Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part II. Progress towards characterization of chemical stressors

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Abstract. An alarming high incidence of deformities in mature and newly metamorphosed *Bufo marinus* was identified in 2002 (29% in adults and 21% in metamorph specimens) in Bermuda. Suspicion of multiple chemical stressors in many of the effected ponds warranted identification of potential causative agents. Short-term embryo-larval, chronic exposure studies through hind limb development, and a battery of toxicity identification studies were performed with *B. marinus* and the surrogate species, *Xenopus laevis*, using sediment and water collected from four target ponds and one reference pond in Bermuda. Short-term embryo larval developmental toxicity studies based on a modified FETAX method indicated that samples from each of the target sites were capable of inducing abnormal development. Malformations induced in the early developmental tests were consistent amongst the species and included abnormal development of the craniofacial region, eye, mouth, and gut. Limb reduction deficit defects were identified in the chronic exposure studies with *B. marinus* and *X. laevis*. Toxicity characterization studies found that the toxicity of sediment extracts was variably filterable and reduced by C₁₈-SPE, cation exchange, and zeolite treatments. Developmentally toxic samples contained elevated levels of petroleum hydrocarbons, metals (Al, As, Sn, Cd, Cr, Cu, Fe, Pb, Hg, Mn, Ni, and Zn), and ammonia. Sediment spiking studies with reference sediment confirmed that the levels of petroleum hydrocarbons and metals were capable of inducing developmental toxicity individually. Joint mixture interaction studies with sediment extracts prepared from the individually spiked reference sediment suggested however, that these extracts were potentially capable of acting synergistically, producing a greater level of toxicity than would normally be predicted based on the response to the individual spiked samples.

Key words: Bermuda; *Bufo marinus*; chemical interaction; chemical stressors; deformities; synergism.
Introduction

The increase in the frequency of amphibian malformations reported over the last several years (Burkhart et al., 1998; Burkhart et al., 2000; Bacon et al., 2006), combined with the more widespread occurrence of ectromelia, ectrodactyly, missing or misplaced eyes, and internal abnormalities, are becoming more widespread globally (Hoppe and Mottl, 1997; Ouellet et al., 1997). The first suggestion of a possible increase in the incidence of abnormal frogs in Minnesota was identified in 1994 (Burkhart et al., 1998). By 1996, the Minnesota Pollution Control Agency (MPCA) had received reports from over 100 locations with approximately 20 sites confirmed by biologists. The abnormalities in six species of frogs and toads were primarily in the form of missing, reduced and misshapen rear limbs, and a few animals with extra limbs or missing eyes. The incidence rates ranged from 4.8-24.3%, with >2,000 specimens collected.

In the late summer of 1996 malformed frogs were reported by the general public to the Vermont Agency of Natural Resources (VTANR) from 12 sites in five counties within the Lake Champlain Basin (Burkhart et al., 2000). Of 290 northern leopard frogs (Rana pipiens) examined, the incidence of malformations averaged 13.1%, ranging from 5-23%. Malformations were primarily missing and partial hind limbs. In late July 1997 1,475 metamorphs of R. pipiens were collected and examined; roughly 8.0% had malformations, with rates ranging from 2.0-45.4%. Categories of malformations were primarily missing/partial limbs and shortened/missing digits. Missing or partial hind limbs comprised 57% of the malformations encountered, followed by 11.2% with shortened hind digits.

Several factors with the potential to produce frog deformities have been proposed. Various groups have supported theories based on changes in predation, endoparasite infestation and disease, ultraviolet (UV) radiation, mineral depletion (e.g. calcium, magnesium), and natural or anthropogenic chemicals to cause malformation under certain conditions. These hypotheses have recently been reviewed by Burkhart et al. (2000). The least understood of these potential causes of malformation is the role of natural or anthropogenic chemicals. Studies by Burkhart et al. (1998) indicated that pond water and sediment samples collected from various sites in Minnesota were capable of inducing early embryo-larval maldevelopment, abnormal limb development, and disruption of metamorphosis in Xenopus laevis. Additional studies by Fort et al. (1999a, 1999b) supported the hypothesis that the effects induced were potentially due to exposure to developmental toxicants present in the environment. Extensive focus in this work was placed on identifying chemicals directly from field samples that were potentially responsible for the deformities induced in X. laevis in the laboratory. However, potential issues relating to the specific application of the FETAX (Frog Embryo Teratogenesis Assay – Xenopus) method used identified by Tietge et al. (2000), warranted further examination of the potential link between the contaminants identified and the effects induced.

The Bermuda Amphibian Project (BAP) was initiated in response to local concerns that the populations of Bermuda’s amphibians were declining. Overall, the