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Introduction

Chairwoman Porter, Ranking Member Gosar and members of the Subcommittee, I am Dr. Jenifer McIntyre, Assistant Professor of Aquatic Toxicology at Washington State University (WSU). Thank you for the opportunity to testify before the Committee today regarding research led by WSU and the University of Washington that discovered the impact of 6PPD-quinone on Coho salmon. Partners for this research included federal researchers at NOAA's National Marine Fisheries Service and the U.S. Fish & Wildlife Service.

WSU is Washington State's land-grant university and a public research university committed to its mission and tradition of service to society. With six campuses[1] across the state of Washington and a presence in every county through its Extension system, WSU has an enrollment of 31,159 students statewide. In FY2019, WSU's total research and development expenditures totaled $345 million.

The research we are discussing today is based out of WSU's Puyallup Research and Extension Center. The facility is located within 50 miles of more than 60 percent of the state's population and is home to the Washington Stormwater Center created by the Washington State Legislature as technical resource center. As a result, the Washington State University Puyallup Research and Extension Center and the University of Washington's Tacoma Center for Urban Waters joined forces to create the Washington Stormwater Center.

In my testimony I will focus specifically on the research we have done to understand the acute toxicity of stormwater runoff to salmon and the role of a new chemical called 6PPD-quinone which can impact waters that serve as habitat for coho salmon.

Research Overview

We have known for about 40 years that urban surface waters can kill coho salmon (*Oncorhynchus kisutch*), one of the 5 species of Pacific salmon. In the 1980s, the salmon hatchery in Bellingham, WA experienced die-offs of its coho salmon if surface water intakes remained open during rain events. In the 2000s in the Seattle area, we noticed that adult coho salmon were dying during rain events in spawning streams where physical habitat had been restored to enable recolonization by Pacific salmon. Hours prior to death, fish were observed behaving abnormally, including disorientation and disequilibrium. Mortalities were widespread among urban-impacted stream systems and recurrent year after year [1]. The mortality was unrelated to water quality conditions, including elevated stream temperature, low water oxygen levels, or pathogens, that have been responsible for other documented fish kills of salmon in the contiguous U.S. Instead the weight-of-evidence implicated the influx of stormwater runoff in urbanized watersheds. Tissue samples confirmed that fish in these streams were exposed to various chemicals commonly measured in urban-impacted waters, but concentrations were below those known to be toxic. Population modeling studies by the National Marine Fisheries Service (NMFS) predicted that even modest rates of mortality would impact coho populations across the region – not just in streams with acute mortality events [2].

Trying to understand why these mortalities were taking place, the U.S. Fish & Wildlife Service and NMFS conducted a land-use and land-cover analysis for the Puget Sound area [3, 4]. The land-use most strongly associated with rates of coho mortality was roads; sub-basins with busier roads had higher rates of mortality (>50%). From there, we began studying roadway runoff. At first, we created mixtures of chemicals commonly measured in roadway runoff and road-impacted streams; heavy metals and hydrocarbons from fossil fuels. These chemicals accumulated in the tissues of the adult salmon we exposed but produced no changes in behavior and did not cause any mortality, even at concentrations higher than those measured in runoff [5]. It wasn't until we collected runoff directly from a busy road that we were able to recreate the acute changes in behavior and mortality of adult salmon. All fish exposed to roadway runoff died within 24 hours, often much less, similar to what we had observed in impacted streams.

We have since shown that other life stages are similarly sensitive to roadway runoff; juveniles and recently hatched salmon also die within 24 hours when exposed [6]. Juveniles are easier to work with than adults, and are available year-round instead of just a few weeks during the autumn. Using juveniles we have shown that there is a progressive display of abnormal behaviors in coho exposed to runoff [7]. Fish begin to swim at the surface of the water, at first intermittently and later continuously. They then lose equilibrium and begin swimming at an angle or in spirals. Fish ultimately become immobile at the bottom of the water column before dying. We have shown that fish that have progressed to the stage of continuous surface swimming will die even if they are transferred to clean water [7]. In still-unpublished research, we have documented that mortality is triggered by very small amounts of runoff: fish died even in clean water that contained just 5% runoff. Also, mortality is triggered early during the

exposure; nearly all fish exposed to roadway runoff diluted with clean water died within 24 hours of a 2-hour exposure to 25% runoff. None of those fish appeared sick at the time they were transferred to clean water.

In fish that have become sick from roadway runoff exposure, behavior and blood physiology suggest they are suffering from cardiorespiratory distress [8]. In particular, we see that the blood of these fish becomes very thick. One reason for this could be that fish are not getting enough oxygen. However, our experiments show that the red blood cells of sick coho are not impaired – there is no impact on their ability to transport oxygen [9]. These experiments confirmed that the blood itself is not a target of stormwater contaminants. Instead, further experiments have shown that the blood of sick coho becomes thick because it is losing plasma – the watery part of blood [10]. We injected a dye into the heart of juvenile coho and allowed it to circulate through the vascular system of the fish (all of the arteries, veins and capillaries). During this time, dye can be observed leaking from the gills of coho that had been in the runoff, but not those that had been in clean water only. We then 'rinsed out' the dye with clean saline water. The only dye remaining in the fish would be where it had leaked out of the vasculature into surrounding tissues. The vascular system of all animals is 'leaky' to various degrees. This allows molecules to be transported from the gut, into the blood stream, and then into the liver, for example. However, the vascular system of certain organs should not be 'leaky'. The brain, for example, is protected from unwanted chemicals in the blood stream by the 'blood-brain barrier'. However, in sick coho we see that the dye we injected has leaked extensively into the brain, in addition to other organs. This may explain the changes in behavior that we see in sick fish and may also be the cause of mortality. We are continuing to study this dysfunction of the blood-brain barrier as a possible cause of mortality but also to determine if there are sub-lethal effects that would be a concern for other animals, including humans, even if they have a more mild reaction to the chemicals coming from roads.

What is it in roadway runoff that triggers the mortality phenomenon in coho salmon? In collaboration with Dr. Edward Kolodziej and his team of environmental chemists at the University of Washington, we examined seven vehicle sources of chemicals that were ending up in roadway runoff. The source most chemically similar to roadway runoff and urban-impacted water samples tires. Specifically, water in which we had soaked particles from the tread of vehicle tires [11]. In other words, the pattern of chemicals that had leached out of tire particles was statistically similar to the pattern of chemicals in roadway runoff and urban-impacted waters. However, the presence in these waters of hundreds of chemicals sourced from tires did not necessarily mean that tires were the source of the toxicity to coho salmon. So, in addition to the chemical analysis, we 'let the animals tell us' if tires could be the source of toxicity. If tires were the source of toxicity, chemicals leached from tires would cause acute mortality in coho salmon, would not appear to affect chum salmon (a closely-related species that does not die from exposure to roadway runoff), and would cause the same changes in coho behavior and physiology as we see from roadway runoff. And that is exactly what we saw [12], confirming that tires were the most likely source of the chemical(s) killing coho in runoff-impacted streams.

Very recently, our research group identified a chemical that leaches from tires that is primarily responsible for the mortality phenomenon [13]. To do so, we soaked tire tread particles in clean water and 'fractionated' the resulting chemical mixture. There were more than 2000 chemicals in the water we made, and these chemicals behave in slightly different ways. It might have taken decades to identify all 2000 chemicals in order to figure out which one(s) were killing coho, so instead we put the water through different kinds of filters and used juvenile coho as a screening tool to track whether the filtered water was still able to kill coho. In this way we were able to reduce the chemical complexity to just 4 chemicals in the 'fraction' that still killed coho. Fractions containing the other ~1996 chemicals did not kill coho. The most abundant chemical of the 4 was ultimately identified as 6PPD-quinone – a previously unknown chemical that is created when ozone interacts with tires or with particles worn from tires.

Specifically, 6PPD-quinone is a transformation product of 6PPD – a very common chemical added to rubber products, including tires, to protect them from ambient ozone. 6PPD is included in tire formulations for the express purpose of scavenging ozone molecules that would otherwise damage the rubber itself. Every tire contains 6PPD or a similar 'anti-ozonant' chemical and this chemical is designed to migrate to the surface of the tire throughout its lifespan in order to prevent degradation from ozone. As a result, after 6PPD reacts with ozone, 6PPD-quinone can leach into water directly from the surface of the tires when it is raining or when cars are washed, as well as from tire particles embedded in the roadway surface (think: skid marks), or tire particles that wash off roads into catch basins or surface waters. We found this chemical in surface waters from the Puget Sound area and several other west coast locations (San Francisco, Los Angeles) as part of the study we published in Science. Researchers around the country and around the world are now reporting that they too are finding this chemical in surface waters, as well sources entering surface waters [14, 15].

Beyond coho salmon, other Pacific salmon species are also sensitive to roadway runoff, including steelhead trout (*O. mykiss*) and chinook salmon (*O. tshawytscha*) – both listed under the U.S. Endangered Species Act, as well as critical food resources for endangered killer whales in North America. Some aquatic invertebrates are also sensitive to roadway runoff (e.g., mayfly nymphs [6]. The toxicity of tires has been studied for decades due to concerns about exposures for aquatic animals, with acute mortality documented for various species, including *O. mykiss* [16]. Ongoing research will determine if toxicity to these organisms is also due to 6PPD-quinone, or if other chemicals in the complicated mixture of roadway runoff are responsible. While the list of species sensitive to stormwater, tire chemicals, or 6PPD-quinone is short, it is important to note that relative to the number of species potentially exposed to 6PPD-quinone, very few have been directly tested.

Finally, tire chemicals are also a concern for the effects of air pollution on human health. The smallest particles that wear from tires contribute to the particle pollution of air. These particles are inhaled when we breathe, and they release chemicals when they settle on the wet surfaces inside our lungs. We know that humans living closer to busy roads have poorer health outcomes due to air pollution [17]. It is important that we learn whether 6PPD-quinone plays a

role in the toxicity documented when human cells are exposed to chemicals released from tires [18].

Even before we knew that 6PPD-quinone was the primary cause of the coho mortality phenomenon, we were studying the ability of non-proprietary treatment systems to filter out chemicals in stormwater runoff and prevent toxicity to aquatic animals. 'Green stormwater infrastructure' (GSI) approaches for treating stormwater were initially intended to address the water *quantity* problem of impervious areas in cities (i.e., to prevent flooding), but can also improve the *quality* of the water running off our buildings and roadways into surface waters. We have known for decades that infiltrating water into soils will filter out many chemical contaminants, including heavy metals and hydrocarbons. Unknown was whether those improvements were enough to prevent toxic impacts to aquatic animals, including coho salmon. We showed that the simple mixture of sand and compost used in GSI in Washington State to treat stormwater runoff prevented all evidence of toxicity in coho salmon, as well as other animals [6, 19-21]. In the short time since our discovery, we have conducted retrospective analyses of water samples taken during previous studies and verified that GSI can be highly effective at removing 6PPD-quinone from water.

However, how much GSI is needed on the landscape to prevent impacts on coho and other sensitive species? Limited modeling of the risks to coho salmon from stormwater runoff suggests that it may not be possible to install enough GSI to protect them [22]. Furthermore, the scale of GSI installation needed to fully protect aquatic ecosystems from stormwater impacts is on the order of billions of dollars per year [23, 24]. However, those costs could decrease if we invest in research to optimize treatment ability, including being able to increase loading rates and lifespan of treatment systems.

Another option for society is to control 6PPD-quinone at the source, primarily that coming from tires. Studies are needed to determine the relative contributions of 6PPD-quinone from tires during their use on vehicles as well as the various end-of-life uses of tires such as crumb rubber in playgrounds and synthetic turf fields. It is feasible that an alternative antiozonant exists or can be designed that that can still meet acceptable standards for treadwear, traction performance, and temperature resistance while not causing acute mortality of a sentinel species. This will not necessarily be a simple process of substitution, but it is important that we avoid a 'regrettable substitution', or a substitution in 'name only' that will cause similar ecological harm. Our labs have begun screening alternative antiozonants, but a significant investment in research is needed if we are to solve the problem of 6PPD-quinone.

Funding:

USTMA states that "We have invested tens of millions of dollars [since 2005] in peer-reviewed research with the World Business Council for Sustainable Development's Tire Industry Project [TIP] to assess the impact of tire materials on the environment, wildlife and human health, including tire and road wear particles". These studies conclude that there are no

environmentally-relevant impacts. Notably, no salmonids have been included in TIP-funded studies of toxicity to aquatic animals, despite the reputation that salmonids have for being sensitive to chemical contamination and reports in the literature since the 1990s that chemicals from tires can kill *O. mykiss* (steelhead, aka rainbow trout).

Our research group has been almost exclusively responsible for research to understand the coho pre-spawning mortality phenomenon. Funding to support this research has been from a patchwork of sources, including federal, state, municipal, and private sector. Over 20 years, our larger research team has invested approximately 5 million dollars to ultimately discover that tires are the source of the acute mortality of an ESA-listed species of salmon that is economically, ecologically, and culturally important throughout the Pacific Northwestern United States.

Conclusion:

What regulations exist to protect a species like coho salmon from a toxic chemical like 6PPD-quinone? The Clean Water Act ensures "no toxics in toxic amounts" in our surface waters. The Endangered Species Act protects from harm species at risk of extinction. Tribal Treaty Rights, following the Boldt Decision, ensure that salmon exist at sufficient levels to enable harvest by Native Americans. Regulators of these and more local legislation have many more questions about 6PPD-quinone than we have answers. There are no answers yet for even simple questions such as what is the formation, fate, and transport of 6PPD-quinone from rubber products including tires? What other species are vulnerable to 6PPD-quinone? What are sub-lethal effects and do we need to be concerned about bioaccumulation to higher trophic levels, including humans? Are humans vulnerable to 6PPD-quinone through air pollution from traffic, playgrounds, or crumb rubber athletic fields? How is 6PPD-quinone captured in green infrastructure treatment systems and can those systems be optimized to enable more treatment per land area? Finally, are there safer anti-ozonants that would protect tire polymers from ozone? These and many other questions require a significant investment in research.

Finally, we are grateful for the committee's leadership on this issue and would like to specifically thank Members of the House of Representatives who unanimously supported the passage of H.R. 1144, the Promoting United Government Efforts to Save Our Sound (PUGET SOS) Act of 2021 sponsored by Congressman Derek Kilmer and Congresswoman Marilyn Strickland who represents the WSU Puyallup Research and Extension Center. We are grateful for the bipartisan support for this legislation. Salmon in the Pacific Northwest are not only a tribal and cultural resource for the Puget Sound region, but an economic resource as well.

I am grateful for the opportunity to present this testimony today and look forward to working with the committee going forward. Thank you.

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