Bull. Environm. Contam. Toxicol. 20,826-833 (1978)

Endosulfan: Lack of Cytogenetic Effects in Male Rats

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Potential genetic damage due to pesticides and other chemical agents is well recognized. Studies of LEGATOR et al., (1969), PALMER et al., (1972), EPSTEIN et al., (1972) and also the report of the Advisory Panel (1969) revealed that certain chemicals do cause chromosome breakage and possess mutagenic properties. There is no report on the cytogenetic effects of several pesticides including endosulfan. Cytogenetic study of pesticides is very important since pesticides are being used extensively in agriculture, food storage and public health programms. Further, the Health, Education and Welfare Committee has recommended that pesticides, drugs and food additives should undergo mutagenicity tests in mammalian systems before their actual use or registration (Advisory Panel on Mutagenicity of Pesticides, 1969).

Cytogenetic assay of somatic cell line is the major recommended method for evaluating chemical mutagenesis in mammalian systems (ROHRBORN, 1971; EPSTEIN and LEGATOR, 1971; MALLING, 1972; DURHAM and WILLIAMS, 1972). The important addition if not an alternative to the dominant lethal test in the mutagenic studies are the in vivo cytogenetic assays (LEGATOR et al., 1969; KATO et al., 1969; SCHLEIERMACHER et al., 1967). Therefore, in continuation of our earlier studies with other pesticides (DIKSHITH, 1973; DIKSHITH and DATTA, 1973) the present paper reports the effects of endosulfan on somatic and germinal cells of male rats.

MATERIALS AND METHODS

Expermental Procedure

Animals: Male albino rats weighing about 160 to 175 g were divided into five groups, with 8 animals in each group. All the animals were given pellet diet[1] and water ad libitum and housed in the air conditioned rooms of the animal house.

[1] Hind liver Laboratory Animals Feeds, India.

0007-4861/78/0020-0826 $01.60

Chemical and Treatment:

Endosulfan, obtained from National Chemical Laboratory, Poona, India, was used throughout the studies. Each rat was treated with endosulfan suspended in peanut oil daily for a period of five days by oral intubation. Thus, rats of group 1, 2, 3 and 4 were administered 11.0, 22.0, 36.60 and 55.0 mg/Kg of endosulfan respectively and the rats of group 5 (control) were given peanut oil alone.

Chromosome analysis:

Colchicine was administered to each rat (4 mg/Kg) by i.p. injection 4 hrs before killing by decapitation. Bone marrow from femur bones and seminiferous tubules were quickly collected in separate tubes in HANK's balanced salt solution (HBBS,pH 7, 2). Cell suspensions were made by careful maceration and subjected to centrifugation (800 rpm). The cells were washed in HBSS and exposed to hypotonic treatment for 10 minutes at 37°C. After recentrifugation, the cell pellet was fixed in methanol acetic acid (3:1) mixture. Air dried and flame fixed cells were stained with Giemsa.

The slides were numbered at random. Scoring was made to determine mitotic index and chromosome damage in bone marrow and spermatogonial cells. The mitotic index was calculated as % cells at metaphase after scoring a total of 100 cells per slide. Fifty metaphases were analysed from each treatment. Selection of metaphase plates for analyses was carried out with low power objective (X10). Chromosome damage was analysed for chromatid breaks, chromosome breaks, exchange figures, under x 1000 mangification. Cells having wide spread chromatids with minimum overlapping were selected for scoring.

RESULTS

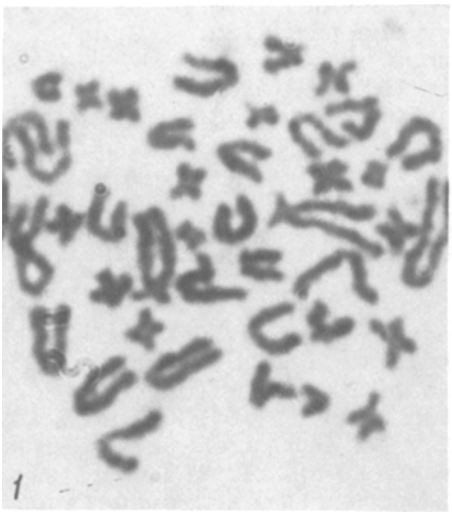
Mortality

Animals died in group 3 and 4 before 24 hrs; two animals died in group 2 after 72 hrs. There were no deaths in groups 1 and 5. The mortality of animals indicates that the dosage of 22.0, 36.60 and 55.0 mg/Kg/day are very toxic to rats.

Chromosome damage and Mitotic index

There were no major chromosomal aberrations either in bone marrow cells or in speramtogonial cells. Chromatid breaks were the only type of aberrations with 1 to 2 exchange figures. These were present in bone marrow cells but not in speramatogonial cells. There was no chromosomal deletion nor formation of large number of fragments (Fig. 1 & 2).

In comparison to control there was no significant mitotic inhibition in any of the treated groups.



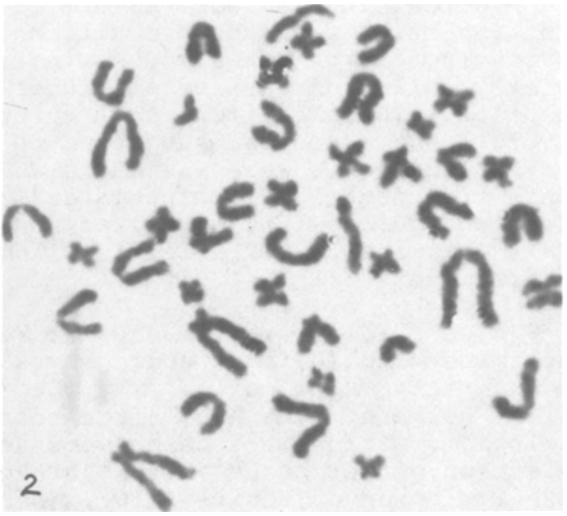


Fig. 1: Metaphase chromosome from bone marrow cells of male rat after daily oral feeding of endosulfan (11.0 mg/Kg) for five days. Ca. x 2000.

Fig. 2: Metaphase chromosome from bone marrow cells of male rats. (control). Ca. x 2000.

DISCUSSION

Assay of chromosome damage in bone marrow cells of mammals *in vivo* has been considered as a standard method for testing the potential mutagenic effects of various toxicants (SCHLEIERMACHER *et al.*, 1967; KATO *et al.*, 1969; LEGATOR *et al.*, (1969). Reports are available on the *in vivo* cytogenicity tests using bone marrow cells of mice (DATTA and SCHLEIERMACHER, 1969; ADLER *et al.*, 1970). We have selected rats as test animal keeping in view certain advantages they have over the mouse. The chromosomes of rat are structurally well differentiated and the

chromosome number (2n=42) nearly approximates that of humans. Further, rats are used successfully in the cytogenetic analysi of bone marrow cells by various workers (CHAUDHURI and LANGENDORFE, 1968; LEGATOR et al., 1969; PETERSEN, 1969), and the use of rat as a test species has been advocated for routine mutagenicity testing of chemical compounds (ROHRBORN, 1971).

The in vivo cytogenetic study designed to elucidate a possible damaging effect of endosulfan has not produced any significant effect on chromosomes. Endosulfan produced little or no effect on somatic as well as on germinal cells. Earlier studies also indicated a similar situation (DIKSHITH, 1975, 1976). Endosulfan produced only few chromatid type aberrations. Reports indicate that dieldrin, another organo-chlorinated insecticide, did not produce any chromosomal aberrations in the bone marrow cells of hamsters in vivo (DEAN et al., 1975) and dominant lethal mutations in male mice (EPSTEIN et al., 1972). Similarly no chromosomal damages were observed in PCB treated rats in vivo (GREEN et al., 1975; DIKSHITH et al., 1975).

Studies with the rat Kangaroo cell line and the chinese hamster cell lines in vitro, however, have shown heavy chromosomal damage due to captan (LEGATOR et al., 1969) and due to p-p'DDT (PALMER et al., 1972; KELLY-GARVERT and LEGATOR, 1973). It is well known that many chemicals that cause chromosome damage in plants and in cell lines in vitro do not induce similar changes in animals. Structural changes and increase of aneuploid cells found in vitro could not be confirmed in vivo (VAN WENT DE VRIED and KRAGTEN, 1975). Similarly no chromosomal aberrations were found in chinese hamsters in vivo due to cyclamates (VAN WENT DE VRIES and KRAGTEN, 1975). In contrast human lymphocyte cultures and monolayers and chinese hamster cells lines showed chromosomal damage due to cyclamates (STOLTZ et al., 1969; DIXON, 1973; KRISTOFFERSSON, 1971).

Two factors seem to be responsible for this difference between in vitro and in vivo. Firstly the role of a latent period between exposure and expressing effects that operates in vivo; secondly thepopulations of cells in mitosis are too small in vivo (VAN WENT DE VRIES and KRAGTEN, 1975). This study therefore, recalls for reconsideration of the practical use and exposure situation and their importance before giving

a meaningful explanation to the toxicological evaluation of pesticides and other chemicals. It has been reported that chromosomal damage is greater during the peak and heavy spray season like summers and the chromatid lesions were seen parimarily among workers who handled herbicides (YODER et al., 1973).

It has been shown that frequency of mutation in an organism increases with the severity of chromosomal aberrations (KAO and PUCK, 1969). DDT as an example neither produced any chromosomal aberration nor increased the rate of mutation in contrast to its metabolite, DDE (KELLY-GARVERT and LEGATOR, 1969). Reports have indicated that endosulfan, in comparison to DDT is less persistent in fat or lipoids of warm blooded animals. It has been also shown that endosulfan is rapidly excreted in feces and urine (MAIER-BODE, 1968; GORBACH et al., 1971; DIKSHITH et al., (1971). The quick elimination of the insecticide from the body of rat seems to be responsible for the lack of effect on chromosomes.

It is well known that chromosomal changes have different consequences depending upon the type of cell and the degree of aberration (KALTER, 1971; MILLER and MILLER, 1971). The present results are, however, not comparable with the "above reports" since endosulfan has not produced any significant chromosome changes either in somatic or germinal cell lines at the concentrations tested. This study is in agreement with the observations of KENNEDY et al.,(1975)and VAN WENT DE VRIES and KRAGTEN (1975) in that it is more accurate to extrapolate cytogenetic damage from animals to man from in vivo studies rather than from lower organisms or from in vitro experiments alone. As has been recommended by WHO, more weight should be attached to the results gathered from mammals than from microbial or non mammalian or isolated cell systems in tests of mutagenic effects of pesticides and different chemicals (WHO Report, 1974).

ACKNOWLEDGEMENT

Authors are grateful to Dr. S.H. Zaidi, Director, and Dr. C.R. Krishna Murti for their keen interest and encouragement. Thanks are due to Mr. M. Ahmed for photomicrography

SUMMARY

Cytogenetic effects of endosulfan (Thiodan^{R}) a member of the chlorinated hydrocarbon insedticide was tested in male albino rats. The insecticide was administered orally to rats at 0, 11.00, 22.00, 36.60 and 55.00 mg/Kg daily for 5 days. The highest doses were associated with clinical signs of insecticide poisoning and death. Cytogenetic analysis of bone marrow cells and spermatogenial cells did not reveal any significant effect of the insecticides on chromosomes. The ratio of mitotic index and frequency of chromatid break in the two cell types had no correlation with the doses tested and was not very different from those of the control group.

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