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Cytogenetic and Hormonal Alteration in Rats Exposed to Recommended "Safe Doses" of Spinosad and Malathion Insecticides

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ABSTRACT

This study evaluated the cytogenetic and reproductive effects of spinosad and malathion on male albino rats. The insecticides were given daily, via oral route, in 60 repetitive doses equaled to Acceptable Daily Intake "ADI" (0.02 & 0.30 mg a.i.kg^{-1} b.w.), No Observed Adverse Effect Level "NOAEL" (9.00 & 29.00 mg a.i.kg^{-1} b.w.) and 1/100 LD_{50} (37.38 & 13.75 mg a.i.kg^{-1} b.w.) of spinosad and malathion respectively. Results of cytogenetic effects indicated that spinosad (37.38 mg a.i. kg^{-1} b.w.) and malathion (13.75 & 29.00 mg a.i.kg^{-1} b.w.) induced significant decrease in mitotic activity (MI %) and increased micronucleated polychromatic erythrocyte (MN %) in rat's bone marrow cells. The MN % recorded 0.36% in control rats, compared to 0.96% for spinosad at 37.38 mg a.i.kg^{-1} b.w. and 1.46 and 1.93% for malathion at 13.75 and 29.00 mg a.i.kg^{-1} b.w., respectively. Except the treatment with 0.02 mg a.i. kg^{-1} b.w. (ADI) of spinosad, the other tested doses of both insecticides showed significant increase in the percentage of chromosomal aberrations in rat's bone marrow cells. The percentage of chromosomal aberration (excluding gaps) recorded 0.66, 1.33 and 3.66% for spinosad at 0.02, 9.00 and 37.38 mg a.i. kg^{-1} b.w., respectively. In case of malathion, such values recorded 3.00, 6.33 and 6.66% at 0.30, 13.75 and 29.00 mg a.i.kg^{-1} b.w., respectively. The obtained cytogenetic effects occurred in a dose-dependent manner. After 45 days of treatment, testosterone concentration significantly decreased ($P \leq 0.05$) at a dose levels of 9.00 and 37.38 mg a.i.kg^{-1} b.w. spinosad and 0.30 mg a.i. kg^{-1} b.w. malathion. At the other tested doses, malathion caused highly significant decrease ($P \leq 0.01$) in testosterone concentration. Moreover, varieties of histopathological lesions were identified in the examined testes. The incidence and severity of these lesions appeared to increase with increasing dose and the most pronounced pathological changes were necrosis of spermatogonial cells, congest in blood vessels and exfoliation of necrotic cells within the somniferous lumen. The tested doses of both insecticides recorded observable significant decrease in the absolute testes weights and the lowest value (2.18 g) was resulted from malathion at the 1/100 LD_{50} (29.00 mg a.i. kg^{-1} b.w.). Except the treatment with 0.02 mg a.i. kg^{-1} of spinosad, the other tested doses of both insecticides showed significant decrease in body weight gains. Generally, cytogenetic and reproductive effects, as well as alteration in testosterone level, were observed in a dose-dependent manner and malathion exhibited more pronounced effect than spinosad.

Key Words: Cytogenetic; Hormones; Safe doses; Spinosad; Malathion; Male rats

INTRODUCTION

Pesticides pose human health concerns, because they are toxic substances, widely released into the environment and major cause of morbidity and mortality especially in developing countries. In Egypt, the organophosphorus (OP) compound malathion is extensively used in agriculture, veterinary, medical and public health practices, because it is relatively cheap and possess low acute toxicity towards mammals. It has been reported that malathion affects activity of esterases and other enzymes and possibly causes a disturbance in cell metabolism leading to a disruption of cell division machinery and pose genetic hazards (Flessel *et al*., 1993).

The new natural compounds such as spinosad are being registered in the country as less hazardous than the traditional synthetic pesticides. Spinosad exhibits wide margins of safety to many beneficial insects and related organisms and thus considered as a selective insecticide. It is primarily a stomach poison with some contact activity and is a neurotoxin with a novel mode of action involving the nicotinic acetylcholine receptor and apparently the GABA receptors as well (Salgado, 1998).

In fact, genotoxic risks of human exposure to pesticides remain a worldwide concern, because many of these chemicals are mutagenic and linked to the development of cancers (Leiss & Savitz, 1995). The concept of chromosomal damage as a biomarker of early carcinogenic effects rests on the evidence of an association between biomarker frequency and cancer risk, in addition to the association between biomarker and exposure to genotoxic agents. Increasing levels of chromosomal aberrations have been associated with increased cancer risk (Hagmar *et al.,* 1994). Some pesticides cause reproductive

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toxicity in animals and human and many authors have reported some biochemical and pathological effects of pesticides on the reproductive system of experimental animals (Hileman, 1994; Salem & Abd Elghaffar, 1998).

Pesticides are usually applied in their formulated forms, where the active ingredient is combined with organic solvents, emulsifying and wetting agents, which affect the pesticide penetration and performance. These additives may synergize or antagonize the toxicity of the active ingredient (Abo-Zeid *et al.,* 1993). In addition, formulated pesticides of poor-quality may contain hazardous substances and impurities that have already been banned or severely restricted elsewhere. Abo-Zeid *et al.* (1993), Mansour and Mossa (2005) found that formulated insecticides (e.g., malathion) were more effective than the pure ones in inhibiting either the acetylcholinesterase activity or the blood serum profile. Therefore, information about toxicity of formulations is needed when evaluating adverse health effects from exposure to pesticides in practice (WHO, 1991). For this reason, the present study was undertaken to assess and compare genotoxic and reproductive effects from exposure of rats to selected low doses of commercial spinosad and malathion insecticides.

MATERIALS AND METHODS

Insecticides. Tracers® (24% SC) is a commercial formulation containing spinosyns A and D (Dow AgroSciences Company). Spinosyn A is 2-[(6-deoxy-2, 3, 4-tri-O-methyl-alpha-L-mannopyranosyl) oxy) -13-[(5-dimethylamino) tetrahydro-6-methyl-2H-pyran-2-yl) oxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a, 16b - tetradecahydro-14-methyl-1H-as-indaceno (3, 2-d) oxacyclododecin-7, 15-dione. Spinosyn D is 2-((6-deoxy-2, 3, 4-tri-O-methyl-alpha-L-mannopyranosyl) oxy) -13-((5-(dimethylamino) tetrahydro-6-methyl-2H-pyran-2-yl) oxy) -9-ethyl2, 3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a, 16b-tetradecahydro-4, 14-dimethyl-1H-as-indaceno (3, 2-d) oxacyclododecin-7, 15-dione. Acute oral LD_{50} = 3738 mg a.i.kg^{-1} for male rats, No Observed Adverse Effect Level "NOAEL" for rats = 9 - 10 mg a.i.kg^{-1} b.w. daily and Acceptable daily intake "ADI" (JMPR) = 0.02 mg a.i.kg^{-1} b.w. (Anonymous, 2005). Malathion® (57% EC) is S-(1, 2-dicarbethoxy) ethyl O, O-dimethylphosphorodithioate (Kafr El-Zayat Pesticides & Chemicals Company, Egypt). Acute oral LD_{50} for rats = 1375-5500 mg a.i.kg^{-1} (the lower LD_{50} is used), NOAEL for rats = 29 mg a.i.kg^{-1} b.w. and ADI (JMPR) = 0.3 mg a.i.kg^{-1} b.w. daily (Anonymous, 2005).

Tested animals and dosing. Rats (*Rattus norvegicus var. albus*), weighing 195 - 205 g obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt were acclimatized under laboratory conditions at room temperature of 22 ± 3.0°C for a week. Food and water were provided *ad-libitum.* Rats (10 rats per dose) were given daily, via oral route, 60 repetitive doses equaled to 0.02 and 0.30 mg a.i.kg^{-1} b.w. (ADI), 9.00 and 29.00 mg a.i.kg^{-1} b.w. (NOAEL) and 37.38 and 13.75 mg a.i.kg^{-1} b.w. (1/100 LD_{50}) of spinosad and malathion, respectively using water as a solvent. Rats of control group were given the same volume of water (0.5 mL rat^{-1}) throughout the experimental durations.

The experimental work on rats was performed with the approval of the Animal Care and Experimental Committee, National Research Centre, Cairo, Egypt and according to the guidance for care and use of laboratory animals (NRC, 1996).

Micronucleus test. Animals were killed by decapitation after 24 h from the last treatment and bone marrow was flushed into a test tube containing calf serum (3 mL) and then centrifuged at 1500 rpm. Smears were made on the slides, air-dried and stained by May-Grunwald Giemsa method (Schmid, 1975). Three thousand polychromatic erythrocytes were scored from animal and four rats were used for each dosage. In this analysis, only polychromatic erythrocytes (PE's) were scored and micronuclei were identified as dark blue staining bodies in the cytoplasm of PEs. Results were expressed in terms of the percentage of micronucleated PEs.

Chromosomal abnormalities in rat bone-marrow cells. After 24 h from the last treatment, rats were injected with 0.6 mg kg^{-1} b.w. colcemid 2 h prior to killing by decapitation. Bone marrow preparations were prepared according to the method of Preston *et al*. (1987). Four rats were used for each dosage, where the structural alterations of chromosomes were evaluated in 75 metaphases per animal.

Mitotic index (MI). The slides prepared for the assessment of chromosomal aberrations were also used for calculating the mitotic index. Random views on the slides were made to determine the number of dividing cells (metaphase stage) and the total number of cells. At least 1000 cells were examined in each preparation. Mitotic activity was estimated according to the following formula: Mitotic index (MI) = [no. dividing cells/total no. of cells] x 100.

Blood collection. Blood samples were taken from retro-orbital venous plexus at beginning of the dosing and after 2, 4, 6 and 8 weeks, centrifuged for 8 min at 2600 rpm, then the clear serum was stored at -20°C until using for testosterone assay.

Testosterone determination. Testosterone hormone was measured according to the method of Trachtenberg (1987), using testosterone ELISA kits (Calbiotech, Inc., 10461 Austin). The absorbance was measured at 450 nm with a microtiter well reader by means of ELISA-ASYS Reader.

Histological examination. After postmortem examination of sacrificed rats, testes were carefully separated and washed by distilled water. Small pieces of testis organ were fixed in 10% neutral buffer formalin and in Bowan's fixative. The fixed samples were dehydrated in ethyl alcohol, processed and embedded in paraffin blocks. Sections of 5 – 7 µ were prepared and stained with heamatoxelin and eosin (Bancroft & Stevens, 1982).

Body and testes weight. Final body weights of animals

Table I. Effect of repeated doses for 60 consecutive days of Spinosad and Malathion on mitotic activity and micronucleated polychromatic erythrocytes in bone marrow cells of male rats

Treatments	Dose mg a.i.kg^{-1} b.w.	Mitotic Index (MI %)	Micronucleated polychromatic erythrocytes	
			No. of micronucleated polychromatic erythrocyte	% PE's with micronuclei
Control	0.00	10.00	44	0.36
Spinosad	0.02	9.40	52	0.43
	9.00	8.90	76	0.63
	37.38	8.00*	116	0.96*
Malathion	0.30	8.70	68	0.56
	13.75	7.80*	176	1.46**
	29.00	7.50**	232	1.93**

N.B.: number of polychromatic erythrocytes (PE) in each treatment = 12000.
Statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.

Table II. Frequencies of abnormalities in affected bone morrow cells of rats after repeated dose treatments for 60 consecutive days of Spinosad and Malathion

Treatments	Dose mg a.i. kg^{-1} b.w.	Structural chromosomal aberrations			% of Chromosomal aberration including gaps ± SE	% of Chromosomal aberration excluding gaps ± SE
		Gap	Fragment	Deletion		
Control	0.00	6	1	1	2.66±0.02	0.66±0.00
	0.02	7	1	1	3.00±0.18	0.66±0.03
Spinosad	9.00	8	2	2	4.00±0.24*	1.33±0.32*
	37.38	9	6	5	6.66±1.36**	3.66±0.02**
	0.30	12	5	4	7.00±1.85*	3.00±0.41*
Malathion	13.75	15	13	6	11.33±1.16**	6.33±1.03**
	29.00	12	14	6	10.66±1.63**	6.66±1.26**

N.B.: number of cells scored in each treatment = 300.
Values are means ± SE; statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.

Table III. Effect of repeated doses for 60 consecutive days of Spinosad and Malathion on testosterone hormone concentration (ng/ml) in the serum of treated male rats

Treatments	Dose mg a.i. kg^{-1} b.w.	Testosterone concentration (ng ml^{-1})					
		0 day	15 day	30 day	45 day	60 day	% of change[(1)]
Control	0.00	5.11±0.12	4.98±0.13	4.75±0.05	4.59±0.15	4.38±0.16	-14.29
	0.02	5.01±0.11	4.73±0.23	4.66±0.13	4.50±0.20	4.16±0.20	-16.97
Spinosad	9.00	5.04±0.21	4.64±0.12	4.35±0.29	3.43±0.07*	3.42±0.12*	-32.14
	37.38	5.13±0.13	4.39±0.19	4.15±0.22	3.26±0.04*	3.23±0.16*	-37.04
	0.30	5.10±0.10	4.48±0.18	4.20±0.14	3.20±0.15*	3.14±0.13*	-38.43
Malathion	13.75	5.08±0.23	4.39±0.08	3.93±0.16*	3.05±0.21**	2.95±0.02**	-41.93
	29.00	5.10±0.09	4.25±0.16	3.54±0.18*	2.78±0.09**	2.46±0.11**	-51.76

Each value is a mean of 10 replicates ± SE; statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.
(1) % of change = [(final testosterone conc. - initial testosterone conc.) / (initial testosterone conc.)] x 100

Table IV. Body and testes weights in rats received 60 consecutive doses of Spinosad and Malathion

Treatments	Dose mg a.i.kg^{-1} b.w.	Body weight (g)	Testes weight (g)	
		Final body weight	Mean ± SE	Relative testes weight (g. kg^{-1} b.w.)[(1)]
Control	0.00	265.00± 14.81	3.08± 0.08	1.16
Spinosad	0.02	251.60±5.04	2.87±0.24*	1.14
	9.00	248.66±3.54*	2.76±0.02*	1.11
	37.38	247.00±4.92*	2.69±0.12*	1.09
Malathion	0.30	245.86±3.15*	2.81±0.22*	1.14
	13.75	220.00±4.39**	2.44±0.08**	1.11
	29.00	207.66±3.39**	2.18±0.06**	1.05

Each value is a mean of 10 replicates ± SE; statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.
(1) Relative testes weight = (testes weight / body weight) x 100

were taken after the last day of treatment, then the animals were killed by cervical dislocation and the testes were dissected out and weighted. To ensure normalization of the data, testes weights were expressed per 100 g body weight.

Statistical analysis. The experimental design was a factorial CRD (Complete Randomized Design) with ten replicates. Statistical analysis of data collected was carried out using a computer program (Cohort Software, 1986).

RESULTS

The present study was carried out to compare cytogenetic and reproductive effects of commercial formulation of a new natural insecticide "Spinosad" and an organophosphorus insecticide "Malathion" administered daily to male rats, via oral route, in 60 repetitive doses equaled to ADI, NOAEL and 1/100 LD_{50}. The data in Table I indicate that spinosad (37.38 mg a.i.kg^{-1} b.w.) and malathion (13.75& 29.00 mg a.i.kg^{-1} b.w.) caused significant decrease in the mitotic activity (MI %) and significantly increased micronucleated polychromatic erythrocyte (MN %) in rat bone-marrow cells. Moreover, binucleate cells were observed at higher doses of spinosad

Fig. 1. Rat bone marrow cells after treatments with spinosad and malathion showing: (A) Normal polychromatic erythrocyte; (B & C) Micronucleated polychromatic erythrocyte; (D) Binucleate [x 100; Giemsa stain]

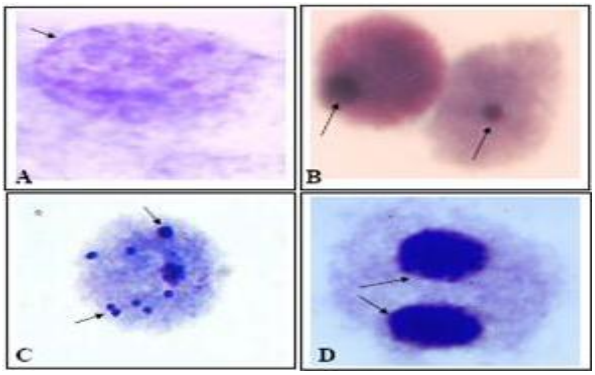


Fig. 2. Rat bone marrow cells after treatments with spinosad and malathion showing: Fragment (F); Gapes (G) and Deletions (D) [x 100; Giemsa stain]

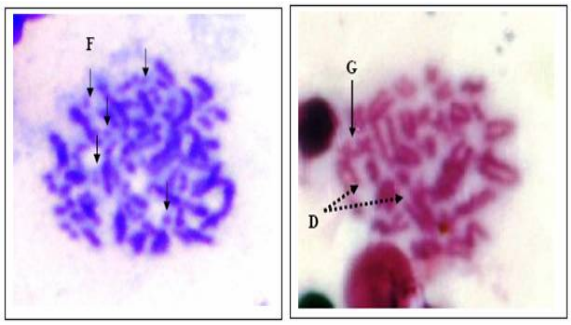
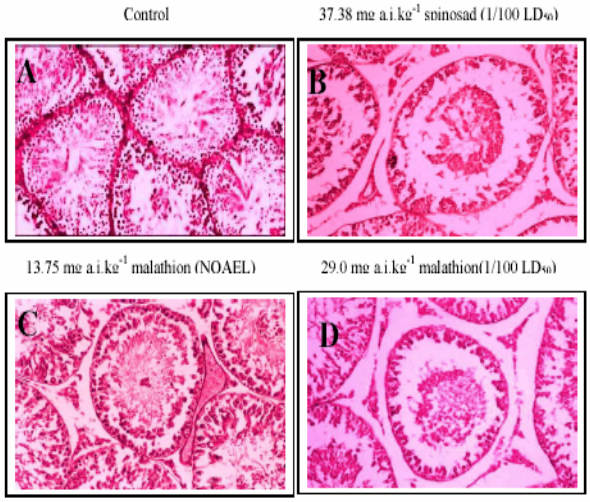


Fig. 3. Rat testis after treatments with spinosad and malathion showing: (A) normal testis; (B) necrosis of spermatogonial cells; (C) congest in blood vessels and (D) **exfoliation of necrotic cells within the somniferous lumen (H & E., X 100)**



and Malathion (Fig. 1).

Both malathion and spinosad treatments induced structural chromosomal aberrations in rat bone marrow cells and gaps (gap was identified as unstained region in the chromatid, which was smaller than the width of the chromatid), fragments and deletions represented the observed aberrations (Fig. 2). The percentage of metaphases with chromosomal aberrations excluding or including gaps was found to be statistically significant in all treatments of malathion and in spinosad treatments with 9.00 and 37.38 mg a.i. kg^{-1} b.w. Chromosomal aberration in control rat group was accounted to 2.66% only. Such data may indicate that the obtained cytogenetic effects occurred in a dose-dependent manner (Table II).

Testosterone levels of animals treated with 13.75 and 29.00 mg a.i.kg^{-1} b.w. malathion after 30 days or with 9.00 and 37.38 mg a.i.kg^{-1} b.w. of spinosad after 45 days were significantly lower ($P \leq 0.05$) than that of the control values (4.75 & 4.59 ng mL^{-1}, respectively at 30 & 45 days) (Table III). With time elapsed, the decrease in testosterone concentration was more pronounced (i.e., highly significant at $P \leq 0.01$), especially in malathion treatments. Generally, the percentage of change in testosterone concentration after 60 repetitive doses ranged between (-16.97% to -37.04%) with spinosad treatments. With malathion treatments, such change ranged between (-38.43% to -51.76%).

The histopathological examination of the testes was performed after exposure to the tested insecticides. Varieties of histopathological lesions were identified in the examined testes. The incidence and severity of these lesions appeared to increase with increasing dose and the most pronounced pathological changes were necrosis of spermatogonial cells, congest in blood vessels and exfoliation of necrotic cells within the seminiferous lumen (Fig. 3).

The mean weight of paired testes of control and treated animals are shown in Table IV. For control, the absolute weight recorded 3.08 g. The tested doses of both insecticides recorded observable significant decrease in the testes weights and the lowest value (2.18 g) was resulted from malathion at the 1/100 LD_{50} (29.00 mg a.i. kg^{-1} b.w.). As relative to body weight, statistical analysis revealed no-significant differences between control and all treatment values. Moreover, except the treatment with 0.02 mg a.i. kg^{-1} of spinosad, the other tested doses of both insecticides showed significant decrease in body weight gains.

DISCUSSION

Genotoxic risks of human exposure to pesticides remain a worldwide concern, because many of these pesticides are mutagenic linked to the development of cancers (Leiss & Savitz, 1995). Several published reports suggested an association between exposure to pesticides and different types of human cancer. Also, increasing levels of chromosomal aberrations has been associated with increased cancer risk (Hagmar *et al.*, 1994). Complementary, some pesticides cause reproductive toxicity in animals and human (Hileman, 1994). Spinosad and malathion, tested in the present study, showed cytotoxic effects represented by significant decrease of mitotic activity (MI), which agree with the earlier findings. Malathion is a very potent cell cycle inhibitor and decreases the mitotic indices in the mice

bone marrow (Salvadori *et al.,* 1988).

Biomarkers such as chromosomal aberrations (CA) analysis and micronucleus test are among the most extensively used markers of genotoxic effects of pesticides. CA is particularly dangerous to the cell, because the physical discontinuity of the chromosome may cause loss of genetic information and even cell death if a housekeeping gene is involved (Carbonell *et al.,* 1995). Also, CA may be used as an early warning signal for cancer development and it has been suggested that the detection of an increase in chromosome aberrations, related to an exposure to genotoxic agents, may be used to estimate cancer risk (Hagmar *et al.,* 1994). Additionally, to assess the degree to which tested doses cause chromosomal damage of developing red blood cells, the incidence of residual chromosome fragments (micronuclei) in polychromatic erythrocytes from femoral bone marrow has to be determined, as this is a well-established biomarker of chromosome breakage due to DNA damage (Schmid, 1975). Many studies have demonstrated the efficiency of the MN assay to detect DNA damage induced by pesticides (e.g., Da Silva Augusto *et al.*, 1997). Our data showed significant increase in chromosomal aberration and micronucleus frequency. Moreover, binucleate cells were observed at higher doses of spinosad and malathion.

The mode of action of spinosad is characterized by an excitation of the nervous system with activation of nicotinic acetylcholine receptors (nAChRs), along with effects on γ-amino butyric acid (GABA) receptor function, GABA-gated chloride channels and can elicit a small-amplitude Cl^- current (Salgado, 1998). On the other hand, spinosad toxicity is consistent with altered phospholipid metabolism, resulting in cellular phospholipidosis. In support of this, spinosad's chemical structure (a hydrophobic ring structure with a hydrophilic side-chain containing a basic amine group) is comparable to other cationic amphiphilic drugs, which produce similar cellular toxicity (Halliwell, 1997). According to EPA (1997), spinosad has no mutagenic activity and the literature offers no additional data in this respect. Recently, Mansour *et al.* (2007) found that spinosad at 9.0; 37.38 mg a.i. kg^{-1} b.w. reduced the total number of erythrocytes and inhibited serum ChE in male rats. In the light of our findings study, we suggest that cytogenetic activity of spinosad may refer to the spinosad's chemical structure and/or certain impurities in the commercial formulation; a matter, which needs further elucidation.

On the other side, the primary molecular mechanism of action of the OP pesticides generally is the inhibition of acetylcholinesterase (AChE), a widely distributed serine esterase. Organophosphorothioate (OPT) insecticides containing the P = S bond (e.g., malathion) are converted to P = O (oxons) by enzymes mixed-function oxidases (MFO) in which the enzyme cytochrome P-450 plays a major role. The oxons are highly toxic compounds, which account for the profound cytotoxic effects of organophosphorus pesticides and can induce cholinergic crisis in mammals (Maroni *et al.,* 2000). They affect activity of esterases and other enzymes and possibly cause a disturbance in cell metabolism leading to a disruption of cell division machinery (Flessel *et al.,* 1993). Also, OP insecticides are reported to have the ability to bind to DNA and several phosphorothioate compounds such as malathion can lead to mutagenesis (Wild, 1975).

In support of our findings, many *in vivo* and *in vitro* studies in humans and animals have investigated the genotoxic effects of malathion. Based on genetic studies conducted between 1978-1995 in 29 different laboratories on various organisms (e.g., bacteria, fruit flies, mice, hamsters, fish & human cell cultures), malathion appeared to be a mutagenic compound (NIOSH, 2002). It induced a dose-dependent significant increase in the chromosomal aberrations and/or micronucleus frequency in mouse bone marrow cells (Amer *et al.*, 2002; Giri *et al*., 2002). In experimental animal studies, using very high doses (ranging between 50 & 2000 mg kg^{-1} b.w.), malathion is reported to produce chromosomal aberrations and micronucleus frequency (Flessel *et al*., 1993). In contrast, some studies showed that malathion produced no cytogenetic effects in a variety of test systems (Degraeve & Moutschen, 1984).

The reported genotoxicity of malathion might, therefore be a consequence of its metabolic biotransformation to malaoxon, formed by oxidation or the presence of malaoxon and/or isomalathion, formed by isomerization, as well as other unspecified impurities in commercial formulations of malathion (Berkman *et al.,* 1993). Impurities, such as isomalathion and various trimethylphosphorothioate esters, present in the technical grade malathion or formed during storage, can potentiate malathion-induced toxicity up to 10-fold (Aldridge *et al*., 1979), and are responsible for other effects, including DNA lesions (Flessel *et al*., 1993; Blasiak *et al.,* 1999).

Pesticides have serious hazard in reducing men fertility and in causing damage of reproductive organs of animals (Whorton *et al.,* 1977). Our findings indicated that repetitive treatment with malathion or spinosad caused significant decrease in the serum testosterone concentration and histopathological lesions to testes in a dose-depended manner. Testosterone is the most important androgen secreted into the blood by the Leydig cells of the testes in males. The changes in the rate of inactivation and elimination of testosterone, due to chemical exposure, can alter the levels of circulating testosterone and thereby disrupt steroid hormone homeostasis. Furthermore, testosterone is metabolically inactivated in the liver by some of the same hepatic enzymes responsible for the detoxification of xenobiotics and the expression of these hepatic enzymes is differentially susceptible to induction or suppression due to xenobiotic exposure (Wilson & LeBlanc, 2000). As a result, alterations in hepatic enzyme activity due to chemical exposure can lead to concomitant changes in the biotransformation of endogenous substrates such as testosterone. The possible mechanism of action of

organophosphorus pesticides leading to reduction of serum testosterone level was referred to secretion of gonadotrophins according to Stewart and Fish (1966). Krause and Homota (1974) added that the decreased testosterone level might be due to a direct damage of Leydig cell or to lowered stimulation of these cells by interstitial cells stimulating hormone. Malathion itself had also been reported to decrease plasma testosterone levels in rats and the histological examination of the testes showed slight reductions of the spermatogentic cells and Leydig cells (Krause, 1977). The observed testicular degenerative changes in our study may be attributed to the direct cytotoxic action on testes.

The overall findings of the present investigation revealed that both commercial malathion and spinosad cause mutagenic and reproductive effects on male albino rats. However, the effect of malathion was much pronounced than spinosad. This may be due to the metabolic biotransformation of malathion to malaoxon or the presence of malaoxon and/or isomalathion, as well as other unspecified impurities and unidentified inert ingredients in the commercial formulation of both insecticides. Supporting this, Cox and Surgan (2006), suggested that pesticide registration and their environmental monitoring should include full assessment of formulations. Furthermore, the present study highlights the necessity of evaluating toxic hazards of formulated pesticides even at the recommended "safe doses" for technical compounds.

REFERENCES

Abo-Zeid, M.M., G. El-Baroty, E. Abdel-Rahim, J. Blankats, C. Dary, A.H. El-Sebae and M.A. Saleh, 1993. Malathion distribution in dermally and orally treated rats and its impact on the blood serum acetylcholine esterase and protein profile. *J. Environ. Sci. Hlth.,* 28: 413–30

Aldridge, W.N., J.W. Miles, D.L. Mount and R.D. Verschoyle, 1979. The toxicological properties of impurities in malathion. *Arch. Toxicol*., 42: 95–106

Amer, S.M., M.A. Fahmy, F.A.E. Aly and A.A. Farghaly, 2002. Cytogenetic studies on the effect of feeding mice with stored wheat grains treated with malathion. *Mut. Res.,* 513: 1–10

Anonymous, 2005. *The E-Pesticide Manual*. (B.C.P.C.) The British Crop Protection Council Software developed by Wise and Loveys Information Services Ltd.

Bancroft, J.D. and A. Stevens, 1982. *Theory and Practice of Histologic Techniques*, 2nd edition, p: 113. Long Man Group Limited

Berkman, C.E., C.M. Thompson and S.R. Perrin, 1993. Synthesis, absolute configuration and analysis of malathion, malaxon, and isomalathion enantiomers. *Chem. Res. Toxicol.,* 718–23

Blasiak, J., P. Jaloszynski, A. Trzeciak and K. Szyfter, 1999. *In vitro* studies on the genotoxicity of the organophosphorus insecticide malathion and its two analogues. *Mut. Res.,* 445: 275–83

Carbonell, E., A. Valbuena, N. Xamena, A. Creus and R. Marcos, 1995. Temporary variations in chromosomal aberrations in a group of agricultural workers exposed to pesticides. *Mut. Res.,* 344: 127–34

Cohort Software, 1986. *Costat User's Manual Virgin 3.03*. Berkley, California, U.S.A

Cox, C. and M. Surgan, 2006. Unidentified inert ingredients in pesticides: implications for human and environmental health. *Environ. Hlth. Perspec*., 114: 1803–6

Da Silva Augusto, L.G., S.R. Lieber, M.A. Ruiz and C.A. De Souza, 1997. Micronucleus monitoring to assess human occupational exposure to organochlorides. *Environ. Mol. Mut.,* 29: 46–52

Degraeve, N. and J. Moutschen, 1984. Genetic and cytogenetic effects induced in the mouse by an organophosphorus insecticide malathion. *Environ. Res.*, 34: 170–4

EPA, 1997. *Spinosad Pesticide Fact Sheet No. HJ 501C*. EPA, Office of Pesticides and Toxic Substances, (www.epa.gov)

Flessel, P., P.E. Quintana and K. Hooper, 1993. Genetic toxicity of malathion: a review. *Environ. Mol. Mut.*, 22: 7–17

Giri, S., S.B. Prasad, A. Giri and G.D. Sharma, 2002. Genotoxic effects of malathion: an organophosphorus insecticide, using three mammalian bioassays in vivo. *Mut. Res.,* 514: 223–31

Hagmar, L., A. Brogger, I.L. Hansteen, S. Heim, B. Hogstedt, L. Knudsen, B. Lambert, K. Linnainmaa, F. Mitelman, I.C. Reuterwall, C. Salomaa, S. Skerfving and M. Sorsa, 1994. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. *Cancer Res.,* 54: 2919–22

Halliwell, W.H., 1997. Cationic amphiphilic drug-induced phospholipidosis. *Toxicol. Pathol.,* 25: 53–60

Hileman, B., 1994. Environmental estrogens linked to reproductive abnormalities and cancer. *Chem. Eng. New*, 31: 19–23

Krause, W. and S. Homota, 1974. Alteration of seminiferous epithelium and Leydig cells of the rat testes after application of dichlorovos (DDVP). *Bull. Environ. Contam. Toxicol.,* 11: 429–33

Krause, W., 1977. Influence of DDT, DDVP and malathion on FSH, LH and testosterone concentration in testis. *Bull. Environ. Contam. Toxicol*., 18: 231–42

Leiss, J.K. and D.A. Savitz, 1995. Home pesticide use and childhood cancer: a case control study. *American J. Publ. Hlth.*, 85: 249–53

Mansour, S.A. and A.H. Mossa, 2005. Comparative effects of some insecticides as technical and formulated on male albino rats. *J. Egyptian Soc. Toxicol.,* 32: 41–54

Mansour, S.A., A.H. Mossa and T.M. Heikal, 2007. Haematoxicity of a new natural insecticide "Spinosad" on male albino rats. *Int. J. Agric. Biol*., 9: 342–6

Maroni, M., C. Colosio, A. Feridi and A. Fait, 2000. Organophosphorus pesticides. *Toxicol.,* 143: 9–37

NIOSH "National Institute for Occupational Safety and Health", 2002. *Registry of Toxic Effects of Chemical Substances: Succinic Acid, Mercapto-,-diethyl Ester, S-ester with O, O-dimethyl-phosphorodithioate*(http://www.cdc.gov/niosh/rtecs/wm802c80.html

NRC, 1996. *National Research Council: Guide for the Care and Use of Laboratory Animals,* p: 125. National Academic Press, Washington,

Preston, R.J., B.J. Deen, S. Galloway, S.H. Holden, A.F. Mc Fee and M. Shelby, 1987. Mammalian in vivo-cytogenetic assays: analysis of chromosome aberrations in bone marrow cells. *Mut. Res*., 21: 187–8

Salem, A.D. and S. Kh. Abd Elghaffar, 1998. Effect of nuvacron on reproductive system of male albino rats. *Assiut Vet. Med. J.*, 39: 130–44

Salgado, V.L., 1998. Studies on the mode of action of spinosad: Insect symptoms and physiology correlates. *Pestici. Bioch. Physiol*., 60: 91–102

Salvadori, D.M., L.R. Eibero, C.A. Pereira and W. Becak, 1988. Cytogenetic effects of malathion on somatic and germ cells of mice. *Mut. Res.,* 204: 283–7

Schmid, W., 1975. The micronucleus test. *Mut. Res*., 31: 9–15

Stewart, A. and M.D. Fish, 1966. Organophosphorus cholinesterase inhibitors and fetal development. *American J. Obstet. Gynaec*., 15: 1148–54

Trachtenberg, J., 1987. Experimental treatment of prostatic cancer by intermittent hormonal therapy. *J. Urol.,* 137: 785–8

WHO, 1991. *Guidline to WHO Recommended Classification of Pesticides by Hazard,* p: 39. IPCS

Whorton, D., R.M. Krause, S. Marshal and T.H. Miby, 1977. Infertility in male pesticide workers. *Lancet*, 2: 1259–61

Wild, D., 1975. Mutagenicity studies on organophosphorus insecticide. *Mut. Res*., 32: 133–50

Wilson, V.S. and G.A. LeBlanc, 2000. The contribution of hepatic inactivation of testosterone to the lowering of serum testosterone levels by ketoconazole. *Toxicol. Sci.*, 54: 128–37

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