Research paper

Capability of some pesticides to induce reproductive toxicity and teratogenicity

ABSTRACT:

Several currently used pesticides, especially organophpsphorus and pyrethroid synthetic compound, are known to adversely impair reproductive competence of males under laboratory, field, clinical. Reduced fertility in males is one of the major end points of reproductive toxicity, so the objective of the present study was to assess the potential impacts of lambada-cyhalothrin, profenofos and chlorpyrifos on fertility and sperm parameters of rats with a view of possible extrapolation of the findings to man, as the processes and regulation of male reproduction are highly conserved in mammals. To assess the effect of tested pesticides on fertility of male rats they administered for 30, 60, 90 consecutive days with different doses of (1/10, 1/40 and ADI LD_{50}). Data suggest a potential association between exposures to tested used pesticides and decreased sperm quality. The present study revealed that increased teratospermic (abnormal sperm morphology). Further support for testicular toxicity comes from studies in laboratory rats that showed associations between exposure tested pesticides and sperm shape abnormalities, as well as dose–response relationships between exposure and a decline in epididymal sperm count and motility and increased abnormal sperm. Finally, we can conclude that tested pesticides can cause male reproductive system abnormalities that include reduced sperm production and/or fertilizing capability. It is also possible that the genetic information of the sperm may potentially be altered prior to fertilization. However, the evidence that such environmental chemicals cause infertility is still largely circumstantial. There are many missing links in the causal chain that would connect receptor binding to changes in reproductive health with decreased fertility.

Key Words: male mice, lambada-cyhalothrin, profenofos, chlorpyrifos, sperm fertility and abnormalities, primary spermatocytes.

1. INTRODUCTION:

The health effects of pesticide exposures on male reproduction are a topic of considerable concern in environmental, occupational and reproductive epidemiology. In recent years, scientists have become more aware that human-made chemicals may disrupt reproductive function in wildlife and humans **Colborn *et al.,* (1993); Golden *et al.,* (1999); Moline *et al.,* (2000)**. Pesticides, as human-made chemicals designed to kill living target organisms, are biologically active. An early insight into how pesticides can act as reproductive toxicants at the population level came from case reports in the 1970s of sterility among men working with the pesticides **Teitelbaum, (1999)**. Despite the ubiquitous use of insecticides and subsequent exposure among the general population **[Centers for Disease Control and Prevention (CDC) (2003); Hill *et al.* (1995); MacIntosh *et al.* (1999)]**, there are limited human studies investigating associations between exposure to contemporary-use insecticides at environmental levels and male reproductive health. Human and animal data suggest a potential association between exposures to some commonly used insecticides and decreased sperm quality. A study found an increased teratospermic (abnormal sperm morphology). Further support for testicular toxicity comes from studies in laboratory rats that showed associations between exposure tested pesticides and sperm shape abnormalities **Luca and Balan (1987)**, as well as dose-response relationships between exposure and a decline in epididymal sperm count and motility and increased abnormal sperm morphology **Pant *et al.* (1995), (1996)**. Recently, the CDC reported chlorpyrifos increase sperm shape abnormalities of males in the United States, **CDC (2003)**. Although both animal toxicology and human epidemiologic studies have shown that pesticides may operate through hormonal or genotoxic pathways to affect spermatogenesis **Toppari *et al.,* (1996)**, a limited number of epidemiologic studies have

been published. The objective of the present review was to evaluate population based studies to determine the weight of evidence for associations between occupational and environmental pesticide exposures and different sperm indicators including semen quality.

2. MATERIALS AND METHODS:

2.1. Animals: 80 male albino mice were used in this investigation, aged 4-5 weeks and of mean weight 20 gram. The animals were randomly housed in appropriate stainless cages in group of 4 animals/cage. The animals were rearranged to classes and subclasses, and group; they were also monitored daily for abnormal symptom and weight change was recorded weekly.

2.2. Chemicals: Lambda-cyhalothrin**:** is a restricted use synthetic pyrethroid insecticide. The active ingredient (Lambda-cyhalothrin 99.8 %Agrochemical Co.,). Profenofos: is an organophosphorus insecticide, cholinesterase inhibitor. Which introduced by Giba- Geigy AG (Novartis) Chlorpyrifos: is organophosphorus insecticide. Commercially introduced by Dow chemical Co. (now Dow Agro Sciences) during 1965.

2.3. Animal treatment schedule: Randomized groups of rats housed in cages containing saw dust as bedding and were allocated into 10 groups, each one contained 8 males, the first one group as a control, while the second, third, and fourth group were treated with Lambda-cyhalothrin at doses 1/10 LD_{50}, 1/40 LD_{50} and daily acceptable in take (ADI) for 30, 60, and 90 days respectively through the oral administration. But the other groups were treated with Profenofos and Chlorpyrifos as a previously mentioned doses and period. Pesticides were given twice dose weekly.

2.4. Sampling: The used procedure follows basically the description given by **Alder (1984)**. The tests were removed by making an incision into the scrotum and fat tissue was cleaned. The tunica was removed, transferred the tubes to a small Petri sodium citrate. The tubes were cut up with forceps several times, and then they were mashed on the fly mesh with flat- top forceps. The fluid containing the cells were transferred to 12 x 100 mm round bottom centrifuge tubes, centrifuged at 1000 r.p.m. for 5 min. Supernatant was completely discarded. The hypotonic solution (1% tris-sodium citrate) was slowly added and centrifuged, after 15-20 min., and then the cells were fixed in (methanol and glacial acetic acid, 3:1).The fixation was changed twice after 10 min., for each by centrifugation between changes.

Slide preparation and staining: Cells in fixation were dropped into very clean glass slides and air dried. The slides were stained at least 10 min., using 10 % Giemsa (PH 6.8) or orcein, washed and allowed to dry for subsequent light microscope analysis.

3. RESULTS AND DISCUSSION:

3.1. Analysis of sperm fertility, measures and abnormalities:

It is evident from the present study that the treatment of male mice with Lambda-cyhalothrin, Profenofos and Chlorpyrifos resulted in profound altered sperm morphology. Various morphology sperm abnormalities Fig (1-10) were observed in control and treated animals. The most common types of abnormalities were amorphous, hookless and big head. Percentage of abnormal spermatozoa is present in Table (1) and illustrated in Fig (1-10). Profenofos as well as Chlorpyrifos caused an increase in abnormal sperm heads and tails not only at all closes level used, but also at different time interval. Their frequencies significantly (P= 0.01) that of the control animals Table (1). Lambda-cyhalothrin, caused the same preivous changes but less than Profenofos and Chlorpyrifos. These present evidence that the percentages of abnormal sperms were significantly affected by treatment and period. The same result was mentioned by **Silva Gomes, (1991)** Cyhalothrin exposed rats had a significantly smaller number of head dips in the whole board test. **Ratnasooriya W.D., *et al.,* (2002)** lambda-cyhalothrin in male rats with different doses had no effect on fertility, but sexual competence was seriously impaired in male rats. **Piña-Guzmán B. *et al.,* (2005)**

Organophosphorus pesticides, are associated with male reproductive effects, including sperm chromatin alterations. **Ai Okamura *et al.,* (2005)** sperm counts and sperm morphology in the rats was decreased when eexposed to Dichlorvos. **Narayana K. *et al.,* (2006)** Methyl parathion organophosphate changes such as epithelial cell morphology and luminal observations, the sperm density was normal in control, moderately decreased in experiment 1 at 3.5 and 7 mg/kg. **Aydogan M., and Barlas N., (2006)** rats treated with organophosphate it has been observed that abnormal sperm percentages in treatment groups increased considerably. **Geetha Mathew *et al.,* (2008)** A dose-related statistically significant increase in the percentage of abnormal sperm observed indicates the genotoxic potency of methyl parathion. **Fatma Gokce Uzun, *et al.,* (2009)** malathion (27 mg/kg; 1/50 of the LD_{50} for an oral dose) and/or vitamin C (200 mg/kg) + vitamin E (200 mg/kg) daily *via* gavage for 4 weeks. By the end of 4th week, rats given malathion alone, or in combination with vitamins C and E, had significantly lower sperm counts and sperm motility, and significantly higher abnormal sperm numbers, than the untreated control rats. The rats given malathion alone or in combination with vitamins also had significantly lower plasma sperm motility, sperm morphology, and testosterone levels than the control rats. **Wang X.-Z. *et al.,* (2009)** showed that three doses of cypermethrin (1, 10, and 20 mg/kg) were administered to male mice for 35 d, with or without vitamin E (20 mg/kg). The moderate (10 mg/kg) and high (20 mg/kg) doses of beta-CYP not only decreased the weight of the testes, but also reduced serum testosterone concentration and the expression of steroidogenic acute regulatory protein, in addition to damaging the seminiferous tubules and sperm development. Furthermore, moderate and high doses of beta-CYP administration decreased sperm number, sperm motility.

Data suggest a potential association between exposures to tested used pesticides and decreased sperm quality. The present study revealed that increased teratospermic (abnormal sperm morphology). Further support for testicular toxicity comes from studies in laboratory rats that showed associations between exposure tested pesticides and sperm shape abnormalities, as well as dose–response relationships between exposure and a decline in epididymal sperm count and motility and increased abnormal sperm. Finally, we can conclude that tested pesticides can cause male reproductive system abnormalities that include reduced sperm production and/or fertilizing capability. It is also possible that the genetic information of the sperm may potentially be altered prior to fertilization. However, the evidence that such environmental chemicals cause infertility is still largely circumstantial. There are many missing links in the causal chain that would connect receptor binding to changes in reproductive health with decreased fertility.

Table (1): Effect on sperm morphology induced by lambda-cyhalothrin, profenofos, and chlorpyrifos at (1/10, 1/40, LD_{50} and ADI) for 30, 60, and 90 days as respectively.

Pesticides	Doses	Period	Total abnormal sperm	Types of sperm abnormalities					
				Amorphous	Without book	Big head	Small head	Tail with 2 head	Others
Control	-	30	6.2	1.4	1.5	1.1	0.8	0.5	0.9
		60	6.0	1.5	1.4	1.3	1.0	0.7	1.2
		90	7.2	1.8	1.7	1.2	1.1	1.0	1.5
Lamba-cyhalothrin	1/10	30	13.5	3.2	2.7	1.9	1.7	1.5	2.5
		60	15.3	3.5	3.0	2.3	1.8	1.9	2.8
		90	16.5	3.8	3.2	2.4	2.0	2.1	3.0
	1/40	30	15.0	2.9	3.0	2.6	2.0	1.7	2.8
		60	17.1	4.1	3.1	2.7	2.4	1.9	2.9
		90	18.4	4.2	3.5	2.9	2.5	2.2	3.1
	ADI	30	10.5	1.7	1.0	1.2	1.3	1.4	1.3
		60	8.3	1.5	1.3	1.2	1.4	1.4	1.5
		90	8.8	1.8	1.4	1.3	1.2	1.6	1.5
Profenofos	1/10	30	18.2	5.1	3.4	2.5	1.9	2.3	3.0
		60	20.9	5.8	3.7	2.8	2.5	2.7	3.4
		90	25.6	7.1	4.4	3.5	2.7	3.2	4.7
	1/40	30	18.9	4.6	3.5	3.1	2.2	2.1	3.4
		60	21.9	5.1	4.2	3.7	2.5	2.8	3.6
		90	23.9	5.5	4.6	4.1	3.1	3.1	3.5
	ADI	30	10.8	2.5	1.9	1.4	1.7	1.5	1.8
		60	11.6	2.7	2.2	1.4	1.6	1.8	1.9
		90	12.3	2.8	2.0	1.7	2.0	1.9	1.9
Chlorpyrifos	1/10	30	18	4.8	3.3	2.4	1.9	2.4	3.2
		60	18.3	5.5	3.9	2.9	2.5	2.8	3.7
		90	25.4	6.1	4.6	3.8	3.3	3.5	4.1
	1/40	30	19.9	5.4	3.6	2.3	2.7	2.1	3.8
		60	23.4	5.9	4.7	3.0	2.9	2.8	4.1
		90	26.9	6.5	5.4	3.2	3.7	3.4	4.7
	ADI	30	10.8	1.9	2.0	1.6	1.6	1.9	1.8
		60	11.5	2.0	2.1	1.6	1.7	2.1	2.0
		90	12.2	2.2	2.2	1.9	1.7	2.2	2.0

100 cells were counted

Fig. (1) : Photomicrograph of mice sperm morphology as a negative control. (X 1000)

Fig. (2): Photomicrograph of mice sperm morphology induced by ambda-cyhalothrin at (1/10 LD_{50}) for 90 days. (X 1000)

Fig. (3): Photomicrograph of mice sperm morphology induced by lambda-cyhalothrin at (1/40 LD_{50}) for 90 days. (X 1000)

Fig. (4): Photomicrograph of mice sperm morphology induced after treated by lambda-cyhalothrin at (ADI) for 90 days (X 1000)

Fig. (5): Photomicrograph of mice sperm morphology induced by profenofos at (1/10 LD_{50}) for 90 days. (X 1000)

Fig. (6): Photomicrograph of mice sperm morphology induced by pofenofos at (1/40 LD_{50}) for 90 days. (X 1000)

Fig. (7): Photomicrograph of mice sperm morphology induced by profenofos at (ADI) for 90 days. (X 1000)

Fig. (8): Photomicrograph of mice sperm morphology induced by chloropyrifos at (1/10 LD_{50}) for 90 days. (X 1000)

Fig. (9): Photomicrograph of mice sperm morphology induced by chloropyrifos at (1/40 LD_{50}) for 90 days. (X 1000)

Fig. (10): Photomicrograph of mice sperm morphology induced chlorpyrifos at (ADI) for 90 day. (X 1000)

3.2. Analysis of mice primary spermatocytes:

The results obtained from the analysis of Diakinesis stage in mice primary spermatocytes after treatment with the lambda-cyhalothrin, profenofos and chlorpyrifos is illustrated in Table (2). Three different types of aberration were observed they are stickiness, exchanges, and univalent of se as well as of autosomal chromosomes were observed in Fig. (11-21). After treatment with tested pesticides stickiness ranged from 4, 4, and 5 in the negative control to 9, 13, and 14 after treatment with the highest tested dose 1/10 LD_{50} for 90 days with the Lambda-cyhalothrin, profenofos and chlorpyrifos as respectively. Univalent involved X, Y and autosomal chromosomes were obtained. The total percent of aberrant cells ranged from 8 to 13 % for the control group. Meanwhile, chlorpyrifos highly significantly decreased by 39, 67, and 19 after treatment with 1/10 LD_{50}, and 1/40 LD_{50} and (ADI) for 90 days respectively. In similar, profenofos caused significant decrease after treatment with 1/10 LD_{50}, 1/40 LD_{50} and (ADI) as recorded 66, 63, and 17 for 90 days respectively. Also, lambda-cyhalothrin caused decrease after treatment with 1/10 LD_{50}, 1/40 LD_{50} and (ADI) as 40, 66, and 23 for 90 days respectively.

It was found that the tested pesticides were capable to cause univalent X, Y as well as autosomal chromosomes. Illustrates stickiness and univalents obtained after treatment with all tested pesticides. Cytological examination proved that in the control group binucleat and multinuclei were not observed. At a low dose level 1/40 the binucleat cells were shown to be 20, 19 and 18 and multinuclei were 19, 18, and 18, While the higher dose 1/10 binucleat cells were estimated to be 18, 22 and 20 and multinuclei were 16, 17, and 16 as well treated with lambda-cyhalothrin, profenofos, and chlorpyrifos for 90 days respectively.

The data revealed that significant deecreased of fertility after administration of all tested pesticides either in hight (1/10 LD_{50}) or low dose (1/40 LD_{50}) within the three post treatment period (30, 60 and 90 days) respectively. In the similar effect between hight dose (1/10 LD_{50}) and low dose (1/40 LD_{50}), while with (ADI) dose the result showed no significant changes with all tested pesticides and all treatment period. Profenofos was proven to induce different types of aberration in mice germinal cells more than lambda-cyhalothrin, and chlorpyrifos.

This finding agree with **Sang-Hee Jeong, Byung et al., (2006)** Chlorpyrifos by the administration of (1, 10 and 100 mg/kg b.w./day) to mature rats (F0) through pre-mating, mating, gestation and lactation period and to their offspring (F1) until 13 weeks age via gavage, its caused decreased in fertility index and numbers of implantation and born pups and a higher male sex ratio of pups.

Dutta *et al.,* (2006) Effect of endosulfan on bluegill testes was studied, the seminiferous tubules were of round or oval shape and contained primary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatozoa, spermatids. After 24 h of exposure, there was evidence of slight signs of connective tissue splintering. The 48-h exposure resulted in breakage of primary spermatocyte walls and separation from the seminiferous tubules. The 72-h testis showed further connective tissue damage and migration of primary spermatogonia into the lumen. After 96 h, there was significant damage to connective tissue and the seminiferous tubules were less pronounced. After 1 and 2 weeks, the seminiferous tubule walls were disrupted and missing in places and the structure of the testis was much disorganized compared to the control testis. Biometric analysis indicated that the diameter of the primary spermatogonia decreased from 24 h to two weeks. These kinds of damage could affect the spermatids and spermatozoa and possibly have a negative impact on spermatogenesis and male fertility.

Amina T. Farag, *et al.,* (2007) Dimethoate was given orally by gavage to male mice for 20 days before mating with untreated females. The percent morphologically normal spermatozoa were unaffected in any of dose groups. However, sperm production and percent motile sperm were decreased in the 15 and

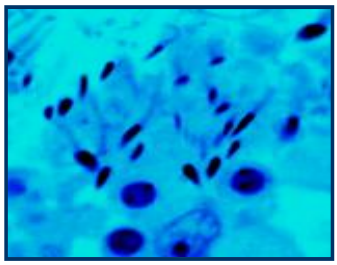
28 mg/kg/day treated groups compared to the control. **Piña-Guzmán B, *et al.*, (2009)** Male mice were exposed to Methyl parathion (20 mg/kg bw, i.p.) and spermatozoa from epididymis-vas deferens were collected at 7 or 28 days post-treatment to assess the effects on maturing spermatozoa and spermatocytes, respectively. In spermatozoa collected at 7 and 28 (dpt), and decreases in sperm quality and induced acrosome reactions were observed; reduced mitochondrial membrane potential and lipoperoxidation were observed at 7 (dpt) only. Negative correlations between lipoperoxidation and sperm alterations were found. Altered sperm functional parameters evaluated either in vitro or in vivo were associated with reduced fertilization rates at both times

Table (2): Effect on mice primary spermatocytes induced by lambda-cyhalothrin, profenofos, and chlorpyrifos at (1/10, 1/40, from LD_{50} and ADI) for 30, 60, and 90 as respectively.

Pesticides	Doses	Period	Stickiness	Univalent				
				XY	Autosomes	Binucleate	Multinuclear	Total percent of aberant cells
Control	-	30	4.0	2	2	0	0	8
		60	4.0	2	1	0	0	7
		90	5.0	3	2	1	2	13
Lamba-cyhalothrin	1/10	30	5.0	4	2	11	10	32
		60	8.0	5	5	15	13	35
		90	9.0	5	7	18	16	40
	1/40	30	6.0	4	3	13	11	51
		60	8.0	6	6	15	16	40
		90	12.0	7	8	20	19	66
	ADI	30	4.0	3	2	4	3	16
		60	4.0	3	2	5	3	17
		90	5.0	4	3	6	5	23
Profenofos	1/10	30	5.0	3	3	14	11	36
		60	8.0	5	6	18	15	52
		90	13.0	6	8	22	17	66
	1/40	30	5.0	4	3	15	13	40
		60	7.0	6	5	17	14	49
		90	10.0	7	9	19	18	63
	ADI	30	3.0	2	2	5	4	16
		60	5.0	2	2	5	5	16
		90	4.0	3	2	6	5	17
Chlorpyrifos	1/10	30	7.0	4	4	15	10	55
		60	11.0	6	6	17	15	39
		90	14.0	7	7	20	16	39
	1/40	30	7.0	4	3	13	12	36
		60	13.0	5	5	16	16	55
		90	17.0	7	7	18	18	67
	ADI	30	4.0	2	2	5	3	16
		60	3.0	1	2	4	3	13

		90	5.0	2	3	5	4	19

100 cells were counted



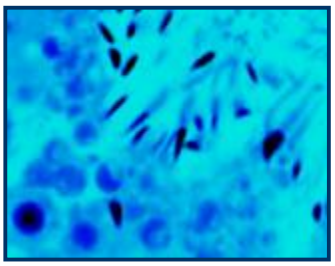


Fig. (11): Photomicrograph of mice primary spermatocytes aberrations as a negative control. (X 1000)

Fig. (12): Photomicrograph of mice primary spermatocytes aberration induced by lambda-cyhalothrin, at (1/10 LD_{50}) for 90 days. (X 1000)

Fig. (13): Photomicrograph of mice primary spermatocytes aberration induced by lambda-cyhalothrin, at (1/40 LD_{50}) for 90 days. (X 1000)

Fig. (14): Photomicrograph of mice primary spermatocytes aberration induced by lambda-cyhalothrin, at (ADI) for 90 days. (X1000)

Fig. (15): Photomicrograph of mice primary spermatocytes aberration induced by profenofos, at (1/10 LD_{50}) for 90 days. (X1000)

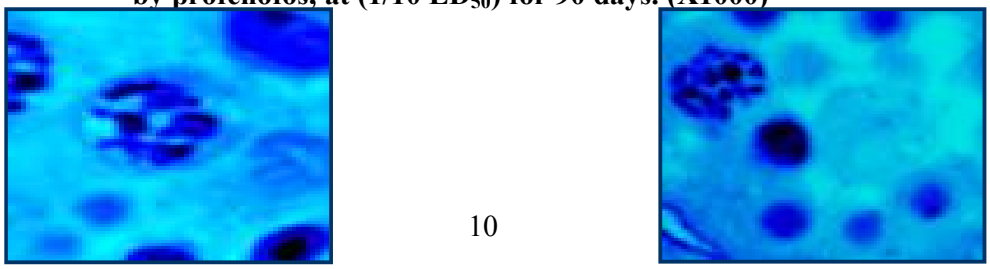


Fig. (16): Photomicrograph of mice primary spermatocytes aberration induced by profenofos, at (1/40 LD_{50}) for 90 days. (X1000)

Fig. (17): Photomicrograph of mice primary spermatocytes aberration induced by profenofos, at (ADI) for 90 days. (X1000)

Fig. (18): Photomicrograph of mice primary spermatocytes aberration induced by chlorpyrifos at (1/10 LD_{50}) for 90 days. (X 1000)

Fig. (20): Photomicrograph of mice primary spermatocytes aberration induced by chlorpyrifos at (1/40 LD_{50}) for 90 days. (X 1000)

Fig. (21): Photomicrograph of mice primary spermatocytes aberration induced by chlorpyrifos at (ADI) for 90 days. (X1000)

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