INDUCTION OF CHROMOSOME ABERRATIONS IN MOUSE GERM CELLS BY THE ORGANOPHOSPHOROUS INSECTICIDES "DURSBAN" and "DICHLORVOS" AND SPERM ABNORMALITIES IN THE TREATED MICE

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ABSTRACT

The ability of the two organophosphorous insecticides "Dursban" and "Dichlorvos" to induce chromosomal abnormalities in mouse spermatocytes was investigated. Male swiss mice were intraperitoneally (i.p) injected with the doses 2, 4, 10 mg/kg^{-1} body wt. of "Dursban" (dissolved in 0.1 DMSO) and 2.8, 3.5, 7 mg/kg^{-1} body wt. of "Dichlorvos" and samples were taken 6, 24 and 48 hrs after the treatment.

The percentage of chromosomal aberrations in diakinesis - metaphase I cells increased by increasing the concentration of both insecticides and reached its maximum 24 hrs following i.p. injection. Its highest value was 19.3 ± 1.61 ($P < 0.01$) and 16.5 ± 0.63 ($P < 0.01$) 24 hrs after treatment with the highest dose of "Dursban" and "Dichlorvos" respectively. Compared with 25.5 ± 0.97 ($P < 0.01$), 24 hrs after i.p. injection with "Mitomycin C" at 1 mg/kg^{-1} body wt. The two insecticides induced abnormal chromosomal associations including univalents (autosomal and x-y univalents) and chains, as well as structural and numerical chromosome aberrations.

Dursban and Dichlorvos induced a dose-dependent increase in the percentage of abnormal sperm heads. Its highest value was 15.48 ± 0.73 ($P < 0.01$) and 10.8 ± 0.89 ($P < 0.01$) after oral treatment with Dursban and Dichlorvos respectively, compared with 18.3 ± 1.10 ($P < 0.01$) after treatment with "Mitomycin C".

INTRODUCTION

The organophosphorous insecticides chlorpyifos "Dursban" and "Dichlorvos" (DDVP) are well known insecticides.

Dursban is effective against a wide range of insects e.g. cockrooches (Rust *et al.*, 1991) and Mosquitos (Sinegre *et al.*,

1990). The insecticide controls insect pests which attach economic corps e.g. potato (Parihar & Singh 1998) and corn (Guillebeau and All, 1991). It is used in Egypt to control several pests (Programme of pest control, Ministry of Agriculture, A.R.E. 1973/1974 - 1980/1981).

Dichlorvos is used mainly for the control of insects in tobacco and other warehouses, mashroom houses, green houses, animal shelters and homes (Hayes, 1982). The insecticide is used also for control of household and public heath pests stored-product insects and mosquitos (Royal Society of Chemistry, 1983).

The cytogenetic effect of "Dursban" was studied in both plant and animal systems. It induced a significant percentage of abnormal cells in root-mitosis of *Vicia faba* plant (Amer and Farah, 1983a) as well as in meiosis of barley and *Vicia faba* plants (El-Metainy and Badr, 1981; Amer and Farah, 1983b).

The potential of "Dursban" as a genotoxic agent on the basis of chromosome damage and rates of sister chromatid exchange in cultured human lymphocytes was determined (Nelson, 1990). Dursban induced a significant percentage of chromosome aberrations in the spleen (Amer *et al.*, 1996) and in cultured spleen cells of the mouse (Amer and Aly, 1992).

Dichlorvos has also received marked attention from the cytogenetic point of view, particularly as a mutagen, but unlickely the results obtained in this respect, were apparently problematic and contradickory (Lofroth, 1970). But nervertheless, a communication was presented by Bignami *et al.*, (1977) indicating that "Dichlorvos" had induced obvious mutations a high frequency of mitotic crossing-over and non-disjunction of the meiotic chromosomes in *Aspergillus didulans* similarly, base-substitute mutations were noticed in Salmonella by Byeon *et al.*, (1976) due to "Dichlorvos" exposure.

Plants were also revealed by some researchers to respond to "Dichlorvos" treatment by the development of chromosome aberrations in the root tips of onion (Sax and Sax, 1968) barley (Bhan and Kaul, 1975) and *Vicia faba* (Amer and Ali, 1986).

Dichlorvos induced a significant percentage of PE with micronuclei (Aboul-Ela, 1985), chromosome aberrations and sister chromatid exchange in bone-marrow, as well as, chromosome aberrations in cultured spleen cells of the mouse (Aboul-Ela, 1992).

The aim of the present experiments is to study the effects of Dursban and Dichlorvos on germ cells and sperm-shape abnormalities of male mice are studied.

MATERIALS AND METHODS

Animals :

Male Swiss mice aged 8 - 10 weeks and weight 25 - 30 gm ± 2 obtained from a closed randome - bred colony at the National Research Centre, were used.

Insecticides :

Purified Dursban and DDVP (purity 100%) were kindly supplied by Prof. S.M.A.D. Zayed Department of Organic Chemistry, National Research Centre.

Treatment :

There were three experimental groups for each insecticide. Mice were i.p. injected with the doses 2, 4 and 10 mg kg^{-1} body wt. of "Dursban" (dissolved in 0.1 ml DMSO) and 2.8, 3.5 and 7 mg kg^{-1} body wt. of "Dichlorvos" (DDVP) diluted with distilled water. LD_{50} of "Dursban" is 40 mg kg^{-1} body wt. for i.p. injection (Fakhr *et al.*, 1982) and 152 mg kg^{-1} body wt. for oral (Hayes, 1982) and the LD_{50} of DDVP for i.p. 28 mg kg^{-1} body wt. (Hayes, 1982) and 145 mg kg^{-1} body wt. for oral (Hayes, 1982).

A non-treated group of animals was used as control and another control group was i.p. injected with 0.1 ml DMSO in the same way for each fixation time. Moreover, a group of animals was i.p. injected with "Mitomycin C" at 1 mg kg^{-1} body wt., killed 24 and 48 hrs after i.p. treatment and was used as positive control.

Each experimental group included 5 males. Mice were killed 6, 24 and 48 hrs. after treatment and were injected with colchicine at 3 mg kg^{-1} body wt. 2 hrs. prior to sacrifice.

For sperm shape abnormalities, 3 groups of animals (5 animals for each group) were treated by gavage with Dursban for 5 consecutive days at dose levels 2.5, 10 and 20 mg kg^{-1} body wt. and 2, 9 and 18 mg kg^{-1} body wt. of DDVP. Animals were killed 35 days after administrating the first dose. A non-treated group of animals was used as a control, another group was i.p. injected with 1 mg "Mitomycin C" kg^{-1} body wt. and used as positive control.

Slide preparation and scoring :

Chromosomal preparations were made from testes according to the technique of Evans *et al.*, (1964) and stained with Giemsa in phosphate buffer (pH 6.8). 50 primary spermatocytes / mouse at diakinesis - metaphases I were analyzed. Types of diakinesis - MI abnormalities recorded included : Chains, sex-chromosomal univalents (x-y Univ.), autosomal univalents (A.U), polyploidy, gaps, breaks and fragments.

For sperm - shape abnormalities, mice were killed, the epididymides excised and minced in isotonic sodium citrate solution (2.2%), dispersed and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with Eosin Y (Wyrobek and Bruce, 1975). At least 500 sperm per animal wrere assessed for morphological abnormalities included

triangular, without hook, banana shape, amorphous, small and big head.

Statistical evaluation :

The significance of the experimental from control data was calculated using student t-test.

Significant ($P < 0.05$).

Highly significant ($P < 0.01$).

RESUTLS

The results indicated that "Dursban" at the tested doses is a potent inducer of chromosome aberrations in spermatocytes. The percentage of metaphases with chromosomal aberrations was found to be statistically significant 6, 24 and 48 hrs after i.p. injection with the concentrations 2, 4 and 10 mg/kg^{-1} body wt. (Table 1).

"Dichlorvos" at the lowest tested concentration induced a low percentage of chromosome aberrations 6h after i.p. injection. This percentage increased, reached 9.2% 24 hrs after the treatment and was found to be statistically significant. The two higher concentrations of the insecticide induced a statistically significant percentage of chromosome aberrations 6, 24 and 48 hrs after the treatment (Table 2).

The percentage of the induced chromosome aberrations was dose dependent, it reached its maximum 24 hrs after treatment with the different concentrations tested of both insecticides. Its highest value was 19.3 ± 1.61 and 16.5 ± 0.63 ($P < 0.01$) after treatment with highest tested concentration of "Dursban" and "Dichlorvos" respectively. These values decreased and reached 14.3 ± 0.97 and 9.03 ± 0.40 ($P < 0.01$) 48 hrs after i.p. injection with "Dursban" and "Dichlorvos" respectively, compared with 25.5 ± 0.97 and 14.25 ± 0.76 ($P < 0.05$) 24 and 48 hrs after i.p. injection with Mitomycine C (Table 1, 2).

The two insecticides induced abnormal chromosome associations including autosomal and x-y univalents as well as structural and numerical chromosomal aberrations (Table 1, 2).

Diakinesis - metaphase I cells with chain IV were observed after treatment with the two higher concentrations of "Dursban" (Table 1).

Sperm head abnormalities were observed in control and treated mice. The two higher concetrations of Dursban and Dichlorvos induced a dose-dependent and statistically significant increase in the percentage of sperm head abnormalities over the that of the control (Table 3, 4).

DISCUSSION

All the used concentrations of "Dursban" and "Dichlorvos" increased chromosome aberrations significantly ($P < 0.01$ at most dose-time points). Dursban

induced chromosome aberrations in bone marrow (Amer *et al.*, 1996) and spleen of the mouse (Amer *et al.*, 1996). Dichlorvos induced breaks, translocations and rings in Chinese hamster fibroblasts (Ishidate, 1976). The insecticide induced also chromosome aberrations in bone marrow and cultured spleen cells of the mouse (Aboul-Ela, 1992).

In the present study, a low percentage of chain IV was observed in diakinesis-MI spermatocytes after treatment with the two higher concentrations of "Dursban". This indicates that Dursban is one of the few chemicals that can induce chromosome damage in germ cells.

Chain IV was not observed in animals treated with Mitomycin C (MMC) at 1 mg/kg^{-1} body wt. This result is in accordance with that of De Luca *et al.*, (1990) who reported that, the frequencies of quadrivalent chains induced by MMC after single treatment (2 mg/kg^{-1} body wt.) were very low (0.62%). Chromosome translocations expressed in diakinesis metaphase I spermatocytes (rings and chains) have been observed in animals whose spermatogonia were treated with ionizing radiations (Van Buul, 1983, 1984). Rarely in those treated with chemicals e.g. adriamycin (Au and Hsu, 1980) and grisofulvin (Fahmy and Hassan, 1996) have been shown to be positive in this respect.

The most common type of abnormality observed in spermatocytes after treatment with both insecticides was the presence of univalents. This may be the result of precocious separation of the chromosomes from the bivalents (Tates and Natarajan, 1976). It was observed that x-y univalents were more often separated than autosomal univalents. This phenomenon was observed in spermatocytes of mice after treatment with uranyl fluoride (Hu and Zhu, 1990) and Pirimiphos-methyl (Aly and Fahmy, 1995).

A statistically significant increase in the percentage of sperm head abnormalities occurred in Dursban and Dichlorvos treated animals. It may be mentioned in this respect that the organophosphorous insecticide Curacron (El-Nahas *et al.*, 1989), tamaron (Abdel-Aziz *et al.*, 1993) and malathion (Hassan *et al.*, 1995) have been reported to increase sperm-head abnormalities in the treated mice.

Sperm morphology assay is said to provide a quantitative method for locating genetic damage in male germline cell (Lock and Soares, 1980). The relevance of this assay in evaluating mammalian germ cell mutagens is well accepted (Wyrobeck *et al.*, 1983).

In the present study, the dose dependent increase in the percentage of chromosome aberrations in spermatocytes and sperm head abnormalities induced by Dursban and Dichlorvos emphasises the positive correlation between cytogenetic damage and sperm abnormality which was previously reported in mice by Lavu *et al.*, (1985) and El-Nahas *et al.*, (1989).

The results indicate the mutagenic potential of the organophosphorous insecticides "Dursban" and "Dichlorvos" in germ cells of mice.

Accordingly much more care should be taken in using "Dursban" and "Dichlorvos" in agricultural aspects.

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Table (1): Mean percentage of chromosomal aberrations, number and percentage of the different types of aberrations in mouse germ cell 6, 24 and 48h. after i.p. injection with different concentrations of "Dursban" dissolved in DMSO.

Treatment	No. of mice	Metaphases with :								Mean% ± S.E.
		Autosomal univalent	x-y univ.	Autosomal univ. + x-y univ.	Frag. and/or break	Gap	Chain	Diplo-idy	Trip-loidy	
I. Non-treated	5	3 (1.2)	4 (1.6)	–	–	–	–	–	–	2.8 ± 0.30
II. DMSO										
6 h	5	5 (2)	7 (2.8)	–	–	–	–	2 (0.8)		5.6 ± 0.39
24 h	5	8 (2.2)	4 (1.6)	–	–	–	–	1 (0.4)	–	5.2 ± 0.48
48 h	5	3 (1.2)	7 (2.8)	–	–	–	–	1 (0.4)	–	4.4 ± 0.39
III. Mitomycin C										
1 mg kg-1 body wt.										
(Positive control)										
24 h	5	64 (16)	21 (5.25)	4 (1)	2 (0.5)	–	–	11 (2.75)	–	25.5 ± 0.79*
48 h	5	38 (9.5)	11 (2.75)	2 (0.5)	–	–	–	6 (0.25)	–	14.25 ± 0.76*
IV. Dursban										
a) 2 mg kg^{-1} body wt.										
6 h	5	8 (3.2)	9 (3.6)	–	1 (0.4)	2 (0.8)	–	4 (1.6)	–	9.6 ± 0.58*
24 h	5	11 (4.4)	12 (4.8)	–	3 (1.2)	–	–	8 (3.2)	–	13.6 ± 0.67**
48 h	5	9 (3.6)	7 (2.8)	–	–	1 (0.4)	–	2 (0.8)	–	7.6 ± 0.50
b) 4 mg kg^{-1} body wt.										
6 h	5	12 (4.8)	11 (4.4)	–	–	–	1 (0.4)	7 (2.8)	–	12.4 ± 0.50**
24 h	5	15 (6)	14 (5.6)	–	–	–	2 (0.8)	7 (2.8)	–	15.2 ± 1.24**
48 h	5	14 (5.6)	6 (2.4)	–	–	–	–	3 (1.2)	–	9.2 ± 0.40*
c) 10 mg kg^{-1} body wt.										
6 h	5	15 (6)	20 (8)	–	8 (3.2)	3 (1.2)	3 (1.2)	9 (3.6)	1 (0.4)	17.3 ± 1.31**
24 h	5	13 (5.2)	18 (7.2)	–	8 (3.2)	4 (1.6)	2 (0.8)	13 (5.2)	4 (1.6)	19.3 ± 1.61**
48 h	5	15 (6)	13 (5.2)	–	7 (2.8)	3 (1.2)	1 (0.4)	7 (2.8)	–	14.3 ± 0.97**

* Significant at ($P < 0.05$) level.

** Significant at ($P < 0.01$) level (t-test).

Table (2): Mean percentage of chromosomal aberrations, number and percentage of the different types of aberrations in mouse germ cell 6, 24 and 48h. after i.p. injection with different concentrations of "Dichlorvos".

Treatment	No. of mice	Metaphases with :							Mean % ± S.E.
		Autosomal univalent	x-y univ.	Autosomal univ. + x-y univ.	Frag. and/or break	Gap	Diploidy	Trip-loidy	
I. Non-treated	5	3 (1.2)	4 (1.6)	-	-	-	-	-	2.8 ± 0.30
II. Mitomycin C 1 mg kg^{-1} body wt. (Positive control)									
24 h	5	64 (16)	21 (5.25)	4 (1)	2 (0.5)	-	11 (2.75)	-	25.5 ± 0.97*
48 h	5	38 (9.5)	11 (2.75)	2 (0.5)	-	-	6 (0.25)	-	14.25 ± 0.76*
III. Dichlorvos									
a) 2.8 mg kg^{-1} body wt.									
6 h	5	5 (2)	7 (2.8)	-	2 (0.8)	-	4 (1.6)	-	7.2 ± 0.50
24 h	5	8 (3.2)	10 (4)	-	3 (1.2)	-	2 (0.8)	-	9.2 ± 0.40**
48 h	5	6 (2.4)	8 (3.2)	-	1 (0.4)		2 (0.8)	-	6.4 ± 0.50
b) 3.5 mg kg^{-1} body wt.									
6 h	5	9 (3.6)	11 (4.4)	-	4 (1.6)	2 (0.8)	6 (2.4)	-	10.66 ± 0.50**
24 h	5	12 (4.8)	16 (6.4)	-	6 (2.4)	4 (1.6)	7 (2.8)	-	12.84 ± 0.45**
48 h	5	7 (2.8)	10 (4)	-	1 (0.4)	-	2 (0.8)	-	8.0 ± 0.58*
c) 7 mg kg^{-1} body wt.									
6 h	5	11 (4.4)	14 (5.6)	-	7 (2.8)	3 (1.2)	6 (2.4)	-	14.14 ± 1.20**
24 h	5	14 (5.6)	12 (4.8)	-	8 (3.2)	8 (3.2)	10 (4)	3 (1.2)	16.5 ± 0.63**
48 h	5	10 (4)	9 (3.6)	-	1 (0.4)	2 (0.8)	6 (2.4)	-	9.03 ± 0.40**

* Significant at ($P < 0.05$) level. ** Significant at ($P < 0.01$) level (t-test).

Table (3): Mean percentage of sperm abnormalities in control and mice treated with different doses of Dursban (dissolved in oil).

Doses	No. of sperm examined	No. of abnormal sperm	Mean% ± S.E.	Types of sperm head abnormalities :					
				amor-phous	without hock	Trian-gular	Banana shape	Small head	Big head
I. Mitomycin C	2970	543	18.3 ± 1.10	111	109	120	96	58	49
II. Control (oil)	2510	100	3.58 ± 0.27	14	36	24	4	13	9
III. Dursban									
2.5 mg kg^{-1} body wt.	2522	125	4.96 ± 0.66	4	30	79	5	6	1
10 mg kg^{-1} body wt.	2528	254	10 ± 0.97*	12	69	146	12	11	4
20 mg kg^{-1} body wt.	2527	391	15.48 ± 0.73*	5	103	248	26	4	5

* Significant at ($P < 0.01$) level.

Table (4): Mean percentage of sperm abnormalities in control and mice treated with different doses of DDVP.

Doses	No. of sperm examined	No. of abnormal sperm	Mean% ± S.E.	Types of sperm head abnormalities :					
				amor-phous	without hock	Trian-gular	Banana shape	Small head	Big head
I. Mitomycin C	2970	543	18.3 ± 1.10	111	109	120	96	58	49
II. Control	2520	113	4.5 ± 0.15	35	40	21	6	6	5
III. DDVP									
2 mg kg^{-1} body wt.	2742	168	6.1 ± 0.53	60	48	28	8	16	8
9 mg kg^{-1} body wt.	2773	259	9.3 ± 0.67*	75	69	64	15	26	10
18 mg kg^{-1} body wt.	2747	296	10.8 ± 0.85*	95	68	78	12	17	26

* Significant at ($P < 0.01$) level.

إحداث شذوذ كروموسومى فى الخلايا التناسلية للفأر بواسطة المبيدين الحشريين الدورسبان والدايكلورفوس وشذوذ رؤوس الحيوانات المنوية فى الفئران المعاملة

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يهدف البحث إلى دراسة قدرة كل من المبيدين الحشريين الدورسبان والدايكلورفوس على إحداث الشذوذ الكروموسومى فى الخلايا التناسلية فى ذكور الفئران . تم الحقن خلال الغشاء البريتونى بالجرعات ٢ ، ٤ ، ١٠ ملليجرام دورسبان / كجم من وزن الجسم (مذاب فى ٠,١ مللتر دايميثيل سلفوكسيد) ، والجرعات ٢,٨ ، ٣,٥ ، ٧ ملليجرام دايكلورفوس / كجم من وزن الجسم ثم أخذت العينات بعد ٦ ، ٢٤ ، ٤٨ ساعة من الحقن .

زادت النسبة المئوية للشذوذ الكروموسومى بزيادة تركيز كل من المبيدين وكان أعلى تركيز للمبيدين بعد ٢٤ ساعة من الحقن وبلغت النسبة ١٩,٣ ± ١,٦١ ، ١٦,٥ ± ٠,٦٣ (وكانت ذات قيمة إحصائية معنوية عالية) بعد حقن الفئران بأعلى تركيز لكل من الدورسبان والدايكلورفوس على التوالى بالمقارنة بالنسبة ٢٥,٥ ± ٠,٩٧ بعد ٢٤ ساعة من الحقن خلال الغشاء البريتونى بالمادة الضابطة الموجبة الميتوميسين (ج) . أما أنواع الشذوذ الكروموسومى فكانت الكروموسومات المنفصلة والسلاسل والكسور والخلل فى عدد الكروموسومات .

أحدث كل من الدورسبان والدايكلورفوس شذوذ فى رؤوس الحيوانات المنوية زادت نسبته بزيادة تركيز كل من المبيدين وكانت أعلى نسبة ١٥,٤٨ ± ٠,٧٣ ، ١٠,٨ ± ٠,٨٩ بعد المعاملة بالدورسبان والدايكلورفوس على التوالى وهى ذات قيمة إحصائية معنوية عالية بالمقارنة بالنسبة ١٨,٣ ± ١,١ بعد المعاملة بالمادة الضابطة الموجبة الميتوميسين (ج) .