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Effects of Altosid and Abate-4E on Deformities and Survival in Southern Leopard Frogs Under Semi-Natural Conditions

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Since 1995 when a group of school children in Minnesota found frogs with supernumerary limbs and missing limbs, there has been widespread interest in the amphibian malformation issue. Despite considerable effort to understand this problem, its extent and seriousness as well as direct causes of these malformations remain unclear. Progress on this issue has been hampered by a scarcity of scientifically reliable information on historical rates of abnormalities under undisturbed conditions and by the normal turnaround times of data collection, sample analysis and publication. One of the very few peer-reviewed publications with field-collected data showed that an average of 12% (range 0–69%) of the frogs inhabiting wetlands in southern Québec, Canada had deformities, most of which consisted of partially or completely missing hind limbs or digits (Ouellet et al. 1997). The use of pesticides was correlated with these deformities, but no specific cause and effect relationships were developed.

The mosquito control agents S-methoprene, an inhibitor of chitin production and growth in insects; and temephos, an organophosphorous pesticide, are widely applied to wetlands in their formulations of Altosid (in 5% or 20% methoprene solutions or briquet form) and Abate-4E (emulsifiable solution containing 44.6% temephos). Since 1995, cooperative research between the U.S. Fish and Wildlife Service and the U.S. Geological Survey on these chemicals has involved both laboratory and field investigations but has focused primarily on non-target invertebrates.

Lethal effects of temephos on insects (Tsai 1978, Sanders et al. 1981), other invertebrates (Ward and Busch 1976), fish (Sanders et al. 1981), amphibians (LC_{50} of 4.64 μl/l, approximately 2 ppm in green frogs, *Rana clamitans*; Sparling et al. 1997), and birds (Franson et al. 1983) have been well documented. Methoprene is effective in controlling certain nuisance species of insects and is thought to be relatively non-toxic to vertebrates. However, methoprene and its derivatives can stimulate gene transcription in vertebrates by acting through the retinoic acid-responsive transcriptors (Harmon et al. 1995). Developmental deformities in the African-clawed frog (*Xenopus laevis*) have been associated with methoprene derivatives (LaClair et al. 1998) and may be related to this transcription activation.

Of great interest, therefore, is whether Altosid or Abate-4E affect free-ranging populations of amphibians. Our ongoing study on these chemicals gave us the opportunity to examine this question in the southern leopard frog (*Rana sphenocephala*).

Our study site consisted of a 24-cell experimental wetland complex that was created at Patuxent Wildlife Research Center, Laurel, MD, USA in 1990. Although a few previous contaminants-related studies have occurred in these wetlands, we have always been very careful to use chemicals with minimal or no persistence (e.g. acidification, glyphosate) and have followed each study with drainage of the wetlands to facilitate degradation and dilution and with analysis for residual chemicals. Predator control in these wetlands includes an outer, electrified, partially buried cyclone fence and inner fence around each wetland to prevent access by medium and large mammals and periodic drainage to control aquatic predators. All wetlands are equally accessible by avian and aerially dispersed invertebrate predators. The complex is surrounded by larger (>1 ha) wetlands and wet forests which serve as sources for adult anurans that naturally emigrate into the complex and breed each year. The wetlands measure approximately 213 m^2 and have a maximum depth of 0.60 m. Eighteen wetlands were selected randomly for treatments consisting of Altosid, Abate-4E, or control; 6 replicates were used for each treatment. Application procedures followed label instructions. During morning hours when winds were light, certified applicators sprayed pesticide solutions three times in June and July, 1997. Label instructions for Altosid give a nominal concentration of methoprene immediately after spraying, as 0.04 ppm in the first 2.54 cm of water. The calculated concentration based on application rate and volume of wetland was 1.6 ppb throughout the water column, assuming that there was no adsorption by organic material. This nominal concentration was verified the following year when we used identical procedures followed by chemical analyses of water (Ross et al. 1994). With Abate-4E, nominal temephos concentrations in the first 2.54 cm of water were 0.13 ppm and as low as 2 ppb through the water column.

Amphibian populations were sampled with dip nets from 27 August to 3 September 1997 for the Altosid-sprayed and control wetlands and on 9 September for the Abate-4E sprayed ponds. Each pond was sampled by taking 50 pulls with an aquatic dip net from shorelines while walking around the perimeter, thus the numbers of frogs collected represented relative abundance population estimates. Each amphibian was identified to species and inspected for abnormal development including abnormal coloration, missing eyes and missing or supernumerary appendages and digits. Water was collected following each spray for temephos measurement and was analyzed with gas chromatography for temephos based on Belisle and Swineford (1988). Temephos concentrations averaged 12 ppb shortly after spraying.

In all, 91 juvenile (tail completely absorbed, hind limbs and forelimbs well developed) and metamorph (hind and forelimbs developed, tail still present) southern leopard frogs were collected from Altosid-sprayed wetlands; 14 (15%) of these displayed at least one major deformity. Ten of these animals had completely or partially missing hind limbs, in 9 only the right hind limb was affected. Two of the remaining tadpoles had missing eyes and two were very light in color (amelanistic). Seventy-nine juveniles and metamorphs were collected from control wetlands with 4 (5%) having missing or partially missing right hind limbs. Only six juveniles or metamorphs were collected from Abate-4E sprayed wetlands and none of these showed deformities. Two of 12 northern cricket frogs (*Acris crepitans*) metamorphs collected from Altosid-sprayed wetlands also had an eye missing; this contrasts with 0 of 22 cricket frog metamorphs collected from control wetlands. Considering only leopard frogs, Altosid-sprayed wetlands had a significantly greater frequency of deformities than control wetlands ($\chi^2 = 6.44$, $P < 0.02$) and the occurrence of 12 of the 13 missing being on the right side was highly different from an equal distribution (binomial test, $P = 0.002$). The mean number of southern leopard frogs captured per pond (tadpoles, metamorphs, and juveniles combined) was: Control 60.2 ± 45.7; Altosid 95.5 ± 44.9; and Abate-4E 31.3 ± 19.8. which significantly differed among treatments (ANOVA, $P = 0.033$) with a posteriori differences (Tukey's HSD test, $P = 0.05$) occurring between Abate-4E sprayed and the other two treatments but not between Altosid and control wetlands. The difference between Abate-4E sprayed wetlands and the other two treatments was confounded by differences in sampling times (see below).

Our data indicate that Altosid can be linked to missing hind limb malformations in *Rana sphenocephala* and possibly with missing eyes and amelanization under the test conditions of label application rates, repeated applications, and water conditions similar to those found in these wetlands. However, most of the reported instances of high frequencies (e.g. >15% of a collected sample) of amphibian deformities involve other species such as mink frogs (*R. septentrionalis*), northern leopard frogs (*R. pipiens*), and green frogs and it is not known if Altosid would affect these species in a similar manner. In many of the areas where high frequencies of deformities have been reported there is no record of Altosid application, thus it is very likely that other causes are also involved in amphibian malformations. Leading candidates include trematode parasitism (Sessions and Ruth 1990, Johnson et al. 1999), other anthropogenic chemicals (Ouellet et al. 1997), and UV light (Blaustein et al. 1997, Ankley et al. 1999). These factors may act singly or in combination with each other.

Our data did not suggest that Altosid decreases amphibian survival or productivity, at least into the metamorph stage, in that there was no significant difference in the total number of amphibians collected between Altosid and control wetlands. However, the malformations observed would almost certainly reduce survival past the metamorph stage by affecting mobility and ability of young frogs to burrow into the mud during hibernation, particularly in more northern climates. We found a significantly lower relative abundance of southern leopard frogs and reduced abundance of northern cricket frogs in ponds sprayed with Abate-4E compared to those sprayed with Altosid or controls. However, sampling in Abate-4E sprayed ponds occurred about 1 week after that in Altosid and control wetlands at a time when frogs could have been emigrating from small ponds to larger ones, and this may have affected comparisons of numbers. The concentrations of temephos measured in water were below the 96 hr LC_{50} values for tadpoles in the pre-hind limb green frog tadpoles (Sparling et al. 1997), but that bioassay was a short term laboratory assessment employing an advanced stage of development of a different species and may not be comparable to a chronic exposure to various life stages of southern leopard frogs under field conditions.

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