Effects of PCB 126 and Ammonia, Alone and in Combination, on Green Frog (Rana clamitans) and Leopard Frog (R. pipiens) Hatching Success, Development, and Metamorphosis

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The Green Bay watershed in Wisconsin is polluted with polychlorinated biphenyls (PCBs), dioxin, heavy metals, ammonia, and over 100 organic contaminants. In this study we exposed embryos and larvae of two rain species commonly occurring in the Green Bay ecosystem, the green frog (Rana clamitans) and the leopard frog (R. pipiens), to PCB 126, 3,3', 4,4', 5-Pentachlorobiphenyl, nominal concentrations 0-50 μg/l, two control treatments: water plus 0.008% acetone as carrier for the PCB, water alone), unammoniated ammonia (0-2 mg/l), and mixtures of both contaminants. Exposure to PCB 126 did not cause significant mortality of embryos before hatching. However, exposure to unammoniated ammonia (NH4 concentrations in excess of 0.6 mg/l (green frogs) or 1.5 mg/l (leopard frogs) caused a decline in hatching success and an increase in prevalence of deformities. PCB 126 and NH3 in combination had a significant negative effect on hatching success. Survival of larvae was significantly reduced at the highest PCB concentration (50 mg/l) for both species. Few skeletal deformities were observed in tadpoles at this concentration, but the incidence of edema was significantly increased. A slowing of growth was also observed in anuran tadpoles exposed to PCB 126. NH3 exposure caused a decrease in the survival and growth of green frog tadpoles. When exposed to mixtures of both chemicals, green frog tadpoles showed a decrease in survival. However, growth was not affected. Fewer tadpoles metamorphosed with increasing PCB 126 and NH3 concentrations. In tadpoles exposed to PCB 126, tissue concentrations of PCB 126 at the end of the experiment increased with increasing nominal concentrations, ranging from 1.2-9600 ng/g wet weight. Our data indicate that ammonia may not be particularly sensitive to NH3 as compared to many fish species, and that water quality criteria determined using data collected on fish species will be protective for many anuran amphibians. At high concentrations, PCB 126 and unammoniated NH3 affected both larval species. However, no sublethal effects were apparent at water concentrations that occur in the Green Bay ecosystem.

INDEX DESCRIPTORS: PCBs, ammonia, amphibians, survival, development, Green Bay.
However, relatively little is known of their bioaccumulation in or possible effects on amphibians (Birge et al. 1978, Niethammer et al. 1984). The direct toxic effects of high environmental NH₃ on anurans are also little understood. There are few data available to determine whether anurans are adequately protected by the U.S. water quality criteria of 0.02mg NH₄/l that has been established to protect aquatic life (US EPA 1977).

A recent study (Jung 1996, Jung and Karasov, unpublished data) determined the percent hatching success of green frog and leopard frog embryos in enclosures located at stites situated along a PCB gradient in the Fox River and Green Bay. This field study found a negative correlation of percent hatching success with PCB concentrations in sediment and un-ionized ammonia concentrations in water.

To test whether this correlation might reflect a cause-effect linkage we exposed amphibian embryos and tadpoles in a static-renewal experiment to increasing concentrations of (1) PCB 126, (2) NH₄, and (3) PCB 126 and NH₄ in combination. We studied the green frog (Rana clamitans) and the northern leopard frog (R. pipiens) because they are common residents of the Green Bay ecosystem and pollutants in the field may impact their populations. We assessed the effects of PCB 126, ammonia, and PCB 126 + ammonia on embryos (hatching success and deformities), tadpoles (survivorship, deformities, edema, growth), and metamorphs (percent metamorphosis). We also measured bicarbonate of PCB 126 in tadpole tissues. Overall, this study is the first to look at sensitivity of North American anurans to a model coplanar PCB compound and it doubles the information available on the sensitivity of North American anurans to ammonia. It is also the first study of the combined effect of ammonia and PCB 126 and provides range-finding data for planning future PCB and ammonia dose-response experiments.

METHODS

Study Organisms

Embryos and tadpoles were staged during the experiment following the table proposed by Gosner (1960). Experiments were carried out at the Water Science and Engineering Laboratory at the University of Wisconsin-Madison. Seven leopard frog egg clutches were purchased from NASCO (Fort Atkinson, Wisconsin). These egg clutches were fertilized the night before or the same morning they were transported to the laboratory in Madison. Three green frog egg clutches were collected by netting, in a pond near Deerfield, Dane County, Wisconsin and a pond near Stoughton, Dane County, Wisconsin. Clutches of eggs collected in the field were identified using the key from Watermolen (1995). Exposure began with embryos in the 4 to 16 cell stage (stages 4 to 6) and the neurula stage (stages 13 to 16). Eggs from the green frog clutch collected in Deerfield were used for the experiment of ammonia exposure in tadpoles. These embryos were raised to hatching in tap water and tadpoles at stage 24 to 26 (spectrum development to early limb bud development) were exposed to treatments.

Exposure of Eggs

PCB 126 (Ultra Scientific and Accu Standard, Inc.) exposure levels were 0.005 (green frog only), 0.05, 0.5, 5, and 50 μg/L, and we had two control treatments. The first control (C+) contained water plus 0.083% acetone (99:99% pure, HPLC grade, Sigma Chemical Co.) as carrier for the PCB and the second control (C−) contained only water. Two egg masses of each species were exposed to PCB 126 for five (green frog) or six (leopard frog) days. For the ammonia exposure experiments, five leopard frog egg masses were exposed for five days to four target concentrations of NH₄ (0, 0.5, 1, 2 mg/L). Green frog embryos (from one egg mass) were exposed for four days to five target concentrations of NH₄ (0, 0.1, 0.2, 0.5 mg/L). Ammonium chloride (NH₄Cl; Sigma Chemical Co.) was used as the source of un-ionized ammonia (NH₃) and the concentrations were adjusted according to tables for aqueous ammonia equilibrium (Thorsten et al. 1979) and our target values of NH₄. In the combined exposure experiment, green frog eggs and tadpoles (from one clutch) were exposed to three PCB 126 concentrations (0, 0.1, 1 mg/L) and three ammonia concentrations (0, 0.1, 0.5 mg/L) arranged in a multifactorial design. All solutions were prepared with dechlorinated, charcoal filtered water (pH 8.0, hardness 324 mg/L as CaCO₃ and dissolved oxygen 11.5 ppm.).

Thirty eggs from each clutch were exposed to 40–70 ml of each treatment solution in 100 × 20 mm glass petri dishes. Petri dishes were placed in a 25°C incubator on a 14:10 h light-dark cycle. Treatment solutions were changed every 24 h (static renewal system). In the ammonia and combined exposure experiments, water temperature (±1°C), pH (±0.02 units), and total ammonia content by nesslerization (sampled mg/L) were measured in each petri dish immediately before (final) and after (initial) the solution was renewed. On the day embryos hatched, hatching success, deformities (bent or asymmetric tails), edema (distension of the body with fluid), and abnormal swimming performance were recorded.

Exposure of Tadpoles

The exposure of tadpoles was carried out in tanks containing 6.0 to 8.0 l of treatment solution. In the PCB 126 and the combined exposure experiments, between 11 and 28 tadpoles that survived after hatching from each petri dish were transferred to the tanks. In the ammonia experiment, 20–40 tadpoles from each of two clutches hatched in tap water were transferred to tanks (clutches kept separate, 9 tadpoles in each tank) containing treatment solutions with 0, 0.01, 0.1, 1 mg/L NH₄ (target concentrations). Tanks were placed in a thermostatically controlled water bath kept at 23–24°C (14:10 h light-dark cycle). Water treatments in tanks were changed and tadpoles were fed every three days. Tadpole food consisted of boiled romaine lettuce blended into a puree and combined with a 9:1 Rabbits Chow:Terras Mix mixture (LM Animal Farms, Pleasant Plain, Ohio; TerraMix Flax Seed, TerraSales, Blacksburg, Virginia). When the front legs of a tadpole emerged, the animal was measured and transferred to a tilted plastic tub containing 1 L of treatment solution. The tilted tubs provided tadpoles with both dry and wet surfaces until they completed metamorphosis. Once placed in the tubs, tadpoles were not fed (metamorphizing tadpoles live off fat stored in the tail) and treatment solutions were changed every 3 days. Tanks were checked every day for mortality and all dead tadpoles were removed and preserved in 10% formalin. Any deformities or abnormal swimming behaviors were recorded every three days. Body length and/or total length were measured in a sample (n = 3–10) of tadpoles chosen randomly from each tank every six or nine days. At metamorphosis (tail length = 2 mm), frogs were weighed, measured for snout-vent length (SVL), and euthanized. Frogs were then dissected to determine masses (±0.0001 g) of liver, kidneys plus gonads, and fat bodies. Time to metamorphosis for each frog was recorded. Tadpoles that failed to metamorphose by the end of the experiment were weighed, measured for total length, staged, and euthanized by immersion in a MS-222 solution (3-aminobenzoic acid ethyl ester, 0.05% solution, Sigma Chemical Co.). After euthanization, tadpoles from the PCB 126 exposure experiment were frozen for contaminant analysis. Tadpoles were analyzed for PCB contaminant levels at the Wisconsin State Lab of Hygiene.
EFFECTS OF PCB AND AMMONIA ON FROGS

(WSLH), University of Wisconsin-Madison. Levels of six PCB congeners, 77 (3,3',4,4'-tetrachlorobiphenyl), 123 (2,3,4,5,5'-pentachlorobiphenyl), 103 (2,3,4,4',5'-pentachlorobiphenyl), 136 (2,3,3',4,4',5'-hexachlorobiphenyl), 157 (2,3,3',4,4',5'-hexachlorobiphenyl) and, 169 (3,3',4,4',5,5'-hexachlorobiphenyl), as well as PCB #126 were analyzed. Organic analyses followed procedures described in "Methods for Organic Analysis" (1993).

Statistical Analyses

Logit values of percent hatching, survival, edema, deformities, and metamorphosis and raw data of tadpole total and body length (ammonia experiments), and body mass, SVL and time to metamorphosis in metamorphosed frogs were analyzed by ANCOVA model. In the PCB 126 experiment the variation in these parameters was tested using nine models conditional on three explanatory variables: species, clutch nested within species, and log [PCB concentration]. Mallow's C2 (1973) was used as the basis for selecting the most appropriate model to explain variation in the data. In the ammonia experiment, we tested the effects of NH3 (a covariate), species (a factor), and NH3 X species. In the combined exposure experiment, we tested for effects of NH3, PCB 126, and NH3 X PCB 126 interactions (model: logit value = constant + NH3 + PCB + NH3 X PCB). In all cases we used the general linear model in SYSTAT (Wilkinson 1992).

The values of total length for tadpoles were compared between treatments using two-way ANOVA with treatment and clutch (PCB experiment) or PCB 126 and NH3 (combined experiment) as factors, keeping species separate. When ANOVA results were significant, Tukey's honestly significant difference test (HSD) for multiple comparisons was used. In the PCB experiment, organ masses for metamorphosed frogs were compared using ANCOVA with species as factor and log [PCB concentration] and SVL as covariates.

In our ammonia experiments we found that within petri dishes or tanks ammonia concentration varied significantly between water changes. Ammonia concentration rose during the 24-72 h in the solutions with low target NH3 concentrations (0-0.2 mg/l), probably due to animal inputs, and fell in the solutions with higher target NH3 concentrations (0.5-2 mg/l), perhaps due to evaporation.

For each petri dish or tank we calculated the initial and final NH3 concentrations using the following equation:

\[
\text{NH3 concentration} = \frac{\text{initial NH3 concentration} \times \text{final volume}}{\text{initial volume}}
\]

\[
\text{NH3 concentration} = \frac{0.5 \times 1000}{1000} = 0.5 \text{ mg/l}
\]

\[
\text{NH3 concentration} = \frac{2 \times 1000}{1000} = 2 \text{ mg/l}
\]

In the experiment, mean NH3 concentrations for the two species for each of the target solution concentrations were 0.128 mg/l (for target of 0 mg/l), 0.173 (for target of 0.1), 0.347 (for target of 0.2), 0.597 (for target of 0.5), 0.808 (for target of 1.0), and 1.911 (for target of 2.0). In the classic exposure experiments, NH3 concentrations for each of the concentration targets were 0.125 (for target 0), 0.112 (for target of 0.01), 0.147 (for target of 0.1) and 1.171 (for target of 1).

P-values <0.05 for main effects and <0.1 for interaction terms were considered statistically significant. P-values for main effects <0.10 and >0.05 were considered to reflect trends.

RESULTS

Acute Exposure of Embryos

PCB 126.—Treatment was not included in the best model to explain hatching success data (Fig. 1A). Hatching success was significantly different between species (F1,18 = 269, P < 0.001), with green frogs eggs hatching at higher percentages than leopard frog eggs. Hatching success of clutches within each species varied significantly (F2,18 = 6.06, P = 0.06). If treatments is added to our model with species and clutch, treatment is not significant differences between groups.

Fig. 1. Hatching success of Rana ridibunda. (A) PCB 126: Leopold frogs and green frogs (two clutches of each species) were exposed to PCB 126 and controls with (+) and without (−) acetone vehicle. (B) Un-ionized ammonia: Two (green frog) and five (leopard frog) replicates, each containing 30 eggs, were exposed to the range of NH3 concentrations. (C) Combined exposure: Three replicates from the same green frog egg clutch were exposed to each combination of PCB 126 and un-ionized ammonia.

(\text{F}_{1,17} = 1.66, P = 0.22). Hence, PCB concentration had no significant effect on hatchability over the concentration range we tested. No deformities were observed in newly hatched tadpoles.

Ammonia.—Over the range of concentrations that we tested, NH3...
Fig. 2. Survival (A), edema (B) and growth (C, D) of tadpoles exposed to PCB 126. In (C) and (D) leopard frog (C) and green frog (D) clutches (each a mean value from six or ten tadpoles in a tank) are pooled within each exposure concentration (except for leopard frogs in the 50 μg/l concentration).

had a negative effect on hatching success (F_{1,31} = 31.151; P < 0.001) but this differed among species (F_{1,31} = 4.465; P = 0.043; Fig. 1B). Green frogs were affected at lower concentrations compared with leopard frogs. Un-ionized ammonia concentration was positive- ly related to the percent deformities in newly hatched tadpoles (F_{1,28} = 39.856, P < 0.001), and this did not differ among species (F_{1,28} = 0.332; P = 0.569). The deformities observed were the same in the two species: body curled up or down, asymmetric body, curved spine, short tail, abnormal tail fins, and deformed tail.

Combination exposure.—PCB 126 and NH₃ (green frog only) had a significant effect on hatching success (F_{1,31} = 3.898, P < 0.022, r² = 0.337), but only when in combination (F_{1,28} = 7.975, P < 0.01). There was not a significant effect of either PCB (F_{1,28} = 0.949, P = 0.340) or NH₃ (F_{1,28} = 0.450, P = 0.509) alone. The combination of chemicals at the highest concentrations produced a decrease of around 60% (average) in hatching success (Fig. 1C). The percent deformities in newly hatched tadpoles was higher in embryos exposed to the highest ammonia concentration for both PCB concentrations (0.1 and 1 μg/l), but the effects of NH₃, PCB 126, or the interaction were not significant (F_{1,23} = 2.322, P = 0.541; F_{1,23} = 0.920, P = 0.347 and F_{1,23} = 1.496, P = 0.234, respectively).

Tadpole Exposure

PCB 126.—Survival of tadpoles evaluated at the end of the experiment was significantly lower in both species at the highest PCB concentration (F_{1,19} = 20.6, P < 0.001; Fig. 2A). Survival also differed between species (F_{1,19} = 5.81, P = 0.026). For the green frog, six tadpoles out of 30 survived after 125 days of exposure to the highest PCB 126 concentration of 50 μg/l. For the leopard frog, no tadpoles survived after 47 days of exposure to this concentration.

The incidence of edema increased significantly in both species at high PCB concentrations (F_{1,20} = 11.373, P = 0.003; Fig. 2B); 100% of the leopard frog tadpoles and 77% of the green frog tadpoles exposed to 50 μg/l PCB 126 exhibited edema at some point during the experiment.

The incidence of skeletal deformities (bent, kinked, or asymmetric tails or asymmetric bodies) in tadpoles of both species exposed to PCB 126 was relatively low, never exceeding 10% in any treatment. Treatment was not included in the best model to explain incidence of deformities. There was no significant difference in deformities between leopard frogs and green frogs (F_{1,18} = 0.48, P = 0.50), however clutches within each species had a significantly different
incidence of deformities ($F_{2,18} = 3.725, P = 0.044$). When treatment was included in the model with species and clutch, it was not significant ($F_{1,17} = 0.19, P = 0.67$).

Leopard frog tadpoles exposed to 50 µg/l were significantly smaller than tadpoles exposed to all other treatments on all dates that both clutches were alive in this treatment (days 13, 19, and 25 after hatching, all $P > 0.002$; Fig. 2C). There were no significant differences in total length of tadpoles in the five remaining treatments throughout the rest of the experiment. Green frog tadpoles exposed to 50 µg/l were smaller than tadpoles exposed to all other treatments on all dates measured ($n = 12$), but the difference was significant only on six dates (days 50, 56, 62, 99, 105, and 117 after hatching, all $P < 0.03$; Fig. 2D).

Ammonia—Tadpole survival (green frog only) evaluated on the last day of the experiment was significantly affected by NH$_3$ concentration ($F_{1,4} = 21.449, P < 0.005$). The effect of clutch was not significant ($F_{4,4} = 4.100, P = 0.113$), however the interaction between clutch and treatment was significant ($F_{1,4} = 8.130, P < 0.057$), probably due to the large difference in survival between clutches at the highest concentration (9% survival in clutch 1 versus 44% in clutch 2; Fig. 3A). Deaths at the higher concentrations of NH$_3$ began after 20 days of exposure.

The prevalence of deformities in chronically exposed green frog tadpoles was low and there was no significant effect of NH$_3$ concentration ($F_{1,4} = 0.911, P = 0.394$).

Growth, as indexed by body length or total length (Fig. 3B), was slower for the highest NH$_3$ concentration compared with the lower concentrations. On the last day of the experiment, tadpoles exposed to the highest concentration had a significantly shorter body and total length than those exposed to the lower concentrations ($F_{1,19} = 6.451, P < 0.05$ and $F_{1,19} = 13.671; P < 0.003$, respectively).

Combined exposure.—The survival of green frog tadpoles after 123 days of joint exposure to PCB 126 and NH$_3$ was significantly reduced by NH$_3$ alone ($F_{1,22} = 8.248, P < 0.05$) but not affected by PCB 126 alone ($F_{1,22} = 0.904, P = 0.352$) and the interaction was not significant ($F_{1,23} = 0.336, P = 0.568$; Fig. 3C). There was
no effect of PCB 126 alone ($F_{1,22} = 3.240$, $P = 0.086$) or NH$_3$ alone ($F_{1,23} = 0.081$, $P = 0.778$) on the percent of deformities in tadpoles, and the interaction was also not significant ($F_{1,22} = 2.893$, $P = 0.103$). Growth as indexed by total length (Fig. 3D) was not different between treatments on all dates tadpoles were measured.

Metamorphs

**PCB 126.** There was a trend for decreased percent metamorphosis in tadpoles at the highest concentration of PCB 126 ($F_{1,20} = 3.97$, $P = 0.00$) for both species. When percent metamorphosis was analyzed without the 50 mg/l group, there was a significant increase in percent metamorphosis with increased concentration of PCB 126 ($F_{5,13} = 7.72$, $P < 0.01$). A higher percentage of leopard frogs metamorphosed than green frogs ($F_{1,13} = 36.7$, $P < 0.001$) and clutches within species had significantly different numbers of tadpoles that metamorphosed ($F_{13,13} = 18$, $P < 0.001$). Four green frog tadpoles and nine leopard frog tadpoles exposed to 0 ppm, 0.05 ppm, 0.5 ppm and 5 ppm died during the period of tail resorption. Exposure to PCB 126 did not significantly affect the time at which tadpoles metamorphosed. There was a significantly higher incidence of edema in leopard frog metamorphs from the 5 ppm treatment.

For both species, Log [PCB concentration] was not a significant covariate for liver mass ($F_{1,138} = 0.017$, $P = 0.989$), kidney-gonad mass ($F_{1,135} = 0.777$, $P = 0.380$), or fat-body mass ($F_{1,137} = 1.65$, $P = 0.249$). There were significant differences between species in liver mass ($F_{1,138} = 5.87$, $P = 0.017$), kidney-gonad mass ($F_{1,135} = 5.59$, $P < 0.001$), and fat-body mass ($F_{1,137} = 25.81$, $P < 0.001$). The effect of SVL as a covariate was significant for the liver organs mass ($F_{1,138} = 112$, $P < 0.001$ for liver; $F_{1,135} = 35.5$, $P < 0.001$ for kidney-gonad; $F_{1,135} = 59.0$, $P < 0.001$ for fat body). The adjusted least squares mean values for green frogs and leopard frogs respectively were: liver: 0.025 ± 0.001 g and 0.022 ± 0.001 g; kidney-gonad: 0.013 ± 0.001 g and 0.009 ± 0.001 g; and fat-body mass 0.006 ± 0.001 g and 0.003 ± 0.001 g.

Ammonia.—Metamorphosis of green frogs was first observed in a control tank 51 days after exposures began. By day 114 the percent metamorphosis observed in the tanks was 49% of tadpoles in control tanks (mean time to metamorphosis 104 ± 9 d, n = 5), 59% in 0.01 mg/l N$_3$ ($F_{1,13} = 7.77$, $P = 0.015$). A higher percentage of leopard frogs metamorphosed than green frogs ($F_{1,13} = 36.7$, $P < 0.001$) and clutches within species had significantly different numbers of tadpoles that metamorphosed ($F_{13,13} = 18$, $P < 0.001$). Four green frog tadpoles and nine leopard frog tadpoles exposed to 0 ppm, 0.05 ppm, 0.5 ppm and 5 ppm died during the period of tail resorption. Exposure to PCB 126 did not significantly affect the time at which tadpoles metamorphosed. There was a significantly higher incidence of edema in leopard frog metamorphs from the 5 ppm treatment.

**PCB 126.** The concentration of PCB 126 in tissues of both green frog and leopard frog tadpoles living in the end of the experiment increased in relation to nominal concentration of treatment water (Fig. 4). The log [concentration of PCB 126 in treatment water] was a significant factor in determining log [concentration of PCB 126 in tadpole tissues] ($F_{1.13} = 116$, $P < 0.01$). The species term and the interaction were also significant ($F_{1.13} = 11.5$, $P = 0.04$ and $F_{1.13} = 0.92$, $P = 0.08$, respectively). Control tadpoles did not have detectable concentrations of any PCB congener. No PCB congener other than #126 was detectable in treated animals, with the exception of the 50 mg/l green frog group, which had very small concentrations of PCB 77 (5.3 ng/g, 0.05% of the total PCB body burden) and PCB 110 (1.1 ng/g, 0.01%of the total PCB body burden). These two congeners may have been impurities in the initial PCB stock solution.

**Ammonia.**—Among green frog tadpoles that failed to metamorphose 100% in both the control and 0.01 mg/l tanks had passed stage 30 (tadpole development) whereas 87% in the 0.1 mg/l tank and 50% in the 1 mg/l tank had passed this stage. Thus, there was a tendency for slowing of development time as well as growth rate in tadpoles exposed to higher NH$_3$ concentrations.

**Combined exposure.**—All the green frog tadpoles that failed to metamorphose had passed stage 30 by the end of the experiment. There was no effect of NH$_3$ alone ($F_{2,21} = 2.200$, $P = 0.115$) or PCB 126 alone ($F_{2,21} = 1.682$, $P = 0.189$) on the total length of these tadpoles and the interaction of chemicals was not significant ($F_{2,21} = 2.115$, $P = 0.08$).

**DISCUSSION**

**PCB 126 Exposure**

**Toxic effects in anurans.**—Newly hatched green frog and leopard frog tadpoles were more susceptible to embryos to the toxic effects of PCB 126. Hatching success of random embryos exposed to PCB 126 throughout the egg stage at concentrations up to 50 mg/l was not significantly lower than controls. However, tadpoles in this group exposed during the egg and larval stages exhibited high mortality. Junge and Walker (1997) also observed increased mortality of tadpoles compared to embryos when exposed leopard frogs during the egg stage to graded doses of waterborne 2,3,7,8-tetrachlorodibenzo-p-dioxid (TCDD), a highly toxic dioxin isomer stereochemically similar to PCB 126. Therefore, it is possible that toxicity of PCB 126 during the egg stage is not manifest until the eggs have hatched into larva. Our results are consistent with those found for other animal
groups and other contaminants (Berrill et al. 1993, Dial et al. 1984, Nebeker et al. 1974, Walker and Peterson 1991, Walker et al. 1991). Our results seemingly differ from those of Jung (1996, Jung and Karasov unpubl.) who found decreased hatching success of anuran eggs maintained in the field (Fox River and Green Bay) in water with concentrations of total PCBs as low as 0.12 μg/L. We suggest two hypotheses to explain these contrasting results. First of all, it is possible that effects of PCB 126 on frogs may be different from effects of the mixture of TDCDD and non-TCD-like total PCB congeners found in higher concentrations in frogs in the field (Huang et al. 1998, Jung 1996), but which is irrespective the environmental factors besides PCBs, differentially influenced the field sites and therefore caused variation in hatching success is very likely. Rosenzweig and Karasov (unpubl.) performed a study to determine if the pattern of hatching success of anuran eggs exposed in the laboratory to water collected along the same pollution gradient in the Fox River would be different from the pattern of hatching success of eggs exposed in the field. Their study minimized the confounding environmental factors present in Jung and Karasov (unpubl.) field study. Rosenzweig and Karasov (unpubl.) found no significant differences in hatching success among sites or between sites and tap water controls in the lab experiment. Therefore, differences in hatching success between sites in the field study were likely due to factors other than contaminants in the water, including PCBs. Another possible explanation is that the more important "toxic" fraction of PCB may be those bound to sediments rather than those present in the water column.

Growth of both green and leopard frog tadpoles was slowed at the highest concentration of PCB 126. By days after hatch (the first day animals were measured), both clutches of leopard frog tadpoles exposed to the highest concentration were already significantly smaller in total length than tadpoles in the other treatments. In green frogs, body length of tadpoles in the highest PCB concentration was also significantly smaller than tadpoles in all other treatments by day 20 after hatch. This suggests that the effect of the contaminant on growth occurs quite early in development. Perhaps contaminated larvae are already at a disadvantage at the onset of hatching. Jung (1996) found a negative correlation between tadpole total length and TCDD dose for green frog 31 days after exposure for 24 hours at the egg stage. This retarded growth of newly hatched tadpoles exposed to TCDD, or coelomic PCBs that likewise bind the A6 receptor, could have detrimental effects on a frog population as a whole. Smaller tadpoles may take a longer period of time to reach metamorphosis than larger ones. Therefore, the time animals remain in an aquatic environment is prolonged, leaving them vulnerable to predators and pond desiccation. The observed trend of decreased percent metamorphosis with increasing PCB 126 concentrations is probably due to a lack of metamorphosis in green frogs exposed to the highest PCB concentration because no leopard frogs tadpoles survived in this treatment. Only six green frog tadpoles in the 50 μg/L treatment survived to the last day of the experiment, therefore we should be cautious in relating this effect to PCB toxicity. Once percent metamorphosis was analyzed without the highest concentration group, it increased with increasing concentration of PCB.

Tadpoles that developed edema during the period of tail resorption of metamorphosis (stage 41 to 45) did not show signs of edema before metamorphic climax began (stage 40). We hypothesize that PCBs present in the far end of the tadpole tail were released and mobilized into the systemic circulation during a surge of stages of metamorphosis or that, the high levels of thyroid hormone, which are maximal at the time of metamorphic climax (Mondul and Kaltenbach 1979, Wet 1986) can identify the toxicity of PCB 126 (Reisman et al. 1984). The edematous response of larvae exposed to high concentrations of PCB 126 was consistent with signs of toxicity seen in other vertebrate classes (Birge et al. 1978, Cecil et al. 1974, Vos and Koenen 1970, Walker et al. 1991). The edematous response may be caused by induction of cytochrome P450A1 in the vascular endothelium resulting in changes in hemodynamic or vascular permeability (Guiney et al. 1990), but this hypothesis has yet to be tested. We suspect that this pathologic response may be useful as a biomedical marker of PCB contamination in amphibians and deserves further examination.

Bioconcentration in tissue.—Leopard frog and green frog tadpoles biocenetrated PCB 126 from treatment water over the course of the experiment. BCFs (bioconcentration factor = PCB 126 concentration in wet tadpole tissue/ nominal PCB 126 concentration in treatment water) ranged from 22 to 28 in leopard frogs and 130 to 500 in green frogs. The difference in bioconcentration between species might be explained by the difference in exposure time (leopard frog = 104 days, green frog = 125 days).

The PCB 126 body burdens we determined from this study (0.0012 to 9.3 μg/g wet mass) are comparable to total PCB body burdens recorded in other studies of anurans in the Green Bay ecosystem (Huang et al. 1998, Jung 1996, Jung and Karasov, unpubl.). The PCB 126 levels in tadpoles exposed to the high concentration treatments (0.14 and 0.75 μg/g wet mass for 5 μg/L and 9.3 μg/L wet mass for 50 μg/L) were similar to levels of total PCBs reported for invertebrates, fish, and birds in the Green Bay watershed (Askley et al. 1993, Call et al. 1991, Sullivan and Defino 1983). It is important to note that more than 100 PCB congeners are included in these "total PCB body burdens". In the case of frogs in the Green Bay ecosystem, PCB 126 and other coplanar congeners occurred at very low or undetectable levels. Furthermore, congeners vary greatly in their ability to cause deleterious effects in organisms (Safe 1987). Therefore, the body burdens of PCB 126 recorded in this study would be expected to cause more toxicity than comparable total PCB body burdens reported for animals in the ecosystem.

Ammonia Exposure

We found declines in embryo survival, increases in prevalence of deformities in newly hatched tadpoles, and a slowing of growth and development in anuran embryos and tadpoles exposed to NH3 concentrations in excess of 0.6 mg/L (green frogs) or 1.5 mg/L (leopard frogs). Our findings are consistent with those of Diamond et al. (1993) who reported 90-h LC50s of 8 (9, 20 °C) of 1.9 mg/L NH3 for leopard frog embryos and 0.09 mg/L for spring peepers (Hyla crucifer). Many of the species of fish that have been tested appear to be more sensitive to NH3 than these anuran amphibians. The highest concentration is not strong enough to cause the rainbow trout (Salmo gairdneri) embryos exhibited transformations when exposed to NH3 concentrations between 0.01 to 0.2 mg/L (Costa Ramusino 1980). Acute and chronic LC50s among fish species are reported to be 0.03 to 0.25 mg/L NH3 and 0.3 to 2.7 mg/L NH3, respectively (Arthur et al. 1987, Colt and Thobangilongos 1978, Diamond et al. 1993, Knoph 1992, Robinette 1976, Ball 1967, Sgrist and Specie 1983, Thurston et al. 1978, Thurston and Russo 1983).

Depressions of growth rate have been observed in fish exposed to un-ionized ammonia concentrations between 0.05 to 0.59 mg/L (Aldrich 1979, Colt and Thobangilongos 1978, Robinette 1976, Sgrist and Specie 1983). Some proposed mechanisms for the effect of un-ionized ammonia on growth in fish include reduction of oxygen uptake due to gill damage, imposition of additional energy demand caused by the use of alternative detoxification pathways, increased
loss of ions by increased urine flow, inhibition of sodium uptake and damage to various tissues (Cote and Tchobanoglous 1978). Combined Exposure to PCB 126 and Un-ionized Ammonia Aquatic organisms are usually exposed to a wide variety of toxicants (Cairns et al. 1990) which may interact with each other or with other environmental parameters and influence a number of animal responses (Voyer and Heltsle 1984). In this experiment, the hatchling success of green frog embryos was reduced and the percent of deformities in newly hatched tadpoles was increased (not significantly) for the combination of PCB and un-ionized ammonia at the highest concentrations. Interaction between chemicals may occur at different levels in chemical and physiological processes (make a chemical more or less available), in physiological processes (influence the quantity of chemical in the body), and in intracellular processes (affect the interaction with receptors at target sites) (Calamari and Albaquer 1980). PCB 126 and NH3 are very different chemicals that produce toxic responses through different biochemical pathways. In this experiment we did not expect any interaction between the chemicals at the chemical or physiological level. Note that we did expect any interaction between the chemicals at the level of cellular receptors. However, the observed effects of increased embryo mortality at the highest combination of both chemicals may be due to an interaction at the physiological level. The damage caused by one of the chemicals may be exacerbated by the other chemical through modifications in absorption, transport, distribution, transformation, accumulation or excretion of the toxicants.

Survival of tadpoles was significantly affected by the highest ammonia concentration independent of the PCB 126 exposure concentration. Therefore we can conclude that chronic exposure to un-ionized ammonia constitutes a hazard, and that embryos may be more susceptible than tadpoles to the joint action of NH3 and PCB. The decrease in survival observed in tadpoles exposed to ammonia and PCB 126 was comparable to that observed in tadpoles exposed to ammonia alone (see above). The current study is insufficient in drawing general conclusions from this combined exposure experiment because we exposed only one clutch of eggs to the two contaminants. However, we can consider these results as a basis for future research in this area. Presumably, there is a lack of information concerning the effects of chemicals in combination on amphibians.

Ecological and Regulatory Implications Waterborne PCB 126 and un-ionized ammonia at high concentrations negatively affected leopard frogs and green frogs, however, no sublethal effects were apparent at concentrations that occur in water in the Green Bay ecosystem (Jung 1995) approximated total PCB concentrations to two highly contaminated sites in the Fox River to be 0.147 and 0.021 mg/l. Therefore, the most contaminated Fox River site had total PCB levels more than one order of magnitude lower than the concentration of PCB 126 that caused the lowest level of observable effects in this study (5 µg/l).

It appears from the few data available that amanurs may not be particularly sensitive to NH3 when compared with many fish species. The criterion established by U.S. EPA to protect fresh water aquatic systems, however, no sublethal effects were apparent at concentrations that occur in water in the Green Bay ecosystem. Jut (1996) approximated total PCB concentrations to two highly contaminated sites in the Fox River to be 0.147 and 0.021 mg/l. Therefore, the most contaminated Fox River site had total PCB levels more than one order of magnitude lower than the concentration of PCB 126 that caused the lowest level of observable effects in this study (5 µg/l).
by birds nesting in the lower Fox River and Green Bay, Wisconsin, USA.


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