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The Effects of Coal Tar Based Pavement Sealer on Amphibian Development and Metamorphosis

PAMELA J. BRYER,[1,2,]* JAN. N. ELLIOTT[1] AND EMILY J. WILLINGHAM[1,3]
[1]*Department of Biology, Texas State University–San Marcos, San Marcos, TX, 78666, USA*
[2]*Department of Environmental Toxicology, The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX, 79416, USA*
[3]*Department of Urology, School of Medicine, University of California, San Francisco, CA, 94143-0330, USA*

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Abstract. Coal tar based pavement sealers are applied regularly to parking lots and contain significant levels of polycyclic aromatic hydrocarbons (PAHs). Recently a connection between elevated levels of PAHs in streams and storm water runoff from parking lots has been identified. We tested the hypothesis that coal tar based pavement sealers could alter the survival, growth, and development of amphibians using a model species, *Xenopus laevis*. Ten fertilized individuals were placed singly into containers containing one of four treatment groups: control, low, medium, and high (respective nominal concentrations 0, 3, 30, and 300 ppm TPAH). All of the individuals in the high exposure group died by the sixth day of exposure. By day 14 there were significant patterns of stunted growth ($p < 0.0001$) and slower development ($p = 0.006$) in the medium and high exposure groups relative to the control and low treatment groups. When the experiment ended on day 52 the control and low-dose individuals had achieved more advanced developmental stages than the medium group ($p = 0.0007$). These data indicate that these commonly used coal tar based pavement sealers may potentially affect the amphibian taxa living in areas that receive storm water runoff.

Keywords: PAHs; *Xenopus*; runoff; non-point source pollution

Introduction

Recent studies initiated by the City of Austin Watershed Protection Department have uncovered a potential new source for polycyclic aromatic hydrocarbons (PAHs) in urban streams. During routine monitoring, the Watershed Protection Department discovered high levels of PAHs in Barton Creek, which serves as both critical habitat for an endangered salamander (*Eurycea sosorum*) and a popular swimming hole for 500,000 people annually (data available City of Austin website http://www.ci.austin.tx.us/salamander/, accessed 12/12/05). Thorough stream sampling isolated a point source for the PAH levels to a storm-water runoff gully for a parking lot sealed with a coal tar based pavement sealer (http://www.ci.austin.tx.us/watershed/bartonsprings.htm, accessed 12/12/05). In a series of simulations, Mahler et al. (2005) demonstrated that light rainfall can transport significant quantities of PAHs from parking lot surfaces into nearby waterbodies. Mahler et al. (2005) suggest that a large portion of the current PAH

*To whom correspondence should be addressed:
Tel.: +1-806-885-4567; Fax: +1-806-885-4577;
E-mail: pamela.bryer@tiehh.ttu.edu

loading seen in urban freshwater bodies potentially originates from coal tar sealed parking lots.

The negative effects of PAHs have been established for a variety of freshwater species including embryonic and larval amphibians. Benzo(a)pyrene, a frequently encountered PAH, was lethal to newt larvae at low levels (50 ppb) (Fernandez and Lharidon, 1994). Sadinski et al. (1995) showed that *Xenopus laevis* had slower development and growth after exposure to 249 nM benzo(a)pyrene, a sublethal concentration equivalent to approximately 60 ppb. Hatch and Burton (1998) compared the sensitivity of three species of amphibians and found that *X. laevis* was more sensitive than either *Ambystoma maculatum* or *Rana pipiens* to a combined exposure of fluoranthene and sunlight. By comparison, sediment TPAH (a sum of 16 PAHs) levels found in Barton Creek immediately upstream from the area of salamander habitat ranges from 1.7 to 31.9 ppm (data from the City of Austin online database http://www.ci.austin.tx.us/wrequery/db_query_form.cfm, accessed 12/12/05).

In spite of the data on PAHs and amphibians, there is little known of the relationship between PAHs originating from coal tar and their bioavailability. PAHs tend to be highly hydrophobic and adsorb strongly to organic carbon and small particulate matter such as charcoal, silt, and clay (Klassen, 2001). The question of interest in this study is whether or not the PAHs that originate from coal tar based pavement sealant move into another compartment – such as water, food, or animal tissues – and pose a risk to aquatic species. Because of documented proximity of an endangered amphibian to a known source of coal tar based pavement sealant and elevated concentrations of PAHs in the stream sediments, we initiated a study to explore whether the coal tar might affect the growth and development of a model amphibian, *X. laevis*.

Methods

Treatments

We chose four coal tar sealant treatment levels: control, low, medium, and high. We set the treatment levels to approximate the range of sediment TPAH (total PAH, a sum of 16 parent PAHs) values that are seen regularly in the streams of the City of Austin (data available at http://www.ci.austin.tx.us/wrequery/, accessed 12/12/05). The treatment values were calculated based on the theoretical maximum amount of TPAH present in the coal tar pavement sealant. The coal tar pavement sealant used was 23% TPAH by wet volume, see Table 1. The low treatment values approximated 3 ppm TPAH, the medium approximated 30 ppm TPAH, and the high treatment approximated 300 ppm TPAH. The treatments were created by placing coal tar pavement sealant flake directly into conditioned tap water as dried flakes. The sealant (SealMaster 1080; SealMaster, Sandusky, Ohio) flakes were formed by painting the wet sealant onto glass plates and allowing it to dry for 72 h. Once dry, we attempted to mimic the wear and flaking that occurs outdoors by scraping, the sealant off of the glass, forming a mixture of particle sizes, see Fig. 1. The coal tar sealant flakes were then weighed on a balance and the final weight was recorded and verified. The mean amount of coal tar sealant in the low treatment, in mg, was 1.2 ± SD 0.63; medium treatment was

Table 1. Distribution of the TPAH constituents in the pavement sealer product used in this study. The wet column indicates PAH concentrations for the sealant in the form as it is typically applied; the dry column has values for the product as prepared in this study, that is, after it has been painted onto glass allowed to cure for 72 h and then scraped off. These data were provided by the City of Austin and are available via an online database request at http://www.ci.austin.tx.us/wrequery/

	Wet (mg/kg)	Dry (mg/kg)
Acenapthene	9230	3860
Acenapthylene	0	0
Anthracene	11,800	5770
Benzo(a)anthracene	9950	5450
Benzo(a)pyrene	11,100	5260
Benzo(b)fluoranthene	9260	6090
Benzo(g,h,i)perylene	7660	4450
Benzo(k)Fluoranthene	6880	3190
Chrysene	10,100	5800
Dibenz(a,h)anthracene	670	1250
Flouranthene	41,300	21,400
Flourene	7860	3920
Indeno(1,2,3,-cd)pyrene	5920	3370
Napthelene	18,500	3220
Phenanthrene	49,600	27,700
Pyrene	32,200	17,500
TPAH	232,030	118,230

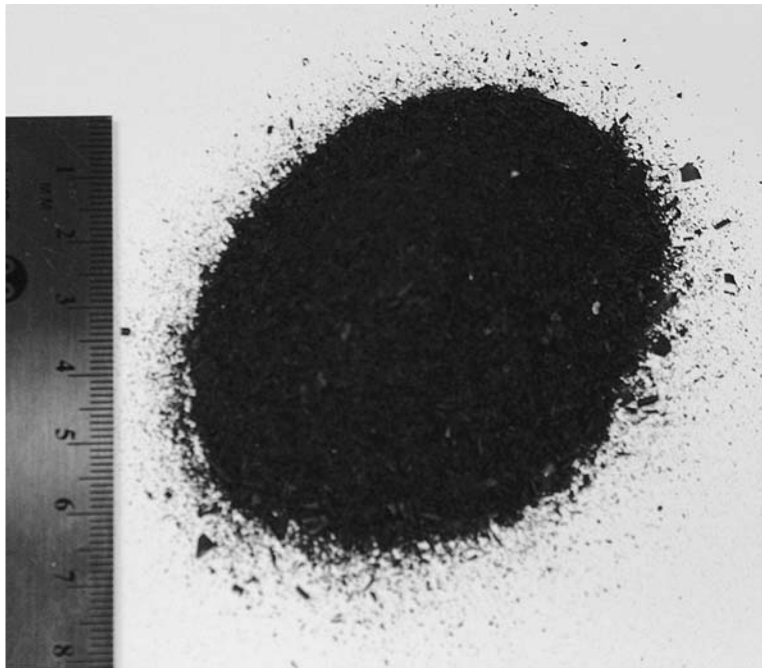

Figure 1. Photograph showing the texture and size of the dried pavement sealant flakes as prepared and used in this study.

10.5 ± SD 0.32; and the high treatment was 104.1 ± SD 0.95. The control treatment contained only conditioned tap water and food. Each of the treatments started with 10 individuals, each housed separately for the duration of the experiment.

Animals

We induced spawning in two *Xenopus laevis* breeding pairs (Nasco, Fort Atkinson, WI). On the day of spawning, we collected the embryos from the bottom of the container and carefully distributed them in a stratified random block design. Eggs were placed singly into the rearing containers that contained the proper amount of dried coal tar sealant and 80 ml of conditioned tap water. The sealant flakes were added to the rearing containers 1 day before the eggs were added. The water was conditioned by passing tap water over an activated carbon and zeolite filter followed by the addition of Stress Coat (Aquarium Pharmaceuticals, Chalfont, PA) and allowed to come up to room temperature. The room temperature varied from 22 to 25 °C. For the first 19 days of the experiment the rearing containers were Dixie brand 207 ml (7 oz) cups, which are made of non-bleached paper coated with food grade wax. Later, as the tadpoles outgrew the smaller cups, we switched the tadpoles, water, and coal tar sealant into one-pint glass ball jars, but maintained the same volume of water. Once active, the tadpoles were fed daily with a suspension of *Xenopus* tadpole chow (Nasco, Fort Atkinson, WI). Water changes were performed every 3–4 days by gently swirling the container and pouring off approximately 1/3 of the total water volume making every effort to not lose the coal tar sealant flakes. The volume pored off was replaced with conditioned tap water. We did not monitor water quality but instead relied on experience to verify that the conditions were adequate for normal tadpole growth. The room photoperiod was set by natural light and was approximately 14 h of light and 10 h dark. All individuals were housed in a dimly lit fume hood with no direct or bright sunlight in order to eliminate any potential photoactivation of the PAHs.

Measurement endpoints

The eggs were checked once daily to record time taken to escape the vitelline membrane. Hatching can be identified as the point at which the embryo completely straightens out after the vitelline membrane ruptures. For the first 2 weeks the tadpoles were also checked daily to monitor for mortality. On day 14 all individuals were staged, using the Nieuwkoop and Faber staging table (Nieuwkoop and Faber, 1994), and total length was measured (0.01 mm) using calipers on live tadpoles under a dissecting microscope. Once metamorphosis neared, the tadpoles were once again checked daily. On post-hatching day 52 the experiment ended and all remaining individuals were staged.

Statistical analysis

The count data concerning hatching success, time to hatching, survivorship, and number of metamorphs were set to 2×4 contingency tables and analyzed with χ^2 distribution. The length data were first tested for homoscedasticity and normality and then analyzed using ANOVA. Dunnett's tests were used for post-hoc comparisons. The developmental stage data were more suitable for non-parametric analysis and thus were analyzed using Kruskal–Wallis ANOVA. One individual in the medium treatment died as a result of experimental error and was excluded from the analyses. All statistical analyses were performed using 'R' Version 1.9.1 (R Development Core Team, 2004).

Results

Hatching success

The morning of the third day post-fertilization, the embryos were beginning to hatch. Three embryos from the low treatment group where noted as dead at this point and were considered to be a part of normal attrition despite the random stratified manner of distributing the eggs. No pattern in hatching success was noticed at 72 h (Table 2) ($\chi^2 = 6.775$, d.f. = 3, $p = 0.079$).

On the evening of the post-fertilization day 3, the proportion of embryos that had completely straightened out lengthwise was recorded. More individuals in the medium and high-dose groups were still curled and/or obviously still wrapped up

Table 2. Developmental phenology for several life history time points by treatment. Each of the treatments started with 10 separately housed individuals. Three embryos in the low treatment failed to hatch and one individual in the medium treatment died to experimenter error on day 47

	Number hatched at 72 h	Number hatched at 82 h	Number of survivors at 10 days	Developmental stage at 14 days	Number of survivors at 52 days	Developmental stage at 52 days
Control	8	10	10	46	9	66
Low	3	7	7	45	6	66
Medium	3	9	8	44	6	58
High	4	5	0	–	–	–
p-Value	n.s.	0.0075	<0.0001	0.006	–	0.0007

in the vitelline membrane. The control and low groups were both completely straightened. The high-dose group, however, had half of its individuals still bound in the vitelline membrane (Table 1) ($\chi^2 = 11.97$, d.f. = 3, $p = 0.0075$).

Growth and developmental endpoints

By the morning of the post-hatching, day 6, all of the individuals in the high treatment group were dead. By day 10 more individuals from the low, medium, and high groups had died relative to the control group (Table 2) ($\chi^2 = 24.21$, d.f. = 3, $n = 40$, $p < 0.0001$). By the end of the second week obvious differences in developmental rate became evident. Tadpoles in the control treatment were larger, measured as total length in mm, than those in the low and medium exposure groups (Fig. 2) (ANOVA $F = 51.67$, d.f. = 1, $n = 25$, $p < 0.0001$; Dunnett's control versus low $p < 0.0001$; and control versus medium $p = 0.028$). Tadpoles that were in the control treatment were also more developed than those in the treatment groups: median control group developmental stage 46, low group 45, and medium group 44 (Table 2) (Kruskal–Wallis = 10.22, d.f. = 2, $n = 25$, $p = 0.006$). At the end of the study the differences between the treatment groups were

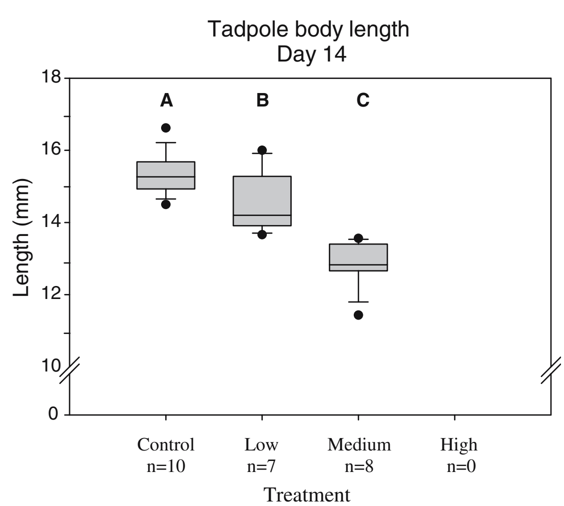

Figure 2. Growth at 2 weeks. Mean ± SE tadpole total length (mm) as measured on day 14. Significance was tested with one-way ANOVA, different letters indicate significant differences. High treatment individuals were not included in the analysis because they all died by day 6.

obvious as most of the surviving frogs in the control and low treatment groups had reached N-F stage 66, i.e., they had metamorphosed, in contrast, none of the remaining medium treatment individuals had reached metamorphosis. The median developmental stage for the control and low groups was stage 66 and stage 58 for the medium treatment (Table 2) (Kruskal–Wallis = 14.62, d.f. = 2, $n = 21$, $p = 0.0007$).

Discussion

Coal tar pavement sealers appear to affect the growth and development of amphibians, even at low part per million concentrations. Frogs exposed to coal tar sealant took longer to hatch and were smaller and developmentally behind control frogs at multiple time points. These results supported our hypothesis that coal tar pavement sealant would alter amphibian development. Coal tar sealant contains a large percentage of PAHs (20–35%, depending on brand), (SealMaster, 2002 and STAR Inc. 1996), which are known to cause developmental effects similar to the ones found in this experiment. Coal tar is a compound widely known for its toxicity. As early as 1309 AD laws were passed in England that banned the burning of coal because of the air pollution it created (Freese, 2003). In the mid 1770s coal soot was recognized to cause cancer in chimney sweeps (Gallo, 2001). Despite this long history, a large portion of the chemistry in coal tar is still unidentified, though the most active compounds have been identified as PAHs (Thami and Sarkar, 2002).

Currently there are no published reports of coal tar sealants and its affect on amphibians, however, the results reported here are in agreement with the existing literature on the effects of PAHs on amphibians. While it is unknown what component of the coal tar sealers affected the *X. laevis* in this study we could suspect PAHs because of their percent weight in coal tar pavement sealers, their known toxicity to amphibians, and recent evidence that PAHs from these coal tar pavement sealers are more mobile than previously believed. In a study with *Rana pipiens* Monson et al. (1999) found that despite the high rate of depuration, 2–10 ppm of fluoranthene in conjunction with common levels of sunlight was highly lethal.

Sadinski et al. (1995) found that when exposed to 248 nM benzo(a)pyrene *Xenopus laevis* tadpoles were smaller and took longer to metamorphose than the controls. The longer development time, in turn, predisposed those individuals to greater frequency of DNA adducts and red blood cells with micronuclei. The Sadinski et al. results are interesting because they only dosed for a portion of development and did not start dosing until the larvae were approximately stage 50. Only those individuals in the highest exposure treatment showed negative effects; however, they did not test them during the life stage where we experienced our greatest tadpole mortality, at approximately stage 45. Overall the nature of the results and direction of the responses were similar between these two studies.

Without chemical analyses on the rearing water it is impossible to say what in the coal tar pavement sealant affected the tadpoles. Because coal tar sealant is 23% PAH by volume, and the results of this study mirror the only other study done on *X. laevis* and PAHs, the indication is that the PAH content of the coal tar sealant affected the development of these amphibians. The other known ingredients of coal tar pavement sealers are Ball clay and water, though some formulations contain elasticizers. In this study the rearing chambers contained only water, food, and varying amounts of dried coal tar sealant, so potential additional stream pollutants did not contribute to the results. By the same token however, a typical stream also contains abundant organic matter and suspended sediments, both of which can bind PAHs making them less bioavailable (Patterson et al., 1996).

The results from this study are potentially useful because the effects observed occurred within environmentally relevant concentrations. Within the City of Austin, stream sampling has shown that 13% of the streams are above the probable effects concentration and 35% are above the threshold effects concentration (22.8 and 1.61 ppm, respectively, of 13 summed PAHs after MacDonald et al., 2000). Our nominal concentrations represent the maximum TPAH value attainable; however, it is unlikely that the exposure ever approached that concentration due to the hydrophobic character of PAHs. Additionally, there could have been losses of available PAH via adsorption to the sides of the rearing containers. Even the concentration used in the high treatment is well within the range of values seen in the environment, for example, in Austin high values of 3400 and 1417 ppm sediment TPAH have been recorded (data from the City of Austin online database http://www.ci.austin.tx.us/wrequery/db_query_form.cfm, accessed 12/12/05). One of the interesting attributes to the streams in this area is how prone to scouring they are, such that from one storm event to the next the sediment TPAH concentration may change radically. This makes assessing risk to stream organisms in this area even more difficult.

A better assessment of the environmental occurrences of pavement sealant runoff is necessary to understand the potential effects on amphibians and other aquatic organisms. Mahler et al. (2004, 2005) of the U.S. Geological Survey documented the potential for a variety of parking surfaces to release PAHs into storm water runoff. Their work showed that coal tar based pavement sealant significantly increased PAH concentrations in storm-water running off of them. They simulated a light rain washing over a cordoned off section of different parking lots and found that coal tar pavement sealants released 65 times more PAHs (mean concentration of 3500 ppm TPAH) than a similar simulation over unsealed asphalt and cement parking lots (Mahler et al., 2005). Their experimental design included both areas that were in active use by vehicles and areas created specifically for the study that were never driven on (Mahler et al., 2004, 2005). Automobile exhaust also contributes PAHs and a variety of contaminants to the storm water runoff in urban areas (Van Metre et al., 2000).

This study opens the door for future research into the nature of urban runoff by documenting the possibility that coal tar pavement sealants may cause harmful developmental effects in amphibians. In this study, we showed that *X. laevis* experienced slower growth and development at sublethal levels of pavement sealant exposure and death at higher levels of exposure. Although it is understood that PAHs can harm larval amphibians, it was previously thought that these pavement sealant products would not be able to desorb PAHs (or any of the other hydrocarbon components) and that they thus posed no problem to aquatic life. Given that coal tar pavement sealant products themselves can be toxic to amphibians, additional studies are necessary to better understand the environmental fate of these products.

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