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RESEARCH ARTICLE

# Effects of Malathion in Fetal Kidney Tissues in Pregnant Rats: Teratogenic Effects Induced by Different Doses

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## Summary

The aim of this study was to investigate the teratogenic effects of Malathion (ML) induced by different doses on fetal kidney tissues in pregnant rats. A total of 28 Sprague-Dawley pregnant rats were randomly divided into 4 groups of 7 rats each. Depending on ML dose, four groups were formed, including (I) control, (II) ML 2.5 (ML 2.5 mg/kg/day, orally), (III) ML 5 (5 mg/kg/day, orally), and (IV) ML 10 (10 mg/kg/day, orally). ML application started when the male and female were put together (when mating started). Daily ML application was continued until birth. It was determined that in parallel with dose of ML, ML resulted in toxic effects on serum enzymes (acetyl-cholinesterase (AChE), amylase and lipase) and kidney tissues of pregnant rats, and also -regardless of ML dose in fetal kidneys- it led to teratogenic effects in all the doses. Biochemical data wasconfirmed by histopathologic data. We concluded that ML leads to kidney damage in both pregnant and fetal rats as a result of its teratogenic and toxic effects.

***Keywords:*** *Fetus, Pregnant rat, Organophosphate, Prenatal exposure, Teratogenic effect*

# Gebe Ratlara Farklı Dozlarda Uygulanan Malathionun Fetüs Böbrek Dokusu Üzerine Teratojenik Etkileri

## Özet

Bu çalışmanın amacı gebe ratlara düşük dozlarda subakut uygulanan Malathion (ML)'un fetüs böbrek dokusu üzerine teratojenik etkisini araştırmaktır. Toplam 28 Sprague-Dawley gebe rat randomize olarak her grupta 7 adet gebe rat olacak şekilde 4 gruba ayrıldı. ML'un dozuna bağlı olarak, gruplar; kontrol, ML 2.5 (2.5 mg/kg/gün dozunda oral yoldan (p.o) ML uygulandı), ML 5 (5 mg/kg/gün, p.o) ve ML 10 (10 mg/kg/gün, p.o) olmak üzere 4 gruba ayrıldı. ML uygulamsı erkekler ve dişilerin aynı ortama konulmasından itibaren başladı (çiftleşmeden itibaren). Günlük ML uygulamasına doğuma kadar devam edildi. ML'un, dozuna paralel bir şekilde gebe ratlarda böbrek dokusu ve serum enzimleri (asetilkolinesteraz (AChE), lipaz, amilaz) üzerine toksik etkiler oluşturduğu, ayrıca yavru rat böbreklerinde ise ML'un dozuna bağlı olmayarak, tüm dozlarda teratojenik etkiye neden olduğu belirlendi. Histopatolojik veriler biyokimyasal verileri doğruladı. ML'un düşük dozlarının bile hem anne hem de yavru böbrekleri üzerine toksik ve teratojenik böbrek hasarına neden olduğu sonucuna varıldı.

***Anahtar sözcükler:*** *Fetüs, Gebe rat, Organofosfat, Prenatal maruziyet, Teratojenik etki*

## INTRODUCTION

Toxicologic evidences indicate that low-level exposure to organophosphate pesticides (OP) may affect neuro-development and growth in developing animals and children. Several studies have revealed that fetotoxicity

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and marked neurochemical changes may occur due to repeated exposures to OP during gestation [1-9]. Recent biological studies on females and children have reported that there is a widespread OP exposure [10-15]. There are concerns about the potential health effects of pre- and post-natal exposure of children to pesticides, therefore the Food Quality Protection Act (FQPA) established a stringent health-based standard for pesticide residues in food to assure protection from pesticide exposure and to strengthen health protection from pesticide risks for sensitive populations [1,12].

Children are particularly sensitive to the health risks of pesticide exposure since their internal organs are not fully developed. For instance, their immune systems may not be able to protect them against pesticides, and their excretory systems may not be able to excrete these toxic chemicals. Indeed, pesticide exposure may permanently affect development negatively by blocking the absorption of nutrients. Especially children of farmworkers are at risk for pesticide exposure. Their parents may bring pesticide residues from the agricultural fields into the house and thus the pesticides may drift from fields into areas where children play [16].

Long-term exposure of the pregnant women to OP can cause toxic effects both on their bodies and their fetuses. However, the number of studies on subacute toxicity of OP on maternal and fetal organs is scarce. Also, there are no studies which compare maternal and fetal tissues in this sense. Malathion (ML) (O,O-dimethyl-S-1.2-bis ethoxy-carbonyl ethyl phosphorodithioate), which is a common OP in the world, was selected as the toxic material of the present study since it usually has lower toxicity than other pesticides. Malaoxon is the bioactivation metabolite of ML, causing higher toxicity. Malaoxon inhibits acetyl-cholin-esterase (AChE), causing the accumulation of acetylcholine within synapses and consequently leading to the over-stimulation of postsynaptic receptors and producing rapid twitching of voluntary muscles, incoordination, convulsions, paralysis and ultimately death [17-23]. The aim of this study was to investigate the teratogenic effects of ML induced by subacute low doses on fetus kidney tissues in pregnant rats.

## MATERIAL and METHODS

The study was approved by Dicle University Animal Ethical Committee and was carried out in accordance with the "Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by Dicle University Animal Ethical Committee". Female Sprague-Dawley rats (250±50 g) were obtained from the Animal laboratory at Dicle University. The rats were housed in clean polypropylene cages (having six rats per cage) and maintained under controlled room temperature (23±2°C) with a photoperiod of 12 h light and 12 h dark cycle. The rats were given standard pellet diet and water *ad libitum* throughout the experimental period.

In the study, at first, a total of 40 Sprague-Dawley female rats were divided into 4 groups of 10 rats each. ML was applied in various low doses (2.5-5 and 10 mg/kg/day, orally). The rats were caged for 3 days with one male and one female in each cage. Once ML was applied, the rats were mated. After 3 days, male rats were removed from the cages and vaginal smear was applied to the female rats to determine pregnancy. Only the pregnant females were studied. A total of 28 Sprague-Dawley pregnant rats were randomly divided into 4 groups of 7 rats each. Depending on dose, the rats were divided into 4 groups including (I) control, (II) ML 2.5 (2.5 mg/kg/day, orally), (III) ML 5 (5 mg/kg/day, orally), and (IV) ML 10 (10 mg/kg/day, orally). ML application started when the male and female were put together (when mating started). Daily ML application was continued until birth.

After the birth and anesthesia with ketamine, (85 mg/kg, intraperitoneal, Ketalar, Pfizer), blood samples were obtained from mother rats using intracardiac puncture in sterile tubes without anticoagulant. After a one-hour clotting in the room temperature and centrifugation (1500 g, 10 minutes, 4°C), the sera were carefully harvested and stored at -20°C till biochemical analysis.

***Biochemical Analysis***

The enzyme amylase, lipase and AChE activities in serum were determined with Roche Cobas Integra 800 autoanalyser via enzymatic colorimetric method using Roche brand commercial kits in 409/659 nm. The enzyme activities were expressed as U / L. Measurement range of the tests were 3-2000 U/L (0,05-33 µkat/L) for amylase, 200-14000 U/L (3,34-234 µkat/L) for AChE and 3-1200 U/L (0,05-5,01 µkat/L) for lipase. All analyses were performed in Biochemistry Department of Dicle University Medical Faculty.

***Histopathologic Analysis***

Immediately after the death of the rats, kidney tissues were processed in 10% formaldehyde solution via cassette autotechnic tissue processing equipment (Leica ASP 300). Once the processing was completed, the tissues were embedded in paraffin blocks and the sections (5 µm in thickness) were taken by microtome instrument onto lysine laminin. The preparations stained with haematoxylin and eosin (H&E) were evaluated under a light microscope at x100 magnification (Olympus BX51) by a pathologist blinded to the study groups.

Histopathologic evaluation consisted of tissue damages including the intensity of cellular hydropic degeneration along with neutrophil and mononuclear cell infiltration, degenerative changes, nuclear loss, necrosis and fibrosis. Each organ was graded [23] on a scale *(Fig. 1)*. For the kidney, for instance, the occurrence of tubular epithelium damage

and proteinous accumulation in the tubular lumen due to the filtration failure were examined and graded. Each parameter was scored between 0 and 3 (0: normal, 1: mild, 2: moderate, and 3: severe) depending on the situation of lesions *(Table 1)*.

***Statistical Analysis***

Activities of enzymes were analyzed using One-way ANOVA and Tukey test. A value of $P<0.05$ was considered statistically significant.

## RESULTS

In the present study, depending on ML dose, histopathologically vacuolar degeneration, necrosis and spills were observed in kidney tubular epithelium of the ML groups. In the F1 and P1 groups, swelling of the tubular cells with brush border loss were present. In the P2 and F2 groups, in addition to swelling and brush border loss of the tubular cells, nuclear losses in the tubular cells were seen. Apart from these complications, the P3 and F3 groups presented with degenerations and numerous nuclear losses in the tubular cells *(Fig. 1)*. Moreover, regardless of ML dose, widespread degenerations and necrosis (nuclear loss) were observed in all the fetal groups. The lesions were scored depending on the scale *(Fig. 1)*. The results conclusively indicated that Malathion results in toxic effects on kidney tissues in pregnant rats depending on the dose, and it causes teratogenic effects in fetal kidneys at all doses. In the biochemical evaluation of pregnant rats, a significant difference was observed between the control group and the ML group in terms of enzyme results. It was determined that ML leads to a decrease in amylase and cholinesterase activities and an increase in lipase activities. Also, the in-group comparisons among ML groups revealed that, depending on dose, the use of ML inhibits AChE and amylase activities while increasing lipase activity *(Table 2)*.

## DISCUSSION

Widely used in agriculture, OP has several important features such as environmental safety, limited persistence, and selective toxicity to insects with respect to mammals [24]. Therefore, ML was selected as the toxic material for the present study. Previous studies argued that ML inhibits AChE, causing the accumulation of acetylcholine within synapses, and consequently leading to overstimulation of postsynaptic receptors. Among these, Asini *et al*.[25] demonstrated that repeated ML administrations cause a decrease in the circulating AChE activity in rats. Akhgar *et al*.[26] and Rezg *et al*.[27] also observed that the plasma AChE activity in rats was seriously decreased in the case of subchronic exposure to ML.

It has been accepted that a 20% AChE inhibition causes the deleterious effects of OP [28], and when the ratio is greater than 50%, there is a life-threatening situation [29]. Thus, a decrease in the AChE activity is considered as a significant marker for OP poisoning. In the present study, depending on dose, the AChE inhibition ratio reached 10.4% in ML 2.5 group, 36.1% in ML 5, and 44% in ML 10. These results indicated that the doses of 5 mg/kg/day and 10 mg/kg/day may cause deleterious effects while the doses greater

***Table 1.*** *Grades for kidney lesions*
***Tablo 1.*** *Böbrek lezyonlarının dereceleri*

| Grades | Evaluation |
|---|---|
| Grade 0 | No Lesion |
| Grade 1 | Swelling of tubular cells coupled to the loss of brush border, Alteration of the tubule up to a third with nuclear loss without nuclear thickening and epithelial cells into the lumen |
| Grade 2 | Swelling of tubular cells coupled to the loss of brush border Alteration of the tubule up to 2 thirds with nuclear loss and proteinous mass into the lumen |
| Grade 3 | Cell degeneration Tubular alterations in more than 2 thirds with nuclear loss, proteinous mass into the lumen and neutrophil/mononuclear cell infiltrates in the interstitium |

***Table 2.*** *Comparison of serum enzymes among groups (mean ± Standard deviation)*
***Tablo 2.*** *Çalışma gruplarında serum enzimlerinin karşılaştırılması*

| Group | Amylase (U/L) | Lipase (U/L) | Cholinesterase (U/L) |
|---|---|---|---|
| Control | 2752±361 | 4.24±0.58 | 1066±52 |
| Malathion 2.5 | 2019±273[a] | 5.22±0.35 | 956±98 |
| Malathion 5 | 1801±102[b] | 5.70±0.62[b] | 681±91[b,d] |
| Malathion 10 | 1688±99[c] | 7.74±1.30[c,e,f] | 596±29[c,e] |

***a, b, c;*** *difference between control with Malathion 2.5, 5, 10 respectively*
***d, e;*** *difference between Malathion 2.5 with Malathion 5, 10 respectively*
***f;*** *difference between Malathion 5 with Malathion 10*
*for* ***a,c,d,e,f values:*** $P<0.001$, ***for b value:*** $P = 0.011$

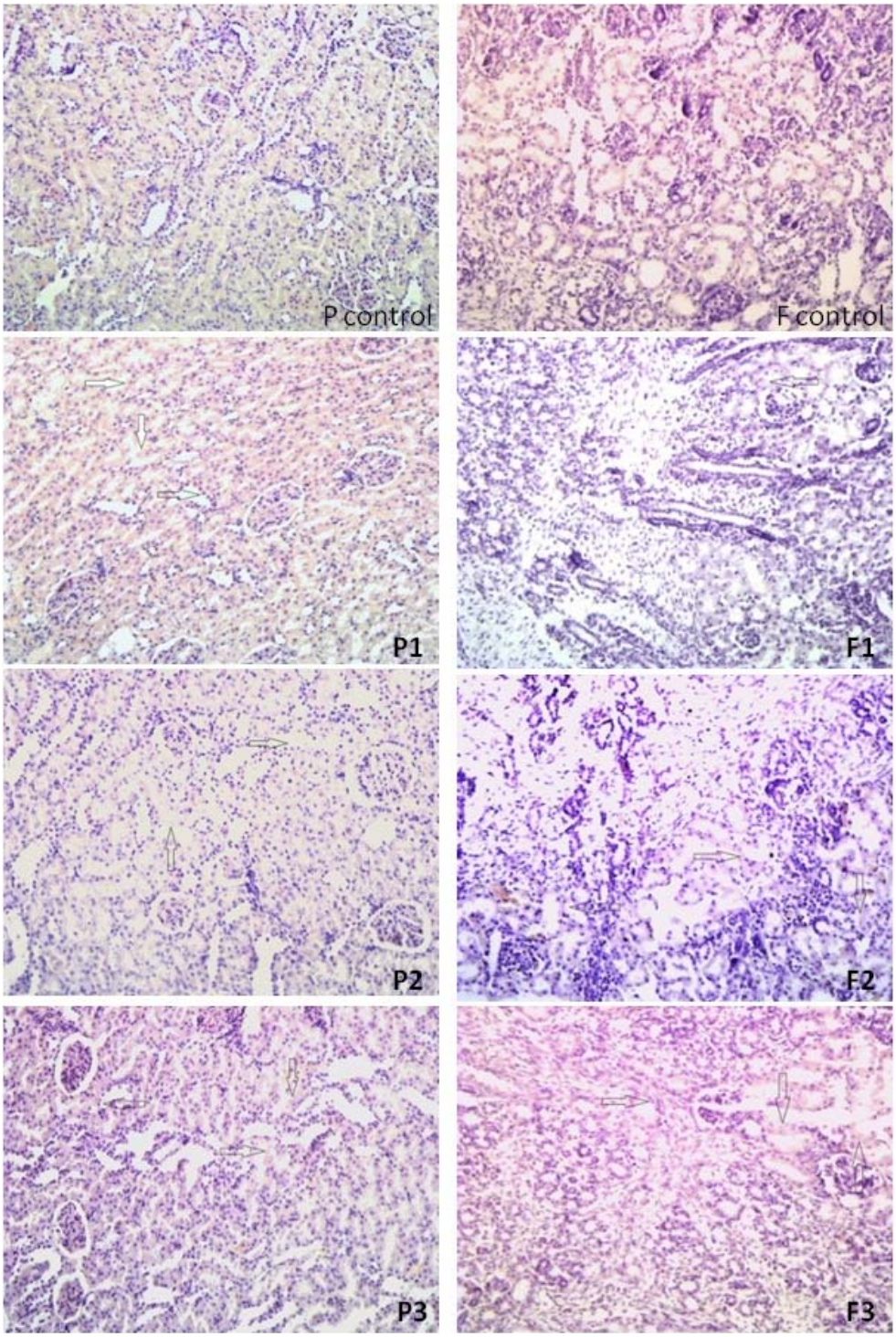


**Fig 1.** Fetal (F) and Pregnant (P) rat kidney tissues (H&E stain, x100). Normal histomorphological appearance of the pregnant and fetal kidney tissues of control groups ("P control" and "F control" respectively). Swelling of the tubular cells (down and left arrows) with brush border loss (right arrows) [P1 and F1 respectively]. Swelling and brush border loss of the tubular cells (right arrows) with some nuclear loss (up and down arrows) [P2 and F2 respectively]. In addition to swelling and brush border loss (right and left arrows), degeneration and many nuclear loss of the tubular cells (up and down arrows) are seen [P3 and F3]

**F:** Fetal rats; **F1:** Fetal rats treated with Malathion (2.5 mg/kg/day, p.o), **F2:** Fetal rats treated with Malathion (5 mg/kg/day, p.o), **F3:** Fetal rats treated with Malathion (10 mg/kg/day, p.o), **P:** Pregnant rats**; P1:** Pregnant rats treated with Malathion (2.5 mg/kg/day, p.o), **P2:** Pregnant rats treated with Malathion (5 mg/kg/day, p.o), **P3:** Pregnant rats treated with Malathion (10 mg/kg/day, p.o)

**Şekil 1.** Fetus (F) ve gebe (P) sıçan böbrek dokuları (H&E boyama, x100). Kontrol gruplarına ait fetal ve gebe sıçan böbrek dokularında normal histomorfolojik görünüm (sırasıyla "P control" ve "F control"). Fırçamsı kenar kayıpları (aşağı ve sol oklar) ile birlikte tubuler hücrelerde şişme (sağ oklar) (Sırasıyla P1 ve F1)). Tubuler hücrelerde bazı nükleer kayıpların ( yukarı ve aşağı oklar) eşlik ettiği fırçamsı kenar kaybı ve şişme (sağ oklar) (Sırasıyla P2 ve F2). Tubuler hücrelerde fırçamsı kenar kaybı ve şişme (sağ ve sol oklar) yanı sıra dejenerasyon ve birçok hücrede nükleer kayıp (yukarı ve aşağı oklar) görülmektedir (sırasıyla P3 ve F3)

**F: Fetal sıçan; F1:** Malathion uygulanmış fetal sıçan (2.5 mg/kg/gün, oral), **F2:** Malathion uygulanmış fetal sıçan (5 mg/kg/gün, oral), **F3:** Malathion uygulanmış fetal sıçan (10 mg/kg/gün, oral), **P: Gebe sıçan; P1:** Malathion uygulanmış gebe sıçan (2.5 mg/kg/gün, oral), **P2:** Malathion uygulanmış gebe sıçan (5 mg/kg/gün, oral), **P3:** Malathion uygulanmış gebe sıçan (10 mg/kg/gün, oral)

than 10 mg/kg/day may be life-threatening. However, the toxic indicators of ML are not only limited to inhibition of AChE. OP may cause several effects on other parameters such as lipase and amylase activities. Precise measurement of these enzymes is very important in that they indicate organ damages. In previous studies, it was revealed that OP may change lipase and amylase activities depending on pancreatitis and other organ damages. Alp *et al.* found that ML (200 mg/kg, single dose, orally) markedly depletes the AChE activity while significantly increasing the GGT (g-glutamyltransferase) and amylase activities [23]. Gokalp *et al*.[30] reported that high doses of diazinon, used as an OP, significantly inhibits and decreases serum amylase activities in rats due to pancreatitis. In the present study, in parallel with dose of ML, it was found that ML inhibits AChE activity while increasing lipase activity as suggested by Gokalp *et al*.[30], and decreasing amylase activity as contrary to the result by Alp *et al.*[23].

According to the histopathologic analyses in previous studies, OP is likely to cause kidney damage. Alp *et al.*[23] stated that degenerative changes associated with necrosis and inflammatory cell infiltration were evident in the kidneys of the rats intoxicated with ML. In a study by Kalender *et al*.[31], vascular dilation and glomerular atrophy were observed in kidney tissues 4 weeks after the administration of methyl parathion as OP to rats. In another study, it was observed that diazinon leads to tubular swelling, hyperplasia and cell infiltration in rabbit kidneys [32]. Sulak *et al*.[33] also administered the OP methidathion to male rats over a 4-week period, concluding that methidathion causes a reduction in AChE activity and kidney damage, together with tubular epithelial cell degeneration, focal tubular necrosis, fibrosis and infiltration. In the present study, similar to the studies aforementioned, it was revealed that ML, in parallel with the dose, causes vacuolar degeneration, necrosis and spills in kidney tubular epithelium. In addition, it leads to mono-nuclear cell infiltrations and intertubular bleeding. Regardless of ML dose, wide spread degenerations and necrosis was observed in all the fetal kidneys of the ML group. In the present study, similar to histopathologic results, it was found that ML, in parallel with dose of ML, inhibits AChE activity while increasing lipase activity and decreasing amylase activity. The biochemical results were verified by histopathologic results.

According to these results, we conclude that even low doses of ML have strong toxic effects on both pregnant and fetal kidney tissues, causing teratogenic kidney damage.

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