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LEAD ACETATE AND LEAD NITRATE INDUCED SEX-LINKED RECESSIVE LETHAL MUTATIONS IN MATURE SPERM OF *Drosophila melanogaster*

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ABSTRACT

The mutagenic effects of two lead salts were investigated: acetate and nitrate on mature sperm *of Drosophila melanogaster*. Oregon R adult males were injected intraperitoneally with different concentrations of the salts: 650, 750 and 850 ppm for lead acetate and 25, 50 and 75 ppm for lead nitrate. Treated males were mated with virgin BASC females and the F_2 scored for the induction of sex-linked recessive lethal mutations. The higher doses of both salts were found to induce a significant number of these mutations at 5% level on the Kastenbaum-Bowman test (1970), lead acetate being less toxic and less mutagenic than lead nitrate.

RESUMEN

Se investigaron los efectos mutagénicos de dos sales de plomo: acetato y nitrato en espermatozoides maduros de *Drosophila melanogaster*. Machos adultos Oregon R fueron inyectados intraperitonealmente con diferentes concentraciones de las sales: 650, 750 y 850 ppm de acetato de plomo y 25, 50 y 75 ppm de nitrato de plomo. Los machos tratados fueron cruzados con hembras virgenes BASC y la F_2 fue examinada para valorar la inducción de mutaciones letales recesivas ligadas al sexo. Ambas sales produjeron estas mutaciones en un nivel de 5% en la prueba de Kastenbaum-Bowman (1970), siendo el acetato de plomo menos tóxico y menos mutagénico que el nitrato de plomo.

INTRODUCTION

Drosophila melanogaster has been used for mutagenic studies ever since H. J. Müller developed the sexlinked recessive lethal test (SLRL) to detect the mutagenic effects of X rays (Muller 1927). He used the C1B chromosome which was later replaced by the Muller-5 or BASC chromosome. Using *Drosophila,* Auerbach and Robson (1946) were the first to discover the mutagenic effects of mustard gas. Since then a great number of chemicals have been tested for mutagenic activity in *Drosophila* (Lee *et al.* 1983).

Among the heavy metals, lead, has been shown to be widely distributed in the atmosphere, water, soils and foods (Beliles 1975). At the cellular level, lead inhibits the activity of enzymes that are dependant on the presence of free sulphydryl groups (SH). The clearest manifestation of these effects is the disturbance on the biosynthesis of heme, which in humans is accompanied by abnormalities in porphyrin metabolism (Valle and Ulmer 1972).

Lead acetate is used as a topical astringent and is found to be a renal carcinogen in rats (Bolyland *et al.* 1962, Van Esch *et al.* 1962, Roe *et al.* 1965, Mao and Molnar 1967, Choie and Richter 1974, Furst *et al.* 1976). In the Syrian hamster lead induces neoplastic changes in the bronchio-alveolar area (Kobayashi and Okamoto 1974, ICPEMC 1984). It also produces infertility in mice (Varma *et al.* 1974) and reduces the reproductive ability of rats (Stowe and Goyer 1971, Hackett *et al.* 1982, Hess and Sikov 1982).

Lead nitrate is employed in the manufacture of matches and explosives, as a mordant in dyeing of textiles, as well as a sensitizer in photography. It is embryotoxic to rats (McClain and Becker 1975) but not teratogenic (McClain and Siekier 1975). In *Drosophila melanogaster* lead induces enzymatic alterations in esterase and triose phosphate isomerase (Lower *et al.* 1976) and affects non disjunction (Ramel 1973).

Information about the mutagenic effects of lead salts in humans who are occupationally exposed to them and information obtained from in vitro studies are contradictory (Maki-Paakkanen *et al.* 1981).

We report the mutagenic effects of two lead salts, namely, acetate and nitrate on the induction of SLRL in mature sperm of *Drosophila melanogaster*.

MATERIALS AND METHODS

Lead acetate [$(CH_3\ COO)_2\ Pb \cdot 3H_2O$] (Baker) was employed at concentrations of 650, 750 and 850 ppm. Lead nitrate [$Pb(NO_3)_2$] (Baker) was used at concentrations of 25, 50 and 75 ppm. The highest concentration of both salts is their LD_{50}. The salts were dissolved in distilled water.

Oregon R adult males were injected intraperitoneally (Felix and Rodriguez-Arnaiz 1968) with almost 0.2 μl of the salts solutions (Sega and Lee 1970) and after treatment the males were mated with BASC virgin females in a proportion of one male to two females, for three days. F_1 females fertilized by F_1 males were put into fresh medium, one per vial, and the F_2 scored for the induction of SLRL mutations by means of the absence of wild males. Only mature sperm were sampled (Vogel 1975). Controls were injected with 0.2 μl of 5% sucrose solution (Vogel and Sobels 1976). The Kastenbaum-Bowman test (1970) was employed in order to calculate the statistical significance of the results.

RESULTS AND DISCUSSION

Chemical agents which induce genetic damage constitute a risk for all populations, including human beings. The sex-linked recessive lethal test is capable of detecting chemicals which are capable of inducing heritable lethal mutations which may range from changes in only one base of the DNA, that is a point mutation, to small

deletions or chromosome aberrations. The SLRL test simultaneously examines 600 to 800 genes, which represents about 80% of the loci of the X-chromosome or roughly one fifth of the *Drosophila* genome (Lee *et al.* 1983).

Both lead salts induced SLRL (Table I), lead acetate was less toxic and less mutagenic than lead nitrate.

TABLE I. INDUCTION OF SEX-LINKED RECESSIVE LETHALS BY LEAD ACETATE AND LEAD NITRATE ON MATURE SPERM OF *Drosophila melanogaster*

Concentration (ppm)	*Number of tested chromosomes*	*Number of induced lethals*	*Percentage of lethals*
Control	1 178	7	0.59
Lead acetate			
650	1 285	11	0.86
750	1 061	10	0.94
850	1 327	28	2.11 *
Lead nitrate			
25	1 205	15	1.24
50	1 326	21	1.58 *
75	1 584	34	2.15 *

* Significant at 5% level on Kastenbaum-Bowman test (1970).

In the *Tradescantia* micronucleus test, the mutagenic effect of lead nitrate was found to be significant while lead acetate gave a borderline response (Ma *et al.* 1984).

In cattle, lead does not produce severe chromosome abnormalities (Leonard *et al.* 1974). In monkeys with a low calcium diet, lead acetate induces dicentrics, translocations and exchanges, while in groups receiving lead, irrespecive of the diet, lead acetate produce gaps and fragments (Deknudt *et al.* 1977). In humans, reports about the induction of chromosomal aberrations in lead exposed individuals are contradictory. Some have reported positive results (Garza-Chapa *et al.* 1977, Hogstead *et al.* 1979, Forni *et al.* 1980) while others have not found any increase in rates of chromosome aberrations (Schmid and Bauchinger 1973, O'Riordan and Evans 1974, Bijlsma and De France 1976, Horvat and Prpić-Majić 1977, Maki-Paakkanen *et al.* 1981. Qazi *et al.* (1980) observed a temporary increase in chromosome breakage in an infant exposed to lead while in utero. In some experiments, human lymphocytes exposed to lead salts in vitro, have been reported as unaffected (Deknudt and Deminatti 1978, Bauchinger and Gasiorek 1980), although Beek and Oboe (1974) obtained increased frequencies of chromosome aberrations.

In *Drosophila* we have found a statistical significant increase in the frequency of SLRL, mutations. However other experiments are needed to show the mode of action of these mutagens (Valencia *et al.* 1984).

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