Mutagenicity Assay of an Organophosphate Pesticide, Monocrotophos in Mammalian *in vivo* Test System

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Amongst the environmental chemical pollutants with possible health hazards to man, no doubt the pesticides play an important role, since residues of these chemicals may remain in food and being ingested by humans. The great number of pesticides and their different formulations used today make it difficult to select those that are genetically active i.e. induce mutations or recombinations. Mutagenicity testing has assumed increasing importance in the context of society's responsibility not only to conserve the genetic material as a whole but also to protect our own hereditary material from an artificial increase in the frequency of detrimental mutations.

Genotoxicity of a number of organophosphate pesticides has been studied in plants and animals (Epstein and Legator 1971, Ahmed and Grant 1972, Shirasu *et al.* 1976, Nehez *et al.* 1982, Wild 1975, Bhunya and Behera 1984, Hanna and Dyer 1975, Amer and Fahmy 1982, Behera and Bhunya 1987). Monocrotophos is one of the commonly used organophosphorus insecticides having acaricidal property too. Recently monocrotophos has been reported to be mutagenic in peanuts (Jaganmohan *et al.* 1985). As far as the knowledge of the present authors goes, mutagenicity of monocrotophos has not been studied in mammalian system. The present communication provides the information regarding the cytogenetic effects of monocrotophos (Nuvacron) on the bone marrow cells and sperm shape of mice.

Materials and methods

Test Animal: Male and female individuals of inbred mice (Swiss strain) of age group 10-12 weeks were used in the present investigation.

Test Chemical: Technical form of monocrotophos (Nuvacron), O, O-dimethyl-O (2 methyl carbanmoyl-1-methyl vinyl) phosphate was donated by Hindustan Ciba-Giegy India Limited. The structure of this compound is as follows. The solution of the chemical was prepared by glass double distilled water. Equal volume of g.d.d. water served as control.

Structure of monocrotophos

Dose: Three different doses like 5, 2.5 and 1.25 mg/kg b.w. have been used.

Bone Marrow Chromosome Assay

For this assay all the three doses were used through i.p. and the highest dose (5 mg/kg)

through p.o. and sc also. The time of exposures were 6, 24 and 48 hrs for acute dosing and 120 hrs for chronic dosing. In chronic series the highest dose was fractionated into 5 equal parts and each part was administered repeatedly for 5 times at the interval of 24 hrs in between and animals were sacrificed after 24 hrs following the last dose. Cytological slides were prepared following usual colchicine—sodium citrate—air drying—Giemsa schedule.

Micronucleus Assay (MN)

All the doses of the chemical were administered i.p. Each individual received the dose twice with a time gap of 24 hrs in between and was sacrificed after 6 hrs following the second dosing. Slides were prepared from the bone marrow cells according to Schmid (1976) modified by Das and Kar (1980). Assessment for the incidence of MN was done separately for Polychromatic erythrocytes (PCEs), Normochromatic erythrocytes (NCEs) and nucleated cells and per animal 1000 cells of each type were examined.

Sperm Shape Abnormality Assay (SSA)

Each individual was injected i.p. subcutely 5 times for all three doses, with an interval of 24 hrs in between and specimens were sacrificed after 35 days following the 1st injection. The caudae epididymides were dissected out and placed into a petridish containing 0.9% saline. After dicing with a scissors, the suspension was gently pipetted five to six times. Slides were prepared from this suspension by spreading a drop over the slide with the edge of another slide and stained in 1% eosin Y (BDH). Per animal 500 sperms were scored; the sperm abnormalities were categorised, wherever possible, according to Wytobek and Bruce (1975).

Bone marrow chromosome aberration, MN and SSA were scored from slides under code. The proportion test (Z value) was used for all treated results to see whether the treated values deviated significantly from control or not.

Results

In the treatment group different general effects like corrosiveness, chromatin stretching and centromeric separation were observed in the bone marrow chromosomes. Aberration like chromatid gaps and breaks, isochromatid gaps, rings and fragments have also been recorded (Fig. 1). Quantitatively highest dose induced highest percentage of aberration (5.66%) after 24 hrs exposure. Regarding route sensitivity chemical has been found to be most effective when treated through i.p. The fractionated dose when treated repeatedly (chronic) produced less effect than the equivalent acute dose. All the quantitative data of bone marrow chromosome aberration have been presented in Table 1.

Table 2 shows the frequency of MN in the bone marrow cells. There were no significant differences from control for any of the treatment groups. The shape of the micronuclei were either dot or ring shaped (Fig. 2). The size of the micronuclei in different cell types ranges from 1/10th to 1/5th of the cell size. Quantitatively frequency of MN was highest in PCEs and lowest in nucleated cells.

Frequency of SSA in all treated groups has been summarized in Table 3. Various types of sperm head abnormalities like amorphous, banana type, beak shaped acrosomal end, forked acrosomal end and some with double tailed type were noticed (Fig. 3). Quantitatively the highest frequency of sperm abnormalities was induced by the highest dose and least by the lowest dose. In this assay all the treated values significantly differed from the control.

Table 1. Frequency distribution of chromosome aberrations induced by monocrotophos by different doses and by different routes

Route	Dose mg/kg	Hrs of exposure	No. of cells studied	Chromatid		Isoch- ronatid gaps	Fragment		Stretch- ing	Poly- ploidy	Total	% of aberration	Z value
				Gaps	Breaks		Single	Double					
i.p.	5.00	6	300	3	2	—	—	—	—	—	5	1.66±0.72	1.53
"	5.00	24	300	3	8	1	4	1	—	—	17	5.66±0.54	5.60
"	5.00	48	300	5	4	1	1	1	—	—	13	4.33±0.27	4.48
"	2.50	24	300	1	1	—	4	—	—	2	8	2.66±0.54	2.79
"	1.25	24	300	1	1	—	2	—	—	1	5	1.66±0.27	1.53
"	Chronic (1 mg×5)	120	300	2	3	—	1	1	—	2	11	3.66±0.98	3.85
"	Control		1000	5	2	—	—	—	—	—	7	0.70±0.26	—
sc	5.00	24	300	2	2	—	3	2	—	1	10	3.33±1.51	3.04
"	Control		600	4	—	—	—	—	—	—	4	0.66±0.33	—
p.o.	5.00	24	300	2	—	—	1	—	1	1	5	1.66±0.54	1.42
"	Control		600	3	1	—	—	—	—	—	4	0.66±0.33	—

Value is statistically significant when $Z \geq 1.96$.

i.p. = Intraperitoneal, sc = Subcutaneous, p.o. = Oral.

Discussion

It is evident from the present results that monocrotophos is a mutagen in the present test system. Significant variations of the treated results from controls have been recorded for bone marrow chromosome aberrations and SSA but not for MN. However, some of the MN results are higher than the controls (Table 2). Decrease of chromosome aberration frequency in late hours and with lower doses, was probably due to the non-availability of critical concentration of the chemical at the target molecules (DNA and protein) and due to elimination of the metabolites from the body with time. Out of the 3 routes of administration the

Figs. 1-3. 1. Chromosomal aberration. (a), (b), chromatid gap and fragment; (c), (d), chromatid break; (e), double fragment of unknown origin; (f), ring chromosome. 2. Photomicrographs showing micronucleated erythrocytes and nucleated cells. (a)-(d), PCEs with MN; (e)-(f), NCEs with MN; (g)-(h), nucleated cells with MN. 3. Photomicrographs showing sperm shape abnormalities. (a), double tailed sperm; (b), forked acrosomal end; (c), beak shaped acrosomal end; (d), banana shaped head; (e), amorphous type head.

compound is cytogenetically most active when treated through i.p. and least through oral (Fig. 4). Production of least effect through oral may be due to the least absorption in the oral route for particular pharmacodynamic properties of the compound. The mechanism of action of monocrotophos at nucleic acid level is not properly known. However, organophosphorus pesticides have alkylating properties (Wild 1975). So DNA alkylation might be

Table 2. Incidence of micronucleated bone marrow cells induced by monocrotophos

Dose mg/kg	No. of animals	% of polychromatic erythrocytes (PCEs) with MN $\pm$ SE (A)	% of normochromatic erythrocytes (NCEs) with MN $\pm$ SE (B)	% of PCEs + NCEs with MN $\pm$ SE (A+B)	% of nucleated cells with MN $\pm$ SE (C)	Total % of MN (A+B+C)
5.00	3	0.40 $\pm$ 0.04	0.16 $\pm$ 0.02	0.28 $\pm$ 0.01	0.13 $\pm$ 0.03	0.23 $\pm$ 0.00
2.50	3	0.16 $\pm$ 0.05	0.10 $\pm$ 0.05	0.13 $\pm$ 0.05	0.13 $\pm$ 0.07	0.13 $\pm$ 0.07
1.25	3	0.10 $\pm$ 0.05	0.06 $\pm$ 0.03	0.08 $\pm$ 0.01	0.03 $\pm$ 0.03	0.06 $\pm$ 0.00
Control	3	0.20 $\pm$ 0.05	0.10 $\pm$ 0.05	0.15 $\pm$ 0.05	0.06 $\pm$ 0.03	0.12 $\pm$ 0.01

All the values are insignificant statistically.

Table 3. Monocrotophos induced sperm shape abnormality in mice

Dose mg/kg	No. of animals	Total no. of sperms studied	Total no. of abnormal sperms	% of abnormal sperms $\pm$ SE	Z value
5.00	3	1500	129	8.60 $\pm$ 2.61	10.25
2.50	3	1500	98	6.53 $\pm$ 1.10	7.62
1.25	3	1500	77	5.13 $\pm$ 1.01	5.60
Control	3	3000	62	2.06 $\pm$ 0.66	—

Value is statistically significant when $Z \geq 1.96$.

Fig. 4. Monocrotophos induced route dependent bone marrow chromosome aberration.

Fig. 5. Monocrotophos induced bone marrow chromosome aberration (□—□), micronucleus (Δ—Δ) and sperm shape abnormality (O—O).

one of the reasons for the production of chromosomal aberrations and so also for MN formation. Effects like stretching and corrosive action of chromosomes indicate that the pesticide may have acted upon the protein moiety of the chromosome. Occurrence of chromatid type aberrations indicates that the chemical acted upon the stages following G₁ phase of cell cycle.

Sperm abnormality is due to changes in the genetic components controlling the process of spermatogenesis (Wyrobek and Bruce 1975, 1978, Krzanowska 1976a, b, Hugenholtz and Bruce 1979, and Kar and Das 1983). This view is contemplated by differential spontaneous incidences of sperm abnormalities in different strains of mice (Bruce *et al.* 1974, Krzanowska 1976a, Buda and Krzanowska 1978). So the production of higher percentage of abnormalities by monocrotophos may be a measure of the genetic damage caused in the germline cells.

Out of the three assays the relative sensitivity has been found to be: sperm shape abnormalities > chromosomal aberration > micronucleus (Fig. 5).

Since monocrotophos has been found to be genotoxic in the present test system, its restricted use and cautious handling are suggested.

Summary

In the present study an endeavour has been made to assay the mutagenicity of an organophosphorus pesticide, monocrotophos (Nuvacron) in mouse cytogenetic test system. Cytogenetic assays like somatic chromosome aberration, micronucleus test and sperm shape abnormalities have been done. Different doses (5, 2.5 and 1.25 mg/kg), different exposure times (6, 24, 48 and 120 hours) and different routes (i.p., p.o. and sc) of administration have been employed. The effects are mostly dose, time and route dependent. Comparison of acute and chronic treatments revealed that the chemical has no cumulative effect. Relative sensitivity of cytogenetic assays has been found to be as sperm shape abnormality > chromosome aberration > micronucleus. Monocrotophos has been found to be mutagenic in the present test system.

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