenital heart disease.^{5,7}

In 1948 two positive teratogens, Trypan blue and Nitrogen mustard were identified.⁹ Female mice, when placed in a low pressure chamber for five hours at 260-280 mm of Hg from 2nd to 18th day of gestation,

anomalies such as fused ribs, cleft palate and cranioschisis resulted, and anoxia was identified as the cause of fetal death and congenital malformations.⁹

During the same period it was reported that congenital anomalies could be produced on administration of cortisone to pregnant mice during gestation.⁹ Various studies were conducted with X-ray as teratogenic tool during World War II. Study with drugs soon followed. Soon Thalidomide was shown to be teratogenic.

During the World War II, severely deformed children were born to mothers who received Thalidomide to relieve morning sickness during pregnancy. About 8000 children were born in 28 countries of the world with birth defects due to Thalidomide.⁷ Various workers have worked on the cases of Thalidomide. Animal experiments were also reported. In humans this drug

caused limb reduction anomaly such as phocomelia.^{5,6,10} In animals Thalidomide was administered to rabbits, rats, hamsters and mice during gestation period, Fratta et al., (1964)¹¹ observed that when rabbits were administered with 50, 150, and 300 mg/kg Thalidomide, dose dependant skeletal anomalies were observed. No fetal anomalies were produced in the fetuses of the mice treated with 200 mg/kg and 150 mg/kg in hamsters, neither did it produce anomalies in the rats. These results were confirmed by Goran Jonsson (1972).¹² This lack of teratogenic hazard in rats and hamsters were attributed to the slower absorption of the compound.⁵ The results of Thalidomide catastrophe were confirmed by the birth of almost 20,000 defective children following rubella epidemic in the U.S.A.⁷.

Congenital malformation is one of the leading causes of infant mortality and morbidity. The food, water, air, occupational and recreational environment can be heavily contaminated with pesticide pollutants. Hence suspected pesticide teratogens can be a potential threat to pregnant females as they may be present in every component of the environment. Pesticides are highly toxic in contrast to other pollutants in one important respect that they are deliberately added to the environment. The increasing concern about teratogenic risks is

therefore to women of the child bearing age.

Teratogenic effects of various classes of pesticides have been reported by various workers. The important pesticide of the organochlorine class, DDT, does not produce any teratogenic effect, but a DDT carrier women may show frequent abortions and complications during pregnancy.¹³ In a study conducted by Alice Ottoboni (1968)¹⁴ it was observed that DDT produced no gross teratogenic effects in the rats with 20 and 200 ppm doses. In another study McLachlan and Dixon (1972)¹⁵ administered p' p' DDT to pregnant mice at the rate of 1 mg/kg on days 10, 12 to 17 of gestation and did not observe any gross teratogenic effects.

Aldrin, Dieldrin and Endrin at 1/2 LD₅₀ doses were teratogenic and produced embryocidal effects in experimental animals.¹⁶ Single oral dose of Aldrin (50 mg/kg), Dieldrin (30 mg/kg) and Endrin (5 mg/kg) administered to pregnant golden hamsters on day 7, 8 or 9 of gestation, caused high incidence of fetal death, congenital anomalies and growth retardation. Defects such as cleft palate, open eye and webbed foot were also noted frequently. Mice treated on day 9 of gestation, given equivalent dose, showed similar anomalies. However, Chlordane was not found to be teratogenic.¹³ When Endosulfan, a pesticide of the organochlorine class was

administered in rats at 10 mg/kg, incidence of maternal mortality, fetal death and resorption were observed.¹³ In a study with carbamate pesticides, Carbaryl upto 500 mg/kg in diet resulted in no teratogenicity, but at 500 mg/kg dose there was reduction in maternal weight gain and fetal death.¹⁷

Organophosphates are important class of pesticides used all over the world. Teratogenic studies with various class of these compounds have been reported.¹⁸ Fish (1966)¹⁹ treated albino rats with isofluorophate (DFP), Parathion and Methyl Parathion. Isofluorophate when administered at the doses of 1, 2, 4 mg/kg on days 8, 9 and 12 of gestation had no effect on fetal weight. During later stages of implantation and primitive streak formation, low dose treatment with DFP had no effect on birth weight or fetal development. Parathion when administered as split and single doses of 2.4, 5, 0.77, 0.63 and 0.5 mg/kg on days 8, 9, 15 and 16 of gestation, there was no difference between treated and control animals. Similarly single dose of Methyl parathion at 4 and 6 mg/kg administered on days 9 and 15 of gestation produced negative results. Large sub-cutaneous hematomas were found in several offspring of the Parathion and Methyl parathion treated groups but no significant developmental defects were observed. High still birth and neonatal death rates were also reported in the offspring

of all Parathion and Methyl parathion treated animals which were allowed to deliver.¹⁹

Parathion was embryocidal to pregnant mice at 12 mg/kg dose on days 12 to 14 of gestation period and produced incidence of 90 % death in utero and also significantly reduced fetal body weight (Harbison Raymond D., 1975).²⁰ Another important organophosphate pesticide, Dipterex was found to be teratogenic at the dose of 80 mg/kg during the critical period of embryogenesis but not at 8 mg/kg. Imidan at 30 mg/kg once on day 9 of gestation, and at the dose of 1.5 mg/kg daily throughout the gestation, increased post implantation mortality of the embryos. The dose of 30 mg/kg of Imidan on day 9 or 13 of gestation caused developmental anomalies including hypognathia and hydrocephaly (Martson L.V., 1976).²¹ Staples et al. (1976) found that Dipterex administration with the diet at 432 and 519 mg/kg from day 6 to 15 of gestation caused major external and skeletal anomalies, but not with 145 mg/kg.²²

Technical grade and formulations of Linuron, Methoxychlor and technical grade of Malathion were studied for teratogenecity. Formulations of Linuron, from one supplier at 200 mg/kg were not teratogenic, while the same formulation, from another supplier was teratogenic

at 200 mg/kg but not at 100 mg/kg. Treatment with technical grade or formulations of Methoxychlor resulted in reduced maternal weight gain at the doses of 50 to 400 mg/kg during gestation. At 200 and 400 mg/kg they were fetotoxic and produced a dose related increase in the incidence of wavy ribs at 100, 200 and 400 mg/kg/day. Malathion at doses as high as 300 mg/kg was not teratogenic (Khera K.S. et al.).²³ In a study with Biphenyl (99.9%), Ethoxyquine (unknown purity), Piperonyl butoxide (80%) or Mertect (45 % Thiobendazole) at 0, 125, 250 or 500 mg/kg when administered from day 6 to 15 of gestation, by oral route, were not found to be teratogenic. Similarly Zolone (30 % Phosalone) at the doses of 0, 12.5, 25 or 50 mg/kg and Benesan (50 % Lindane) at 0, 6.25, 12.5 or 25 mg/kg were also not teratogenic in the rats. Doses of Karmex (80 % Diuron) at 250 mg/kg and 500 mg/kg (Maternally toxic dose) was associated with increased incidence of wavy ribs (Khera K.S. et al., 1979)²⁴

Chlorpyrifos at the dose of 25 mg/kg in mice produced maternal and fetal toxicity. (Deacon M.M., 1980).²⁵ In a mice study by Budreau and Singh where Demeton was administered as single intraperitoneal dose at 7 to 10 mg/kg or as three consecutive doses of 5 mg/kg to mice between days 7 to 12 of gestation was found to be embryotoxic with

mild teratogenic potential.²⁶ Decreased fetal weight and fetal mortality were the main embryotoxic effects. Similar results were obtained with 40 and 80 mg/kg doses of Fenthion.²⁶ In one study where Thimet was studied for teratogenic effects by inhalation route, rats were exposed at 1.94 ± 0.48 mg/m from day 7 to day 14 of gestation. Thimet caused increased fetal mortality and decrease in fetal weight.²⁷

Teratological studies with Dimethoate technical and formulations have been reported. The toxicological evaluation of Dimethoate technical of 95 % purity in mice showed a very high incidence of maternal mortality with 40 and 80 mg/kg doses. However, at 10 and 20 mg/kg doses, there was decrease in maternal weight gain, but there were no significant anomalies when compared to the control.⁴ In a five generation mice study where Dimethoate was incorporated in drinking water at 60 ppm, there was reduced pregnancy rate and decreased survival and growth of pups.²⁸

Teratogenic studies with Dimethoate formulation (47.3 % EC) at 12 and 24 mg/kg was associated with a statistically significant increase in the number of anomalous litters and wavy ribbed fetuses. There were also other non-significant anomalies such as extra ribs,

fused sternebrae, runted fetuses, hydroureter and dilated urinary bladder.²⁹

With the aftermath of Thlidomide tragedy, protocols for teratogenic screening were set. In 1960 a wide number of publications from different committees and individuals suggested various protocols.³⁰ The Commission on Drug Safety in its meeting in June 1963 placed emphasis on the know-how of teratogenecity to be studied.³⁰ The general guidelines for teratogenecity testing were laid down in 1967 where choice of animal model, dosage, duration of treatment, route of administration, care and breeding of animals were specified.⁵ The protocol was designed to evaluate the safety of new drugs and environmental pollutants. Teratological study involves treatment during organogenesis period. For example, from day 6 to day 15 of the gestation in the rat. This is the period of maximum susceptibility to teratogenic agents.

The pre-differential period is resistant to teratogenic agents (from 0 to 6 days of gestation) except in conditions like hypoxia, hypothermia and with Actinomycin D. This resistance is because of a small cell population, there may be cell death resulting in no term fetuses or small but normal fetuses. The fetal

period is refractory to teratogens though there are exceptions as lead, methyl mercury etc.⁷

Wilson has described the susceptibility of embryonic organ system to teratogenic agents during organogenesis. He has reported that anomalies of various degrees can be attained if the dam is treated with teratogens during different days of organogenesis.³¹ A positive control such as Aspirin at 250 mg/kg is used for comparison. Josef Warkany (1959)³² had produced congenital malformations in female rats by administering salicylates during early stages of pregnancy. Defects such as craniorachischisis, exencephaly, facial clefts, eye defects, vertebral and rib defects were observed. Aspirin though is teratogenic in rodents, is not teratogenic in primates.⁷ This may be because of higher concentration and longer duration of concentration in embryo of the rats on day to day basis (Wilson et al., 1977).³⁴

Differences also exist among strains for the susceptibility to teratogens. In a study, differences were reported in mice strains with respect to the incidence of cortisone induced cleft palate.³⁴ Depass and Weaver in 1982³⁵ found that the frequency of soft tissue malformations including cleft palate and severe

cardiac malformations was much higher in Wistar rats as compared to the Fischer 344 strains on administration of single oral dose of Aspirin at 500 and 625 mg/kg on day 10 of gestation. Similar results were observed with an intraperitoneal injection of Hydroxyurea at 500 mg/kg on day 11 of gestation.³⁵

It is believed that teratogens act on genetic locus or loci which controls the toxicokinetics of the teratogen, or the target tissue or organ is directly susceptible for teratogenic action. A teratogen therefore increases the incidence of previously existing malformation.⁷ After administering the test material from day 6 to day 15 of the gestation period, dams are sacrificed one day before the anticipated date of parturition to deliver the fetuses by cesarean section. Relevant data such as number and position of the implantation sites, live and dead fetuses, fetal weight, fetal length, and sex is recorded. One third of the fetuses are examined for soft tissue defects and two thirds are stained for skeletal examination. This protocol is used all over the world as guide lines for teratogenicity tests. Research to improve the existing tests and develop new tests is in progress.

Many substances are known to be teratogenic in

one or more species of mammals including man.^{36,37} Human teratogens have been discovered from anecdotal observations and on examinations of epidemiological studies. Researchers suggest that if a substance is found to be teratogenic in two or more species of mammals, it is also suspected to be teratogenic in humans. Alcohol, antibiotics, anticonvulsants, metals like lead are some examples.⁷

The most important part of teratogenic studies is perhaps extrapolation of the animal data to human beings. This part is difficult as there does not exist a perfect animal model that is similar to the humans in all respects. The first important aspect is to select the animal model that is closest to the human metabolism so as to extrapolate the possible hazard.⁷ The problem that interferes with application of animal data to the humans are variability of the response between the species.³⁰

Species difference in metabolism plays an important role in differences in toxicological manifestation. As far as possible there should be a similarity between the test system and the humans. Genetic heterogeneity is also equally important. Dose levels tested in animals and expected dose that may cause insult to the human biological system may differ markedly.

Variability in placental transfer is also important. Also there are some malformations that are restricted to animal species under experimentation. For example, absence of tail cannot be correlated in the human beings, whereas, absence of the limbs can be easily correlated. Adequate physiological, anatomical and metabolic information and correct correlation are the basis of correct extrapolation. Rat is one of the choice species of animals for teratogenic studies.⁵

3. CHOICE OF ANIMAL MODEL

CHOICE OF ANIMAL MODEL

In teratogenic studies, animal models are used, because of the social and ethical implications in the use of human subjects. Teratogenic studies are usually carried out in animals during the gestation period and fetal development. It is terminated prior to littering to observe structural deviations. In selection of animal models, various complex factors are taken into consideration such as their biological systems and their numbers available. These varying complexities are important in selection of experimental model that has similarity with human development, in order to extrapolate results to human situation.

Various animal models are used in teratogenic testing of drugs and chemicals. These are the avians, amphibians, and the mammals. Some of the developmental stages of the avians are similar to those of the humans.⁵ This factor may be important if initial embryologic development is desired to be studied. The disadvantage being that they do not possess a placenta and the entry of outside influences may not be possible due to presence of a shell. There are varied differences in the developmental and other characteristics of the humans and the amphibians.⁵

Mammals have been extensively used in teratogenicity studies. Mammals consisting of rodents, non-rodents and primates have all been used. Amongst mammals, rats and rabbits have been used most frequently in teratogenic studies. They have a distinct advantage that they are available in large numbers, they possess a placenta and their embryo is in dynamic equilibrium with the environment.⁵

Rat has been the animal of choice for various teratogenic studies. The rat has a high fertility and a short gestation period of about 21 days. Embryology of rat is well known and also its placental and hormonal characters are similar to those of the humans.⁵ In teratogenic studies, the teratogens are administered during the period of organogenesis, hence the mating time becomes important. The time of copulation or mating can be measured accurately in the rat, and also in this species, the spontaneous occurrence of malformations has been well documented.⁵

Another species, namely the rabbit is used frequently for teratogenic testing of compounds. The rabbit also serves as a good model because of its high fertility and a short gestation period. Its embryological development and the spontaneous occurrence of malformations

is also well documented. For some teratogenic compounds the rabbits are sensitive, similar to the humans which indicates its similarity with human metabolism. Hamsters also practically have the same advantages as the rodent species, but they do not offer any specific advantages.⁵

The model closest to the humans are the higher simians. The disadvantages being that they are not available in large numbers, they have a long gestation period with small number of litters and are difficult and expensive to maintain. These factors preclude their use in teratogenic studies. Using large animals like dogs, cats, pigs is expensive and their breeding and maintenance is difficult. Large amounts of compound is also required for dosing. Rat therefore is one of the choice species in teratogenic studies.

4. MATERIALS AND METHODS

MATERIALS AND METHODS

Test compounds : Dimethoate formulation as Rogor 30 EC and Aspirin I.P. These were obtained from the local market.

Acute toxicity :

Acute oral toxicity of Dimethoate 30 EC was carried out in albino rats (Haffkine Strain), weighing 80 to 100 gms. Ten rats (five males and five females) were taken per dose. The test compounds were given by oral intubation in different concentrations. Ten rats were also taken as control. All animals were fasted overnight prior to dosage, but water was given ad libitum. Aspirin was administered as a suspension in distilled water with Tween-80, whereas control group for Aspirin received distilled water with Tween-80. Dimethoate was diluted with distilled water and its control group received distilled water only. All the animals were observed for a period of 7 days. The final test was terminated 7 days after dosing as no delayed symptoms or mortality was observed. All animals dead during the experimental period, and those that were alive at the end of experimental period were postmortemed for gross pathological changes. LD₅₀ values were obtained by the method of Litchfield and Wilcoxon.³⁹

Teratological study :

Female virgin albino rats (Haffkine strain) of 100 to 120 days age were used. Twenty female rats were taken per group. Females and males were paired in the evening. Three females were mated with one male. Females were examined for the presence of sperms in the vaginal smears the next day in the morning. The vaginal smear was prepared by flushing the vagina with little saline with the help of a blunt sterile pasture pipette and sucking back the saline with vaginal contents. The day on which the sperm was found in the vaginal smear of the female rat was considered as day zero of the gestation period.⁴⁰ Females were housed in polypropylene cages with paddy husk after mating. They were allowed pelleted food and water ad libitum. All females were housed individually from day 18 of gestation. Twenty mated rats were allotted at random to all experimental or control groups.

After the review of acute oral toxicity data regarding the lethal dose a range finding study was performed on three female rats of 100 to 120 days age using the LD₅₀ obtained in the acute oral toxicity study as a guideline. The range study was conducted to obtain the least maternal toxic dose so that fetal toxic doses could be identified for subsequent final teratogenic studies.

Three control groups were also taken, one group of female rats received normal diet only (Negative control). Second group received Aspirin at 250 mg/kg (Positive control)^{5,40} and the third group received equivalent amount of Tween 80 without Aspirin (Vehicle control for Aspirin). The test materials were administered from day 6 to day 15 of the gestation period.

One day prior to term i.e., on day 20 of gestation, fetuses were delivered by cesarean section under ether anaesthesia. It is reported that newly delivered maternal rats, tend to eat their defective offspring.⁴¹ Hence pregnant rats were sacrificed a day before full term to accurately evaluate a teratological result.

After opening the abdominal wall of the pregnant rats, both the horns were promptly exposed and the number and position of implantation sites were counted (Fig.1). This was done to find out live and dead fetuses and resorptions, if any. Animals such as rats have multiple implantations and resorb dead conceptuses in situ, hence counting the number of resorbed or dead fetuses along with the implantation sites may provide an interesting correlate to the extent of teratogenicity.³²

After recording the above parameters and removing

the fetuses from the amniotic sac, the fetuses were gently dried with folds of filter paper, to remove the excess amniotic fluid (Fig. 2). Fetal weights which indicate the developmental state of the entire litter, were taken. External examination of each fetus was conducted to record the external anomalies. Crown rump measurement, indicating the fetal length was also recorded. Sex of all the fetuses was determined by observing the distance between the anus and the genital tubercle ⁴² (Fig.17).

On completion of external observation one third of the fetuses randomly selected, were fixed in Bouin's fluid⁴³ for Wilson razor blade sections. The composition of Bouin's fluid was as follows :

Saturated aqueous Picric acid		75 ml
Formaldehyde		25 ml
Glacial acetic acid		5 ml

The Zenker's fluid is also usable, but Bouin's fluid has the advantages causing less shrinkage and as it is sufficiently acidic it calcifies bony structures within a week or two.^{30,45} Before sectioning, the fetuses were again observed for external malformations. The legs and tail were clipped off with scissors. The neck was firmly held between fingers and the fetus was placed in a supine position on a block. A sharp razor blade was

inserted between the jaw to examine the palate. One mm transverse sections of the head and trunk were cut and subjected to observation under magnifying lens of high order.

The remaining two thirds of the fetuses were subjected for examination of skeletal anomalies. Fetuses were fixed in 95% alcohol for a period of 48-72 hours. They were placed in a solution of 1% aqueous KOH, till the bones were clearly visible through the surrounding tissue. The fetuses were then stained. A dilute solution of Alizarin red S in KOH (one part of Alizarin red S to 10,000 parts of 1% KOH) was used as a staining solution. The specimen were kept in the contact with stain for 4-6 hours. Then the staining solution was removed. After removal of the staining solution three types of clearing solutions were added to the stained fetuses for clearing. The clearing solutions were :

20% Clearing solution : Water 79 parts, Glycerine
20 parts and KOH one part.

30% Clearing solution : Water 69 parts, Glycerine
30 parts and KOH one part.

40% Clearing solution : Water 59 parts, Glycerine
40 parts and KOH one part.

The stained and cleared specimen were preserved in pure Glycerine, for examination of skeletal anomalies.⁴⁴

The results of the studies were expressed as mean $\pm$ S.E. and were statistically analysed.

5. RESULTS

R E S U L T S

In the selection of doses for teratological studies, it is essential to select doses which are not maternally toxic but toxic to the fetus.⁴⁷ This is because we are looking for toxic effects on the fetus at dose levels below those producing toxic effects in the mother. Toxic doses to mother may directly affect the mother resulting even in maternal mortality and resorption. For this purpose an indication is obtained regarding the maternal toxic doses from an acute toxicity study of the test sample. From the acute toxicity data, a preliminary range finding study is carried out for selection of subsequent doses for teratogenic studies.

In the present study, the acute oral toxicity of the test materials was carried out (Dimethoate 30 EC, Aspirin and Tween 80). Results show that the LD₅₀ of Dimethoate 30 EC was found to be 776 mg/kg body weight with fiducial limits between 662.4 (lower limit) and 905.6 (higher limit). The pattern of symptoms noted in Dimethoate acute toxicity were tremors, hyperexcitation, convulsions, respiratory disturbances and death. It has been reported that mothers show tendency of spontaneous malformation, normally which cannot be attributed to chemical insult.⁴⁶ Under such circumstances, it becomes

essential to take two control groups of animals which serve as negative control (rats on normal diet only) and positive control (Aspirin at 250 mg/kg). These groups were taken to compare the spontaneous anomalies and the anomalies occurring due to chemical insult.

The LD₅₀ of Aspirin (Positive control) in our study was found to be 1600 mg/kg with fiducial limits between 1386.7 (lower limit) and 1844.8 (higher limit) (Table-1). In the LD₅₀ study with Tween 80 the vehicle control for Aspirin, no mortality and toxic symptoms were observed even with 50 ml/kg and hence the LD₅₀ seemed to be more than 50 ml/kg body weight which is practically non-toxic.⁴⁵ From the range finding study, based on the LD₅₀ data, the doses were calculated for the final study. Animals administered with 77.6 mg/kg (1/10th of LD₅₀) and 51.73 mg/kg (1/15th LD₅₀) of Dimethoate 30 EC resulted in maternal toxic symptoms such as tremors, hyperexcitations, gasping, and convulsions, and produced 100 % resorptions. The final doses selected for fetal anomalies were :

38.8	mg/kg	...	1/20th LD ₅₀	...	High dose
25.87	mg/kg	...	1/30th LD ₅₀	...	Intermediate dose
15.52	mg/kg	...	1/50th LD ₅₀	...	Low dose

The body weight of all the dams from different groups were recorded from day 6 to day 20th of gestation period. The body weight of dams from day 6 to 20 of gestation period increased significantly in all the groups irrespective of the treatment they received (Table - 5). However, when the percent weight gain between the Dimethoate treated groups and the negative control group were compared, there was a marked difference (Graph - 1). This was also observed with Aspirin and its vehicle control Tween 80 (Graph - 2). The percent gain in body weight of the dams receiving 38.8 mg/kg (high dose) was significantly lower than those observed in the negative control group. Similarly the percent weight gain in the dams treated with Aspirin was significantly lower than its control (Tween 80). The percent weight gain in dams of the low dose group (15.52 mg/kg) and the intermediate dose (25.87 mg/kg) were comparable with the negative control (Table - 2) (Graph - 1).

One rat from the positive control group and one rat from the high dose group died before term. The pregnancy rate at necropsy was similar in all groups of dams (75 to 80 % in each group) (Table - 5). The number of implantation sites was not similar in all the groups receiving Dimethoate. The position of implantation sites was also variable in the right and left uterine horn in

different groups. A high percentage of resorption was observed in the high dose group of Dimethoate at 38.8 mg/kg (62 %) as well as in the positive control group, Aspirin at 250 mg/kg (80 %) (Fig. - 3). The percentage of resorption in the dams receiving Dimethoate was found to be dose related. As the dose of Dimethoate decreased so also the percent resorption (45 % in the intermediate dose group and 29 % in the low dose group). The negative control group showed 17 % and the vehicle control group 20 % resorption respectively (Table 5).

Amongst the fetuses of rats receiving Dimethoate there were severe growth retardation, runted fetuses, syndactyly (webbed or fused digits) short and abnormal tail, hydrocephalus, cleft palate, microphthalmia (abnormal smallness of the eye), micromelia (abnormal smallness of the limb), ectrodactyly (congenital absence of digits), drop foot and twisting of the limb. External examination of the fetuses receiving high dose of Dimethoate 30 EC showed marked reduction in body size (Table - 5). Two fetuses of the high dose group and one from the positive control group exhibited meningocoele (Fig. 4). Amongst the fetuses of the rats receiving high and intermediate dose, there was severe growth retardation (Fig. 5). The incidence of runted fetuses was comparatively higher in the high dose group. The limb

size of the growth retarded fetuses was abnormally small (micromelia) with one fetus of Dimethoate high dose group with amelia (congenital absence of limbs) (Fig. 6). Seventeen percent of the fetuses receiving high dose of Dimethoate had webbed paws (Fig. 7). The incidence of webbed paws was also observed in fetuses of the positive control group though of low percentage (5.26 %) (Table - 4; Fig. 8). In small sized fetuses of each group external organs such as ears and eyes were not well developed. The anomaly namely, twisting of the limb was observed in the high dose group of Dimethoate and the positive control group (Fig. 4). In the Dimethoate high dose group, fetuses with curled and short tail constituted 17.14 % (Table - 4) (Fig. 4). Other non significant anomalies such as depigmented skin were observed in one fetus of the high dose group and two fetuses of the positive control group. However, the incidence of these external anomalies was low in the intermediate and the low dose group in comparison with high dose group (Table - 4).

The length and the weight of the fetuses of the dams which received Dimethoate in different doses were significantly lower than their controls (Table - 5; Graph - 3, 5). When both these parameters were expressed as percent of their control, a pattern like dose response relationship was found to be evident. The weights of the

fetuses of dams receiving 38.8 mg/kg, 25.87 mg/kg and 15.52 mg/kg were 43 %, 70.11 % and 88.04 % respectively of the control fetal weights. Similarly the lengths of the same fetuses were 40.47 %, 67.92 % and 93.63 % respectively of the control fetal length values. In case of Aspirin, the fetal weights and lengths were 60.78 and 59.43 % of the control values.

Fetuses stained with Alizarin red S were observed for skeletal defects (Fig. 9). The defects of the skull such as missing and underdeveloped bones, cranioschisis were observed (Fig. 10). A high percentage of skull defects was round to be evident in the high dose group of Dimethoate (65.21 %) and in the positive control group (83.33 %) when compared with respective control values of 6.06 and 5.88 % (Table - 6).

Vertebral defects such as extra vertebrae, missing caudal and thoracic vertebrae and spina bifida were observed (Fig. 11). The incidence of missing and underdeveloped sternum, missing sternebrae, wavy, missing and extra ribs were observed in each of the Dimethoate test group. Incidence of missing sternum and other sternal defects was much higher in the high dose group (60.87 %) as compared to the intermediate dose group (20.51 %) and 3.87 % in the low dose group (Table - 6). High incidence

of sternal defects were also observed (75 %) in the Aspirin group as compared to its control (3.92 %) (Fig. 12) Tween 80. Majority of the fetuses of the high dose group and the positive control group were characterized by the absence of digits of the both the hind limbs and the fore limbs. The absence of phalanges was common amongst Dimethoate test groups (Fig. 12). The percentage wise hind limb defects were 78.26 % in the high dose group, 20.51 in the intermediate dose group, 3.85 % in the low dose group as compared to the negative control with zero percent anomalies. Similarly the percentages for fore limb defects were 73.91 % in case of high dose group, 20.51 % with intermediate group and 3.85 % in the low dose group. The incidence of skeletal anomalies decreased with the decrease in dose of Dimethoate 30 EC. A very low incidence of skeletal anomalies were observed in the negative control group and the vehicle control group.

Fetuses preserved in Bouin's fluid were sectioned by the Wilson razor blade technique. Evaluation of visceral anomalies showed underdeveloped organs. Incidence of cleft palate was as high as 42.86 % in the positive control group and 16.67 % in the high dose group of Dimethoate 30 EC as compared with their respective controls. (Figs. 13 & 14). The occurrence

of brain defects were 50 % in the high dose group of Dimethoate 38.8 mg/kg, 15 % in the intermediate dose and 3.85 % in the low dose group. The negative control group showed 3.33 % brain defects. Hydrocephaly was seen in two fetuses of the high dose group of Dimethoate and in one fetus of the positive control group. This anomaly was not evident in any other group.

Sections passing through the lung and the oesophagus revealed underdeveloped organs. The percentage was 41.67 % in high dose group with 15 % in the intermediate dose group and 3.85 % in the low dose group with a control value of 3.33 % (Fig. 16). On the other hand, Aspirin showed 42.86 % and its control group 6.90 %. One fetus from the high dose group and one from the positive control group exhibited a missing septum in the heart (Fig. 16). None of the fetuses of the other groups revealed this anomaly. In small fetuses size of the organs were comparatively small. Similarly, sections passing through the liver, stomach, kidney, or the urinary bladder showed underdevelopment (Fig. 15).

All the organs were observed at their respective positions and the viscera intact. The visceral anomalies decreased with the decrease in dose of the test material. The percentage of visceral anomalies was of a very low incidence in the control groups (Table - 7).

TABLE-1 : Acute oral toxicity of Dimethoate 30 EC,
Aspirin and Tween-80 in rats.

Product	Lower * limits (mg/kg)	LD ₅₀ (mg/kg)	Higher * limits (mg/kg)
1. Dimethoate 30 EC	662.4	776.0	905.6
2. Aspirin	1387.7	1600.0	1844.8
3. Tween-80	More than	53250 mg/kg (50 ml/kg)	

* At 95% confidence levels.

TABLE-2 : Comparative percent weight gain during the gestation period of Control and Treated Rats.

Group No.	Treatment	No. of observations	Percent Weight gain in gms. (Mean $\pm$ S.E.)
1.	Negative control	20	16.90 $\pm$ 1.66
2.	Positive control (Aspirin 250 mg/kg)	19	9.68 $\pm$ 0.92
3.	Vehicle control	20	14.43 $\pm$ 1.67
4.	High Dose Dimethoate 30 EC 38.8 mg/kg	19	* 8.82 $\pm$ 0.86
5.	Intermediate Dose Dimethoate 30 EC 25.87 mg/kg	20	13.83 $\pm$ 1.50
6.	Low Dose Dimethoate 30 EC 15.52 mg/kg	20	14.72 $\pm$ 1.59

* Percent weight gain was significantly lower than that observed in the negative control. ($p < 0.05$).

TABLE-3 : Implantation sites and live fetuses of control and treated rats.

Sr. No.	Group/Treatment	Number of observations	Implantation sites	Percent Alive
1.	Negative Control	16	7.19 $\pm$ 0.43	81.81 $\pm$ 8.30
2.	Positive Control (Aspirin 250 mg/kg)	15	6.60 $\pm$ 0.36	17.38 $\pm$ 9.37
3.	Vehicle Control (Tween-80)	15	6.80 $\pm$ 0.63	73.48 $\pm$ 9.65
4.	Test : High Dose Dimethoate 38.8 mg/kg (1/20th LD ₅₀)	14	7.21 $\pm$ 0.57	28.55 $\pm$ 11.33
5.	Intermediate Dose - Dimethoate 25.87 mg/kg (1/30th LD ₅₀)	16	6.75 $\pm$ 0.60	43.89 $\pm$ 11.85
6.	Low Dose - Dimethoate 15.52 mg/kg (1/50th LD ₅₀)	16	6.94 $\pm$ 0.59	69.79 $\pm$ 10.99

TABLE-4 : Incidence of anomalies in fetuses from Control & Treated Rats.

No.	Group/Treatment	Meningo- cele	Depigmen- ted skin	Micro- melia	Amelia	Webbed paws	Short and coiled tail
1	Negative Control	0/96 (0%)	0/96 (0%)	1/96 (1.04%)	0/96 (0%)	0/96 (0%)	1/96 (1.04%)
2	Positive Control (Aspirin 250 mg/kg)	0/19 (0%)	2/19 (10.53%)	2/19 (10.53%)	0/19 (0%)	1/19 (5.26%)	2/19 (10.53%)
3	Vehicle Control (Tween-80)	0/80 (0%)	0/80 (0%)	1/80 (1.25%)	0/80 (0%)	0/80 (0%)	1/80 (1.25%)
4	Dimethoate- 32.8 mg/kg High dose $\frac{1}{20}$ th LD ₅₀	1/35 (2.86%)	1/35 (2.86%)	6/35 (17.14%)	1/35 (2.86%)	6/35 (17.14%)	6/35 (17.14%)
5.	Dimethoate- 25.87 mg/kg Intermediate dose : $\frac{1}{30}$ th LD ₅₀	0/59 (0%)	0/59 (0%)	3/59 (5.08%)	0/59 (0%)	1/59 (1.69%)	1/59 (1.69%)
6	Dimethoate- 15.52 mg/kg Low dose $\frac{1}{50}$ th LD ₅₀	0/78 (0%)	0/78 (0%)	1/78 (1.28%)	0/78 (0%)	0/78 (0%)	1/78 (1.28%)

TABLE-5 : Prenatal Data of Control and Treated Rats on day 6 through 15 of Gestation.

TREATMENT	D A M S				No. of impl- ants	FETUSES					RESORPTIONS	
	Total per group	Preg- nant at necro- psy per group (%)	Spont- aneous death per group	Weight gain % Day 6-20		Normal	Mal- form- ed	Dead	Mean body wt. (gms)	Mean body size (cms)	Total	%
Negative Control	20	16 (80%)	-	16.90 $\pm$ 1.66	115	96	-	-	3.911	3.835	19	16.52
Positive Control (Aspirin 250mg/kg)	20	15 (75%)	1	9.68 $\pm$ 0.92	99	16	3	1	2.06	2.03	79	79.80
Vehicle Control (Tween-80)	20	15 (75%)	-	14.43 $\pm$ 1.67	102	82	-	-	3.885	3.412	20	19.61
Test : High dose Dimethoate 30 EC 38.8 mg/kg 1/20th LD ₅₀	20	15 (75%)	1	8.82 $\pm$ 0.86	101	31	4	3	1.675	1.56	63	61.76
Test: Intermediate Dose Dimethoate 30 EC 25.87mg/kg 1/30th LD ₅₀	20	16 (80%)	-	13.83 $\pm$ 1.50	108	55	2	2	2.618	2.47	49	45.37
Test: Low dose Dimethoate 30 EC 15.52 mg/kg 1/50th LD ₅₀	20	16 (80%)	-	14.72 $\pm$ 1.59	111	78	-	1	3.443	3.58	32	28.83

TABLE-6 : Incidence of Skeletal Anomalies in Fetuses of Control & Treated Rats.

No.	Group/Treatment	Skull	Vertebrae & Verte- bral column	Ribs	Sternum	Hind limb	Fore limb
1.	Negative Control	4/66 (6.06%)	1/66 (1.52%)	0/66 (0%)	0/66 (0%)	0/66 (0%)	0/66 (0%)
2.	Positive Control (Aspirin 250 mg/kg)	10/12 (83.33%)	9/12 (75 %)	3/12 (25%)	9/12 (75%)	8/12 (66.67%)	10/12 (83.33%)
3.	Vehicle Control (Tween-80)	3/51 (5.88%)	6/51 (11.76%)	4/51 (7.84%)	2/51 (3.92%)	1/51 (1.96%)	0/51 (0%)
4.	TEST: High Dose Dimethoate 38.8mg/kg 1/20th LD ₅₀	15/23 (65.22%)	12/23 (52.17%)	10/23 (43.48%)	14/23 (60.87%)	18/23 (78.26%)	17/23 (73.91%)
5.	TEST : Intermediate Dimethoate 25.87mg/kg 1/30th LD ₅₀	10/39 (25.64%)	19/39 (48.72%)	7/39 (17.95%)	5/39 (20.51%)	8/39 (20.51%)	8/39 (20.51%)
6.	TEST : Low Dose Dimethoate 15.52mg/kg 1/50th LD ₅₀	3/52 (9.62%)	3/52 (5.77%)	6/52 (11.54%)	2/52 (3.85%)	2/52 (3.85%)	2/52 (3.85%)

Note : 1) The percentage of abnormality reported pertains to the defect which was observed in maximum numbers of fetuses of the total fetuses examined.

2) Defective fetuses/Total fetuses examined.

TABLE-7 : Incidence of Visceral Anomalies in Fetuses of Control & Treated Rats.

Group/Treatment	Palate	Brain	Thorax	Lung	Oeso-phagus	Heart	Liver	Stomach	Intest-ine	Kidney	Urinary bladder & Ureter
Negative Control	0/30 (0%)	1/30 (3.33)	1/30 (3.33)	1/30 (3.33)	1/30 (3.33)	1/30 (3.33)	1/30 (3.33)	1/30 (3.33)	1/30 (3.33)	1/30 (3.33)	1/30 (3.33)
Positive Control Aspirin 250mg/kg	* 3/7 (42.86)	3/7 (42.86)	2/7 (42.86)	3/7 (42.86)	3/7 (42.86)	3/7 (42.86)	3/7 (42.86)	3/7 (42.86)	3/7 (42.86)	3/7 (42.86)	3/7 (42.86)
Vehicle Control Tween-80	0/29 (0)	2/29 (6.90)	2/29 (6.90)	2/29 (6.90)	2/29 (6.90)	2/29 (6.90)	2/29 (6.90)	2/29 (6.90)	2/29 (6.90)	2/29 (6.90)	2/29 (6.90)
TEST: High Dose Dimethoate 38.8 mg/kg (1/20th LD ₅₀)	* 2/12 (16.67)	6.12 (50.0)	3/12 (25.0)	5/12 (41.67)	4/12 (33.33)	4/12 (33.33)	5/12 (41.67)	4/12 (33.33)	4/12 (33.33)	4/12 (33.33)	2/12 (16.67)
TEST: Intermed- iate Dose Dimethoate 25.87 mg/kg (1/30th LD ₅₀)	1/20 (5.0)	3/20 (15.0)	2/20 (10.0)	3/20 (15.0)	3/20 (15.0)	3/20 (15.0)	3/20 (15.0)	3/20 (15.0)	3/20 (15.0)	2/20 (10.0)	0/20 (0.0)
TEST: Low Dose Dimethoate 15.52 mg/kg (1/50th LD ₅₀)	1/26 (3.85)	1/26 (3.85)	0/26 (0.0)	1/26 (3.85)	1/26 (3.85)	2/26 (7.69)	3/26 (11.54)	2/26 (7.69)	2/26 (7.69)	2/26 (7.69)	2/26 (3.85)

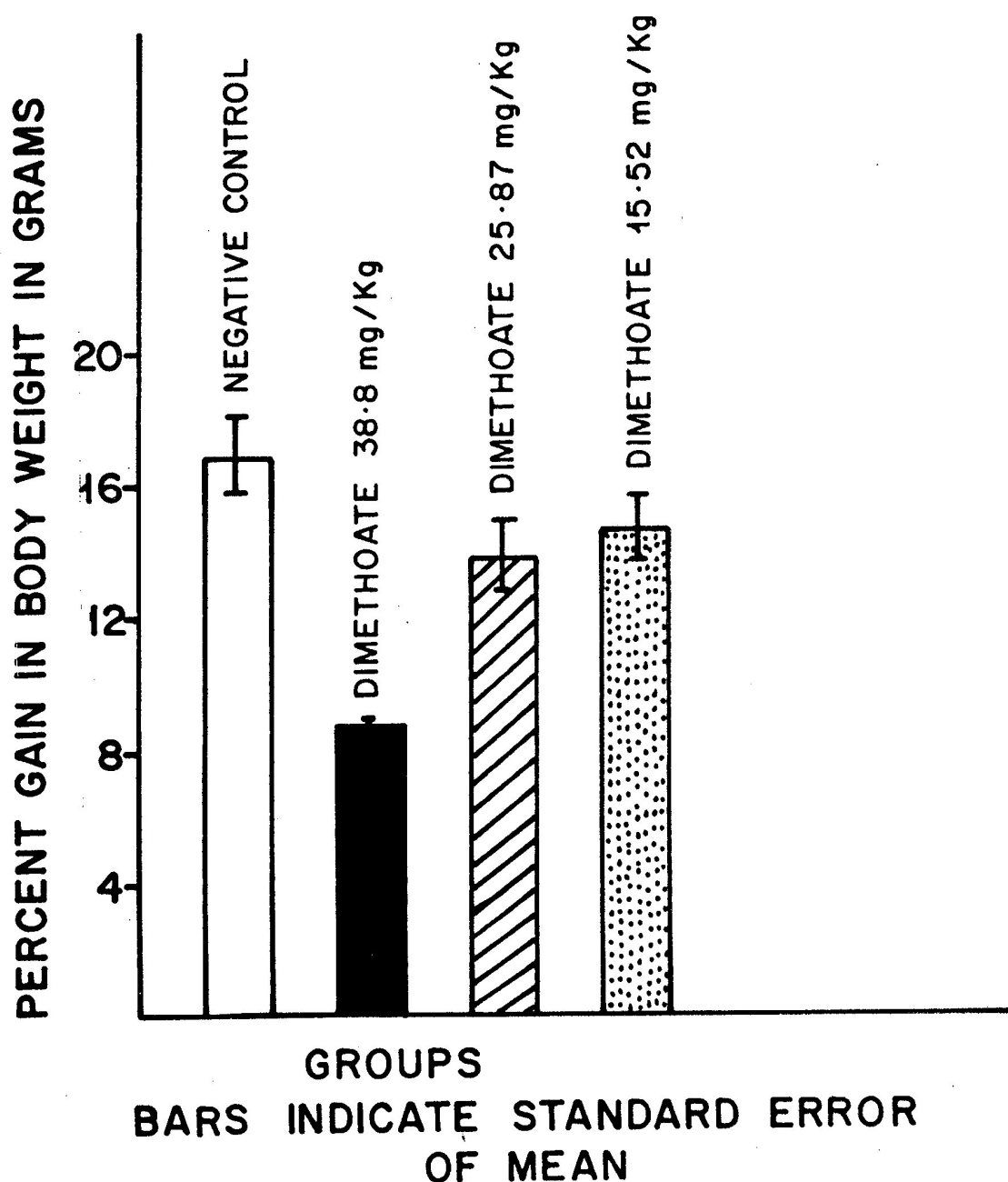
* Cleft Palate.

Note : Fetus exhibiting under developed organs was treated as anomalous fetus.

The figures in the parentheses denote percentage.

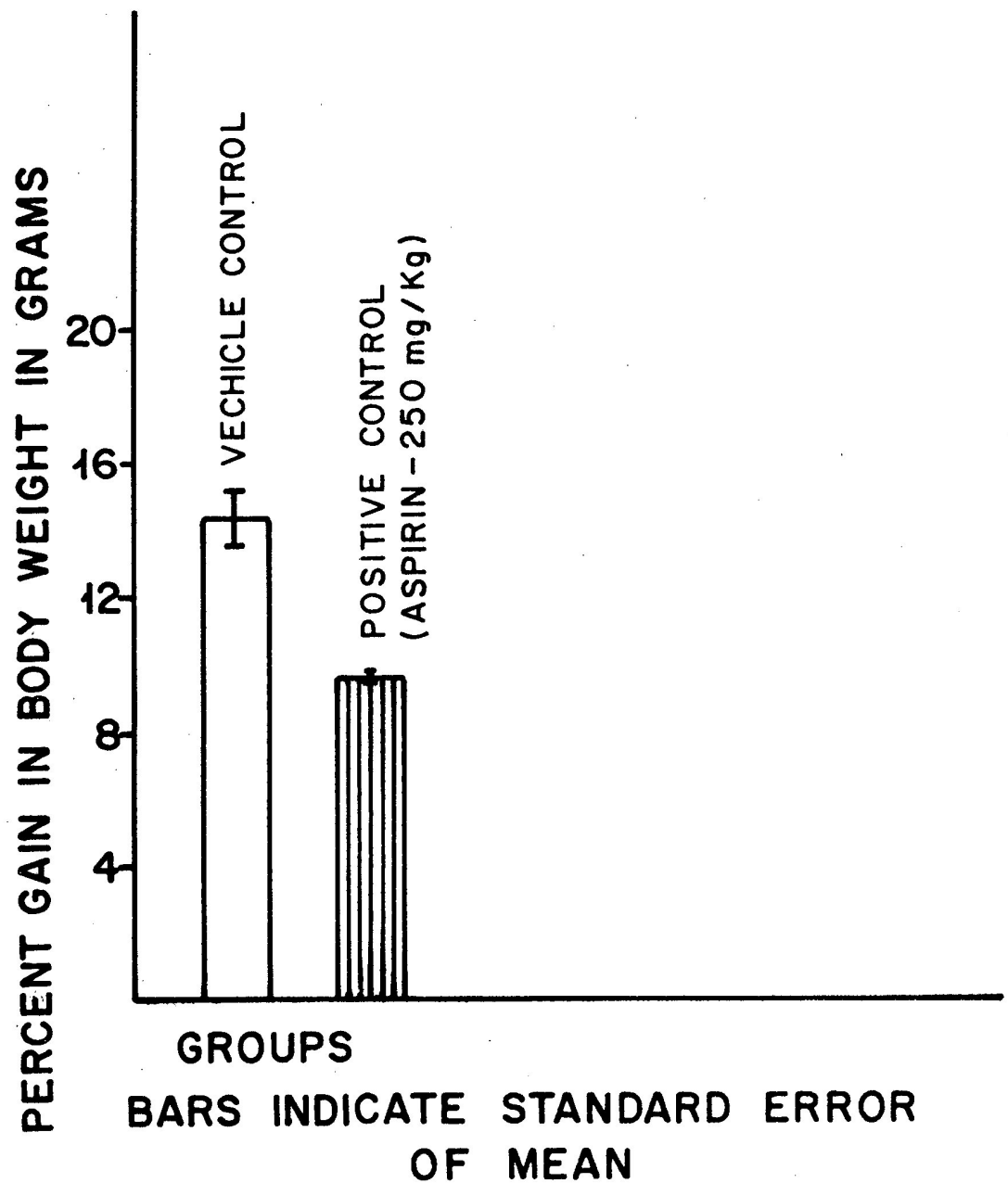
PERCENT GAIN IN BODY WEIGHTS OF
NEGATIVE CONTROL AND DIMETHOATE
30 EC TREATED RATS.

GRAPH - 1



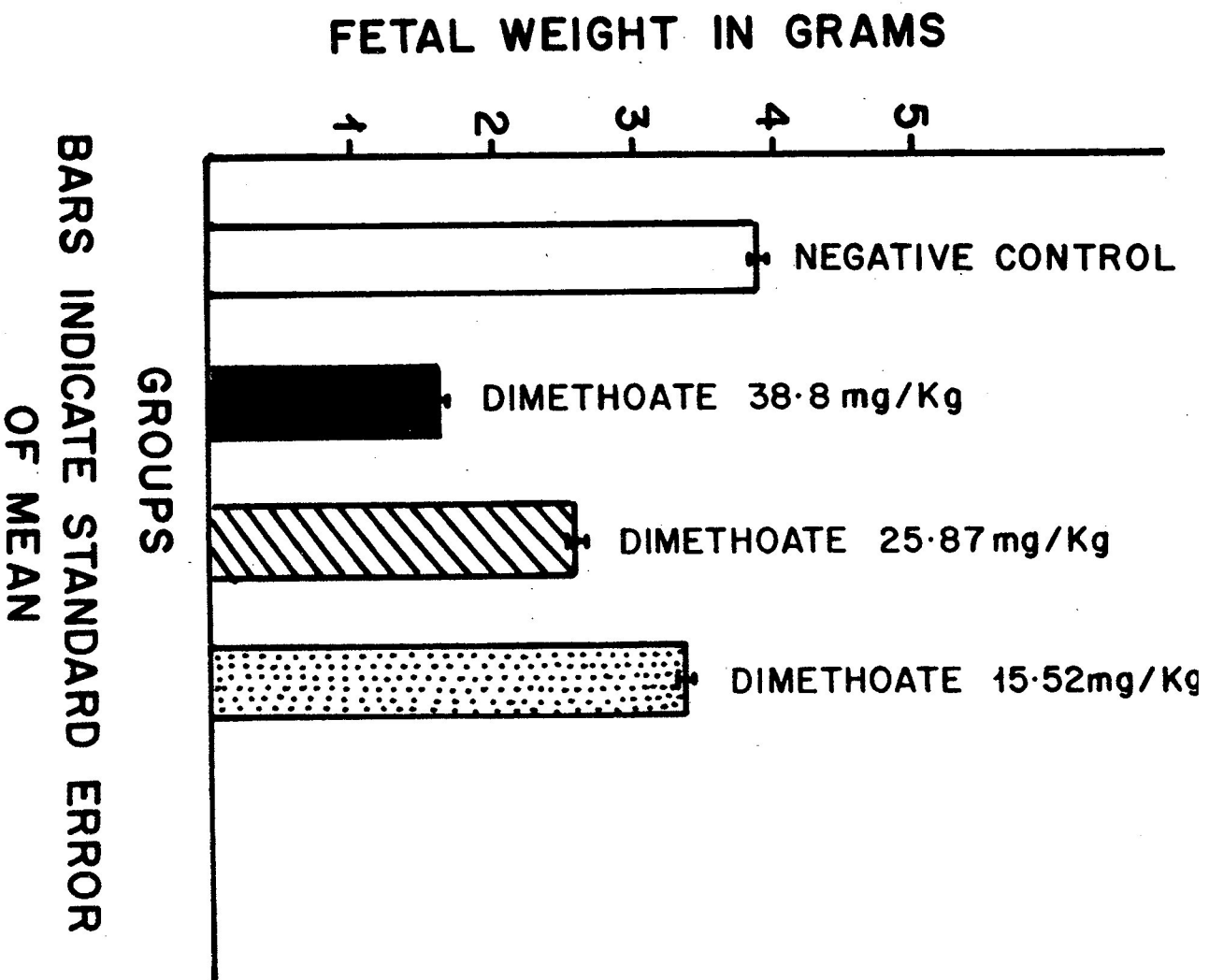
PERCENT GAIN IN BODY WEIGHT OF
VEHICLE CONTROL AND ASPIRIN
TREATED RATS.

GRAPH - 2



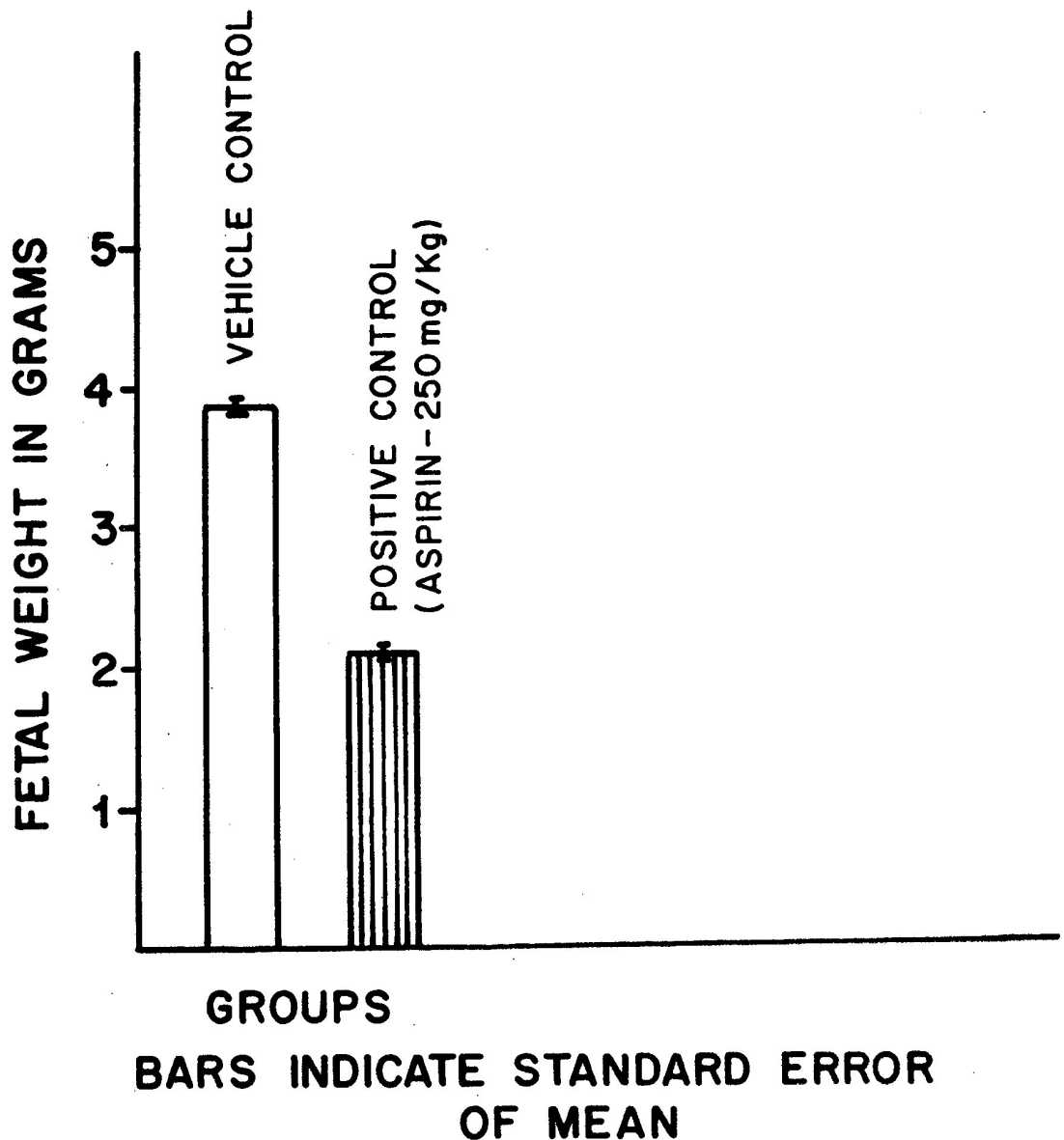
FETAL WEIGHTS OF CONTROL AND DIMETHOATE 30 EC TREATED RATS

GRAPH-3



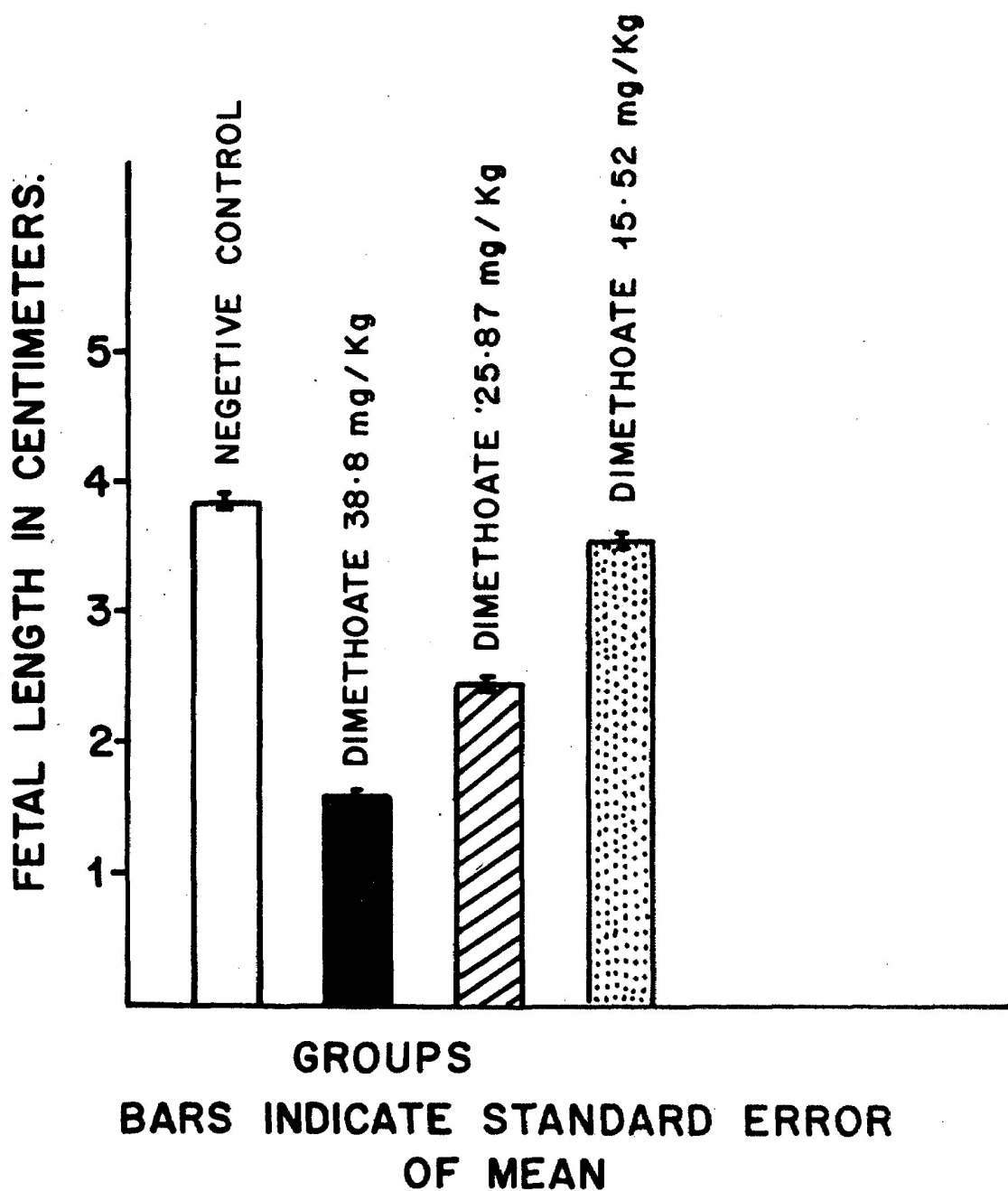
FETAL WEIGHTS OF VEHICLE CONTROL AND ASPIRIN TREATED RATS.

GRAPH - 4



FETAL LENGTHS OF NEGATIVE CONTROL AND DIMETHOATE 30 EC TREATED RATS

GRAPH - 5

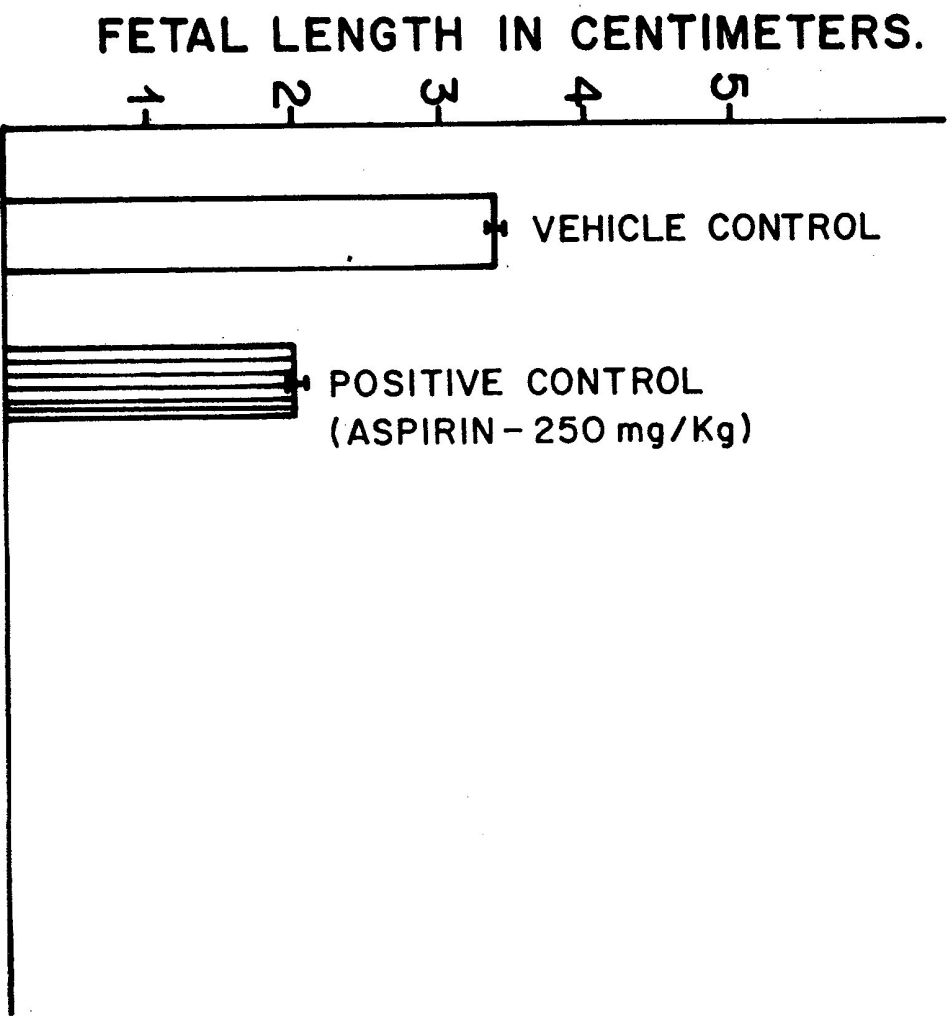


FETAL LENGTH IN CENTIMETERS.

GROUPS

BARS INDICATE STANDARD ERROR OF MEAN

Group	Mean Fetal Length (cm)
VEHICLE CONTROL	~3.2
POSITIVE CONTROL (ASPIRIN - 250 mg/Kg)	~2.0



6. DISCUSSION

DISCUSSION

Teratological studies have become an important part of toxicological studies and safety data requirements for regulatory purposes. It is estimated that about 130 million children are born every year all over the world and at least 2 to 3 % of them are born with birth defects.⁶

The thalidomide disaster stimulated teratological research all over the world. Since then various workers have correlated the use of Thalidomide in pregnant women and the increase in limb reduction anomalies in infants. Epidemiological and experimental investigations have revealed that congenital anomalies amongst humans are estimated as 20-30 % may be due to genetic chromosomal aberration, 10-20 % may be due to extrinsic factors like drug interactions, toxins and environmental pollutants and the rest may be due to a complex interaction of genetical and extrinsic factors.^{5,7,36,37}

The knowledge regarding teratogens can be obtained from case studies and anecdotal observations. Laboratory animals are presently utilized in the investigations of teratogenicity, since ethical and social factors prevent the use of human subjects. In

selecting the species for this study, various factors are taken into consideration such as their gestation period, availability in large numbers, ease of maintenance in the laboratory and economics. Animals should have a fairly short gestation period, a known oestrous cycle, an easily determinable time of conception and high fertility for teratological studies. The animals should bear large number of litters to make different studies possible, their embryological development and the occurrence of spontaneous anomalies must be well documented. These criteria are probably best met by the rat, mouse, rabbits and the hamsters.⁵

Amongst mammals, the rodents are the animals of choice. The rat has a short gestation period of about 21 days, the time of copulation or mating can be measured accurately, its embryology is well known and its placental and hormonal characters are similar to those of the humans.⁵ Also in this species, the spontaneous occurrence of malformations has been well documented. Rats bear large number of litters, so that a teratologist can couple studies such as skeletal anomalies and visceral anomalies together. It is also economic and easy to maintain. Though for some compounds, the rats are less susceptible as compared to the humans because of differences in absorption, metabolism and excretion, the

rat nevertheless is one of the choice species.⁵

In the present study with rats, virgin animals of the conceiving age were used. Virgin females are used so that there are less chances of increasing the incidence of previously existing malformations by the teratogens. The test material was administered from day 6 to day 15 of the gestation period. This is the period of organogenesis and is the most susceptible period for chemical insult.^{5,7,31,49}

The pre-implantation period is characterized by fertilization, blastulation and gastrulation. This stage is susceptible to embryoletality but rarely to teratogenecity. Insult during this period may result in embryonic death or repair of the damaged cells.⁴⁸ This period is the first six days in the rat and mice and six days in the hamsters and rabbits. The pre-implantation period is followed by the period of organogenesis, which is from day 6 to 15 in rats and mice, day 6 to 10 in hamsters, day 6 to 18 in the rabbits and approximately third to 8th week in the humans.^{5,7,48} During this period, migration and association of cells and tissues into organ rudiments takes place, hence this is the most vulnerable period for production of structural anomalies. Insult during this period can lead to structural birth

defects and embryoletality. Many workers have produced anomalies in animals by chemical insult during the period of organogenesis.^{36,49} It has been reported that the birth defects due to Thalidomide occurred when the drug was consumed by pregnant women from day 27 to 40 of gestation period, the critical period of organogenesis.³⁷ The fetal period after the period of organogenesis is increasingly resistant to teratogens though there are exceptions.

Two control groups are also taken for comparison. The negative control group is used to study the occurrence of spontaneous malformations under normal conditions of maintenance. The positive control group is used to compare the effects due to chemical insult.

In hamsters and rats the positive control is Aspirin at 250 mg/kg, whereas, in mice Aspirin at 150 mg/kg is used. In rabbits, the positive control used is 6-amino-nicotinamide at 2.5 mg/kg and is administered on day 9 only.⁵

The route of administration is an important factor. It is selected on the basis of human exposure. The route of administration selected in our study was the oral route. Twenty mated animals are allotted to each group

Various end points are recorded and the data obtained from animal studies are then extrapolated to the human beings. This is a very important process and a difficult one too. No single animal model is close to the human system and hence no single animal test provides assurance of safety to the human beings. This is mainly because of variation in species response. Many other factors are involved in chemical induced teratogenesis which cause difficulty in extrapolating results from animal studies to human beings. These factors include differences in genotype, genetic heterogeneity, variation in body size, diet and conditions of exposure.^{30,48} Teratogenic testing in animals can be regarded as an indication of the probability that the test substance will or will not produce teratogenicity in humans. Also from animals tests, teratogens that cannot be identified by epidemiological studies can be identified.

In the present study, the acute oral toxicity of Dimethoate 30 EC was carried out. The LD₅₀ value observed in the acute study serves to provide a convenient way to classify pesticides into toxicity classes and the value also serves as a guide for selecting subsequent doses for long term studies. The LD₅₀ of Dimethoate 30 EC was found to be 776 mg/kg body weight as compared to Dimethoate technical which is 320-380 mg/kg. In a study,

the LD₅₀ of Dimethoate technical of 95 % purity in rats by oral route was reported to be 130-325 mg/kg⁵⁰ and in another study it was found to be 200-300 mg/kg body weight.⁵¹ In a study regarding susceptibility between sexes, it was concluded that there was no significant difference between sexes to acute lethal effects of Dimethoate.⁵⁰ Based on known comparative toxicity data and degradation in liver in other species, the acute oral LD₅₀ of Dimethoate to humans was predicted to be about 30 mg/kg.⁵² In our study the pattern of symptoms noted in the acute oral toxicity of Dimethoate 30 EC were tremors, hyperexcitation, convulsions, respiratory disturbances and death.

The acute oral toxicity of the positive control, Aspirin and its vehicle control Tween 80 was also carried out (Table 1). The LD₅₀ of Aspirin was found to be 1600 mg/kg which correlates with the reported value.⁵³ The LD₅₀ of Tween 80 was found to be more than 50 ml/kg as no mortality and toxic symptoms were observed at this dose. As a suspending agent, Tween 80 was nontoxic, as per the classification of toxicity classes.⁴⁵

In teratogenic studies, a toxic dose that is maximally toxic to the fetus is of importance as we are observing the toxic effects on the fetus and not on the

mother. Maternal toxic doses may cause resorptions and even maternal mortality. Maternally toxic doses of the teratogenic agents may kill too many cells to permit survival and cause embryoletality. Therefore on the basis of the LD₅₀ data and variation in animal response, a range finding study was carried out to obtain an indication of the maternal toxic doses and the fetotoxic doses.

In our study animals administered with 77.6 mg/kg (1/10th of LD₅₀) and 51.73 mg/kg (1/15th of LD₅₀) of Dimethoate 30 EC resulted in maternal toxic symptoms such as tremors, hyperexcitation, gasping, convulsions and death and produced 100 % resorptions. Therefore the doses below these levels were tried and the final doses selected for fetal anomalies were -

38.8 mg/kg	... 1/20th LD ₅₀	... High dose
25.87 mg/kg	... 1/30th LD ₅₀	... Intermediate dose
and 15.52 mg/kg	... 1/50th LD ₅₀	... Low dose.

In a study with Dimethoate formulation 47.3 % EC in rats the dose of 24 mg/kg was found to be maternally toxic dose. This dose produced maternal toxic symptoms such as clonic spasms and muscular tremors.²⁹ Gasping and tremors were also observed in our study where rats were administered with doses of 77.6 mg/kg and 51.73 mg/kg.

Similarly in another study in mice dose levels of Dimethoate at 40 and 80 mg/kg produced a high incidence of maternal mortality.⁴ In our study maternal mortality was observed at 77.6 mg/kg dose.

Regular measurements of body weights during the gestation period is important as it may provide a reference point to judge the effect on fetal size. In our study, the body weights were recorded from day 6 to day 20 of the gestation period as the test materials were administered from day 6 of the gestation period. The body weight of the dams increased significantly in all the groups of dams irrespective of the treatment they received (Table - 5, Graphs - 1 & 2). However, when the percent weight gain between the Dimethoate treated groups and its control group were compared, there was a marked difference. Dose of 38.8 mg/kg resulted in decrease in maternal weight gain. The percent weight gains of 25.87 mg/kg and 15.52 mg/kg doses were comparable with the control (Graph 1). In a study with Dimethoate formulation of 47.3 % EC in rats at 24 mg/kg resulted in reduction in body weight²⁹ as found in our study with Dimethoate 30 EC. In another mice study with Dimethoate, dose levels of 10 and 20 mg/kg produced significant decrease in maternal weight gain.⁴ The decrease in maternal weight gain at the dose of 38.8 mg/kg in our study suggests that

Dimethoate had an effect on maternal weight gain. The percent weight gain in dams treated with Aspirin was significantly lower than its control in our study (Tables - 2,5, Graph 2).

One rat from the positive control group and one rat from the high dose group died before term. Post-mortem did not reveal any gross pathology. This can be attributed to spontaneous death. As the rodents tend to eat their offspring at birth, the fetuses were delivered by cesarean section (Fig. 1). Also by delivering the fetuses by cesarean, the number and position of implantation sites can be counted. In the reported study, Dimethoate formulation of 47.3 % EC and technical grade pesticide (95%) did not alter the number of implantations.^{4,29} Study with other organophosphate pesticides Dipterex and Imidan did not reveal any effect on the implantation sites too.^{21,22} In our study there was no significant difference in the implantation sites in Dimethoate treated groups.

The percentage of resorption in the dams receiving Dimethoate in our study was found to be dose related. A high percentage of resorption was found in the high dose group (62%). As the dose of Dimethoate decreased, so also the percent resorption (45% in the intermediate dose and 29% in the low dose group) (Fig. 3). High

percentages of resorption and embryonic death have been reported in teratogenecity studies with organophosphate pesticides. A high incidence of still births and neonatal death rates was reported in dams treated with parathion, Methyl parathion and Isofluorophate (DFP).¹⁹ In another study, Parathion at 12 mg/kg dose when administered on days 12, 13, 14 of pregnancy produced 90% resorptions.²⁰

In a study with Dipterex, it was observed that Dipterex resulted in an insignificant increase in embryonic death at the dose of 80 mg/kg ($1/10$ th of LD_{50}) when administered to rats on day 9 of gestation. Imidan produced increase in post implantation mortality of the embryos at the doses of 1.5 mg/kg and 30 mg/kg when given throughout the course of the pregnancy and on day 9 of gestation respectively.²¹ An interesting result was obtained while observing Parathion for teratogenecity. When Parathion was administered on days 12, 13 and 14 of gestation period to mice at the dose of 12 mg/kg, produced 90% resorptions, whereas the same dose on days 9 and 10 of gestation produced 27% resorptions.²⁰ This study confirms the importance of time and duration of administration of teratogens. A high percentage of resorption was observed in our study with Aspirin, the positive control. It is reported that Aspirin at the doses of

250 mg/kg and above increases the incidence of resorption.^{35,40,56}

Amongst the fetuses of rats receiving Dimethoate, there were severe growth retardation (Fig. 5), runted fetuses, syndactly (webbed or fused digits), short and abnormal tail, hydrocephalus, cleft plate, microphthalmia (abnormal smallness of the eye), micromelia (abnormal smallness of the limb) (Fig. 6), ectrodactly (congenital absence of digits), drop foot and twisting of the limb. These anomalies were observed on a large percentage in the high dose group (Table - 4). Anomalies such as meningocoele was observed in two fetuses of the high dose group but were not observed in any other groups (Fig. 4). Fetuses that exhibited a small body size showed under-developed eyes and ears, specially in the high dose group. Nonsignificant anomalies as depigmented skin were observed in one fetus of the high dose group and the positive control group.

In earlier reported teratological studies of Dimethoate technical and formulations, fetuses exhibited severe growth retardation. The incidence of external anomalies was nonsignificant.^{4,29} Other organophosphates, Dipterex at the dose of 80 mg/kg on day 9 of gestation and Imidan at the dose of 30 mg/kg resulted in anomalies

as exencephaly, non-closing eyelid symptoms, hypognathia, dislocation of extremities and hematomas.²¹ When Imidan was administered at the dose of 1.5 mg/kg daily throughout the gestation, subcutaneous haemorrhages were observed.²¹ When Dipterex was administered in the diet at dose levels of 432 and 519 mg/kg, 33 of the 109 malformed fetuses revealed exencephaly, meningocoele and hydrocephaly.²² Dipterex gavage at the dose of 480 mg/kg three times daily in rats resulted in generalized oedema, hematoma and various types of herniation of the brain. A similar type of response was obtained after administration of Dipterex to hamsters from days 7 to 11 of gestation at 400 mg/kg dose.⁵⁵ In a study with Chlorpyrifos in mice, at the dose of 1 mg/kg exencephaly was observed.²⁵ The above anomaly and anomalies as hematomas and hypognathia were not observed in any of our test groups.

The length and weights of the fetuses of dams receiving Dimethoate in different doses were significantly lower than their control (Graphs - 3,5). When both these parameters were expressed as the percent of their control, a pattern like dose response relationship was found to be evident. The fetal lengths and weights of the dams receiving high and intermediate dose were less than that of their control, whereas the fetal length was comparable

with the control in case of the low dose group. Various workers have reported the effects of pesticides on fetal weights. In a mice study with Dimethoate, it has been reported that the fetal weights for the 20 mg/kg dose levels were significantly lower than its control, and has been correlated to the reduction in maternal weight. In our study the weight of the fetuses were lower with the dose of 38.8 mg/kg of Dimethoate 30 EC which also reflected on the maternal weight gain, and hence may be related. Similarly teratogenic studies with other pesticides as Methyl parathion, Parathion, Isofluorophate, Thimet, Dipterex and Imidan have resulted in reduction in fetal weights.^{19,22,27} The fetal weights for Aspirin, the positive control in our study were also less than its control Tween 80 (Graphs- 4 & 6).

Fetuses stained with Alizarin red S were observed for skeletal defects (Table-6). A high incidence of skeletal defects were observed with the high dose of Dimethoate 30 EC. These defects were dose related. As the dose of Dimethoate decreased, so also the skeletal defects. Defects of the skull such as improper fusion of the skull bones were found to be evident in high dose group with a high percentage (65.22%) (Figs. 10 & 11). Vertebral defects such as extra vertebrae, missing caudal and thoracic vertebrae and spina bifida were also observed.

The incidence of missing and underdeveloped sternum, missing sternebrae, wavy missing and extra ribs were observed in each of the Dimethoate test group. The percentage of these defects were comparatively higher in the high dose group (Table-6; Figs. 10 & 11). Incidence of sternal defects, absence of digits of the both the limbs, with absence of phalanges being a common defect were higher in the high dose group of Dimethoate. The skeletal defects in case of the low dose group were comparable with the negative control group (Table- 6).

The causes and mechanisms of pesticide induced skeletal defects is not fully understood. Defects in the fetuses are believed to occur if there is interference in the stages of development. Also administration of the chemical during the period of organogenesis, may result in deviations in development from normality and produce malformations. For example, the defect spina bifida results from the failure of closure of the posterior neuropore.⁵⁷ This neuropore closure in the rat takes place upto the 12th day of gestation period. Administration of the teratogen during this period may increase the susceptibility of the embryo to the occurrence of spina bifida.

Administration of organophosphate pesticides during the period of organogenesis and the resulting skeletal

anomalies are reported and correlate with the findings of our study. Dimethoate decreased significantly the number of calcified centres of the fore paws at the doses of 20 mg/kg. The incidence of missing ribs was found to be dose related and was significant at dose levels of 40 and 80 mg/kg.⁴ In another study with Dimethoate formulation of 47.3 % EC it was observed that 12 and 24 mg/kg doses were associated with a significant increase in the number of anomalous litters and wavy ribbed fetuses. Other anomalies observed were, presence of extra ribs and fused sternebrae.²⁹ In our study too, high incidences of these anomalies were observed with the high dose group of 38.8 mg/kg.

Skeletal anomalies have been reported with other organophosphate pesticides. Dipterex with the dose of 80 mg/kg resulted in wavy ribs.²¹ The same pesticide at the dose of 480 mg/kg severely shortened the radius and ulna, caused hypophalangism and resulted in fused sternebrae and ribs.⁵⁵ Chlorpyrifos at the doses of 1, 10, 25 mg/kg resulted in minor skeletal variants.²⁵ In our study with Aspirin, the positive control exhibited number of skeletal anomalies. Skeletal anomalies of the skull spina bifida, sternal defects, rib and vertebral defects of various degrees were observed, which correlate with the reported findings.^{35,40,56} (Fig. 12).

Irregularities of ribs and vertebrae with salicylate poisoning of maternal rats during early embryonic developmental stage has been reported.³² Aspirin at 500 and 625 mg/kg doses on day 9 and 10 of gestation respectively induce a wide variety of skeletal variations such as extra ribs, fused or missing ribs, extra and missing vertebrae.⁵⁶ Similar defects were reported in another study with Aspirin and with similar doses.³⁵ A low incidence of skeletal anomalies was also observed in the control group of our study and correlates with earlier documentation. In an earlier reported study 1430 normal fetuses out of total 2155 rat fetuses were studied for the incidence of teratological anomalies. The most common skeletal anomalies found were improper ossification of skull bones. This incidence was found to be 11.05 %.⁴⁶ In our study, this incidence with the control group was found to be 7 % (Table - 7).

The visceral evaluation of the fetuses was done by Wilson's technique.⁴¹ It revealed a high incidence of underdevelopment, specially in the high dose group of Dimethoate 30 EC 38.8 mg/kg. The incidence of specific anomaly namely cleft palate was observed (Fig. 13). It has been explained that the palatal shelves rotate from a vertical position along the edges of the tongue to a horizontal position above the tongue. These shelves then fuse at the base of the skull to form the palate.

If this closure is delayed beyond a critical time cleft palate results.⁴⁸ It has been reported that in man, 80 % of the isolated cleft palate occurs due to environmental factors.⁵⁴ In our study cleft palate was observed only with high dose of Dimethoate 30 EC (38.8 mg/kg) and was not associated with cleft lip (Fig. 13). This anomaly was not observed with low and intermediate dose groups. When Dipterex was administered to rats, hamsters and mice on days 10 to 14 or 12 to 14 of gestation period at doses of 400 mg/kg, there was increase in the incidence of cleft palate.⁵⁵ The positive control, Aspirin in our study exhibited a high percentage of cleft palate (42.86 %) when compared with its control (Fig. 14). Various workers have reported the incidence of Aspirin induced cleft palate in rats at different doses.^{35,40,56}

The brain defect namely hydrocephaly was observed in two fetuses of the high dose Dimethoate group. This anomaly was not evident in any other test group. Sections passing through various organs showed underdeveloped organs (Figs. 15, 16). One fetus of the high dose group of Dimethoate 38.8 mg/kg exhibited missing septum in the heart. In a study with Dimethoate formulation of 47.3% EC at the doses of 12 and 24 mg/kg nonsignificant anomalies as hydroureter and dilated urinary bladder were reported.²⁹ Hydrocephaly was reported with Imidan.²²

7. CONCLUSION

C O N C L U S I O N

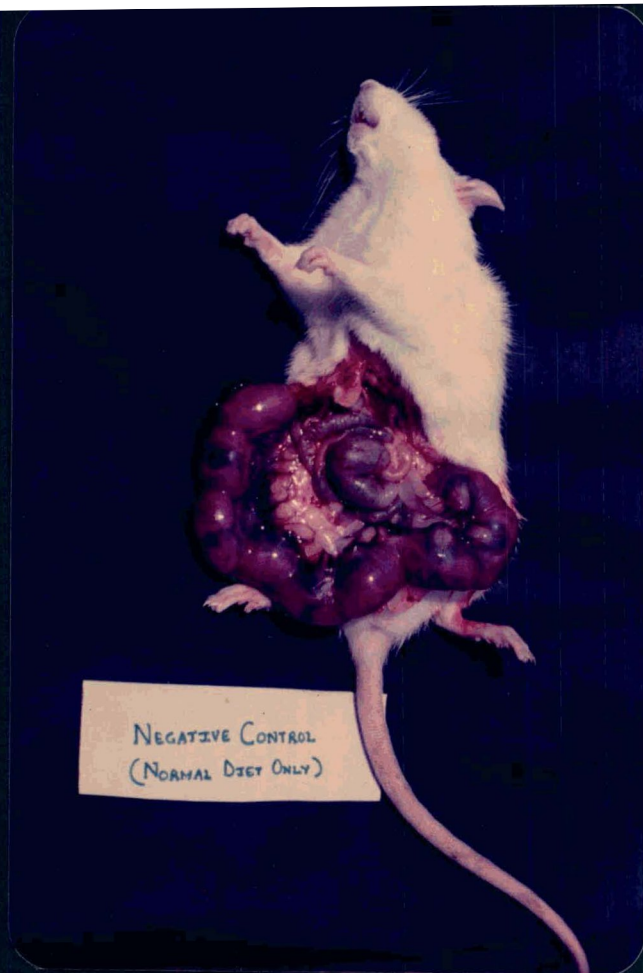
Teratological study was conducted with Dimethoate 30 EC, an organophosphate pesticide. Based on the results and discussions it may be concluded that :

The acute oral toxicity of Dimethoate 30 EC (LD_{50} 776 mg/kg body weight) was found to be less than that of the reported acute oral toxicity of Dimethoate technical (LD_{50} 130-325 mg/kg body weight). These results therefore suggest that the technical grade material is more toxic than the formulation. This may be probably attributed to the presence of less amount of technical grade material (30 %) and the presence of 70% of non-pesticidal ingredients.

Dimethoate 30 EC at the dose of 38.8 mg/kg was found to be teratogenic in rats, similar to that produced by Aspirin at 250 mg/kg. The doses above 38.8mg/kg were found to be maternally toxic. The dose of 38.8 mg/kg of Dimethoate 30 EC reduced percent maternal weight gain during gestation. It also reduced the fetal weight and fetal size. A high percentage of resorption was observed at the dose of 38.8 mg/kg and also in the positive control Aspirin group. A dose response relationship was found to be evident when the fetal lengths and weights were expressed as the percent of their control.

Several external anomalies and growth retardation were caused by Dimethoate 30 EC at the high dose of 38.8 mg/kg. The skeletal anomalies were found to be dose related and decreased with the decrease in dose. A high incidence of skeletal anomalies was observed with the dose of 38.8 mg/kg of Dimethoate 30 EC and also with the positive control group. Visceral anomalies showed underdeveloped organs specially in the high dose group of Dimethoate 30 EC (38.8 mg/kg). Specific anomalies such as cleft palate and hydrocephaly were of a low incidence in the high dose group and were not observed in low and intermediate dose groups. These anomalies were also exhibited by the positive control group.

The low dose group of Dimethoate 30 EC at 15.52 mg/kg did not show any signs of teratogenicity except for 29 % resorptions which was observed as 17 % in the negative control group. Other parameters as maternal weight gain, fetal weight and lengths, skeletal and visceral anomalies were comparable with the negative control group.



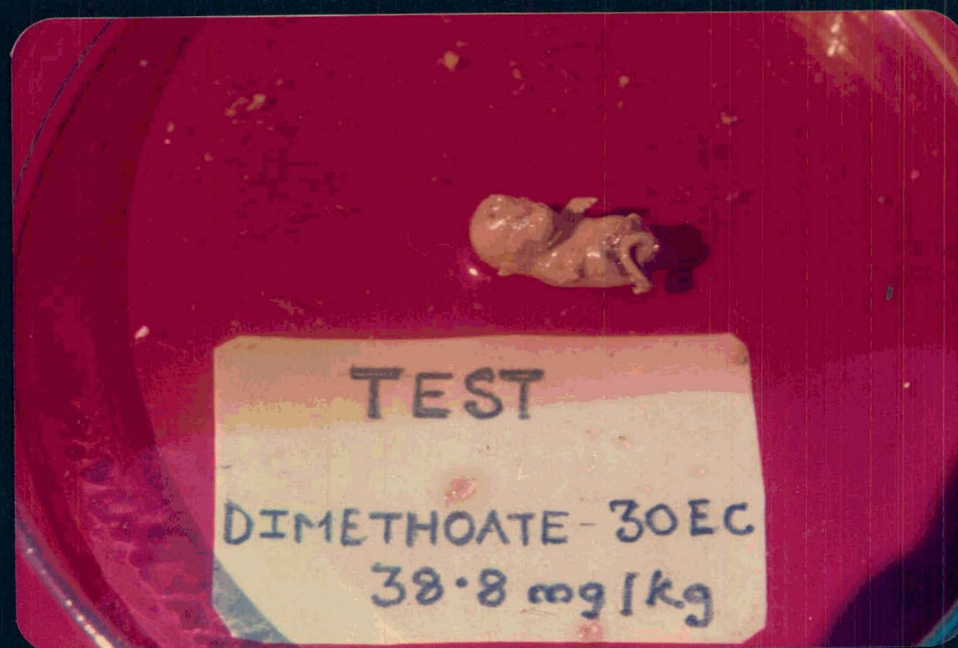
PHOTOGRAPH-1 : Arrangement of fetuses in the uterine horn of the female rat at necropsy (day 20 of the gestation period).



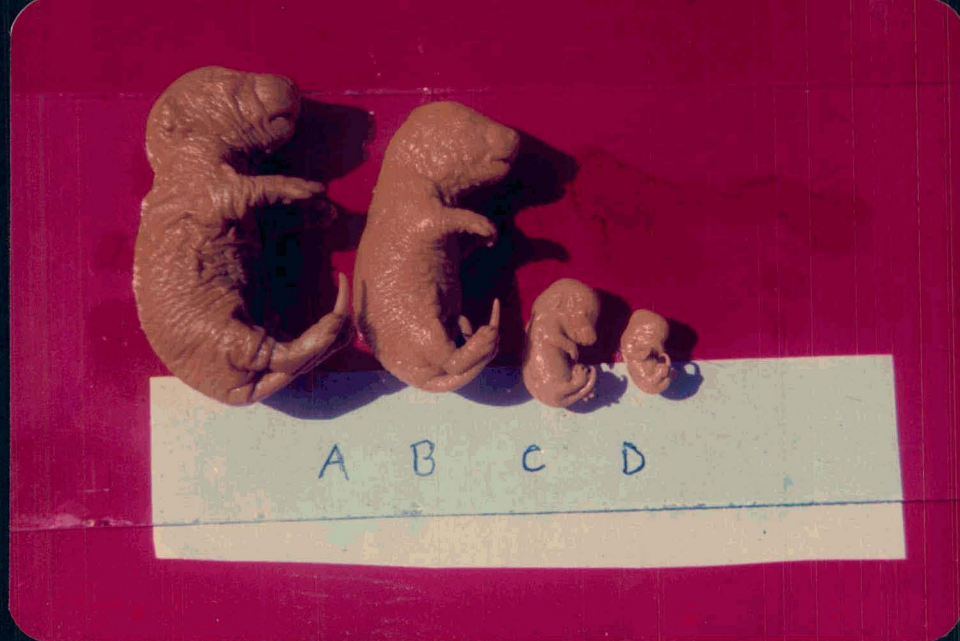
PHOTOGRAPH-2 : Normal fetuses after removal from the uterus of the female rat at necropsy (day 20 of gestation).



PHOTOGRAPH-3 : Resorption observed in the left uterine horn of the female rat at necropsy (day 20 of gestation).



PHOTOGRAPH-4 : Fetus exhibiting meningocele, twisted hind limb and webbed paws.



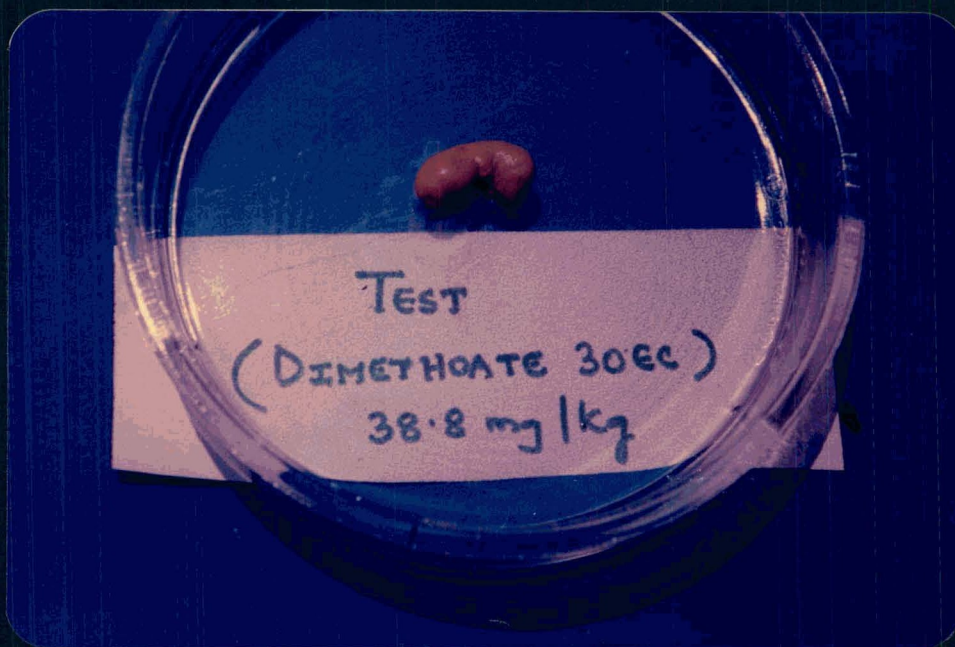
PHOTOGRAPH - 5 : Comparative body sizes of the fetuses of treated and control groups.

A - Negative control.

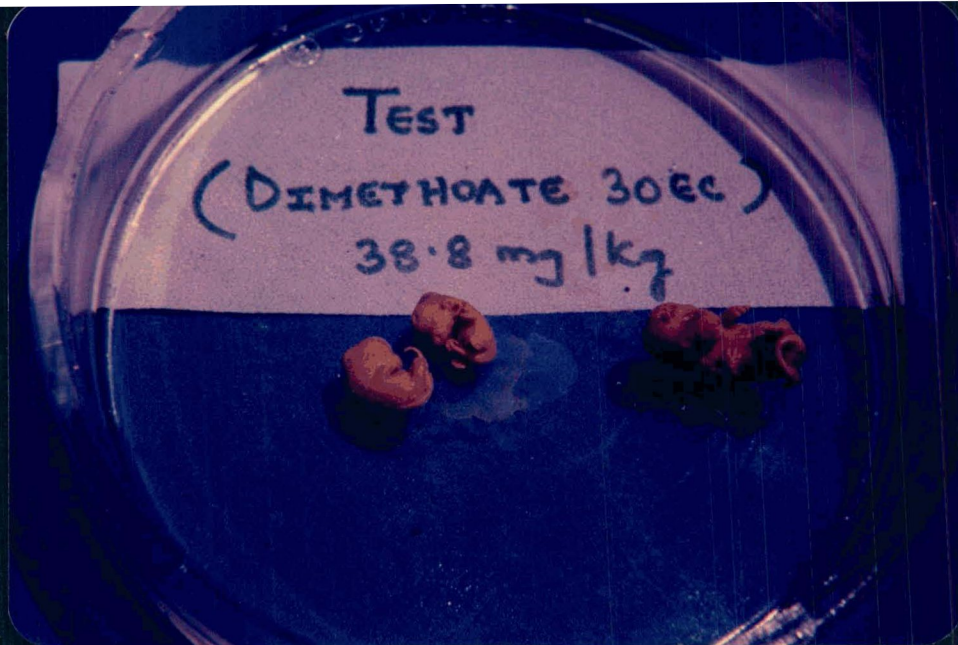
B - Test low dose - Dimethoate 30 EC - 15.52 mg/kg.

C - Test intermediate dose - Dimethoate 30 EC - 25.87 mg/kg.

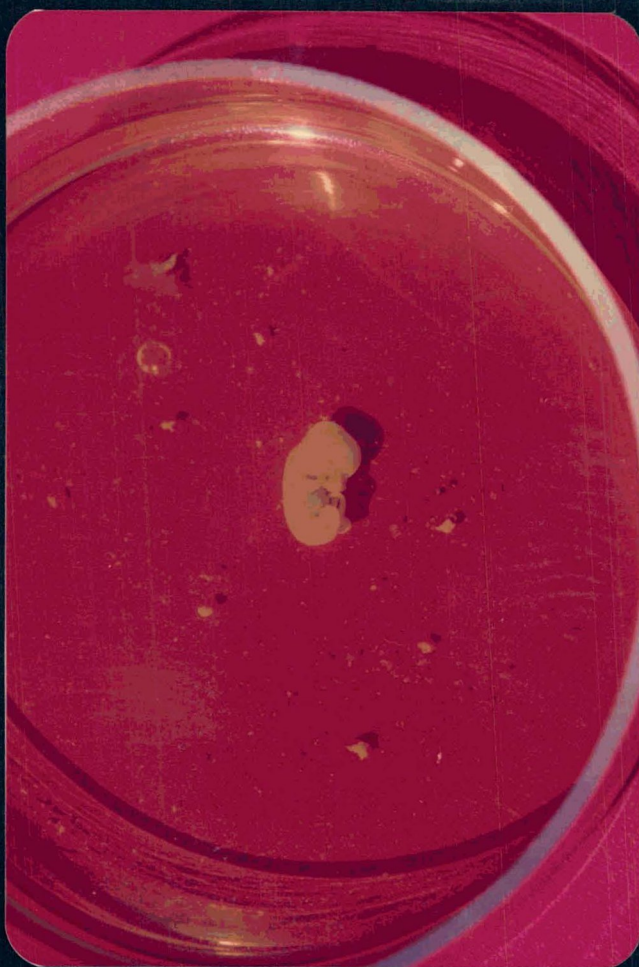
D - Test high dose - Dimethoate 30 EC - 38.8 mg/kg



PHOTOGRAPH-6 : Fetus exhibiting absence of eyes, ears, amelia and micromelia.



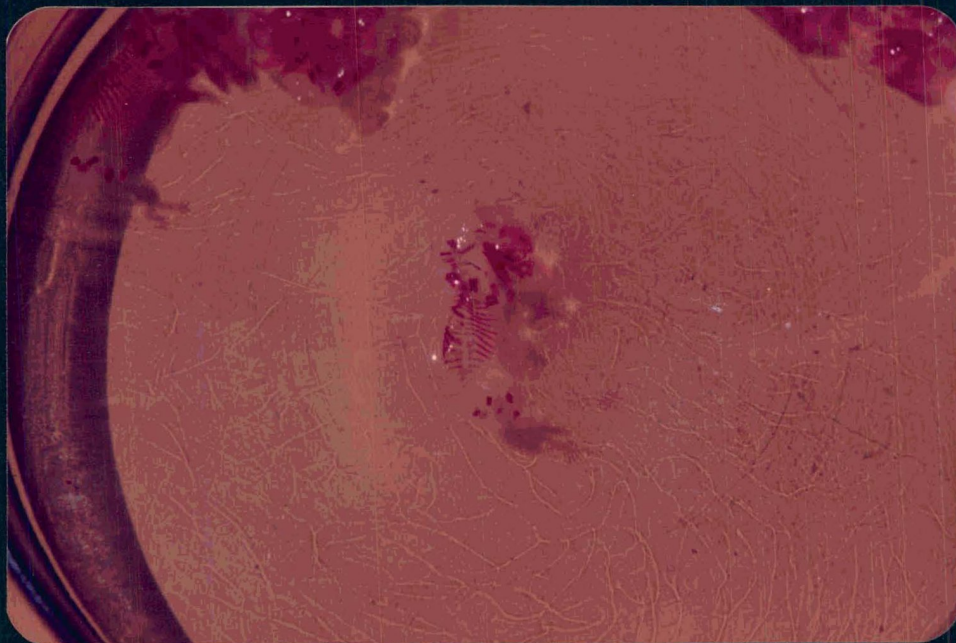
PHOTOGRAPH- 7 : Fetuses exhibiting webbed paws and coiled tail.



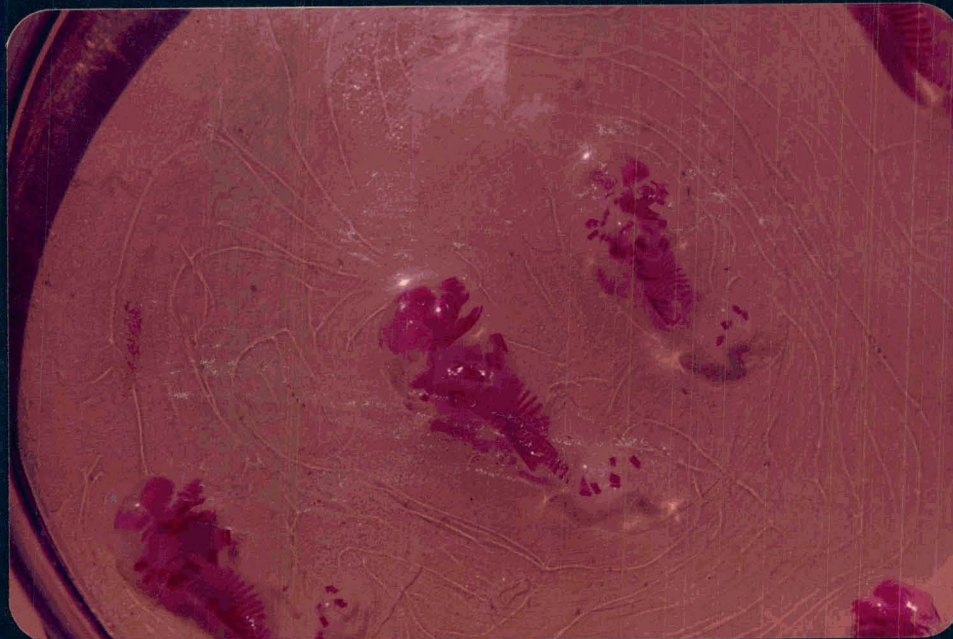
PHOTOGRAPH- 8: Fetus of the positive control group (Aspirin 250 mg/kg) exhibiting webbed paws.



PHOTOGRAPH-9 : Skeletal staining of normal fetus.



PHOTOGRAPH-10 : Stained fetus exhibiting improper ossification of skull bones, split and wavy ribs, spina bifida, absence of sternum and limb digits (Test High Dose- Dimethoate 30 EC- 38.8 mg/kg).



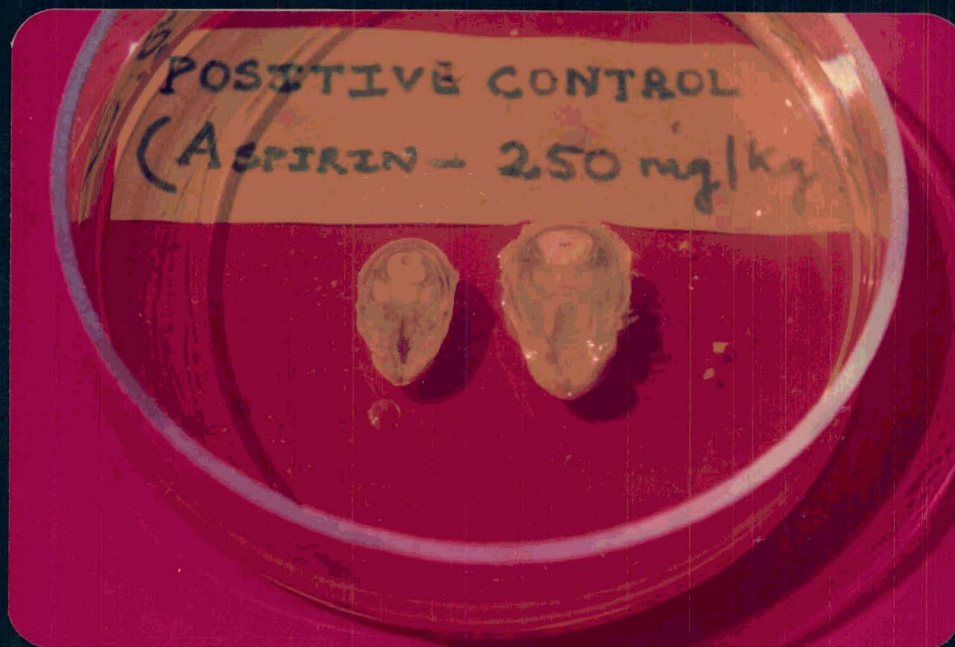
PHOTOGRAPH-11 : Stained fetus exhibiting improper ossification of skull bones, split and wavy ribs, spina bifida, absence of sternum and limb digits (Test High Dose- Dimethoate 30 EC- 38.8 mg/kg).



PHOTOGRAPH-12 : Stained fetuses of the positive control group (Aspirin- 250 mg/kg) exhibiting improper ossification of skull bones, split and wavy ribs and absence of limb digits.



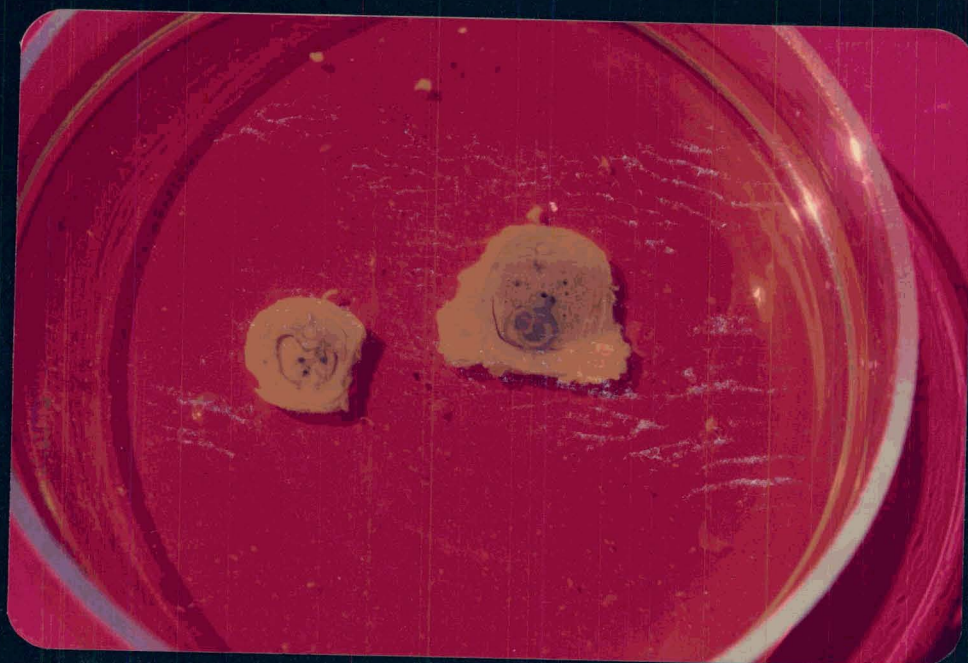
PHOTOGRAPH-13 : Cleft Palate.



PHOTOGRAPH-14 : Cleft Palate.



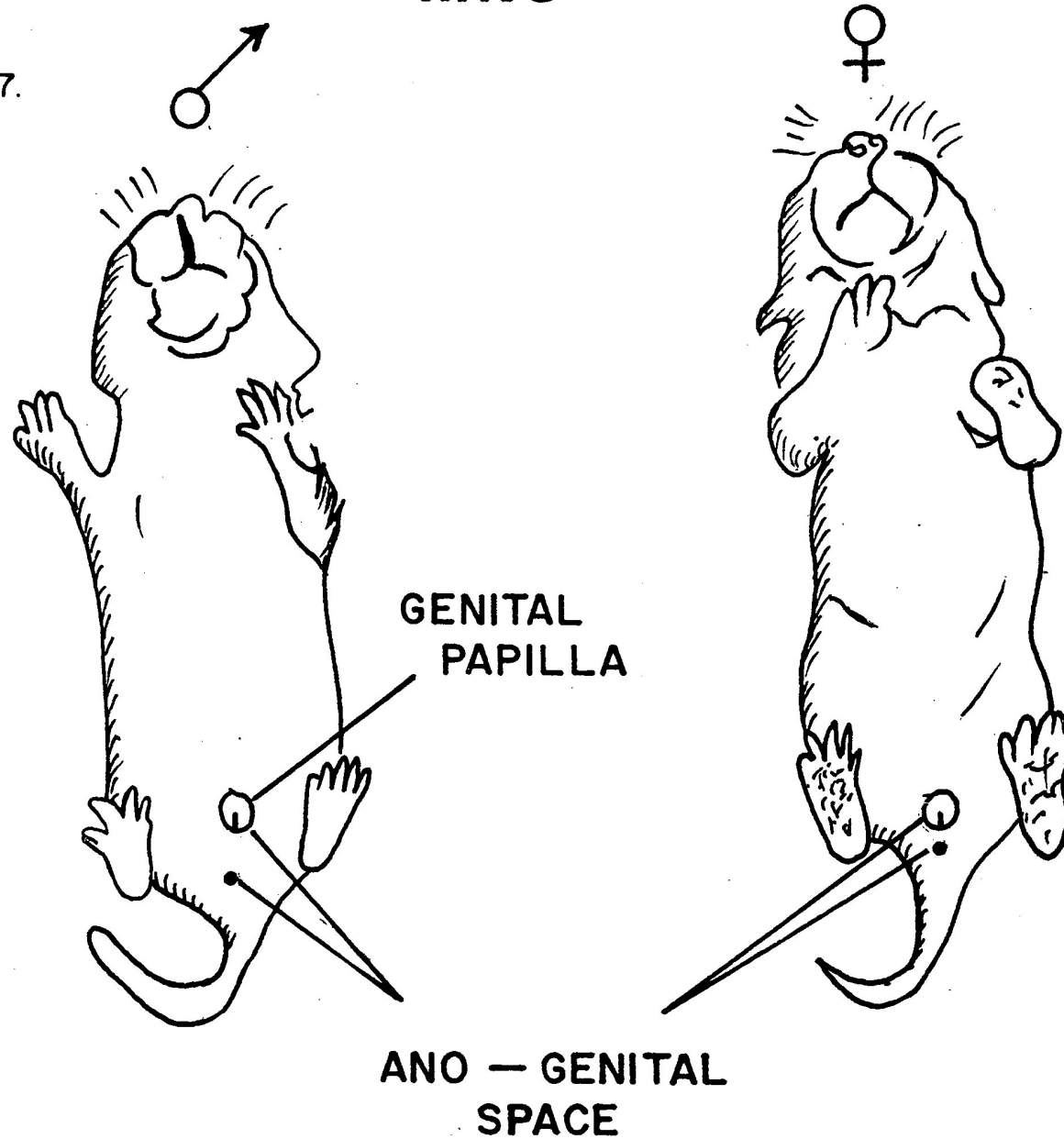
PHOTOGRAPH-15 : Section passing through the liver, stomach and kidney of the fetus. Negative control at the right and Test high dose - Dimethoate 30 EC 38.8 mg/kg at the left.



PHOTOGRAPH-16 : Section passing through the lung and heart of the fetus. Negative control at the right and test high dose- Dimethoate 30 EC 38.8 mg/kg at the left.

EXTERNAL SEX DIFFERENCES IN YOUNG RATS

FIG.— 17.



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9. APPENDIX

PRENATAL DATA OF CONTROL AND TREATED RATS FROM DAY 6 THROUGH DAY 15 OF GESTATION

APPENDIX - 1 : NEGATIVE CONTROL

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of im-plants	No. of fetuses alive	No. of fetuses dead	Weight in gms	Length in cms	Sex	Other anomalies
1.	186	222	9 (4R-5L)	9	-	1.002 1.122 1.00 1.071 1.074 1.070 1.075 1.027 1.015	1.5 1.5 1.4 1.5 1.5 1.4 1.6 1.5 1.5	Could not be indenti-fied because of small size	Nil
2.	195	238	7 (3R-4L)	6	1 resor-bed	4.380 4.097 4.481 4.531 4.230 4.195	4.2 4.0 4.3 4.4 4.2 4.1	M F M F F F	Nil
3.	180	230	5 (3R-2L)	5	-	4.921 4.645 4.823 4.901 4.861	4.5 4.4 4.5 4.5 4.4	M M F F M	Nil

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
4.	180	225	8 (4R-4L)	8	-	3.726	3.6	F	Nil
						3.513	3.4	F	
						3.638	3.6	F	
						3.523	3.4	F	
						3.736	3.5	M	
						3.605	3.5	M	
						3.615	3.5	F	
						3.683	3.5	M	
5.	190	238	9 (6R-3L)	9	-	3.981	3.6	M	Nil
						3.872	3.5	M	
						4.201	3.9	F	
						3.825	3.7	M	
						3.955	3.8	F	
						4.082	3.9	F	
						3.828	3.7	F	
						3.905	3.8	F	
6.	200	218	8 (4R-4L)	All re-sorbed	All re-sorbed	-	-	-	
7.	195	208	-	-	-	-	-	-	Not pregnant
8.	200	238	8 (5R-3L)	7	1 Re-sorbed	4.217	4.1	M	Nil
						4.325	4.2	M	
						4.197	4.1	M	
						4.316	4.2	F	
						4.258	4.1	F	
						4.223	4.2	F	
						4.162	4.1	F	

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of im-plants	No. of fetuses alive	No. of fetuses dead	Weight in cms.	Length in cms.	Sex	Other anomalies
9.	192	202	-	-	-	-	-	-	Not pregnant
10.	197	232	7 (4R-3L)	6	1 re-sorbed	4.612	4.5	M	Nil
						4.549	4.3	M	
						4.516	4.3	F	
						4.601	4.4	F	
						4.492	4.3	M	
11.	193	238	8 (4R-4L)	8	-	4.554	4.3	M	Nil
						4.325	4.2	F	
						4.219	4.2	M	
						4.415	4.3	F	
						4.361	4.2	M	
						4.529	4.3	F	
						4.302	4.2	F	
						4.423	4.3	F	
12.	186	218	7 (3R-4L)	7	-	4.191	4.1	M	Nil
						3.929	4.0	M	
						4.125	4.1	F	
						4.216	4.2	F	
						4.195	4.1	F	
						4.224	4.2	M	
						4.137	4.2	F	
13.	202	232	7 (4R-3L)	7	-	4.162	4.2	M	Nil
						3.825	3.9	F	
						3.919	3.9	F	
						3.926	3.9	F	
						3.891	3.9	M	
						3.842	3.8	M	
						4.012	4.0	F	
						3.995	4.0	M	

Sr. No.	Wt. of Mother on day 6 in gms.	Wt. of mother before cesarean	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
14.	172	182	-	-	-	-	-	-	Not pregnant
15.	182	215	4 (2R-2L)	3	re-sorbed	4.215 4.316 4.327	4.2 4.3 4.3	M F F	Nil
16.	200	252	10 (6R-4L)	10	-	4.012 3.927 4.129 4.215 4.160 4.059 4.291 4.163 4.210 4.132	4.1 3.9 4.2 4.3 4.2 4.1 4.2 4.1 4.2 4.1	F F F M M F M M F F	Short & coiled tail Nil
17.	198	242	8 (4R-4L)	8	-	4.321 4.465 4.348 4.621 4.219 4.359 4.560 4.297	4.0 4.1 4.0 4.3 4.0 4.1 4.2 4.1	M F F M F M F F	Nil
18.	196	228	4 (2R-2L)	3	1 re-sorbed	4.921 4.823 4.895	4.6 4.5 4.5	M F F	

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
19.	182	200	6 (4R-2L)	-	All re-sorbed	-	-	-	
20.	186	208	-	-	-	-	-	-	Not pregnant

PRENATAL DATA OF CONTROL AND TREATED RATS FROM DAY 6 THROUGH DAY 15 OF GESTATION

APPENDIX-2 : POSITIVE CONTROL
(Aspirin 250 mg/kg)

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of im-plants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
1.	170	185	8 (5R-3L)	-	All re-sorbed	-	-	-	
2.	210	223	6 (3R-3L)	-	All re-sorbed	-	-	-	
3.	185	202	7 (4R-3L)	-	All re-sorbed	-	-	-	
4.	200	218	7 (4R-3L)	-	All re-sorbed	-	-	-	
5.	200	240	8 (4R-4L)	6	1 Dead 1 Re-sorbed	1.995 2.397 2.174 2.832 2.594 2.063	2.0 2.5 2.3 2.5 2.3 2.2	M F F F M F	Eyes and skin not developed
6.	170	192	8 (4R-4L)	-	All re-sorbed	-	-	-	
7.	190	205	4 (2R-2L)	-	All re-sorbed	-	-	-	More fat.
8.	195	206	-	-	-	-	-	-	Not pregnant
9.	170	180	-	-	-	-	-	-	Not pregnant

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
10.	190	208	8 (5R-3L)	-	All re-sorbed	-	-	-	
11.	210	245	7 (4R-3L)	7	-	2.550 2.537 2.624 2.850 2.672 2.862 2.626	2.2 2.2 2.3 2.4 2.4 2.5 2.4	F* M F* M M F F**	* One leg absent, soft skin all over. ** Micromelia
12.	190	228	8 (5R-3L)	6	2 re-sorbed	1.140 1.045 1.032 1.066 1.128 0.970	1.5 1.4 1.4 1.4 1.5 1.2	) Short and coiled tail) Could not be identified due to small size Webbed paws	
13.	190	205	6 (3R-3L)	-	All re-sorbed	-	-	-	
14.	160	170	-	-	-	-	-	-	Not pregnant
15.	165	175	-	-	-	-	-	-	Not pregnant
16.	190	208	6 (4R-2L)	-	All re-sorbed	-	-	-	

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
17.	185	202	4 (4R-LO)	-	All re-sorbed	-	-	-	
18.	180	198	7 (3R-4L)	-	All re-sorbed	-	-	-	
19.	190	205	5 (3R-2L)	-	All re-sorbed	-	-	-	
20.	175	Dead on day 4 of gestation, gross pathology did not reveal any abnormality.							

M = Male; F = Female; R = Right; L = Left. NWB = Not well developed.

PRENATAL DATA OF CONTROL AND TREATED RATS FROM DAY 6 THROUGH DAY 15 OF GESTATION.

APPENDIX-3 : VEHICLE CONTROL

Sr.No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of im-plants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
1.	210	252	9 (6R-3L)	9	-	3.364 3.418 2.710 3.490 3.760 3.735 3.780 3.910 3.566	3.3 3.5 3.0 3.4 3.5 3.5 3.5 3.8 3.5	F F F M M F M F M	Nil
2.	195	232	9 (4R-5L)	9	-	3.350 3.440 3.587 3.688 3.605 3.480 3.310 3.494 3.519	3.8 3.9 3.9 4.0 3.9 3.8 3.7 3.8 3.8	M F M F M M F F M	Nil
3.	180	218	5 (3R-2L)	3	2 Re-sorbed	1.212 1.123 1.025	1.5 1.4 1.4	Sex could not be identified due to small size - Short and coiled tail	
4.	182	198	6 (3R-3L)	-	All re-sorbed	-	-	-	

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in gms.	Sex	Other anomalies
5.	175	185	-	-	-	-	-	-	Not pregnant
6.	200	250	10 (6R-4L)	10	-	3.567 3.582 3.602 3.616 3.625 3.569 3.514 3.609 3.551 3.491	3.3 3.4 3.5 3.5 3.6 3.5 3.4 3.5 3.4 3.2	F F F M M F F F M M	Nil
7.	198	235	9 (6R-3L)	9	-	3.325 3.219 3.215 3.316 3.382 3.419 3.501 3.205 3.309	3.1 3.1 3.0 3.2 3.2 3.3 3.4 3.2 3.2	F M F M F F F M F	Nil
8.	186	196	-	-	-	-	-	-	Not pregnant

Contd...

Sr. NO.	Wt. of mother on day 6 in gms.	Wt of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
9.	178	220	10 (5R-5L)	10	-	2.935 3.495 3.570 3.805 3.650 3.808 3.910 3.848 3.573 3.275	2.8 3.3 3.5 3.8 3.5 3.8 4.0 3.8 3.6 3.4	M F F M F F M F M F	Nil
10.	185	210	5 (3R-2L)	2	3 Re- sorbed	0.915 1.101	1.2 1.3	Could not be identified due to small size	
11.	202	212	4 (2R-2L)	2	2 Re- sorbed	1.050 1.048	1.8 1.7	M F	
12.	200	210	5 (2R-3L)	-	All re- sorbed	-	-	-	-
13.	195	242	6 (3R-3L)	5	1 Re- sorbed	3.825 3.910 4.012 3.995 3.895	3.6 3.7 3.8 3.8 3.7	M M F F F	Nil
14.	190	210	2 (1R-1L)	2	-	4.210 4.219	4.2 4.2	F M	Nil

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
15.	188	230	9 (5R-4L)	8	1 Re-sorbed	4.123 4.216 4.019 4.128 4.205 4.166 4.008 4.192	4.3 4.3 4.1 4.2 4.3 4.2 4.1 4.3	F F M M F F M M	Nil
16.	180	208	6 (4R-2L)	6	-	3.925 4.105 2.981 1.125 3.992 3.879	3.8 4.0 3.1 1.5 3.9 3.8	M F E F M	Nil Could not be identified due to their small size
17.	196	240	7 (3R-4L)	7	-	3.562 3.429 3.551 3.610 3.512 3.601 3.529	3.6 3.4 3.5 3.7 3.5 3.6 3.5	M F F F F M M	Nil
18.	200	212	-	-	-	-	-	-	Not pregnant
19.	192	210	-	-	-	-	-	-	Not pregnant
20.	186	200	-	-	-	-	-	-	Not pregnant

M = Male; F = Female; R = Right; L = Left; NWD = Not well developed.

PRENATAL DATA OF CONTROL AND TREATED RATS FROM DAY 6 THROUGH DAY 15 OF GESTATION

APPENDIX - 4 : DIMETHOATE 30 EC - 38.8 mg/kg

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
1.	200	232	9 (6R-3L)	8	1	2.567 2.321 2.716 2.625 2.451 2.690 2.511 2.323 1.209	2.1 1.9 2.3 2.2 2.0 2.2 2.1 1.9 1.1	F M** M F M F F M* F*	** Short coiled tail. Micromelia. *Depigmented skin.
2.	200	212	6 (3R-3L)	-	All re-sorbed	-	-	-	
3.	194	205	7 (4R-3L)	-	All re-sorbed	-	-	-	Excessive fluid and more fat.
4.	195	208	7 (4R-3L)	-	All re-sorbed	-	-	-	
5.	205	235	8 (6R-2L)	7	1 Re-sorbed	2.384 2.295 2.463 2.362 2.591 1.802 1.213	2.1 2.0 2.2 2.1 2.3 1.7 1.3*	M M F F F F M*	*Micromelia and Meningococle.

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
6.	182	192	-	-	-	-	-	-	Not pregnant
7.	177	188	-	-	-	-	-	-	Not pregnant
8.	195	210	-	-	-	-	-	-	Not pregnant
9.	186	205	9 (5R-4L)	-	All re-sorbed	-	-	-	
10.	175	190	8 (4R-4L)	-	All re-sorbed	-	-	-	
11.	200	235	10 (5R-5L)	9	1 dead	2.210	1.9	M	
						1.892	1.8	F	
						1.910	1.9	F	
						2.325	2.1	F	
						2.190	2.0	M	
						2.121	2.1	M	
						1.762	1.8	F	
						1.235	1.1	F	
						1.410	1.4	F*	*Micromelia, short coiled tail.
12.	200	238	9 (5R-4L)	9	-	1.515	1.4	F	
						1.672	1.5	M	
						1.732	1.5	F	
						1.819	1.6	M	
						1.535	1.5	F	
						1.710	1.5	F	
						0.982	1.0)	Could not be identified due to small size.	
						0.861	1.0)		
						1.010	1.1)		

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms	Length in cms.	Sex	Other anomalies
13.	188	Dead on day 9 of gestation, gross pathology did not reveal any abnormality							
14.	183	200	6 (3R-3L)	2	4 Re-sorbed (Fetal)	1.523 1.861 0.927 0.857 0.890 1.102	1.2 1.4 1.0 *1.0 *1.0 1.2	M F Could not be identified due to their small size	*Webbed paws, ears & eyes NWD
15.	193	215	9 (5R-4L)	-	9 Fetal resorptions	0.980 0.991 1.005 1.004 1.116 0.898 0.873 0.910 0.923	1.0 1.0 1.1 1.1 1.2 0.9 0.9 1.0 1.0	- - F F M - - - -	Small and NWD Could not be identified due to small size
16.	189	200	-	-	-	-	-	-	Not pregnant
17.	175	185	-	-	-	-	-	-	Not pregnant
18.	203	218	2 (2R-LO)	-	1 Resorbed. 1 Dead	-	-	-	Entangled in fat -5.423 gms more fluid.
19.	200	214	6 (6R-LO)	-	All re-sorbed	-	-	-	
20.	191	205	5 (3R-2L)	-	5 resorbed	-	-	-	

M = Male; F = Female; R = Right; L = Left; NWD = Not well developed.

PRENATAL DATA OF CONTROL AND TREATED RATS FROM DAY 6 THROUGH DAY 15 OF GESTATION

APPENDIX-5 : TEST - DIMETHOATE 30EC - 25.87 mg/kg.

Sr. No.	Wt. of mother on day 6 in gms.	Wt of mother before cesarean in gms.	No. and position of im-plants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
1.	198	245	10 (6R-4L)	10	-	3.797	3.2	M	Nil
						3.825	3.3	F	
						3.865	3.4	M	
						3.692	3.2	M	
						3.580	3.1	F	
						3.670	3.2	F	
						3.725	3.2	F	
						3.820	3.3	M	
						3.850	3.4	M	
3.670	3.2	F							
2.	187	238	10 (7R-3L)	10	-	3.07	3.0	F	Nil
						3.532	3.3	M	
						3.412	3.2	F	
						3.195	3.1	F	
						3.362	3.2	F	
						3.415	3.2	M	
						3.621	3.4	M	
						3.442	3.2	F	
						3.672	3.4	M	
3.419	3.3	F							
3.	182	222	9 (5R-4L)	8	1 Re-sorbed	3.215	3.2	F	Nil
						3.412	3.3	F	
						3.116	3.1	F	
						3.219	3.2	M	
						3.325	3.2	M	
						3.205	3.2	F	
						3.119	3.1	M	
						3.238	3.2	F	

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
4.	178	218	10 (5R-5L)	9	1 Re-sorbed	1.024 1.020 1.180 1.121 1.185 1.091 1.065 1.042 1.172 1.178	1.3 1.2* 1.4 1.4 1.4 1.3 1.3 1.3** 1.3 1.4‡	Could not be identified due to their small size.	* Small eyes and ears, under-development. ** Micromelia, short & coiled tail. ‡ Webbed paw
5.	195	225	5 (3R-3L)	2	3 resorbed (1 fetal)	0.834 0.901 0.412	0.9* 1.0 0.5*		*Skin, eyes, ears NWD.
6.	180	195	7 (4R-3L)	-	All resorbed	-	-	-	
7.	173	190	6 (3R-3L)	-	All resorbed	-	-	-	
8.	200	215	8 (5R-3L)	-	All resorbed	-	-	-	
9.	183	194	-	-	-	-	-	-	Not pregnant
10.	197	208	-	-	-	-	-	-	Not pregnant

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
11.	204	238	7 (3R-4L)	7	2.5	2.562 2.812 2.915 2.803 2.810 2.895 2.719	2.3 2.5 2.6 2.5 2.5 2.5 2.4	M F F F F M M	Nil
12.	186	218	6 (3R-3L)	6	-	2.859 3.261 3.315 3.201 3.198 2.798	2.5 3.0 3.1 3.1 3.0 2.5	M F M F M F	Nil
13.	192	228	6 (3R-3L)	5	1 re-sorbed	2.995 3.012 3.112 3.115 1.123	2.8 3.0 3.1 3.1 1.4	F F M F *	Nil * NWD
14.	175	208	3 (1R-2L)	-	All re-sorbed	-	-	-	
15.	180	215	2 (0R-2L)	-	All re-sorbed	-	-	-	
16.	182	194	-	-	-	-	-	-	Not pregnant

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
17.	190	202	-	-	-	-	-	-	Not pregnant
18.	176	202	8 (4R+4L)	-	All re-sorbed	-	-	-	
19.	183	198	6 (3R-3L)	-	All re-sorbed	-	-	-	
20.	191	205	5 (3R-2L)	-	All re-sorbed	-	-	-	

M = Male; F = Female; R = Right; L = Left; NWD = Not well developed.

PRENATAL DATA OF CONTROL AND TREATED RATS FROM DAY 6 THROUGH DAY 15 OF GESTATION

APPENDIX-6 : TEST - DIMETHOATE 30 EC - 15.52 mg/kg.

Sr. NO.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of im-plants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
1.	198	245	10 (6R-4L)	10	0	3.987	4.0	M	Nil
						4.102	4.2	M	
						4.119	4.4	F	
						4.215	4.5	F	
						4.198	4.3	F	
						4.085	4.2	M	
						4.173	4.3	F	
						3.991	4.0	M	
						4.201	4.4	F	
						4.139	4.3	F	
2.	200	234	8 (4R-4L)	8	0	3.125	3.5	F	Nil
						3.026	3.4	M	
						3.219	3.6	F	
						3.031	3.4	M	
						3.228	3.7	F	
						3.319	3.8	F	
						3.061	3.4	F	
						3.114	3.5	M	
3.	186	215	6 (4R-2L)	6	0	3.981	3.9	M	Nil
						4.005	4.1	F	
						4.127	4.2	F	
						4.089	4.1	F	
						3.997	3.9	M	
						4.039	4.0	M	

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms	Length in cms.	Sex	Other anomalies
4.	192	240	10 (6R-4L)	9	1 Re-sorbed	3.918	4.0	M	Nil
						3.825	3.9	F	
						4.013	4.1	F	
						3.995	4.0	M	
						3.862	4.0	F	
						4.005	4.2	F	
						3.961	4.0	F	
						3.823	3.9	M	
5.	202	228	7 (3R-4L)	7		3.919	3.9	M	* Short and coiled tail. Micromelia.
						1.799	2.5	M	
						1.808	2.5	F	
						2.000	2.7	M	
						1.689	2.5	F	
						1.733	2.4	F	
						1.830	2.4	M	
6.	195	230	9 (4R-5L)	9		1.902	2.6	F*	
						3.217	3.5	M	Nil
						3.115	3.4	F	
						3.190	3.4	F	
						3.098	3.4	F	
						3.009	3.3	F	
						3.118	3.3	M	
						3.121	3.5	M	
						3.200	3.5	F	
						3.169	3.4	M	

Contd...

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
7.	173	188	7 (3R-4L)	-	All re-sorbed	-	-	-	
8.	182	202	10 (6R-4L)	-	All re-sorbed	-	-	-	
9.	170	180	-	-	-	-	-	-	Not pregnant
10.	165	175	-	-	-	-	-	-	Not pregnant
11.	195	240	8 (4R-4L)	8	-	3.813 3.773 3.810 3.820 3.294 3.815 3.612 3.787	3.5 3.5 3.5 3.6 3.2 3.5 3.4 3.5	F M M M F F M M	Nil
12.	188	215	4 (2R-2L)	2	2 re-sorbed	3.213 3.309	3.2 3.2	F F	
13.	190	238	9 (4R-5L)	9	0	3.418 3.323 3.391 3.386 3.372 3.299 3.401 3.367 3.293	3.4 3.4 3.4 3.3 3.3 3.2 3.4 3.3 3.2	M M F M F F F F M	Nil

Contd..

Sr. No.	Wt. of mother on day 6 in gms.	Wt. of mother before cesarean in gms.	No. and position of implants	No. of fetuses alive	No. of fetuses dead	Weight in gms.	Length in cms.	Sex	Other anomalies
14.	195	212	6 3R-3L)	-	All re-sorbed	-	-	-	More fluid
15.	200	236	3 0R-3L)	3	0	4.261 4.385 4.332	4.3 4.5 4.5	M F F	
16.	175	198	3 2R-1L)	2	1 dead	0.983 1.023 1.100	1.0 1.1 1.2	Could not be identified due to their small size	Small, under-developed
17.	182	240	5 3R-2L)	5	-	4.516 4.520 4.541 4.516 4.433	4.6 4.6 4.7 4.7 4.6	M M F F	Nil
18.	186	202	6 3R-3L)	-	All re-sorbed	-	-	-	
19.	200	210	-	-	-	-	-	-	Not pregnant
20.	198	212	-	-	-	-	-	-	Not pregnant

M = Male; F = Female; R = Right; L = Left; NWD = Not well developed.

APPENDIX - 7INCIDENCE OF SKELETAL ANOMALIES IN CONTROL AND
TREATED RATS.DEFECTS OF SKULL

No.	<u>Group</u> Treatment		Improper fusion of cranial bones	Under- developed bones	Missing bones
1.	Aspirin 250 mg/kg Positive Control	1)	5/5	0/5	0/5
		2)	3/3	0/3	0/3
		3)	2/4	0/4	0/4
		Total :-	10/12	0/12	0/12
2.	High Dose - Dimethoate 30 EC 38.8 mg/kg 1/20th LD ₅₀	1)	2/5	2/5	3/5
		2)	2/5	0/5	0/5
		3)	2/2	2/2	0/2
		4)	0/6	0/6	0/6
		5)	4/5	3/5	0/5
		Total :-	10/23	7/23	3/23
3.	Intermediate Dose - Dimethoate 30 EC 25.8 mg/kg 1/30th LD ₅₀	1)	3/6	0/6	0/6
		2)	2/6	5/6	0/6
		3)	0/2	0/2	0/2
		4)	2/6	0/6	0/6
		5)	0/6	0/6	0/6
		6)	0/6	0/6	0/6
		7)	2/4	0/4	0/4
		8)	1/3	0/3	0/3
		Total :-	10/39	5/39	0/39

Contd...

No.	Group Treatment		Improper fusion of cranial bones	Under- developed bones	Missing bones
4.	Low Dose Dimethoate 30 EC 15.52 mg/kg 1/50th LD ₅₀	1)	0/5	0/5	0/5
		2)	1/5	0/5	0/5
		3)	0/6	0/6	0/6
		4)	0/2	0/2	0/2
		5)	0/1	0/1	0/1
		6)	1/2	0/2	0/2
		7)	0/5	0/5	0/5
		8)	0/6	0/6	0/6
		9)	0/4	0/4	0/4
		10)	1/4	2/4	0/4
		11)	1/6	0/6	0/6
		12)	1/6	0/6	0/6
Total :-			5/52	2/52	0/52
5.	Vehicle control Tween-80	1)	0/6	0/6	0/6
		2)	0/6	0/6	0/6
		3)	1/6	0/6	0/6
		4)	0/2	0/2	0/2
		5)	0/1	0/1	0/1
		6)	0/3	0/3	0/3
		7)	0/6	0/6	0/6
		8)	2/6	0/6	0/6
		9)	0/3	0/3	0/3
		10)	0/6	0/6	0/6
		11)	0/3	0/3	0/3
		12)	0/3	0/3	0/3
Total :-			3/51	0/51	0/51

Contd..

No.	Group Treatment	Improper fusion of cranial bones	Under- developed bones	Missing bones
6. Negative Control	1)	0/6	0/6	0/6
	2)	0/5	0/5	0/5
	3)	0/3	0/3	0/3
	4)	1/6	0/6	0/6
	5)	1/6	0/6	0/6
	6)	0/5	0/5	0/5
	7)	0/4	0/4	0/4
	8)	0/5	0/5	0/5
	9)	0/5	0/5	0/5
	10)	0/5	0/5	0/5
	11)	0/2	0/2	0/2
	12)	1/7	1/7	1/7
	13)	0/5	0/5	0/5
	14)	0/2	0/2	0/2
Total :-		3/66	1/66	1/66

355

APPENDIX-8INCIDENCE OF SKELETAL ANOMALIES IN CONTROL AND
TREATED RATS.DEFECTS OF VERTEBRA & VERTEBRAL COLUMN.

No.	Group Treatment	Missing caudal verte- brae	Extra verte- brae	Spina bifida	Under- devel- oped VC	Missing verte- brae
1.	Positive Control	1) 5/5	1/5	4/5	0/5	0/5
	Aspirin	2) 3/3	0/3	0/3	0/3	0/3
	250 mg/kg	3) 1/4	0/4	0/4	0/4	0/4
Total :-		9/12	1/12	4/12	0/12	0/12
2.	Dimethoate	1) 3/5	0/5	2/5	2/5	2/5
	38.8 mg/kg	2) 3/5	0/5	2/5	0/5	1/5
	High Dose	3) 0/2	0/2	0/2	0/2	0/2
	1/20th LD ₅₀	4) 3/6	0/6	1/6	0/6	2/6
		5) 3/5	0/5	1/5	0/5	1/5
Total :-		12/23	1/23	6/23	2/23	6/23
3.	Intermediate	1) 0/6	0/6	0/6	0/6	0/6
	Dose : Dimetho-	2) 5/6	0/6	2/6	0/6	0/6
	ate 25.87 mg/kg	3) 0/2	0/2	0/2	0/2	0/2
	1/30th LD ₅₀	4) 0/6	0/6	0/6	0/6	0/6
		5) 0/6	0/6	0/6	0/6	0/6
		6) 6/6	0/6	0/6	0/6	0/6
		7) 5/5	0/5	0/5	0/5	0/5
		8) 3/3	0/3	0/3	0/3	0/3
Total :-		19/39	0/39	2/39	0/39	0/39

VC = Vertebral column

Contd..

No.	Group Treatment		Missing caudal verte- brae	Extra verte- brae	Spina bifida	Under- devel- oped VC	Missing verte- brae
4.	Dimethoate 15.52 mg/kg 1/50th LD ₅₀	1)	0/5	0/5	0/5	0/5	0/5
		2)	1/5	0/5	0/5	0/5	0/5
		3)	0/6	0/6	0/6	0/6	0/6
		4)	0/2	0/2	0/2	0/2	0/2
		5)	0/1	0/1	0/1	0/1	0/1
		6)	0/2	0/2	0/2	0/2	0/2
		7)	0/5	0/5	0/5	0/5	0/5
		8)	0/6	0/6	0/6	0/6	0/6
		9)	0/4	0/4	0/4	0/4	0/4
		10)	2/4	0/4	2/4	0/4	0/4
		11)	0/6	0/6	0/6	0/6	0/6
		12)	0/6	0/6	0/6	0/6	0/6
Total :-			3/52	0/52	2/52	0/52	0/52
5.	Vehicle Control Tween 80	1)	0/6	0/6	0/6	0/6	0/6
		2)	0/6	0/6	0/6	0/6	0/6
		3)	0/6	0/6	0/6	0/6	0/6
		4)	0/2	0/2	0/2	0/2	0/2
		5)	0/1	0/1	0/1	0/1	0/1
		6)	0/3	0/3	0/3	0/3	0/3
		7)	0/6	0/6	0/6	0/6	0/6
		8)	4/6	0/6	0/6	0/6	0/6
		9)	2/3	0/3	0/3	0/3	0/3
		10)	0/6	0/6	0/6	0/6	0/6
		11)	0/3	0/3	0/3	0/3	0/3
		12)	0/3	0/3	0/3	0/3	0/3
Total :-			6/51	0/51	0/51	0/51	0/51

Contd....

No.	Group Treatment	Missing caudal verte- brae	Extra verte- brae	Spina bifida	Under- devel- oped VC	Missing verte- brae
6. Negative Control		1) 0/6	0/6	0/6	0/6	0/6
		2) 0/5	0/5	0/5	0/5	0/5
		3) 0/3	0/3	0/3	0/3	0/3
		4) 0/6	0/6	0/6	0/6	0/6
		5) 0/6	0/6	0/6	0/6	0/6
		6) 0/5	0/5	0/5	0/5	0/5
		7) 0/4	0/4	0/4	0/4	0/4
		8) 0/5	0/5	0/5	0/5	0/5
		9) 0/5	0/5	0/5	0/5	0/5
		10) 0/5	0/5	0/5	0/5	0/5
		11) 0/2	0/2	0/2	0/2	0/2
		12) 1/7	0/7	1/7	0/7	1/7
		13) 0/5	0/5	0/5	0/5	0/5
		14) 0/2	0/2	0/2	0/2	0/2
Total :-		1/66	0/66	1/66	0/66	1/66

APPENDIX - 9INCIDENCE OF SKELETAL ANOMALIES IN CONTROL AND
TREATED RATS.DEFECTS OF RIBS

No.	<u>Group</u> Treatment	Missing ribs	Extra ribs	Fused ribs	Wavy ribs
1.	Positive Control	1) 0/5	1/5	1/5	1/5
	Aspirin	2) 0/3	0/3	0/3	1/3
	250 mg/kg	3) 0/4	0/4	1/4	1/4
Total :-		0/12	1/12	2/12	3/12
2.	Dimethoate	1) 2/5	0/5	0/5	2/5
	38.8 mg/kg	2) 2/5	0/5	0/5	0/5
	High dose :	3) 1/2	0/2	0/2	0/2
	1/20th LD ₅₀	4) 4/6	1/6	1/6	3/6
		5) 1/5	0/5	0/5	1/5
Total :-		10/23	1/23	1/23	6/23
3.	Dimethoate	1) 0/6	0/6	0/6	0/6
	25.87 mg/kg	2) 2/6	0/6	0/6	1/6
	Intermediate Dose:	3) 0/2	0/2	0/2	0/2
	1/30th LD ₅₀	4) 0/6	0/6	1/6	1/6
		5) 0/6	0/6	0/6	0/6
		6) 0/6	0/6	0/6	0/6
		7) 0/4	0/4	0/4	2/4
		8) 3/3	0/3	1/3	3/3
Total :-		5/39	0/39	2/39	7/39

Contd...

No.	Group Treatment		Missing ribs	Extra ribs	Fused ribs	Wavy ribs
4.	Dimethoate	1)	0/5	0/5	0/5	0/5
	15.52 mg/kg	2)	0/5	0/5	1/5	1/5
	Low dose -	3)	0/6	0/6	1/6	0/6
	1/50th LD ₅₀	4)	0/2	0/2	0/2	0/2
		5)	0/1	0/1	0/1	1/1
		6)	0/2	0/2	0/2	1/2
		7)	0/5	0/5	2/5	2/5
		8)	0/6	0/6	0/6	0/6
		9)	1/4	0/4	0/4	0/4
		10)	0/4	0/4	1/4	1/4
		11)	0/6	0/6	0/6	0/6
		12)	0/6	0/6	0/6	0/6
Total :-			1/52	0/52	5/52	6/52
5.	Vehicle Control	1)	0/6	0/6	0/6	0/6
	Tween 80	2)	0/6	0/6	0/6	0/6
		3)	0/6	0/6	0/6	0/6
		4)	0/2	0/2	0/2	0/2
		5)	0/1	0/1	0/1	0/1
		6)	1/3	0/3	1/3	0/3
		7)	1/6	1/6	2/6	0/6
		8)	0/6	0/6	0/6	4/6
		9)	0/3	0/3	0/3	0/3
		10)	0/6	0/6	0/6	0/6
		11)	0/3	0/3	0/3	0/3
		12)	0/3	0/3	0/3	0/3
Total :-			2/51	1/51	3/51	4/51

Contd...

No.	Group Treatment	Missing ribs	Extra ribs	Fused ribs	Wavy ribs
6.	Negative Control	1) 0/6	0/6	0/6	0/6
		2) 0/5	0/5	0/5	0/5
		3) 0/3	0/3	0/3	0/3
		4) 0/6	0/6	0/6	0/6
		5) 0/6	0/6	0/6	0/6
		6) 0/5	0/5	0/5	0/5
		7) 0/4	0/4	0/4	0/4
		8) 0/5	0/5	0/5	0/5
		9) 0/5	0/5	0/5	0/5
		10) 0/5	0/5	0/5	0/5
		11) 0/2	0/2	0/2	0/2
		12) 1/7	0/7	1/7	1/7
		13) 0/5	0/5	0/5	0/5
		14) 0/2	0/2	0/2	0/2
Total :-		1/66	0/66	1/66	1/66

APPENDIX - 10INCIDENCE OF SKELETAL ANOMALIES IN CONTROL AND TREATED RATS.DEFECTS OF STERNUM

No.	Group Treatment	Missing sternum	Fused sterne- brae	Missing sterne- brae	Under- developed sternum
1.	Positive Control	1) 1/5	0/4	4/4	4/4
	Aspirin 250 mg/kg	2) 0/3	0/3	1/3	2/3
		3) 0/4	1/4	0/4	3/4
Total :-		1/12	1/11	5/11	9/11
2.	Dimethoate	1) 5/5	-	-	-
	38.8 mg/kg;	2) 0/5	0/5	3/5	3/5
	High dose 1/20th	3) 0/2	0/2	0/2	0/2
	LD ₅₀	4) 4/6	0/2	0/2	0/2
		5) 5/5	-	-	-
Total :-		14/23	0/9	3/9	3/9
3.	Dimethoate	1) 0/6	0/6	0/6	0/6
	25.87 mg/kg,	2) 5/6	0/1	0/1	0/1
	Intermediate dose	3) 0/2	0/2	0/2	0/2
	1/30th LD ₅₀	4) 0/6	0/6	0/6	0/6
		5) 0/6	2/6	0/6	2/6
		6) 0/6	0/6	0/6	0/6
		7) 0/4	0/4	0/4	0/4
		8) 3/3	-	-	-
Total :-		8/39	2/31	0/31	2/31

Contd..

No.	Group Treatment	Missing sternum	Fused sterne- brae	Missing sterne- brae	Under- developed sternum	
4.	Dimethoate 15.52 mg/kg; Low dose - 1/50th LD ₅₀	1)	0/5	0/5	0/5	
		2)	0/5	0/5	0/5	
		3)	0/6	0/6	0/6	
		4)	0/2	0/2	0/2	
		5)	0/1	0/1	0/1	
		6)	0/2	0/2	2/2	
		7)	0/5	0/5	0/5	
		8)	0/6	1/6	2/6	0/6
		9)	0/4	0/4	0/4	0/4
		10)	2/4	0/2	0/2	0/2
		11)	0/6	0/6	0/6	0/6
		12)	0/6	0/6	0/6	0/6
Total :-		2/52	1/50	2/50	2/50	
5.	Vehicle Control Tween 80	1)	0/6	0/6	0/6	0/6
		2)	0/6	0/6	0/6	0/6
		3)	0/6	0/6	0/6	0/6
		4)	0/2	0/2	0/2	0/2
		5)	0/1	0/1	0/1	0/1
		6)	0/3	0/3	0/3	0/3
		7)	0/6	0/6	0/6	0/6
		8)	0/6	0/6	0/6	0/6
		9)	2/3	0/1	0/1	0/1
		10)	0/6	0/6	0/6	0/6
		11)	0/3	0/3	0/3	0/3
		12)	0/3	0/3	0/3	0/3
Total :-		2/51	0/49	0/49	0/49	

Contd...

No.	Group Treatment	Missing sternum	Fused sterne- brae	Missing sterne- brae	Under- developed sternum
6.	Negative Control	1) 0/6	0/6	0/6	0/6
		2) 0/5	0/5	0/5	0/5
		3) 0/3	0/3	0/3	0/3
		4) 0/6	0/6	0/6	0/6
		5) 0/6	0/6	0/6	0/6
		6) 0/5	0/5	0/5	0/5
		7) 0/4	0/4	0/4	0/4
		8) 0/5	0/5	0/5	0/5
		9) 0/5	0/5	0/5	0/5
		10) 0/5	0/5	0/5	0/5
		11) 0/2	0/2	0/2	0/2
		12) 0/7	1/7	1/7	0/7
		13) 0/5	0/5	0/5	0/5
		14) 0/2	0/2	0/2	0/2
Total :-		0/66	1/66	1/66	0/66

APPENDIX-11INCIDENCE OF SKELETAL ANOMALIES IN CONTROL AND TREATED RATS.DEFECTS OF FORE LIMB

No.	Group Treatment	Missing digits	Fused bones	Missing limb	Missing scapula	Short bones
1.	Aspirin - 250 mg/kg Positive control	1) 5/5 2) 3/3 3) 2/4	0/5 0/3 0/4	0/5 0/3 0/4	0/5 0/3 0/4	0/5 0/3 0/4
Total :-		10/12	0/12	0/12	0/12	0/12
2.	Dimethoate - 38.8 mg/kg 1/20th LD ₅₀ High dose	1) 3/5 2) 3/5 3) 0/2 4) 6/6 5) 5/5	0/5 0/5 0/2 2/6 0/5	0/5 0/5 0/2 0/6 0/5	2/5 0/5 0/2 1/6 0/5	2/5 0/5 0/2 4/6 0/5
Total :-		17/23	2/23	0/23	3/23	6/23
3.	Dimethoate - 25.87 mg/kg 1/20th LD ₅₀ Intermediate dose	1) 0/6 2) 5/6 3) 0/2 4) 0/6 5) 0/6 6) 0/6 7) 0/4 8) 3/3	0/6 0/6 0/2 0/6 0/6 0/6 0/4 0/3	0/6 0/6 0/2 0/6 1/6 0/6 0/4 0/3	0/6 1/6 0/2 0/6 0/6 0/6 0/4 0/3	0/6 5/6 0/2 0/6 0/6 0/6 0/4 3/3
Total :-		8/39	0/39	1/39	1/39	8/39

Contd...

No.	Group Treatment	Missing digits	Fused bones	Missing limb	Missing scapula	Short bones
4.	Dimethoate - 15.52 mg/kg 1/50th LD ₅₀	1) 0/5 2) 0/5 3) 0/6 4) 0/2 5) 0/1 6) 0/2 7) 0/5 8) 0/6 9) 0/4 10) 2/4 11) 0/6 12) 0/6	0/5 0/5 0/6 0/2 0/1 0/2 0/5 0/6 0/4 0/4 0/6 0/6	0/5 0/5 0/6 0/2 0/1 0/2 0/5 0/6 0/4 0/4 0/6 0/6	0/5 0/5 0/6 0/2 0/1 0/2 0/5 0/6 0/4 0/4 0/6 0/6	0/5 0/5 0/6 0/2 0/1 0/2 0/5 0/6 0/4 2/4 0/6 0/6
Total :-		2/52	0/52	0/52	0/52	2/52
5.	Vehicle con- trol - Tween 80	1) 0/6 2) 0/6 3) 0/6 4) 0/2 5) 0/1 6) 0/3 7) 0/6 8) 0/6 9) 0/3 10) 0/6 11) 0/3 12) 0/3	0/6 0/6 0/6 0/2 0/1 0/3 0/6 0/6 0/3 0/6 0/3 0/3	0/6 0/6 0/6 0/2 0/1 0/3 0/6 0/6 0/3 0/6 0/3 0/3	0/6 0/6 0/6 0/2 0/1 0/3 0/6 0/6 0/3 0/6 0/3 0/3	0/6 0/6 0/6 0/2 0/1 0/3 0/6 0/6 0/3 0/6 0/3 0/3
Total :-		0/51	0/51	0/51	0/51	0/51

Contd...

353

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No.	Group Treatment	Missing digits	Fused bones	Missing limb	Missing scapula	Short bones
6.	Negative Control	1) 0/6	0/6	0/6	0/6	0/6
		2) 0/5	0/5	0/5	0/5	0/5
		3) 0/3	0/3	0/3	0/3	0/3
		4) 0/6	0/6	0/6	0/6	0/6
		5) 0/6	0/6	0/6	0/6	0/6
		6) 0/5	0/5	0/5	0/5	0/5
		7) 0/4	0/4	0/4	0/4	0/4
		8) 0/5	0/5	0/5	0/5	0/5
		9) 0/5	0/5	0/5	0/5	0/5
		10) 0/5	0/5	0/5	0/5	0/5
		11) 0/2	0/2	0/2	0/2	0/2
		12) 0/7	0/7	0/7	0/7	0/7
		13) 0/5	0/5	0/5	0/5	0/5
		14) 0/2	0/2	0/2	0/2	0/2
Total :-		0/66	0/66	0/66	0/66	0/66

APPENDIX - 12INCIDENCE OF SKELETAL ANOMALIES IN CONTROL AND TREATED RATSDEFECTS OF HIND LIMB

No.	Group Treatment		Missing digits	Fused tibia- fibula	Missing bones	Short bones
1.	Positive Control	1)	5/5	0/5	1/5	0/5
	Aspirin -	2)	3/3	0/3	0/3	0/3
	250 mg/kg	3)	0/4	0/4	0/4	0/4
Total :-			8/12	0/12	1/12	0/12
2.	Dimethoate -	1)	3/5	3/5	0/5	3/5
	38.8 mg/kg	2)	3/5	0/5	0/5	0/5
	High Dose !	3)	2/2	0/2	0/2	0/2
	1/20th LD ₅₀	4)	6/6	0/6	0/6	2/6
		5)	4/5	0/5	1/5	0/5
Total :-			18/23	3/23	1/23	5/23
3.	Dimethoate -	1)	0/6	0/6	0/6	0/6
	25.87 mg/kg	2)	5/6	0/6	0/6	5/6
	Dose 1/30th LD ₅₀	3)	0/2	0/2	0/2	0/2
	Intermediate	4)	0/6	1/6	0/6	0/6
	dose	5)	0/6	0/6	0/6	0/6
		6)	0/6	0/6	0/6	0/6
		7)	0/4	0/4	0/4	0/4
		8)	2/3	0/3	0/3	3/3
Total :-			7/39	1/39	0/39	8/39

Contd...

No.	Group Treatment	Missing digits	Fused tibia- fibula	Missing bones	Short bones
4.	Dimethoate - 15.52 mg/kg Low dose - 1/50th LD ₅₀	1) 0/5 2) 0/5 3) 0/6 4) 0/2 5) 0/1 6) 0/2 7) 0/5 8) 0/6 9) 0/4 10) 2/4 11) 0/6 12) 0/6	0/5 0/5 0/6 0/2 0/1 0/2 0/5 0/6 0/4 1/4 0/6 0/6	0/5 0/5 0/6 0/2 0/1 0/2 0/5 0/6 0/4 0/4 0/6 0/6	0/5 0/5 0/6 0/2 0/1 0/2 0/5 0/6 0/4 2/4 0/6 0/6
Total :-		2/52	1/52	0/52	2/52
5.	Vehicle Control Tween-80	1) 0/6 2) 0/6 3) 0/6 4) 0/2 5) 0/1 6) 0/3 7) 0/6 8) 0/6 9) 1/3 10) 0/6 11) 0/3 12) 0/3	0/6 0/6 0/6 0/2 0/1 0/3 0/6 0/6 0/3 0/6 0/3 0/3	0/6 0/6 0/6 0/2 0/1 0/3 0/6 0/6 0/3 0/6 0/3 0/3	0/6 0/6 0/6 0/2 0/1 0/3 0/6 0/6 0/3 0/6 0/3 0/3
Total :-		1/51	0/51	0/51	0/51

Contd...

No.	Group Treatment	Missing digits	Fused tibia- fubula	Missing bones	Short bones
6.	Negative Control	1) 0/6	0/6	0/6	0/6
		2) 0/5	0/5	0/5	0/5
		3) 0/3	0/3	0/3	0/3
		4) 0/6	0/6	0/6	0/6
		5) 0/6	0/6	0/6	0/6
		6) 0/5	0/5	0/5	0/5
		7) 0/4	0/4	0/4	0/4
		8) 0/5	0/5	0/5	0/5
		9) 0/5	0/5	0/5	0/5
		10) 0/5	0/5	0/5	0/5
		11) 0/2	0/2	0/2	0/2
		12) 0/7	0/7	0/7	0/7
		13) 0/5	0/5	0/5	0/5
		14) 0/2	0/2	0/2	0/2
Total :-		0/66	0/66	0/66	0/66

APPENDIX - 13INCIDENCE OF VISCERAL ANOMALIES IN CONTROL AND TREATED RATS..

Group : Negative Control (Normal diet only)

Sr. No.	Palate & nasal cavity	Brain	Thorax	Lung	Oeso-phagus	Heart
1.	N	N	N	N	N	N
2.	N	N	N	N	N	N
3.	N	N	N	N	N	N
4.	N	N	N	N	N	N
5.	N	N	N	N	N	N
6.	N	N	N	N	N	N
7.	N	N	N	N	N	N
8.	N	N	N	N	N	N
9.	N	N	N	N	N	N
10.	N	N	N	N	N	N
11.	N	N	N	N	N	N
12.	N	N	N	N	N	N
13.	N	N	N	N	N	N
14.	N	N	N	N	N	N
15.	N	N	N	N	N	N
16.	N	N	N	N	N	N
17.	N	N	N	N	N	N
18.	N	N	N	N	N	N
19.	N	N	N	N	N	N
20.	N	N	N	N	N	N
21.	N	N	N	N	N	N
22.	N	N	N	N	N	N
23.	N	N	N	N	N	N
24.	N	N	N	N	N	N
25.	N	N	N	N	N	N
26.	N	N	N	N	N	N
27.	N	N	N	N	N	N
28.	N	N	N	N	N	N
29.	N	N	N	N	N	N
30.	N	N	N	N	N	N

N = Normal

APPENDIX-13 (contd..)

Sr. No.	Liver	Stomach	Intestine	Kidney	Urinary bladder & ureter
1.	N	N	N	N	N
2.	N	N	N	N	N
3.	N	N	N	N	N
4.	N	N	N	N	N
5.	N	N	N	N	N
6.	N	N	N	N	N
7.	N	N	N	N	N
8.	N	N	N	N	N
9.	N	N	N	N	N
10.	N	N	N	N	N
11.	N	N	N	N	N
12.	N	N	N	N	N
13.	N	N	N	N	N
14.	N	N	N	N	N
15.	N	N	N	N	N
16.	N	N	N	N	N
17.	N	N	N	N	N
18.	N	N	N	N	N
19.	N	N	N	N	N
20.	N	N	N	N	N
21.	N	N	N	N	N
22.	N	N	N	N	N
23.	N	N	N	N	N
24.	N	N	N	N	N
25.	N	N	N	N	N
26.	N	N	N	N	N
27.	N	N	N	N	N
28.	N	N	N	N	N
29.	N	N	N	N	N
30.	N	N	N	N	N

N = Normal

APPENDIX - 14INCIDENCE OF VISCERAL ANOMALIES IN CONTROL & TREATED RATS

Group : Positive Control - Aspirin 250 mg/kg.

Sr.No.	Palate & nasal cavity	Brain	Thorax	Lung	Oeso- phagus	Heart
1.	Cleft	NWD	NWD	NWD	NWD	NWD
2.	Cleft	NWD	NWD	NWD	NWD	NWD
3.	N	N	N	N	N	N
4.	N	N	N	N	N	N
5.	Cleft	NWD	N	NWD	NWD	NWD
6.	N	N	N	N	N	N
7.	N	N	N	N	N	N

N = Normal;

NWD = Not well developed.

APPENDIX - 14 (contd...)

Sr.No.	Liver	Stomach	Intestine	Kidney	Urinary bladder ureter
1.	NWD	NWD	NWD	NWD	NWD
2.	NWD	NWD	NWD	NWD	NWD
3.	N	N	N	N	N
4.	N	N	N	N	N
5.	NWD	NWD	NWD	NWD	NWD
6.	N	N	N	N	N
7.	N	N	N	N	N

N = Normal;

NWD = Not well developed.

APPENDIX-15INCIDENCE OF VISCERAL ANOMALIES IN CONTROL & TREATED RATS

Group : Vehicle Control - Tween 80

Sr. No.	Palate & nasal cavity	Brain	Thorax	Lung	Oeso-phagus	Heart
1.	N	N	N	N	N	N
2.	N	N	N	N	N	N
3.	N	N	N	N	N	N
4.	N	N	N	N	N	N
5.	N	N	N	N	N	N
6.	N	N	N	N	N	N
7.	N	N	N	N	N	N
8.	N	N	N	N	N	N
9.	N	N	N	N	N	N
10.	N	N	N	N	N	N
11.	N	N	N	N	N	N
12.	N	N	N	N	N	N
13.	N	N	N	N	N	N
14.	N	N	N	N	N	N
15.	N	N	N	N	N	N
16.	N	N	N	N	N	N
17.	N	NWD	NWD	NWD	NWD	NWD
18.	N	NWD	NWD	NWD	NWD	NWD
19.	N	N	N	N	N	N
20.	N	N	N	N	N	N
21.	N	N	N	N	N	N
22.	N	N	N	N	N	N
23.	N	N	N	N	N	N
24.	N	N	N	N	N	N
25.	N	N	N	N	N	N
26.	N	N	N	N	N	N
27.	N	N	N	N	N	N
28.	N	N	N	N	N	N
29.	N	N	N	N	N	N

N = Normal; NWD = Not well developed.

APPENDIX-15 (Contd..)

Sr. No.	Liver	Stomach	Intestine	Kidney	Urinary bladder & ureter
1.	N	N	N	N	N
2.	N	N	N	N	N
3.	N	N	N	N	N
4.	N	N	N	N	N
5.	N	N	N	N	N
6.	N	N	N	N	N
7.	N	N	N	N	N
8.	N	N	N	N	N
9.	N	N	N	N	N
10.	N	N	N	N	N
11.	N	N	N	N	N
12.	N	N	N	N	N
13.	N	N	N	N	N
14.	N	N	N	N	N
15.	N	N	N	N	N
16.	N	N	N	N	N
17.	NWD	NWD	NWD	NWD	NWD
18.	NWD	NWD	NWD	NWD	NWD
19.	N	N	N	N	N
20.	N	N	N	N	N
21.	N	N	N	N	N
22.	N	N	N	N	N
23.	N	N	N	N	N
24.	N	N	N	N	N
25.	N	N	N	N	N
26.	N	N	N	N	N
27.	N	N	N	N	N
28.	N	N	N	N	N
29.	N	N	N	N	N

N = Normal; NWD = Not well developed.

APPENDIX - 16INCIDENCE OF VISCERAL ANOMALIES IN CONTROL & TREATED RATS

Group : Dimethoate 30 EC : 38.8 mg/kg.
 High Dose $\frac{1}{20}$ th LD₅₀

Sr. No.	Palate & nasal cavity	Brain	Thorax	Lung	Oeso-phagus	Heart
1.	Cleft	Hydro-cephaly	N	NWD	NWD	NWD
2.	N	NWD	NWD	NWD	N	N
3.	N	N	N	N	NWD	NWD
4.	N	N	N	N	N	N
5.	Cleft	NWD	NWD	NWD	NWD	NWD
6.	N	NWD	NWD	NWD	NWD	NWD
7.	N	NWD	N	NWD	NWD	NWD
8.	N	NWD	N	NWD	N	N
9.	N	N	N	N	N	N
10.	N	N	N	N	N	N
11.	N	N	N	N	N	N
12.	N	N	N	N	N	N

N = Normal; NWD = Not well developed.

APPENDIX-16 (contd..)

Sr. No.	Liver	Stomach	Intestines	Kidney	Urinary bladder & ureter
1.	NWD	NWD	NWD	NWD	N
2.	NWD	NWD	NWD	NWD	N
3.	NWD	N	N	N	N
4.	N	N	N	N	N
5.	NWD	NWD	NWD	NWD	NWD
6.	N	N	N	N	N
7.	NWD	NWD	NWD	N	NWD
8.	N	N	N	N	N
9.	N	N	N	NWD	N
10.	N	N	N	N	N
11.	N	N	N	N	N
12.	N	N	N	N	N

N = Normal;

NWD = Not well developed.

APPENDIX - 17INCIDENCE OF VISCERAL ANOMALIES IN CONTROL AND TREATED RATS.

Group : Dimethoate 30 EC - 25.87 mg/kg.
Intermediate Dose $\frac{1}{30}$ th LD₅₀

Sr. No.	Palate & nasal cavity	Brain	Thorax	Lung	Oeso-phagus	Heart
1.	N	N	N	N	N	N
2.	N	N	N	N	N	N
3.	N	N	N	N	N	N
4.	N	N	N	N	N	N
5.	N	N	N	N	N	N
6.	N	N	N	N	N	N
7.	N	N	N	N	N	N
8.	N	N	N	N	N	N
9.	N	NWD	NWD	NWD	NWD	NWD
10.	N	NWD	NWD	NWD	NWD	NWD
11.	N	N	N	N	N	N
12.	N	N	N	N	N	N
13.	N	N	N	N	N	N
14.	N	N	N	N	N	N
15.	N	N	N	N	N	N
16.	N	N	N	N	N	N
17.	N	N	N	N	N	N
18.	N	N	N	N	N	N
19.	N	N	N	N	N	N
20.	NWD	NWD	N	NWD	NWD	NWD

N = Normal; NWD = Not well developed.

APPENDIX-17 (Contd...)

Sr. No.	Liver	Stomach	Intestines	Kidney	Urinary bladder & ureter
1.	N	N	N	N	N
2.	N	N	N	N	N
3.	N	N	N	N	N
4.	N	N	N	N	N
5.	N	N	N	N	N
6.	N	N	N	N	N
7.	N	N	N	N	N
8.	N	N	N	NWD	N
9.	NWD	NWD	NWD	NWD	N
10.	NWD	NWD	NWD	N	N
11.	N	N	N	N	N
12.	N	N	N	N	N
13.	N	N	N	N	N
14.	N	N	N	N	N
15.	N	N	N	N	N
16.	N	N	N	N	N
17.	N	N	N	N	N
18.	N	N	N	N	N
19.	N	N	N	N	N
20.	NWD	NWD	NWD	NWD	N

N = Normal; NWD = Not well developed.

APPENDIX - 18INCIDENCE OF VISCERAL ANOMALIES IN CONTROL & TREATED RATS.

Group : Dimethoate 30 EC - 15.52 mg/kg;
 Low dose - $\frac{1}{50}$ th LD₅₀ -

Sr. No.	Palate & nasal cavity	Brain	Thorax	Lung	Oeso-phagus	Heart
1.	N	N	N	N	N	N
2.	N	N	N	N	N	N
3.	N	N	N	N	N	N
4.	N	N	N	N	N	N
5.	N	N	N	N	N	N
6.	N	N	N	N	N	N
7.	N	N	N	N	N	N
8.	N	N	N	N	N	N
9.	N	N	N	N	N	N
10.	N	N	N	N	N	N
11.	N	N	N	N	N	N
12.	N	N	N	N	N	N
13.	N	N	N	N	N	N
14.	N	N	N	N	N	N
15.	N	N	N	N	N	N
16.	N	N	N	N	N	N
17.	N	N	N	N	N	N
18.	N	N	N	N	N	N
19.	N	N	N	N	N	N
20.	N	N	N	N	N	N
21.	N	N	N	N	N	N
22.	N	N	N	N	N	N
23.	N	N	N	N	N	N
24.	N	NWD	N	N	N	NWD
25.	N	N	N	NWD	NWD	NWD
26.	N	NWD	N	NWD	NWD	NWD

N = Normal; NWD = Not well developed.

APPENDIX-18 (Contd..)

Sr. No.	Liver	Stomach	Intestines	Kidney	Urinary bladder & ureter
1.	N	N	N	N	N
2.	N	N	N	N	N
3.	N	N	N	N	N
4.	N	N	N	N	N
5.	N	N	N	N	N
6.	N	N	N	N	N
7.	N	N	N	N	N
8.	N	N	N	N	N
9.	N	N	N	N	N
10.	N	N	N	N	N
11.	N	N	N	N	N
12.	N	N	N	N	N
13.	N	N	N	N	N
14.	N	N	N	N	N
15.	N	N	N	N	N
16.	N	N	N	N	N
17.	N	N	N	N	N
18.	N	N	N	N	N
19.	N	N	N	N	N
20.	N	N	N	N	N
21.	N	N	N	N	N
22.	N	N	N	N	N
23.	N	N	N	N	N
24.	NWD	N	N	N	N
25.	NWD	NWD	NWD	NWD	N
26.	NWD	NWD	NWD	NWD	NWD

N = Normal; NWD = Not well Developed.

355

10. SYNOPSIS

SYNOPSIS OF THE THESIS TO BE SUBMITTED BY

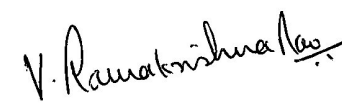
MR. A.B. SHIRGAONKAR

TO THE UNIVERSITY OF BOMBAY

FOR THE DEGREE OF

MASTER OF PHARMACY IN PHARMACOLOGY

* * * * *

- 1) Title of the Thesis : Teratogenic Effects of Organo-phosphate Pesticide Dimethoate 30 EC in Rats.
- 2) Name of the Candidate : Mr. Atul B. Shirgaonkar
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- 5) No. and Date of Registration : 26th March, 1985.
- 6) Signature of the Student : 
- 7) Signature of the Guide : 

INTRODUCTION

Past couple of decades have seen widespread use of pesticides for improving agricultural practices, in health programmes and for household purposes. The fear of adverse effects in man, domestic animals and wild life have become a matter of public concern. Toxicity may be the result of access to treated areas, stored or discarded pesticide containers or contaminated food, water and air.

Dimethoate [O,O dimethyl S-(N-methyl, carbomoyl methyl) phosphorodithioate] also known as Rogor, Cygon, Daphene, De-Fend, Trimethion, a selective insecticide, is extensively used for the control of several insects that infest variety of vegetables and fruits. It is one of the largest pesticide used in India (1). It is used as a residual spray in farm building, for the control of house flies and a wide range of insects and mites on food grains, cash crops, vegetables, fruits and ornamental plants (2). The retail use of Dimethoate is in the form of emulsifiable concentrates (EC).

Pesticides are generally used as their formulations during their actual usage. Because of variation in percentage of technical grade material in different

EC formulations, the impurity levels vary and also the possible toxicity. Formulations consist of different levels of technical grade material along with solvents, emulsifiers, stabilizers and other additives. The percentage of varying impurities in the form of solvents, emulsifiers, stabilizers, additives may result in various degrees of insult to the biological system on exposure to these pollutants. The data regarding the potential teratogenic hazards of the formulation to pregnant females is inadequate.

There are various toxicity studies for assessing the possible hazards of pesticide chemicals. One of the important study is teratogenic study which investigates the effects of chemicals on the fetus itself or birth defects. The study is concerned with the generation, cause and manifestations of structural and functional alterations in fetal development. The recorded account of malformation dates back to as early as 5,000 years ago in Egyptain wall paintings (3). Since then mankind's interest in birth defects has spanned centuries. Though birth defects such as deficiencies and deformities have been known for centuries (3), it was mainly during the last 50 years that scientific attempts have been made to understand the basis for such birth defects.

Earlier credit for extrapolation of malformations to teratogenic agents goes to Hale and in 1940 the influence of dietary factors in fetal developments was established (4). Congenital malformation is one of the leading causes of infant mortality and morbidity. Suspected teratogenic agents can therefore be a potential threat to pregnant females since these agents may be present in air, food, water as well as occupational and recreational environments.

The present study was undertaken to evaluate the teratogenic effects in rats of a widely used indigenously manufactured formulation Dimethoate 30 EC and compare the effects with Aspirin, a known teratogen (positive control (4,5)).

CHOICE OF ANIMAL MODEL

Teratogenic studies are usually carried out in animal models during gestation period and fetal development. It is terminated prior to the littering to observe the structural deviations. In selection of animal models, various complex factors are taken into consideration such as their biological system and numbers available. Amongst animals, rodents have been used extensively, which are available in large numbers, possess a placenta and the embryo is in dynamic equilibrium with the environment.

Rat has been the most frequently used model for teratogenic studies. It has a short gestation period of about 21 days, the time of copulation or mating can be measured accurately, its embryology is well known and the placental and hormonal characteristics are similar to the humans (4). Also in this species, the spontaneous occurrence of malformations has been well documented. Though for some teratogenic compounds the rats are less susceptible as compared to humans because of differences in absorption, metabolism and excretion of these compounds, the rat nevertheless is one of the choice species.

MATERIALS AND METHODS

TEST COMPOUNDS :- Dimethoate formulation (30 EC)
Aspirin I.P.

These were obtained from local market.

ACUTE TOXICITY :

Acute oral toxicity of Dimethoate 30 EC and Aspirin was carried out in albino rats (Haffkine strain) weighing 80-100 gms. Ten rats (five males and five females) were taken per dose and ten for control. All animals were fasted overnight prior to dosage but water was given ad libitum. Aspirin was administered as a suspension in distilled water with Tween-80. Control group

of Dimethoate received only distilled water. Whereas control group for Aspirin received distilled water with Tween-80. The final test was terminated seven days after dosing as no delayed symptoms or mortality were observed. All animals dead during the experimental period and those that were alive at the end of the experimental period were postmortemed for gross pathological changes. The LD₅₀ values were obtained by the method of Litchfield and Wilcoxon (6).

TERATOLOGICAL PROTOCOL :

Female virgin albino rats (Haffkine strain) of 100-120 days age were used. Twenty female rats were taken per group. Females and males were paired in the evening. Three females were mated with one male. All the animals received pelleted food and water ad libitum. The presence of sperm was checked by vaginal smear on the following morning. The day on which the sperm was found in the vagina of the female rat was considered as day zero of gestation (4,7). Pregnant female rats were allotted at random to all control or experimental groups. All females were weighed throughout the gestation period.

After the review of acute oral toxicity data regarding the lethal dose and variation in animal response,

a dose range finding was performed on three female rats of 100-120 days of age, using the LD₅₀ obtained in the acute oral study. The range study was conducted to obtain the least maternal toxic dose so that fetal toxic doses could be identified for subsequent final teratogenic studies.

All the final doses were calculated on the basis of body weights on day 6 of gestation period. The test material was administered by oral intubation (gavage) from day 6 to day 15 of gestation period. Three doses were selected for the final study.

38.8 mg/kg	High dose
25.87 mg/kg	Intermediate dose
15.52 mg/kg	Low dose

Three control groups were also taken. One group of female rats received normal diet only (Negative control), the second group received Aspirin at 250 mg/kg (Positive control) (4), and the third group received equivalent amount of Tween-80 without Aspirin (Vehicle control for Aspirin).

One day prior to term i.e., on day 20 of gestation, fetuses were delivered by Cesarean section under ether anaesthesia. The number and position of implantation

sites and fetuses dead or alive were recorded. Fetuses were weighed and examined for malformations. Fetal sex and crown rump measurement of each group were recorded. One third of the fetuses, randomly selected, were fixed for visceral anomalies by Wilson razor blade sections (8). The remaining two third of the fetuses were examined for skeletal anomalies after clearing with Alizarin red S (9).

RESULTS AND DISCUSSION

Teratological study has become an important part of toxicological studies and safety data requirements for regulatory purposes so as to prevent the recurrence of the Thalidomide catastrophe. Various workers have recorded the correlation between the use of Thalidomide in pregnant women and increase in limb reduction anomalies in infants (4,12,13). Epidemiological and experimental investigations have revealed that congenital anomalies amongst humans are estimated as 20-30 % may be due to genetic chromosomal aberration, 10-20 % may be due to extrinsic factors like drug interaction, toxins and environmental pollutants. The rest may be due to a complex interaction of genetical and extrinsic factors (4,10,11).

In selection of doses for teratological study, it is essential to select doses which are not maternally toxic but fetotoxic. For this purpose, indication is obtained regarding maternal toxic doses from an acute toxicity study of the test sample.

In the present study acute oral toxicity of the test material Dimethoate 30 EC was carried out. Results show that LD_{50} of Dimethoate 30 EC was 776 mg/kg body weight as compared to Dimethoate Technical which is 320-380 mg/kg. In a study the LD_{50} of Dimethoate Technical (94 % purity) in rats by oral route was^{*} reported to be 130-325 mg/kg (14) and in another it was 200-300 mg/kg (15). In a study regarding the susceptibility between sexes, it was concluded that there was no significant difference in susceptibility between sexes to acute lethal effect of Dimethoate (14). The pattern of symptoms noted in Dimethoate acute oral toxicity were tremors hyperexcitation, convulsions, respiratory disturbances and death. The LD_{50} obtained for Aspirin, the positive control, was 1600 mg/kg with fiducial limits between 1386.7 and 1844.8 mg/kg, which correlates with the reported value (18).

Teratological studies with Dimethoate Technical and Formulations have been reported (2,16). The toxicological evaluation of Dimethoate Technical (95% purity)

in mice showed a very high incidence of maternal mortality with 40 and 80 mg/kg doses (2). However, at 10 and 20 mg/kg doses, there was decrease in maternal weight gain but there was no significant difference in incidence of anomalies when compared to control. In a five generation mouse study, where Dimethoate was incorporated in drinking water at 60 ppm, there was reduced pregnancy rate and decreased survival and growth of pups (17). Teratogenic study with Dimethoate formulation (47.3 % EC) at 12 and 24 mg/kg was associated with a statistically significant increase in number of anomalous litters and wavy ribbed fetuses. There were also other non-significant anomalies such as extra ribs, fused sternebrae, runted fetuses, hydroureter, and dialated urinary bladder (15).

In our study animals administered 77.6 mg/kg ($1/10$ th of LD_{50}) and 51.73 mg/kg ($1/15$ th of LD_{50}) of Dimethoate 30 EC resulted in maternal toxic symptoms and produced 100 % resorptions. The final doses selected for fetal anomalies were 38.8 mg/kg ($1/20$ th of LD_{50}), 25.87 mg/kg ($1/30$ th of LD_{50}) and 15.52 mg/kg ($1/50$ th of LD_{50}).

The body weight of dams (from day 6 to day 20) increased significantly in all the groups irrespective of

the type of treatment they received. However, when the percent weight gain in body weights between the treated groups and control groups (negative and positive) were compared, there was marked difference. The percent gain in body weight of dams receiving 38.8 mg/kg (high dose) was significantly lower than those observed in the negative control. Similarly the percent weight gain in Aspirin treated dams was significantly lower than its control (vehicle control). The percent weight gain in dams of the low dose group (15.52 mg/kg) and intermediate dose group (25.87 mg/kg) were comparable with negative control.

The pregnancy rate at necropsy was similar in all the groups of dams. The number of implantation sites were not similar in all groups receiving Dimethoate. A high percentage of resorption was observed in the high dose group of Dimethoate 38.8 mg/kg (62 %) as well as in the positive control group (Aspirin 250 mg/kg) (80 %). As the dose of Dimethoate decreased so also the percent resorption (45%) in the intermediate dose and 29 % in the low dose). The negative control group showed 17 % and the vehicle control group 20 % resorption respectively. The fetal weight and size decreased in the high dose group of Dimethoate. This was also seen in the positive control group.

The length and weight of fetuses of the dams which received Dimethoate in different doses were significantly lower than their control. When both these parameters (lengths and weights) were expressed as per cent of control, the pattern like dose response relationship was found to be evident.

The weights of the fetuses of the dams receiving 38.8 mg/kg, 25.87 mg/kg and 15.52 mg/kg were 43.00 %, 70.11 % and 88.04 % respectively of the control fetal weights. Similarly the lengths of the same fetuses were 40.47 %, 67.92 % and 93.36 % respectively of the control fetal length values. In the case of Aspirin, the weights and lengths were 60.78 % and 59.43 % of the control values.

Fetuses stained with Alizarin Red S were observed for skeletal defects. The defects of the skull such as missing and under developed bones and Cranioschisis were observed with high dose of Dimethoate 30 EC. Defects such as extra vertebrae, missing caudal and thoracic vertebrae and spina bifida were also observed. The incidence of missing and under developed sternum, missing sternebrae, wavy, missing and extra ribs was observed in each Dimethoate test group. The incidence of skeletal anomalies decreased with the decrease in dose of Dimethoate 30 EC. A low incidence of anomalies was

observed in the control group.

Amongst the fetuses of rats receiving Dimethoate there were severe growth retardation, runted fetuses, syndactyly (webbed or fused digits) short and abnormal tail, hydrocephalus, cleft palate, microphthalmia (abnormal smallness of the eye), micromelia (abnormal smallness of the limb), ectrodactyly (congenital absence of digits), drop foot and twisting of limb. Fetuses subjected for evaluation of visceral anomalies showed underdeveloped organs.

CONCLUSION

Teratological study was conducted with Dimethoate 30 EC an organophosphate pesticide. Based on the results it may be concluded that :

Acute oral toxicity of Dimethoate 30 EC (LD_{50} 776 mg/kg) was found to be less than the reported acute oral toxicity of Dimethoate Technical (LD_{50} 130-325). Technical grade material is more toxic than its formulation. Doses higher than 38.8 mg/kg were found to be maternally toxic. Dimethoate 30 EC at 38.8 mg/kg dose was teratogenic, similar to that produced with Aspirin at

250 mg/kg. A high percentage of resorption was observed in both the groups. The percentage of resorption was lower in low dose groups. The incidence of skeletal anomalies was high in the Dimethoate 30 EC group receiving 38.8 mg/kg dose. This was also observed in the Aspirin group. Visceral anomalies showed under-developed organs specially in high dose group.

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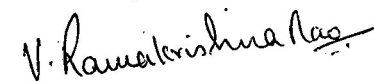
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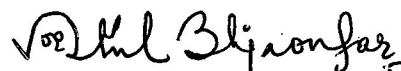

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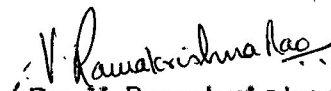
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355

