From Dogs to Frogs: How Pets, Laboratory Animals, and Wildlife Aided in Elucidating Harmful Effects Arising from a Hazardous Dumpsite

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Abstract

The medical literature contains many examples of cases in which serendipitous observations have led to important findings. In the example described in this article, laboratory and field observations conducted at the Mohawk Nation Community of Akwesasne led to the important and unexpected finding that frogs once plentiful in the area were no longer observed. Laboratory tests comparing river sediments from Akwesasne to pristine sediment from Ithaca, NewYork, indicated multiple adverse health effects on developing frogs. Some of the behavioral changes observed in the laboratory were similar to those described by residents of Akwesasne before the onset of amphibian decline. The magnitude of changes paralleled frog body burden of polychlorinated biphenyls acquired from the Akwesasne sediment. The impact of these findings on the identification of a hazardous waste site and global amphibian decline are discussed.

Key Words: dogs; frogs; hypothyroidism; immunosuppression; Mohawk Nation; neurological abnormalities; PCBs; polychlorinated biphenyls

Introduction

Biomedical research has many examples of cases in which serendipitous findings have led to truly important discoveries. In this article, we describe another such serendipitous finding, which grew from our studies elucidating the harmful effect of polychlorinated biphenyls (PCBs[1]) in unintentionally exposed pet dogs. Blood was collected from these animals over a period of 10 yr at the Mohawk Nation Community of Akwesasne. During one of these visits, we noticed that frogs that previously had been numerous were no longer observed. Follow-up studies using sediment collected at the site and fertilized frog eggs collected from a pristine waterway confirmed the association between larval toxicity and PCBs in the contaminated sediment.

Indeed, recent history is replete with other examples of important discoveries that have resulted from serendipitous findings. Although descriptions of these discoveries are beyond the scope of this article, we urge readers who are not acquainted with the following exemplary cases to search the literature: (1) The observation made by a laboratory assistant that flies gathered only on the urine of depancreatinized dogs led Von Mering and Minkowski to discover the urine contained high concentrations of sugar and the pancreas played a role in regulating blood sugar (Comroe 1977). (2) Christiaan Eijkman, while studying the human disease called beri-beri, noticed that chickens housed near his laboratory displayed similar clinical signs. Investigations of the diet fed to those chickens led to the discovery of thiamin as the cause of the disease (Eijkman 1965). (3) The observations by Benoist and Mathis who, after crossing a TCR transgenic mouse to the NOD strain, discovered the progeny carrying both the TCR transgene and the I-A^{g7} MHC class II molecules developed a disease similar to human rheumatoid arthritis (Matsumoto et al. 1999; McDevitt 2000).

Serendipity was also demonstrated by several community members of Akwesasne when they observed changes in the environment in which they lived. Katsi Cook, a Native American woman working at Akwesasne freedom school adjacent to "contaminant cove" at the General Motors (GM[1]) site, became aware of local dairy cattle displaying signs of brittle teeth and bones, birth defects, and low milk production. In her role as midwife, she also observed more and more infants being born with cleft palates, deafness, and intestinal problems. Concerned by these observations, she asked the New York State Department of Environmental Conservation (NYSDEC[1]) to test local wildlife for toxicants. This request led to the discovery of widespread contamination in fish, wildlife, water, and sediment. With help from the Quakers, Ms. Cook requested the New York State Department of Health (NYSDOH[1]) to test for chemical contaminants (PCBs and pesticides) in the breast milk of nursing Mohawk women. This action led to the observation that Mohawk women had high levels of PCBs in their breast milk, with a congener pattern similar to that seen in the fish (further details are provided below).

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[1]Abbreviations used in this article: EPA, Environmental Protection Agency; GM, General Motors; IG, immunoglobulin; IUPAC, International Union of Pure and Applied Chemistry; NYSDEC, New York State Department of Environmental Conservation; NYSDOH, New York State Department of Health; PCB, polychlorinated biphenyl.

Description of the Test Site

For thousands of years, Mohawk people have inhabited an area along the St. Lawrence River between northern New York State and southeastern Canada known as Akwesasne. The St. Lawrence, Raquette, and Grasse River ecosystems have provided many of the natural resources needed to support Mohawk life. Traditional Mohawk culture emphasized the unity of people, animals, plants, land, and water. The residents derived their livelihood from the land and water, where fish were not only a source of revenue but also an important component of their diets. Additionally, this ecosystem supported the recreational activities of the Mohawks.

During the 1950s, the St. Lawrence Seaway and hydropower dams were constructed. The availability of abundant and inexpensive electricity encouraged many industries, including GM, Reynolds Metals, and the Aluminum Company of America (ALCOA), to locate here. The GM Central Foundry was located next to the St. Lawrence River and less than 0.3 km from homes at Akwesasne. It was known that between 1959 and 1974, the facility used PCB-based hydraulic fluids in its die-casting machines. The predominant PCB type used was Aroclor 1248 (Bush and Kadlec 1995).

In the late 1970s, Akwesasne Mohawks reported their health concerns to the NYSDOH and NYSDEC. When studies of regional fish and wildlife were conducted, high levels of PCBs and other organic chemicals and metals were identified in the tissues of fish. Fish captured near a cove located adjacent to the GM Corporation had levels approximately three-fold higher than fish caught from all other areas of Akwesasne (Sloan and Jock 1990). As a result, health advisories were placed on fish consumption in 1986. Samples of blood and breast milk of Akwesasne residents showed elevated levels of PCBs, which diminished over the next 10 yr following the fish consumption advisories (Fitzgerald et al. 1995, 1998, 1999).

In 1988, Mary Fadden, a student of veterinary medicine whose family members resided at Akwesasne, made the observation that pet dogs of the Mohawks appeared to have decreased longevity compared with dogs seen in the area of Ithaca, New York. This observation led to a survey of recently deceased pets at Akwesasne and 10 miles away at Messena, New York. Results revealed that the average life expectancy of recently deceased dogs at Akwesasne was considerably shorter than Messena dogs (2.7 vs. 10.5 yr). This information prompted the investigation of pet dogs at Akwesasne.

During the summer of 1989, Mary Fadden worked in conjunction with the NYSDEC and collected frogs, turtles, and masked shrews from the St. Lawrence River at the cove site and 0.3, 1.5, and 8.0 km downstream from the site. The average levels of PCBs found in the tissues of animals in the area of the cove (and GM facility) were collected for snapping turtles (*Chelydra serpentina*), green frogs (*Rana clamitans*), bullfrogs (*Rana catesbieana*), leopard frogs (*Rana* pipiens), and shrew (*Sorex cinereus*). The levels expressed in ppm on a lipid weight basis appear in Table 1. In each species, a dramatic decrease in PCB concentration characterized the animals collected downstream, and most levels were not detectable (0.1 ppm) at 1.5 km.

Investigation of Dogs, Rats, and Finches

Beginning in 1990, our laboratory staff visited the Akwesasne Nation. At the time, many dogs still had fish or local wild mammals as a part of their diets. Samples of whole blood were collected and transported at 4°C to the laboratory for processing. Serum was analyzed for immunoglobulin levels (by radial immunodiffusion) and total thyroxine concentration (by radioimmunoassay) (Reimers et al. 1981). PCBs were quantitated by gas capillary chromatography using a congener-specific method (Bush et al. 1982), and results were compared with the 95% confidence intervals established on normal laboratory dogs and pets from the Ithaca area.

During our first two visits, samples were taken from 13 Akwesasne dogs, of which 10 were fish eaters. The average age of those eating fish was 5.5 yr compared with 8.16 yr for non-fish eaters. Serum PCB levels averaged 23.44 ppb for those eating fish compared with 3.4 ppb for those that did not (30 laboratory-reared dogs had an average of 3.9 ppb after 2 yr; Table 2). Compared with the 95% lower confidence intervals for normal dogs, dogs at Akwesasne had decreased total thyroxine (46%), serum immunoglobulin (Ig^1)M (35%), and serum IgA (64%). Among those ingesting fish as a principal portion of their diet, the thyroxine values were decreased in 55%, and abnormal Ig levels were observed in 90%. In addition, serum thyroxine levels had a negative linear correlation with serum PCB levels ($Y = 38.47\text{-}14.55x$; $R = 0.8390$); however, no similar correlation existed between serum PCB levels and either IgM or IgA concentration (Fadden 1994). Decreased levels of thyroxine, IgM, and IgA have also been described in humans accidentally exposed to PCB by ingestion (Chang et al. 1981; Higuchi 1976).

Table 1 Mean (standard deviation [SD]) polychlorinated biphenyl levels (in ppm ± SD) in carcasses of selected wildllife collected within 0.3 and 1.5 km of the contaminated cove

Animal	No. (n)	<0.3 km	No. (n)	1.5 km
Snapping turtle	3	1750 ± 1169	1	23
Green frog	3	561 ± 722	1	<0.1
Bull frog	3	30360 ± 6460	3	<0.1
Leopard frog	9	405 ± 729	2	<0.1
Shrew	11	1570 ± 3621	2	<0.1, 69

Table 2 Serum immunoglobulin (Ig) and polychlorinated biphenyl (PCB) concentrations from Akwesasne dogs

Dog no.	Sex	Breed[a]	Age (yr)	Diet	PCB (ppb)	Ig (mg/dL) IgG	IgM	IgA	T4 µg/dL
1	M	G. Shep	4	F	20.0	1000	45[b]	20[b]	1.25[b]
2	F	B. Lab.	3	F	—[c]	900	90	25[b]	1.87
3	M	B. Lab	1	F	8.9	900	90	25[b]	1.84
4	M	Mix.	7	F	10.1	1080	90	45	1.19[b]
5	F	Laboratory-reared mix.	17	F	24	1080	45[b]	45	1.27[b]
6	F	G. Shep.	4	F	22.0	2500	62[b]	25[b]	1.50[b]
7	M	S. Husk.	4	F	18.8	1600	90	25[b]	0.99[b]
8	M	B. Shep.	10	F	14.4	1600	62[b]	25[b]	1.69
9	F	B. Lab.	2	F	69.4	2400	62[b]	15[b]	—[c]
10	M	Beagle	3	F	23.4	2400	100	15[b]	—[c]
11	M	Chow	4	C	5.8	1600	90	45	2.14
12	F	G. Shep.	10.5	C	2.0	1700	125	15[b]	—[c]
13	M	Mix.	10	C	2.4	1165	104	35	—[c]
Akwesasne means			5		18 ± 17.9	1466	80	27	1.54 ± 0.36
Laboratory beagles			1.5	C	3.9 ± 2.1	1185	133	40	
95% confidence interval normals			5.0	C	0.2-6.6	700-2000	75-200	30-160	1.5-3.0

[a]G. Shep., German shepherd; B. Lab., black Labrador; mix., mixed breed; S. Husk., Siberian husky.
[b]Below the 95% confidence level for normal dogs.
[c]Test not conducted.

To validate that the observed changes were attributable to PCBs, we performed a feeding trial in 9-mo-old male and female laboratory mixed breed dogs. Dogs were divided into three groups and fed 0 ppm (n = 8), 5 ppm (n = 8), or 25 ppm Aroclor 1248 in the diet. The level of PCBs in the diet was consistent with the levels observed by Sloan and Jock (1990), who reported levels of 35 ppm of PCBs in brown bullheads caught adjacent to the GM site. The results reported in Table 3 confirm observations made in the field that ingestion of PCBs was associated with a decrease of serum IgM and IgA. These findings under laboratory conditions are similar to those described in rhesus monkeys receiving similar doses of PCBs (Tryphonas et al. 1991).

When the dogs were evaluated for total thyroxine, a significant ($p < 0.03$) decrease was seen between dogs fed 25 ppm compared with controls after 8 wks of feeding, and thyroid-stimulating hormone levels in the 25 ppm group were increased 100% (Table 4). Although dogs consuming 5 ppm of Aroclor as a group did not have significant decreases in thyroxine levels, female dogs had significantly lower levels than male dogs (66 vs. 113% in pre-exposure levels). Once again, laboratory studies confirmed the likely role of PCBs in decreasing the levels of thyroxine in pet dogs at Akwesasne. After 20 wk of exposure, dogs consuming 5 ppm of Aroclor had a mean level of 4.98 ppb of PCBs

Table 3 Changes in serum immunoglobulins at 8 wk (% of pretreatment levels ± standard deviation)

	Polychlorinated Biphenyl Dose (ppm)		
	0	5.0	25.0
Immunoglobulin G	97.6 ± 12	105.4 ± 22	87.2 ± 8
Immunoglobulin M	135.1 ± 49	92.3 ± 25[a]	88.7 ± 15[b]
Immunoglobulin A	111.9 ± 27	95.6 ± 14	62.2 ± 8.2[b]

[a]$p < 0.10$.
[b]$p < 0.05$.

Table 4 Total serum thyroxine and thyroid-stimulating hormone levels in control and polychlorinated biphenyl-exposed dogs after 8 wk of feeding

	Controls	25 ppm
T4	2.44 ± 0.54	1.17 ± 0.60[a]
Thyroid-stimulating hormone	2.10 ± 1.5	4.9 ± 1.6[b]

[a]$p < 0.01$.
[b]$p < 0.05$.

in their serum, and those consuming 25 ppm had 9.86 ppb of PCBs in their serum (Korytko et al. 1999). Both groups had lower average PCB blood levels than Akwesasne dogs. Furthermore, levels of the hepatic P450 cytochrome monooxygenase CYP1A1 and ethoxyresorufin-o-deethylase activity were maximally elevated even in dogs that received the lowest dose of Aroclor, suggesting the likelihood that these microsomal enzymes in pet dogs at Akwesasne were also elevated. We are continuing to investigate the cellular and molecular basis for the decreases in immunoglobulins and thyroxine.

Parallel to the studies in dogs, our laboratory at Cornell University teamed with co-investigators from the State University of New York at Albany and Cortland to investigate the consequences of inhalation or oral exposure of rats to Aroclor 1242. In contrast to findings in dogs, rats administered PCB by inhalation had elevated thyroxine and triiodothyronine levels and histopathological changes consistent with increased secretory activity in thyroid epithelial cells. Changes also included thymic atrophy (a change seen in dogs) and urinary bladder epithelial cell hyperplasia (Casey et al. 1999). Dramatic changes occurred in the behavior of rats exposed orally to Aroclor 1248 or by ingestion of St. Lawrence River fish, with increased open field activity and impulsiveness compared with controls (Berger et al. 2001). Interestingly, increased agitation was also observed in our laboratory dogs exposed to PCBs as well as dogs residing at Akwesasne.

Finally, other researchers had reported elevated levels of PCBs in the eggs of wild birds in the Akwesasne area (Bishop et al. 1999). Almira Hoogesteijn, another graduate student who was working in the DeVoogd Laboratory at Cornell University, examined the reproductive performance of zebra finches exposed to low levels of Aroclor 1248. Compared with controls, exposed birds had significantly increased numbers of clutches laid, nests constructed per pair, and incubation time per pair, as well as an increased rate of hatchling mortality (Hoogesteijn et al. 2005). These findings are consistent with the hypothesis that low levels of PCBs are associated with endocrine disruption and suggest that wild songbirds similarly exposed may have reproductive problems.

A Chance Observation at Akwesasne

Between 1990 and1996, we made repeated trips to Akwesasne to examine dogs. During a 1998 trip, we included a visit to the cove where frogs had been previously collected and analyzed for PCB concentrations. To our amazement, we were unable to find amphibians of any species. The same phenomenon has been documented in other parts of Akwesasne (Rae and Witherspoon 2001). Samples of sediment were collected from the cove, refrigerated, and later submitted to 10,000 Gy gamma irradiation to kill microorganisms. This sediment was then compared with a similarly treated sample collected from Fall Creek in Ithaca for its toxicity to developing frogs.

We collected one clutch of wood frog (*Rana sylvatica*) eggs from Bull Pasture Pond in Ithaca and temporarily housed the embryos in a 12-L tank until they hatched. At the moment of hatching, we randomly divided the 480 healthy tadpoles into one of three treatment groups: aquaria containing 40 g of uncontaminated sediment [Fall Creek], 20 g of PCB-contaminated sediment [St. Lawrence River], or 40 g of PCB-contaminated sediment). Each treatment was replicated in four blocks. Tadpoles were housed in aquaria containing 3 L of dechlorinated municipal water (pH 7.3-7.5; hardness, 160 μg/g as $CaCO_3$). Water was changed every 3 to 5 days without disturbing the animals. Tadpoles were raised through metamorphosis under constant temperature. Aquaria were divided in half with a flow-through divider, and the tadpoles in each replicate tank were randomly assigned to each side. Tadpoles on one half of each tank were placed in direct contact with sediment, and in the other half, tadpoles were separated from the sediment by a 500-μm flow-through mesh barrier raised 2.5 cm above the sediment. All tadpoles were fed boiled romaine lettuce every day, and the uneaten portion was removed before subsequent feeding. During the 8-wk experiment, we monitored tadpoles for mortality, deformities, snout-vent length, and weight and developmental stages (determined at 7-day intervals). We quantified differences in foraging activity and locomotor function at one discrete time point early in the exposure. At the end of the experiment, we conducted congener-specific PCB analyses on the tadpoles from each group and exposure.

During the first 20 days of the study, 70% of tadpoles having contact with the contaminated sediment died, compared with 10 to 24% of tadpoles that had no contact with contaminated sediment. Mortality among tadpoles on noncontaminated sediment was less than 4%. These differences were highly significant. At the end of 42 days, the body weights of control tadpoles were significantly lower than the weights of those exposed to contaminated sediment. Tadpoles that had contact with contaminated sediment were heavier than noncontacts, but Gosner stages were not significantly different between the groups.

Activity levels at day 7 in PCB treatments were significantly reduced compared with control sediment treatment. Activity levels did not differ between contact and noncontact contaminated sediment groups. At day 12, swimming speeds were lower in all PCB sediment groups compared with controls. In addition to reduced foraging, reduced activity levels included reduced movement to the surface to respire. Other abnormalities observed in tadpoles exposed to PCB sediment included movement over short distances, locomotor arrest, nonlinear swimming trajectory, and generalized lethargy.

When analyzed, the sediment from Akwesasne contained 325 μg/g (ppm) of total PCB. The PCB content of this sediment was six times greater than the hazardous waste level of 50 ppm. The sediment contained a highly dechlorinated pattern from the original Aroclor 1248 used at the GM site. The congener profile was dominated by lower

chlorinated congeners (mono- and di-). In particular, the following congeners had low weight percentages in Aroclor 1248 but were highly concentrated in our sediment: 2/2′ (International Union of Pure and Applied Chemistry [IUPAC[1]] 4), 2,6 (IUPAC 10), 4/4′ (IUPAC 15), 2,6/2 (IUPAC 19), and 2,3,6 (IUPAC 24) (Frame et al.1996). Among sediment contact tadpoles, those exposed to 40 g of sediment (termed 40-g tadpoles) accumulated approximately 5.7 times the concentration of 20-g tadpoles. In addition, 40-g noncontact tadpoles accumulated 5.3 times the levels found in 20-g noncontacts. The PCB burdens in contacts were approximately four times the level in noncontacts in either the 40-g- or 20-g contaminated sediment groups. Furthermore, 20-g tadpoles in the noncontact group had 211-fold higher concentrations than the control groups (in contact with the Fall Creek sediment). Among PCB-treated tadpoles, the congener profile was similar; however, noncontacts had a higher percentage of lower chlorinated congeners consistent with exposure only through the water table (and not ingestion) (Savage et al. 2002).

When this study was repeated using the same sediments but monitoring leopard frog (*Rana pipiens*) tadpole development, 98% of the larvae died on the 40-g contaminated sediment compared with 11% of controls. As a highly contaminated sediment-exposed tadpole went through metamorphic climax, forelimb emergence order was reversed and there was a delay in the development of mouth parts. The few contaminant-exposed tadpoles that survived metamorphosis were unable to right themselves (turn over after being placed on their backs). Tadpoles exposed to PCB-laden sediment also accumulated a disproportionate percentage of ortho-substituted, lower chlorinated, hydrophilic congeners known to be neurotoxic (Fischer et al. 1998).

This study demonstrated conclusively that sediments contaminated with PCBs at levels found at the polluted site at Akwesasne were sufficient to cause significant mortality in early life history stages of wood frogs and leopard frogs. The sublethal effects evident from our experiment can also potentially decrease the fitness of the frogs in their natural environment inasmuch as reduced foraging and movement may have strong effects on tadpole survivorship. Likewise, changes seen during metamorphoses and early adulthood would have predisposed these animals to predation.

Postscript

In 1984, the Environmental Protection Agency (EPA[1]) designated the 270-acre GM site a National Priority Listing as a Superfund site. Testing through monitoring wells and through on-site soil and sediment samples from the St. Lawrence River indicated that wastewater discharges from the site contaminated the St. Lawrence and Raquette River sediments with PCBs. In addition, soil and sediment on the St. Regis Mohawk Reservation has been contaminated by runoff from the site. In 1987, remediation began on the GM site, and a temporary cap was placed on the industrial landfill to prevent further migration of pollutants. In 1991, dredging of the St. Lawrence River sediments commenced, and by 1995, 23,000 tons of PCB-contaminated sediment from the St. Lawrence had been removed. In addition, 14,500 tons of PCB-contaminated site soil were excavated. Additional cleanup activities began in 1999 and included the installation of the groundwater collection and treatment system, dredging of the Raquette River, and removal of stockpiled sediments (EPA 2002). Dredging of sediments continued on the Akwesasne Mohawk reservation, including the cove, and in January 2005, the EPA informed the Akwesasne Task Force on the Environment that the remediation was complete.

Our study did not contribute directly to the present remediation efforts because data provided by the NYSDEC and NYSDOH clearly demonstrated widespread contamination and human health effects of PCBs. Nevertheless, our data did contribute as part of the irrefutable background. The long-term collaboration between the Akwesasne Task Force for the Environment and the State University of New York at Albany provided insight into human health effects and assisted in their diagnosis and treatment (Schell and Tarbell 1998). Our study results illustrate the toxicity of PCBs for amphibian populations and its potential impact in a natural setting. It may serve to illustrate the contribution of environmental pollutants to the general problem of amphibian decline, although it is clear that many other chemicals in addition to PCBs are contributing to this problem (Fort et al. 2001). Clearly these investigations based on serendipitous early observations contributed results that are useful to society.

Human health effects attributed to PCB exposure include chloracne (a skin condition), hypothyroidism, immunosuppression, and learning deficits (WHO 1993). Among Quebec Inuit children, who have some of the highest recorded PCB body burdens reported, increased susceptibility to infection has led to a high rate of respiratory and inner ear infections and deafness (Dallaire et al. 2004; Dewailly et al. 2000). Studies of children born in Germany, The Netherlands, and Michigan show a correlation between high exposure to PCBs and learning difficulties and hyperactivity (Schantz et al. 2003). Hypothyroidism, immunosuppression, and central nervous system abnormalities all were documented in our studies and suggest a role for pets and wildlife as sentinels for potential environmental health effects in humans.

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