SDI Paper Template Version 1.6 Date 11.10.2012

Spermatogenic Alterations Induced by Organophosporus Compounds Profenofos, Chlorpyrifos and Synthetic Pyrethroid Lambada-cyhalothrin in Mice

H. M. El-bendary[1], A. A. Saleh[2], S. E. Negm[2], M. E. Khadey[2] and F. A. Hosam Eldeen[2]

[1] Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt
[2] Pesticides Department, Faculty of Agriculture, Mansoura University, Egypt

.

ABSTRACT

Aims: 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 sperm ~~morphology~~fertility, motility, and nuclear changes of ~~sperm shape abnormalities, and~~ primary spermatocytes of~~n~~ male albino mice.

Study design: To assess the effect of tested pesticides on sperm morphology~~fertility~~ of male albino mice ~~they~~ treated ~~administered~~ for 30, 60 and 90 consecutive days with different doses of pesticides (1/10, 1/40 and ADI LD_{50}). ~~; respectively.~~

Place and Duration of Study: Institute of animal health, Ministry of Agriculture, Egypt, between May 2011 and March 2012.

Results: Data suggest a potential association between exposures to tested ~~used~~ pesticides and decreased sperm quality and ~~. The present study revealed that~~ increased teratospermia~~c~~ (abnormal sperm morphology). Further support for testicular toxicity comes from studies in laboratory albino mice 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.

Conclusion: 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. Both the concentrations of the tested pesticides decreased sperm count associated with increase in the number of morphologically abnormal spermatozoa of treated mice; sperm motility was decreased with the highest concentration of the tested pesticides. However their still lake of knowledge of the environmental effect of tested chemicals. There are many missing links in the causal chain that would connect receptor binding to changes in reproductive health with

Comment [u1]: This is similar to the conclusion section, pls authors should recast this section with specificchanges in the study.

Comment [u2]:

Comment [u3]: Delete here. preconcieved

decreased fertility.

Keywords: male albino mice, lambada-cyhalothrin, profenofos, chlorpyrifos, sperm fertility, sperm motility, sperm shape abnormalities, primary spermatocytes.

1. INTRODUCTION

The health ~~effects of pesticide exposures on male reproduction~~ effects of pesticide exposures on male reproduction are ~~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 pesticide~~s~~ (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 mice 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 that 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 this investigation is to evaluate the effect of tested pesticides on sperm fertility, sperm motility, sperm shape abnormalities, and primary spermatocytes in male albino mice, in order to recognize the computability of these insecticides to the environment and to determine the draw bakes of such chemicals on humans.

2. EXPERIMENTAL DETAILS:

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 groups of 8 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, and Chlorpyrifos are an organophosphorus insecticides. Commercially were kindly provided from Central Agricultural Pesticide Laboratory (Dokki, Giza, Egypt) and all compounds were of 99 % purity.

Comment [u4]: This is not correct: c/f Bolognesi C. Genotoxicity of pesticides: a review of human biomonitoring studies. Mutation Research/Reviews in Mutation Research 2003; 543(3): 251–272**;** Gary M. Williamsa, Robert Kroes, Ian C. Munroc**.** Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans. Regulatory Toxicology and Pharmacology 2000; 31(2): 117–165; Maurizio Clementia, Gian Mario Tibonib, Roberto Causinc, Cinzia La Roccad, Francesca Maranghid, Francesco Raffagnatoa, Romano Tenconia. Pesticides and fertility: An epidemiological study in Northeast Italy and review of the literature. Reproductive Toxicology Volume 26, Issue 1, September 2008, Pages 13–18.

Comment [u5]: Not in reference

Comment [u6]: Are you arguring in support for an epidermiological study ?

Comment [u7]: Means?

Comment [u8]: What is the reason for this stratification?

2.3. Animal treatment schedule: Randomized groups of albino mice 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~~ per week~~ly~~, as mentioned in Table (1).

Table (1): The treatment schedule and design

Treatment	Group No.	Doses mg/kg./b.wt.	Period	Dose/weak
___	Group (1)		As a control	
Lambda-cyhalothrin	Group (2)	1/10 LD_{50} = 9.5	30, 60, and 90 days	twice dose
	Group (3)	1/40 LD_{50} = 2.37		
	Group (4)	(ADI) = 0.005		
Profenofos	Group (5)	1/10 LD_{50} = 35	30, 60, and 90 days	twice dose
	Group (6)	1/40 LD_{50} = 8.95		
	Group (7)	(ADI) = 0.01		
Chlorpyrifos	Group (8)	1/10 LD_{50} = 15	30, 60, and 90 days	twice dose
	Group (9)	1/40 LD_{50} = 3.75		
	Group (10)	(ADI) = 0.01		

2.4. Sampling: The testes were removed by making an incision into the scrotum and fat tissue was cleaned as previously described in Alder (1984). Then, the tunica was removed and the tubes were transferred into small Petri dishes containing 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.

Comment [u9]: Whcih tubes?

2.5. Slide preparation and staining: Separated cells were transferred gently on slides then 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.

2.6. Sperm analysis: Sperm motility and sperm morphological analysis according to the method described by Jeong *et al.*, (2005). State briefly what was done here.

2.7. Staistical analysis: Data are expressed as Means using the program SPSS 12 by performing on-way ANOVA with post hoc comparisons between control group and each of the treated group. A p-value less than 0.05 was considered satatistically significant.

Comment [u10]: These were not shown in the results? B

3. RESULTS AND DISCUSSION

3.1. Analysis of sperm fertility, measures and abnormalities:

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 (2) and illustrated in Fig (1-9). Profenofos as well as Chlorpyrifos caused an increase in abnormal sperm heads and tails not only at all doses level used, but also at different time interval. Their frequencies significantly (P= 0.01) in comparison with the control animals Table (1). Lambda-cyhalothrin caused less significant changes. These ~~present~~ evidence suggests that the percentages of abnormal sperms were significantly affected by treatment and period~~. These findings~~ and agrees with Silva Gomes, (1991). Cyhalothrin exposed mice had a significantly smaller number of head dips in the whole board test. Ratnasooriya ~~W.D.~~, *et al*., (2002) lambda-cyhalothrin in male mice exposed to different doses had no effect on fertility. 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 mice was decreased when eexposed to Dichlorvos, also Narayana K. *et al.,* (2006) found abnormalities in sperm density using 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) mice 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, mice 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 mice. The mice given malathion alone or in combination with vitamins also had significantly lower plasma sperm motility, sperm morphology, and testosterone levels than the control mice. 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.

Results showed that there was a correlation between Chlorpyrifos and Profenofos administration and the highly significant decrease of reproductive performance in male mice that ~~is~~ agrees with Ahmed ~~A. Hendawy~~ et al., (2012). The reduction in fertility index may simply represent the effects of Chlorpyrifos exposure on sperm parameters. Therefore, the effects of Chlorpyrifos on the fertility can be attributed to its ability to reduce sperm morphology and motility. Finally we cane concluded that both the concentrations of the tested pesticides decreased sperm motility associated with increase in the number of morphologically abnormal of treated mice; however sperm motility was significantly decreased with the highest concentration of the tested pesticides.

Comment [u11]: These are all reports of various authors which needs to be bput in proper context by authors; in this present form, it is very clumsy and does not add any value for clarity or explaining the principles of the study! Paragraphing style is very poor.

Comment [u12]: Not shown!

Table (2): 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
Con.		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.	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
Chlorpyrfos	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

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 mice 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 say that this is a preliminary work that shows some abnormalities in sperm structure, motility and nuclei morphology, and we suggest some important future studies; whole male reproductive organs sampled fertility tests must be done, to give a full picture of the caused male reproductive system abnormalities can be done using tested pesticides. 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.

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)

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 (3). 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. (10-18). 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 mice (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.

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 mice at both times.

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. The results showed that decrease in concentrations of spermatozoaas the same described with Muftau Shittu *et al.,* (2013).

Table (3): 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
Cont.		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-	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

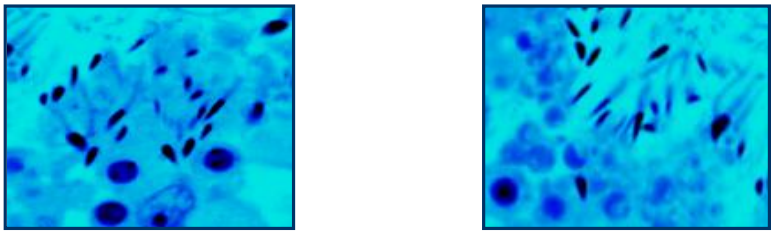

Fig. (10): Photomicrograph of mice primary spermatocytes aberrations as a negative control. (X 1000)

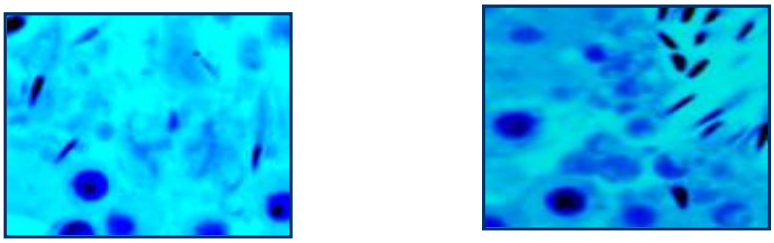

Fig. (11): Photomicrograph of mice primary spermatocytes aberration induced by lambda-cyhalothrin at (1/10 LD50) for 90 days. (X 1000)

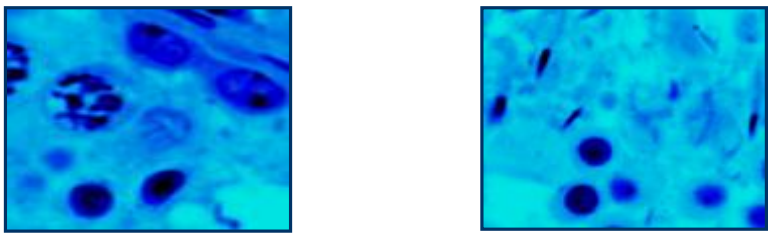

Fig. (12): Photomicrograph of mice primary spermatocytes aberration induced by lambda-cyhalothrin at (1/40 LD50) for 90 days. (X 1000)

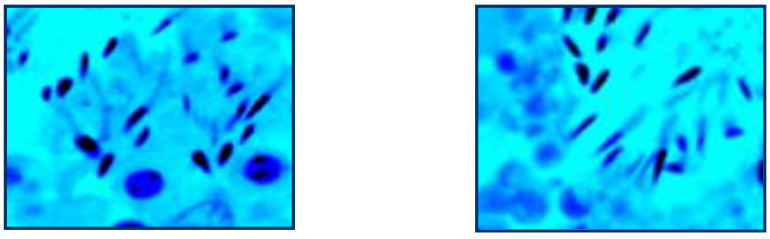

Fig. (13): Photomicrograph of mice primary spermatocytes aberration induced by

lambda-cyhalothrin at (ADI) for 90 days. (X1000)

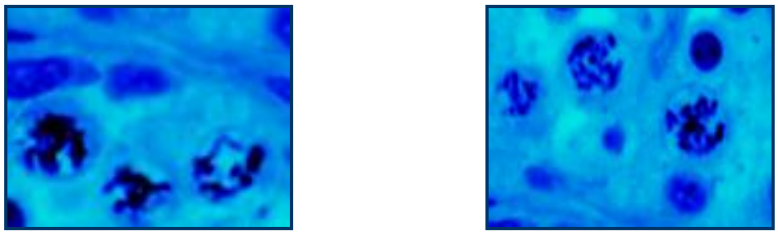

Fig. (14): Photomicrograph of mice primary spermatocytes aberration induced by profenofos at (1/10 LD50) for 90 days. (X1000)

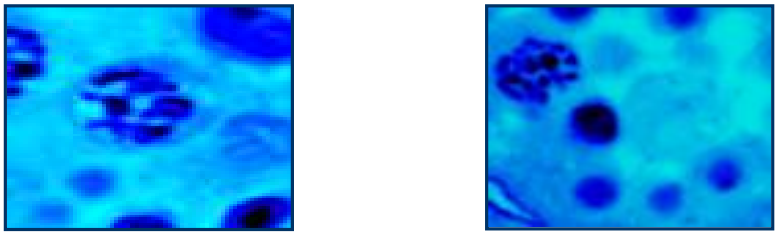

Fig. (15): Photomicrograph of mice primary spermatocytes aberration induced by profenofos at (1/40 LD50) for 90 days. (X1000)

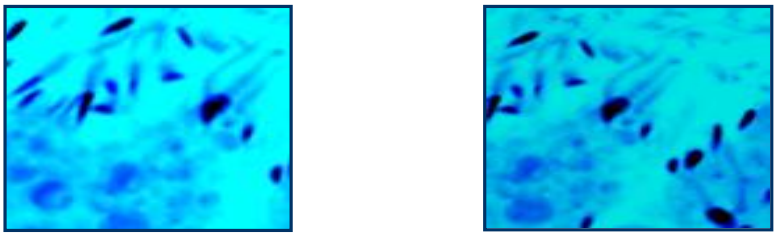

Fig. (16): Photomicrograph of mice primary spermatocytes aberration induced by profenofos at (ADI) for 90 days. (X1000)

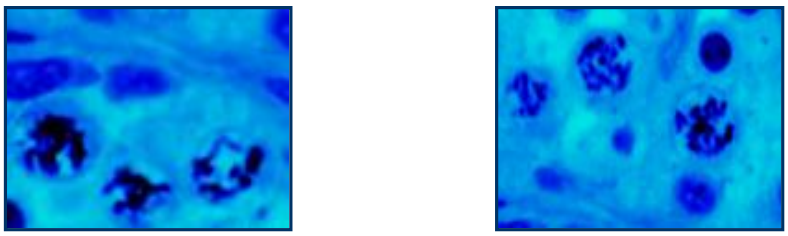

Fig. (17): Photomicrograph of mice primary spermatocytes aberration induced by chlorpyrifos at (1/10 LD50) for 90 days. (X 1000)

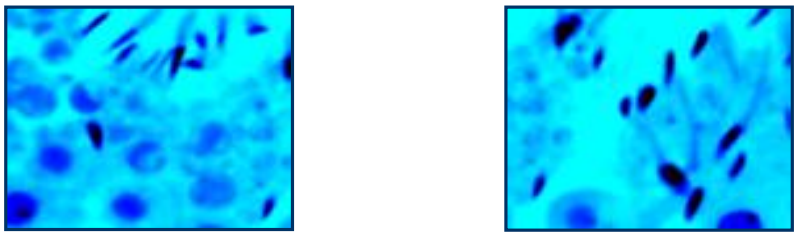

Fig. (18): Photomicrograph of mice primary spermatocytes aberration induced by chlorpyrifos at (1/40 LD_{50}) for 90 days. (X 1000)

4. CONCLUSION:

This preliminary investigation gave us clear picture of abnormalities in sperm structure, motility and nuclei morphology, can be caused by tested pesticides, so that we suggest that these pesticides should be used at recommended doses only if necessary.

AUTHORS' CONTRIBUTIONS

Authors may use the following wordings for this section: "H. M. El-bendary 1, designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'S. E. Negm 2, A. A. Saleh 3, M. E. Khadey 4 and F. A. Hosam Eldeen 5 managed the analyses of the study. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

REFERENCES

Ai Okamura, Michihiro K, Eiji Sh, Katsumi O, Kenji T, Jun U, Yukari W, Minoru O, Hailan W, Gaku I, Takaaki K and Tamie N. Acetylcholinesterase inhibition and increased food consumption rate in the zebrafish, Danio rerio, after chronic exposure to parathion. Reproductive Toxicology. 2005; 13:132-218.

Ahmed AH, Mansour HZ, E l-Sayed AA, Abd El-Aziz AD, and Reham ZH. Ameliorative Role and Antioxidant Effect of Propolis and Ginseng against Reproductive Toxicity of Chlorpyrifos and Profenofos in Male Rats. Life Sci J. 2012; 9 (3):2557-2567.

Alder ID. Cytogenic tests in mammals in Mutagenicity Testing. A Partical Approach, Venitt, S. and Parry, J. M, Eds, IRL Press, Oxford. 1984; 275-306.

Field Code Changed

Amina TF, Ahmed FE, and Nasra AS. Assessment of reproductive toxicity of orally administered technical dimethoate in male mice. Reproductive Toxicology. 2007; 23: 232-238.

Aydogan M, and Barlas N. Effects of maternal 4-tert-octylphenol exposure on reproductive tract of male rats at adulthood. Annals of the New York Academy of Sciences. 2006; 1076 (1): 925–941.

CDC. Second National Report on Human Exposure to Environmental. Chemicals. Atlanta, GA:Centers for Disease Control and Prevention.2004.

Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect. 1993; 101:378–384.

Dutta, HM, Dogra VJ, Singh KN, and Richmonds C. Malathion induced changes in serum proteins and hematological parameters of an Indan Catafish Heteropeustes fossilis (Bloch). Bull. Environ. Contam.Toxicol.1992; 49:91-97.

Fatma GU, Suna K, Dilek D, Filiz D, Yusuf K. Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. Food and Chemical Toxicology, In Press, Corrected Proof, Available online 13 May 2009.

Geetha M, Vijayalaxmi k, and Abdul Rahiman M. Methyl parathion-induced sperm shape abnormalities in mouse. Mutation Research/Genetic Toxicology.2008; 280 (3):169-173.

Golden AL, Moline JM, Bar-Chama N. Male reproduction and environmental and occupational exposures: a review of epidemiologic methods. Salud Publica Mex. 1999; 41: S93–S105.

Hill RH, Head SL, Baker S, Gregg M, Shealy DB, Bailey SL. Pesticide residues in urine of adults living in the United States: reference range concentrations. Environ Res. 1995; 71:99–108

Luca D, and Balan M. Sperm abnormality assay in the evaluation of the genotoxic potential of carbaryl in rats. Morphol Embryol (Bucur). 1987; 33:19–22.

MacIntosh DL, Needham LL, Hammerstrom KA, Ryan PB. A longitudinal investigation of selected pesticide metabolites in urine. J Expo Anal Environ Epidemiol.1999; 9:494–501.

Muftau Sh, Suleiman FA, Joseph OA, Mohammed YF, Mohammed MS, Lukuman SY. Evaluation of chronic chlorpyrifos-induced reproductive toxicity in male Wistar rat: protective effects of vitamin C. Exp Integr Med. 2013; 3 (1): 23-30

Moline JM, Golden AL, Bar-Chama N, Smith E, Rauch ME, Chapin RE, Perreault SD, Schrader SM, Suk WA, Landrigan PJ. Exposure to hazardous substances and male reproductive health. Environ Health Perspect. 2000; 108:803–813.

Narayana K, Hawes A, Michaels J. Precautionary worker health and safety for emerging technologies. Annals of the New York Academy of Sciences. 2006; 1076 (1): 925–941.

Field Code Changed

Field Code Changed

Pant N, Srivastava SC, Prasad AK, Shankar R, Srivastava SP. Effects of carbaryl on the rat's male reproductive system. Vet Hum Toxicol.1995; 37:421–425.

Piña-Guzmán B, Solís-Heredia MJ, and Quintanilla-Vega B. Diazinon alters sperm chromatin structure in mice by phosphorylating nuclear protamines. Toxicology and Applied Pharmacology. 2005; 202: 189-198.

Piña-Guzmán B, Sánchez-Gutiérrez M, Marchetti F, Hernández-Ochoa I. Solís-Heredia MJ, Quintanilla-Vega B. Methyl-parathion decreases sperm function and fertilization capacity after targeting spermatocytes and maturing spermatozoa.Toxicology and Applied Pharmacology. 2009; 238: 141-149.

Ratnasooriya WD, Ratnayake SSK, Jayatunga YNA. Effects of pyrethroid insecticide (lambda cyhalothrin) on reproductive competence of male rats. Asian J Andro. 2002; 4: 35-40.

Sang-Hee J, Byung-Yong K, Hwan-Goo K, Hyun-Ok Ku, and Joon-Hyoung Ch. Effect of chlorpyrifos-methyl on steroid and thyroid hormones in rat F0- and F1-generations. Toxicology. 2006; 220 (2-3): 189-202.

Silva Gomes. The physical and behavioral effects of cyhalothrin were studied in rats. Toxicol. 1993; 33 (4): 315 -317.

Teitelbaum DT. The toxicology of 1,2-dibromo-3-chloropropane (DBCP): a brief review. Int J Occup Environ Health. 1999; 5:122–126.

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, Jegou B, Jensen TK, Jouannet P, Keiding N. Male reproductive health and environmental xenoestrogens. Environ Health Perspect. 1996; 104 (4):741–803.

Wang XZ. Liu S S, Sun Y, W u JY, Zhou Y L, Hang Z h. Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress. Theriogenology, In Press, Corrected Proof, Available online 4 June 2009

Field Code Changed

Field Code Changed