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UNITED STATES DISTRICT COURT
FOR THE WESTERN DISTRICT OF KENTUCKY
PADUCAH DIVISION

FOREST SERVICE EMPLOYEES FOR ENVIRONMENTAL ETHICS, and PAUL SCHAEFER, Plaintiffs, vs. UNITED STATES FOREST SERVICE, an agency of the U.S. Department of Agriculture, Defendant.	Case No: 5:06-CV-92-TBR **DECLARATION OF RICK RELYEA** (Electronically Filed)

I, Dr. Rick Relyea, declare:

1. I am an Associate Professor of Biological Sciences at the University of Pittsburgh where I study and teach aquatic ecology, toxicology, evolution and behavior. My research program focuses on the effects of pesticides on amphibians and aquatic communities, the ecology and evolution of phenotypic plasticity, including how predators and competitors affect an organism's behavior, morphology, and life history, and long-term monitoring of aquatic communities.

2. I am the author of several scientific studies on the subject of pesticides and their effects on amphibians, including: (1) Relyea, R.A., N.M. Schoeppner, and J.T. Hoverman, 2005, "Pesticides and Amphibians: The Importance of Community Context," *Ecological Applications,* 15(4), pp. 1125–1134; (2) Relyea, R.A., 2005, "The Impact of Insecticides and Herbicides on the Biodiversity and Productivity of Aquatic Communities," *Ecological Applications,* 15(2), pp. 618–627; (3) Relyea, R.A., 2005, "The Lethal Impact of Roundup on Aquatic and Terrestrial Amphibians," *Ecological Applications,* 15(4), pp. 1118–1124); and (4) Thompson, et al., 2006, "The Impact of Insecticides and Herbicides on the Biodiversity and Productivity of Aquatic Communities," Letter and Response Thereto by R.A. Relyea, *Ecological Applications*, 16(5), pp. 2022–2027.

3. The plaintiffs in this case have asked me to explain the potential effects of pesticides associated with corn and soybean farming on aquatic communities, particularly amphibians.

4. Assessing the impact of pesticides on amphibian populations is quite difficult because for most pesticides, we have few data on concentrations in nature, and we have few studies on pesticide impacts on amphibians. The reason for this paucity of data is that federal regulations for registering pesticides require testing birds, mammals, fish, and aquatic invertebrates but not amphibians. As a result, we often estimate the toxicity to amphibians based on other groups (e.g., fish). However, the impact of a pesticide on amphibians can be very different from the impacts on fish or aquatic invertebrates. Because current regulations do not require testing of amphibians, many globally common pesticides have rarely been tested on amphibians.

5. I tested the effects of the herbicide Roundup (including its surfactant POEA which is particularly toxic to tadpoles) on six species of larval amphibians from North America (wood frogs, Rana sylvatica; leopard frogs, R. pipiens; green frogs, R. clamitans; bullfrogs, R. catesbeiana; American toads, Bufo americanus; and gray tree frogs, Hyla versicolor). Four of these species (R. clamitans, R. catesbeiana, H. versicolor and B. americanus occur at LBL.

Although these species are not currently known to be decreasing, they are a diverse set of species that span a large geographic range and can provide insights into the lethality of Roundup.

6. Roundup had significant effects on the survival of all six amphibian species. Five species experienced 0% survival with 5 to 20 mg AI/L ("active ingredient per liter"). At 1.0 and 0.1 mg AI/L, survival improved and was similar to controls ($p > 0.1$). Based on the current classification scheme, this means that Roundup is moderately toxic (1 to 10 mg AI/L) to highly toxic (0.1 to 1 mg AI/L) to these amphibians. Previous work had concluded that Roundup is only slightly to moderately toxic to larval amphibians, but this work was based on relatively few species.

7. In addition to the acute toxicity of Roundup alone, in one of the six species (wood frogs), Roundup became twice as toxic when combined with predator-induced stress. The mechanism underlying this synergism is unknown, but not unique to Roundup, having been also observed when amphibians are exposed to the insecticide carbaryl (which is not used at LBL) in the presence of amphibian predators. Synergism between malathion and amphibian predators has also been documented for the Gray treefrog. We also conducted experiments with Roundup under more natural community conditions in outdoor mesocosms and found similar results, with some species experiencing 70% declines at only 1 mg a.e./L, which is within the range of concentrations measured in water bodies nearby to commercial farming.

8. The discovery of synergistic interactions between predator cues and Roundup indicates that the phenomenon is not restricted to carbaryl and malathion (and possibly other carbamates and organophosphates). Rather, it suggests that predatory stress may make a variety of pesticides more deadly to amphibians. This is important because most amphibians live with the stress of predators.

9. In addition to Roundup, I understand that the other pesticides used by LBL farm permittees in 2006 are Callisto (mesotrione), Celebrity Plus (dicamba, diflufenzopyr, nicosulfuron), Distinct (diflufenzopyr, dicamba), Dual II Magnum (S-metolachlor), Extreme (imazethapyr, glyphosate), and Lightning (imazethapyr, imazapyr). Studies of the type I carried

out for Roundup have not been performed for these pesticides. Thus we know little about the lethal effects of these chemicals, either direct or synergistic, on amphibians.

10. However, adverse effects to the northern leopard frog (LBL includes three species of the same frog genus) short of outright lethality have been shown for very low concentrations (0.1 part per billion) of two of the LBL pesticides – nicosulfuron and S-metolachlor (the other pesticides were not included in this study), singly and in combination with other pesticides. Each pesticide individually affected amphibian development by retarding growth before metamorphosis. Combinations of pesticides in this study were often more deleterious than were individual pesticides. See, Hayes, T.R., et al., 2006, "Pesticide Mixtures, Endocrine Disruption, and Amphibian Declines: Are We Underestimating the Impact?" *Environmental Health Perspectives*, Vol. 114, Number S-1.

11. In conclusion, pesticides used at LBL in concentrations comparable to those that would result from regular commercial farming application have been shown to significantly and adversely affect some LBL amphibian species. However, what we know is much less than what we do not know about the effects pesticides may have on amphibians.

I declare under penalty of perjury that the foregoing is true and correct. Dated this 9th day of December, 2006, in Pittsburgh, Pennsylvania.

/s/ Dr. Rick Relyea
Dr. Rick Relyea
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(original signature retained by attorney Marc Fink)

CERTIFICATE OF SERVICE

I hereby certify that on December 11, 2006, I electronically filed this document through the ECF system, which will send a notice of electronic filing to Jeffrey Dillen, United States Department of Justice (Jeffrey.Dillen@usdoj.gov).

s/ Marc D. Fink
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