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Organochlorine insecticides, polychlorinated biphenyls, and metals in water, sediment, and green frogs from southwestern Michigan

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Abstract

In an attempt to explain the etiology of frog deformities and population declines, many possible causative factors have been examined, including the input of synthetic chemicals into aquatic systems, where frogs spend much of their lives, including their entire developmental stages. Deformities in populations of green frogs in wetlands of southwestern Michigan that are influenced by agricultural, urban, or industrial inputs were assessed in this study. Of the 1445 green frogs (*Rana clamitans*) examined, only four (0.3%) exhibited morphological deformities. This deformity rate is less than the recognized background level of deformities for this species, which is approximately 1%. Concentrations of organochlorine insecticides, polychlorinated biphenyls (PCBs), and metals were determined in water, sediment, frog eggs, tadpoles, and adult green frog tissues. Concentrations of all individual organochlorine insecticides in tissue were less than 6 ng/g, wet wt. Concentrations of $\sum$PCBs in tissue did not exceed 100 ng/g, wet wt. Concentrations of toxic metals were less than the limits of detection. Because no significant numbers of green frog deformities were observed in this region, it can be assumed that at these low concentrations, physical malformations in green frogs should not be observed.

Significance of study. This study provides information on the incidence of deformities in green frog populations in southwestern Michigan and offers background data on chemical residues in green frogs and their environment. © 2001 Published by Elsevier Science Ltd.

Keywords: Amphibians; Frogs; Malformations; Chemicals; DDT; PCBs; Metals

1. Introduction

Amphibians belong to a class of about 4550 species of caecilians, salamanders, toads, and frogs (Stebbins and Cohen, 1995). They occupy a dual role in the ecosystem, serving as predators to many insects and other small animals while acting as prey for a large number of animals, including reptiles, birds, and mammals. Due to the important role of amphibians in the ecosystem, a decline in their populations can have deleterious effects upon many different populations of animals and, thus, upon the many different ecosystems that they inhabit

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(Stebbins and Cohen, 1995). Furthermore, amphibians serve as excellent sentinels for the effects of chemical stressors during development. This is because, due to their complex life histories in both the aquatic and terrestrial environments and the permeability of their skin to water and electrolytes (Stebbins and Cohen, 1995), they are sensitive to a variety of factors. Therefore, a decline in amphibian populations or the expression of developmental abnormalities could be indicative of environmental changes, including exposure to xenobiotics.

Amphibian populations have been declining in various parts of the world for at least two decades (Wake, 1991). In February 1990, a workshop sponsored by the National Research Council's Board on Biology documented declines over the past twenty years in certain amphibian populations around the world. These declines include almost complete extirpation of boreal toads (*Bufo boreas boreas*) from their traditional breeding range in the West Elk Mountains of Colorado (Carey, 1993), and golden toads (*Bufo periglenes*) and the harlequin frog (*Atelopus varius*) from Costa Rica's Monteverde Cloud Forest Preserve in the mid-1980s (Pounds and Crump, 1994).

Recently, anatomical deformities of frogs have attracted international attention from both the scientific community and the public. In 1995, a group of Minnesota schoolchildren encountered a large number of northern leopard frogs (*Rana pipiens*) with hypermorphic (multiple digits and/or limbs) and hypomorphic (missing digits and/or limbs) limb abnormalities in a farm wetland in southern Minnesota (Kaiser, 1997). Malformed frogs and other amphibians had been studied previously in different regions; however, this incident brought deformed frogs into the spotlight of many environmental and scientific causes.

Deformed mink frogs (*Rana septentrionalis*) collected from a lake in Minnesota in 1996 and 1997 exhibited numerous limb abnormalities, the most notable being the presence of more than the normal number of hind limbs (Gardiner and Hoppe, 1999). Out of the 23 frogs examined by skeletal analysis, there were a total of 101 hind limbs. The majority of these hind limbs were abnormal due to skeletal dysplasia, characterized by triangles, hypomorphism, split limbs, and supernumary distal elements, and due to skin webbing (inter-limb, inter-segmental, and intra-segmental webbing). The authors speculated that the deformities were caused by exposure to degraded products of the insecticide methoprene that can mimic retinoic acid (Vitamin A), which is known to cause similar deformities under laboratory conditions (LaClair et al., 1998).

Populations of some of the 13 endemic frog and toad species in Michigan have declined in recent years (Michigan Department of Natural Resources MDNR, 1996), based upon frog call surveys at known and historical breeding locations. The Blanchard's cricket frog (*Acris crepitans blanchardi*) and the boreal chorus frog (*Pseudacris triseriata maculata*) are listed as "special concern" (Michigan Natural Features Inventory MNFI, 1999). In 1996, the northern leopard frog was observed at only 9% of its expected breeding sites. Likewise, the green frog (*Rana clamitans*), wood frog (*Rana sylvatica*), and the bullfrog were found at an average of 53, 47, and 4%, respectively, of their expected breeding sites (MDNR, 1996).

Several hypotheses have been proposed to explain these population declines and anatomical malformations. Several possible causative agents have been suggested to cause frog deformities. The proposed mechanisms can be classified as chemical, physical, or biological. Specifically, these hypotheses include the following: (1) chemical contamination in the water (Burkhart et al., 1998), (2) acid precipitation (Gannon, 1997), (3) increased levels of ammonia in the water (Jofre and Karasov, 1999), (4) increased ultraviolet radiation due to the thinning ozone layer (Ankley et al., 1998), and (5) parasites encysting in the developing limb buds (Sessions and Ruth, 1990). The aforementioned factors can also contribute to population declines, as well as general diseases, habitat destruction, climate changes, introduction of predators and competitors, and human consumption (Wake, 1991).

It is possible that the observed effects are caused by not one, but a combination of factors acting in concert (Wake, 1991). Furthermore, while the occurrence of deformities and declines in amphibian populations seem to be global in nature, there is probably no single universal factor to explain all of the population declines and deformities. For example, exposure to synthetic chemicals may be affecting some populations whereas increased UV irradiance may be involved in another region. Most of the proposed hypotheses suggest that humans are contributing to the demise of amphibians and may be indicative of environmental changes. However, most studies of amphibian populations have been conducted relatively recently. Without long-term census data, it will be difficult to determine whether population declines are due to natural fluctuations or anthropogenic factors (Pechmann et al., 1991). Amphibian deformities have been reported since the 1700s (Van Valen, 1974), which suggests that the deformities currently being observed are a natural phenomenon. Perhaps the increased attention on deformed frogs is leading to the greater number of sightings of frogs with developmental abnormalities. Therefore, more research will be required to fully understand the scope of the amphibian dilemma.

The UV radiation hypothesis has received some support, particularly in regions where the ozone layer has decreased (Gannon, 1997). A less dense ozone layer allows more UV radiation to reach the earth, where it can cause more damage to life (Berner and Berner, 1996). Deformed frogs have been found in the northern

latitudes, where increases in UV-B light have been shown to occur in late spring, early summer, and coinciding with the amphibian breeding season (Ankley et al., 1998). A recent study produced hind-limb deformities in leopard frog embryos by exposing them to UV-B radiation under laboratory conditions for more than 24 days (Renner, 1998). However, this study has also been the center of a great deal of debate, because the laboratory setting for this study does not duplicate actual environmental settings, as far as physical parameters in the water column and the amount of UV-B radiation received by the frog embryos. Another study indicated that treatment of leopard frogs with UV-B light can induce bilateral and often symmetrical ectromelia and ectrodactyly (missing limbs and digits). The authors of this study also admit that these conditions and effects are not representative of those in nature (Ankley et al., 1998).

The biological (parasite) explanation involves one or more types of parasite contributing to the deformities through a variety of mechanisms (Sessions and Ruth, 1990; Johnson et al., 1999). Two major studies have found that naturally occurring trematodes form cysts in amphibian limb buds, which potentially disrupts limb development and leads to a range of deformities. Recently, a fungus known as chytrid skin fungus was found in dead and dying frogs in Arizona (Milius, 1998; Lips, 1999). This fungus has not been known to infect vertebrates, but is the same fungus that is considered responsible for recent amphibian die-offs in Australia and Central America. While this fungus appears to be more responsible for amphibian deaths than deformities, it is still representative of the wide variety of parasites that can infect frogs. One possibility is that the fungal infections are secondary to lesions caused by the chemical or biological etiologies.

Chemicals, primarily pesticides and industrial byproducts, are ubiquitous in the environment. Even remote areas experience some degree of contamination due to atmospheric transport of pollutants, such as polychlorinated biphenyls (PCBs), DDT, and mercury compounds (Wania et al., 1998). Frogs spend the greater part of their developmental stages in the aquatic environment; hence, they can be directly exposed during their critical growth period to any chemicals that may be in the water (Stebbins and Cohen, 1995). Thus, some of these chemicals have the capacity to cause growth anomalies. For instance, a recent study in the Quebec region found more frogs with deformed hind limbs in areas where historically pesticides have been used than from areas that have not been exposed to pesticides (Ouellet et al., 1997). However, no concentrations of residues were measured. Thus, no correlations or gradients of deformities and exposure to chemicals could be established. One pesticide shown to cause life-threatening deformities in leopard frogs is methoprene (Ankley et al., 1998). Degradation products of methoprene have been suggested to affect the retinoid-signaling pathway via interaction with one or more retinoid receptors (Ankley et al., 1998). Many of the compounds currently being studied in relation to the deformity issue have been characterized as environmental endocrine disruptors, compounds that disrupt the normal functioning of the endocrine system.

To document deformities in southwestern Michigan, an amphibian deformity survey was initiated in 1998. This deformity survey was a part of a larger study that attempted to link deformities to mechanical disruption and chemical contamination. The objectives of this study were to: (1) assess the incidence of green frog (*Rana clamitans*) deformities in southwestern Michigan; (2) determine concentrations of organochlorine insecticides, PCBs, and metals in adult green frogs, juveniles, tadpoles, and egg tissues; (3) determine the levels of these compounds in the water and/or sediment inhabited by green frogs; and (4) correlate the incidence of deformities to chemical concentrations in the frogs and/or their environment.

2. Materials and methods

The study region was southwestern Michigan (Fig. 1). This region was chosen because of the variety of habitats

Fig. 1. Counties of southwestern Michigan from which green frogs were collected, 1998.

available. The sampling locations ranged from agricultural areas, reference locations, and locations with known historical and current PCB/pesticide contamination (Table 1). Other regions of Michigan are not as likely to have the available green frog habitat amidst these different locations. These seven locations were chosen to give a wide variety of sampling sites with a wide range of potential chemical influences. The reference locations (REF1, REF2, and REF3) are within the Allegan State Game Area in Allegan County, an area of successional forests that is not currently directly impacted by agriculture or urban activity. The agricultural areas (AGR1, AGR2, and AGR3) are located in St. Joseph and Cass Counties, in a region characterized by cash crop farming of corn and the usage of triazine herbicides, most notably atrazine. The Kalamazoo River passes through urbanized and industrialized areas as well as agricultural areas. The sampling location for this area (KLMZR) is a marsh created by a nearby dam on the river in Kalamazoo County. Specific location ranges are given for the reference and Kalamazoo River sampling locations (Table 1). Specific locations cannot be given for the agricultural sampling locations based on request made by the farmers who own the land.

There were four sampling dates during the summer of 1998 – June 5, June 23, July 22, and August 26. Sampling for tadpoles took place during the daylight hours, while sampling for adults occurred between sunset and sunrise. The sampling dates were chosen based on agricultural applications of triazine herbicides, which would potentially impact the herbicide concentrations of the agricultural locations. The sampling was conducted early in the summer after triazine applications, then later in the summer when less herbicides, if any, were applied (Mehne, personal communication).

The green frog (*R. clamitans*) was selected for study for three reasons. First, the green frog is one of the most abundant frogs in the Great Lakes region (Harding, 1997); thus, these frogs are relatively easy to locate and the study will not be negatively impacting a species with an already small population. Second, green frogs tend to remain in one aquatic location exhibiting less of a tendency to migrate to upland, dryer areas than other frogs (Harding, 1997). Therefore, these frogs are more likely to be affected by chemicals in the body of water where they are caught. Lastly, green frogs are relatively large frogs that eat a variety of prey from plant material to insects to smaller frogs and snakes (Harding, 1997). Since part of their diet places them at a higher trophic level, residues have the potential to bioaccumulate in their bodies, with the possibility of being passed along through the eggs to their offspring (Lu, 1991).

Tadpoles, juveniles, and adults were collected by either dip net or hand. Most adults were collected after sunset. Tadpoles were identified according to Watermolen and Gilbertson (1996). All frogs were examined for anatomical deformities, such as malformations of the tail, eyes, and mouth. Additionally, adults and juveniles were examined for hypomorphic and hypermorphic limb abnormalities. Possible deformities were carefully examined to ensure that the deformity was not simply a wound or scar tissue. For instance, a missing limb may have at first appeared to be a hypomorphic limb abnormality. However, with more careful scrutiny, the skin in the region of where the limb should be may have scar tissue, which would indicate that this ''deformity'' was

Table 1
Sampling location characteristics

Site	Characteristics
REF1	118th pond, T.2N. R.14W., section 17. Permanent pond, depth 0–5 ft, various grasses border the pond edge, lily pads, submergent vegetation present. This sampling location is surrounded by mixed hardwood forest.
REF2	Crooked Lake, T.2N. R.15W., section 36. Small lake, depth 0–8 ft, cattails and various grasses border the lake edge, submergent vegetation present. This sampling location is surrounded by mixed hardwood forest.
REF3	Swan Creek, T.2N R.14W., section 20. Marsh in a drainage area, depth of 0–8 ft, various grasses border the marsh edge, submergent vegetation is sparse. This sampling location is surrounded by mixed hardwood forest.
KLMZR	Kalamazoo River, T.2N R.14W., section 10. Marsh in a drainage area, formed from a man-made dam along the Kalamazoo River, depth 0–6 ft, cattails, various grasses, and mixed hardwoods border the marsh edge, submergent vegetation present.
AGR1	Temporary pond, depth 0–5 ft, cattails, saw grass, and other various grasses border the pond edge, submergent vegetation present. Surrounded by corn fields in an agricultural region.
AGR2	Permanent pond, depth 0–3 ft, cattails and various grasses border the pond edge, submergent vegetation present. Surrounded by corn fields in an agricultural region.
AGR3	Drainage ditch, depth 0–3 ft, cattails and various grasses border the ditch edge, no submergent vegetation. This sampling location is located in an agricultural region.

actually a wound caused by predation. The majority of frogs captured were immediately released, after examination, back into the same locations from which they were captured. Only the minimal number of frogs necessary for future analyses was kept.

PCBs and OC pesticides were extracted following methods described elsewhere (Kannan et al., 1995; Khim et al., 1999). PCBs and OC pesticides in the water and tissue samples were quantified using a gas chromatograph (Perkin Elmer series 600) equipped with ^{63}Ni electron capture detector (GC-ECD), as described by Khim et al. (1999).

Concentrations of Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Ni, Pb, Sc, Sr, Ti, and Zn were determined in sediment and tissue by nitric acid digestion followed by ICP-MS (Platform ICP-MS, Micromass, Inc., Manchester, England). Samples were introduced with a concentric nebulizer that was connected to a CETAC ASX-500 auto-sampler. Data were treated statistically to test for the significance of differences in mean concentrations ($\alpha = 0.05$).

3. Results

3.1. Deformities

A total of 1445 green frog adults, juveniles, and tadpoles were examined from all four sampling dates and all the sampling locations (Table 2). Of these, four were deformed. One adult had six toes on its right hind leg, one juvenile had a shortened forelimb, one juvenile had only three legs, and another juvenile had a malformed rear foot (Table 2).

A greater number of frogs from REF1 were deformed than from the other sampling locations. More frogs were collected from REF1 than from any of the other sampling locations; therefore, the overall percentage of deformed frogs from this site (0.34%) was actually less than the overall percentage of deformed frogs from KLMZR (0.38%) (Table 3). The overall deformity rate among the green frogs collected from southwestern Michigan was 0.28% (Table 3). This percentage is less than observed deformity rates from other

Table 2
Number of green frogs collected, including number of deformities, from southwestern Michigan, 1998

Location	Adult	Juvenile	Tadpole
5 June 1998, N = 154			
REF1	17, 1 deformed[a]	0	20
REF2	0	0	12
REF3	3	0	20
KLMZR	18	0	20
AGR1	0	0	21
AGR2	0	0	22
AGR3	1	0	0
23 June 1998, N = 417			
REF1	68	8	202
REF3	7	7	20
KLMZR	11	0	30
AGR1	24	0	0
AGR2	2	0	20
AGR3	14	0	4
22 July 1998, N = 636			
REF1	52	145, 1 deformed[b]	230
KLMZR	48	24	29
AGR1	3	0	0
AGR2	3	38	0
AGR3	3	61	0
26 August 1998, N = 238			
REF1	8	30, 1 deformed[c]	98
KLMZR	3	47, 1 deformed[d]	35
AGR2	10	4	0
AGR3	2	1	0
Total frogs = 1445			

[a] Six toes on right hind leg.
[b] Shortened forelimb.
[c] Missing hind limb.
[d] Malformed rear foot.

Table 3
Prevalence of deformities, and total number of green frogs by location, in southwestern Michigan, 1998

Location	Overall frog count	Number deformed	Percent deformed
REF1	878	3	0.34%
REF2	12	0	0
REF3	57	0	0
Total REF	947	3	0.32%
KLMZR	265	1	0.38%
AGR1	48	0	0
AGR2	99	0	0
AGR3	86	0	0
Total AGR	233	0	0

regions. For instance, 50% of the leopard frogs caught by the school children in the Minnesota wetland in 1995 were deformed (North American Reporting Center for Amphibian Malformations NARCAM, 2000). Of the 853 anurans observed at the agricultural locations near Quebec, 12% exhibited physical malformations (Ouellet et al., 1997).

It is important for the accuracy of the deformity survey to look at all the life stages of the frogs. For instance, if a tadpole were to have extreme deformities, it may not survive to adulthood. The same is true for juvenile frogs. If it were missing a leg, then it may be preyed upon and not survive to adulthood. Therefore, a more accurate deformity count was conducted by examining frogs of all life stages.

3.2. Organochlorine residues

For many of the frog samples, there were no significant differences in concentrations of organochlorines among locations. Concentrations of the isomers of HCH, the isomers of chlordane, and p,p'-DDT and its metabolites in all water samples were less than the method detection limit (MDL) of 1.0 ng/l. For individual isomers and metabolites of HCH, chlordane, and DDT, concentrations of p,p'-DDE and p,p'-DDD were greatest in adults (0.61 ng/g, wet wt for each) (Table 4). There were no significant differences among sampling locations in concentrations of any of the OCs in adult tissues.

Tadpoles contained lesser concentrations of OC insecticides than did the adults. Most concentrations of individual isomers were less than the MDL (0.01 ng/g, wet wt), with p,p'-DDD being the greatest at 0.09 ng/g, wet wt (Table 4). There were no significant differences in concentrations of OCs in juvenile tissues among locations.

Some differences in concentrations of residues in tadpole tissue among locations were observed. Concentrations ranged from less than 0.01 ng/g, wet wt for p,p'-DDT to 0.29 ng/g, wet wt for p,p'-DDD (Table 4), but were not statistically different among locations. Concentrations of OC insecticides in eggs ranged from less than 0.01 ng/g wet wt for many of the residues to 5.86 ng/g wet wt for p,p'-DDE (Table 4).

Concentrations of PCBs in all water samples were less than the MDL of 1.0 ng/l. PCBs were detected in tissues of adult, juvenile, tadpole, and eggs of the green frogs. There were no significant differences in concentrations of PCBs among locations. The least concentration (7.63 ng/g, wet wt) was observed in tadpoles while the greatest concentration (79.11 ng/g, wet wt) was observed in eggs (Table 4).

There were no significant differences in metal concentrations between the upper and lower layers of sediments. Therefore, sediment layers were analyzed as individual samples. Concentrations of five of the 20 elements analyzed (Al, Ca, Co, Mg, Sr) in sediments differed significantly among locations when the MDL was substituted for non-detectable concentrations (Table 1). For those elements that did not differ significantly among locations, the least concentrated were Cd ($\leqslant 0.21$ μg/g dry wt), Hg ($\leqslant 0.22$ μg/g dry wt), Ni ($\leqslant 2.0$ μg/g dry wt), Sc ($\leqslant 2.4$ μg/g dry wt), and As ($\leqslant 3.8$ μg/g dry wt). The most concentrated elements in sediment that did not differ significantly among locations were Fe (9.5×10^2 μg/g dry wt), Mn (3.6×10^2 μg/g dry wt), K (2.3×10^2 μg/g dry wt), Ba (1.0×10^2 μg/g dry wt), and Cr (42 μg/g dry wt) (Tables 5 and 6).

Concentrations of four of the elements analyzed (Ba, Ca, Mg, and Ti) in adult livers differed significantly among locations when the MDL was substituted for non-detectable concentrations (Table 6). Concentrations of elements that did not differ significantly among location included Cd ($\leqslant 0.08$ μg/g dry wt), As ($\leqslant 0.10$ μg/g dry wt), Co ($\leqslant 0.13$ μg/g dry wt), and Sc ($\leqslant 0.23$ μg/g dry wt) at the low end to K (3.3×10^3 μg/g dry wt), Fe (1.1×10^3 μg/g dry wt), Cu (65 μg/g dry wt), Al (58 μg/g dry wt), and Zn (27 μg/g dry wt) (Table 6).

Juvenile tissues were most concentrated with Ca (2.8×10^4 μg/g dry wt), Fe (1.9×10^4 μg/g dry wt), K (4.2×10^3 μg/g dry wt), Mg (1.0×10^3 μg/g dry wt), and Al (3.7×10^2 μg/g dry wt). Metals that were least concentrated in juvenile tissues were Cd ($\leqslant 0.03$ μg/g dry wt), Hg ($\leqslant 0.17$ μg/g dry wt), Co ($\leqslant 0.20$ μg/g dry wt), and As ($\leqslant 0.30$ μg/g dry wt) (Table 6).

Tadpole tissues contained the greatest concentrations of Fe (6.5×10^4 μg/g dry wt), Ca (2.3×10^4 μg/g dry wt), K (5.8×10^3 μg/g dry wt), Mg (1.0×10^3 μg/g dry wt), and Al (5.9×10^2 μg/g dry wt). Metals that were least concentrated in tadpole tissues were Cd (0.23 μg/g dry wt), Hg ($\leqslant 0.24$ μg/g dry wt), Co ($\leqslant 0.41$ μg/g dry wt), and Sc ($\leqslant 0.80$ μg/g dry wt) (Tables 6 and 7).

Table 4
Concentrations (ng/g, wet wt, mean and standard deviation, in parentheses) of organochlorine insecticides and total PCBs in green frog tissue from southwestern Michigan, 1998

Insecticide	Adult[a]	Juvenile[b] ($n=2$)	Tadpole[b] ($n=10$)	Egg[c] ($n=5$)
Values calculated with 0.00 substituted in for the non-detects				
α-HCH	0.02 (0.03)	0.04 (0.04)	0.07 (0.09)	0.00*
β-HCH	0.01 (0.02)	0.00*	0.08 (0.15)	0.00*
γ-HCH	0.06 (0.04)	0.00*	****	0.16 (0.19)
δ-HCH	0.03 (0.07)	0.00*	0.15 (0.27)	0.00*
α-chlordane	0.01 (0.01)	0.00*	****	0.18 (0.30)
γ-chlordane	0.04 (0.04)	0.00*	****	0.10 (0.08)
p,p'-DDT	0.03 (0.07)	0.00*	0.00*	****
p,p'-DDE	0.61 (0.97)	0.01 (0.01)	0.08 (0.14)	5.86 (2.92)
p,p'-DDD	0.61 (0.51)	0.09 (0.01)	0.29 (0.31)	1.71 (2.96)
∑HCH	0.12 (0.12)	0.04 (0.04)	0.33 (0.50)	0.34 (0.37)
∑chlordane	0.05 (0.05)	0.00*	****	0.29 (0.32)
∑DDT	1.24 (1.19)	0.10 (0.01)	0.37 (0.40)	7.91 (5.32)
∑PCBs	19.62 (41.01)	19.12 (5.74)	7.63 (8.35)	79.11 (89.82)
Values calculated with the MDL substituted in for the non-detects				
α-HCH	0.03 (0.03)	0.04 (0.04)	0.08 (0.08)	0.01 (0.00)
β-HCH	0.02 (0.01)	0.01 (0.00)	0.08 (0.14)	0.01 (0.00)
γ-HCH	0.06 (0.04)	0.01 (0.00)	****	0.16 (0.19)
δ-HCH	0.04 (0.07)	0.01 (0.00)	0.16 (0.27)	0.01 (0.00)
α-chlordane	0.01 (0.01)	0.01 (0.00)	****	0.19 (0.30)
γ-chlordane	0.04 (0.04)	0.01 (0.00)	****	0.11 (0.07)
p,p'-DDT	0.03 (0.07)	0.01 (0.00)	0.01 (0.00)	****
p,p'-DDE	0.61 (0.97)	0.02 (0.00)	0.09 (0.14)	5.86 (2.92)
p,p'-DDD	0.61 (0.51)	0.09 (0.00)	0.29 (0.30)	1.71 (2.96)
∑HCH	0.12 (0.12)	0.04 (0.04)	0.34 (0.50)	0.34 (0.37)
∑chlordane	0.05 (0.05)	0.01 (0.00)	****	0.29 (0.32)
∑DDT	1.24 (1.19)	0.10 (0.01)	0.37 (0.39)	7.98 (5.32)
∑PCBs	19.62 (41.01)	19.12 (5.74)	7.63 (8.35)	79.11 (89.82)

[a] $n=21$ for insecticide analysis and $n=22$ for PCB analysis.
[b] These were pooled samples.
[c] Two of the five samples were pooled.
* No standard deviations are presented here because the values were below the MDL and none could be calculated.
**** At the 95% confidence level, there were significant differences among locations so a total mean could not be calculated.

Table 5
Concentration (ng/g, wet wt, mean and standard deviation, in parentheses) of organochlorine insecticides and total PCBs in green frog tissue from sampling locations that are significantly different at the 95% confidence level in southwestern Michigan, 1998

	REF1	REF2	REF3	KLMZR	AGR1	AGR2	AGR3
Tadpole							
γ-HCH	0.04 (0.05)	0.01*	0.01 (0.00)	0.01 (0.00)	0.25*	0.01*	0.04*
α-chlordane	0.01 (0.00)	0.04*	0.02 (0.02)	0.01 (0.00)	0.06*	0.14*	0.01*
γ-chlordane	0.01 (0.00)	0.02*	0.01 (0.01)	0.01 (0.00)	0.03*	0.01*	0.01*
∑-chlordane	0.01 (0.00)	0.06*	0.02 (0.03)	0.01 (0.00)	0.09*	0.14*	0.01*
Egg							
p,p'-DDT	0.01 (0.00)	NA[a]	NA[a]	0.01 (0.00)	1.40*	NA[a]	0.75*

[a] NA = Not analyzed.
* No standard deviation is presented because $n=1$ pool for these samples.

4. Discussion

The fact that few deformities were observed during this survey may be indicative of a relatively stable green frog population in southwestern Michigan and that the residues measured were either not the causative agent of deformities observed or at least not occurring at sufficient concentrations at these locations to cause

Table 6
Metal concentrations (μg/g, dry wt, mean and standard deviation, in parentheses) in sediment and green frog tissue from southwestern Michigan, 1998

Metal	Adult[a]	Juvenile[b]	Tadpole[c]	Sediment[d]
Values with 0.0 substituted for non-detects				
Al	57 (1.2×10^2)	3.7×10^2 (3.3×10^2)	5.9×10^2 (2.1×10^2)	****
As	0*	0*	0*	0*
Ba	****	30 (27)	35 (36)	66 (85)
Ca	2.4×10^2 (3.3×10^2)	2.8×10^4 (1.1×10^4)	2.0×10^4 (1.4×10^4)	2.0×10^4 (2.2×10^4)
Cd	0*	0*	0*	0.18 (0.28)
Co	0*	0*	0*	0*
Cr	0*	0*	0*	0*
Cu	65 (30)	1.4 (2.5)	1.8 (3.7)	2.1 (7.3)
Fe	1.1×10^3 (2.3×10^3)	1.9×10^4 (2.7×10^4)	6.5×10^4 (5.8×10^4)	6.8×10^2 (1.6×10^3)
Hg	0*	0*	0*	0*
K	****	4.3×10^3 ($6.5\times10^{2)}$	5.5×10^3 (3.6×10^3)	1.5×10^2 (5.0×10^2)
Mg	****	1.0×10^3 (4.5×10^3)	9.2×10^2 (6.5×10^2)	****
Mn	0*	50 (86)	2.8×10^2 (4.6×10^2)	3.6×10^2 (7.0×10^2)
Ni	0.61 (2.5)	0*	0*	2.0 (5.6)
Pb	0*	3.9 (3.6)	0*	20 (14)
Sc	0*	0*	0*	0*
Sr	0*	5.8 (10)	4.0 (11)	****
Ti	1.3 (1.5)	17 (8.2)	22 (8.0)	8.2 (14)
Zn	23 (14)	49 (25)	30 (32)	0*
*Values with MDL** substituted for non-detects*				
Al	58 (1.2×10^2)	3.7×10^2 (3.3×10^2)	5.9×10^2 (2.1×10^2)	****
As	0.10 (0.14)	0.30 (0.25)	1.3 (0.82)	3.8 (3.1)
Ba	****	34 (21)	45 (28)	1.0×10^2 (59)
Ca	****	2.8×10^4 (8.0×10^2)	2.3×10^4 (2.0×10^3)	****
Cd	0.08 (0.11)	0.03 (0.04)	0.23 (0.37)	0.21 (0.27)
Co	0.13 (0.17)	0.20 (0.21)	0.41 (0.27)	****
Cr	8.3 (18)	8.8 (5.6)	9.5 (8.4)	41 (61)
Cu	65 (30)	4.0 (2.3)	4.2 (2.7)	13 (7.2)
Fe	1.1×10^3 (2.3×10^3)	1.9×10^4 (2.7×10^4)	6.5×10^4 (5.8×10^4)	9.5×10^2 (1.5×10^3)
Hg	0.43 (0.79)	0.17 (0.12)	0.24 (0.17)	0.22 (0.34)
K	3.3×10^3 (7.2×10^2)	4.3×10^3 (6.5×10^2)	5.8×10^3 (3.1×10^3)	2.3×10^2 (5.6×10^2)
Mg	****	1.0×10^3 (4.5×10^2)	1.1×10^3 (5.1×10^2)	****
Mn	2.9 (4.8)	73 (73)	3.2×10^2 (4.4×10^2)	3.6×10^2 (7.0×10^2)
Ni	0.9 (2.6)	2.9 (0.01)	2.9 (0.01)	2.0 (5.6)
Pb	0.76 (0.01)	3.9 (3.6)	1.6 (1.5)	24 (9.9)
Sc	0.23 (0.18)	0.53 (0.23)	0.80 (0.39)	2.4 (0.88)
Sr	0.31 (0.69)	11 (6.4)	13 (8.5)	****
Ti	****	17 (8.2)	22 (8.2)	19 (11)
Zn	27 (7.9)	49 (25)	46 (20)	24 (18)

[a] $n=16$ livers.
[b] $n=3$ pooled samples.
[c] $n=7$ pooled samples.
[d] $n=12$ samples of top and bottom layers.
* No standard deviations are presented here because the values were below the MDL and none could be calculated.
** The MDL is a function of sample mass and recovery, and varies from sample to sample. The range of concentrations is presented in this table by substituting in both 0.0 and the MDLs for non-detected concentrations.
**** At the 95% confidence level, there were significant differences among locations so a total mean could not be calculated.

deformities. While some Michigan frog populations have been reported to have declined (MDNR, 1996), reports of deformities have been rare. Other reasons for the low deformity rate observed could be the possibility that any deformities caused lethality to the frogs earlier in the season or in earlier life stages before they could be recorded.

The differences observed among locations of OC insecticide concentrations for tadpole and egg tissues can be due to a variety of factors. First, general experimental

Table 7
Metal concentrations (μg/g dry wt, mean and standard deviation, in parentheses)[a] in sediment and green frog tissue from sampling locations that are significantly different at the 95% confidence level in southwestern Michigan, 1998

	REF1	REF3	KLMZR	AGR1	AGR2	AGR3
Adult						
Ba	12 (17)	37*	4.2 (5.11)	1.8 (2.7)	16 (6.7)	NS[b]
Ca	1.8×10^2 (30)	$8.6\times10^{2*}$	1.1×10^2 (32)	3.7×10^2 (9.9)	6.0×10^2 (3.1×10^2)	NS
Mg	2.5×10^2 (55)	$4.8\times10^{2*}$	2.6×10^2 (26)	3.0×10^2 (28)	2.2×10^2 (25)	NS
Ti	3.3 (0.74)	4.1*	2.8 (0.28)	3.2 (0.38)	2.2 (0.63)	NS
Sediment						
Al	3.3×10^3 (1.2×10^3)	3.1×10^3 (1.4×10^3)	3.7×10^3 (6.9×10^2)	NS	9.9×10^3 (2.7×10^3)	3.9×10^3 (3.0×10^2)
Ca	1.3×10^4 (3.5×10^3)	1.3×10^4 (1.8×10^2)	1.7×10^5 (2.8×10^4)	NS	3.7×10^3 (2.0×10^3)	6.5×10^3 (1.5×10^3)
Co	0.09 (0.04)	1.9 (0.42)	2.5 (1.0)	NS	6.4 (2.4)	1.5 (0.06)
Mg	1.2×10^3 (2.4×10^2)	1.9×10^3 (1.8×10^2)	4.6×10^3 (7.3×10^2)	NS	1.3×10^3 (6.2×10^2)	1.1×10^3 (1.8×10^2)
Sr	21 (8.9)	28 (12)	96 (21)	NS	13 (1.3)	9.5 (0.78)

[a] Values using the MDL for non-detects.
[b] NS = Not sampled.
* No standard deviations could be calculated for these because $n = 1$.

variation can be involved. Second, it is possible that the differences among locations observed for these two life stages are due to the greater lipid content of the eggs and tadpoles. Third, tadpoles may have a greater exposure to residues in the water and sediment, because they feed exclusively on aquatic materials and do not leave the aquatic system. Lastly, perhaps older life stages may have the ability to metabolize and excrete the residues at a faster rate.

When all of the locations were analyzed together, the egg tissue contained significantly greater concentrations of α-chlordane, γ-chlordane, p,p'-DDE, p,p'-DDT, ∑DDT, and ∑chlordane than did tadpoles, juveniles, and adults. This is likely due to the greater lipid content of the eggs. OCs tend to accumulate in lipids because of their low polarity and high octanol–water partitioning coefficient (K_{ow}). For instance, the concentrations of ∑DDT in adult, juvenile, tadpole, and egg lipid tissue were 119, 5.4, 14, and 75 ng/g, wet wt, respectively. Therefore, because the adults have a lower lipid content than the eggs (by an average factor of 10.3), ∑DDT is actually more concentrated in the lipids of the adults than in the lipids of the eggs. A similar pattern in concentrations of γ-chlordane, p,p'-DDE, p,p'-DDT, and ∑chlordane was observed. Adult green frogs consume a variety of prey items, including aquatic and terrestrial insects, other invertebrates, smaller frogs, snakes, and hatchling turtles (Harding, 1997). Therefore, their diet allows them to be comparatively high on the food chain. Thus, they have the capacity to bioaccumulate organochlorine residues. Also, adults, being older than the other life stages analyzed, have had a longer time to accumulate such residues.

Concentrations of OCs in green frogs in the present study were less than those reported in adult spring peepers (*Pseudacris crucifer*) collected from Point Pelee National Park in southern Ontario in April 1993 (Russell et al., 1995). Historically, DDT had been applied extensively to this park as mosquito control. ∑DDT concentrations reported in this study were as great as 1188 ng/g, wet wt. The authors hypothesized that these relatively high concentrations were due primarily to historical applications of DDT. Half of the amphibian fauna once present at this location has disappeared over the last 50 years. The authors attributed these disappearances to toxic concentrations of pesticides. A later study in neighboring locations of southern Ontario near Point Pelee found lesser concentrations of pesticide residues in adult green frog tissue (Russell et al., 1997). Concentrations of p,p'-DDE in green frog tissue ranged from 0.58 to 45 ng/g, wet wt. At these locations, green frogs were the dominant anuran, indicating that at these OC concentrations the green frog population appeared to not be depleted. The lesser concentrations, in that study, are more similar to the concentrations of p,p'-DDE in this study.

Concentrations of α-HCH, β-HCH, lindane, p,p'-DDD, p,p'-DDE, heptachlor, dieldrin, o,p'-DDD, o,p'-DDE, o,p'-DDT, p,p'-DDT, heptachlor epoxide, aldrin, and endrin in tissues of heron adults, nestlings, and eggs and in the tissues of their primary prey, frogs (*Rana* spp.) collected in May and June of 1992 and 1993 from wetlands of Thermaikos Gulf, Macedonia, Greece were measured (Albanis et al., 1996). Concentrations of residues in water ranged from <0.01 to 0.21 μg/l. Few residues were detected in adult frogs, with the exception of

α-HCH, β-HCH, lindane, *p,p′*-DDD, *p,p′*-DDE, and heptachlor. Average concentrations of α-HCH, β-HCH, lindane, *p,p′*-DDD, and *p,p′*-DDE in the frogs were 2.45, 0.56, 3.64, 0.49, and 0.29 ng/g, dry wt, respectively. Generally, the wet weight to dry weight ratio is five. Therefore, these reported values could be divided by five to compare to the concentrations from the present study. Overall, the concentrations of HCH in the frogs in the study in Greece would still be greater by a factor of about 10 on average, while the concentrations of DDD and DDE in Greece would be less by a factor of about 8.

Twenty OC pesticides and 39 PCB congeners were determined in mudpuppies and snapping turtles collected from 1988 to 1992 from the St. Lawrence River in Canada, a river that is greatly impacted by human activities and synthetic chemicals (Bonin et al., 1995). Concentrations of DDD, DDE, DDT, α-HCH, lindane, *trans*-chlordane, and *cis*-chlordane in whole mudpuppies were 1.2 to 24.8, 0.3 to 90.0, ND to 8.3, ND to 6.8, ND to 3.3, ND to 7.1, and 0.3 to 13.9 ng/g, wet wt, respectively. Concentrations of PCBs in whole mudpuppies ranged from 92 to 1082 ng/g, wet wt. These values are slightly greater than the concentrations observed in the present study.

Concentrations of PCBs and OC pesticides were determined in green frogs and leopard frogs collected along the north shores of Lake Erie and Lake Ontario in Ontario, Canada (Gillan et al., 1998). Sediments were also collected in an effort to determine biota-sediment accumulation factors (BSAFs), as well as to determine the cytotoxicity and genotoxicity of the sediments. Concentrations of *p,p′*-DDE in green frogs ranged from 19 ng/g lipid wt in a large pond in a public park area to 754 ng/g lipid wt from a small pond in an agricultural region. Concentrations of *p,p′*-DDT in green frogs ranged from ND in a large pond in a public park area to 60 ng/g lipid wt in a large marsh in a conservation area. Concentrations of *p,p′*-DDE in leopard frogs ranged from 60 ng/g lipid wt in a shallow creek in a conservation area to 659 ng/g lipid wt from a large pond in a provincial region. Concentrations of *p,p′*-DDT in green frogs ranged from ND in a few sites to 80 ng/g lipid wt in a small pond in an agricultural area. In the present study, the mean percent lipid content of the adult frogs was 1.04%. Therefore, if concentrations were normalized by lipid weight, the mean concentration of *p,p′*-DDE in adult green frogs from southwestern Michigan is 57.86 ng/g, lipid wt, and that of *p,p′*-DDT is 2.86 ng/g lipid wt. The value for DDE are within the ranges of the green frogs studied in the Ontario study, and slightly lower than the concentrations in leopard frogs. The value for DDT is within the concentration ranges for both green and leopard frogs in the Ontario study.

Concentrations of PCBs measured in green frogs in regions of southern Ontario were in the low ng/g range (Russell et al., 1997). PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzo-*p*-furans (PCDFs) were measured in northern leopard frogs collected from the lower Fox River and Green Bay regions of Wisconsin in the summers of 1994 and 1995. The concentrations of total PCBs ranged from 3 to 154 ng/g, wet wt. One PCDD and two PCDFs at concentrations of 6–8 pg/g were also found in the frogs from one site (Huang et al., 1999). The concentration of total PCBs measured in the present study are comparable to those in Wisconsin. The small concentrations of TEQs in this study were probably due to the fact that concentrations of PCBs were also small.

OC pesticides were determined in fat bodies of breeding male green frogs in 1993 and 1994 from orchard wetlands in rural areas of southern Ontario (Harris et al., 1998). Concentrations of DDD, DDE, and DDT ranged from ND to 0.21 μg/g, 0.05 to 2.81 μg/g, and ND to 0.46 μg/g, respectively. As calculated previously, the mean lipid concentrations of *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT of adult green frogs in the present study were 57.86, 57.86, and 2.86 ng/g, respectively. Because of the large range of concentrations in the Ontario study, the values for the current study in southwestern Michigan are similar, albeit at the very low end.

Hepatic ethoxy-resorufin-*o*-deethylase (EROD) activity is a metabolic response that is mediated by the Ah-receptor upon exposure to dioxins and dioxin-like compounds (Huang et al., 1999). In an aforementioned study (Huang et al., 1999), no significant correlation was found between EROD activity and PCB concentration. This result was consistent with the fact that the frogs from Wisconsin contained relatively small concentrations of PCBs (<152 ng/g wet wt) compared with what was required for induction in the laboratory (ED50 for EROD is between 700 and 2300 ng/g wet wt). Concentrations of PCBs in green frog tissue from the present study in southwestern Michigan were lesser than those observed in frog tissue from Wisconsin (<20 ng/g wet wt in adults); hence, it would be unlikely that significant EROD induction would be observed in these tissues.

The toxicities of some insecticides to amphibians have been determined. Static bioassays to determine the relative acute toxicities of pesticides to western chorus frog (*Pseudacris triseriata*) and Fowler's toad (*Bufo fowleri*) tadpoles found that endrin was the most toxic OC insecticide to the chorus frog tadpoles and the second most toxic insecticide to the Fowler's toad tadpoles (Sanders, 1970). Lindane was the least toxic insecticide to both frog and toad 4 and 5 week old tadpoles, with a 96 h TL50 value of 4.4 mg/l for toads and a 96 h TL50 value of 2.7 mg/l for frogs. Median tolerance limit (TL50 or LC50) values for toad tadpoles decreased with age (Sanders, 1970). For example, the 96 h TL50 value for 1 week old Fowler's toad tadpoles exposed to waterborne

exposures of DDT was measured at 0.75 mg/l, while the 96 h TL50 for 7 week old tadpoles was determined to be 0.03 mg/l. This indicates that DDT might be most toxic to advanced tadpole developmental stages (Sanders, 1970). Dieldrin was observed to have approximately the same 96 h TL50 values as DDD in Fowler's toad tadpoles (0.15 mg/l) but was found to be eight times more toxic than DDT to western chorus frog tadpoles (0.10 mg/l versus 0.80 mg/l) (Sanders, 1970). These values are much greater than those concentrations measured in the water samples in the present study. Exposure of tadpoles to dieldrin resulted in structural abnormalities, lesser rates of development, and changes in behavior (Cooke, 1972).

DDT has been shown to adversely impact the development and behavior of common European frogs (Cooke, 1970). Uncoordinated activity, weight loss, and restricted development were observed in tadpoles following 1 h exposures to 0.1, 1.0, and 10.0 mg/l aqueous concentrations of DDT. After treatment, tissue concentrations of DDT ranged from 140 to 180 μg/g (Cooke, 1970). Tadpoles exposed for 24 and 48 h to aqueous concentrations of DDT (ranging from 0 to 0.5 mg/l) have exhibited hyperactive behavior either just before or just after developing limb buds (Cooke, 1972). Tissue concentrations of whole tadpoles with this behavior ranged from 2000 to 4000 μg/kg (Cooke, 1972). Exposures to aqueous DDT at 0.1 mg/l for 2 d produced histological changes in mandibles of some tadpoles (Osborn et al., 1981). This has been attributed to the disruptive effect DDT has on the development of skin glands in the region above the upper mandible and to the hyperactivity that DDT causes in tadpoles (Osborn et al., 1981). Effects of DDT exposure were more severe for acute exposures than for chronic exposures even though the two types of exposure resulted in the same concentrations in tadpoles (Cooke, 1973). The degree of crowding in laboratory populations was positively related to the severity of sub-lethal effects, including hyperactivity and mandibular deformities, due to exposure to DDT (Cooke, 1979). Hyperactivity caused by sublethal exposure to DDT in tadpoles has been postulated to potentially result in greater risk from predation (Cooke, 1971). None of these previously observed abnormalities were observed in the tadpoles of the present study in southwestern Michigan, as would be expected due to the lower water concentrations of DDT.

Many of the metals found at relatively low concentrations in sediments, such as Cd, Hg, Ni, Sc, As, and Sr, are usually not present in most sediments and are generally considered to be toxic, at high enough concentrations (Bunce, 1994). However, because most were below the limit of detection, and may even be bound in the soil, which reduces their bioavailability, they are not considered to be a potential threat to the wildlife, such as frogs, of the aquatic system. The more concentrated metals, such as Al, Ca, Mg, Fe, Mn, and K are components of biological systems and/or sediments; therefore, the high concentrations are not alarming. As stated above, the greatest concentrations of Al and Co were observed in the sediment at AGR2. This may be due to natural variation, because these concentrations were within acceptable levels. The same is true for the increased concentrations of Ca, Mg, and Sr at KLMZR.

A similar pattern of metal concentrations is observed in frog tissues. Concentrations of toxic metals, such as Pb, Cd, As, Co, Hg, Cr, Sc, and Sr, in adult livers were relatively low. Essential metals, such as Ca, Mg, Al, Fe, and K exhibited the greatest concentrations in adult livers. There appears to be no pattern of accumulation within locations based on concentrations in each matrix. For instance, in livers that differed among locations, the location where the greatest concentrations of Ca and Mg were observed was REF3, whereas the location where the greatest concentrations of those elements were observed in sediment was KLMZR. The significantly greatest concentrations of Ba, Ca, Mg, and Ti were observed at REF3; however, this data is based on only one frog liver, and as such, may not be representative of the entire population.

As with the adult livers, essential metals were most concentrated in the tissues of juveniles and tadpoles. Ca, Fe, K, Mg, and Al exhibited the greatest concentrations in each, while Ni, Cd, Hg, Co, As, Sc, Cr, and Sc concentrations were relatively low in each. Pb was much less concentrated in tadpole tissues and adult livers than in juvenile tissues.

Mercury was determined in mudpuppies and snapping turtles collected from 1988 to 1992 from the St. Lawrence River in Canada, a river that is greatly impacted by human activities and synthetic chemicals (Bonin et al., 1995). Concentrations ranged from <20 to 45 ng/g, wet wt in mudpuppies and 50 to 180 ng/g, wet wt, in turtle eggs (Bonin et al., 1995). Unlike the present study, concentrations in this study were not specifically measured in liver tissue. Generally, the wet weight to dry weight ratio is 5. Therefore, these reported values can be multiplied by 5 to compare to the concentrations in dry weight from the present study. The mercury concentration reported in frog livers in the present study ($\leqslant 430$ ng/g, dry wt) is comparable to the values of those in the St. Lawrence River study. Even though these species are different from the green frog, these data indicate that a variety of aquatic organisms are capable of absorbing mercury.

Concentrations of Cd, Pb, Zn, and Cu in soil and wildlife within 30 km of a zinc smelter site in eastern Pennsylvania were determined seven years after smelting was terminated in 1980 (Storm et al., 1994). Concentrations of Cd, Pb, Zn, and Cu in green frog tadpole whole body tissue ranged from 0.3 to 1.5, 2.3 to 5.0, 23.1 to 117.0, and 0.3 to 0.8 mg/kg, wet wt, respectively.

When calculated on a dry weight basis, the concentrations of Cd and Pb are higher from the smelter study by at least a factor of about 7 each when compared to the values for tadpole tissue in the present study. Concentrations of Zn and Cd are more comparable between the two studies, with Zn being at least 2.5 times more concentrated at the smelter site and Cd levels being similar. Overall, these metal concentrations were similar for adult red-backed salamander and eastern newt tissue (Storm et al., 1994).

5. Conclusion

The study presented here attempted to link deformities in green frog populations of southwestern Michigan to metals in their environment and tissues. However, only 0.3% of green frogs exhibited obvious physical abnormalities. Concentrations of organochlorine insecticides, PCBs, and metals were measured in sediment and tissues of tadpoles, juveniles, and adults. Overall, the low levels of the chemicals can be used as reference background levels, from which it can be assumed that at or below these measured concentrations, no significant frog deformities should be observed.

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