Spring 2018

Assessing the Effects of Chemical Mixtures using a Bayesian Network-Relative Risk Model (BN- RRM) Integrating Adverse Outcome Pathways (AOPs) in Four Watersheds

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By

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Accepted in Partial Completion

Of the Requirements for the Degree

Master of Science

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MASTER'S THESIS

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5/25/18

Assessing the Effects of Chemical Mixtures using a Bayesian Network-Relative Risk Model (BN-RRM) Integrating Adverse Outcome Pathways (AOPs) in Four Watersheds

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

Of the Requirements for the

Degree Master of Science

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May 25, 2018

ABSTRACT

Chemical mixtures are difficult to assess at the individual scale and are more challenging at the population scale. I have conducted a regional-scale ecological risk assessment by evaluating the effects of chemical mixtures on populations with a Bayesian Network- Relative Risk Model (BN-RRM) in four Washington state watersheds (Lower Skagit, Nooksack, Cedar and Lower Yakima). Organophosphate pesticides (diazinon, malathion and chlorpyrifos) were chosen as the chemical stressors and the Puget Sound Chinook and Middle Columbia Chinook salmon (*Oncorhynchus tshawytscha)* Evolutionary Significant Units (ESUs) were chosen as the population endpoints. Laboratory tests found that organophosphate pesticide mixtures act synergistically and impair acetylcholinesterase activity leading to a change in swimming behavior and mortality. I have generated exposure-response equations for single chemicals, binary and ternary mixtures of organophosphates. The equations were incorporated into the BN-RRM framework to predict risk to a population. Dissolved oxygen and water temperature were chosen as ecological stressors to place the population in environmental context. The Puget Sound Partnership's management goal of Puget Sound Chinook is no net loss. A generic ocean-type Chinook salmon population model was used in this risk assessment. Each of the population model simulations started with 500,000 fish. Any number below 500,000 was defined as a net loss. Risk was defined the probability of not achieving the management goal of 500,000 fish. Calculations indicate synergism does not occur with measured concentrations. This is because malathion, the known synergist, was not found in concentrations that induced a greater than additive response. However, at malathion concentrations of 3-15 µg/L, synergism with the other OPs is predicted to occur and does increase risk. My research demonstrates that mixture toxicity can be incorporated into a probabilistic model that estimates the risk of mixtures to populations.

ACKNOWLEDGEMENTS

The laboratory data used for this project were provided by the Northwest Fisheries Science Center at NOAA and had been published in peer-reviewed literature. The environmental data were provided from various contributing agencies including USGS, WA Department of Agriculture, WA Department of Ecology, City of Bellingham, Nooksack Salmon Enhancement Association, King County DNR, King Conservation District, Skagit Conservation District, Yakima Tribal DNR, Samish Tribal DNR. Abigail Nickelson of the WA Department of Agriculture provided pesticide data used in this research. Cathy Laetz of NOAA Fisheries provided laboratory toxicity data for chemical mixtures. Julann Spromberg of NOAA Fisheries provided guidance of the population model in this project. Chris Trines of Huxley College of the Environment created the maps and provided land cover data. April Markiewicz of the Institute of Environmental Toxicology provided additional scientific guidance. My family, friends and my NOAA Office of Response and Restoration colleagues provided much needed emotional support and guidance.

Funding for this research was provided by the USEPA STAR Grant RD-83579501. Funding for travel to conferences was provided by the Institute of Environmental Toxicology at Western Washington University, the Dean’s Fund for Sustainability Studies and Ross Travel Grant

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ACRONYMS AND ABBREVIATIONS

AChE	Acetylcholinesterase
AOP	Adverse Outcome Pathway
BN	Bayesian Network
BN-RRM	Bayesian Network- Relative Risk Model
CA	Concentration Addition
CPT	Conditional Probability Table
DO	Dissolved Oxygen
ERA	Ecological Risk Assessment
ESA	Endangered Species Act
ESLOC	Endangered Species Level of Concern
ESU	Evolutionary Significant Unit
IA	Independent Action
KE	Key Event
MIE	Molecular Initiating Event
NOAA	National Oceanic and Atmospheric Administration
OP	Organophosphate pesticide
PSP	Puget Sound Partnership
WADOE	Washington State Department of Ecology
USEPA	United States Environmental Protection Agency

1.0 INTRODUCTION

In this research, I added data for chemical mixtures to an established Bayesian Network-Relative Risk Model (BN-RRM) framework incorporating an Adverse Outcome Pathway (AOP) (Landis et al. 2018, submitted). This framework examined risk to Chinook salmon (*Oncorhynchus tshawytscha)* populations in four Washington State watersheds from chemical and environmental stressors. I incorporated organophosphate pesticide mixtures to this BN-RRM framework. Risk was defined in this instance as the probability of not reaching the management goal of no net loss to the population. In each of the study watersheds, there was a high probability of not meeting the management goal. This risk assessment demonstrated that chemical mixtures can be incorporated to predict effects on a population.

1.1 Integrating Chemical Mixtures on Populations

Laboratory toxicity tests typically examine the effects of chemical mixtures on individuals (Barata et al. 2006, Laetz et al. 2009, 2013), but not on a population. Assessing risk at the individual scale and translating it to the population scale presents a long-standing challenge (Hinton et al. 2005). Individuals in populations interact over different spatial and temporal scales with a variety of ecological systems (Landis 2006). A framework is needed to predict the effects of chemical mixtures on populations across different spatial and temporal scales.

1.2 Ecological Risk Assessment and the Bayesian Network- Relative Risk Model

In an ecological risk assessment (ERA), impacts to the environment are calculated using several endpoints from various stressors (Suter 2007). The Relative Risk Model (RRM) is a method of ERA used to quantify the relative risk of an impact at a regional level over large spatial and temporal scales (Wiegers et al. 1998, Landis and Wiegers 2005, Colnar and Landis 2007) (Figure 1). The Bayesian Network- Relative Risk Model (BN-RRM) was developed by

Ayre and Landis (2012) to apply the Relative Risk Model (RRM) using a Bayesian Network (BN).

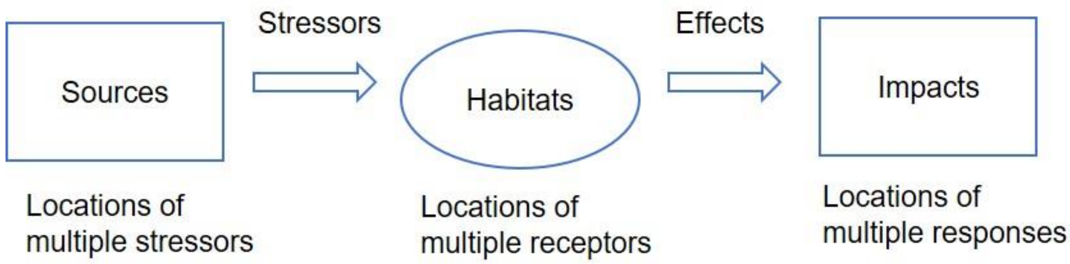

Figure 1. The relative risk model based on Landis and Wiegers (1997, 2005)

Bayesian Networks (BNs) are directed acyclic graphical models that use probabilistic calculations to describe ecological variables and the interactions. A BN model consists of nodes and linkages to represent cause and effect relationships, which represent the variables and the causal pathways, respectively. Each BN has parent nodes and child nodes. Parent nodes do not have inputs. Child nodes receive input from the parent nodes. Conditional probability tables (CPTs) within each node describe the interactions between parent and child nodes (Woodberry et al. 2004, Marcot et al. 2006, Carriger and Newman 2011). The BN-RRM has been used in a variety of ecological contexts, including forest management (Ayre and Landis 2012), whirling disease in cutthroat trout stocks (Ayre et al. 2014), nonindigenous species for the marine estuary in Padilla Bay, Washington, USA (Herring et al. 2015), and a mercury contaminated site in the South River, Virginia, USA (Landis et al. 2017a, 2017b, Johns et al. 2017, Harris et al. 2017). In my research, I introduced chemical mixtures on an already established BN-RRM framework using an Adverse Outcome Pathway (AOP) (Landis et al. 2018, submitted).

1.3 Adverse Outcome Pathway

An Adverse Outcome Pathway (AOP) is a cause-effect model that uses existing knowledge of the linkage of exposure and outcomes to organisms across all biological levels (Ankley et al.

2010). The AOP can enhance the risk assessment process by providing a mechanistic basis for linking key biological events at the molecular and cellular levels to risk assessment endpoints (Ankley et al 2010). Each AOP starts with a molecular initiating event (MIE) in which a chemical interacts with a biological target followed by a series of higher order effects or key events (KE) to produce an adverse outcome (Ankley et al. 2010, Russom et al. 2014). The current AOP structure, however, has a shortcoming in that the AOP does not provide ecological context. In this risk assessment, I applied the effects of mixtures through an AOP within four Washington state watersheds.

1.4 Mixtures, Toxicity and Risk

In the environment, chemicals exist as mixtures in all types of media (i.e. air, water, soil, sediment) (Monosson 2005). The U.S. Environmental Protection Agency defines chemical mixtures as “containing two or more identifiable components, but few enough that the mixture toxicity can be adequately characterized by a combination of the components toxicities and the component interactions” (USEPA 2000). There are three types of mixture interactions: additive, antagonistic and synergistic. Additive interactions are defined as when the toxicity of the chemicals is equivalent to the sum of the individual chemical toxicities on a per mole basis. Antagonistic interactions are defined as a situation in which the toxicity of the mixtures is less than the sum of the individual chemical toxicities (Monosson 2005). For example, metal mixtures such as cadmium and copper are less toxic at high concentrations than the predicted additive toxicity (Barata et al. 2006). Synergistic interactions are defined as a situation in which the toxicity of the mixtures is greater than the sum of the individual chemical toxicities. For example, the organophosphate pesticides (OP) mixtures such as malathion and chlorpyrifos are more toxic at high concentrations than the predicted additive toxicity (Laetz et al. 2009, 2013)

Predicting the toxicity of synergistic or antagonistic mixtures is challenging (Lydy et al. 2004). Two reference models are used to predict mixture toxicity: Concentration Addition (CA) and Independent Action (IA) (Loewe and Muischnek 1926, Bliss 1939). The CA assumes that all chemicals in a mixture act on the same biological target site (Loewe and Musichnek 1926). The IA considers chemicals that do not affect organisms at the same biological target site (Bliss 1939). Deviations from either the CA or IA model represent synergism or antagonism (Loewe and Muischnek 1926, Bliss 1939).

The CA and IA approaches have been successful at predicting toxicity from chemical mixtures in the laboratory setting (Barata et al. 2006, Laetz et al. 2009), but risk from chemical mixtures to the population scale has not been yet calculated. My thesis demonstrated that risk from chemical mixtures can be calculated in a multiple-stressor framework at the population scale.

1.5. Organophosphate Pesticides

Organophosphate Pesticides (OPs) are commonly used in agricultural and urban settings. In salmonid bearing streams in the Pacific Northwest, OPs are frequently detected as mixtures (Gilliom 2007, Anderson and Duggar 2008, Tuttle 2014). Many studies demonstrate that OPs cause neurotoxicity in salmonids (Sandahl et al. 2005, Tierney et al. 2007, Laetz et al. 2009, 2013). These OPs are known to inhibit the enzyme acetylcholinesterase (AChE) or the MIE by binding irreversibly to cysteine residues in the active site. This prevents AChE from cleaving free acetylcholine, a neurotransmitter in the neurosynaptic cleft. The buildup of the neurotransmitter leads to an excitatory response in the muscle and the brain. This effect can lead to neurotoxic death (KE2-KE5) (Russom et al. 2014). Laetz et al. (2009, 2013) demonstrated that OP mixtures are synergistic with the CA assumption. In my research, the toxicity of three OPs (diazinon, chlorpyrifos and malathion) were estimated in single chemical, binary and ternary mixtures.

1.6 Population Model with Chinook Salmon

Chinook salmon are an iconic species in the Pacific Northwest (PNW) because they are spiritually and culturally valued to local indigenous tribes and are economically important fisheries. The Puget Sound Partnership (PSP) uses Chinook salmon as an indicator species for the health of Puget Sound because these species are listed under the Endangered Species Act (ESA) (SSDC 2015, NOAA Fisheries 2017). Using a modeling approach, risk was modeled for a Chinook salmon population based upon water quality and pesticide use characteristics of four watersheds. The population model used in my research is derived from a Leslie matrix developed by Baldwin et al. (2009). This model is a generic model for ocean-type Chinook salmon.

1.7. Study Objectives

There were three primary objectives of this research:

(1) Derive a method to integrate potentially synergistic mixtures into the BN-RRM incorporating an AOP

(2) Compare change in risk to a Chinook salmon population from potentially synergistic mixtures compared to single chemical and additive mixtures

1.8 Summary of Outcomes

Based on my study objectives, the three major outcomes of this research are:

(1) I successfully described mixture toxicity using an AOP in a BN-RRM.

(2) The model results indicated that synergism did not occur with measured environmental concentrations in single, binary and ternary exposures, but measured environmental concentrations of OPs increased risk of not meeting the management goal.

(3) Synergism increased risk at high concentrations of 3-15 µg/L malathion compared to an additive model but, synergism rarely occurred (less than 6% probability) in the model.

The BN-RRMs created for my research provided a mechanistic understanding of the effects of chemical mixtures on a population.

2.0 METHODS

This section first provides an overview of the case study and then describes how the BN-RRMs were constructed and applied to this risk assessment for Chinook salmon in four watersheds. The four watersheds described different risk regions and thus different site-specific inputs. I constructed a total of seven BN models in Netica (Norsys Software Corp. 2014) represented by single OPs (malathion, chlorpyrifos and diazinon), binary mixtures (malathion and chlorpyrifos, malathion and diazinon, and diazinon and chlorpyrifos) and ternary mixtures (malathion, chlorpyrifos and diazinon). Each of the models were built with the same physical BN structure, but different equations were used to accommodate different chemicals and mixtures. Site specific data were used to incorporate pesticide concentrations and environmental conditions.

2.1 Case Study

The case study is based on four watersheds in Washington State, USA: Nooksack, Lower Skagit, Cedar and Lower Yakima Rivers (Figure 2). Chinook salmon populations in the Nooksack, Lower Skagit and Cedar River watersheds are part of the Puget Sound Chinook Evolutionary Significant Unit (ESU). An ESU is a population or group of populations that is considered distinct, usually separated by geographic regions, for conservation purposes (Waples 1991). The Lower Yakima watershed is part of the Middle Columbia Chinook ESU. More information about these watersheds can be found in the Supplemental Information (Section S1.0).

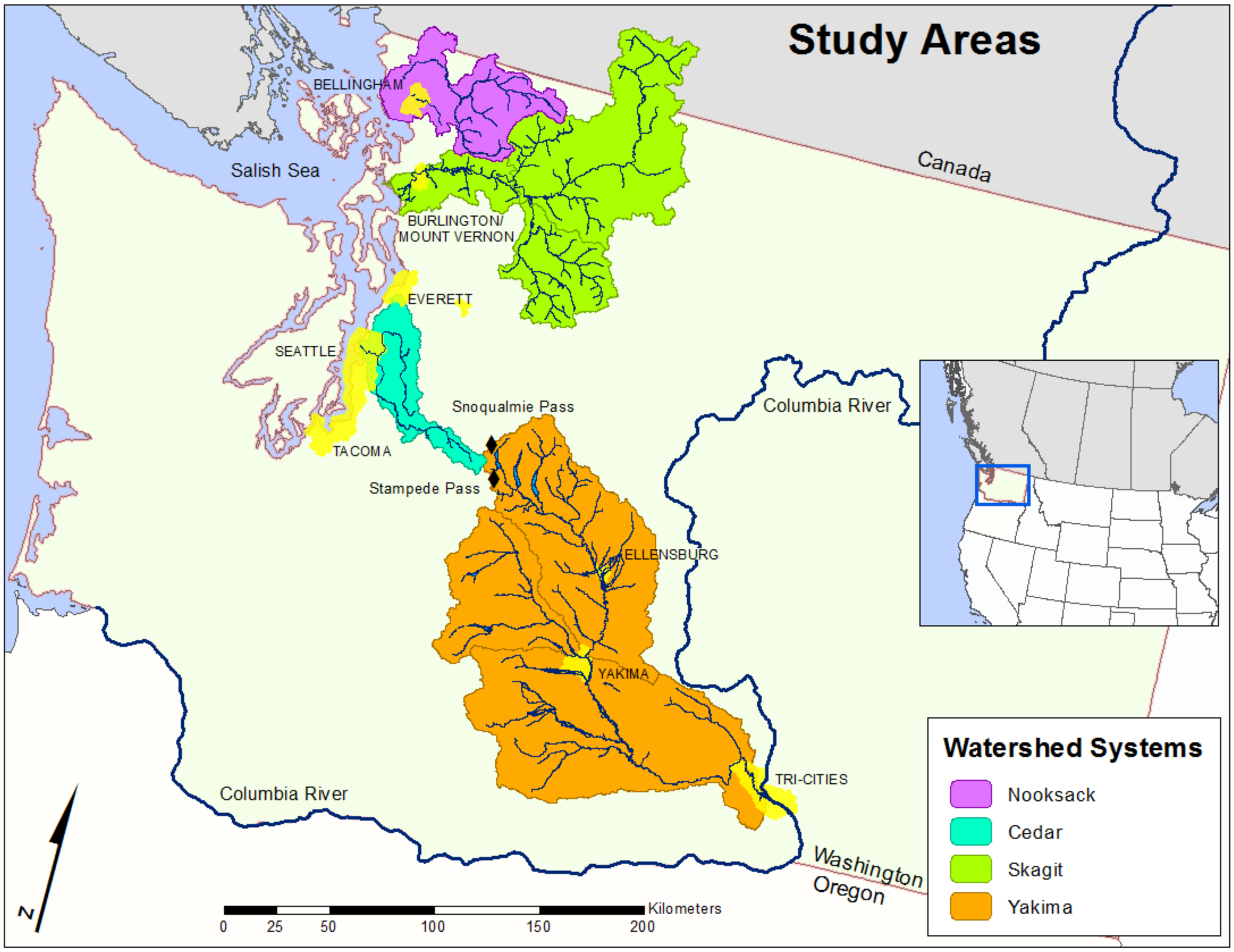

Figure 2. Map of watershed systems in Washington State. The Nooksack, Cedar and Skagit River watersheds are part of the Puget Sound Evolutionary Significant Unit. Yellow areas represent urbanized areas.

2.1.1 Lower Skagit River

The Skagit River Basin (SRB) is in southwestern British Columbia, Canada and northwestern Washington, USA (Figure 2). This river system is composed of many tributaries and drains an area of the Cascade Range into Puget Sound. Of all the drainages in Puget Sound, the SRB is the largest and produces the greatest abundance of salmonids with multiple salmon runs (Smith et al. 2003). The SRB consists of three watersheds: Upper Skagit, Sauk and Lower Skagit. Principle land uses in the Lower Skagit watershed include 72% forest, 15% agriculture and 10% developed areas.

2.1.2 Nooksack River

The Nooksack River Basin (NRB) drains an area of the Cascade Range around Mount Baker and empties into Bellingham Bay and to the Pacific Ocean via the Strait of Georgia (Beamer et al. 2016). Before the 20th century, the Nooksack River emptied into Lummi Bay, but a river delta formed blocking the channel to Lummi Bay. Salmonid spawning habitats in the NRB are subject to sedimentation, most originating from landslides (Brown et al. 2005). Principle land uses of the Nooksack River watershed include 66% forest, 12% agriculture and 11% developed areas.

2.1.3 Cedar River

The Cedar River Basin (CRB) drains to highly populated areas of the Seattle-metropolitan area (Figure 2). The CRB contains Lake Washington, which complicates salmon life history in this area as salmon will rear in the lake in addition to streams and tributaries (Greene 2017). Principle land uses of the Cedar River watershed include 48% developed, 43% forest, and 1% agriculture.

2.1.4 Lower Yakima River

The Yakima River Basin (YRB) is different from Puget Sound rivers because it is on the east side of the Cascades and is influenced more by snowmelt than rainfall (Furher et al. 2004) (Figure 2). In addition, salmon in the Yakima migrate through the lower Columbia River to the Pacific coast (Astoria), whereas all Puget Sound populations migrate out through some part of the Salish Sea. The YRB composes of three watersheds: Upper Yakima, Naches and Lower Yakima Rivers (Hoffarth 2017). Principle land uses in the Lower Yakima watershed include 68% forest, 24% agriculture and 7% developed.

2.2 Bayesian Network Relative Risk Model Overview

The steps involved in developing a BN-RRM are outlined in Herring et al. (2015), Johns et al. (2017) and Harris et al. (2017). This section describes the steps in sequential order.

2.2.1 Model Structure

The structure of the BN-RRMs for this study (Figure 3) was developed based on the original RRM framework on multiple endpoints over large spatial and temporal scales (Landis and Wiegers 2005, Colnar and Landis 2007, Herring et al. 2015, Johns et al. 2017, Harris et al. 2017) with modifications based on Chinook salmon life stage. Input variables or nodes represented the sources of the stressors based on the watershed and season. Each of the nodes were set up with ranks to describe distributions. The stressors to the salmon in these BN-RRMs were multiple OPs, dissolved oxygen (DO) and water temperature. Habitats were the Chinook habitat in each watershed represented by toxicological and ecological pathways, respectively. The effects of Chinook survival were described by life stages: egg to emergence, juveniles and adults. The impact was defined as the change in the probability of Chinook Population Size from the initial population of 500,000 fish in each river.

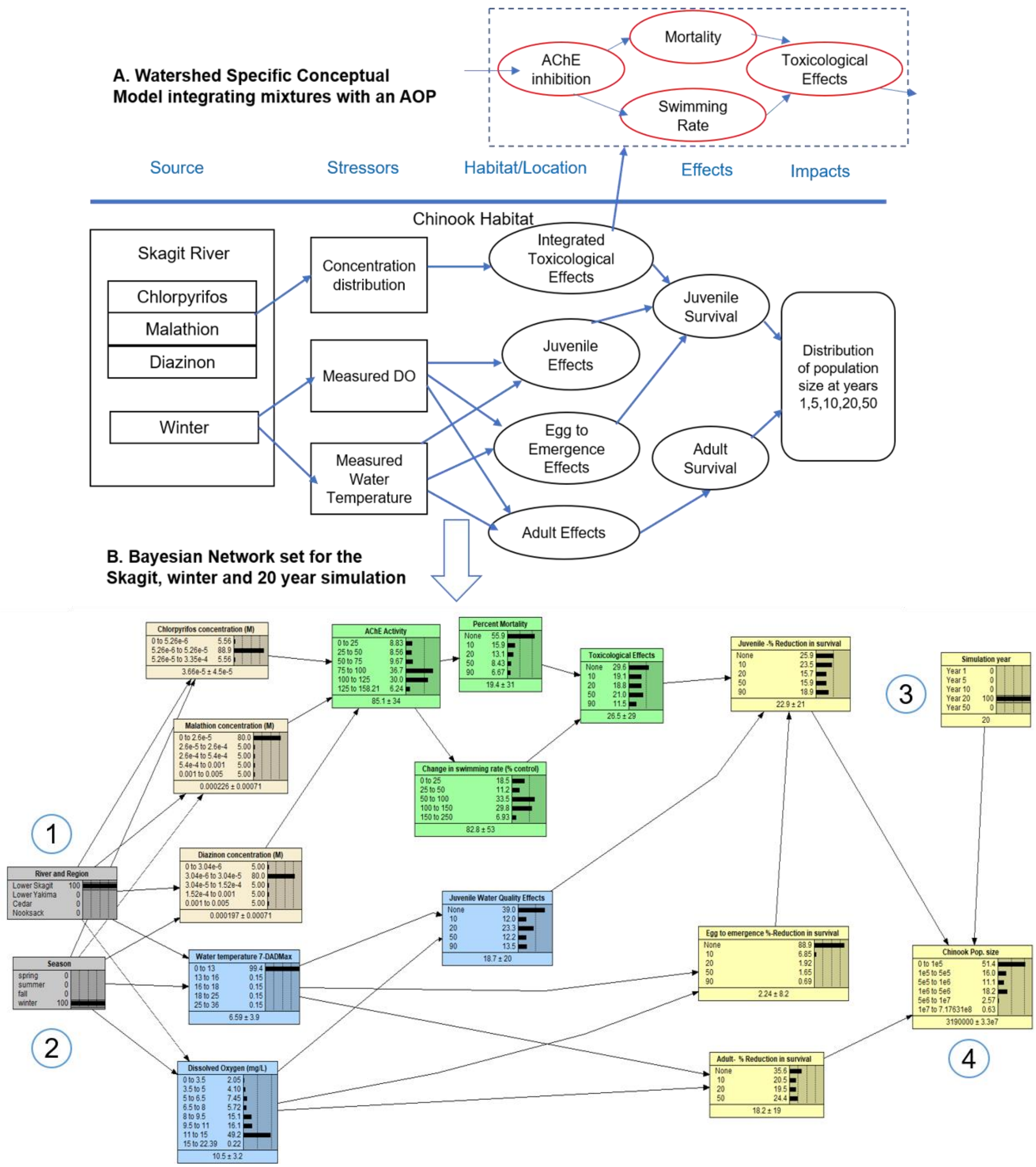

Figure 3 The conceptual model for the Skagit River introducing mixtures to a Bayesian Network (BN) using an Adverse Outcome Pathway (AOP). **A.** The conceptual model for the Skagit River introducing mixtures to an AOP **B.** The Bayesian Network- Relative risk model based on the (1) Skagit river in the (2) winter (3) year 20 (4) Chinook Population Size. The network structure is the same for each OP model

2.2.2 Selection of Endpoint- Chinook Salmon

Endpoints can be defined as entities and their attributes, where attributes describe the characteristics or qualities of an endpoint (USEPA 1998). For example, the endpoint selected in my risk assessment was Chinook salmon. The attribute was population size compared to the PSP management goal of no net loss. The initial population size in each of the Baldwin et al. (2009) simulations was arbitrarily set at 500,000 fish. Thus, a number below 500,000 fish was defined as a net loss. Puget Sound Evolutionary Significant Unit (ESU) populations are listed as threatened under the Endangered Species Act (ESA) (NOAA Fisheries 2017) while Middle Columbia ESU populations are not yet listed. The same criterion was used for the Lower Yakima watershed although the Chinook populations are not yet listed.

2.2.3 Identification of Stressors, Sources of Stressors and Habitat

In these BN-RRMs, the stressors were multiple OPs and water quality parameters. The sources of the stressors were the river systems during each season. The habitats were the rivers within the four watersheds containing Chinook salmon. Spatial relationships between the sources were depicted by habitats within the watersheds from the site-specific water quality and OP concentration data. In Washington State, juvenile Chinook rear in small streams often proximate to agricultural land, leading to both chronic and acute exposure to OP pesticides (Macneale et al. 2010, NMFS 2008). The Nooksack, Lower Skagit and Lower Yakima River watersheds are influenced by agricultural land use, while the Cedar watershed is influenced by urbanization (Tuttle 2014).

2.3 Model Construction

Each of the BN-RRMs used in this study was constructed in the same manner, but with different equations accommodating different mixtures. These BN-RRMs used many types of data to 1) set up ranks and 2) build the CPTs. The methods of construction relating to these two categories are described below.

2.3.1 Ranks

Each node in the BN-RRMs was set up with ranks from states and ranges to represent groupings of the model output distribution. The stressor nodes were ranked based on regulatory criteria and exposure response breaks in the dose response curves. The river and watershed nodes were based on the four watersheds to model distributions of OP and ecological stressors for the selected watershed. The season node was based on months to set the site-specific data for the selected watershed. The concentrations of the OPs were converted from µg/L to moles/L and the ranks were based on regulatory criteria and exposure response breaks in Laetz et al. (2009) data (Table 1). Each of the chemicals had its own ranking. For example, malathion concentrations were ranked with these justifications: 2.6E-5 M is the Maximum Daily Load (MDL), 5.4E-4 M is the endangered species level of concern (ESLOC) for freshwater fish, 0.001 M is the 0.05 EC50 unit published in Laetz et al. (2009) and 0.005 M is the 0.2 EC50 unit published in Laetz et al. (2009). Dissolved oxygen and water temperature stressors were ranked with regulatory criteria (WAC 2011a, 2011b) and survival data (Brett 1952, Carter 2008, Carter 2005, Geist et al. 2006, Jager 2011, McCullough 1999, McCullough 2001, Peery 2010, Richter and Kolmes 2005). A complete table of ranks and the criteria used to set them can be found in the Supplemental Information (Section S2.0, Table S1).

Table 1. Summary Table describing the stressor nodes, rankings and justifications for pesticides, water temperature and dissolved oxygen.

Node	Description	Ranking	Justification	Units / Descriptor
Diazinon Concentrations	Measured concentrations of diazinon over a ten-year period in each of the river's major waterways.	0 - 3.04e-6, 3.04e-6 - 3.04e-5, 3.04e-5 - 1.52e-4, 1.52e-4 - 0.001, 0.001 - 0.005	Values were converted from µg/l to M. 3.04e-6 is the Maximum Daily Load (MDL). 1.52e-4 is the Endangered Species level of concern (ESLOC) for Freshwater fish. 0.001 is the 0.025 EC50 published in Laetz et al. (2009) 0.005 is the 0.1 EC50 published in Laetz et al. (2009). Distribution is based on downloaded data for each of the watersheds	Moles
Malathion Concentrations	Measured concentrations of malathion over a ten-year period in each of the river's major waterways.	0 - 2.6e-5, 2.6e-5 - 2.6e-4, 2.6e-4 - 5.4e-4, 5.4e-4 - 0.001, 0.001 - 0.005	Values were converted from µg/l to M. 2.6e-5 is the Maximum Daily Load (MDL). 5.4e-4 is the Endangered Species level of concern (ESLOC) for Freshwater fish. 0.001 is the 0.05 EC50 published in Laetz et al. (2009). 0.005 is the 0.2 EC50 published in Laetz et al. (2009). Distribution is based on downloaded data for each of the watersheds	Moles
Chlorpyrifos Concentrations	Measured concentrations of chlorpyrifos over a ten-year period in each of the river's major waterways.	0 - 5.26e-6, 5.26e-6 - 5.26e-5, 5.26e-5 - 3.35e-4	Values were converted from µg/l to M. 5.26e-6 is the Maximum Daily Load (MDL), 5.26e-5 is the Endangered Species level of concern (ESLOC) for Freshwater fish. 3.35e-4 is the 0.5 EC50 published in Laetz et al. (2009) Distribution is based on downloaded data for each of the watersheds.	Moles
Water Temperature	Measured water temperature over a ten-year period in each of the river's main waterways.	0 to 13, 14 - 18, 19 -25, >25	Temperature ranges specific to salmonids based on Table 200 (1)(c) Aquatic Life Temperature Criteria in Fresh Water and survival data. Distribution is based on downloaded data for each of the watersheds.	°C (7-day average of the daily maximum temperature)
Dissolved Oxygen	Measured oxygen concentrations over a ten-year period in each of the river's main waterways.	0 - 3.5, 3.5 - 5, 5 - 9, 9.5 - 15, >15	Ranges specific to salmonids based on Table 200 (1)(d) Aquatic Life Temperature Criteria in Fresh Water and survival data. Distribution is based on downloaded data for each of the watersheds.	mg/L

2.3.2 Conditional Probability Tables

Conditional Probability Tables (CPTs) were built by using empirical evidence, curve fitting, simulation models, expert judgement and case file learning (Marcot et al. 2006; Pollino et al. 2006; Chen and Pollino 2012). A case file is a compilation of a set of empirical data that provide information about the variables. *Case learning* is a function of Netica (Norsys Software Corp. 2014) that finds relationships to create a distribution of values based on empirical data. The

AOP pathway constructed in this model mostly used curve fitting from exposure response curves via a Netica function called *Equation to table* to generate CPTs (Norsys Software Corp. 2014). *Equation to table* used inputted equations to build CPTs (Twardy et al. 2004). Chinook Population Size was constructed with population model simulations and *case learning* to incorporate the simulations. The remaining nodes were derived with case learning functions and empirical data.

2.4 Toxicology and Adverse Outcome Pathway

This section describes how the Adverse Outcome Pathway (AOP) was constructed for single, binary and ternary OP mixtures. An AOP is a cause-effect model that uses existing knowledge of the linkage between a molecular initiating event (MIE) and key events (KE) to organisms at all biological levels (Ankley et al. 2010). An AOP of OPs started with the MIE of acetylcholinesterase (AChE) inhibition followed by KEs. Single, binary and ternary OP exposures connected the MIE and subsequent KEs. The KEs were in the form of sublethal and lethal toxicological effects. Because of this, toxicological effects were focused on the early development stages of the salmon (fry, parr and smolt) before they migrate to the ocean because juvenile salmon tend to rear in streams that are proximate to agricultural lands (Scholz et al. 2000, Baldwin et al. 2009, Laetz et al. 2009, 2013, 2014).

2.4.1 Single, binary and ternary OPs and the MIE

Single OP exposures were evaluated in single exposure-response relationships. Using data provided from Laetz et al. (2009), exposure-response relationships were generated for AChE inhibition as a function of exposure to diazinon, chlorpyrifos and malathion exposure concentrations. Exposure-response equations were developed with the *drc* package in R statistical software (R Core Team 2017) and then incorporated in the BN.

In this research, binary mixture exposures were modified by converting Laetz et al. (2009, 2013) binary mixture data into moles/L. Mole fractions are the units, which are defined as the amount of a chemical divided by the total amount of all chemicals in a mixture (Taylor and Thompson 2008). Binary mixtures (malathion +chlorpyrifos, malathion + diazinon, chlorpyrifos + diazinon) were fit into one exposure response dimension. I standardized the units to moles/L with the following steps:

1. Using data from Laetz et al. (2009, 2013), I converted effect concentration fifty percent (EC50) chemical concentrations to moles/L to help facilitate the fit.
2. From Laetz et al. (2009, 2013), OP binary mixture exposure concentrations were derived from individual respective median EC50 units expressed in 0.1, 0.4, 1.0 or low, medium and high exposures
3. Moles/L from each chemical from all binary mixture EC50 units were summed.
4. Exposure-response relationships were generated for OP-induced AChE inhibition as a function of exposure to binary mixtures concentrations.

Exposure-response equations for binary mixtures were developed with the *drc* package in R statistical software (R Core Team 2017) and then inputted in the BN using the *Equation to Table* command. Within R, multiple dose response equations were tested for fit for each of the OP pesticide dose-response relationships (Ritz et al. 2015). An example of an equation and figure for an OP binary mixture can be found in the Supplemental Information (Section S3.1, Figure S5).

There were no data currently available for ternary OP mixtures (chlorpyrifos + malathion+ diazinon). As a result, the ternary mixture AChE activity node was extrapolated based on binary mixture information from all three combinations of binary mixtures (malathion + diazinon, malathion + chlorpyrifos, diazinon + chlorpyrifos).

2.4.2 *MIE to Change in Swimming Speed and Percent Mortality*

The Change in Swimming Speed node was defined as individual sublethal effects linked from AChE inhibition. Data for swimming speed were derived from NOAA Fisheries (Sandahl et al. 2005, Laetz, unpublished, and Tierney et al. 2007). A case file consisting of 87 cases were entered into Netica using the *Learn from Case File* function to set the CPT.

The Percent Mortality node was created with a dose response curve using Laetz et al. (2009) data with AChE values and mortality data. The dose response equation was evaluated for model fit in R statistical software (R Core Team 2017) using the *lmtest* package and then inputted into the BN using the *Equation to Table* command. The dose response equation and figure can also be found in the Supplemental Information (Section S3.2, Figure S6).

2.4.3 *Combining Swimming Speed and Percent Mortality to Toxicological Effects*

Toxicological Effects was a summary node that combines both sublethal and lethal effects. Combined sublethal and lethal effects of single OPs and mixtures provided a more accurate population response to estimate risk, since sublethal effects may have important population-level consequences. High and low sublethal and lethal effects were well-documented in the literature (Sandahl et al. 2005, Laetz, unpublished, and Tierney et al. 2007, Laetz et al. 2009). However, the intermediate sublethal and lethal effects were less defined in the literature, necessitating the use of an extrapolation approach called “peg the corners” (Marcot 2017) to fill in the data gap. The “peg the corners” approach was used by establishing the minimum and maximum values in the corners of the CPT and estimating the intermediate values.

2.5. Ecological Pathway

The ecological segment incorporated pathways based on site-specific water quality data from each of the watersheds. Different temperature and dissolved oxygen regimes were given in the different distributions by season in each watershed. Connections were made from water temperature and dissolved oxygen to generate Juvenile Water Quality Effects, Egg to Emergence %-Reduction in Survival and Adult %-Reduction in Survival nodes. These connections were made based on case file learning functions generated from the literature (Brett 1952, Carter 2008, Carter 2005, Geist et al. 2006, Jager 2011, McCullough 1999, McCullough 2001, Peery 2010, Richter and Kolmes 2005). Juvenile Water Quality and Egg to Emergence %-Reduction in Survival then connected to Juvenile %-Reduction in the Survival node.

2.6 Population Parameters and Modeling Chinook Population Size

This section first describes the BN population parameter nodes of Juvenile and Adult %-Reduction in Survival. Then this section describes how the Baldwin et al. (2009) population model was used to calculate Chinook salmon abundance. Baldwin et al. (2009) model simulations were run in RAMAS GIS 6 (Akçakaya and Root 2013) by Chelsea Mitchell at Washington State University-Puyallup.

2.6.1. Juvenile and Adult %-Reduction in Survival

Juvenile %-Reduction in Survival was constructed with a “peg the corners” approach assuming additivity linked from the Toxicological Effects, Juvenile Water Quality Effects and Egg to Emergence %-Reduction in Survival nodes. The “peg the corners” approach was used because there was a large data uncertainty with the interactions between chemical and ecological stressors. Juvenile %-Reduction in Survival node then linked to the Chinook Population Size node. Adult %-Reduction in survival was constructed with water quality effects from a case file learning function generated from the literature (Jager 2011; McCullough 1999; McCullough et al. 2001; Peery 2010; Richter and Kolmes 2005). Adult %-Reduction in Survival node also linked to the Chinook Population Size node.

2.6.2 The Baldwin et al. (2009) Model and Chinook Population Size

The Baldwin et al. (2009) model was an age-based Leslie matrix population projection for ocean-type Chinook salmon. This model is generic and was developed from demographic information from multiple Pacific Northwest Chinook populations from the Columbia River Basin, Skagit River Basin and the Oregon coast from natural origin data. The transition elements in the matrix reflect an anadromous and semelparous life history strategy where the maximum female age is 5, the sex ratio is 1:1, and reproductive maturity occurs at ages 3, 4, and 5.

With the Baldwin et al. (2009) model, RAMAS GIS 6 (Akçakaya and Root. 2013) was used to run the simulations for a 50-year period assuming no density-dependence. Two hundred replications of each simulation were performed for each possible combination of conditions from the Juvenile %-Reduction in Survival and Adult %-Reduction in Survival nodes. The initial population size for each model simulation was arbitrarily set at 500,000. The raw output of each simulation was collected at 1, 5, 10, 20 and 50 years. All model simulations were constructed into a case file resulting in 24,388 cases. The case file was then inputted into the BN using case learning to the Chinook Population Size node.

2.6.3 Extinction

The case learning algorithm did not account for population extinction in the model simulations, thus modifications were made in the Chinook Population Size CPT. Population extinction was defined in model simulations as having a population of zero fish at any simulation year. Extinction was generally observed at longer simulation years (e.g. 20 or 50 years) and higher values of percent reduction in survival in both juveniles and adults (e.g. 50 or 90% reduction). I edited the population size case file derived CPT to reflect the knowledge that extinct populations in closed models cannot return using specific rules. The rules are found in the Supplemental Information (Section S4.0).

2.7 Risk Calculation and Characterization

Each input of the BN-RRMs represented different scenarios to calculate risk to the endpoint. For each scenario, Netica used probabilistic methods to calculate a population distribution to the endpoint (Norsys Software Corp. 2014). The endpoint in my study was the population abundance of Chinook salmon for a specific population model simulation year. The output of Chinook population abundance reported six different population distribution bins: 0-100,000; 100,000-500,000; 500,000-1,000,000; 1,000,000-5,000,000; 5,000,000-10,000,000; and 10,000,000-720,000,000. The simulation years are for years 1, 5, 10, 20 and 50 from the Simulation Year node.

Estimations of risk to the endpoint was based on the management goal. For Chinook salmon, the defined goal was no net loss of the population. In the population simulations, the starting point for the population was arbitrarily set at 500,000 fish and a number below that was defined as a net loss. Risk was defined as the probability that a population was below the management goal of 500,000 fish. The total probability of not meeting the management goal was made by summing up the probabilities of the Chinook Population Size bins of less than 500,000 fish. The results were presented in Simulation Year 20 because the population size distribution starts to anchor in this year. I defined the following scenario categories to calculate risk to Chinook populations at the twenty-year simulation time:

1) *The Baldwin model* was defined in the BN model as set to 100% probability of 0% reduction in survivorship in both the Juvenile %-Reduction in Survival and Adult %-Reduction in Survival nodes
2) *20 percent reduction* in survivorship was defined in the BN model as set to 100% probability of 20% reduction in both the Juvenile %-Reduction in Survival and Adult %-Reduction in Survival nodes
3) *Only environmental stressors* were defined in the BN model as set to 100% probability of no Toxicological Effects node leaving only environmental stressors for each watershed

4) *Measured concentrations* were defined in the BN model as set to the amount of OP concentrations (single, binary or ternary) given in each watershed.
5) *Modeled synergistic concentrations* were defined in the BN as set to 100% probability of 3-15 µg/L or 0.001-0.005 M malathion and diazinon and 0.15-1 µg/L or 5.26e-5 to 3.35e-4 M chlorpyrifos derived from Table 2 and equations below.
6) *Synergistic and additive exposures* was defined as set to *modeled synergistic concentrations* in the BN with each binary OP mixture (malathion + diazinon, malathion + chlorpyrifos, chlorpyrifos + diazinon) and an additive malathion + diazinon exposure from derived from Table 2 and equations below.

The equations used to model synergistic concentrations was derived from a log-logistic 3-parameter model given in R statistical software's *drc* package (Ritz et al. 2015):

$$f\,(x;\, b,\, d,\, e) = \frac{d}{1 + \exp(b(\log(x)-\log(e)))}$$

Table 2. Parameters of the binary mixture exposure response curves. Data from Laetz et al. (2009, 2013)

Mixture concentrations (x)	d	b	e
Synergistic- Malathion + Diazinon	121	8.88	0.000897
Synergistic- Malathion + Chlorpyrifos	102	2.02	0.00296
Synergistic- Diazinon + Chlorpyrifos	102	0.63	0.00803
Additive- Malathion + Diazinon	153	0.83	0.268

2.7.1 Uncertainty Analysis

Epistemic uncertainty was described in the probability distributions of BNs as well as the model inputs (Spiegelhalter and Riesch 2011). In these BN-RRMs, uncertainty in any model input was incorporated into the variation of that node. When data were unavailable for an input parameter, equal probabilities were assigned to the each of the states for that node. Both the uncertainty in the model inputs and exposure response relationships were translated through the model as wider probability distributions of the intermediate and endpoint nodes.

2.7.2 Sensitivity Analysis

A sensitivity analysis was performed to assess the importance of each parameter to the endpoint of Chinook Population Size at Simulation Year 20. The *Sensitivity to Findings* tool within Netica was used to run this analysis. *Sensitivity to Findings* measured mutual information between each of the input nodes calculated to the endpoint node (Norsys Software Corp. 2014, Pollino et al. 2006). Mutual information was a function of both the findings in the node (input frequency) and the relationship described in the CPT (Marcot 2012, Norsys Software Corp. 2014). A high value of mutual information for an input indicated more influence on the endpoint node (Marcot 2012).

3.0 RESULTS

3.1 Understanding the Model Output with an Example

Each BN concluded with the endpoint node of Chinook Population Size. For each scenario, Netica used the model inputs and probabilistic methods to calculate a distribution at specific time points. From the distribution, probabilities of less than 500,000 fish were summed to calculate risk. As examples, I compared the probability distributions of each size category for year 20 of the *Baldwin model*, *20 percent reduction*, *only environmental stressors* in the Skagit-winter and *measured concentrations of* OP stressors in the Skagit-winter (Figure 4). Table 3 compared risk from these four scenarios. All scenarios had a probability of not meeting the management goal. For the Baldwin model, the probability is 2%, whereas the *20 percent reduction* scenario was at a 92% probability. *Only environmental stressors* in the Skagit-winter had a 54% probability. Adding *measured concentrations* of OPs in the Skagit-winter increased risk (67% probability or a 13% probability increase).

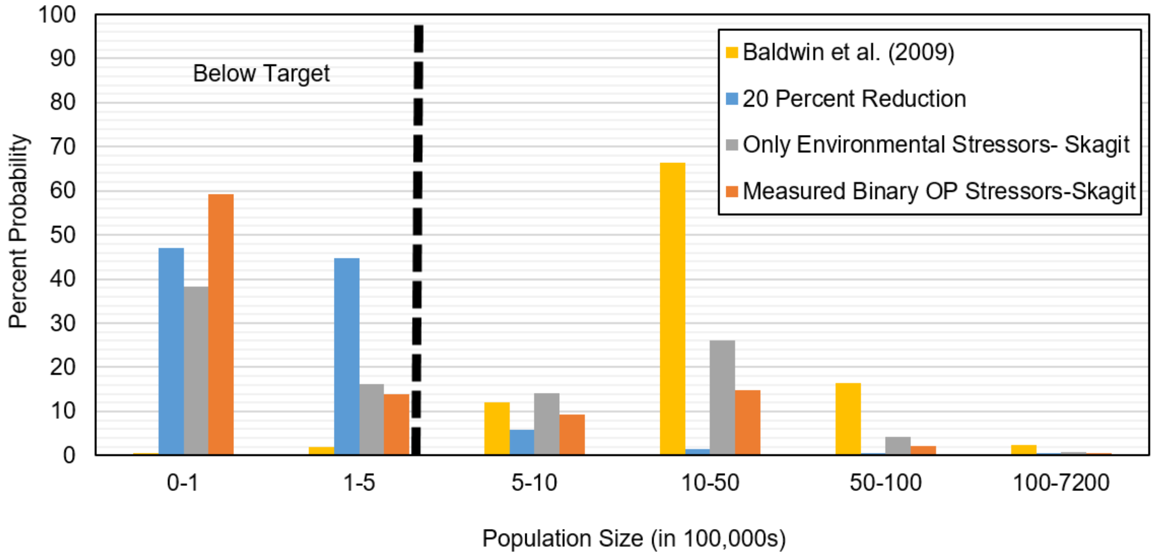

Figure 4. Chinook Population Size distribution outputs with various exposure inputs represented by Baldwin et al. (2009), 20 percent reduction, only environmental stressors, measured binary OP stressors. The dashed line represents the management goal of 500,000 fish.

Table 3. Risk from various exposure inputs (in percent probability)

Scenario	Risk
Baldwin Model	2
20 Percent Reduction	92
Only Environmental Stressors- Skagit River	54
Measured Binary OP Stressors- Skagit River	67

3.2. Risk from Measured Concentrations by Watershed

Risk was calculated with *measured concentrations* of binary mixtures combined with environmental stressors of temperature and dissolved oxygen. Table 4 compared risk for each of the four watersheds between winter and summer conditions. Risk varied more among seasons than watersheds or *measured concentrations*. Risk was highest with binary *measured concentrations* stressors in the Nooksack-summer (93% probability) and lowest with ternary *measured concentrations* stressors in the Cedar-winter (66% probability). Although the contribution of environmental stressors was greater than toxicity in both the winter and summer, the contribution of average measured OP mixtures concentrations was greater in the winter than the summer. The average proportion of risk due to toxicity was greatest in the Cedar-winter (22% probability) and the lowest in the Nooksack-summer (3% probability).

Risk exhibited a similar pattern between watersheds because risk was lower in the winter and greater in the summer. In the winter, the change in risk between watersheds was about 14% probability from adding OP stressors. In the summer, the change in risk between watersheds was smaller at about a 6% probability from adding OP stressors (Table 4). There were no differences in risk between *measured* binary or ternary OP stressors between watersheds during all seasons. In fact, the differences in risk between *measured* binary or ternary OP stressors was only about 1% (Table 4).

Table 4. Winter and summer risk for measured concentrations by watershed (in percent probability)

Scenario	Measured Ternary OP Stressor Risk	Measured *Binary OP Stressor Risk	Only Environmental Stressors Risk	Change in Risk (from adding OP stressors)	Proportion of Risk Due to Toxicity	Proportion of Risk Due to Environmental Stressors
Skagit- winter	67	68	55	13	19	81
Skagit - summer	80	80	73	8	9	91
Nooksack- winter	69	69	55	14	20	80
Nooksack- summer	92	93	90	3	3	97
Cedar-winter	65	66	51	14	22	78
Cedar-summer	82	82	75	7	9	91
Yakima- winter	67	67	53	14	20	80
Yakima- summer	85	85	80	6	7	93
*Binary OP Stressor is malathion and diazinon						

3.3. Risk from OP Concentrations in the Skagit River

Risk was calculated with *measured* and *modeled synergistic concentrations* of OP mixtures as well as environmental stressors in the Skagit River. Table 5 compared risk from various OP concentrations and environmental conditions in the Skagit River during the winter. Risk was at a 55% probability with *only environmental stressors* in the winter. Adding *measured concentrations* of single, binary or ternary OP stressors to environmental stressors, increased risk (67, 68 or 67% probability, respectively; Table 5) with no difference between measured single, binary or ternary OPs. Once *modeled synergistic concentrations* of binary and ternary OP mixtures were added, the risk increased even more (75 or 74% probability, respectively; Table 5) from *only environmental stressors*. *Modeled synergistic concentrations* of binary and ternary OP mixtures increased risk about 7 or 8% from *measured concentrations* and 19 or 20% from *only environmental stressors* (Table 5). Table 6 compared risk from various OP concentrations and environmental conditions in the Skagit during the summer. The contribution of *measured* and *modeled synergistic concentrations* of binary and ternary OP mixtures in the summer was less than the winter. The proportion of risk due to toxicity was about 10% with *measured concentrations* and 14% with *modeled synergistic concentrations* in the summer (Table 6) compared to 19% and 27%, respectively in the winter (Table 5)

Table 5. Winter Skagit River risk from OP concentrations (in percent probability)

Scenario	OP Exposure Type	Risk	**Change in Risk** (from adding OP stressors)	Proportion of Risk Due to Toxicity	Proportion of Risk Due to Environmental Stressors
Only Environmental Stressors	None	55	-	-	100
*Single OP Stressor	Measured	67	12	18	82
*Binary OP Mixture	Measured	68	13	19	81
Ternary OP Mixture	Measured	67	12	18	82
*Binary OP Mixture	Modeled Synergistic	75	20	27	73
Ternary OP Mixture	Modeled Synergistic	74	19	26	74
*Binary OP Mixture is malathion and diazinon, single OP stressor is chlorpyrifos					

Table 6. Summer Skagit River risk from OP concentrations (in percent probability)

Scenario	OP Exposure Type	Risk	**Change in Risk** (from adding OP Stressors)	Proportion of Risk Due to Toxicity	Proportion of Risk Due to Environmental Stressors
Only Environmental Stressors	None	73	-	-	100
*Single OP Stressor	Measured	80	8	10	90
*Binary OP Mixture	Measured	80	8	10	90
Ternary OP Mixture	Measured	80	8	10	90
*Binary OP Mixture	Modeled Synergistic	85	12	14	86
Ternary OP Mixture	Modeled Synergistic	85	12	14	86
*Binary OP Mixture is malathion and diazinon, single OP stressor is chlorpyrifos					

3.4. Risk from Additive and Synergistic Exposures in the Skagit River

Risk was calculated with *additive and synergistic exposures* in the Skagit River. Synergism was observed in the diazinon and malathion mixture as well as the malathion and chlorpyrifos mixture (Tables 7 and 8). No synergism was observed with the diazinon and chlorpyrifos mixture (Tables 7 and 8). Table 7 compared risk from *additive and synergistic exposures* in the Skagit during the winter. The proportion of risk due to synergism was 11% in the diazinon and malathion mixture and 3% in the malathion and chlorpyrifos mixture (Table 7). Table 8 compared risk to *additive and synergistic exposures* in the Skagit during the summer. Synergism was still observed in the summer, but less risk contributed from OP mixtures than in the winter. The proportion of risk due to synergism is 6% in the diazinon and malathion mixture and 1% in the malathion and chlorpyrifos mixture (Table 8). Overall, synergism did not contribute much more risk.

Table 7. Winter Skagit River risk from additive and synergistic exposures (in percent probability)

Scenario	**OP Exposure Type**	**Risk**	**Change in Risk** (from additive)	**Proportion of Risk Due to Synergism**
Additive- Diazinon + Malathion	Modeled Synergistic	67	-	-
Synergistic- Diazinon + Malathion	Modeled Synergistic	75	8	11
Synergistic- Malathion + Chlorpyrifos	Modeled Synergistic	69	2	3
Synergistic- Diazinon + Chlorpyrifos	Modeled Synergistic	67	-	-

Table 8. Summer Skagit River risk from additive and synergistic exposures (in percent probability)

Scenario	OP Exposure Type	Risk	**Change in Risk** (from additive)	Proportion of Risk Due to Synergism
Additive- Diazinon + Malathion	Modeled Synergistic	80	-	-
Synergistic- Diazinon + Malathion	Modeled Synergistic	85	5	6
Synergistic- Malathion + Chlorpyrifos	Modeled Synergistic	81	1	1
Synergistic- Diazinon + Chlorpyrifos	Modeled Synergistic	80	-	-

3.5 Uncertainty Analysis

The BN-RRMs were successful at calculating risk to a Chinook salmon population, but there were several sources of uncertainty. One source of uncertainty was that the toxicological pathway (found in the AChE Activity, Change in Swimming Rate, and Percent Mortality nodes) are not species-specific. Coho salmon instead of Chinook salmon were used due to the ESA-listed status of Chinook (Laetz et al. 2009, 2013, Tierney et al. 2007, Sandahl et al. 2005, NOAA Fisheries 2017). Another source of uncertainty was that the ecological pathway was sometimes not site-specific to the four watersheds. These uncertainties are highlighted in the Supplemental Information (Table S1).

Variability in the exposure response curves and population model was another source of uncertainty. In the toxicity pathway, each of the exposure response curve equations were evaluated with confidence intervals. An example is in the Supplemental Information (Figure S5). Environmental and demographic stochasticity was implemented in the Baldwin et al. (2009) population model. Environmental stochasticity was implemented by selecting survival and reproduction values from a lognormal distribution, based on values from a standard deviation matrix. Demographic stochasticity was implemented by sampling the number of survivors in each iteration from a binomial distribution, and the number of offspring from a Poisson distribution (Akçakaya and Root 2013).

Another source of uncertainty was lack of knowledge. A “peg the corners” method was used to construct both the Juvenile %-Reduction in Survival and Toxicological Effects nodes. This “peg the corners” method was cited as an extrapolation method in Marcot (2017), but information about the intermediate effects were unknown, necessitating the use. These data uncertainties are addressed further in the Discussion section.

3.6 Sensitivity Analysis

A sensitivity analysis was conducted to measure which model parameters most affected the endpoint at Simulation Year 20. Sensitivity analyses used mutual information as the metric (Marcot 2012). The top two nodes in each model parameter category are presented below in Tables 9-14. Juvenile and Adult %-Reduction in Survival were ranked the highest by mutual information in all scenarios because these are the two nodes adjacent to the Chinook Population Size node. In addition, these two nodes were critical variables in the calculation of population dynamics. Percent Mortality and Change in Swimming Rate were ranked the highest in the toxicity pathway because those nodes were the lethal and sublethal effects driving the pathway. The following results indicated that the stressor nodes were different between scenarios. Results for each stressor scenario in the winter and summer are described below. A complete ranking of the model parameters is found in the Supplemental Information (Section S5.0)

3.6.1 Sensitivity to Endpoint by Watershed from Measured Concentrations

Sensitivity results with *measured concentrations* of the diazinon and malathion mixture by watershed showed that the stressors with the most mutual information vary by season. In the winter, the sensitivity analysis indicated that Toxicological Effects was the stressor that was the most common top ranked by mutual information in all watersheds. Dissolved Oxygen was the second top ranked in the Skagit, Cedar and Yakima River watersheds. Water Temperature was the second top ranked in the Nooksack River (Table 9). In the summer, the sensitivity analysis indicated that Water Temperature was the stressor that was the most common top ranked by mutual information in all watersheds. Toxicological Effects was the second top ranked in the Nooksack and Yakima River watersheds; while Dissolved Oxygen was the second top ranked in the Skagit River and Cedar River watersheds (Table 10).

Table 9. Winter sensitivity analysis results from measured concentrations by watershed (in mutual information). The top two nodes in each parameter category for each watershed are listed. The order of importance with the top two nodes differed among watersheds.

Parameter Category	Node	Sensitivity by Watershed			
		Skagit River	Nooksack River	Cedar River	Yakima River
Population Parameters	Juvenile %-Reduction in Survival	X	X	X	X
	Adult %-Reduction in Survival	X	X	X	X
	Egg to Emergence %-Reduction in Survival				
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)				
Stressors	Dissolved Oxygen	X		X	X
	Water Temperature		X		
	Toxicological Effects	X	X	X	X
Toxicological Pathway	Percent Mortality	X	X	X	X
	Change in Swimming Rate	X	X	X	X
	AChE Activity				
	Malathion				
	Diazinon				

Table 10. Summer sensitivity analysis results from measured concentrations by watershed (in mutual information). Top two nodes in each parameter category for each watershed are listed. The order of importance with the top two nodes differed among watersheds.

Parameter Category	**Node**	**Sensitivity by Watershed**			
		Skagit River	**Nooksack River**	**Cedar River**	**Yakima River**
Population Parameters	Juvenile %-Reduction in Survival	X	X	X	X
	Adult %-Reduction in Survival	X	X	X	X
	Egg to Emergence %-Reduction in Survival				
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)				
Stressors	Dissolved Oxygen	X		X	
	Water Temperature	X	X	X	X
	Toxicological Effects		X		X
Toxicological Pathway	Percent Mortality	X	X	X	X
	Change in Swimming Rate	X	X	X	X
	AChE Activity				
	Malathion				
	Diazinon				

3.6.2 Sensitivity to Endpoint from OP Concentrations in the Skagit River

Sensitivity results from various OP concentrations in the Skagit River indicated that the stressors with the most mutual information vary by season. In the winter, all model parameters were identical in ranking from each of OP concentration scenarios (Table 11). However, in the summer *modeled synergistic* concentrations of binary and ternary OP stressors increased ranking in the Toxicological Effects node. Dissolved Oxygen was the still the most common top ranked stressor by mutual information from all OP concentrations (Table 12).

Table 11. Winter Skagit River sensitivity analysis results from OP concentrations (in mutual information). The top two nodes in each parameter category for each OP concentration are listed. The order of importance with the top two nodes did not differ between OP concentrations.

Parameter Category	Node	Sensitivity by OP concentration				
		Measured Single OP	**Measured Binary OP**	**Measured Ternary OP**	**Modeled Synergistic Binary OP**	**Modeled Synergistic Ternary OP**
Population Parameters	Juvenile %-Reduction in Survival	X	X	X	X	X
	Adult %-Reduction in Survival	X	X	X	X	X
	Egg to Emergence %-Reduction in Survival					
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)					
Stressors	Dissolved Oxygen	X	X	X	X	X
	Water Temperature					
	Toxicological Effects	X	X	X	X	X
Toxicological Pathway	Percent Mortality	X	X	X	X	X
	Change in Swimming Rate	X	X	X	X	X
	AChE Activity					
	Malathion					
	Diazinon					
	Chlorpyrifos					

Table 12. Summer Skagit River sensitivity analysis results from OP concentrations (in mutual information). The top two nodes in each parameter category for each OP concentration are listed. The order of importance with the top two nodes differed between OP concentrations.

Parameter Category	Node	Sensitivity by OP concentration				
		Measured Single OP	Measured Binary OP	Measured Ternary OP	Modeled Synergistic Binary OP	Modeled Synergistic Ternary OP
Population Parameters	Juvenile %-Reduction in Survival	X	X	X	X	X
	Adult %-Reduction in Survival	X	X	X	X	X
	Egg to Emergence %-Reduction in Survival					
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)					
Stressors	Dissolved Oxygen	X	X	X	X	X
	Water Temperature	X	X	X		
	Toxicological Effects				X	X
Toxicological Pathway	Percent Mortality	X	X	X	X	X
	Change in Swimming Rate	X	X	X	X	X
	AChE Activity					
	Malathion					
	Diazinon					
	Chlorpyrifos					

3.6.3 Sensitivity to Endpoint from Additive and Synergistic Exposures in the Skagit River

Sensitivity results from *additive and synergistic exposures* in the Skagit River indicated that stressors with the most mutual information vary by season. In the winter, all model parameters were identical with all the scenarios in ranking from additive and synergistic exposures (Table 13). However, in the summer, synergistic concentrations of diazinon and malathion increased ranking in the Toxicological Effects node (Table 14). Dissolved Oxygen was the still the most common top ranked stressor by mutual information from all *additive and synergistic exposures.*

Table 13. Winter Skagit River sensitivity analysis results from additive and synergistic exposures (in mutual information). The top two nodes in each parameter category for each additive and synergistic exposure is listed. The order of importance with the top two nodes did not differ between additive and synergistic exposures

Parameter Category	Node	Sensitivity by Additive and Synergistic Exposures			
		Additive-Malathion + Diazinon	**Synergistic-Diazinon + Malathion**	**Synergistic-Malathion + Chlorpyrifos**	**Synergistic-Diazinon + Chlorpyrifos**
Population parameters	Juvenile %-Reduction in Survival	X	X	X	X
	Adult %-Reduction in Survival	X	X	X	X
	Egg to Emergence %-Reduction in Survival				
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)				
Stressors	Dissolved Oxygen	X	X	X	X
	Water Temperature				
	Toxicological Effects	X	X	X	X
Toxicological Pathway	Percent Mortality	X	X	X	X
	Change in Swimming Rate	X	X	X	X
	AChE Activity				
	Malathion				
	Diazinon				
	Chlorpyrifos				

Table 14. Summer Skagit River sensitivity analysis results from additive and synergistic exposures (in mutual information). The top two nodes in each parameter category for each additive and synergistic exposure is listed. The order of importance with the top two nodes differed between additive and synergistic exposures

Parameter Category	Node	Sensitivity by Additive and Synergistic Exposures			
		Additive- Malathion + Diazinon	**Synergistic- Diazinon + Malathion**	**Synergistic- Malathion + Chlorpyrifos**	**Synergistic- Diazinon + Chlorpyrifos**
Population parameters	Juvenile %-Reduction in Survival	X	X	X	X
	Adult %-Reduction in Survival	X	X	X	X
	Egg to Emergence %-Reduction in Survival				
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)				
Stressors	Dissolved Oxygen	X	X	X	X
	Water Temperature	X		X	X
	Toxicological Effects		X		
Toxicological Pathway	Percent Mortality	X	X	X	X
	Change in Swimming Rate	X	X	X	X
	AChE Activity				
	Malathion				
	Diazinon				
	Chlorpyrifos				

4.0. DISCUSSION

The objectives of this research were to first derive a methodology for integrating potentially synergistic mixtures into an ERA framework to the population scale and then to evaluate the results with single chemical or additive models. I successfully integrated chemical mixtures in an already established BN-RRM framework (Landis et al. 2018, submitted) incorporating an AOP in four watersheds. Mixture results can be used to inform management decisions for Puget Sound Chinook salmon.

4.1. Quantitative AOPs and BN-RRMs

The quantitative AOP (qAOP) used biologically based modelling to quantify the relationships between the MIE and subsequent KEs to assess the probability of an adverse outcome (Conolly et al. 2017). However, the examples presented in Conolly et al. (2017) did not give actual probabilities. The population size output was illustrated as the proportion of carrying capacity, which is not defined. Risk was also not clearly defined in the examples. In these BN-RRMs, risk was defined as the probability of not meeting the management goal of 500,000 fish. Probability was also clearly defined in each risk calculation (Tables 4-8).

The examples in Conolly et al. (2017) did not clearly address exposure response with actual exposure response curves. There were data presented, but there were no error terms. These BN-RRMs have confidence intervals presented in each of the exposure response relationships and variability was also presented in the distributions of the nodes. With these BN-RRMs, I defined exposure-response curves incorporating mixtures to connect the MIE of AChE inhibition exposure and subsequent KEs (Table 2).

Ecological context was not addressed in Conolly et al. (2017). Laboratory tests were used to examine the adverse outcome of a reduction in population size. However, populations interact with differences over space and time (Landis 2006). These BN-RRMs provided site-specific OP concentration and water quality data in four watersheds, incorporating ecological context over space and time.

These BN-RRMs were more developed than the qAOPs presented in Conolly et al. (2017) because probability, risk, and exposure response were addressed to calculate risk to populations. In addition, mixtures and ecological context were addressed, allowing for more risk calculations and thus more of an understanding of risk at the population scale. In the future, more BN-RRMs incorporating AOPs, mixtures and ecological context can be created to facilitate more management decisions.

4.2. Risk Assessments with Chemical Mixtures to Populations

A risk assessment linking chemical mixture exposure to population impacts has not been completed. There are many experimental, modeling and predictive ERA approaches to predicting toxicity of chemical mixtures to individuals, all having potentials and obstacles. The lack of guidance, data and expertise on how to use these approaches exacerbates the challenge with chemical mixtures (Kienzler et al. 2016, Beyer et al. 2014). In addition to a lack of a consistent framework, extrapolating data available from mixture toxicity to higher levels of biological organization such as populations or communities is even more challenging (Altenburger et al. 2013).

These BN-RRMs achieved the objective of completing a risk assessment with chemical mixtures to a population in four watersheds. This risk assessment included a mechanistic basis of chemical mixtures through an AOP by defining an exposure response curve at the MIE and subsequent KEs to a population of Chinook salmon. Site-specific concentrations of OPs, water temperature and dissolved oxygen data were indicated by each of the four watersheds, making these BN-RRMs spatially-explicit.

4.3 Data Uncertainties

Even though these BN-RRMs successfully produced calculations of risk to a Chinook salmon population, there were some data uncertainties with monitoring data, toxicological and ecological interactions and linking changes in behavior. The BN-RRMs relied heavily on monitoring studies of OPs and water quality in each of the watersheds (WADOE 2016abc). Monitoring data had shown that most of current use pesticides in surface waters are detected at concentrations below 0.1 µg/L (Gilliom 2007). Monitoring measurements only measured OPs and other pesticides at a single point in time and did not consider half-lives. Malathion has a short half-life of between 2 to 18 days depending on temperature and pH (Gervais et al. 2009). Diazinon and chlorpyrifos have longer half-lives at 12 days to 4 weeks (Harper et al. 2009, Christensen et al. 2009). OPs should be found in higher concentrations, specifically right after storm events (Trac et al. 2016). In dry weather, pesticides can accumulate on the application sites from the household products. During storm events, the accumulated pesticides runoff into the aquatic environments exposing salmon to pesticides such as OPs (Trac et al. 2016, Laetz et al. 2009, 2013). Overall, monitoring measurements would detect high applications after spray drift events (WSDA 2016) and during the first precipitation event after application.

Monitoring studies also did not necessarily take measurements in areas where OP exposures and thermal stress can occur, increasing uncertainty. Juvenile salmon are more likely to be exposed to OPs in proximal, low volume side channel habitats (Laetz et al. 2014). Stream order data of the OP monitoring studies indicated that the OPs were only sometimes measured in headwater streams in the watersheds. Headwater streams can represent areas that are side channels or small tributaries, but these areas may or may not be proximate to agricultural fields or urban areas (Laetz et al. 2014, WSDA 2016). Thermal stress also is more likely to occur in side channels and tributaries. The degree of thermal stress also depends on riparian shading, groundwater inflow and other factors (Laetz et al. 2014, Beechie et al. 2013).

There was a lack of data from toxicological and ecological interactions. Toxicological and ecological interactions were most apparent in connecting Juvenile %-Reduction in survival. Many studies confirmed that increasing temperatures do increase the chemical uptake and metabolism resulting in increased toxicity from numerous chemicals (Cairns et al. 1974, Lydy et al. 1999, Hooper et al. 2013). As a result, extreme effect values of toxicological and ecological interactions are supported by the literature, but intermediate effect values were not known. Thus, a “peg the corners” approach was used to connect toxicological and ecological interactions in the Juvenile %-Reduction in Survival node.

These BN-RRM successfully linked adverse outcomes from individual level effects to populations through survival but, linking individual behavioral effects to population fitness was not fully understood. The only behavioral change modeled in these BN-RRMs was the Change in Swimming Rate because AChE inhibition impaired swimming performance (Little and Finger 1990, Beauvais et al. 2000, Brewer et al. 2001, Sandahl et al. 2005, Groh et al. 2015). Change in Swimming Rate was modeled as the sublethal effect because swimming can lead to reduced predator avoidance, prey capture success and migration ability leading to ecological death (Sandahl et al. 2005, Mesa et al. 1994). However, more connections were not made from change in swimming because there were no clear exposure response relationships. Instead, a Toxicological Effects summary node connected Change in Swimming and Percent Mortality. Thus, the Toxicological Effects node may have underestimated sublethal effects.

4.4. The Endangered Species Level of Concern (ESLOC)

The Endangered Species Level of Concern (ESLOC) developed by the EPA is a limit that is assumed to protect endangered species like the Chinook salmon (USEPA 1998). The OP ESLOC values for fisheries were calculated with a factor of 1/20 of the lethal concentration 50 percent (LC50) value of rainbow trout *(Oncorhynchus mykiss*) (Tuttle 2014). These BN-RRMs relied on monitoring data in which most measured concentrations were below 0.1 µg/L, which is below the ESLOC for malathion and diazinon (Tuttle 2014). Adding measured concentrations that were below the ESLOC still increased risk to Chinook salmon (Tables 4,5,6). This was because sublethal effects were also incorporated into these BN-RRMs, which led to increased risk. The results from these BN-RRMs support Baldwin et al. (2009) and Spromberg and Meador (2005) that low concentrations of OPs do contribute risk to Chinook salmon populations.

Synergism in mixtures are also not incorporated into the ESLOC value. Modeled synergistic concentrations increased risk to Chinook (Tables 5, 6, 7 and 8). Synergism in these results supported Laetz et al. (2009, 2013). However, the mixture of diazinon and chlorpyrifos did not support Laetz et al. (2009) lab data because concentrations of 3-15 µg/L diazinon and 0.15-1 µg/L chlorpyrifos were too low to trigger synergism.

4.5 Risk to Ecosystem Services

These BN-RRMs can serve as aides in decision making to protect Chinook salmon populations. From these results, managers are informed that environmental stressors accounted for more risk than toxicological stressors during all seasons at all watersheds (Tables 4-6). Though, measured and synergistic concentrations of OPs increased risk (Tables 4-8) and the proportion of risk due to toxicity was greater in the winter than the summer (Tables 4-6). Sensitivity analysis results indicated that toxicological and ecological stressors were ranked higher depending on the season and OP concentration (Tables 9-14). These BN-RRMs indicated that both environmental and toxicological stressors should be included in decision making, improving upon Spromberg and Meador (2005) and Baldwin et al. (2009).

Habitat improvements such as reduced grazing, reconnecting floodplains and planting more vegetation can reduce risk from environmental and toxicological stressors and allow for improved population abundance (SSDC 2015, Beechie et al. 2013, WSDA 2015). According to the 2017 Puget Sound Partnership State of our Sound report, Chinook salmon populations are not recovering even though the habitat restoration efforts have occurred (PSP 2017). Thus, risk to Chinook have not been reduced. Improving population abundance for Chinook salmon will take a collaborative effort with more societal and economic costs (Lackey 2017). A decision network could be added to these BN-RRMs to evaluate more habitat restoration options and enhance decision making (Carriger and Newman 2011). Water quality and OP concentration data from the chosen habitat restoration option could easily be updated into the BN-RRM. As more habitat restoration efforts are implemented, these BN-RRMs can be used to evaluate risk and thereby facilitates adaptive management (Landis et al. 2017b)

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S 1.0 Watershed Characterization

S 1.1 Skagit River Basin

Of the drainages in Puget Sound, the Skagit River Basin (SRB) is the largest and produces the greatest abundance of salmonids and the greatest number of salmonid stocks (Figure S1). The SRB is the origin of the most abundant wild Chinook salmon populations in Puget Sound (Smith et al. 2003). The primary river draining is the Skagit River. Principle land uses include cropland, forestland, urban and built-up areas. Dairy, farming and row cropping are widespread. Other agricultural operations include berries, bulbs and tree nurseries. Much of the low-lying areas are diked and drained, and several pump stations discharge water from the draining districts into the SRB. Major resource issues are streambank erosion, impaired water quality, forest health issues, invasive weeds and urban encroachment on agricultural areas (NRCS 2006).

The Lower Skagit sub-basin contains the most highly degraded freshwater salmonid habitat in the Skagit basin. Degradation mostly has been caused by dikes and riprap. Road density in the Lower Skagit is 3.3 mi/mi^2 indicating a high level of development contributing to sedimentation problems, fish blockage impacts, and hydrologic changes. These high levels of development also contribute to degraded levels of water quality including elevated nutrients, very warm water temperatures in the summer months, low dissolved oxygen levels and increased turbidity. Sediment sampling has indicated levels of lead, copper and zinc above water quality criteria. Many of the Lower Skagit tributary watersheds also have impaired flow conditions (Smith et al. 2003)).

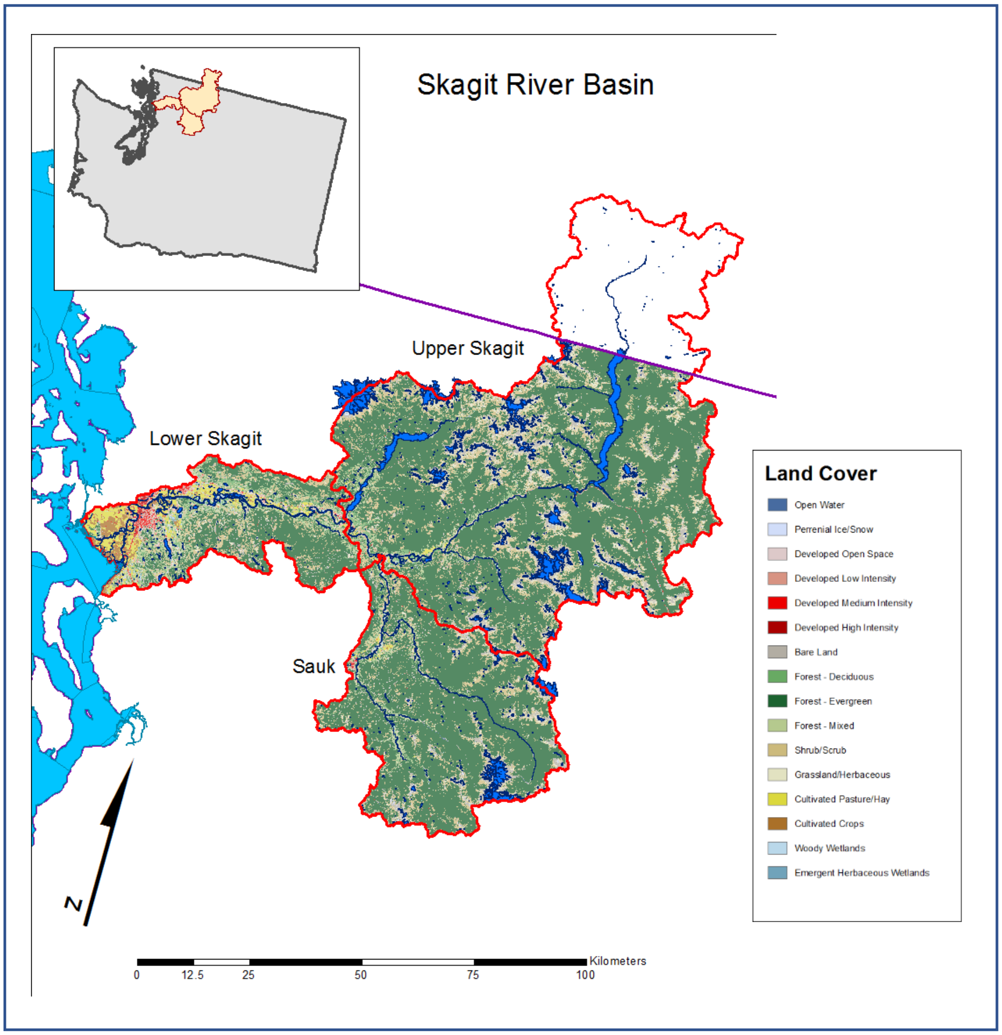

Figure S1. Map of the Skagit River Basin.

S1.2 Nooksack River Basin

The Nooksack River Basin (NRB) branches into three forks: North Fork, Middle Fork and South Fork Nooksack (Figure S2). The river drains to Bellingham Bay/Lummi Bay, and salmon migrate into Strait of Georgia and Northern Salish Sea (Beamer et al. 2016). The NRB is mostly rural and dominated by forestlands. The land use of the mainstem below the confluence of the three forks is primarily agricultural with small towns and cities. Agricultural areas are undergoing a shift from low pesticide use dairy farming to high intensity pesticide crops such as blueberries (Tuttle 2014)

Salmonid spawning habitat in the NRB have been impacted by considerable sedimentation problems, most originating from landslides. The NRB also has problems with high road densities and warm water temperatures. Potentially low stream flows are also a limiting factor (Smith 2002).

Salmon habitat has been degraded by forestry and agricultural practices (NWIFC 2016). From 1890 to 1925, increased logging, coal mining, and clearing of 130,000 acres of lowlands to agricultural lands changed the landscape. By 1938, nearly all the forests and numerous wetlands in the delta and the lower mainstem were converted to agricultural land and more than 2,000 coarse woody debris were cleared from the NRB. After 1950, commercial activity greatly increased (Smith 2002). Now land-use practices have improved, but water quality and quantity continue to be challenged by human population growth (NWIFC 2016).

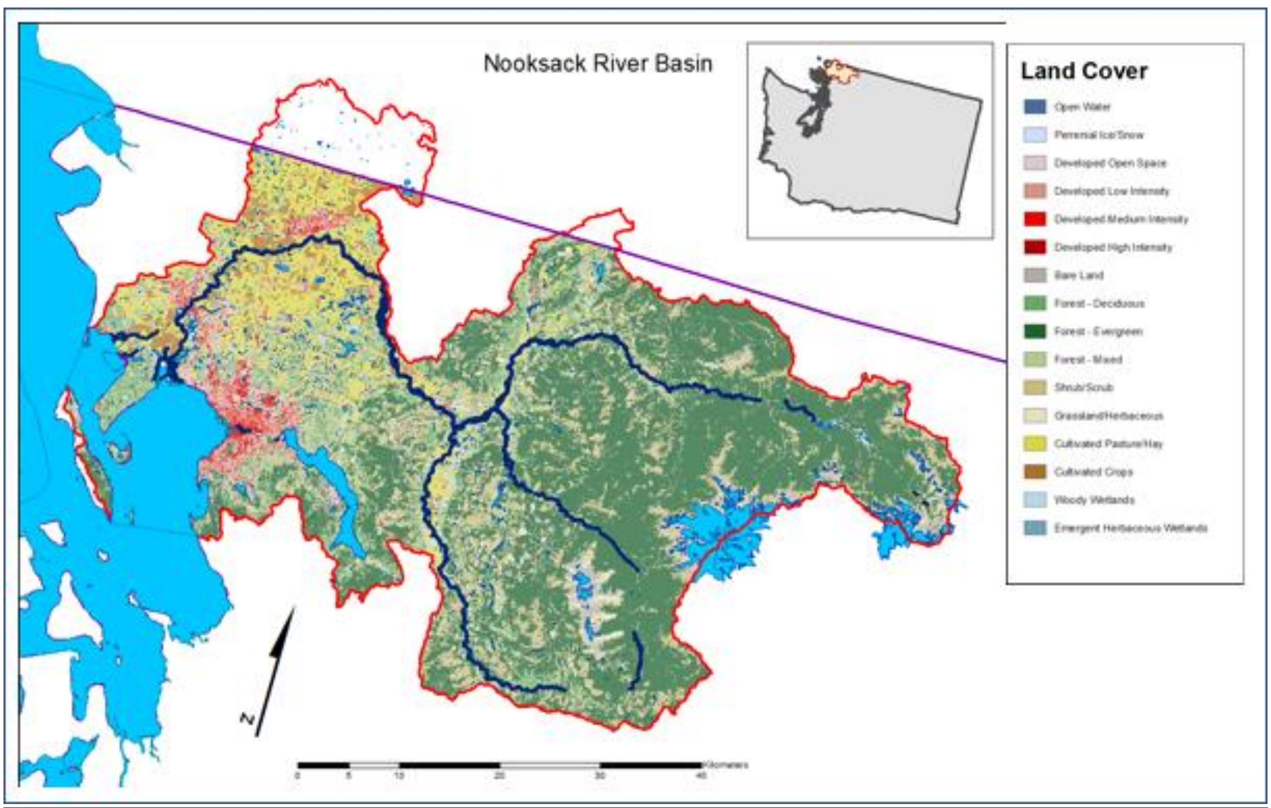

Figure S2. Map of the Nooksack River Basin.

S1.3 Cedar River Basin

The Cedar River Basin (CRB) drains highly populated areas of Seattle, Redmond, Kirkland, Bothell, Bellevue, Issaquah, and other large metropolitan areas (Figure S3). The CRB contains Lake Washington, which complicates salmon life history in this area as they will rear in the lake in addition to streams and tributaries (Greene 2017). The Eastern, mountainous portion of the Cedar watershed occupies the Cascade Range and is the only portion of the CRB with snowpack and seasonal snowmelt. Seattle's water supply is generated from the upper (Eastern) portion of the CRB. The Western portion of the CRB consists of Puget Sound lowlands, and it relies on groundwater for flow in the summer and early fall (King County 2015). The heavily urbanized areas in the Western portion of the CRB have "very poor" Stream Biological Condition (determined by Benthic Index for Biological integrity (B-IBI)), whereas the rural and forested areas in the Eastern portion of the watershed have "very good" Stream Biological Condition (determined by B-IBI) (King County 2015).

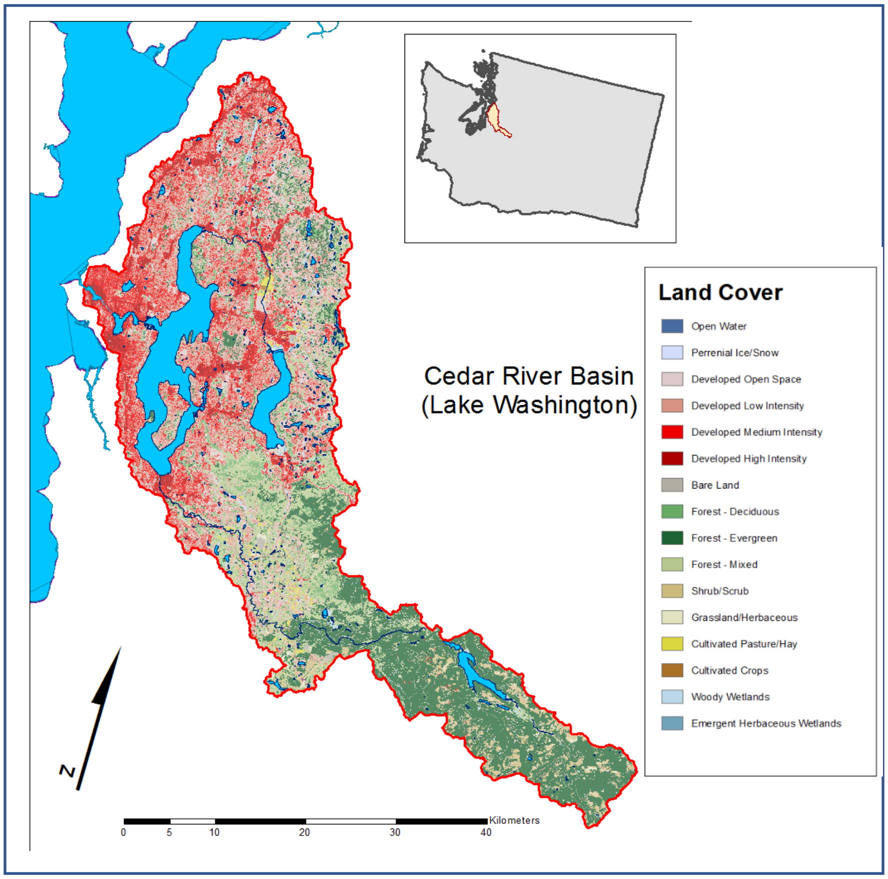

Figure S3. Map of the Cedar River Basin.

S1.4 Yakima River Basin

The Yakima River Basin (YRB) has multiple dams, including Prosser, a diversion dam for agricultural irrigation on the lower Yakima which all YRB salmon must pass in their outmigration. There are also several dams on the Lower Columbia River (the McNary Dam just after the Yakima/Columbia confluence, and the Bonneville Dam just before the mouth of the Columbia) that must be passed by all Yakima River Basin salmon. YRB salmon migrate through Lower Columbia to coast (Astoria), whereas all Puget Sound populations migrate out through some part of the Salish Sea. The Lower Yakima is warmer and more productive than the Upper Yakima, so eggs emerge earlier and fish rear more quickly when hatched here compared with the Upper Yakima (Hoffarth 2017).

The climate in the YRB ranges from high precipitation, alpine in the headwaters of the Eastern Cascades, to semi-arid in the lower elevation basin. Because of the diversion irrigation systems, the river flow is regulated by reservoir storage, and flows are lower than natural in spring, and higher than natural during summer (Pearsons et al. 2008). About 50% of the water withdrawn for irrigation re-enter the river system downstream after being used for irrigation and hydropower (Fast et al. 1988).

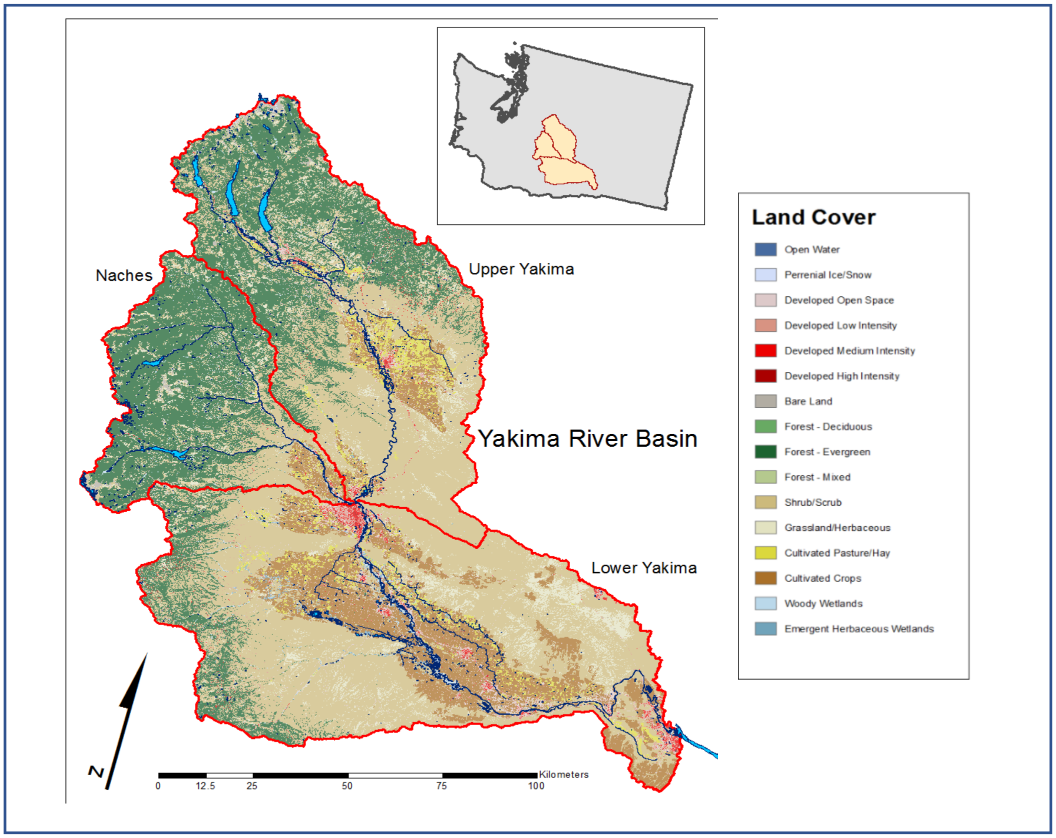

Figure S4. Map of the Yakima River Basin.

S2.0 Characterization of Nodes

Table S1 presents a summary of the information contained in each node in this BN-RRM. First, a brief description of each node is presented along with the categories used to rank each. The ranking criteria and justifications are presented in the next column followed by the references and quality of the sources. The quality of the sources and CPTs are ranked with criteria listed on the bottom. I present Dissolved Oxygen and Adult %-Reduction in Survival nodes as examples.

For the Dissolved Oxygen node, the ranges were related to the water quality criteria and the survivorship of the fish in freshwater. In the case of Dissolved Oxygen, there was not a need for a typical CPT. The setting of the river name and season resulted in the placing of data specific to that river and season into the node. Therefore, the result was derived directly from observation.

For the Adult % Reduction in Survival node, the ranks were from literature describing the effects of dissolved oxygen and temperature and are limited to no more than 50 percent mortality. In the case of Adult %-Reduction in Survival, the CPT had to be derived from an evaluation of the relevant literature from the references listed.

The final column evaluated the credibility and quality of the data used to build the node. In the instance of the Dissolved Oxygen the ranking was High because the data were from direct observations from state governmental sources using standard methods from each of the four watersheds. Medium was the ranking for the Adult %-Reduction in Survival node. The literature was not site-specific. Extrapolation of information from numerous sources and multiple sites that may not be typical of the four rivers in this study. The information was obtained from many reliable sources and a portion were peer reviewed.

Table S1. Summary of information, ranking schemes, justifications, and units used to construct the nodes in the Adverse Outcome Pathway- Bayesian network

Node	Description	Ranking	Justification	References	Source Creditability/ Data Quality
River and Watershed	Four Bayesian networks: one for the Lower Skagit, Lower Yakima, Cedar, and Nooksack rivers and their watersheds, populated with site-specific data for each.	Deterministic. Select a specific river watershed BN to model the dose-response results for that watershed (WRIA boundary).	Large, recreational, economically, culturally, environmentally important river systems in Washington state, e.g. salmon, water, irrigation. Most have historical and current data available compared to other systems. Each river and its surrounding watershed is delineated by the boundaries of the WRIA in which it is located.	Tuttle 2014; Washington Administrative Code 2011a, 2011b; Washington State Department of Ecology 2016a, 2016b	High[1a]
Season	Temporal changes in each river affecting uses, water quality (temp, DO), and salmon presence in and utilization of it associated with its life-cycle stage.	Spring (months 3 - 5), summer (months 6 - 8), fall (months 9 - 11), winter (months 12 - 2)	Water quality, salmon, and uses in all watersheds changes over time. For example, different salmon species have different spawning seasons accounting for habitat conditions throughout the year.	Washington Administrative Code 2011a, 2011b; Washington State Department of Ecology 2016a, 2016b	High[1a]
Diazinon Concentrations	Measured concentrations of diazinon over a ten-year period in each of the river's major waterways.	0 - 3.04e-6 moles/L, 3.04e-6 - 3.04e-5 moles/L, 3.04e-5 - 1.52e-4 moles/L, 1.52e-4 - 0.001 moles/L, 0.001 - 0.005 moles/L	Values were converted from µg/l to M. 3.04e-6 is the Maximum Daily Load (MDL). 1.52e-4 is the Endangered Species level of concern (ESLOC) for Freshwater fish. 0.001 is the 0.025 EC50 published in Laetz et al. (2009) 0.005 is the 0.1 EC50 published in Laetz et al. (2009). Distribution is based on downloaded data for each of the watersheds	Tuttle 2014; Washington State Department of Ecology 2016a; Laetz et al. (2009)	High[1a]

Information Source Ranking Criteria

1 HIGH: Site- and/or species-specific information. Peer reviewed in a journal or with external reviewers. Includes description of uncertainty or provides access to dataset. Data acquired using specific standardized protocols.
2 MEDIUM: Information for similar location/site or closely related species. Government report or similar reliable information source. Some description of data uncertainty. Not as clear information regarding protocols used to acquire data, no access to dataset
3 LOW: General information not site- and/or species-specific. Gray literature with no verification of dataset or conclusions. No clear description of data uncertainty. No clear information on sampling or experimental protocols, no dataset.

CPT Construction Ranking Criteria

a HIGH: Mathematical or case-based derivations of the relationship used, such as in a dose-response curve or modeled relationship.
b MEDIUM: Used estimates based on relationships reported in the literature or by extrapolation from other sites or species.
c LOW: Used judgement, either expert or by the investigator, to peg the corners and fill in the CPT.

Table S1 (continued)

Node	Description	Ranking	Justification	References	Source Creditability/ Data Quality
Malathion Concentrations	Measured concentrations of malathion over a ten-year period in each of the river's major waterways.	0 - 2.6e-5 moles/L, 2.6e-5 - 2.6e-4 moles/L, 2.6e-4 - 5.4e-4 moles/L, 5.4e-4 - 0.001 moles/L, 0.001 - 0.005 moles/L	Values were converted from µg/l to M. 2.6e-5 is the Maximum Daily Load (MDL). 5.4e-4 is the Endangered Species level of concern (ESLOC) for Freshwater fish. 0.001 is the 0.05 EC50 published in Laetz et al. (2009). 0.005 is the 0.2 EC50 published in Laetz et al. (2009). Distribution is based on downloaded data for each of the watersheds	Tuttle 2014; Washington State Department of Ecology 2016a; Laetz et al. (2009)	High[1a]
Chlorpyrifos Concentrations	Measured concentrations of chlorpyrifos over a ten-year period in each of the river's major waterways.	0 - 5.26e-6 moles/L, 5.26e-6 - 5.26e-5 moles/L, 5.26e-5 - 3.35e-4 moles/L	Values were converted from µg/l to M. 5.26e-6 is the Maximum Daily Load (MDL), 5.26e-5 is the Endangered Species level of concern (ESLOC) for Freshwater fish. 3.35e-4 is the 0.5 EC50 published in Laetz et al. (2009). Distribution is based on downloaded data for each of the watersheds.	Tuttle 2014; Washington State Department of Ecology 2016a; Laetz et al. (2009)	High[1a]
Water Temperature	Measured water temperature over a ten-year period in each of the river's main waterways.	0 to 13 °C, 13 - 16°C, 16 - 18°C, 18 -25°C, 25-36°C	Temperature ranges specific to salmonids based on Table 200 (1)(c) Aquatic Life Temperature Criteria in Fresh Water and survival data. Distribution is based on downloaded data for each of the watersheds.	Washington Administrative Code 2011a; Washington State Department of Ecology 2016b	High[1a]
Dissolved Oxygen	Measured dissolved oxygen concentrations over a ten-year period in each of the river's main waterways.	0 - 3.5 mg/L, 3.5 - 5 mg/L, 5 - 6.5 mg/L, 6.5-8 mg/L, 8-9.5 mg/L, 9.5 - 15 mg/L, 15 mg/L-22 mg/L	Ranges specific to salmonids based on Table 200 (1)(d) Aquatic Life Temperature Criteria in Fresh Water and survival data. Distribution is based on downloaded data for each of the watersheds.	Washington Administrative Code 2011b; Washington State Department of Ecology 2016b	High[1a]

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Table S1 (continued)

Node	Description	Ranking	Justification	References	Source Creditability/ Data Quality
Water Quality Effects - Juvenile Salmonids	Mortality to juvenile salmonids due to water quality in each river.	0%, 10%, 20%, 50%, 90%	Temperature related changes to water quality where severe high temperatures result in severe DO depletions in the water column. (E.g., low water quality = high temperature 25- 36 °C, resulting in low 0 to 3.5 mg DO/L to cause juvenile mortality.) Ranking is based on data from the literature. This CPT was completed using the literature and a case file learning function.	Brett 1952; Carter 2005, 2008; Geist et al. 2006; Warren et al. 1973	Medium[2b]
AChE activity	AChE activity measured in salmonids when exposed to OP concentrations dissolved in water under laboratory conditions.	0 - 25%, 25 - 50%, 50 - 75%, 75 - 100%, 100 to 125%, 125 to 200%	AChE activity was quantified as milli optical density (mOD) per minute per gram of tissue and reported as a percentage of the baseline enzyme activity for fish exposed to carrier alone.	Laetz et al. 2009; 2013	High[1a]
Toxicological Effects (Direct) - Percent Mortality	Mortality directly due to AChE activity	0%, 10%, 20%, 50%, 90%	AChE values of 5-90% reported in Laetz et al. (2009) were linked to mortality at high levels	Laetz et al. (2009)	High[1a]
Toxicological Effects (Indirect) - Change in Swimming Rate	Change in salmonid swimming rate due to increased AChE activity after exposure to OP concentrations dissolved in water under laboratory conditions	0 to 25%, 25 to 50%, 50 to 75%, 75 to 100%, 100 to 150%, 150 to 250%	Chlorpyrifos causes increased AChE activity in salmonids ranging from slight to measurable effects on swimming, breathing, foraging/feeding and other behaviors that can adversely impact survival, growth, and reproduction. AChE inhibits brain and muscle function which can be linked to a change in swimming based on (Laetz et al. 2009). Ranking is set as equal intervals up until 100%. >100% indicates a faster swimming speed. This CPT was completed using the literature and a case file learning function	Laetz et al. 2009, 2013; Sandahl et al. 2005, Tierney et al. 2007	High[1a]

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Table S1 (continued)

Node	Description	Ranking	Justification	References	Source Creditability/ Data Quality
Summed Toxicological Effects (Direct and Indirect)	Summation of toxicological effects due to acute mortality and change in swimming rate.	None, 10%, 20%, 50%, 90%	Combined direct and indirect toxicological effects of OPs on salmonids provide a more accurate population response to estimate risk. Ranking based on a peg the corners approach in which minimum and maximum values for each were used to establish the corners of the CPT. Data and information from the literature were then used to populate the CPT.	Coppage et al. 1975; Duangsawasdi 1977; Fulton and Key 2001; Laetz et al. 2009; Weiss 1961; Wheelock et al. 2005	Medium[2c]
Egg to Emergence % Reduction in Survival	Effects specific to eggs and larval salmonids, specifically the decline in survivorship of eggs to hatch due to water quality effects.	0%, 10%, 20%, 50%, 90%	Temperature related changes to water quality where severe high temperatures result in severe DO depletions in the water column. (E.g., low water quality = high temperature 25- 36 degrees Celsius resulting in low 0 to 3.5 mg DO/L to cause juvenile mortality. Ranking is based on data from the literature. This CPT was completed using data and information from the literature.	Carter 2005, 2008; Geist et al. 2006; Jager 2011; McCullough 1999; McCullough et al. 2001; Richter and Kolmes 2005	Medium[2b]
Juvenile % Reduction in Survival	Reduction in juvenile salmonid survivorship due to all effects.	0%, 10%, 20%, 50%, 90%	Reduction in juvenile survival is a function of OP induced toxicological effects, water quality (temp, DO) effects on juveniles, and reduction in survivors from egg to emergence life stages that become juveniles. Ranks are identical to the ranks used in the Toxicological Effects, Water Quality Effects to Juveniles, and Egg to Emergence nodes. The CPT was constructed using a peg the corners approach due to lack of data in the literature, with the highest (100%) probability of risk set at 270 (the summed maximum percent in each of the three nodes (90+90+90= 270)). to cause a 90% reduction in juvenile survival.	Brett 1952; Carter 2005, 2008; Coppage et al. 1975; Duangsawasdi 1977; Fulton and Key 2001; Geist et al. 2006; Jager 2011; Laetz et al. 2009; McCullough 1999; McCullough et al. 2001; Richter and Kolmes 2005; Warren et al. 1973; Weiss 1961; Wheelock et al. 2005;	Medium[2c]

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Table S1 (continued)

Node	Description	Ranking	Justification	References	Source Creditability/ Data Quality
Adult % Reduction in Survival	Reduction in adult salmonid survivorship due to water quality effects	0%, 10%, 20%, 50%	Low water quality (low DO (0 to 3.5 mg/L) and high temperatures (25- 36 °C) causes up to 50% mortality to all life-cycle stages from egg to adult based on data from the literature. Ranking is based on data from the literature. This CPT was completed using the literature and a case file learning function.	Jager 2011; McCullough 1999; McCullough et al. 2001; Peery 2010; Richter and Kolmes 2005	Medium[2b]
Simulation Year	Year selected for model simulation of salmonid population size distribution.	1, 5, 10, 20, 50 Year	The maximum model simulation year is 50. The years are based on progression of these simulation years generated by RAMAS® GIS 6.0 software	Applied Biomathematics. 2017	Addressed in uncertainty and sensitivity analyses
Chinook Pop. Size*	The probability of different population levels in a given year based on model simulations.	0 to 10000, 10000 to 50000, 50000 to 100000, 100000 to 500000, 500000 to 1000000, 1000000 to 7631067e8 fish	CPT compiled from case file learning using RAMAS® GIS 6.0 software population modeling scenarios.	Applied Biomathematics 2017	Addressed in uncertainty and sensitivity analyses

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S3.0 Binary Mixture Exposure Response Curves

An example of a binary mixture exposure response curve will be given with malathion and diazinon. Binary mixture exposure response curves were completed with similar methods for malathion and chlorpyrifos as well as chlorpyrifos and diazinon mixtures from Laetz et al. (2009, 2013).

S3.1. Malathion and Diazinon to AChE Activity

I analyzed malathion and diazinon binary mixtures from Laetz et al. (2013). Data from the individual chemical concentrations were converted to moles/L and then fitted to exposure response curves with R statistical software (R Core Team 2017) and the *drc* package (Ritz et al. 2015). Data were converted from EC50 nominal chemical concentrations to moles/L. Then the moles/L of each compound were summed. Model fit was evaluated using the t-test of coefficients (in R, coeftest () command), and the F-test for overall significance of regression (in R, modelFit () command). A 3-parameter log-logistic equation was selected for this binary mixture of malathion and diazinon (Figure S5). This equation generated from the exposure-response curve in R and inputted was into the BN using the *Equation to Table* command. The equation used to model exposure-response is given below (Ritz et al. 2015) and the parameters are given in Table S2.

$$f(x; b, d, e) = \frac{d}{1 + \exp(b(\log(x) - \log(e)))}$$

Table S2. Parameters of the malathion + diazinon mixture exposure response curve

Mixture (x)	d	b	e
Synergistic-Malathion + Diazinon	121	8.88	0.000897

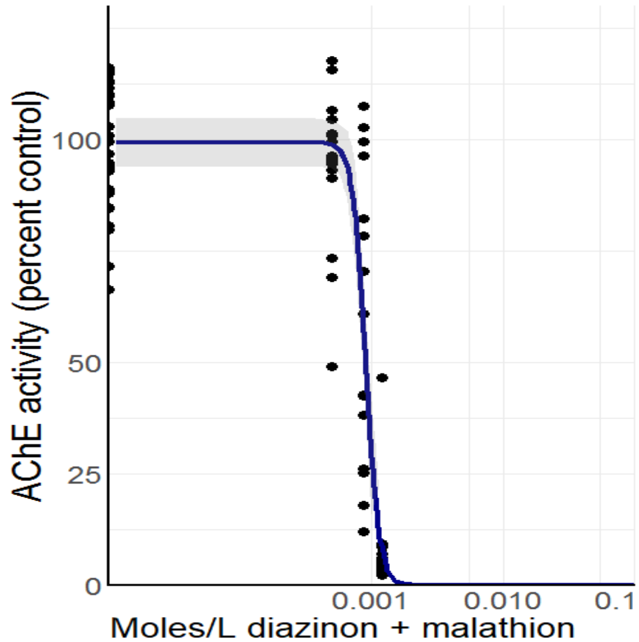

Figure S5. Binary mixture exposure response curve for diazinon and malathion mixture (blue) with 95% confidence intervals (gray) as a function of AChE activity (percent of control). A 3-parameter log-logistic equation was selected for model fit. Data from Laetz et al. (2013)

S3.2 Percent Mortality Exposure Response Curve

Percent mortality was the metric used for measuring lethal effects in the AOP section of the BN-RRM. Percent mortality was a function of AChE activity. A mixture exposure response equation was generated for the Percent Mortality node based on Laetz et al. (2009).

The binary mixture equation was based on AChE values reported in dead fish reported in Laetz et al. (2009). There were dead fish in the 0.4 and 1.0 EC50 exposures of diazinon and malathion and 1.0 EC50 exposures of chlorpyrifos and malathion. No fish were reported as dead in the chlorpyrifos and diazinon exposures. A logarithmic relationship provided the best fit for the data ($F=289.5$, $p<<0.05$). The equation is given below as y= Percent Mortality, x= AChE activity

$$y = -21.97\ln(x) + 100.49$$

S4.0 Population Modeling Modifications

The case learning algorithm did not account for population extinction in the model simulations, thus modifications were made in the Chinook Population Size CPT. Population extinction was defined in model simulations as having a population of zero fish at any simulation year. Once a population was extinct in such a model it cannot return. I edited the case file derived CPT to reflect the knowledge that extinct populations in closed models did not return using specific rules with the methods described below.

The rules were derived from making count functions of the number of simulations per year for every possible combination of Juvenile (0, 10, 20, 50, 90%) and Adult %-Reduction in survival (0, 10, 20, 50%) as well as each Simulation Year (1, 5, 10, 20, 50). Each of the simulations with each Juvenile and Adult %-Reduction survival combination in RAMAS had 200 replications. Any frequencies less than 200 assumed that an extinction had occurred in the simulation year. Table S3 is a summary of each survival combination per simulation year. Rules were assigned arbitrarily to the Chinook Population Size node from the frequencies that were less than 25 counts. The rules were then applied manually to the Chinook Population Size CPT. The rules were for these six different population distribution bins: 0-100,000; 100,000-500,000; 500,000-1,000,000; 1,000,000-5,000,000; 5,000,000-10,000,000; and 10,000,000-720,000,000

- Rule 1: <25 cases for any frequency apply these probabilities for the bins: 97.48, 0.51, 0.51, 0.51, 0.51, 0.51
- Rule 2: <10 cases for any frequency apply these probabilities for the bins: 98.71, 0.26, 0.26, 0.26, 0.26, 0.26
- Rule 3: =1 case for any frequency apply these probabilities for the bins: 99.5, 0.1, 0.1, 0.1, 0.1, 0.1
- Rule 4: =0 case for any frequency apply these probabilities for the bins: 100, 0, 0, 0, 0, 0

Table S3. Frequency summary of all the survival combinations of Juvenile and Adult %- Reduction in Survival per Simulation Year. Any frequency below 200 indicates extinction had occurred in the simulation year

Year 1	Adult %-Reduction in Survival			
Juvenile %-Reduction in Survival	0	10	20	50
0	200	200	200	200
10	200	200	200	200
20	200	200	200	200
50	200	200	200	200
90	200	200	200	200

Year 5	Adult %-Reduction in Survival			
Juvenile %-Reduction in Survival	0	10	20	50
0	200	200	200	200
10	200	200	200	200
20	200	200	200	200
50	200	200	200	200
90	200	200	200	200

Year 10	Adult %-Reduction in Survival			
Juvenile %-Reduction in Survival	0	10	20	50
0	200	200	200	200
10	200	200	200	200
20	200	200	200	200
50	200	200	200	200
90	192	176	170	92

Year 20	Adult %-Reduction in Survival			
Juvenile %-Reduction in Survival	0	10	20	50
0	200	200	200	200
10	200	200	200	200
20	200	200	200	200
50	200	191	191	104
90	10	6	3	0

Year 50	Adult %-Reduction in Survival			
Juvenile %-Reduction in Survival	0	10	20	50
0	200	200	200	24
10	200	200	200	9
20	200	200	199	3
50	150	1	1	0
90	0	0	0	0

S5.0 Sensitivity Analysis

The sensitivity analysis used mutual information as a measure of which model parameters were driving risk to the endpoint (Marcot 2012). This sensitivity analysis provided a more complete ranking of which model parameters were driving risk to the endpoint. Results for each stressor scenario in the winter and summer are described below. A full summary of the model parameters in the sensitivity analysis can be found in Tables S4-S9.

S5.1 Sensitivity to Endpoint by Watershed from Measured Concentrations of OP Stressors

Sensitivity results with measured concentrations of binary OP stressors by watershed showed that Juvenile and Adult %-Reduction in Survival were the highest ranked model parameters by mutual information in both the winter and summer (Tables S4, S5). In the winter, the sensitivity analysis indicated that Toxicological Effects was the third highest ranked model parameter in all watersheds, followed by Juvenile Water Quality Effects in all watersheds. The ranking of the rest of the model parameters varied by watershed (Table S4). In the summer, the sensitivity analysis indicated that Egg to Emergence %-Reduction in Survival was the third highest ranked model parameter in all watersheds. Water Temperature was the fourth highest ranked parameter in the Nooksack, Cedar and Lower Yakima River watersheds; while Dissolved Oxygen was the fourth highest ranked in the Skagit River. The ranking of the rest of the model parameters varied by watershed (Table S5).

Table S4. Winter sensitivity analysis ranking results from measure concentrations by watersheds (in mutual information). The order of importance between model parameters differed among watersheds.

Parameter Category	Node	Sensitivity by Watershed			
		Skagit River	Nooksack River	Cedar River	Yakima River
Population parameters	Juvenile %-Reduction in Survival	1	1	1	1
	Adult %-Reduction in Survival	2	2	2	2
	Egg to Emergence %-Reduction in Survival	7	5	10	8
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)	4	4	4	4
Stressors	Dissolved Oxygen	6	9	8	7
	Water Temperature	10	7	11	11
	Toxicological Effects	3	3	3	3
Toxicological Pathway	Percent Mortality	5	6	5	5
	Change in Swimming Rate	8	8	6	6
	AChE Activity	9	10	7	9
	Malathion	11	11	9	10
	Diazinon	12	12	12	12

Table S5. Summer sensitivity analysis ranking results from measure concentrations by watersheds (in mutual information). The order of importance between model parameters differed among watersheds.

Parameter Category	Node	Sensitivity by Watershed			
		Skagit River	Nooksack River	Cedar River	Yakima River
Population parameters	Juvenile %-Reduction in Survival	1	1	1	1
	Adult %-Reduction in Survival	2	2	2	2
	Egg to Emergence %-Reduction in Survival	3	3	3	3
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)	6	6	6	6
Stressors	Dissolved Oxygen	4	8	5	9
	Water Temperature	5	4	4	4
	Toxicological Effects	7	5	7	5
Toxicological Pathway	Percent Mortality	8	7	8	7
	Change in Swimming Rate	9	9	9	8
	AChE Activity	10	10	10	10
	Malathion	11	11	11	11
	Diazinon	12	12	12	12

S5.2 Sensitivity to Endpoint from OP Concentrations in the Skagit River

Sensitivity results from various OP concentrations in the Skagit River indicated that Juvenile and Adult %-Reduction in Survival were the highest ranked model parameters in both the winter and summer (Table S6, S7). In the winter, all model parameters were identical in ranking from various OP concentrations (Table S6). In the summer, Egg to Emergence and Dissolved oxygen were the third and fourth highest ranked model parameters, respectively. Modeled synergistic binary and ternary OP stressors changed the importance in Toxicological Effects as the fifth highest ranked compared to Water Temperature in the measured concentration exposures. Juvenile Water Quality Effects and Toxicological Effects were the sixth and seventh highest ranked model parameters in the measured exposures; while Water Temperature and Juvenile Water Quality Effects were the sixth and seventh highest ranked model parameters in the modeled synergistic exposures (Table S7).

Table S6. Winter Skagit River sensitivity analysis ranking results from OP concentrations (in mutual information). The order of importance between model parameters did not differ among OP concentrations.

Parameter Category	Node	Sensitivity by OP concentration				
		Measured Single OP	Measured Binary OP	Measured Ternary OP	Modeled Synergistic Binary OP	Modeled Synergistic Ternary OP
Population parameters	Juvenile %-Reduction in Survival	1	1	1	1	1
	Adult %-Reduction in Survival	2	2	2	2	2
	Egg to Emergence %-Reduction in Survival	7	7	7	7	7
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)	4	4	4	4	4
Stressors	Dissolved Oxygen	6	6	6	6	6
	Water Temperature	10	10	10	10	10
	Toxicological Effects	3	3	3	3	3
Toxicological Pathway	Percent Mortality	5	5	5	5	5
	Change in Swimming Rate	8	8	8	8	8
	AChE Activity	9	9	9	9	9
	Malathion	11	11	11	11	11
	Diazinon	12	12	12	12	12
	Chlorpyrifos	-	-	13	-	13

Table S7. Summer Skagit River sensitivity analysis ranking results from OP concentrations (in mutual information). The order of importance between model parameters differed among OP concentrations.

Parameter Category	Node	Sensitivity by OP concentration				
		Measured Single OP	Measured Binary OP	Measured Ternary OP	Modeled Synergistic Binary OP	Modeled Synergistic Ternary OP
Population parameters	Juvenile %-Reduction in Survival	1	1	1	1	1
	Adult %-Reduction in Survival	2	2	2	2	2
	Egg to Emergence %-Reduction in Survival	3	3	3	3	3
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)	6	6	6	7	7
Stressors	Dissolved Oxygen	4	4	4	4	4
	Water Temperature	5	5	5	6	6
	Toxicological Effects	7	7	7	5	5
Toxicological Pathway	Percent Mortality	8	8	8	8	8
	Change in Swimming Rate	9	9	9	9	9
	AChE Activity	10	10	10	10	10
	Malathion	11	11	11	11	11
	Diazinon	12	12	12	12	12
	Chlorpyrifos	-	-	13	-	13

S5.3 Sensitivity to Endpoint from Additive and Synergistic Exposures in the Skagit River

Sensitivity results from various additive and synergistic exposures in the Skagit River indicated that Juvenile and Adult %-Reduction in Survival were the highest ranked parameters in both the winter and summer (Table S8, S9). In the winter, the ranking between synergistic malathion and diazinon as well as malathion and chlorpyrifos were identical. The ranking between synergistic diazinon and chlorpyrifos and additive malathion and diazinon were also identical (Table S8). With the synergistic malathion exposures, Toxicological Effects were the third highest ranked followed by Juvenile Water Quality Effects; when it was the opposite with the synergistic diazinon and chlorpyrifos and additive exposures (Table S9). In the summer, Egg to Emergence %-Reduction in Survival was the third highest ranked. However, the fourth highest ranked model parameter was Water Temperature in the malathion and chlorpyrifos exposure and Dissolved Oxygen was the fourth highest ranked parameter in the other exposures. The other rankings varied between the synergistic diazinon and malathion and malathion and chlorpyrifos exposures. The additive and synergistic diazinon and chlorpyrifos exposures were identical in ranking (Table S9).

Table S8. Winter Skagit River sensitivity analysis ranking results from additive and synergistic exposures (in mutual information). The order of importance between model parameters differed among additive and synergistic exposures.

Parameter Category	Node	Sensitivity by Additive and Synergistic Exposures			
		Additive-Malathion + Diazinon	**Synergistic-Diazinon + Malathion**	**Synergistic-Malathion + Chlorpyrifos**	**Synergistic-Diazinon + Chlorpyrifos**
Population parameters	Juvenile %-Reduction in Survival	1	1	1	1
	Adult %-Reduction in Survival	2	2	2	2
	Egg to Emergence %-Reduction in Survival	7	9	9	7
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)	3	4	4	3
Stressors	Dissolved Oxygen	6	6	6	6
	Water Temperature	10	10	10	10
	Toxicological Effects	4	3	3	4
Toxicological Pathway	Percent Mortality	5	5	5	5
	Change in Swimming Rate	8	7	7	8
	AChE Activity	9	8	8	9
	Malathion	11	11	11	-
	Diazinon	12	12	-	11
	Chlorpyrifos	-	-	12	12

Table S9. Summer Skagit River sensitivity analysis ranking results from additive and synergistic exposures (in mutual information). The order of importance between model parameters differed among additive and synergistic exposures.

Parameter Category	Node	Sensitivity by Additive and Synergistic Exposures			
		Additive-Malathion + Diazinon	Synergistic-Diazinon + Malathion	Synergistic-Malathion + Chlorpyrifos	Synergistic-Diazinon + Chlorpyrifos
Population parameters	Juvenile %-Reduction in Survival	1	1	1	1
	Adult %-Reduction in Survival	2	2	2	2
	Egg to Emergence %-Reduction in Survival	3	3	3	3
	Juvenile Water Quality Effects (contributing to Juvenile %-Reduction in Survival)	6	7	7	6
Stressors	Dissolved Oxygen	4	4	5	4
	Water Temperature	5	6	4	5
	Toxicological Effects	7	5	6	7
Toxicological Pathway	Percent Mortality	8	8	8	8
	Change in Swimming Rate	9	9	9	9
	AChE Activity	10	10	10	10
	Malathion	11	11	11	-
	Diazinon	12	12	-	11
	Chlorpyrifos	-	-	12	12

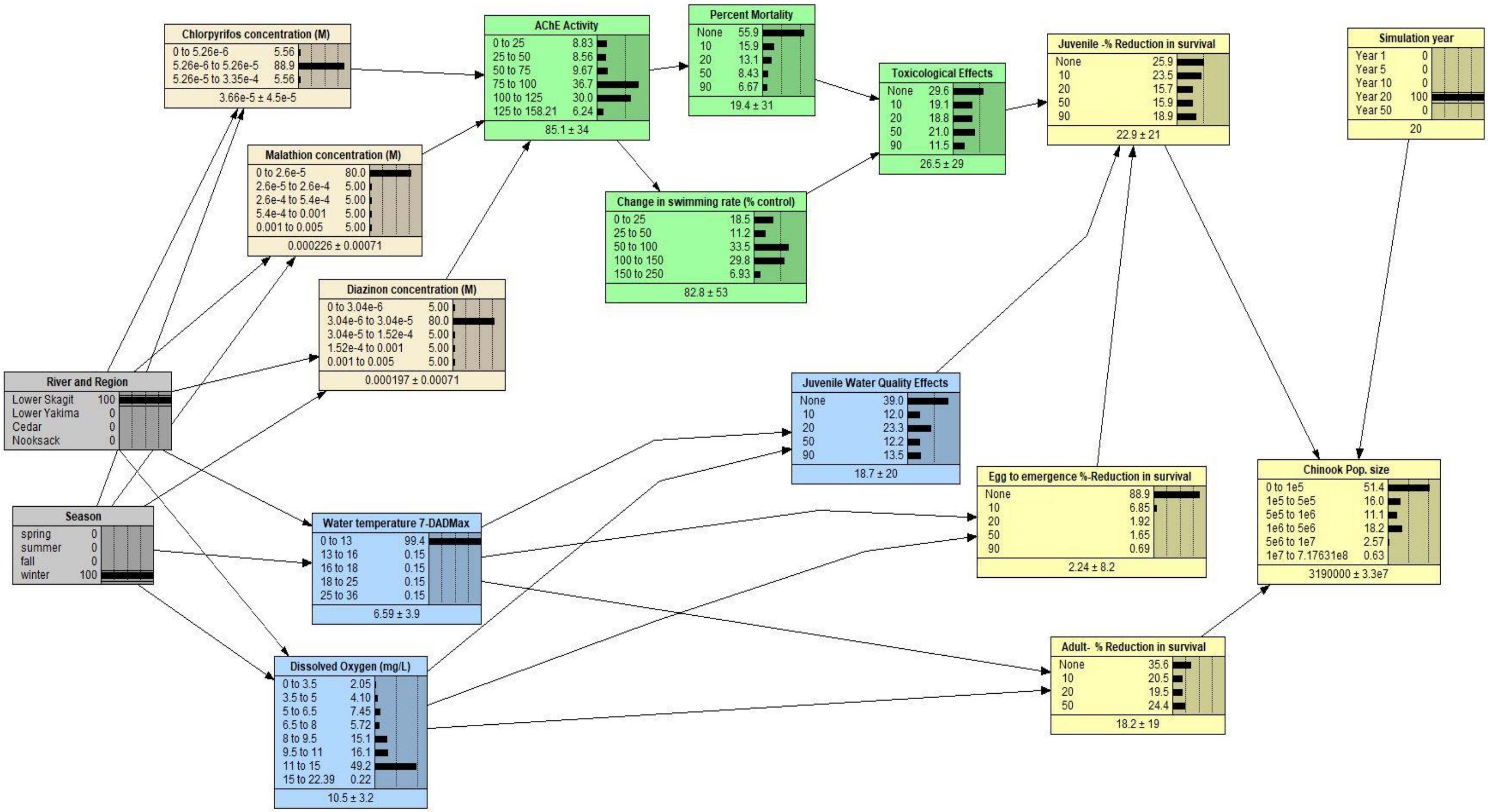

Figure S6. BN-RRM showing the output with the Skagit River-Winter measured concentrations.

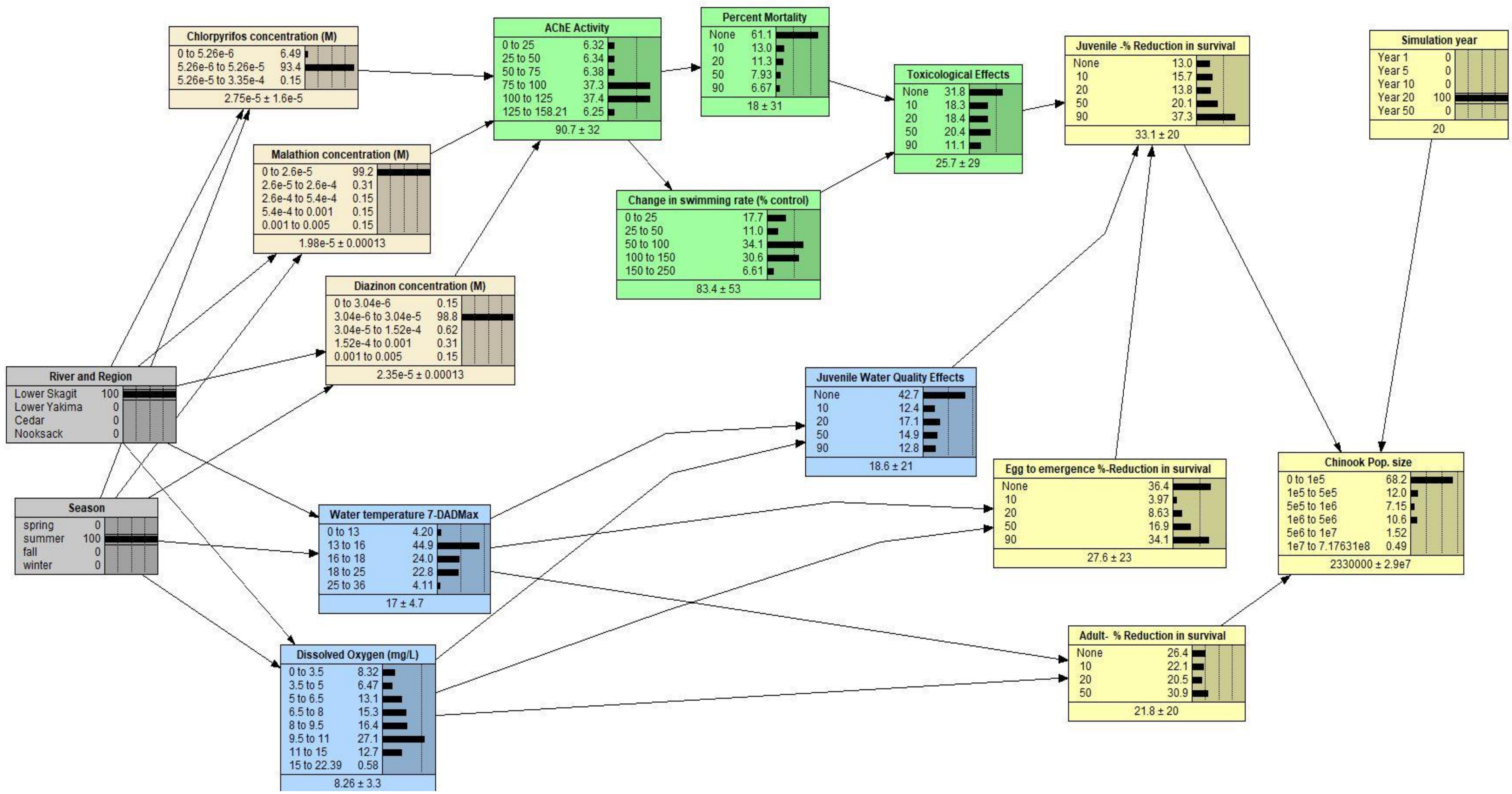

Figure S7. BN-RRM showing the output with the Skagit River-Summer measured concentrations.

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