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Effects of Pesticide Use on Semen Quality among Farmers in Rural Areas of Sabah, Malaysia

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Abstract: Effects of Pesticide Use on Semen Quality among Farmers in Rural Areas of Sabah, Malaysia: Feroz Hossain, *et al*. School of Medicine, University Malaysia Sabah, Malaysia—Objectives: To determine the relationship between semen quality and exposure to pesticide residues. **Methods:** A cross-sectional study was conducted among male farmers from 3 different communities in Sabah, Malaysia. A total of 152 farmers participated in this study of whom 62 farmers had been exposed to either paraquat or malathion or both to varying extents. Questionnaires were designed to record a history of pesticides exposure and other potential risk factors among farmers. All semen samples were collected, processed and analyzed by qualified personnel based on WHO guidelines. Volume, pH, sperm concentration, motility, morphology and WBC count were examined and recorded. The association between pesticide exposure and semen parameters was highly significant. **Results:** The mean values of volume, pH, sperm concentration, motility, and WBC count were significantly less in the exposed group than in compared with the non-exposed group, with $p<0.005$. Those who were exposed to pesticides had greater risk of having abnormal semen parameters than those in with the non exposed group, with p values of less than 0.05. The comparison between semen qualities such as lower sperm count, motility and higher percentage of sperm abnormality of those exposed to different types of pesticides (paraquat and malathion) showed no significant differences. **Conclusion:** The results showed a significant decline in semen quality with a decline in sperm count, motility and higher percent of teratospermia among subjects with pesticide exposure, and those who were exposed to pesticides had significantly 3 to 9 times greater risk of having abnormal semen parameters.
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Declining semen quality and sperm count are growing risks for the fertility index. Epidemiologic studies have indicated a decline in sperm count and semen quality of almost a half over the last five decades among healthy men all over the world. Pesticides have been proposed as one of the major causes for this decline. Pesticides contaminate the environment, the soil and the agricultural produce food. As a result, human beings are exposed to pesticides through food, water, air, via tainted breast milk, playing in the fields and later bringing pesticides into their homes or through skin contact.

There have been many studies of farmers with the goal of determining the effects of pesticide exposure. A recent study has shown that pesticide exposure causes lower semen quality[1)]. Also, there has been a decline in the concentration and motility of sperm and in the percentage of morphologically normal spermatozoa in fertile men that is independent of the age of the men[2)]. One study showed a significant decline in semen quality over the past 50 yr[3)]. Many other international studies have provided significant evidences of reduction in semen quality or lower sperm count being associated with exposures to agricultural pesticides[4)].

Malathion, a pesticide in the organophosphate chemical family, is the most commonly used insecticide in the world. Many studies have been conducted on organophosphate insecticides in relation to their adverse reproductive effects on humans. Known studies on organophosphate pesticide, such as malathion[5, 6)] and methyl parathion[7)], have demonstrated their adverse effects on male and female reproductive system, with alterations in sexual behavior, decrease in fertility/sperm count, loss of fetus during pregnancy, lactation problems as well as premature menopause being the potential manifestations. Laboratory rodent model studies have accumulated a significant body of evidence confirming data regarding the reproductive toxicity of low doses of pesticides[7, 13)].

The adverse health effect of pesticide exposure on male reproduction is 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 both wildlife and humans[8]. Recent discoveries of the hormone disrupting properties of some pesticides at low exposures[9] have raised interest in how low-dose acute or chronic pesticide exposures—the types of exposures that can currently occur both occupationally and environmentally—impact human spermatogenesis. Both animal toxicology and human epidemiologic studies have shown that pesticides may operate through a hormonal or genotoxic pathway to affect spermatogenesis[10]. Most of the pesticides induce result in disruption in the normal endocrine axis and there are many rodent studies in the literature. In the 1930s, studies involving laboratory animals have demonstrated the estrogenic properties of a number of industrial chemicals, including pesticides such as paraquat, methyl parathion, endosulphan and dichlorodiphenyltrichloroethane (DDT)[11]. Paraquat is highly toxic to animals and has serious and irreversible delayed effects if absorbed. As little as one teaspoonful of the active ingredient is fatal and death occurs up to 30 days after ingestion. Paraquat is also toxic if absorbed through the skin but at spray strength paraquat has of relatively low acute toxicity. A study of Malaysian plantation sprayers cited health problems after exposure to paraquat[12]. Dermal exposures to low doses of paraquat in rats resulted in cytotoxic and genotoxic germ cell sequelae in male rats[13]. There are number of animal studies highlighting the adverse effects of low doses of pesticides on reproductive functions and spermatogenesis. Laboratory studies paves the way for the determination of the toxicity profiles of these pesticides and chemicals, community and clinical studies among the human population are necessary to evaluate the full extent of the adverse effect, especially among the pesticide exposed farming communities.

Most of the farming practices in the Sabah state of Malaysia involve usage of a variety of pesticides on vegetables, fruits, paddy and palm oil plantations. Farmers use these pesticides without proper protective measures and their risk of exposure is quite high. Though paraquat and other organophosphate pesticides are banned by the government, their illegal usage of the same is common. Random interviews before the actual study suggested various risks of exposure to pesticide residues. Based on these findings, a cross-sectional study was conducted to explore into the toxicity effects of pesticides among on farmers, semen quality among the farmers in the Telupid, Kundasang and Papar districts of Sabah, Malaysia.

Materials and Methods

1) Research design and methodology

A cross-sectional study was undertaken to investigate the relationship between pesticide exposure and their effects on semen parameters. Ethical clearance for the conduct of the study was obtained from the School of Medicine, University Malaysia Sabah. Before being enrolled in the study, male farmers were informed of the purpose of the study, procedures, benefits and possible risks involved. They were also told that all information as well as biological samples and data obtained for the study would remain confidential. Written consent to participation was voluntarily obtained from all of the participants in the study prior to their participation.

2) Selection of study area

This study was conducted among male farmers from 3 different communities in Sabah, Malaysia, namely Kundasang, Telupid and Papar. A total of 152 farmers participated in this study after we had obtained their informed consent and all of them successfully provided semen samples. Participation in the study by a number of subjects was impeded by local larger group was difficult as their religious practices and cultural beliefs did not allow them, since people were less literate. Based on a statistician's advice, the appropriate numbers of voluntarily willing participants were randomly selected as a statistically viable sample for the study. The present study may pave the way for highlighting the need for extensive studies world-wide.

3) Grouping of human beings subjects

Based on the occupation and exposure farmers were divided into two groups:

The farmers who were exposed to pesticides were allocated to the exposed group. The number of exposed group n=62; and the farmers who gave no history of exposure to pesticides were allocated to the non exposed group and the number of non-exposed group n=90.

4) Semen collection and seminal fluid analysis

Semen collection:

A semen sample was collected by masturbation into a sterile wide-mouth glass container in a laboratory at each community health station. Sexual abstinence of 2–3 days prior to collection of semen was advised to the participants. It was adequate for the accurate assessment of semen quality. Information on date, time and spillage were recorded and coded on each sample. The samples were kept in an incubator at 36–37℃ until liquefaction and subsequently examined. All semen samples were processed and analyzed by qualified personnel based on WHO guidelines (WHO 1992, 1999)[14, 15]. All samples were examined twice for the authenticity. Volume, pH, sperm concentration, motility, morphology and WBC count were examined and recorded. If any discrepancy and error were noticed, the semen analysis including the count was repeated for a third time to assure the accuracy.

Gross examination of semen:

Semen volume: The volume was measured with a measuring pipette and it was recorded in m*l*.

pH of collected semen: A pH indicator strip was put in the container to assess the pH of the seminal fluid.

Microscopic analysis:

Sperm morphology and motility:

A drop of seminal fluid was applied into a clean glass slide, and mounted with a glass cover and examined at 400x magnification. An improved Neubaur Counting Chamber (hemocytometer) was used to determine the sperm morphology. At 400x magnification, sperm motility was also determined and recorded.

WBC count: WBC count was examined at 100 x magnifications under a microscope and the average was recorded using an improved Neubaur counting chamber (hemocytometer).

Sperm concentration: A drop of semen was then mixed with 19 drops of lytic agent and it was kept for the time left to stand to kill the sperm. Then a drop of the mixed semen was placed on a clean glass slide, and after mounted with a glass cover and examined at 400x magnification. The counting chamber was also used to determine the sperm count.

Personal interviews were done with farmers who participated in this study, using questionnaires designed to record their history of pesticide exposure and other potential risk factors. Population profile (age, marital status, number of children and residency), exposure pattern (type of pesticide use, duration of use, spraying and cleaning of pesticide cans) and other risk behaviors (smoking, drinking alcohol) were recorded.

5) Statistical analysis

The final data obtained from the experiments were checked and analyzed using SPSS version 16 (SPSS Inc. 2007) (16). Frequency analyses, descriptive data, group statistics, Mann-Whitney test and the chi-square test were performed. Values of $p<0.05$ were considered as statistically significant.

Results

1) Background of the samples

A total of 152 persons participated in this study; 90 volunteers who were not exposed to pesticides and 62 volunteers who were exposed. Of the 62 exposed participants, 39 were exposed to paraquat, 15 were exposed to malathion, and 8 were exposed to both paraquat and malathion.

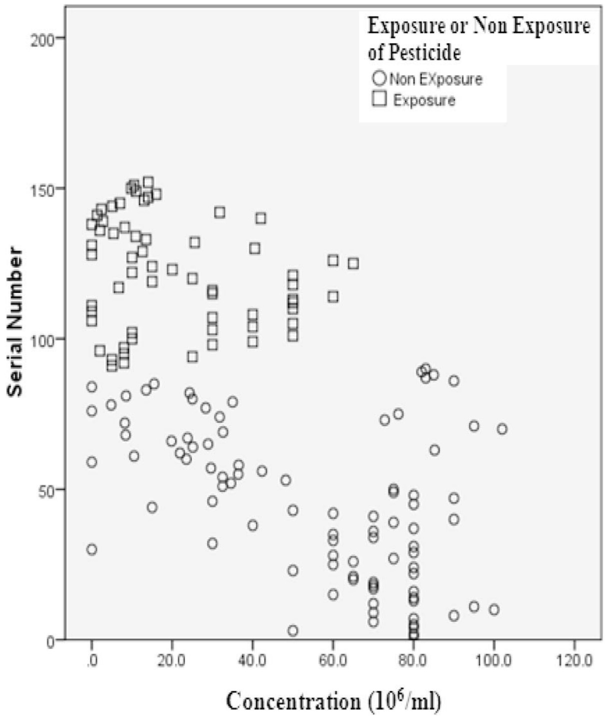

Fig. 1. Distribution of sperm concentration among those exposed and not exposed to pesticides.

2) Semen qualities

Abnormal semen parameters were found for volume in 35 samples, for pH in 26 samples, for concentration in 50 samples, for motility in 19 samples, for morphology in 19 samples and for WBC count in 18 samples.

3) Distribution of sperm concentrations across the exposed and non exposed groups (Fig. 1).

Figure 1 showed sperm concentrations were higher in non exposed group than in exposed group.

4) Summary statistics of seminal fluid parameters in relation to exposure and non-exposure status

The mean values of all seminal fluid parameters showed significant differences between the exposed and non-exposed groups with their p values of < 0.05 (Table 1).

5) Semen quality differences between the exposed and non exposed groups

Statistically significant differences were seen in (a) volume of semen (p=0.000), (b) pH of seminal fluid (p=0.000), (c) sperm concentration (p=0.000), (d) motility of the sperms (p=0.000), (e) morphology of the sperms (p=0.000) and (f) WBC count of the seminal fluid (p=0.011) between the exposed group and non exposed groups (Table 2).

Table 1. The summary statistics of seminal fluid parameters in relation to pesticide exposure and non-exposure status

		Volume (m*l*)	*p*	pH	*p*	Concentration (10^6/m*l*)	*p*	Motility (%)	*p*	Morphology (%)	*p*	WBC (10^6/m*l*)	*p*
Non exposure n=90	Mean	3.46		7.60		55.00		66.50		72.90		0.60	
	SD	1.60		0.20		28.60		27.70		22.40		0.30	
Exposure n=62	Mean	2.16	0.00	7.40	0.00	20.70	0.00	40.80	0.00	43.90	0.00	0.80	0.00
	SD	1.21		0.20		18.60		23.60		23.60		0.50	
Total n=152	Mean	2.93		7.50		41.00		56.00		61.10		0.60	
	SD	1.58		0.20		30.10		28.90		26.90		0.40	

SD: Standard deviation.

Table 2. Semen qualities of the exposed and non exposed groups

	Volume (m*l*)	pH of the specimen	Concentration (10^6/m*l*)	Motility (%)	Morphology (%)	WBC (10^6/m*l*)
Mann-Whitney U	1,340.500	1,489.000	982.500	1,272.000	909.500	2,117.500
Wilcox on W	3,293.500	3,442.000	2,935.500	3,225.000	2,862.500	6,212.500
Z	–5.455	–4.929	–6.784	–5.708	–7.081	–2.544
P	0.000	0.000	0.000	0.000	0.000	0.011

6) Abnormal semen quality in the exposed and non-exposed groups with their proportion of respondents

Those who were exposed to pesticides had significantly greater risk of having abnormal semen quality in volume (p=0.000), in pH (p=0.04), in concentration (p=0.000), in motility (p=0.000), in morphology (p=0.002), and in WBC count (p=0.004). They had 6.5 times risk of having lower semen volume (OR=6.5, 95% CI=2.7–15.2), 2.66 times risk of having abnormal pH (OR=2.66, 95% CI=0.1–0.9), 8.7 times risk of having low concentration of sperm (OR=8.7, 95% CI=4–19), 5.1 times risk of having less motile sperm (OR=5.1, 95% CI=2.5–10.5), 4.9 times risk of having abnormal sperm (OR=4.9, 95% CI=1.6–14.6), 4.5 times risk of having abnormal WBC count (OR=4.5, 95% CI=1.5–13.4) in compared to the non exposed group (Table 3).

7) Group statistics of different types of pesticides in relation to different parameters of semen

No significance differences were observed in mean duration of pesticide exposure or volume, pH, morphology or WBC count of the seminal fluid with regard to the three types of pesticide exposure groups with p values of 0.14, 0.94, 0.28, 0.11 and 0.72 respectively. And all mean values of the above parameters were within the normal range of WHO protocol 1992 and 1999[14, 15].

A significant difference was found for the mean sperm concentrations of the three exposure subgroups. In the malathion and combined subgroups the mean value was lower than normal range with a p value of 0.04. A slightly significant difference was also observed for the mean motilities of the three exposure subgroups. In the combined group it was lower than normal reference range of WHO protocol 1992 and 1999[14, 15] with a p value of 0.05 (Table 4).

8) Potential abnormal semen qualities in relation to smoking, alcohol drinking, marital status and children

No significance differences were seen in semen quality between smokers and non-smokers group (p=0.226), between those taking alcohol and those who did not (p=0.449), between married and unmarried subjects (p=0.653) or between participants having children and those who did not (p=0.552), Table 5.

Discussion

Pesticides, mainly the organophosphorus compounds are known to affect the general health. Laboratory studies have confirmed the adverse low dose effects of paraquat, endosuphan, methyl parathion and malathion on the male reproductive system of rodent models (7, 13). Paraquat at a very low dose, delivered through dermal exposure has resulted in a cytotoxic damage of germ cells in the

Table 3. Proportion of respondents with abnormal semen quality in the exposed and non exposed groups

Semen quality	Exposure status	Normal	Abnormal	Total	Chi-square value	*p* value	Odds ratio	95% Confidence interval
Volume	Non-exposure	81	9	90	21.12	0.000	6.5	2.7–15.2
	Exposure	36	26	62				
pH of the specimen	Non-exposure	70	20	90	4.07	0.04	2.66	0.1–0.9
	Exposure	56	6	62				
Concentration	Non-exposure	77	13	90	34.02	0.000	8.766	4–19
	Exposure	25	37	62				
Motility	Non-exposure	70	20	90	21.97	0.000	5.18	2.5–10.5
	Exposure	25	37	62				
Morphology	Non-exposure	85	5	90	9.729	0.002	4.958	1.6–14.6
	Exposure	48	14	62				
WBC	Non-exposure	85	5	90	8.353	0.004	4.51	1.5–13.4
	Exposure	49	13	62				

Table 4. The summary statistics of seminal fluid parameters among subjects with exposure to different pesticides

Variables	Type of pesticides	Number	Mean	SD	F	*p* value
Duration of exposure (yr)	Paraquat	39	5.92	2.71	1.99	0.14
	Malathion	15	6.07	2.63		
	Combined	8	8.00	2.77		
Volume (m*l*)	Paraquat	39	2.14	1.23	0.05	0.94
	Malathion	15	2.13	1.29		
	Combined	8	2.3	1.08		
pH of the specimen	Paraquat	39	7.44	0.22	1.27	0.28
	Malathion	15	7.34	0.13		
	Combined	8	7.4	0.31		
Concentration (in 10^6/m*l*)	Paraquat	39	24.67	18.73	3.32	0.04
	Malathion	15	17.62	19.61		
	Combined	8	7.55	6.48		
Motility (%)	Paraquat	39	45.38	22.43	2.97	0.05
	Malathion	15	37.67	22.02		
	Combined	8	24.38	27.05		
Morphology (%)	Paraquat	39	48.59	21.48	2.24	0.11
	Malathion	15	37.47	21.17		
	Combined	8	33.12	33.48		
WBC (in 10^6/m*l*)	Paraquat	39	0.85	0.49	0.32	0.72
	Malathion	15	0.74	0.54		
	Combined	8	0.9	0.61		

male Sprague Dawley rats[13]. Also methyl parathion has produced seminiferous tubular atrophy as an evidence of a decline in sperm count, in a rodent model[7]. Despite of a number of experimental studies, evidence for the adverse effects of pesticide exposure in human subjects is scanty. This may be due to the major poorly educated farming folks and their cultural beliefs hindering the collection of semen samples for the cause of research. Our efforts in this direction among the farmers of three districts of Sabah, Malaysia, have yielded a response

Table 5. Potential abnormal semen qualities in relation to smoking, alcohol drinking, marital status and children

Dependent variables	Independent variable			Total	Chi-square value	p value	Odds ratio	95% Confidence interval
Overall semen quality based on volume, pH,	Smoker/non-smoker	Smoker 39	Non-smoker 113	152	1.46	0.22	0.50	0.16–1.56
concentration, motility, morphology,	Alcoholic/non-alcoholic	Alcoholic 33	Non-alcoholic 119	152	0.57	0.44	0.64	0.20–2.02
WBC count	Married/ Unmarried	Married 136	Un-married 16	152	0.20	0.65	1.42	0.30–6.69
	Children				7.82	0.55		

which may form a scientifically a basis for future studies and provide a statistically a sound sample number for investigating of hypotheses about pesticide exposures of the farming men.

This is the first of its kind pioneering study in Malaysia. It evaluated the semen quality of the farmers living in the vicinity of farming practices in Sabah state. The results provide strong initial evidence that pesticide exposure is associated with reduced seminal quality in the population of rural areas of Sabah, Malaysia. This study involved quite a substantial number of participants[152], all of whom consented to provide the semen. The major findings of the semen analysis of the exposed group were reduced semen volume, abnormal pH, decreased sperm concentration, reduced semen motility, reduced sperm morphology and abnormal WBC count. Thus, the findings of this study indicated that pesticide exposure is significantly associated with abnormal semen characteristics. In a similar study, there was a correlation with regard to the decreased semen quality and exposure to toxic chemicals was found among 61 cases of Croatian male subjects in a group of 123 subjects exposed to pervasive environmental exposure to heavy metals and chemicals[17] compared to our study.

The WHO guidelines for the examination of human semen were strictly followed (WHO, 1992, 1999)[14, 15] for the semen analysis and all the semen specimens were evaluated within one hour of collection. After recording the clinical and occupational history, and semen was analyzed by qualified personnel. Each semen sample was kept at 37℃ for liquefaction which took about 20–30 min.

The interviews were performed privately and strict confidentiality was maintained. All related biases were ruled out, and interviewers were not informed of the semen characteristics of the farmers before they were being interviewed. The interviewer tried to get information that was as specific as possible from the male farmers by the detailed questionnaire. By interviews and questionnaire we found the mean ages were similar in the exposed and non exposed groups, and their socio-economic conditions, numbers of family members, residency and educational levels were more or less the same in both groups. There was no chronic disease, especially testicular diseases among the farmers who participated in this study. The percentages of risk behaviors such as smoking, and drinking alcohol were similar among farmers in the exposed pesticide and non exposed pesticide groups. All the semen parameters showed significant differences between the exposed and non exposed groups. Increase in sperm abnormality and declines in both motility and sperm numbers correlate well with the experimental rodent studies[7, 13] confirming the adverse effect of pesticide exposure on the pesticide users.

The present results provide sufficient evidence for concern that there is a strong relation between pesticide exposure and male semen quality. All parameters of semen analysis showed significance differences between the exposure and non exposure groups. One study showed pesticide exposure causes lower semen quality[1]. Some other studies that showed a possible decline in semen characteristics[2]. There is an evidence of reduction in semen quality or lower sperm count associated with exposure to agricultural pesticides[4]. Associations have been found between semen quality (morphology, sperm concentration, motility) and exposure to pesticide including DDT[18], organochlorine, including organophosphorus[19] and mixtures of herbicides, fungicides and insecticides[1]. Exposure to pesticides was related to lower sperm count[20]. The results of our study are comparable with those of the studies cited above. We could not find any major differences in semen quality between malathion and paraquat exposures farmers.

Paraquat is banned in Malaysia nowadays, but farmers are still using paraquat in diluted form. May be it is the one of reason that why paraquat and malathion exposures farmers had nearly similar effects, though paraquat is more toxic than malathion.

Possibly paraquat and most of the organophosphate pesticides induce reactive oxygen species. The mechanism of action of paraquat involves the formation of the free radicals by reduction of the ion and subsequent auto oxidation to yield the original ion. During the auto oxidation of the paraquat free radical to the ion; H_2O_2 (hydrogen peroxide), O_2 (–) (superoxide radical), *OH (hydroxyl radical), and ‘O_2 (singlet oxygen) are formed. Each of these by-products is potentially phytotoxic. However, recent research suggests that the hydroxyl radical is responsible for paraquat-induced lipid peroxidation and related phytotoxic symptoms. Lipid degradation induced by paraquat causes cell death. Secondly Seminiferous tubular sloughing and atrophy were confirmed in an experimental rodent model on exposed to paraquat and methyl parathion[7, 13)] and further gonadal hormone levels may be involved in the possible mechanism of decline in semen quality which need to be addressed. These results suggested that pesticide exposure really does have an adverse effect on the various parameters of seminal fluid of the farmers who are at greater risk of having abnormal semen parameters. Especially, our results suggested that men have poor semen quality as well as greater risk of having abnormal semen parameters.

This study is a valuable tool for predicting the possible semen quality status of men engaged in agriculture with pesticide use and shall be extended further to different areas of Sabah, so that a semen quality prediction formula for Sabah state can be formulated. As most of the area of Sabah is classified as agricultural zone, restriction of extensive pesticide use in agricultural practices could be exercised effectively based upon the findings of this study. Future in-depth research into the relationship between semen quality and exposure to different types of pesticides with a bigger number of participants is recommended so as to facilitate the safe use of pesticides in agricultural practices. In conclusion, this study confirmed that farmers exposed to pesticides, such as paraquat and malathion, exhibit a significant decline in sperm concentration and motility with an increase in sperm abnormality. In addition the semen volume and an overall quality of semen were abnormal compared to the national averages and those of the non exposed groups. This highlights the previous hypothesis as well as fact of declining semen quality among the male population in rural farming population of Sabah. Occupational pesticide exposure is a major threat which has to be addressed. We need to be extended this study by including a more representative and investigating the possible involvement of the hypothalomo-pituitary-gonadal axis and to see if there are any abnormalities in hormone levels.

Overall the decline in semen quality may adversely affect the fertility index of exposed subjects.

References

1) Swan SH, Kruse RL, Liu F, et al. Semen quality in relation to biomarkers of pesticide exposure. Environ Health Perspect 2003; 111: 1478–84.
2) Auger J, Kunstmann JM, Czyglik F, Jouannet P. Declines in semen quality among fertile men in Paris during the past 20 years. N Engl Med 1995; 332: 281–5.
3) Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. Bmj 1992; 305: 609–13.
4) Tuc VP, Wangsuphachart V, Tasanapradit P, Fungladda W, Van Trong P, Nhung NT. Impacts of pesticide use on semen characteristics among rice farmers in Kienxuong district, Thaibinh Province, Vietnam. Southeast Asian J Trop Med Public Health 2007; 38: 569–75.
5) Espinoza-Navarro O, Bustos-Obregón E. Effect of malathion on the male reproductive organs of earthworms, Eisenia foetida, Asian J Androl 2005; 7: 97–101.
6) Eskenazi B, Harley K, Bradman A, et al. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect 2004; 112: 1116–24.
7) Narayana K, Prashanthi N, Nayanatara A, Baily LK, D’Souza UJ. An organophosphate insecticide methyl parathion (0-0- Dimethyl 0-4- Nitrophenyl phosphorothioates) induces cytotoxic damage and tubular atrophy in the testes despite elevated testosterone level in the rat. J Toxicol Sci 2006; 31: 177–89.
8) Colborn T, Saal vom FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 1993; 101: 378–84.
9) Kelce WR, Monosson E, Gamcsik MP, Laws SC, Gray LE Jr. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. Toxicol Appl Pharmacol 1994; 126: 276–85.
10) Toppari J, Larsen JC, Christiansen P, et al. Male reproductive health and environmental xenoestrogens. Environ Health Perspect 1996; 104: 741–803.
11) Solomon GM, Schettler T. Environment and health: endocrine disruption and potential human health implications CMAJ 2000; 163: 1471–6.
12) Arumugam V. Victims without Voice. Paraquat fact sheet. Tenaganita and PAN Asia Pacific, Malaysia. 1992.
13) D’Souza UJA, Zain A, Raju S, Nizam HM, Narayana K, Noriah O. Dermal exposure to the herbicide-paraquat results in genotoxic and cytotoxic damage to

germ cells in the male rat. Folia Morphol 2006; 65: 6–10.
14) WHO, 1992. Laboratory Manual for the Examination of Human Semen and Sperm—Cervical Mucus Interaction. Cambridge University Press. 3rd ed.
15) WHO, 1999. Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction (Paperback). 4th ed.
16) SPSS for Windows, Rel. 16.0.1. 2007. Chicago: SPSS Inc.
17) Jurasovic J, Cvitkovic P, Pizent A, Colak B, Telisman S. Semen quality and reproductive endocrine function with regard to blood cadmium in Croatian male subjects. Bio Metals 2004; 17: 735–43.
18) Dalvie MA, Myers JE, Thompson ML, et al. The long-term effects of DDT exposure on semen, fertility, and sexual function of malaria vector-control workers in Limpopo Province, South Africa. Environmental Research 2004; 96: 1–8.
19) Padungtod C, Savitz DA, Overstreet JW, Christiani DC, Ryan LM, Xu X. Occupational pesticide exposure and semen quality among Chinese workers. J Occup Environ Med 2000; 42: 982–92.
20) Lerda D, Rizzi R. Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2, 4-D). Mutation Research 1991; 262: 47–50.