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Effects of Dimethoate on Tree Frog (*Hyla arborea*) Larvae

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Abstract: Considering the global decline of amphibian populations, the present study aimed to investigate the sensitivity of tree frogs to a common pesticide, dimethoate. Our study reports the effects of dimethoate on 21st- and 25th-stage *Hyla arborea* larvae under standardized laboratory conditions in an acute toxicity test using the static system. Specimens used for testing were obtained from the eggs of mating pairs collected at a local natural pond. Each experimental group contained 10 healthy larvae exposed to 5 different concentrations of technical grade dimethoate for 96 h. For each concentration, including the controls, 3 replicates were used. The concentrations of dimethoate causing 50% mortality (LC_{50}) after 96 h were estimated using a probit analysis program. Regarding the lethal concentrations, 21st-stage larvae were more sensitive (LC_{50} = 20.27 ppm) than the 25th-stage larvae (LC_{50} = 37.37 ppm). Malformations such as edema and tail deformaities were observed in 21st- and 25th-stage larvae, respectively. Retardation of growth was also observed in dimethoate-exposed 21st-stage larvae. Certain signs of toxicity, such as initial hyperactivity symptoms, followed by loss of balance, motionlessness, and finally death, were observed.

Key Words: Acute toxicity, organophosphorus pesticides, dimethoate, Amphibia, *Hyla arborea*, anuran larvae

Dimethoate'ın Ağaç Kurbağası, *Hyla arborea* Larvaları Üzerine Etkisi

Özet: Amfibi populasyonlarında gözlenen global azalmalar göz önüne alındığında; mevcut çalışma, ağaç kurbağasının yaygın bir pestisit olan dimethoate'a olan hassasiyetini ortaya koymada katkı sağlamaktadır. Çalışmamız; standart laboratuvar koşulları altında, statik sistem esas alınarak yapılan akut tosisite denemeleriyle dimethoate'ın 21. ve 25. evre *Hyla arborea* larvaları üzerindeki etkilerini ortaya koymaktadır. Denemede kullanılan larvalar, doğal bir lokal göletten toplanan amplexus halindeki çiftlerin yumurtalarından elde edilmiştir. Sağlıklı on larvadan oluşan herbir deneme grubu, 96 saatlik deney süresince, ticari dimethoate'ın beş farklı konsantrasyonuna maruz bırakılmıştır. Her bir konsantrasyon ve kontrol grubu için üç tekrar yapılmıştır. Probit analiz programı kullanılarak 96 saatte, %50 ölüme neden olan dimethoate konsantrasyonları (LC_{50}) hesaplanmıştır. Letal konsantrasyonlara göre; 21. evre larvalar (LC_{50} = 20.27 ppm) 25. evre larvalardan (LC_{50} = 37.37 ppm) daha hassastır. 21. evre larvalarda ödem ve 25. evre larvalarda da kuyruk deformasyonları şeklinde morfolojik anomaliler gözlenmiştir. Ayrıca, 21. evre larvalarda gelişmede gecikme de gözlenmiştir. Dimethoate uygulanan hayvanların davranışlarında; sırasıyla hiperaktivite semptomları, denge kaybı, hareketsizlik ve son olarak da ölüm gibi bazı toksisite belirtileri gözlenmiştir.

Anahtar Sözcükler: Akut toksisite, organofosforlu pestisitler, dimethoate, Amphibia, *Hyla arborea*, anur larvası

Introduction

There has been growing concern about worldwide declines in amphibian populations since the late 1980s (Blaustein and Wake, 1990; 1995; Houlahan et al., 2000; Gaizick et al., 2001). At present, it is an accepted fact that amphibian decline is a global problem and a variety of explanations have been offered. One of the most important proposed causes for the decline is agrochemicals, especially pesticides (Phillips, 1990; Berrill et al., 1994; Materna et al., 1995; Ouellet et al., 1997). Amphibians are sensitive to environmental contaminants because they inhabit aquatic and terrestrial realms, and their thin, highly permeable skin rapidly absorbs toxic substances (Duellman and Trueb, 1986; Materna et al., 1995). They can be exposed to waterborne and airborne pollutants in their breeding and foraging habitats. Amphibians are key components of many ecosystems and the impacts of pollution upon them are indicative of ecosystem health. Moreover, amphibian tadpoles have been used as bioindicators for contamination monitoring

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because their skin is highly permeable, both in water and air (Dumpert and Zeitz, 1984; Beiswenger, 1988; Williams et al., 1989). Retardation of growth, limb degeneration, and teratogenesis are some of the biomarkers used to evaluate the degree of pollution (Henle, 1981; Duellman and Trueb, 1986; Nebeker et al., 1994a; 1994b). Most studies have been conducted in consideration of the effects of pesticides on frogs; however, only a few studies have been carried out on tree frogs of the family Hylidae which is one of the most diverse and widely distributed frog family in the world. Some hylid species from South America seem to be very sensitive to habitat degradation (Guillermo et al., 2000). Considering the global decline of amphibian populations, the present study aimed to investigate the sensitivity of the hylid species to dimethoate, an organophosphorus insecticide, by means of laboratory toxicity bioassays using larval stages of *Hyla arborea*. Organophosphorus pesticides are preferred over many other pesticides because they are less persistent in the environment; however, they are cholinesterase inhibitors and adversely affect the nervous system (Chambers and Levi, 1992; Barron and Woodburn, 1995). The purpose of this study was to determine the LC_{50} of dimethoate and to describe its acute toxic effects on *H. arborea* larvae.

Materials and Methods

Hyla arborea, a member of the family Hylidae, were used in this study. Mating pairs were collected from a clean, permanent pond located near Bornova, İzmir, Turkey and transferred to the laboratory. They were captured during amplexus and placed in glass jars containing water and vegetation until egg laying was observed. Eggs obtained by this procedure were reared in the laboratory at 22 ± 1 °C, with natural lightning, in gently aerated and dechlorinated tap water. Experiments were performed on pre-feeding larvae in the 21^{st} and 25^{th} fully aquatic stage. The stages of larvae were determined according to the table of Gosner (1960). Many investigators have justified using tadpoles for toxicity tests because they were described as the most sensitive life stage in some studies (Freda and McDonald, 1993). Formulation grade of the pesticide dimethoate (Korumagor 40 EC, Koruma Tarım A.Ş.), was used. Dimethoate is a systemic insecticide and acaricide with contact and stomach action, which is used to control a wide range of coleoptera, diptera, homoptera, and acari in grain, cotton, vegetable, and fruit crops. Dimethoate was diluted in dechlorinated tap water in order to reach the following test concentrations; 22, 26, 30, 34, and 38 ppm for 21^{st}-stage, and 16, 26, 36, 46, 56 ppm for 25^{th} -stage larvae. The concentrations of formulation grade dimethoate were calculated from the percentage of the active ingredient. Solutions were freshly made immediately before use. The static system was used in each acute toxicity test as a method of exposure. Ten healthy larvae were randomly selected and transferred to glass petri dishes containing 100 ml of dimethoate solution of the desired concentration for 96 h of experimentation; they were covered with glass plates in order to obtain a negligible air-water interface and to minimize evaporation. Controls were maintained without insecticide under the same condition. For each concentration, including the controls, 3 replicates were used. Observations were made at 24-h intervals through 96 h of exposure. Lethality was considered the endpoint. Every 24 h the number of dead animals was recorded, which were then removed from the containers and their external aspects registered. Also, the morphological and behavioral characteristics of the live animals were observed. Mortality data from the replicate samples from each dimethoate concentration was pooled prior to calculating LC_{50} and 95% confidence intervals. The 96-h LC_{50} values and the 95% confidence intervals were determined through probit analysis with SPSS version 10.0 for windows. All regressions were significantly linear.

Results and Discussion

While no mortality occurred in the control groups throughout the 96-h experimental period, mortality of exposed larvae correlated with increasing dimethoate concentration. It is known that the effects of the insecticide are always graded proportionally according to its concentration and to incubation time. The 96-h LC_{50} values for dimethoate in 21^{st}- and 25^{th} -stage larvae were 20.27 and 37.37 ppm, respectively (Table 1).

Regarding these results, 21^{st}-stage larvae were more sensitive to dimethoate than the 25^{th}-stage larvae. The sensitivity to dimethoate was greater in younger larvae. It was reported that the 96-h LC_{50} values observed for dimethoate in *Rana hexadactyla* tadpoles was 7.82 ug/l (Khangarot et al., 1985); however, to date, information

Table 1. Lethal concentrations (LC_{10}, LC_{50}, and LC_{90}) of dimethoate (ppm) in exposed *H. arborea* larvae.

	LC_{10} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)
21^{st}-stage larvae	9.60 (-12.66 - 16.22)	20.27 (10.84 - 23.59)	30.95 (28.21 - 37.09)
25^{th}-stage larvae	14.73 (-5.51 - 23.73)	37.37 (30.51 - 42.46)	60.01 (53.05 - 74.67)

CI = Confidence interval

on the acute toxicity of dimethoate on hylid species is not available.

Dimethoate at all concentrations caused growth retardation in 21^{st}-stage larvae. An increase in dimethoate concentration caused large numbers of larvae to remain in the 24^{th} stage, while the controls were in the 25^{th} stage. Delayed metamorphosis, reduced growth rates, and mortality of tadpoles have been documented after exposure of eggs and larvae to dimethoate (Mizgireuv et al., 1984) and fenitrothion, another organophosphorus pesticide (Mohanty-Hejmandi and Dutta, 1981). Retardation of growth may place larvae at greater risk of predation and dehydration as ephemeral ponds dry out through the year. Moreover, delays in growth increase the exposure of larvae to pesticide runoff.

Some morphological alterations were observed in the exposed groups. They were characterized by edema, especially on the head and trunk of 21^{st}-stage larvae, and tail deformities in 25^{th}-stage larvae. Additionally, the length of dimethoate treated larvae was shorter than the controls. The malformations found in dimethoate-treated 21^{st}-stage larvae are presented in Figure 1, which shows a normal larva (a) and others suffering from edema (b and c). While Figure 2 shows untreated larvae, Figures 3-5 show dimethoate-treated 25^{th}-stage larvae that were greatly malformed. Except for the group exposed 16 ppm concentration, tail deformities were observed in all concentration groups. Larvae in the 16 ppm concentration group displayed normal larvae morphology, but they were shorter in length than the controls. Another type of tail abnormality was a bent tail, which was observed at the end of the tail in a few of the treated larvae (Figure 5). It was reported that malathion, another organophosphorus insecticide, caused morphological abnormalities in the head, trunk, and tail in *Microhyla ornata* (Pawar et al., 1983). Similarly, tail deformations

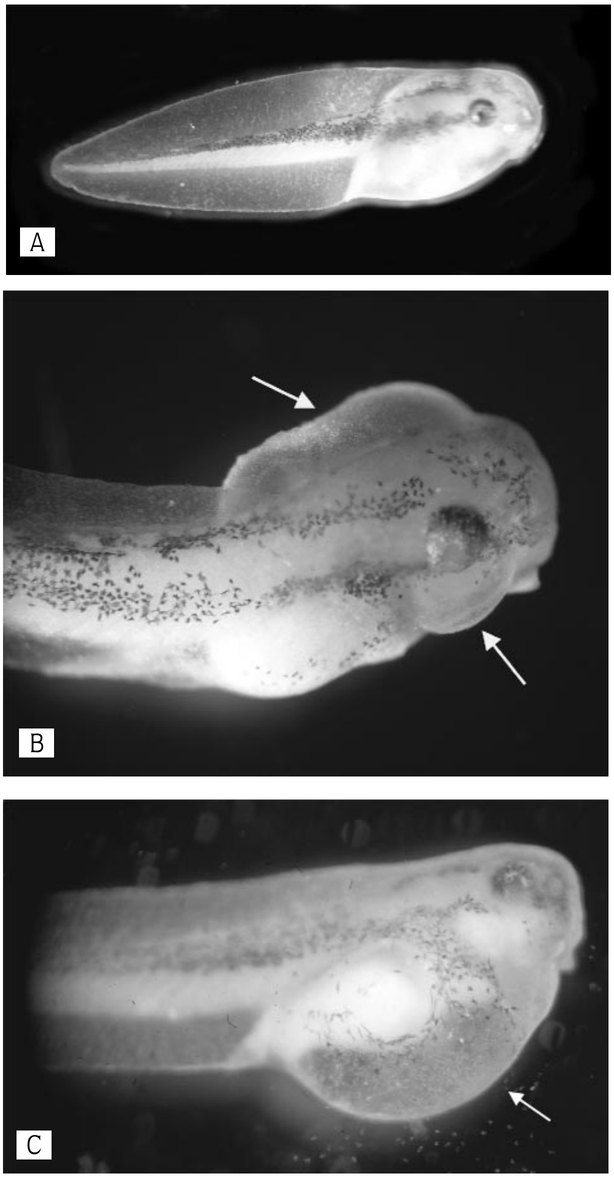

Figure 1. (A) Lateral view of untreated *H. arborea* larva (0.8X). (B) Dorso-lateral view (2X), and (C) lateral view (2X) of malformed larvae exposed to 30 and 38 ppm dimethoate concentrations, respectively. Note the edema (→).

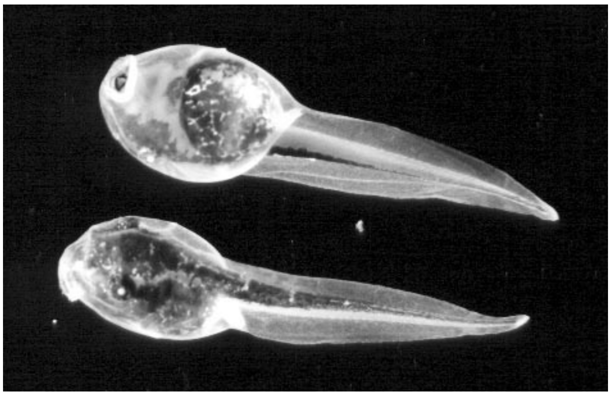

Figure 2. Ventral and dorsal view of untreated *H. arborea* larvae (0.8X).

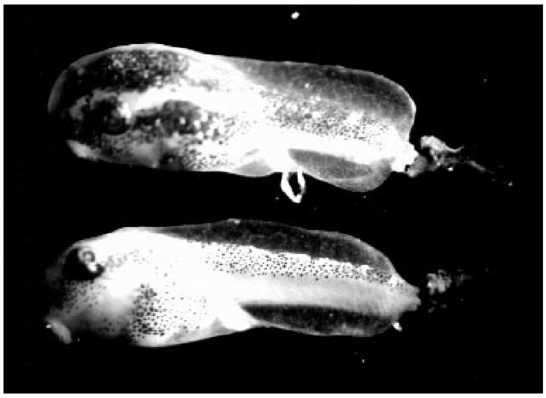

Figure 3. Lateral view of malformed larvae exposed to 36 ppm dimethoate concentration (0.8X). Note the tail deformation.

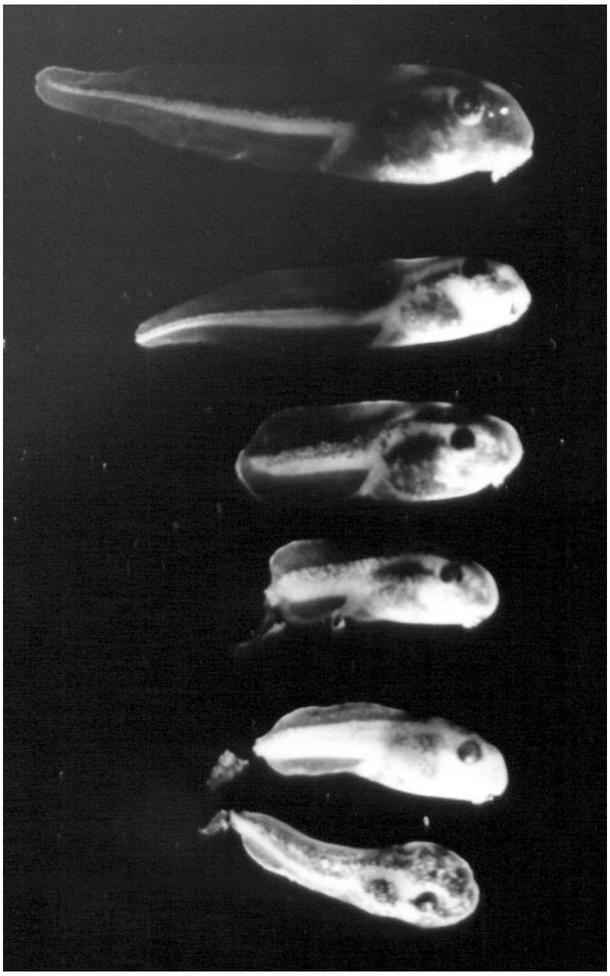

Figure 4. Figure demonstrates decreased larval length and tail abnormalities observed in many exposed larvae (0.8X). From largest to smallest: Control and16, 26, 36, 46, and 56 ppm dimethoate exposure.

Figure 5. Dorsal view of malformed larvae exposed to 36 ppm dimethoate concentration (2X). Note the tail deformation.

and edema occurred when tadpoles of *Rana temporaria* and *Xenopus laevis* were exposed to carbaryl (Bishop, 1992). Growth retardation and morphologic alterations, especially edema, similar to those described for dimethoate and discussed here, were reported in MCPA-(4-chloro-2-methylphenoxyacetic acid) treated *X. laevis* (Bernardini et al., 1996). Tadpoles of *X. laevis* exposed to lindane show morphological abnormalities (Marchal-Segault and Ramade, 1981). Tail abnormalities, such as narrow margins, bent or drooped, and stunted growth, were observed in *Rana pipiens* tadpoles exposed to paraquat (Dial and Bauer, 1984).

All exposed animals showed signs of toxicity. At the beginning of the experiments, signs of toxicity were characterized by hyperactivity symptoms, then loss of balance, motionlessness, and finally death, which was defined as the lack of response to mechanical stimuli. Similar behavioral effects of laboratory exposures to deltamethrin, carbaryl, carbofuran, and malathion in amphibian larvae were reported (Bishop, 1992; Salibián, 1992; Sayım and Akyurtlaklı, 1999). These behavioral effects are not surprising because most of these pesticides are neurotoxic. There is no doubt that those types of behavioral changes may subject larvae to a greater hazard from predation.

The biota may be stressed by discharges of pesticides, and therefore, it is essential to know their potential effects on non-target organisms before irreversible change occurs. Moreover, the study of the effect of different contaminants, such as pesticides, on amphibians will help to identify the various causes of declining populations.

References

Barron, M.G. and Woodburn, K.B. 1995. Ecotoxicology of chlorpyrifos. Reviews of Environmental Contamination and Toxicology, 144: 1-93.

Beiswenger, R.E. 1988. Integrating anuran amphibian species into environmental assessment programs. U.S. Forest Service. General Technical Report R.M., 166: 159-165.

Bernardini, G., Spinelli, O., Presutti, C., Vismara, C., Bolzacchini, E., Orlandi M. and Settimi, R. 1996. Evaluation of the developmental toxicity of the pesticide MCPA and its contaminants phenol and chlorocresol. Environmental Toxicology and Chemistry, 15-5: 754-760.

Berrill, M.S., Bertram, S., McGillivray, L., Kolohon, M. and Paull, B. 1994. Effects of low concentrations of forest-use pesticides on frog embryos and tadpoles. Environmental Toxicology and Chemistry, 13: 657-664.

Bishop, C.A. 1992. The effects of pesticides on amphibians and the implications for determining causes of declines in amphibian populations. Canadian Wildlife Service, 76, Canada, 67-70.

Blaustein, A.R. and Wake, D.B. 1990. Declining amphibian populations: A global Phenomenon? Trends in Ecology & Evolution, 5: 203-204.

Blaustein, A.R. and Wake, D.B. 1995. The puzzle of declining amphibian populations. Scientific American, 272: 52-63.

Chambers, J.E. and Levi, P.E. 1992. Organophosphates: chemistry, fate and effects. Academic Press, New York.

Dial, N.A. and Bauer, C.A. 1984. Teratogenic and lethal effects of Paraquat on developing frog embryos (*Rana pipiens*). Bulletin of Environmental Contamination and Toxicology, 33: 592-597.

Duellman, W.E. and Trueb, L. 1986. Biology of amphibians. McGraw-Hill, New York, NY.

Dumpert, K. and Zeits, C.A. 1984. Platanna (*Xenopus laevis*) as a test organism for determining the embryotoxic effects of environmental chemicals. Ecotoxicology and Environmental Safety, 8: 55-74.

Freda, J. and McDonald, D.G. 1993. Toxicity of amphibian breeding ponds in the Sudbury region. Canadian Journal of Fisheries & Aquatic Sciences, 50: 1497-1503.

Gaizick, L., Gupta, G. and Bass, E. 2001. Toxicity of chlorpyrifos to *Rana pipiens* embryos. Bulletin of Environmental Contamination and Toxicology, 66: 386-391.

Guillermo, S.N., Basso, N.G. and Ronco, A. E. 2000. Effect of Cr (VI) on early life stages of three species of hylid frogs (Amphibia, Anura) from south America. John Wiley & Sons, Inc. 509-512.

Gosner, K.L. 1960. A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification, Herpetologica, 16: 183-190.

Henle, K. 1981. A unique case of malformations in a natural population of the Green Toad (*Bufo viridis*) and its meaning for environmental politics. British Herpetological Society Bulletin, 4: 48-49.

Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Meyers, A.H. and Kuzmin, S.L. 2000. Quantitative evidence for global amphibian population declines. Nature, 404: 752-755.

Khangarot, B.S., Sehgal, A. and Bhasin, M.K. 1985. Toxicity of Selected Pesticides to Frog Tadpole *Rana hexadactyla* (Lesson). Acta Hydrochimica et Hydrobiologica, 13(3): 391-394.

Marchal-Segault, D. and Ramade F. 1981. The effects of lindane, an insecticide, on hatching and postembryonic development of *Xenopus laevis* anuran amphibian. Environmental Research, 24: 250-258.

Materna E.J., Rabeni C.F., LaPoint T.W. 1995. Effects of the synthetic pyrethroid insecticide, esfenvalerate, on larval Leopard frogs (*Rana* sp.). Environmental Toxicology and Chemistry, 14 (4): 613-622.

Mizgireuv, I.V., Flax, N.L., Borkin L.J. and Khudoley V.V. 1984. Dysplastic lesions and abnormalities in amphibians associated with environmental conditions. Neoplasma, 31: 175-181.

Mohanty-Hejmandi, P. and Dutta S.K. 1981. Effects of some pesticides on the development of the Indian bull frog *Rana tigrina*. Environmental Pollution Series, A 24: 145-161.

Nebeker, A.V., Schuytema G.S. and Ott S.L. 1994a. Effect of cadmium on growth and bioaccumulation in the Northwestern salamander *Ambystoma gracile*, Archives of Environmental Contamination and Toxicology 29, 492-499.

Nebeker, A.V., Schuytema G.S. and Ott, S.L. 1994b. Effect of cadmium on limb regeneration in the Northwestern salamander *Ambystoma gracile*. Archives of Environmental Contamination and Toxicology, 27, 318-322.

Ouellet, M., Bonin, J., Rodrigue, J., DesGranges, J-L. and Lair, S. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. Journal of Wildlife Diseases, 33: 95-104.

Pawar, K.R., Ghate, H.V., Katdare M. 1983. Effect of malathion on embryonic development of the frog *Microhyla ornata*. Bulletin of Environmental Contamination and Toxicology, 31: 170-176.

Phillips K., 1990. Where have all the frogs and toads gone? Bio-Science, 40:422-424.

Salibián A. 1992. Effects of deltamethrin on the South American Toad, *Bufo arenarum*, tadpoles. Bulletin of Environmental Contamination and Toxicology, 48:616-621.

Sayım F. and Akyurtlaklı N. 1999. Acute toxicity of malathion on the 25^{th} stage larvae of *Rana ridibunda*. Journal of Fisheries and Aquatic Sciences. 16(1-2):19-29.

Williams B., Marcy S. and Gerould S. 1989. Water quality to protect wildlife resources EPA 600/3-89-067. U.S. E.P.A. Corvallis Environmental Research Laboratory, Corvallis, OR.