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Dimethoate Induced Intrauterine Growth Retardations in Mice

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Abstract: Dimethoate is an organophosphorus insecticide with contact and systemic action. It was introduced in 1956 and is produced in many countries. It is a general-use chemical for use against a broad range of insects in agriculture and also for the control of the housefly. This study was designed to evaluate and assess the possible embryo toxic potentials and neurodevelopmental impairment of Dimethoate on the intrauterine growth of mouse embryos. Fifty female mice (CD1) were given Dimethoate at sublethal doses of 9, 13, 18 and 33 mg/kg body weight /day respectively, by oral gavage, from zero day of gestation (GD0) up to 20th day of gestation (GD20). Body weights were recorded daily and at the end of the experiment mice were sacrificed, uteri were removed and dissected to evaluate the intrauterine growth retardations as well as skeletal malformation. The Student's "t"-distribution were adopted for assessment of significant changes occurring between the groups. Results revealed that Dimethoate induced reduction in the maternal and foetal body weight, reduction in number with asymmetrical distribution of implantation sites, increase in both mortality and resorption rates. Dimethoate induced decrease in the embryonic crown rump values, decreases in male anogenital distance and skewed sex ratio toward females and induced severe skeletal anomalies. These alterations were statistically significant ($P \leq 0.01$).In conclusion. this study shed more light on the potentials of embryo toxicity of Dimethoate as of one of the most common organophosphorus compounds. It also sends an alarm to mankind to exert more efforts at national and international levels to overcome the problem of food contaminated with the organophosphorus compounds to save ourselves and our offspring from the serious harmful effects of such compounds.

Key words: Dimethoate · Embryo Teratogenicity · Skeletal Malformations · Mice

INTRODUCTION

Recently, the increased usage of organophosphorus pesticides in agriculture resulted in environmental and hence, food and water contamination which may threaten the reproductive health of humans and wildlife. Dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithio-ate; 2-dimethoxyphosphinothioylthio-N-methylacetamide) is an organophosphorus insecticide with a contact and systemic action [1].

It is widely used against a broad range of insects including aphids, thrips, plant hoppers and whiteflies in ornamental plants, alfalfa, apples, corn, cottons, grapefruits, grapes, lemons, melons, oranges, pears, pecans, safflower, sorghum, soybeans, tangerines, tobacco, tomatoes, watermelons, wheat and several vegetables [2,3]. It is also used as residual wall spray in farm buildings for houseflies. Dimethoate has been demonstrated to be useful in livestock for the control of botflies [4]. In Egypt, women in villages work on farms beside their husbands to improve their families' life since the poverty level in Egypt is higher than 40% of the population [5] and sometimes those women become pregnant which represents a major socioeconomic problem.

Dimethoate was found in 358 of 6391 food samples analyzed in the USA; 96% of the samples had levels at or below 2 mg/kg [6]. IPCS on 1989 has estimated a total

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daily intake from food of 0.001 ìg/kg of body weight [7]. The toxicity of Dimethoate, however, is attributed to its biotransformation to Omethoate, which is a more toxic pesticide and plays a dominant role in the toxicity of this compound in insects and mammals [8].

Several investigators worldwide studied the teratogenic potentials of the organophosphorus insecticide[9 -15]. Repeated exposure of rats at dose levels of 7 and 28 mg/kg/day to dimethoate, as a neurotoxicant, on gestation days (GD) 5-15 considerably altered the functioning of the central nervous system [16,17].

Groups of pregnant female rats received 0, 3, 6, 10, 18, 20 and 40 mg Dimethoate / kg bw. by gavage from day 6-15 and 7-19 of gestation. Salivation and hair loss was observed in females at all dose levels. Females of the highest dose group also had brown facial staining, small rounded feces, hypersensitivity, body tremors and unsteady gait. Body weight gain and food consumption were significantly reduced. No malformations were found. There was no obvious indication of an adverse effect of Dimethoate treatment on mean incidences of both visceral and skeletal anomalies, although at all dosages an unspecific increase was observed relative to control values [9].

Organophosphorus insecticides (OPIs) are one of the most widely used ones and consequently their tendency to produce developmental neurotoxicity remains a major concern [18 - 21]. Pregnant women could be exposed to OPIs under circumstances that do not elicit outward signs of intoxication [22,23] and in light of recent findings such exposures can produce long term cognitive impairment in their children [24,25], the mechanisms and consequences of OPIs developmental neurotoxicity remain a major environmental concern.

The OPIs are known to affect the nervous system by inhibiting acetyl cholinesterase (AChE), the enzyme that modulates the amount of the neurotransmitter acetylcholine [26].

Unfortunately, many pregnant women are often exposed to different OPIs in different dosages at different or overlapping times. However, the developing embryos remain more vulnerable than adults due to chronic cholinergic intoxication by OPIs. These agents affect the process of neural development itself, leading to permanent deficits in the architecture of the nervous system. Despite the increasing recognition of the need to evaluate developmental neurotoxicity in safety assessments [27 -29], only very few of the mixtures of commercial chemicals in current use have been examined with respect to neurodevelopmental effects [30]. The inhibition of AChE results in the accumulation of acetylcholine that over- stimulates cholinergic receptors, which in turn stimulates neurological activity[31]. Indeed, a number of studies utilizing neural cell lines or micromass cultures have clearly demonstrated direct effects of organophosphates on neurodevelopment[32- 40].

Dimethoate caused adverse reproductive effects on mating success, survival of pups and growth of surviving pups in male and female mice after exposure to 60 mg/l for 30 days before mating; however, no teratogenic effects were observed [41]. No fetotoxic effects were observed in rats treated with Dimethoate for doses up to 15 mg/kg through GD 6-20 [42]. At doses up to 20 mg/kg/day, Dimethoate did not produce fetotoxicity, fetal lethality, or malformations in the mice [43].

Although Dimethoate still remains one of the most widely used insecticides in the world, there is limited information regarding the developmental toxicity of this compound [17,42]. Earlier studies in animals exposed to Dimethoate during gestation are limited and scant or insufficient. Therefore, the present investigation was undertaken to assess the possible maternal-foetal toxicity potentials of Dimethoate on the intrauterine growth of mouse embryos at GD20th.

MATERIALS AND METHODS

Chemicals: Dimethoate was obtained from the Pesticide Center Institute, Dokki, Cairo. Dimethoate was of commercial grade of (35% E.C.) with the common name: O, O dimethyl S-(N-methyl carbamoyl-methyl) phosphorodithioate.

Animals, Mating and Condition: This study was carried out using sexually mature male and female mice (CD1), 2 months old, from the central farm for experimental animals of Vaccera, Giza, Egypt. All mice were examined for health status and acclimated to the laboratory environment for 2 weeks before use. Temperature and relative humidity were maintained at 23±2°C and approximately 50% respectively, with a 12 hr : 12 hr light : dark photoperiod. They were housed in stainless steel cages and given a standard diet and water ad libitum throughout the study and fed a standard rodent pellet diet to acclimate for two weeks. The proestrous females cohabited in a ration of (male / two females). On the next morning sperm-positive females and females with a vaginal semen plug were considered to be in Day 0 of pregnancy [44,45].

Experimental Groups: Fifty bred females (29 - 35 g) were divided into five treatment groups (10 pregnant mice / group) according to approximately equal mean body weight. Corn oil served as the vehicle for the control group, while the other four groups were treated with Dimethoate at sublethal doses of 9, 13, 18 and 33 mg/kg body weight respectively.

Maternal-Foetal Investigation: Maternal-Fetal investigations were conducted according to the US Environmental Protection Agency (EPA) TSCA (Toxic Substances Control Act) Test Guidelines [46]. All animals were observed twice daily for mortality and toxicological effects. Body weight and feed consumption values were recorded during treatment. Post exposure observations were performed approximately a half hour after exposure. On (GD 21st), all females were euthanized via carbon dioxide inhalation and a cesarean section and a macroscopic postmortem examination were performed on each. During the macroscopic postmortem examination, uteri, ovaries, liver, kidneys, adrenal, spleen, lungs and heart weights were recorded. The uteri of apparently non pregnant mice were stained with 10% sodium sulfide [47] and examined for evidence of implantation sites. Fetuses were removed from uteri, weighed, sexed and examined for external malformations, measured for crown rump values and anogenital distance. Implantation sites, live, dead, resorbed fetuses indices were calculated. After recording all measurements and parameters, fetuses were divided into two groups. The first group was fixed in Bouin's solution [48], while the second group was placed in acetone to dissolve the body fats, then transferred to potassium hydroxide (1%) to clear the skeletons and applying Alizarin red S to stain the ossified skeletal bones [49,50]. After staining, the fetuses were examined for skeletal anomalies. Skeletal alterations were evaluated according to the atlas of skeletal anomalies [51].

Statistical Procedure: Data were expressed as mean ± standard error of mean (S.E). Student's t-test was applied to determine the significance of differences between treated and control means[52].

RESULTS

The Data obtained from this study revealed that there were no deaths or abortions during the course of the treatment. Signs of cholinergic toxicity including tremors, diarrhea, weakness and salivation were noted in dams at 18 and 33 mg/kg/day Dimethoate compared to the control and the other treated groups.

Maternal Body and Organ Weights: Maternal body change and organ weights are presented in Table 1. The data showed that pregnant female mice treated with Dimethoate exhibited remarkable reduction in body and organ weights when compared to control and these reductions were dose dependent and statistically significant.

Fetal Observations: Developmental parameters and fetal weights are summarized in Table 2 and Plat. 1. The number of the implants per litter was significantly altered in the Dimethoate treated groups with approximately (-39.60 %) preimplantation loss compared to (- 8.18 %) in the control group. Dimethoate produced a significant increase in embryo lethality in the treated groups with approximately (- 44.26 %) postimplantation loss compared to (- 3.96 %) in the control group. Total resorption of litters was significantly increased in the treated groups which approximately reached (+ 8.19 %) compared to the control group. A significant decrease was observed in the number of live fetuses as noted from the results of live birth index which showed maximum reduction reached (- 44.26%).

Although significant reduction was observed in the crown rump values of fetuses from treated groups as shown in Table 3. Mean fetal weight was decreased significantly as compared with control. Referring to teratogenic potentials of Dimethoate the obtained data after examining mouse fetuses revealed that Dimethoate significantly induced decrease in male anogenital distance as compared with control and skewed the sex ratio of male toward female which approximately reached (65.52%) in female.

Assessment of Bone Calcification and Anomalies: Examining alizarin red S stained skeletal bones using dissecting microscope revealed that Dimethoate induced reduction in the ossification of the skull bones and these reductions were dose and time dependent. The most affected bones were skull, Clavical and scapular, ossification reached (34.52 %, 22.51 % and 35.14 %) in these bones respectively. Referring to ribs Dimethoate induced several abnormalities of ribs of mouse embryos represented by missing of some ribs (maximum ~ 23.89 %), shortness of ribs (maximum ~ 29.75 %) and rib fusion (maximum ~ 34.63 %) as seen in Table (4) and Plate (2).

Bones of Sternebrae were observed completely ossified in control group. While examination of embryos from treated groups revealed that, the percentage of fused sternebrae reached (maximum ~ 39.16 %) the missed sternebrae its percentage in the treated groups reached (maximum ~ 37.81 %). Vertebral centra of embryos from all

Table 1: Effect of Dimethoate on body and organ weight in pregnant mice.

		Groups of Pregnant Mice				
	Groups	GI	GII	GIII	GIV	GV
Relative	Dose (mg/kg)	Control	9 mg/kg	13 mg/kg	18 mg/kg	33 mg/kg
Weight/100g	Body Weight	43.12 + 1.77	37.19* + 1.12	35.23* ± 1.27	32.82* ± 1.19	29.28* ± 1.61
BW	Liver (g)	4.34 ± 0.52	4.16 ± 0.13	3.89* ± 0.24	3.22* ± 0.14	3.02* ± 0.19
	Ovaries (mg)	28.41 ± 0.51	26.06* ± 0.11	23.22* ± 0.71	19.77* ± 0.52	18.05* ± 0.81
	Uterus (mg)	489.30 ± 7.52	473.42* ± 8.16	461.33* ± 7.62	453.38* ± 6.35	444.23 ± 7.25
	Kidneys (mg)	1.89 ± 0.14	1.73* ± 0.14	1.38* ± 0.31	0.92* ± 0.01	0.87 ± 0.03
	Adrenal (mg)	31.81 ± 0.81	30.76* ± 0.83	29.77* ± 0.51	27.47* ± 0.62	25.44 ± 0.88
	Spleen (mg)	405.4 ± 2.21	400.6* ± 5.03	397.5* ± 4.34	382.2* ± 5.13	375.4 ± 4.11
	Thymus (mg)	87.02 ± 0.21	83.20* ± 1.02	79.13* ± 1.11	62.81* ± 1.51	57.22 ± 1.30
	Thyroid (mg)	15.24 ± 0.02	14.96* ± 0.12	14.65* ± 0.03	13.69* ± 0.01	13.15 ± 0.02

Data expressed as mean ± SD. * = Significant.

Table 2: In utero exposure of mouse embryos to Dimethoate

	Groups of Pregnant Mice				
Groups	GI	GII	GIII	GIV	GV
Dose (mg/kg)	Control	9 mg/kg	13 mg/kg	18 mg/kg	33 mg/kg
Implantation Sites	(101) 10.10 ± 1.03	(91) 9.90* ± 1.12	(79) 7.91* ± 1.30	(68) 6.80* ± 0.98	(61) 6.10* ± 1.17
Corpora Lutea	(110) 11.00 ± 0.10	(108)10.80 ± 0.33	(105) 10.50 ± 0.41	(103) 10.30 ± 0.11	(101) 10.10 ± 0.32
Pre implantation Loss	- 8.18 %	- 15.74%	- 24.76 %	- 33.98 %	- 39.60 %
Implantation Index	91.81 %	84.25 %	75.23 %	60.01 %	50.39 %
Live Birth Index	(97) 96.03 %	(70) 76.92 %	(53) 67.08 %	(37) 54.41 %	(29) 47.54 %
Postimplantation Loss	(4) 3.96 %	(18) 19.78 %	(21) 26.58 %	(26) 35.29 %	(27) 44.26 %
Resorption Index	0.00	(3) 3.29 %	(5) 6.32 %	(5) 7.35 %	(5) 8.19 %

Data expressed as mean ± SD. * = Significant

Table 3: Fetal dimensions and Sex ratio of mouse embryos exposed to Dimethoate *In Utero*.

	Gestation Days (GD0 to GD 20)				
Groups	GI	GII	GIII	GIV	GV
Dose (mg/kg)	Control	9 mg/kg	13 mg/kg	18 mg/kg	33 mg/kg
Crown-rump (cm)	2.51 ± 0.01	2.37* ± 0.05	1.05* ± 0.01	0.97* ± 0.02	0.85* ± 0.01
Body Weight (gm)	2.68 ± 0.21	2.48* ± 0.07	2.26* ± 0.11	1.97* ± 0.05	1.48* ± 0.02
Sex Ratio (M/F)	55/42	37/33	25/28	18/19	10/19
% of Males	56.71 %	52.85 %	47.16 %	48.64 %	34.48 %
% of Females	43.29 %	47.15 %	52.84 %	51.36 %	65.52 %
Male Anogenital Distance (mm)	2.66 ± 0.04	2.51* ± 0.07	1.99* ± 0.01	1.79* ± 0.06	1.62* ± 0.05

Data expressed as mean ± SD. * = Significant.

Table 4: Percentage of Ossification of Skeletal System of Mouse Embryos Exposed to Dimethoate *In Utero*.

Skeletal System Parts	GII	GIII	GIV	GVI
Skull Ossification	71.13	53.29	41.94	34.52
Clavical Ossification	45.29	37.20	33.10	22.51
Scapular Ossification	71.30	53.61	43.82	35.14
Missed Ribs	14.11	18.35	21.16	23.89
Short Ribs	17.27	20.32	26.91	29.75
Fused Ribs	21.12	24.82	29.83	34.63
Missed Sternebrae	22.39	26.25	31.75	37.81
Fused Sternebrae	18.29	26.71	34.29	39.16
Fused Vertebral Centra	10.91	15.21	21.18	26.63
Missed Vertebral Centra	36.81	41.19	49.73	54.19
Humeral Ossification	47.28	42.58	36.64	32.21
Ulnar Ossification	51. 82	46.74	41.46	38.29
Radial Ossification	42.68	36.95	34.91	31.20
Femoral Ossification	45.13	41.69	38.10	34.33
Tibial Ossification	47.32	43.91	39.00	32.91
Fibular Ossification	46.46	43.21	38.55	32.16

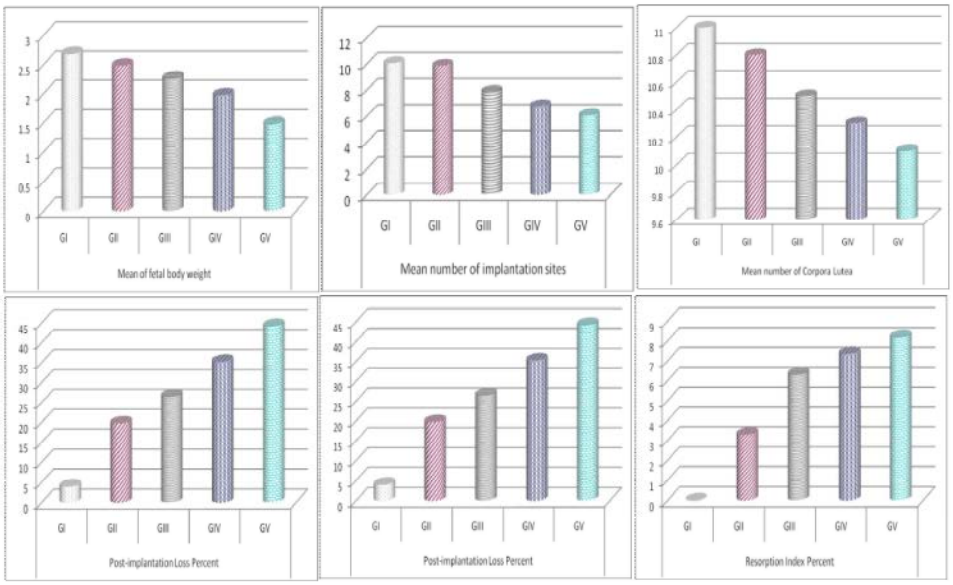

Plate (1): Effect of Dimethoate on pregnancy status of female mice

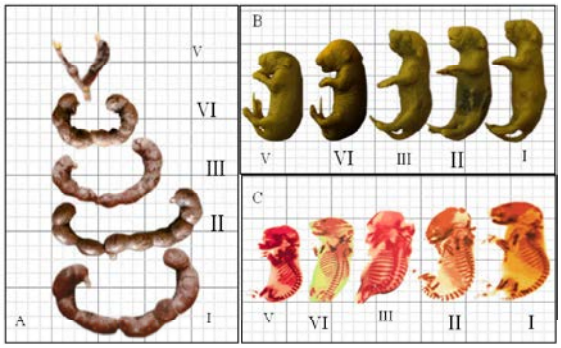

Plate. (2): (A) A photograph of isolated uteri. (B) A photograph showing fetus's size. (C) A photograph showing a lateral view of fetus's skeleton obtained from control and treated pregnant mice after 20 days of gestation.

treated groups were found to be less ossified than those in control as seen in Table (4). Some of cervical, thoracic, lumbar and caudal vertebrae were missing. The most common abnormalities were manifested in missing of central discs (maximum ~ 54.19 %) as well as fused (maximum ~ 26.63 %) of vertebral columns.

The bones of both fore- and hind limbs were completely ossified in control rat embryos. Fore and hind limb bones examined in embryos from Dimethoate-treated groups showed reduction in ossification of Humerus (maximum ~ 32.21 %), ulna (maximum ~ 38.29 %) and radius (maximum ~ 31.20 %). Also, sever reduction in ossification of femur (34.33 %), tibia (32.91 %) and fibula (32.16 %). Finally, Dimethoate doses induced incomplete ossification in the proximal bones in both fore-and hind limbs as carpals, metacarpals, fore phalanges, tarsal, metatarsal and hind phalanges. In addition, all bones of the treated fetuses showed retardation in their length and size when compared with those of control.

DISCUSSION

In the present developmental toxicity study, treatment of the pregnant mice with Dimethoate from GD 0-20 produces maternal and fetal toxicity manifested in reduction of body and organs weight. These results are in accordance with those obtained bymany authors [52-60] for dimethoate insecticide. Reductions in body weight were sensitive indices of toxicity after exposure to toxic substances[61]. Moreover, the reduction of body weight gain of pregnant rats treated with ethylenethiourea might be due to the effect of the compound on the thyroid gland [56].

Regarding to developmental toxicity and teratogenicity of Dimethoate the data of the present investigation showed significant decrease in the crown rump values and fetal weight as compared with control. Also, decrease in male anogenital distance skewed the in the sex ratio of male toward female as well as several skeletal malformation. These effects are in agreement with those obtained previously [43,55, 62- 65].

Sublethal dose of Dimethoate was able to inhibit of implantation completely; since such toxic agent might act directly on the gonadotrophins to alter indirectly the secretion of FSH and LH of the pituitary gland [66].

Exposure to xenobiotic that eventually disrupt ovarian steroid secretion would directly result in inadequate uterine decidulization and receptivity [67]. Fetuses were often susceptible to toxicant due to their fragile developmental state and lack of adequate defense mechanisms [68]. Also, the complex nature of the reproductive regulatory process allows for numerous target sites and accounts for the various mechanisms through which toxins operate to exert their adverse effects. In addition to development of a receptive uterus, estrogen and progesterone play a key role in synchronizing oviductal transport of the pre-implantation mouse embryo [67]. Foetal weight reduction explained by Budreau and Singh [69] who referred this reduction to the impact of Dimethoate by the inhibition of esterases, found in placenta which causes reduction in nutrition.

Foetal Skeletal malformation reported in this study are in accordance with those reported by several authors [57,70] however treated dams with Dimethoate their fetuses exhibited skeletal variations such as absence of important bones or parts of bones, shortenings, bandings, asymmetry, fusions or clefts [71]. The present study data demonstrate that Dimethoate can produce fetotoxicity and teratogenic effects in mouse embryos. Maternal toxicity at 13 mg/kg/day appeared several signs of toxicity ; the 15 mg/kg/day dose is about 1,500 times the acceptable daily intake (ADI) for humans (ADI50.01 mg/kg/day) [72]. So the authors of this study are concerned that such exposure might occur occupationally, particularly for applicators and farmers.

The study also, sends alarm to the mankind to exert more efforts at national and international levels to overcome the problem of food contaminated with the organophosphorus compounds to save ourselves and our offspring from the serious harmful effects of such compounds.

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REFERENCES

1. Sanderson, D.M. and E.F. Edson, 1964. Toxicological properties of the organophosphorus insecticide Dimethoate. Brit. J. industr. Med., 21: 52-64.
2. Mandal, D.M., S. Mandal and N.K. Pal, 2002. Evaluation of bioremediation potential of organophosphorus pesticide dimethoate 30% EC by heavy metal and antibiotic resistant Proteus vulgaris isolated from Ganges at Sreerampore. Research Journal of Chemistry and Environment, 6: 49-52.
3. Mandal, D.M., M. Shyamapada and K. Nishith, 2008. Potential metabolites of dimethoate produced by bacterial degradation. World Journal of Microbiology and Biotechnology, 24: 69-72.
4. FAO (Food Agriculture Organization) 1965. Evaluation of the toxicity of Pesticide Residues in Food. FAO Meeting Report. 10: 27-65.
5. Hundley, H.K., 1988. Pesticide residue findings by the Luke method in domestic and imported foods and animal feeds for fiscal years 1982-1986. Journal of the Association of Official Analytical Chemists, 71(5): 875.
6. IPCS (International Programme on Chemical Safety), 2010. Characterization and application of physiologically-based pharmacokinetic models in risk assessment. Available from: http://www. who. int/ipcs/methods/harmonization/areas/pbpk_models.pdf
7. WHO (World Health Organization), 2001. The WHO recommended classification of pesticides by hazard and guidelines to classification 2000-2002. Geneva, World Health Organization, International Programme on Chemical Safety (WHO/PCS/01.5).
8. Edwards, J.A., N.M. Leeming and R. Clark, 1984. Effect of Dimethoate on pregnancy of the rat. Unpublished report no.: DTF d.d. April 13: 3/84245.
9. Brooker, A.J. and A. Stubbs, 1990. Dimethoate: Dietary range finding study in mature male and female rats and their juvenile offspring, Huntingdon, Cambridgeshire, England :Huntingdon Research Center Ltd.
10. Brooker, A.J. and A. Stubbs, 1991. Dimethoate: dietary range finding study in mature male and female rats and their juvenile offspring. Huntingdon Research Center. DTF Doc No: '453-004' [CHA; sub: 12564, Ref: 3-21/Vol 3-7].

11. Brooker, A.J., B.A. Homan, C.A. Parker, J.M. Offer, A. Anderson and I.S. Dawe, 1992. The effect of dimethoate on reproductive function of two generations in the rat, Huntingdon, Cambridgeshire, England : Huntingdon Research Center Ltd.
12. Brooker, R.W., R.M. Callaway, L. Cavieres, Z. Kikvidze, C. Lortie, R. Michalet, P. Pugnaire, A.Valiente-Banuet and T.G. Whitham, 2009. Don't dissintegration: a comment on Ricklefs' disintegrating communities. Am Nat., 174: 919-927.
13. Mellert, W., J. Hellwig, C. Gembardt, K. Deckert and B. van-Ravenzwaay, 2003. Dimethoate—Two-generation reproduction toxicity study in Wistar rats: Administration in the diet, Ludwigshafen/Rhein, Germany : Experimental Toxicology and Ecology, BASF Aktiengesellschaft.
14. Akhtar, N., M. K. Srivastava and R. B. Raizada, 2006. Transplacental disposition and teratogenic effects of chlorpyrifos in rats. J. Toxicol. Sci., 31: 521-527.
15. Nagymgtenyi, L., H. Schulz, A. Papp and I. Desi, 1998. Developmental neurotoxicological effects of lead and dimethoate in animal experiments. Neurotoxicology, 19: 617-622.
16. Farag, A.T., T.A. Karkour and A. El-Okazy, 2006. Developmental toxicity of orally administered technical dimethoate in rats. Birth Defects Research (Part B), 77: 40-46.
17. Colborn, T., 2006. A case for revisiting the safety of pesticides: a closer look at neurodevelopment. Environ Health Perspect. 114: 10-17.
18. Costa, L.G., T.B. Cole, A. Vitalote and C.E. Furlong, 2005. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. Clin. Chim. Acta. 352: 37-47.
19. Landrigan, P.J., L. Claudio, S.B. Markowitz, G.S. Berkowitz, B.L. Brenner, H. Romero, J.G. Wetmur, T.D. Matte, A.C. Gore and J.H. Godbold, 1999. Pesticides and inner-city children: exposures, risks and prevention. Environ Health Perspect. 107: 431-437.
20. Weiss, B., S. Amler and R.W. Amler, 2004. Pesticides. Pediatrics, 113:1030-1036.
21. De Peyster, A., W.O. Willis, C.A. Molgaard, T.M. MacKendrick and C.Walker, 1993. Cholinesterase and self-reported pesticide exposure among pregnant women. Arch Environ Health, 48: 348-352.
22. Gurunathan, S., M. Robson, N. Freeman, B. Buckley, A, Roy and R. Meyer, 1998. Accumulation of chlorpyrifos on residential surfaces and toys accessible to children, Environ Health Perspect. 106: 9-16.
23. Rauh, V.A., R. Garfinkel, F.P. Perera, H.F. Andrews, L, Hoepner and D.B. Barr, 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics, 118: 1845-1859.
24. Rohlman, D.S., T.A. Arcury, S.A. Quandt, M. Lasarev, J. Rothlein and R. Travers, 2005. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon.
25. Fukuto, T.R., 1971. Relationships between the structure of organophosphorus compounds and their activity as acetylcholinesterase inhibitors. Bull World Health Organ., 44(1-2-3): 31-42.
26. Claudio, L., W.C. Kwa, A.L. Russell and D. Wallinga, 2000. Testing methods for developmental neurotoxicity of environmental chemicals. Toxic Pharmacol., 164: 1-14.
27. Kackar, R., M.K. Srivastava and R.B. Raizada, 1997. Induction of gonadal toxicity to male rats after chronic exposure to mancozeb. Ind. Health, 35: 104-111.
28. Eriksson, P., 1997. Developmental neurotoxicity of environmental agents in the neonate. Neurotoxicology, 18: 719-726.
29. Grandjean, P. and P.J. Landrigan, 2006. Developmental neurotoxicity of industrial chemicals. Lancet, 368: 2167-2178.
30. Gallo, M.A. and N.J. Lawryk, 1991. Organic phosphorus pesticides. In: Hayes WJ and Laws ER, editors, Handbook of Pesticide Toxicology, New York, Academic Press, pp: 3-5.
31. Bagchi, D., M. Bagchi, E.A. Hassoun and S.J. Stohs, 1995. In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology, 104: 129-140.
32. Daruich, J., F. Zirulnik and M.S. Gimenez, 2001. Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses. Environ. Res., 85: 226-231.
33. Crumpton, T.L., F.J. Seidler and T.A. Slotkin, 2000. Developmental neurotoxicity of chlorpyrifos in vivo and in vitro: effects on nuclear transcription factor involved in cell replication and differentiation. Brain Res., 857: 87-98.

34. Das, K.P. and S. Barone, 1999. Neuronal differentiation in PC12 cells is inhibited by chlorpyrifos and its metabolites: is acetylcholinesterase inhibition the site of action? Toxicol Appl Pharmacol., 160: 217-230.
35. Jameson, R.R., F.J. Seidler, D. Qiao and T.A. Slotkin, 2006. Chlorpyrifos affects phenotypic outcomes in a model of mammalian neurodevelopment: critical stages targeting differentiation in PC12 cells. Environ Health Perspect. 114(5): 667-72.
36. Monnet-Tschudi, F., M.G. Zurich, B. Schilter, L.G. Costa and P. Honegger, 2000. Maturation-dependent effects of chlorpyrifos and parathion and their oxygen analogs on acetylcholinesterase and neuronal and glial markers in aggregating brain cell cultures. Toxicol Appl. Pharmacol., 165: 175-183.
37. Qiao, D., F.J. Seidler and T.A. Slotkin, 2001. Developmental neurotoxicity of chlorpyrifos modeled in vitro: comparative effects of metabolites and other cholinesterase inhibitors on DNA synthesis in PC12 and C6 cells. Environ Health Perspect. 109: 909-913.
38. Qiao, D., F.J. Seidler and T.A. Slotkin, 2005. Oxidative mechanisms contributing to the developmental neurotoxicity of nicotine and chlorpyrifos. Toxicol. Appl. Pharmacol., 206: 17-26.
39. Slotkin, T.A., E.A MacKillop, I.T. Ryde, C.A. Tate and F.J. Seidler, 2007. Screening for developmental neurotoxicity using PC12 cells: comparisons of organophosphates with a carbamate, an organochlorine and divalent nickel. Environ Health Perspect. 115: 93-101.
40. Song, X., J.D. Violin, F.J. Seidler and T.A. Slotkin, 1998. Modeling the developmental neurotoxicity of chlorpyrifos in vitro: macromolecule synthesis in PC12 cells. Toxicol. Appl. Pharmacol., 151: 182-191.
41. Budrea, C.H. and R.P. Singh, 1973. Effect of fenthion and dimethoate on reproduction in mouse. Toxicol. Appl. Pharmacol., 26: 29.
42. Srivastava, M.K. and R.B. Raizada, 1996. Developmental effect of technical dimethoate in rats: maternal and fetal toxicity evaluation. Indian J. Exp. Biol., 34: 329-333.
43. Courtney, K.D., J.E. Andrews, J. Springer and L. Dalley, 1985. Teratogenic evaluation of the pesticides baygon, carbofuran, dimethoate and EPN. J Environ Sci Health Bull, 20: 373-406.
44. Gray, L.E., 1991. Delayed effects on reproduction following exposure to toxic chemicals during critical periods of development. In: Cooper, R.L. Goldman, J.M. Harbin, T.J. Aging and Environmental Toxicology: Biological and Behavioral Perspectives. Johns Hopkins University Press, Baltimore. pp: 183-210.
45. Ali, M.O., E.E. El-Nahass, M.O. Diamond and G. Desouki, 1989. Embryo toxic effect of Diabetes mellitus. Al-Azhar Medical J., 17(4): 421-428.
46. EPA, 1998. Health Effects Test Guidelines OPPTS 870.3800 Reproduction and Fertility Effects.
47. Salewski, E., 1964. Staining method for a macroscopic test for implantation points in the uterus of the rat. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol., 247: 367.
48. Wilson, J.G. and J. Warkany, 1965. Teratology: principles and techniques. Chicago: University of Chicago Press.
49. Peters, P.W.J., 1965. Double staining of fetal skeletons for cartilage and bone. In: Wilson JG, Wurkany J, editors. Chicago: University of Chicago Press. pp: 153.
50. Hayes, A.W., 1994. Principle and method of toxicology. New York: Raven Press. Health Canada (1991) Dimethoate. In: Guidelines for Canadian drinking water quality- documents. Available at http://www.hc-sc.gc.ca/hecs-sesc/water/ dwgsup. htm.
51. Chahoud, I., 1997. Atlas of External and Skeletal Anomalies in Rats. CD-ROM. Leipzig, Berlin.
52. Steel, R.G. and J.H. Torrie, 1981. Principle and Procedure of Statistics. A Biometric Approach, Second ed. McGraw- Hill Book Company, New York, USA.
53. WHO Report, 1994. International programme on chemical safety. In: Health & Safety Guide-54 Lindane (WHO,Geneva).
54. Eliane, D., D.M. Fabiana, S.C. Ricardo, J.D. Pereiraa, P.R. Dalsenter and A. Langeloh, 2003. The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. Toxicology Letter, pp: 142: 45.
55. Matokhnyuk, L.A., 1971. Toxicity of the fungicide maneb on inhalation. Hyg. Sanit. 36(5): 195-199.
56. Lu, M.H. and R.E. Staples, 1978. Teratogenicity of ethylenethiourea and thyroid function in the rat. Teratology, 17: 171-178.

57. Lu, M.H. and Kennedy.1986.Teratogenic evaluation of mancozeb in the rat following inhalation exposure. Toxicol. Appl. Pharmacol., 84: 355.
58. Vachkova-Petrova, R., L. Vassileva, G. Antov, N. Choumkov, N. Dontchev, M. Stavreva, E. Tyagounenko, S. Dinoeva, J. Halkova, T. Ivanova and N. Dontchev, 1991. Toxicologic evaluation of propyneb on the Wistar rat. J. Toxicol. Clin. Exp., 11: 407-16.
59. Guven, K., D. Engin, A. Osman, O. Abdurrahman and P. David, 1998.The accumulation and histological effects of organometallic fungicides Propineb and Maneb in the kidneys of fetus and female rats during pregnancy. Toxicology Letters, 99: 91.
60. Baligar, P.N. and B.B. Kaliwal, 2002. Induction of gonadal toxicity to female rats after chronic exposure to mancozeb. Ind. Health, 39: 235-243.
61. Teo, S., D. Stirling, S. Thomas, A. Hoberman, A. Kiorpes and V. Khetani, 2002. A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague-Dawley rats. Toxicology, 174: 183.
62. Dallegrave, E., F.D. Mantese, R.T. Oliveira, A.J. Andrade, P.R. Dalsenter and A. Langeloh, 2002. Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Arch. Toxicol., 81: 665-673.
63. Martson, L.V., 1969. Effect of maneb on embryonal development and generative function of rats Farmkol. Toksikol. Moscow, 32: 731.
64. Janes, J.M. and M. Blair, 1980. Teratology Study in Rats: FMC 35001 Technical, Unpublished report No. ACT 265.33 from International Research and Development Corporation. Submitted to WHO by FMC Corporation.
65. Janes, J.M., D.E. Rodwell and M. Blair, 1980. Teratolgy Study in Rabbits: FMC 35001 Technical, Unpublished report No. ACT 265.33 from International Research and Development Corporation. Submitted to WHO by FMC Corporation.
66. Dickarson, R., L. Howie and S. Safe, 1992. The effect of 6-nitro-1,3,8-trichlorodibenzofuran as a partial estrogen in the female rat uterus. ibid. 113: 55.
67. Matt, D.W. and J.F. Borzelleca, 1995. Toxic effects on the female reproductive system during pregnancy, parturition and lactation. In: Witorsch, Raphael J. (Ed.), Reproductive Toxicology, second ed. Raven Press Ltd. New York, NY, USA, pp: 175-193.
68. Kimberly, P.M., C. Borgeest, D.T. Greenfeld and J.A. Flaws, 2004. In utero effects of chemicals on reproductive tissues in females. Toxicol. Appl. Pharmacol., 198: 111 198.
69. Budreau, C.H. and R.P. Singh, 1973. Effect of fenthion and dimethoate on reproduction in the mouse. Toxicol. Appl. Pharmacol., 26: 29-38.
70. Souza, C.A., R.R. De-Carvalho, S.N. Kuriyama, I.B. Araujo, R.P. Rodrigues, R.S. Vollmer, E.N. Alves and F.J. Paumgartten, 1997. Study of the embryofeto-toxicity of Crown-of-Thorns (*Euphorbia milii*) latex, a natural molluscicide. Braz, J. Med. Biol. Res., 30: 1325.
71. Lorke, D., 1977. Evaluation of Skeleton. In: D. Neubert, H.J. Merker, T.E. Kwasigroch, Methods in Prenatal Toxicology, Evaluation of Embryotoxic Effects in Experimental Animals. Georg Thieme Ver-lag, Stuttgart, Germany, pp: 52-71.
72. Lue, F.C., 1995. A review of the acceptable daily intakes of pesticides assessed by the WHO. Regul. Toxicol. Pharmacol., 21: 351-364.