

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

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ABSTRACT

Aims: Several currently used pesticides, especially organophosphorus 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 fertility, motility, sperm shape abnormalities, and primary spermatocytes on male albino mice.

Study design: To assess the effect of tested pesticides on fertility of male albino mice they administered for 30, 60 and 90 consecutive days with different doses of (1/10, 1/40 and ADI LD₅₀); 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. The present study revealed that increased teratospermic (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 decreased fertility.

20 *Keywords:* male albino mice, lambda-cyhalothrin, profenofos, chlorpyrifos, sperm fertility,
21 sperm motility, sperm shape abnormalities, primary spermatocytes.

22 1. INTRODUCTION

23 The health effects of pesticide exposures on male reproduction are a topic of considerable
24 concern in environmental, occupational and reproductive epidemiology. In recent years,
25 scientists have become more aware that human-made chemicals may disrupt reproductive
26 function in wildlife and humans Colborn *et al.*, (1993); Golden *et al.*, (1999); Moline *et al.*,
27 (2000). Pesticides, as human-made chemicals designed to kill living target organisms, are
28 biologically active. An early insight into how pesticides can act as reproductive toxicants at
29 the population level came from case reports in the 1970s of sterility among men working with
30 the pesticides Teitelbaum, (1999). Despite the ubiquitous use of insecticides and
31 subsequent exposure among the general population [Centers for Disease Control and
32 Prevention (CDC) (2003); Hill *et al.* (1995); MacIntosh *et al.* (1999)], there are limited human
33 studies investigating associations between exposure to contemporary-use insecticides at
34 environmental levels and male reproductive health. Human and animal data suggest a
35 potential association between exposures to some commonly used insecticides and
36 decreased sperm quality. A study found an increased teratospermic (abnormal sperm
37 morphology). Further support for testicular toxicity comes from studies in laboratory mice
38 that showed associations between exposure tested pesticides and sperm shape
39 abnormalities Luca and Balan (1987), as well as dose-response relationships between
40 exposure and a decline in epididymal sperm count and motility and increased abnormal
41 sperm morphology Pant *et al.* (1995), (1996). Recently, the CDC reported chlorpyrifos
42 increase sperm shape abnormalities of males in the United States, CDC (2003). Although
43 both animal toxicology and human epidemiologic studies have shown that pesticides may
44 operate through hormonal or genotoxic pathways to affect spermatogenesis Toppari *et al.*,
45 (1996), a limited number of epidemiologic studies have been published. The objective of this
46 investigation is to evaluate the effect of tested pesticides on sperm fertility, sperm motility,
47 sperm shape abnormalities, and primary spermatocytes in male albino mice, in order to
48 recognize the computability of these insecticides to the environment and to determine the
49 draw bakes of such chemicals on humans.

50 2. EXPERIMENTAL DETAILS:

51 **2.1. Animals:** 80 male albino mice were used in this investigation, aged 4-5 weeks and of
52 mean weight 20 gram. The animals were randomly housed in appropriate stainless cages in
53 group of 8 animals/cage. The animals were rearranged to classes and subclasses, and
54 group; they were also monitored daily for abnormal symptom and weight change was
55 recorded weekly.

56 **2.2. Chemicals:** Lambda-cyhalothrin: is a restricted use synthetic pyrethroid insecticide.
57 The active ingredient (Lambda-cyhalothrin 99.8 % Agrochemical Co.). Profenofos, and
58 Chlorpyrifos are an organophosphorus insecticides. Commercially were kindly provided from
59 Central Agricultural Pesticide Laboratory (Dokki, Giza, Egypt) and all compounds were of 99
60 % purity.

61 **2.3. Animal treatment schedule:** Randomized groups of albino mice housed in cages
62 containing saw dust as bedding and were allocated into 10 groups, each one contained 8
63 males, the first one group as a control, while the second, third, and fourth group were treated
64 with Lambda-cyhalothrin at doses 1/10 LD₅₀, 1/40 LD₅₀ and daily acceptable in take (ADI) for
65 30, 60, and 90 days respectively through the oral administration. But the other groups were

66 treated with Profenofos and Chlorpyrifos as a previously mentioned doses and period.
 67 Pesticides were given twice dose weekly, as mentioned in Table (1).

68 **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		
	Group (5)	1/10 LD ₅₀ = 35		
Profenofos	Group (6)	1/40 LD ₅₀ = 8.95	30, 60, and 90 days	twice dose
	Group (7)	(ADI) = 0.01		
	Group (8)	1/10 LD ₅₀ = 15		
Chlorpyrifos	Group (9)	1/40 LD ₅₀ = 3.75	30, 60, and 90 days	twice dose
	Group (10)	(ADI) = 0.01		

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70 **2.4. Sampling:** The tests were removed by making an incision into the scrotum and fat
 71 tissue was cleaned Alder (1984). Then, the tunica was removed and the tubes were
 72 transferred into small Petri dishes containing sodium citrate. The tubes were cut up with
 73 forceps several times, and then they were mashed on the fly mesh with flat- top forceps. The
 74 fluid containing the cells were transferred to 12 x 100 mm round bottom centrifuge tubes,
 75 centrifuged at 1000 r.p.m. for 5 min. Supernatant was completely discarded. The hypotonic
 76 solution (1% tris-sodium citrate) was slowly added and centrifuged, after 15-20 min., and
 77 then the cells were fixed in (methanol and glacial acetic acid, 3:1).The fixation was changed
 78 twice after 10 min., for each by centrifugation between changes.

79 **2.5. Slide preparation and staining:** Separated cells were transferred gently on slides
 80 then air dried. The slides were stained at least 10 min., using 10 % Giemsa (pH 6.8) or
 81 orcein, washed and allowed to dry for subsequent light microscope analysis.

82 **2.6. Sperm analysis:** Sperm motility and sperm morphological analysis according to the
 83 method described by Jeong *et al.*, (2005).

84 **2.7. Staistical analysis:** Data are expressed as Means using the program SPSS 12 by
 85 performing on-way ANOVA with post hoc comparisons between control group and each of
 86 the treated group. A p-value less than 0.05 was considered atatically significant.
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88 3. RESULTS AND DISCUSSION

89 3.1. Analysis of sperm fertility, measures and abnormalities:

90 Various morphology sperm abnormalities Fig (1-10) were observed in control and treated
 91 animals. The most common types of abnormalities were amorphous, hookless and big head.
 92 Percentage of abnormal spermatozoa is present in Table (2) and illustrated in Fig (1-9).
 93 Profenofos as well as Chlorpyrifos caused an increase in abnormal sperm heads and tails

94 not only at all doses level used, but also at different time interval. Their frequencies
95 significantly ($P= 0.01$) in comparison with the control animals Table (1). Lambda-cyhalothrin
96 less significant changes. These present evidence that the percentages of abnormal sperms
97 were significantly affected by treatment and period. These findings agree with Silva Gomes,
98 (1991) Cyhalothrin exposed mice had a significantly smaller number of head dips in the
99 whole board test. Ratnasooriya W.D., *et al.*, (2002) lambda-cyhalothrin in male mice
100 exposed to different doses had no effect on fertility. Piña-Guzmán B. *et al.*, (2005)
101 Organophosphorus pesticides, are associated with male reproductive effects, including
102 sperm chromatin alterations. Ai Okamura *et al.*, (2005) sperm counts and sperm morphology
103 in the mice was decreased when exposed to Dichlorvos, also Narayana K. *et al.*, (2006)
104 found abnormalities in sperm density using Methyl parathion organophosphate changes
105 such as epithelial cell morphology and luminal observations, the sperm density was normal
106 in control, moderately decreased in experiment 1 at 3.5 and 7 mg/kg. Aydogan M., and
107 Barlas N., (2006) mice treated with organophosphate it has been observed that abnormal
108 sperm percentages in treatment groups increased considerably. Geetha Mathew *et al.*,
109 (2008) A dose-related statistically significant increase in the percentage of abnormal sperm
110 observed indicates the genotoxic potency of methyl parathion. Fatma Gokce Uzun, *et al.*,
111 (2009) malathion (27 mg/kg; 1/50 of the LD₅₀ for an oral dose) and/or vitamin C
112 (200 mg/kg) + vitamin E (200 mg/kg) daily via gavage for 4 weeks. By the end of 4th week,
113 mice given malathion alone, or in combination with vitamins C and E, had significantly lower
114 sperm counts and sperm motility, and significantly higher abnormal sperm numbers, than the
115 untreated control mice. The mice given malathion alone or in combination with vitamins also
116 had significantly lower plasma sperm motility, sperm morphology, and testosterone levels
117 than the control mice. Wang X.-Z. *et al.*, (2009) showed that three doses of cypermethrin (1,
118 10, and 20 mg/kg) were administered to male mice for 35 d, with or without vitamin E
119 (20 mg/kg). The moderate (10 mg/kg) and high (20 mg/kg) doses of beta-CYP not only
120 decreased the weight of the testes, but also reduced serum testosterone concentration and
121 the expression of steroidogenic acute regulatory protein, in addition to damaging the
122 seminiferous tubules and sperm development. Furthermore, moderate and high doses of
123 beta-CYP administration decreased sperm number, sperm motility. Results showed that there
124 was a correlation between Chlorpyrifos and Profenofos administration and the highly significant
125 decrease of reproductive performance in male mice that is agree with Ahmed A. Hendawy *et al.*,
126 (2012). The reduction in fertility index may simply represent the effects of Chlorpyrifos exposure on
127 sperm parameters. Therefore, the effects of Chlorpyrifos on the fertility can be attributed to its ability
128 to reduce sperm morphology and motility. Finally we can concluded that both the
129 concentrations of the tested pesticides decreased sperm motility associated with increase in
130 the number of morphologically abnormal of treated mice; however sperm motility was
131 significantly decreased with the highest concentration of the tested pesticides.

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Table (2): Effect on sperm morphology induced by lambda-cyhalothrin, profenofos, and chlorpyrifos at (1/10, 1/40, LD₅₀ and ADI) for 30, 60, and 90 days as respectively.

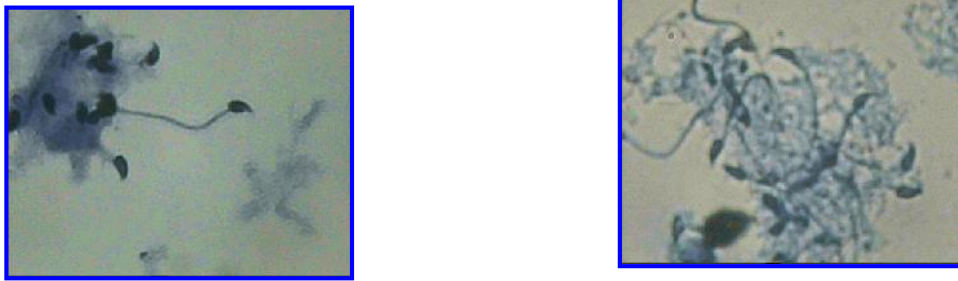
Pesticides	Doses	Period	Total abnormal sperm	Types of sperm abnormalities					
				Amorphous	Without book	Big head	Small head	Tail with 2 head	Others
Con.		30	6.2	1.4	1.5	1.1	0.8	0.5	0.9
		60	6.0	1.5	1.4	1.3	1.0	0.7	1.2
		90	7.2	1.8	1.7	1.2	1.1	1.0	1.5
Lamba.	1/10	30	13.5	3.2	2.7	1.9	1.7	1.5	2.5
		60	15.3	3.5	3.0	2.3	1.8	1.9	2.8
	1/40	90	16.5	3.8	3.2	2.4	2.0	2.1	3.0
		30	15.0	2.9	3.0	2.6	2.0	1.7	2.8
	ADI	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
30		10.5	1.7	1.0	1.2	1.3	1.4	1.3	
Profenofos	1/10	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
		30	18.2	5.1	3.4	2.5	1.9	2.3	3.0
	1/40	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
		30	18.9	4.6	3.5	3.1	2.2	2.1	3.4
	ADI	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
		30	10.8	2.5	1.9	1.4	1.7	1.5	1.8
Chlorpyrifos	1/10	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
		30	18	4.8	3.3	2.4	1.9	2.4	3.2
	1/40	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
		30	19.9	5.4	3.6	2.3	2.7	2.1	3.8
	ADI	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
		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	

148 100 cells were counted

149 Data suggest a potential association between exposures to tested used pesticides and
150 decreased sperm quality. The present study revealed that increased teratospermic
151 (abnormal sperm morphology). Further support for testicular toxicity comes from studies in
152 laboratory mice that showed associations between exposure tested pesticides and sperm
153 shape abnormalities, as well as dose–response relationships between exposure and a
154 decline in epididymal sperm count and motility and increased abnormal sperm. Finally, we
155 can say that this is a preliminary work that shows some abnormalities in sperm structure,
156 motility and nuclei morphology, and we suggest some important future studies; whole male
157 reproductive organs sampled fertility tests must be done, to give a full picture of the caused
158 male reproductive system abnormalities can be done using tested pesticides. It is also
159 possible that the genetic information of the sperm may potentially be altered prior to
160 fertilization. However, the evidence that such environmental chemicals cause infertility is still

161 largely circumstantial. There are many missing links in the causal chain that would connect
162 receptor binding to changes in reproductive health with decreased fertility.

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173 **Fig. (1): Photomicrograph of mice sperm morphology as a negative control. (X 1000)**
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179 **Fig. (2): Photomicrograph of mice sperm morphology induced by lambda-cyhalothrin**
180 **at (1/10 LD₅₀) for 90 days. (X 1000)**

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191 **Fig. (3): Photomicrograph of mice sperm morphology induced by lambda-cyhalothrin**
192 **at (1/40 LD₅₀) for 90 days. (X 1000)**

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

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Fig. (5): Photomicrograph of mice sperm morphology induced by profenofos at (1/10 LD₅₀) for 90 days. (X 1000)



Fig. (6): Photomicrograph of mice sperm morphology induced by pofenofos at (1/40 LD₅₀) 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 LD₅₀) for 90 days. (X 1000)



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Fig. (9): Photomicrograph of mice sperm morphology induced by chlorpyrifos at (1/40 LD₅₀) for 90 days. (X 1000)

271 **3.2. Analysis of mice primary spermatocytes:**

272 The results obtained from the analysis of Diakinesis stage in mice primary spermatocytes
273 after treatment with the lambda-cyhalothrin, profenofos and chlorpyrifos is illustrated in Table
274 (3). Three different types of aberration were observed they are stickiness, exchanges, and
275 univalent of se as well as of autosomal chromosomes were observed in Fig. (10-18). After
276 treatment with tested pesticides stickiness ranged from 4, 4, and 5 in the negative control to
277 9, 13, and 14 after treatment with the highest tested dose 1/10 LD₅₀ for 90 days with the
278 Lambda-cyhalothrin, profenofos and chlorpyrifos as respectively. Univalent involved X, Y
279 and autosomal chromosomes were obtained. The total percent of aberrant cells ranged from
280 8 to 13 % for the control group. Meanwhile, chlorpyrifos highly significantly decreased by 39,
281 67, and 19 after treatment with 1/10 LD₅₀, and 1/40 LD₅₀ and (ADI) for 90 days respectively.
282 In similar, profenofos caused significant decrease after treatment with 1/10 LD₅₀, 1/40 LD₅₀
283 and (ADI) as recorded 66, 63, and 17 for 90 days respectively. Also, lambda-cyhalothrin
284 caused decrease after treatment with 1/10 LD₅₀, 1/40 LD₅₀ and (ADI) as 40, 66, and 23 for
285 90 days respectively.

286 It was found that the tested pesticides were capable to cause univalent X, Y as well as
287 autosomal chromosomes. Illustrates stickiness and univalents obtained after treatment with
288 all tested pesticides. Cytological examination proved that in the control group binucleat and
289 multinuclei were not observed. At a low dose level 1/40 the binucleat cells were shown to be
290 20, 19 and 18 and multinuclei were 19, 18, and 18, While the higher dose 1/10 binucleat
291 cells were estimated to be 18, 22 and 20 and multinuclei were 16, 17, and 16 as well treated
292 with lambda-cyhalothrin, profenofos, and chlorpyrifos for 90 days respectively.

293 The data revealed that significant decreased of fertility after administration of all tested
294 pesticides either in hight (1/10 LD₅₀) or low dose (1/40 LD₅₀) within the three post treatment
295 period (30, 60 and 90 days) respectively. In the similar effect between hight dose (1/10 LD₅₀)
296 and low dose (1/40 LD₅₀), while with (ADI) dose the result showed no significant changes
297 with all tested pesticides and all treatment period. Profenofos was proven to induce different
298 types of aberration in mice germinal cells more than lambda-cyhalothrin, and chlorpyrifos.

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

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

307 percent motile sperm were decreased in the 15 and 28 mg/kg/day treated groups compared
308 to the control. Piña-Guzmán B, *et al.*, (2009) Male mice were exposed to Methyl parathion
309 (20 mg/kg bw, i.p.) and spermatozoa from epididymis-vas deferens were collected at 7 or
310 28 days post-treatment to assess the effects on maturing spermatozoa and spermatocytes,
311 respectively. In spermatozoa collected at 7 and 28 (dpt), and decreases in sperm quality and
312 induced acrosome reactions were observed; reduced mitochondrial membrane potential and
313 lipoperoxidation were observed at 7 (dpt) only. Negative correlations between
314 lipoperoxidation and sperm alterations were found. Altered sperm functional parameters
315 evaluated either in vitro or in vivo were associated with reduced fertilization mice at both
316 times.

317 Dutta *et al.*, (2006) Effect of endosulfan on bluegill testes was studied, the seminiferous
318 tubules were of round or oval shape and contained primary spermatogonia, primary
319 spermatocytes, secondary spermatocytes, spermatozoa, spermatids. After 24 h of exposure,
320 there was evidence of slight signs of connective tissue splintering. The 48-h exposure
321 resulted in breakage of primary spermatocyte walls and separation from the seminiferous
322 tubules. The 72-h testis showed further connective tissue damage and migration of primary
323 spermatogonia into the lumen. After 96 h, there was significant damage to connective tissue
324 and the seminiferous tubules were less pronounced. After 1 and 2 weeks, the seminiferous
325 tubule walls were disrupted and missing in places and the structure of the testis was much
326 disorganized compared to the control testis. Biometric analysis indicated that the diameter of
327 the primary spermatogonia decreased from 24 h to two weeks. These kinds of damage could
328 affect the spermatids and spermatozoa and possibly have a negative impact on
329 spermatogenesis and male fertility. The results showed that decrease in concentrations of
330 spermatozoas the same described with Muftau Shittu *et al.*, (2013).

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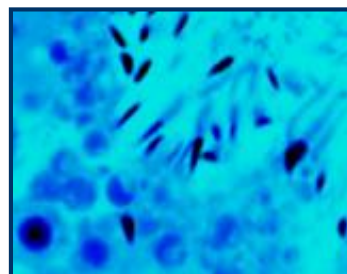
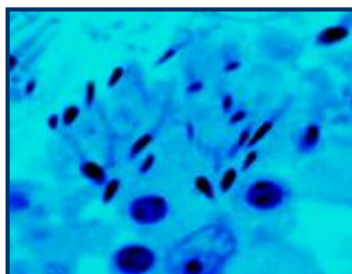
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341 **Table (3): Effect on mice primary spermatocytes induced by lambda-cyhalothrin,**
342 **profenofos, and chlorpyrifos at (1/10, 1/40, from LD₅₀ and ADI) for 30, 60, and 90 as**
343 **respectively.**

Pesticides	Doses	Period	Stickiness	Univalent				Total percent of aberrant cells	
				XY	Autosomes	Binucleate	Multinuclear		
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	
	Profenofos	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
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	
Chlorpyrifos	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	
	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	
		30	4.0	2	2	5	3	16	
		60	3.0	1	2	4	3	13	
		90	5.0	2	3	5	4	19	

344 100 cells were counted

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

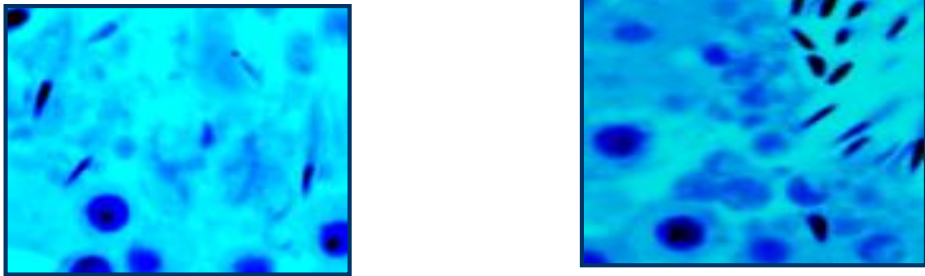


Fig. (11): Photomicrograph of mice primary spermatocytes aberration induced by lambda-cyhalothrin at (1/10 LD50) for 90 days. (X 1000)

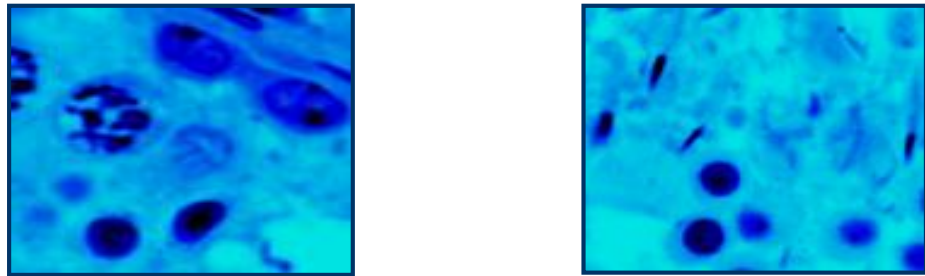


Fig. (12): Photomicrograph of mice primary spermatocytes aberration induced by lambda-cyhalothrin at (1/40 LD50) for 90 days. (X 1000)

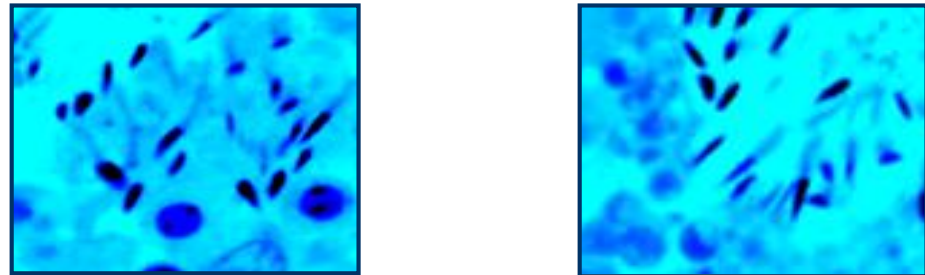
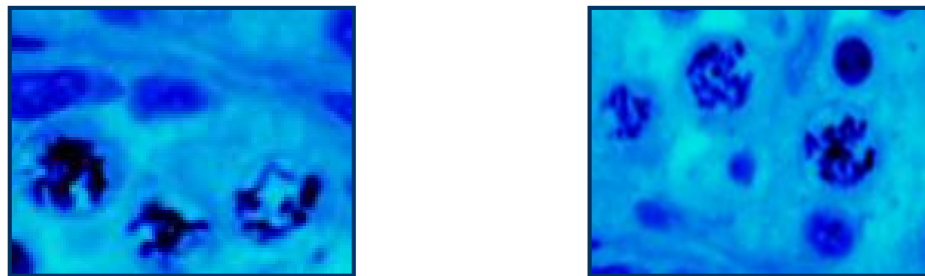
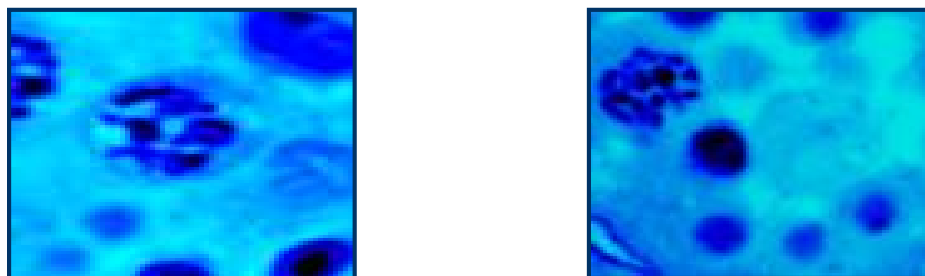


Fig. (13): Photomicrograph of mice primary spermatocytes aberration induced by lambda-cyhalothrin at (ADI) for 90 days. (X1000)



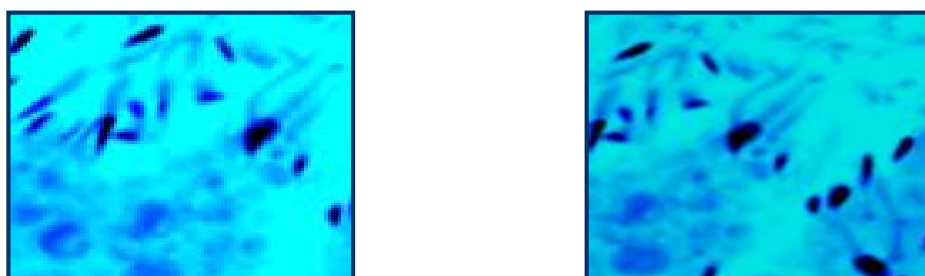
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Fig. (14): Photomicrograph of mice primary spermatocytes aberration induced by profenofos at (1/10 LD50) for 90 days. (X1000)



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



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



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



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Fig. (18): Photomicrograph of mice primary spermatocytes aberration induced by chlorpyrifos at (1/40 LD₅₀) 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.

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