

CHROMOSOMAL ABERRATION IN THE OFFSPRING OF RATS TREATED WITH MALATHION

Ferah SAYIM N. Ülkü KARABAY

Necip TOSUN

Department of Biology
Ege University, Faculty of Science
35100 Bornova- İzmir/TURKEY

Department of Plant Protection
Ege University, Faculty of Agriculture
35100 Bornova-İzmir/TURKEY

ABSTRACT : Malathion is an organophosphate insecticide, one of a class of pesticides that are highly toxic to vertebrates. Mammalian *in vivo* and *in vitro* studies of malathion show a pattern of induction of chromosome damage. In this study, offspring aged 8 weeks from female Wistar albino rats treated chronically with malathion were examined for chromosomal aberrations. The commercial grade of malathion was applied at two doses, 500 and 1000 µl/l. The rats of the control group was fed with a normal diet in pelleted form. Both male and female rats of treated groups had been fed with laboratory chow combined malathion during one month as 6 consecutive day per week before mating. After mating, all females were treated with malathion during pregnancy and until the end of the breast-feeding period. At the end of the treatment, all offspring were killed by cervical dislocation and the organs such as liver, kidney, brain, heart, and lung were excised, cleaned and weighed. The genotoxic effects of malathion were evaluated by the rat bone marrow cytogenetic assay. It was showed that the liver/body weight ratio increased as a result of malathion exposure. In offspring of rats treated with malathion; chromosomal aberrations such as stickiness of chromosome, chromatide separation, aneuploidy, dumbbell-shaped polyploid nuclei, lobbed nuclei, and degenerated nuclei were observed. In conclusion, it is suggested that malathion has some genotoxic effects in rats. Besides, it was observed that frequency of chromosomal aberration increased as dose-dependent.

Keywords: Organophosphate insecticide, malathion, genotoxic effect, chromosomal aberration, rats.

MALATHION İLE BESLENMİŞ SIÇANLARIN DÖLLERİNDEKİ KROMOZOMAL ANOMALİLER

ÖZ: Malathion, omurgalılar için oldukça toksik bir pestisit sınıfı olan organik fosforlu insektisitlerdendir. Memelilerle yapılan *in vivo* ve *in vitro* çalışmalar malathionun kromozomal sapmaları indüklediğini göstermiştir. Bu çalışmada, kronik olarak malathiona maruz bırakılmış dişi Wistar albino siçanların 8 haftalık yavruları, kromozomal sapmalar bakımından incelendi. Malathion preparatı; 500 ve 1000 µl/l'lik iki ayrı dozda uygulandı. Kontrol grubu siçanları pelet formundaki normal besinle beslendi. Uygulama gruplarının dişi ve erkek siçanları eşleştirilmeden bir ay önce, haftada 6 ardışık gün boyunca malathionla kombine edilmiş laboratuvar yemi ile beslendi. Eşleşmeden sonra tüm dişilere hamilelik süresinde ve sütle besleme döneminin sonuna kadar malathion uygulandı. Uygulama sonunda, tüm yavrular servikal dislokasyonla öldürüldü ve karaciğer, böbrek, beyin, kalp ve akciğer gibi organlar çıkarılarak, temizlendi ve tartıldı. Malathionun genotoksik etkileri, siçan kemik iliği sitogenetik yöntemiyle değerlendirildi. Malathion ile muamelenin bir sonucu olarak, karaciğer/vücut ağırlığı oranının artmış olduğu görüldü. Malathion uygulanan siçanların yavrularında; kromozom yapışması, kromatid ayrışması,

aneuploidi, halter şekilli poliploid nukleus, loblu nukleus ve dejenere nukleus gibi kromozomal sapmalar gözlemlendi. Sonuç olarak, malathionun sıçanlar üzerinde bazı genotoksik etkilere sahip olduğu saptandı. Bunun yanında, kromozomal sapmaların frekansının doza bağlı olarak arttığı belirlendi.

Anahtar Sözcükler: Organik fosforlu insektisit, malathion, genotoksik etki, kromozomal sapma, sıçan.

INTRODUCTION

Malathion is one of the most widely used organophosphate insecticides and one of a class of pesticides that are highly toxic to vertebrates and are chemically related to nerve gases used during World War II (Armstrong, 1990). So, its widespread use makes potential exposure high for nontarget organisms. Contributing to its popularity is malathion's relatively low acute mammalian toxicity. But, like DDT and other pesticides that have been found to cause irreparable damage to human and environmental health, malathion may pose a greater risk than the product label. Malathion has a legacy of serious problems such as mutagenicity, carcinogenicity, negative health effects to humans and animals, damaging to nontarget organisms, and containing highly toxic impurities (Brenner, 1992). Mammalian *in vivo* and *in vitro* studies of malathion show a pattern of induction of chromosome damage, as measured by chromosome aberrations, sister chromatid exchanges, and micronuclei (Flessel *et al.*, 1993).

In this study, malathion was examined for its ability to induce chromosomal aberrations in the bone marrow cells of the Wistar albino rats *in vivo*. The aim of this study was to evaluate the effects of malathion on chromosomes in offspring of rats.

MATERIALS AND METHODS

Animals and treatment

The protocols were approved by the Animal Ethical Committee of Ege University, Faculty of Medicine. Adult male ($n=10$) and female ($n=20$) Wistar albino rats (100-150 g) were obtained from Ege University, Faculty of Medicine, Center for Animal Breeding. All animals received 10 days acclimatization before starting experimental procedure. The males and females were assigned randomly to either the unexposed control group or malathion-exposed groups and housed individually in labeled cages until mating. Animals were housed in cages (19x19x12 cm) with solid plastic sides and stainless-steel grid tops and floors in a room designed for control of temperature (approximately $21\pm 1^\circ\text{C}$), humidity (45-75%) and 12 hour light/ dark cycle. Animals of the control group ($n=15$) were orally fed daily with a normal diet in standard laboratory pellet, while animals of the treatment group ($n=$

15) were fed with laboratory pellet food combined 500 and 1000 µl/l malathion. Both male and female rats had been fed with laboratory pellet food combined malathion during one month as 6 consecutive day per week before mating. Tap water was also available ad libitum. Food and water were changed daily (Francis *et al.*, 1990). Commercially available malathion was used in this study.

Mating

During mating period, one male was housed with 2 females. Females were assigned randomly in pairs to each male for the required mating period. After mating, males and females were housed individually and then all females were treated with malathion during pregnancy and until the end of the breast-feeding period.

Post-mortem examination

At the end of the experiment, 10 offspring from each group which were assigned randomly were sacrificed by cervical dislocation and the organs were excised, cleaned and weighed. The genotoxic effect was evaluated by the bone marrow cytogenetic assay (Macgregor and Valey, 1988).

Chromosome preparation

After 4 hours before being humanely killed, animals were given 1 ml/kg of 1 mg/ml colchicine (Sigma C-9754) by intra-peritoneal injection. At post-mortem, bone marrow was aspirated from the femur into 8 ml 0.075 M KCl and cells were swelled by Pasteur pipette. The cell suspension was incubated at 37 °C for 20 mins. The cells were centrifuged (600 rpm for 6 mins) and the supernatant was removed. After centrifugation, cells were suspended in a 7 ml fixative (3:1 methanol: glacial acetic acid). Tubes with cell suspension were kept at +4 °C overnight. The fixative was changed for 4 times after centrifugation (1200 rpm for 5 mins). Slides were prepared by air-drying method. The slides were stained in 8.5 ml 5% Giemsa (in 1.5 ml ammonium hydroxide and 90 ml distilled water) and mounted in Canada balsam. Five slides were prepared for each animal.

Statistical analysis

Statistical analysis was performed using SPSS 6.0 for windows software. *t*-test was used to determine the presence of significant differences among test groups for the liver/ total body weight ratio (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

At post-mortem examination of offspring's organs, no morphologic abnormalities were observed, but there was an increase in the liver/body weight ratio ($p < 0.05$) of treatment groups when compared with that of controls. However, only the increase in liver/body weight ratio of offspring of 1000 $\mu\text{l/l}$ malathion treated rats appeared significant. When compared with an unexposed control group, no change was determined in kidney, brain, heart, and lung weight of treatment groups. The liver/body ratio, the mean of the body and the liver weights of rats in the control group and the malathion-exposed groups is shown at Table 1. Observed increases in the liver/body weight ratio of offspring may be related to the increase of normal physiological function of liver in the detoxification of malathion. In most cases, liver weights generally increase in poisoning chronically with pesticides (Akay *et al.*, 1990). In laboratory animals, malathion exposure has caused increased liver and kidney weights (Anonymous, 1990) and changes in the liver and blood sugar levels (Gowda *et al.*, 1983, Balasubramanian *et al.*, 1990).

Table 1. Total body, liver weight (g) and liver/total body weight ratio ($\bar{X} \pm \text{SEM}$).

Treatments	Total body	Liver	Liver/body
Control	68.99 ± 1.05	3.22 ± 1.45	0.047
500 ppm	45.04 ± 2.72	2.25 ± 0.31	0.049
1000 ppm	54.75 ± 2.76	3.30 ± 0.12	0.060**

** statistically significant at $p=0.05$ level.

Maternal exposure to malathion (when mothers treated with 500 $\mu\text{l/l}$ malathion) caused chromosomal aberrations such as, stickiness of chromosome (Figure 1b), chromatide separation (Figure 1c), dumbbell-shaped polyploid nuclei (Figure 1d), lobbed nuclei (Figure 1e), and degenerated nuclei (Figure 1f) in the offspring. Moreover, aneuploidy was also determined in this study. In case of aneuploidy, the numbers of chromosomes were $2n=40.44$ (Figure 1g, h), while the control rat's chromosome numbers were $2n=42$ (Figure 1a). In offspring of rats treated with 1000 $\mu\text{l/l}$ malathion, chromosomal aberrations such as, stickiness of chromosome (Figure 2a), aneuploidy ($2n=32.36$) (Figure 2b, c), dumbbell-shaped polyploid nuclei (Figure 2d), lobbed nuclei (Figure 2e), and degenerated nuclei (Figure 2f) were observed. Rarely, normal mitosis stages were also observed. The percentage of normal mitosis and aberrations in offspring of rats treated with 500 and 1000 $\mu\text{l/l}$ malathion were shown in Table 2.

Table 2. The percentage distribution of normal mitosis and chromosomal aberrations.

	Total chromosomal aberration (%)							Percentage of cells with chromosomal aberration
	Normal mitosis	Aneuploidy	Stickiness of chromosome	Chromatide separation	Lobbed nucleus	Dumbbell-shaped polyploid nucleus	Degenerated nuclei	
500 µl/l	32	2	16	9	12	8	21	68
1000 µl/l	10	4	21	-	16	9	40	90

In offspring of rats treated with 500 µl/l malathion, the number of normal mitosis stages was much more than that of offspring of rats treated with 1000 µl/l malathion. It was observed that there were much more degenerated nuclei in offspring of rats treated with 1000 µl/l malathion than offspring of rats treated with 500 µl/l malathion. The number of sticky metaphase, dumbbell-shaped polyploid nuclei and lobbed nuclei were about similar in both doses. Chromatide separation was only detected in 500 µl/l. Aneuploidy was rarely observed and so the percentage of it was the least in both doses (Table 2).

The results obtained from the present study are suggested that malathion has some genotoxic effects like chromosomal aberrations in rats. Cytogenetic assays such as the chromosome aberration assay can be used as a reliable biomarker for cellular damage and adverse health risk (Hagmar *et al.*, 1994; Bonassi *et al.*, 1995). Therefore, this biomarker can be used as a mechanistic link between exposure and disease outcome (William *et al.*, 1999). At the end of the present assay, increases in chromosome aberrations were detected in bone marrow cells of offspring of rats chronically treated with malathion. In mice, malathion (30 mg kg⁻¹ body wt.) has caused chromosomal aberrations 24 hour after intra-peritoneal injection (Amer *et al.*, 1996). Similarly, statistically significant increases of chromosomal aberrations in bone marrow cells of mice have occurred after dermal treatment of malathion (500 and 2000 mg/kg body wt.) (Salvadori *et al.*, 1998). Malathion is mutagenic in human, animal, and bacteria cells (Brenner, 1992). Frequencies of chromosomal aberrations were significantly higher in cotton field workers exposed to malathion and other pesticides (Rupa *et al.*, 1989 a, b). Increased chromosome breaks and aberrations occurred following acute malathion human poisonings (Van Bao, 1974) and in human blood cells exposed to malathion (Herath, 1989; Garry, 1990). Malathion caused sister-chromatide exchanges in human blood cells (Sharma and Sobti, 1987; Herath, 1989) and fetal cells (Nicholas *et al.*, 1979). Malathion also caused mutations in laboratory animals, including mice and hamsters. In some cases malathion induced

genetic damage at doses far below acutely toxic levels. Moreover, malathion was also associated with birth defects in domestic and laboratory animals (Brenner, 1992).

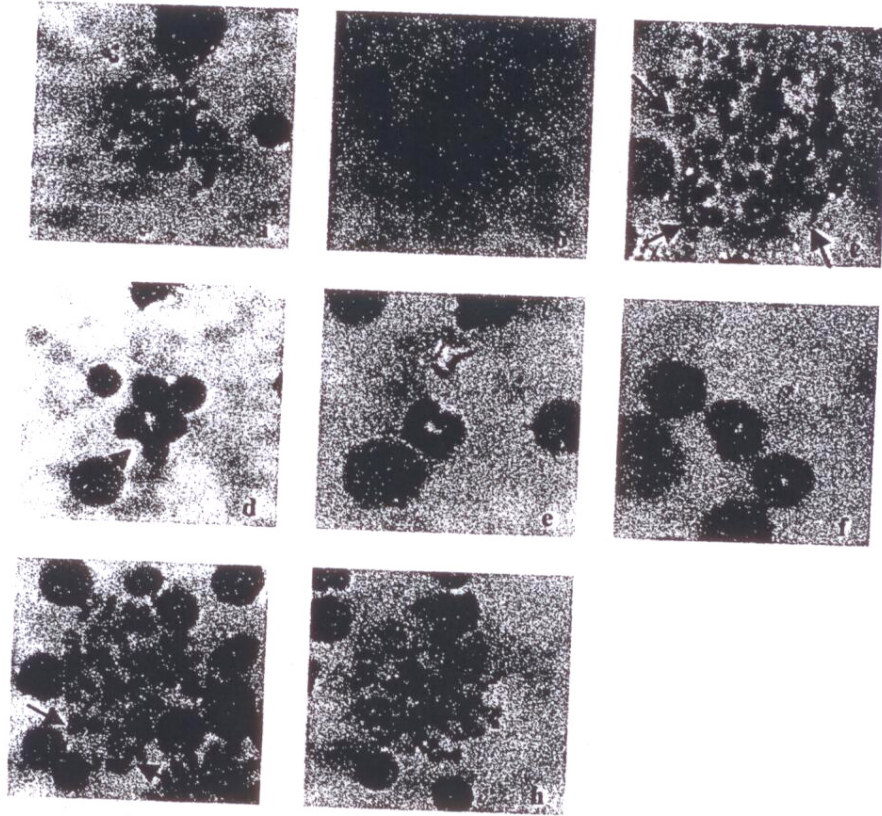


Figure 1. a: Control, chromosomal aberrations. b: Stickiness of chromosome. c: Chromatide separation. d: Dumbbell-shaped polyploid nucleus. e: Lobbed nucleus. f: Degenerated nucleus. g: Aneuploidy ($2n=40$). h: Aneuploidy ($2n=44$).

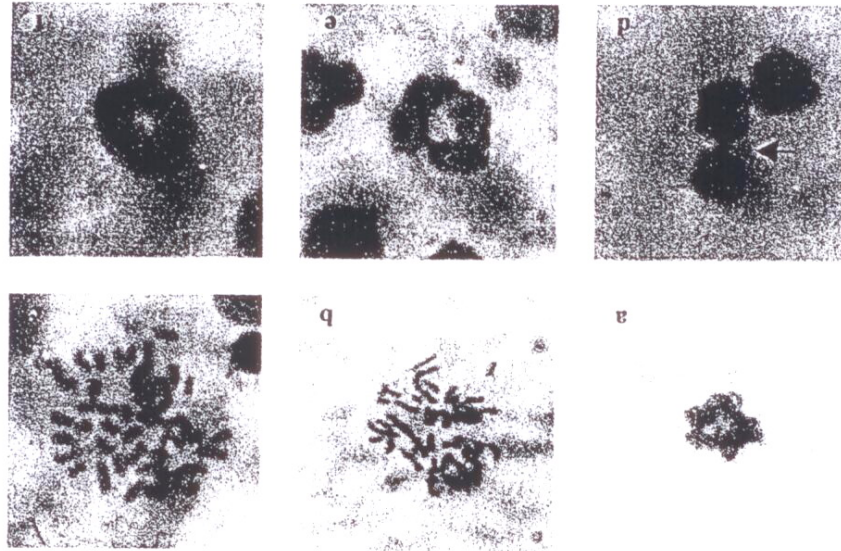


Figure 2. a: Stickiness of chromosome. b: Aneuploidy ($2n=32$). c: Aneuploidy ($2n=36$). d: Dumbbell-shaped polyploid nucleus. e: Lobbed nucleus. f: Degenerated nucleus.

To conclude with, it can be suggested that the results obtained from the present study are indicative of a detrimental influence of malathion upon a mammalian species.

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