



Toxicity of PHOS-CHEK LC-95A and 259F fire retardants to ocean- and stream-type Chinook salmon and their potential to recover before seawater entry



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HIGHLIGHTS

- LC-95A is lethal to 50% of Chinook salmon at 0.23% of the applied concentration.
- 259F is lethal to 50% of Chinook salmon at 0.09% of the applied concentration.
- Delayed mortality occurred in seawater after PHOS-CHEK exposure at smolt stage.
- Chinook salmon exposed to retardants as parr were not seawater-intolerant.
- Chinook salmon sensitivities varied with life stage and life history strategy.

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ABSTRACT

Long-term fire retardants are used to prevent the spread of wildland fire, but have inadvertently entered aquatic habitats and resulted in fish kills. We examined the toxicity of two fire retardant products; PHOS-CHEK 259F and LC-95A, on Chinook salmon with two different life histories, ocean-type and stream-type, at different stages of their development. Ocean-type Chinook outmigrate to the ocean as subyearlings; while, stream-type salmon overwinter in freshwater and outmigrate as yearlings. Ocean-type and stream-type salmon were exposed to the fire retardants prior to their parr to smolt transition (presmolts) as subyearlings (stream-type and ocean-type) and yearlings (stream-type only), as well as during their transition (smolts). The salmon were exposed to eight concentrations of each retardant and a control for 96 h to determine acute toxicity. Lethal concentration curves were modeled by logistic regression for each life history and life stage exposed to the two fire retardants. Among all life histories and life stages tested, PHOS-CHEK 259F was most toxic to stream-type salmon at smolt stage and PHOS-CHEK LC-95A was most toxic to ocean-type salmon at smolt stage. To determine the delayed effects of product exposures on fish health as well as for the potential of recovery, 24-hour seawater challenges were performed immediately after fire retardant exposure, as well as after a recovery period. Previous PHOS-CHEK exposure reduced survival during seawater challenge among salmon from both life histories undergoing the parr-smolt transition and was more pronounced after PHOS-CHEK LC-95A exposure. However, this delayed effect was not observed 34 or more days after either PHOS-CHEK exposure. We conclude that accidental PHOS-CHEK LC-95A or 259F drops during salmon outmigration would have adverse impacts that extend beyond the acute mortality that occurs within the immediate drop and dilution areas.

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1. Introduction

The U.S. Department of Agriculture, Forest Service (USFS) uses the aerial application of fertilizer-based long-term fire retardants to create containment perimeters around wildland fires. Long-term fire retardants are intended to provide a barrier to combustion by coating fuels sources with a long-lasting layer of ammonium salts (Buhl and Hamilton, 1998). An average of almost 11,000 aerial loads, and over 62 million liters, of long-term fire retardants were delivered per year

in the USA from 2008 to 2010 (NMFS, 2011). The USFS currently restricts any application of fire retardants within 91.4 m of all waterways or in mapped avoidance areas of critical habitat and locations of endangered, threatened, or sensitive species (Tidwell, 2011). The total restricted area accounts for 30.8% (240,556 km²) of all U.S. National Forest Lands (Tidwell, 2011), which is almost equivalent to the size of the State of Oregon (254,805 km²; http://en.wikipedia.org/wiki/List_of_U.S._states_by_area). However, fire retardant chemicals still have the potential to enter aquatic ecosystems via surface run-off, misapplication from an aerial drop, or during exceptions to the application restrictions (NMFS, 2011). An exception to the ban on fire retardants within restricted areas occurs when the application of the fire retardant is reasonably expected to alleviate the threat to human life or public safety (Tidwell, 2011). Fire retardants have been misapplied to the habitat of at least nine stocks of Pacific salmon and steelhead, listed under the U.S. Endangered Species Act, between the years 2001 and 2010, including: Snake River spring/summer Chinook salmon (*Oncorhynchus tshawytscha*), Upper Willamette River Chinook, southern Oregon/northern California coast coho (*O. kisutch*), Snake River steelhead (*O. mykiss*), California Central Valley steelhead; northern California steelhead, and Upper, Middle, and Lower Columbia River steelhead (NMFS, 2011). Misapplications of fire retardants have resulted in direct or estimated mortalities of threatened and endangered species, including: over 10,000 Upper Columbia River steelhead when Fire-Trol retardant was misapplied to a five mile stretch of Omak Creek in the state of Washington in 2003 (NMFS, 2011; Ring, 2003); and almost 3000 Snake River steelhead and 2600 Snake River spring/summer Chinook salmon when 1136 to 2271 l of Fire-Trol LCG retardant was misapplied to Nick Creek in the state of Idaho in 2004 (NMFS, 2005, 2011).

Fire retardant formulations are approximately 85% water and 10% inorganic salts (fertilizers) with 5% additives, e.g., gum thickeners, coloring agents, corrosion inhibitors, stabilizers and bactericidal agents (NMFS, 2011). Diammonium phosphates ((NH₄)₂HPO₄) and ammonium polyphosphate salts ((NH₄PO₃)_n) are the principal inorganic salts in currently approved fire retardants. The primary toxic risk to fish associated with fire retardant usage is the formation of un-ionized ammonia in the aquatic habitat (Buhl and Hamilton, 1998, 2000; Calfee and Little, 2003; Gaikowski et al., 1996; Gimenez et al., 2004). However, the other listed and proprietary components in fire retardant formulations may contribute to fish mortality (Buhl and Hamilton, 1998).

As of 2014, twenty-eight Pacific salmon and steelhead Evolutionarily Significant Units (ESUs) in Washington, Oregon, Idaho, and California are listed as either threatened or endangered [http://www.westcoast.fisheries.noaa.gov/publications/protected_species/salmon_steelhead/status_of_esa_salmon_listings_and_ch_designations_map.pdf]. A stock of Pacific salmon is considered an ESU under the U.S. Endangered Species Act if it is reproductively isolated from other stocks and “represent[s] an important component in the evolutionary legacy of the species” (Fox, 1991). Pacific salmon ESUs include Chinook salmon with ocean-type and stream-type life history strategies. Ocean- and stream-type life histories both begin with freshwater rearing, followed by migration to the estuary and then to the ocean, but ocean-type outmigration occurs within the first year of life, and stream-type outmigration occurs after one year of freshwater rearing (Healey, 1991).

While fire retardant toxicity to salmonid early life stages has been previously assessed (Buhl and Hamilton, 1998, 2000; Gaikowski et al., 1996), less is known about the sensitivity of fish to fire retardants undergoing the parr–smolt transition. The parr to smolt transition involves physiological and behavior changes in preparation for the marine environment (Folmar and Dickhoff, 1981; Hoar, 1976), which can be affected by earlier exposure to contaminants (Bjornsson et al., 2011; Deane and Woo, 2009; Lerner et al., 2007; McCormick, 2009) and makes salmon more susceptible to stressors, e.g., pollutants, predators, and disease (Carey and McCormick, 1998; Maule et al., 1987; McCormick et al., 1998; Monette and McCormick, 2008). Previous studies indicate that stream-type salmon undergoing the parr to smolt

transition are more sensitive to PHOS-CHEK 259F than coho salmon exposed as swim-up fry or fingerlings (≤ 1.0 g), prior to their parr to smolt transition (Dietrich et al., 2013; Johnson and Sanders, 1977). Dietrich et al. (2013) also demonstrated that stream-type Chinook salmon exposed as smolts to PHOS-CHEK LC-95A and 259F had delayed mortality upon immediate entry into seawater. However, the cumulative effect (acute plus delayed mortality) due to a PHOS-CHEK exposure was not determined for stream-type Chinook smolts. Chinook salmon's ability to recover from this delayed effect is unknown, as well as if their transition is adversely affected (e.g., timing or success) by exposures prior to the parr to smolt transition.

The aims of the present study were to determine PHOS-CHEK LC-95A and 259F exposure thresholds for acute mortality during misapplication of fire retardants, as well as delayed mortality as exposed salmon enter seawater. The overlap of the fire season and the timing of Chinook salmon freshwater rearing and seaward migration allows for PHOS-CHEK exposure at different Chinook salmon life stages, including varying recovery periods. We investigated the impacts of these fire retardants on Chinook salmon with two distinct life history strategies and with fire retardant exposures occurring as either presmolts or smolts. The laboratory scenarios (Fig. 1) used in the present study reflected ocean-type Chinook salmon that were exposed early in the fire season roughly one month prior to seawater entry (presmolts) or immediately before seawater entry (smolts). Three scenarios for stream-type Chinook salmon included: exposures late in the fire season (presmolts – subyearling) with survivors that would overwinter in freshwater; exposures early in the subsequent fire season before seaward migration (presmolts – yearling); or immediately before seawater entry (smolts). For both ocean- and stream-type Chinook salmon the specific objectives were to:

1. Determine lethal concentration curves for salmon exposed to PHOS-CHEK LC-95A or 259F prior to and during their parr to smolt transition, and
2. Determine the seawater survival of salmon immediately after PHOS-CHEK LC-95A or 259F exposure, as well as after a recovery period.

2. Materials and methods

2.1. Fire retardants

PHOS-CHEK LC-95A and 259F (ICL Performance Products LP; Ontario, CA), are proprietary compounds provided by the USFS Wildland Fire Chemical Systems Program (Missoula Technology Development Center; Missoula, MT, USA). The USFS has approved these two formulations for fighting wildland fires, with PHOS-CHEK 259F being the only long-term fire retardant qualified for use in fixed-tank helicopters (USFS, 2012). PHOS-CHEK 259F is a diammonium phosphate-based dry powder formulation with a manufacturer-recommended mix ratio of 136.6 g per liter of water (1.14 lb per U.S. gallon). At the approved mix ratio, 259F has a specific weight of 1.066 kg/l (8.90 lb/gal), a low viscosity of 75–259 cP, and is 10.9% (w/w) (NH₄)₂HPO₄. The formulation contains a red “fugitive” dye agent that fades over time in sunlight. PHOS-CHEK LC-95A is an ammonium polyphosphate-based liquid concentrate formulation that contains a corrosion inhibitor and iron-oxide color. LC-95A has a specific gravity of 1.473 kg/l (12.29 lb/gal) and a manufacturer-recommended mix ratio of 5.5 parts concentrate to 1 part water. At the approved mix ratio, LC-95A has a specific weight of 1.074 kg/l (8.97 lb/gal), a low viscosity of 75–225 cP, and is 7.6% (w/w) P₂O₅ equivalent.

2.2. Experimental animals

Juvenile Chinook salmon (*O. tshawytscha*) with ocean-type (OT) and stream-type (ST) life histories were used as the experimental stocks of fish in the present study. During rearing, the salmon fry were

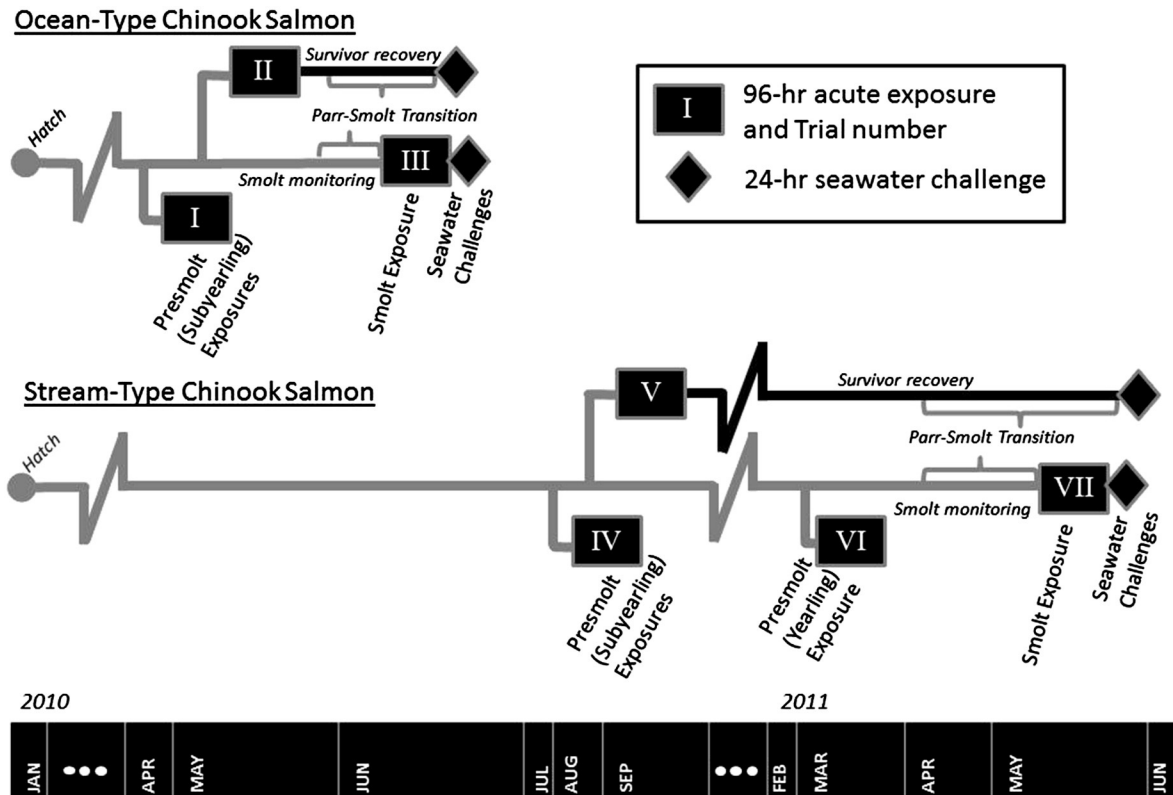


Fig. 1. Graphical characterization of the time course of ocean-type and stream-type Chinook salmon development, PHOS-CHEK exposure trials, recovery, and seawater challenges. Descriptions of exposures and challenges are provided in Tables 1–3.

maintained in flow-through, freshwater, tanks at the National Oceanic and Atmospheric Administration's Newport Research Station (NRS; Newport, OR, USA) and fed a diet of pellets (Bio-Oregon; Longview, WA). OT Chinook salmon originated from Elk River Hatchery (Port Orford, OR) and were transported to the NRS as embryos. These fish represented the 2009 brood year that outmigrated to the ocean in 2010. ST Chinook salmon originated from McKenzie River Hatchery (Leaburg, OR) and were transported to the NRS as fry. These fish represented the 2009 brood year that outmigrated to the ocean in 2011.

2.3. Acute PHOS-CHEK exposures

The acutely lethal effects of both fire retardants on OT and ST Chinook salmon were determined at two life stages (Fig. 1): (1) prior to the parr–smolt transition (“presmolt”); and (2) during the parr–smolt transition (“smolt”). The presmolt exposures included OT (Trial I) and ST (Trial IV) Chinook salmon as subyearlings, as well as ST salmon as yearlings (Trial VI). The smolt exposures (Trial III for OT and Trial VII for ST Chinook salmon) occurred once the parr–smolt transition of the experimental stock fish had progressed sufficiently. The progress of the parr–smolt transition was determined by weekly smoltification status monitoring (described in Section 2.5). Tables 1 and 2 provide descriptions of the exposure trials for OT and ST Chinook life histories, respectively.

In addition, we exposed separate cohorts of presmolts (Trial II for OT and Trial V for ST Chinook salmon) to lower ranges of fire retardant concentrations. The survivors from these trials were then used to assess the sublethal effects of PHOS-CHEK exposure on the parr–smolt transition and their ability to recover before seawater entry. Groups of 120 to 240 fish were exposed to 5 different concentrations of PHOS-CHEK 259F and LC-95A, and a non-chemical control (“PHOS-CHEK Control”) for 96 h, under static conditions (Tables 1 and 2). Greater fish numbers were required at the higher concentrations to ensure sufficient

survivorship for the subsequent holding period and seawater challenge. The survivors were transferred to ca. 300-liter flow-through freshwater tanks and diet of food pellets (1% body weight/d). At the time of transfer, a group of naïve fish (not subjected to the static conditions) was also added to an adjacent tank and treated equally. The recovery period ended once the parr to smolt transition of the experimental stock had progressed sufficiently (described in Section 2.5); i.e. 34 and 266 days post-exposure for OT and ST Chinook salmon, respectively.

Concentrations of the fire retardants were prepared on a mass to exposure volume basis, i.e. milligrams of PHOS-CHEK 259F dry powder or milligrams of PHOS-CHEK LC-95A liquid concentrate. Concentrated stock solutions were prepared with a mechanical mixer less than 24 h before initiating any exposure. The fire retardants were then added to polyethylene exposure vessels and mixed with freshwater immediately prior to water quality sample collection and fish addition. The vessels were placed within larger tanks and a continuously refreshed water jacket maintained a uniform temperature (ca. 10–12 °C). Each tank contained a porous air-stone with constant air supply. Tanks were monitored twice per day and any fish mortalities were recorded and removed. The lethal concentration (LC) curves and subsequent LC50 values (i.e. the PHOS-CHEK concentration predicted to kill 50% of the salmon that are exposed) for each fire retardant and seawater exposure were determined by logistic regression with Systat12© software (Systat Software, Inc.; Chicago, IL).

2.4. Seawater challenge

Seawater challenge tests were conducted as a measure of smolt tolerance for seawater entry (Blackburn and Clark, 1987) as per Dietrich et al. (2013). The challenges occurred in tanks (ca. 300 l) with flow-through, full strength seawater (34 ppt) for 24 h (Table 3). Fish were directly transferred into seawater from their 96-hour smolt exposure vessels or from their freshwater recovery tanks. Replicate tanks of

Table 1
Description of acute PHOSCHEK exposure trials conducted with ocean-type Chinook salmon in 2010.

	Presmolt				Smolt	
	Trial I		Trial II		Trial III	
	LC-95A	259F	LC-95A	259F	LC-95A	259F
Static exposure date	29 April–3 May		17–21 May		20–24 June	
Seawater challenge date	Not applicable		22–23 June		24–25 June	
Concentrations, mg/l	0	0	0	0	0	0
	498	174	154	75	154	75
	582	199	355	122	355	122
	646	224	456	145	456	145
	695	249	561	170	561	170
	749	274	639	188	639 ^a	188
	833	299			700 ^a	200
	917	323			755 ^a	250 ^a
	990	348			1000 ^a	300 ^a
LC50 (mg/l) ^b	656.1	191.9	Not applicable		524.2	186.0
Exposure volume (l)		22	22 and 66 ^c			35
No. of replicates (fish/replicate)		3 (25)	1 to 3 (20) and 1 to 4 (60) ^c			3 (25)
Fish weight at exposure (g)		1.8	2.2			3.4
Fish exposure density (g/l)		1.6	2.0			2.0
Fish age at exposure (days ^d)	85–92	96–103	103–110			137–144

^a These concentrations were not transferred to seawater following the 96-hour smolt test due to high or 100% mortality.

^b LC50 values determined from lethal concentration (LC) curves using logistic regression of the experimental data.

^c The number of fish and replicates varied per dose, in order to ensure a sufficient number of survivors at the higher fire retardant concentrations. The number of fish per replicate volume was preserved to maintain equivalent fish densities, i.e. 20 fish per 22 l and 60 fish per 66 l.

^d Approximate days post-hatch (January 27–February 3, 2010).

naïve fish that had not experienced 96 h of static holding or any fire retardants were also included. Chi-square tests of association (Systat Software, Inc.; Chicago, IL) were used to compare the mean survival among the controls and the PHOS-CHEK concentrations.

2.5. Parr–smolt indicators

The status of the physiological transition from parr to smolt (parr–smolt) was monitored weekly from 25 May to 23 June 2010 for the OT salmon and 9 February to 18 May 2011 for the ST salmon. We monitored the Na⁺–K⁺ ATPase (hereafter ATPase) activity in the gills of 10 stock

fish in freshwater, the survival of 10 additional stock fish placed in full strength seawater for 24 h, as well as the ATPase and sodium concentrations in fish plasma among the weekly survivors. In addition, ATPase and sodium concentrations were determined in 7 to 17 fish per PHOS-CHEK exposure concentration based on the number of survivors following the seawater challenges. An analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test was performed to determine statistically significant differences in the mean ATPase activities and sodium concentrations among the sample groups (i.e., exposure concentration or weekly samples) with significance level of 0.05.

Table 2
Description of acute PHOSCHEK exposure trials conducted with stream-type Chinook salmon in 2010–2011.

	Presmolt subyearling (2010)				Presmolt yearling (2011)		Smolt (2011)	
	Trial IV		Trial V		Trial VI		Trial VII	
	LC-95A	259F	LC-95A	259F	LC-95A	259F	LC-95A	259F
Static exposure date	14–18 Sept.		3–7 Sept.		14–18 March		21–25 May	
Seawater challenge date	Not applicable		31 May–1 June		Not applicable		25–26 May	
Concentrations (mg/l)	0	0	0	0	0	0	0	0
	300	75	30	25	200	75	200	60
	385	122	75	40	300	100	300	80
	470	145	235	80	375	122	375	100
	555	170	400	122	450	145	450	122
	640	188	520	153	525	175	525	145 ^a
	725	200			600	200	600	170 ^a
	810	250			675	250	675	190 ^a
	1000	300			810	300	810	225 ^a
LC50 ^b	557.7	160.0	Not applicable		624.8	136.9	798.6	122.4
Exposure volume (l)	32.4	30.6	31.8 and 95.4 ^c		33.84		102.4	
No. of replicates (fish/replicate)		3 (15)	1 to 2 (15) and 3 to 5 (45) ^c		3 (9)		3 (16)	
Fish weight at exposure (g)	5.4	4.7	5.2		10.9		14.7	
Fish exposure density (g/l)	2.5	2.3	2.5		2.9		2.3	
Fish age at exposure (months ^d)	10	10	10		16		18	

^a These concentrations were not transferred to seawater following the 96-hour smolt test due to high or 100% mortality.

^b LC50 values determined from lethal concentration (LC) curves using logistic regression of the experimental data.

^c The number of fish and replicates varied per dose, in order to ensure a sufficient number of survivors at the higher fire retardant concentrations. The number of fish per replicate volume was preserved to maintain equivalent fish densities, i.e. 15 fish per 31.8 l and 45 fish per 95.4 l.

^d Approximate age post-hatch (specific hatch dates occurred at McKenzie Hatchery and are unknown).

Table 3
Description of seawater challenge conditions conducted with ocean-type and stream-type Chinook salmon.

	Ocean-type				Stream-type			
	Presmolt, Trial II survivors		Smolt, Trial III survivors		Presmolt, Trial V survivors		Smolt Trial VII survivors	
	LC-95A	259F	LC-95A	259F	LC-95A	259F	LC-95A	259F
Seawater challenge date	22–23 June		24–25 June		31 May–1 June		25–26 May	
Recovery period	34 days		0		266 days		0	
Concentrations (mg/l)	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
	154	75	154	75	30	25	200	60
	355	122	355	122	75	40	300	80
	456	145	456	145	235	80	375	100
	561	170	561	170	400	122	450	122
	639	188		188	520	153	525	
				200			600	
							675	
							810	
No. of replicates (fish/replicate)	3 (20)	3 (20)	3 (25) ^b	3 (25) ^b	2 (20)	2 (20)	3 (16) ^c	3 (16) ^c
Fish weight at exposure (g)	2.9		3.1		14.2		13.8	

^a Experimental controls included fish not exposed to fire retardant, but held under equivalent static conditions for 96 h (PHOS-CHEK control) as well as naive fish not exposed to fire retardant or the static conditions.

^b Three replicates of 25 fish represents the maximum sample size. The actual number of replicates and fish per replicate was dependent on the numbers of fish that survived the previous PHOS-CHEK LC-95A and 259F smolt exposures. Any fire retardant concentration replicate that had 10 or more survivors was transferred to seawater.

^c Three replicates of 16 fish represents the maximum sample size. The actual number of replicates and fish per replicate was dependent on the numbers of fish that survived the previous PHOS-CHEK LC-95A and 259F smolt exposures. Any fire retardant concentration replicate that had 10 or more survivors was transferred to seawater.

2.5.1. ATPase activity

ATPase activity was determined from the gills as described in Dietrich et al. (2013). Due to smaller tissue sizes of OT fish, whole gill baskets were collected and a whole gill arch, including cartilage, was analyzed. Assay development indicated that gill cartilage did not affect ATPase activity (data not shown). For ST fish, the first gill arch on the left side was collected and a 2 mm piece of filament was used for analysis. All gill tissues were homogenized with stainless steel beads in a mechanical tissue lyser. ATPase activities in the gill homogenates were corrected for protein content following the bicinchoninic acid (BCA) protein assay (Pierce, Thermo Fisher Scientific). Fish gill-ATPase activities were determined in units of $\mu\text{m ADP/mg protein/h}$ as per McCormick (1993).

2.5.2. Sodium

Blood was collected from the caudal vein and centrifuged for 3 min to separate plasma and red blood cells. Due to the small size of the OT fish, equal volumes of plasma from each surviving fish were pooled for the sample week and stored at $-20\text{ }^{\circ}\text{C}$. Plasma volumes from ST fish were sufficient to be measured individually. Sodium levels were determined using an Orion micro sodium electrode 9811 (Thermo Scientific, Beverly, MA) and a four-step standard plus sample addition method that corrected for sample dilution and interference from the plasma matrix (see supplemental data and equations S1–S4 in Appendix A). Sodium concentrations were determined from four subsamples for each individual ST plasma sample, and from up to six subsamples for each pooled OT plasma sample.

2.6. Water quality measurements

Water quality parameters were assessed at the start and takedown (i.e. upon 100% mortality or at 96 h) of each acute exposure tank. Water quality measurements included instant readings of dissolved oxygen and temperature from a multi-probe (YSI85; YSI Inc., Yellow Springs, OH) suspended in the water column of each tank. In addition, 125 ml grab samples from each tank were analyzed for pH and ammonia. The pH of water samples was determined using a Ross Sure-Flow pH electrode 8172 (Thermo Scientific, Beverly, MA). Samples were stored at $4\text{ }^{\circ}\text{C}$ for less than a week before pH analysis.

Water samples were analyzed for total ammonia by Method 4500-NH₃-D (Eaton et al., 2005) using an Orion ion selective ammonia electrode 9512HP (Thermo Scientific, Beverly, MA). Samples were preserved with 2 ml/l sulfuric acid (96.7% wt/wt) and stored at $4\text{ }^{\circ}\text{C}$ for less than 30 days before analysis. Un-ionized ammonia concentrations were then determined from tables presented in Thurston et al. (1979) based on the calculations of Emerson et al. (1975) using temperature, pH, and total ammonia measurements from each tank.

3. Results

3.1. Acute PHOS-CHEK exposures

OT and ST Chinook salmon had different sensitivities to acute exposures of the two PHOS-CHEK formulations at different life stages. The levels of mortality that occurred at each PHOS-CHEK concentration were well described by logistic regression (LOGIT) models (Fig. 2 and Supplementary data). PHOS-CHEK LC50s are presented in Tables 1 and 2 for OT and ST Chinook exposures, respectively, and a summary of other toxicity endpoints, e.g., no and lowest observable effect concentrations (NOEC and LOEC), are provided in the Supplementary data (Table S.1). The LC-95A LC50s for OT Chinook salmon decreased from 656.1 mg/l to 524.2 mg/l, with a significant translation of the lethal concentration curve to the left as the OT Chinook salmon shift from presmolt (Trial I) to smolts (Trial III; Fig. 2a), indicating increased sensitivity. Similarly, the lethal concentration curve for ST Chinook salmon exposed to 259F translated to the left with life-stage change from presmolts (Trial IV) to smolts (Trial VII; Fig. 2d). The LC-95A lethal concentration curve for ST Chinook salmon was translated in the opposite direction, indicating reduced sensitivity as the fish transitioned from presmolts (Trial IV) to smolts (Trial VII; Fig. 2c). Finally, there was little difference between the lethal concentration curves for OT Chinook salmon exposed to 259 F as presmolts (Trial I) and smolts (Trial III; Fig. 2b).

During the ST Smolt exposure (Trial VII), 10.6% mortality was observed in the PHOS-CHEK control. This was the greatest mortality observed for any PHOS-CHEK control during the 14 static exposures conducted in the present study. In fact, Trial VII was the only 96-hour, static exposure test in which mortalities occurred in the PHOS-CHEK control tanks. The mortalities observed in the three replicate tanks

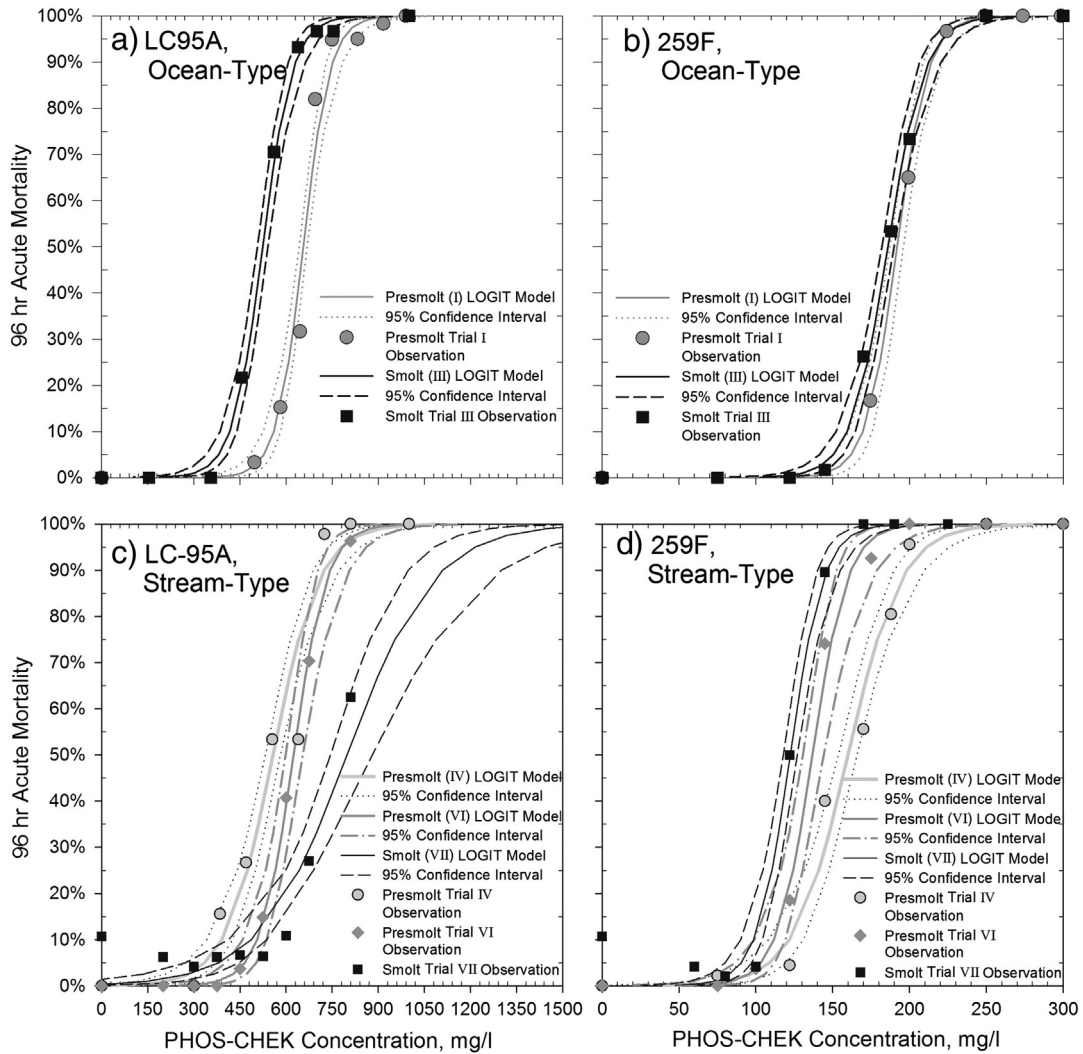


Fig. 2. Lethal concentration curves for ocean-type (a, b) and stream-type (c, d) Chinook salmon exposed to PHOS-CHEK LC-95A (a, c) and 259F (b, d); based on logistic regression models (LOGIT) of observed fish survival. Fish were exposed at presmolt and smolt life stages. Stream-type Chinook salmon were exposed as subyearling and yearling presmolts.

of PHOS-CHEK control were 0% (0/15), 6.3% (1/16), and 25% (4/16), with no striking differences in water quality measurements. The mean level of mortality in the PHOS-CHEK control was roughly greater than, or equal to, the mortality that occurred in PHOS-CHEK LC-95A ≤ 600.0 mg/l and 259F concentrations ≤ 100.0 mg/l.

3.1.1. Presmolt recovery

OT Chinook salmon were exposed to LC-95A and 259F concentrations in Trial II that resulted in 0 to 39.5% or 0 to 26.2% mortality, respectively (data not shown). No mortalities were observed in tanks exposed to ≤ 154.0 of LC-95A or ≤ 122.0 mg/l 259F. OT Chinook salmon that survived were then held for 34 days prior to the seawater challenge to determine their potential for recovery. Low levels of mortality occurred during holding (0 to 5.5%) and were not correlated with concentration or PHOS-CHEK formulation. No mortalities occurred in the PHOS-CHEK controls or the naïve fish.

ST Chinook salmon were exposed to LC-95A and 259 F concentrations in Trial V that resulted in 0 to 4.3% or 0 to 28.5% mortality, respectively (data not shown). No mortalities were observed in tanks exposed to ≤ 235.0 of LC-95A or ≤ 80.0 mg/l 259F. ST Chinook salmon were then held for 266 days to determine their potential for recovery prior to a seawater challenge. Over the 266 days, the greatest mortalities occurred among the naïve fish (27.5%). Mortality among fish previously exposed to LC-95A or 259F, ranged from 3.3 to 20.6% mortality or

6.7 to 18.1% mortality, respectively. There was no indication of a concentration–response in the observed mortalities. Finally, 12.2% mortality occurred among fish in the PHOS-CHEK control treatment.

3.2. Seawater challenge

3.2.1. No adverse effects on seawater acclimation for Chinook salmon exposed as presmolts

The survival of OT Chinook salmon held for 34 days after exposures to PHOS-CHEK 259F or LC-95A in Trial II was not reduced ($p \geq 0.150$) during the 24-hour seawater challenge (Fig. 3a,b). One mortality occurred among PHOS-CHEK controls (1.7%) and no mortalities were observed among the naïve fish that were held for the same recovery period. In addition, no mortalities were observed in OT Chinook salmon exposed to ≤ 639.0 mg/l of LC-95A or ≤ 145.0 mg/l of 259F 34 days earlier.

Similarly, the survival of ST Chinook salmon that were held for 266 days after exposure to PHOS-CHEK 259F or LC-95A as subyearlings in Trial V was not reduced ($p \geq 0.206$ or 0.086, respectively) during the 24-hour seawater challenge (Fig. 3c,d). Low background mortality was observed in the PHOS-CHEK controls (5.9%) and naïve (4.9%) fish that were held for the same recovery period. No mortality was observed in ST Chinook salmon exposed to ≤ 75.0 mg/l of LC-95A; but 12.5%, 12.5%, and 10.3% mortalities were observed with fish previously

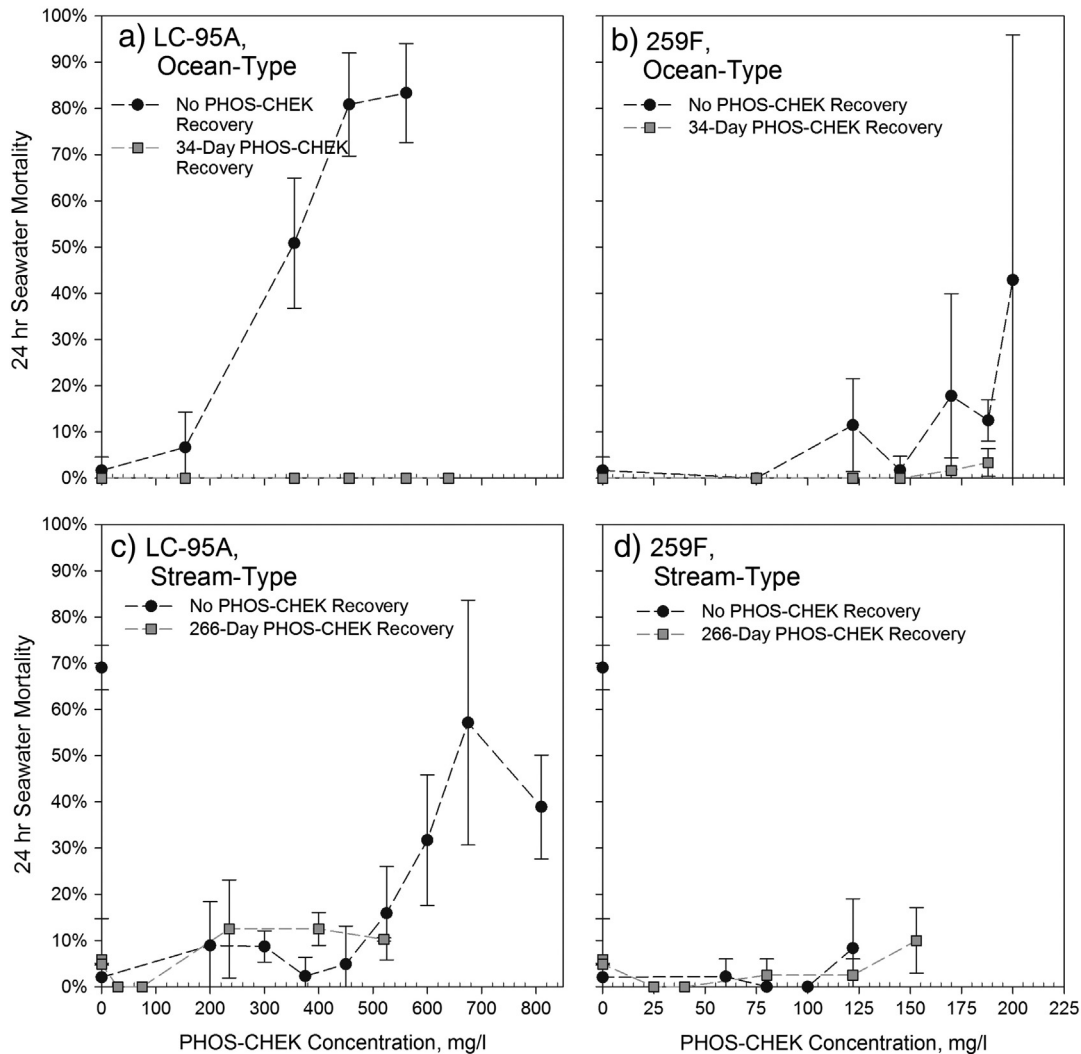


Fig. 3. Lethal concentration curves for ocean-type (a, b) and stream-type (c, d) Chinook salmon during the seawater challenge that were previously exposed to PHOS-CHEK LC-95A (a, c) and 259F (b, d); based on observed fish survival after 24 h. Fish experienced the seawater challenge either immediately after the smolt PHOS-CHEK exposure (no recovery), or after the survivor cohorts' recovery period (34 to 266 days).

exposed to 235.0, 400.0, and 520.0 mg/l of LC-95A, respectively. Likewise, no mortality was observed in ST Chinook salmon exposed to ≤ 40.0 mg/l of 259F; but 2.5%, 2.5% and 10.0% mortalities were observed with fish previously exposed to 80.0, 122.0 and 153.0 mg/l of 259F, respectively.

3.2.2. OT smolt seawater tolerance was significantly affected by LC-95A and 259F exposure

The survivors of acute exposure to LC-95A and 259F as smolts in Trial III had trends of increasing mortality during the seawater challenge with increasing PHOS-CHEK concentration (Fig. 3a,b). However, the level of mortality did not increase with each successive 259F concentration. One mortality (1.7%) occurred in each of the naïve fish and the PHOS-CHEK control treatments during the challenge. Smolts previously exposed to 154 mg/l of LC-95A or 75 and 145 mg/l of 259F had mortality levels that were not significantly different from the PHOS-CHEK control ($p \geq 0.171$). The LC-95A and 259F LOECs were 355 mg/l (50.8% mortality; $p \leq 0.001$) and 122 mg/l (11.5% mortality; $p \leq 0.030$), respectively.

3.2.3. ST smolt seawater tolerance was significantly affected by LC-95A exposure

The survivors from Trial VII's acute exposure to LC-95A as smolts had a concentration-dependent mortality during the seawater challenge

(Fig. 3c) that did not occur among the survivors of 259F exposure (Fig. 3d). One mortality (2.0%) among the naïve fish occurred during the challenge, but the PHOS-CHEK control treatment had 69.4% mortality. The latter fish had approximately 10% mortality during the static holding, suggesting the cause of mortality was exacerbated during the seawater challenge. Chi-square analysis indicated that the mortality levels in LC-95A (2 to 57%) and 259F (0 to 8.3%) treatments were significantly less than or similar to the mortality in the PHOS-CHEK control. Smolts in Trial VII exposed to the four lowest concentrations of LC-95A (≤ 450 mg/l) and all concentrations of 259F (≤ 122 mg/l) had similar mortality levels relative to the naïve fish (0 to 8.9%; $p \geq 0.100$). The 525 mg/l concentration of LC-95A was the LOEC that was significantly different from the naïve fish ($p < 0.017$) with 15.9% mortality.

3.3. Parr-smolt transition

The OT and ST salmon were prepared for surviving seawater entry at the times of their respective seawater challenges based on the ATPase activities in their gills, sodium in their plasma, and their survival in seawater (Supplementary data, Figs. S.1 and S.2, respectively). In addition, PHOS-CHEK LC-95A and 259F exposure did not affect the ATPase activities of OT or ST survivors as presmolts or smolts (Supplementary data, Figs. S.3, S.4, S.5 and S.6). Similarly, sodium concentrations

among ST survivors were unaffected by previous LC-95A or 259F exposure (Supplementary data, Figs. S.7 and S.8). In contrast, sodium concentrations in OT smolts were decreased at higher concentrations of LC-95A and increased at higher concentrations of 259F relative to the controls and lower concentrations ($p \leq 0.025$; Supplementary data, Fig. S.9). OT salmon previously exposed to the lowest 259F concentration in Trial II had significantly lower sodium levels after seawater challenge, but no differences were found among OT salmon previously exposed to LC-95A (Supplementary data, Fig. S.10).

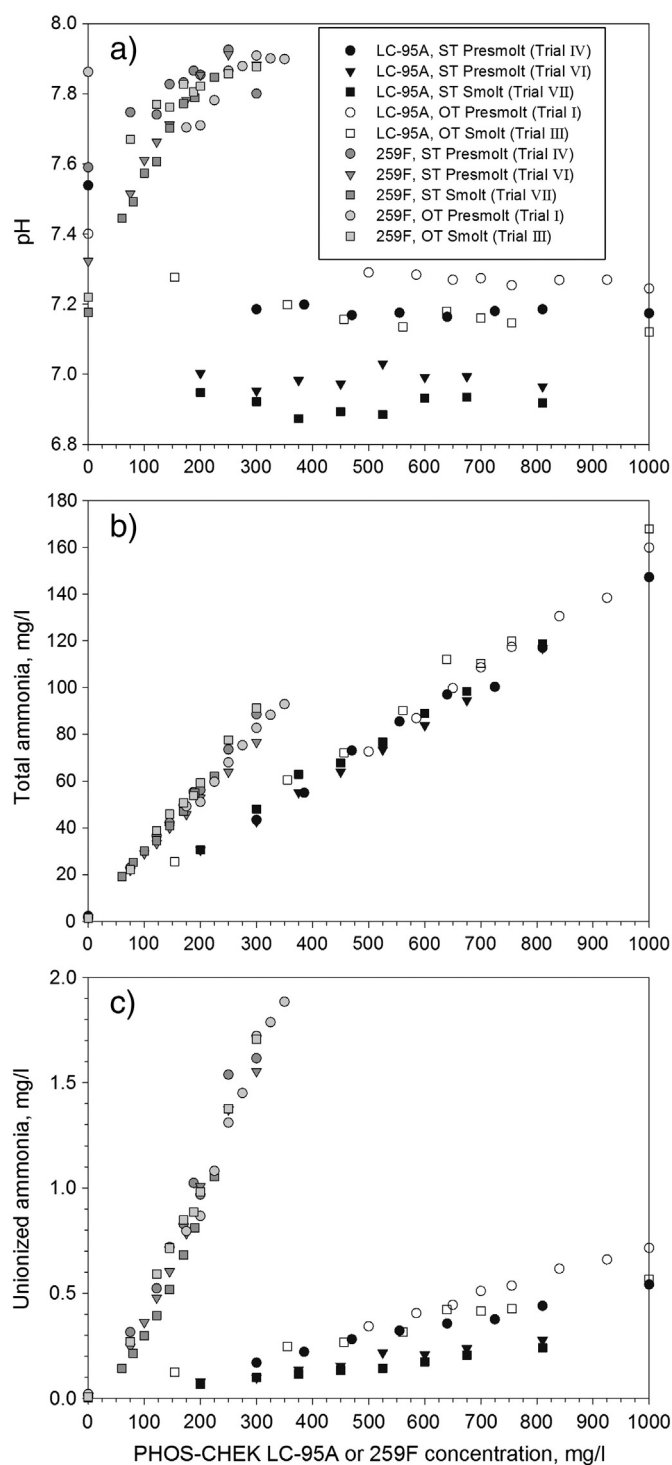


Fig. 4. Water quality measurements of (a) pH and (b) total ammonia, and the resulting un-ionized ammonia concentrations calculated for ocean-type and stream-type Chinook salmon exposed to PHOS-CHEK LC-95A and 259F.

3.4. Water quality measurements

3.4.1. Temperature and dissolved oxygen

The minimum optimal dissolved oxygen level for maintaining salmon health is 8.8 mg/l at 11.3 °C (Wedemeyer, 1996). During the exposure trials, mean temperatures ranged from 10.6 to 11.9 °C (Supplementary data, Table S.2). Mean DO concentrations ranged from 8.9 to 10.9 mg/l (Supplementary data, Table S.2).

3.4.2. pH

Unlike temperature and DO, pH in exposure tanks varied among PHOS-CHEK formulations, concentrations, and during the exposure. The pH ranged from 6.6 to 8.1 across all of the PHOS-CHEK concentrations and from 7.17 to 7.81 across the controls (Fig. 4a and Supplementary data, Tables S.3–S.9). The pH varied little with LC-95A concentration, but increased asymptotically to about 8.0 with increasing 259F concentration (Fig. 4a). Finally, the pH decreased in tanks containing 259F and increased in tanks containing LC-95A during the exposure.

3.4.3. Ammonia

Total ammonia in the water increased as concentrations of both PHOS-CHEK formulations increased (Fig. 4b and Supplementary data, Tables S.3–S.9). Total ammonia levels in PHOS-CHEK 259F concentrations were greater than LC-95A concentrations on an equivalent mass per volume basis, and the difference increased with increasing concentration (Fig. 4b). A consistent increase among the controls (i.e., 0 to 2.4–4.4 mg/l) during each of the exposures is most likely due to accumulation of fish excreta during the static holding. Minimal change in total ammonia levels occurred in tanks containing either of the fire retardants during the exposure.

Un-ionized ammonia concentrations were most influenced by variation in pH and total ammonia concentrations (Fig. 4c and Supplementary data, Tables S.3–S.9). In the LC-95A treatments, pH was low and invariant, but the total ammonia increased with increasing LC-95A concentration. The result was a fairly uniform and slightly increasing range of un-ionized ammonia concentrations across all LC-95A treatments and exposures (0.06 to 0.72 mg/l; Fig. 4c). In contrast, both the pH and total ammonia increased with increasing 259F concentration. The result was a steep increase in un-ionized ammonia concentrations with increasing 259F concentrations across all exposures (0.3 to 2.32 mg/l). Un-ionized ammonia concentrations in all of the PHOS-CHEK controls were less than 0.04 mg/l.

4. Discussion

4.1. Acute PHOS-CHEK Exposures

PHOS-CHEK 259F and LC-95A are acutely toxic to OT and ST Chinook salmon as presmolts and smolts at concentrations below the suggested mix ratios. Consequently, an accidental drop over salmon habitat of either PHOS-CHEK retardant, prepared within concentration guidelines, could result in salmon mortality. According to the manufacturer's mix ratios, the suggested working concentrations of LC-95A and 259F are roughly 226,590 and 136,600 mg/l, respectively. These are the concentrations that would immediately enter a water body during an accidental drop and then begin to be diluted. The estimated 96-hour LC50 concentrations for OT and ST Chinook salmon are presented in Table 4 alongside the required volume to dilute 1 l of the fire retardant, at its working concentration, to the LC50 concentration. Table 4 also includes the estimated LC01, i.e. the concentration at which 99% of the salmon are predicted to survive, and the required dilution of the working concentration to the LC01 concentration. The volumes range from 284 to 432 l (0.35 to 0.23%) and 712 to 1116 l (0.14 to 0.09%) to dilute PHOS-CHEK LC-95A and 259F to their LC50 concentrations, respectively; and 497 to 1527 l and 940 to 1882 l to dilute PHOS-CHEK LC-95A and 259F to their LC01 concentrations, respectively (Table 4; Fig. 5).

Table 4
Lethal concentrations of PHOS-CHEK LC-95A and 259F determined for OT and ST Chinook salmon at presmolt and smolt life stages and volumes of water required to dilute 1 l of each of the fire retardants at their working concentrations^a.

PHOS-CHEK	Life stage (trial number)				
	Presmolt			Smolt	
	OT (I)	ST, subyearling (IV)	ST, yearling (VI)	OT (III)	ST (VII)
LC-95A					
LC50, mg/l	656.1 (0.29)	557.7 (0.25)	624.8 (0.28)	524.2 (0.23)	798.6 (0.35)
(% of working concentration)					
Dilution required ^b , l	345	406	363	432	284
LC01, mg/l	455.7 (0.20)	209.9 (0.09)	376.9 (0.17)	300.4 (0.13)	148.4 (0.07)
(% of working concentration)					
Dilution required, l	497	1080	601	754	1527
259 F					
LC50, mg/l	191.9 (0.14)	160.0 (0.12)	136.9 (0.10)	186.0 (0.14)	122.4 (0.09)
(% of working concentration)					
Dilution required, l	712	854	998	734	1116
LC01, mg/l	145.3 (0.11)	80.4 (0.06)	84.5 (0.06)	130.6 (0.10)	72.6 (0.05)
(% of working concentration)					
Dilution required, l	940	1699	1617	1046	1882

^a The working concentrations are based on manufacturer-recommended mix ratios and are roughly 226,590 and 136,600 mg/l for LC-95A and 259F, respectively.

^b Volume of water (and percent of recommended mix ratio) required to dilute the working concentration of 1 l of PHOS-CHEK fire retardant to the calculated LC50 and LC01 concentrations.

Larger volumes of water are required to dilute an accidental drop of 259F than LC-95A, implying that the 259F formulation is environmentally more acutely toxic than the LC-95A, despite the higher working concentration of LC-95A. Chinook salmon's increased sensitivity to 259F relative to both life histories and life stages is graphically depicted in Fig. 5. The level of actual mortality and dilution that could occur in the environment would depend on the physical (e.g., water volume and flow rate), chemical (e.g., pH buffering capacity), and biological characteristics (e.g., species density) of the water body that received the accidental drop, as well as the time of year in which the drop occurred.

Buhl and Hamilton (1998) describe the misapplication of 1012 l of fire retardant (no longer in use by the USFS) into a tributary of the South Fork John Day River, OR that resulted in the mortality of 23,000 fish along a 2700-m reach of the river in 1995. The fire retardant was dropped along a 55-m length of the creek that was 4.6 m wide and 0.2 m deep. Buhl and Hamilton (1998) estimated the peak concentrations in the application zone as well as downstream of the tributary's confluence with the South Fork John Day River, assuming instantaneous mixing. If PHOS-CHEK LC-95A or 259F had been used at the recommended mix ratio,

the peak concentrations in the application zone would have been 4443 or 2678 mg/l, respectively; values estimated to cause 100% mortality in OT and ST Chinook salmon at both life stages examined in this study. The peak concentrations of LC-95A or 259F downstream of the confluence would have been 667 or 402 mg/l, respectively. Once again, the peak 259F concentration would cause 100% mortality in OT and ST Chinook salmon at both life stages examined in this study, but the peak LC-95A concentration within the range of LC50 concentrations (524 to 799 mg/l). The total volumes that would have been required to dilute this historic misapplication to the lowest 1% mortality (ST Chinook salmon) estimate are $1.545 \cdot 10^6$ or $1.904 \cdot 10^6$ l if LC-95A or 259F had been dropped, respectively, which would encompass a 1.68 or 2.07 km length at the given tributary's dimensions.

Changes in life-stage sensitivity of Chinook salmon to PHOS-CHEK fire retardants varied with life history strategy and fire retardant (Fig. 5). OT Chinook salmon sensitivity to 259F was the only life history and formulation combination that was not altered as the fish transitioned from presmolts to smolts. In contrast, ST Chinook salmon sensitivity to 259F increased at smolt stage. Chinook salmon's sensitivity to LC-95A also increased among smolts with an OT life history, but decreased among smolts with an ST life history. For ST Chinook salmon, the presmolt PHOS-CHEK exposures as yearlings (Trial VI) provided an intermediate data set, in terms of fish age and PHOS-CHEK sensitivity. Both PHOS-CHEK LC-95A and 259F ST presmolt yearling exposures had lethal concentration curves that fell between the presmolt (subyearling, Trial IV) and smolt (Trial VII) lethal concentration curves.

In comparisons with other salmonids, OT Chinook salmon as presmolts and smolts were less sensitive to 259F than swim-up rainbow trout (148 mg/l; 168 mg/l; 94–165 mg/l; (Buhl and Hamilton, 2000; USFS, 2011)) and coho salmon (170–250 mg/l, reported in Buhl and Hamilton (2000)). However, ST Chinook salmon were similar or slightly more sensitive to 259F. Chinook salmon (OT and ST) were less sensitive to LC-95A exposure at presmolt and smolt life stages compared to rainbow trout (435 mg/l) at 60 days post-hatch (USFS, 2011). In our previous study, the LC50's determined for yearling ST Chinook salmon were 339.8 mg/l and 140.5 mg/l for LC-95A and 259F, respectively (Dietrich et al., 2013). Based on the LC50's, the ST Chinook salmon in the present study appear to be less sensitive. The most striking difference from the present study was the mean fish density of about 2.3 g fish per liter during the static exposures compared to 12 g fish per liter in Dietrich et al. (2013). In the earlier study, we observed an accumulation of ammonia in the PHOS-CHEK controls and sought to minimize that in the present

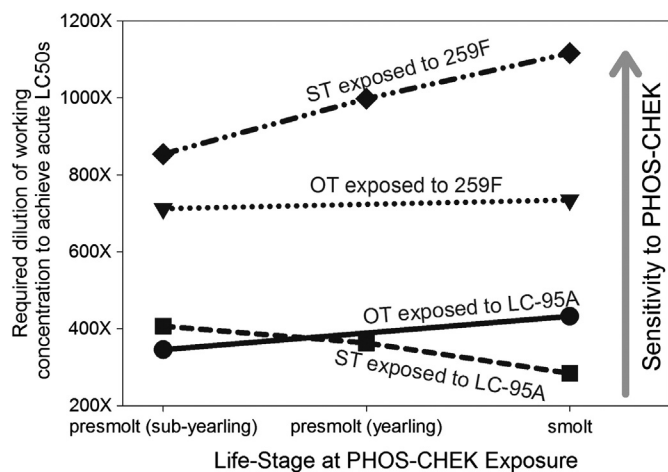


Fig. 5. Variation in sensitivity of Chinook salmon to PHOS-CHEK fire retardants with life stage and life history strategy, represented in terms of how many times a volume of the fire retardant, at the working concentration, must be diluted to reach the acute LC50s.

study. Greater fish densities may have also increased fish stress levels and contributed to an increased sensitivity to the fire retardants.

4.2. Water quality measurements

Greater un-ionized ammonia concentrations in the 259 F treatments may have resulted in more ammonia-associated mortality and greater PHOS-CHEK 259F sensitivity than occurred in salmon exposed to the LC-95A formulation. Once fire retardants are introduced into water, the ammonia within the formulation equilibrates between ionized and un-ionized forms dependent upon water pH and temperature (NMFS, 2007). The un-ionized ammonia form is more toxic to fish (Colt and Tomasso, 2001). Increased pH with increasing 259F concentrations resulted in a greater ratio of un-ionized ammonia to total ammonia than in the LC-95A treatments. Acute toxicity tests of ammonia on juvenile Chinook salmon, performed by other researchers, determined LC50 values ranging from 0.399 to 0.476 mg/l for water pH's between 7.8 and 8.0 (Servizi and Gordon, 1990; Thurston and Meyn, 1984). Given this range, the levels of un-ionized ammonia in PHOS-CHEK treatments were sufficient to induce mortality. In general, un-ionized ammonia levels in treatments that bracketed the LC-95A LC50 values were less than or equal to the un-ionized ammonia LC50. In some LC-95A exposures, 100% mortality occurred when un-ionized ammonia concentrations were less than 0.4 mg/l. In contrast, the un-ionized ammonia in treatments that bracketed the 259F LC50 values were always greater than or equal to the un-ionized ammonia LC50. Our findings are consistent with Dietrich et al. (2013) and we conclude that un-ionized ammonia was likely the principal contributor to the observed mortality during 259F exposures, and additional factor(s) such as the additives in the fire retardants may have contributed to mortality of fish exposed to PHOS-CHEK LC-95A. Consequently, aquatic environments with buffering capacities that maintain pH levels near 7.0 may have a moderating effect on PHOS-CHEK 259F toxicity, but not LC-95A

4.3. Seawater challenge

In addition to acute mortality, Chinook salmon smolts exposed to PHOS-CHEK LC-95A and 259F had delayed mortality upon immediate entry into seawater. Smolts that survived exposure PHOS-CHEK fire retardants in Trials III and VII had up to 83% mortality in seawater. High seawater survival among the naïve fish (ST and OT) indicated that the salmon identified as 'smolts' were prepared for seawater entry at the time of acute exposure and seawater challenge. ST Chinook salmon that survived acute exposure to 259F in Trial VII were the only smolts tested that did not exhibit reduced seawater survival relative to naïve fish. This may have been due to the steep concentration-response during the acute exposure (i.e. high direct mortality), but even survivors from the LC50 concentration did not have significant seawater mortality (~8%). The most significant delayed mortality occurred among ST and OT Chinook salmon exposed to LC-95A as smolts. Increasing seawater mortality with increasing LC-95A exposure was observed among both life history strategies. OT Chinook salmon had a steeper concentration-response and lower LOEC (355 mg/l) in seawater than ST Chinook salmon previously exposed to LC-95A (525 mg/l).

OT and ST Chinook salmon exposed in Trials II and V, respectively, did not exhibit significant PHOS-CHEK delayed mortality in seawater after their respective holding periods. The 34-day recovery period was sufficient to reduce the level of seawater mortality among OT Chinook salmon by 12 to 18% among survivors of 259F exposures \leq 188 mg/l. The same recovery period resulted in even more dramatic results for OT Chinook salmon exposed to LC-95A. OT Chinook salmon exposed to 561 and 639 mg/l of LC-95A as presmolts in Trial II, and left to recover for 34 days, had over 80% less mortality in seawater than OT Chinook salmon exposed to the same concentrations with no recovery period. ST Chinook salmon exposed as presmolts in Trial V and given 266 days to recover also had high survival during seawater challenge.

High survival in seawater among ST presmolts exposed to 259F was expected since no delayed effects were observed among ST Chinook salmon that had no recovery time prior to the seawater challenge. Finally, the seawater survivals of ST Chinook salmon exposed to \leq 520 mg/l of LC-95A as presmolts and allowed to recover for 266 days were similar to the seawater survivals of ST Chinook salmon smolts exposed to \leq 525 mg/l of LC-95A with no recovery period.

In general, the seawater challenge results not only indicated sensitivity to subsequent stress, but also indicated specific sensitivity to seawater. However, no disruptions to specific parr-smolt indicators were found. Dietrich et al. (2013) identified damaged gill tissues following PHOS-CHEK exposure that included: respiratory epithelial hypertrophy and exfoliation; epithelial necrosis at 206 mg/l of 259 F; and high prevalence of phagosomes at \geq 340 mg/l of LC-95A and 206 mg/l of 259F. Similarly damaged rainbow trout gills have been associated with un-ionized ammonia exposure (Hofer et al., 1995; Smart, 1976); while the occurrence of phagosomes suggested phagocytosis of fire retardant compounds or particles (Dietrich et al., 2013). The outcomes from both studies suggest that the seawater sensitivity may be due to physical damages during PHOS-CHEK exposure. The potential of reduced oxygen diffusion across damaged gills could ultimately result in reduced survival (Smart, 1976).

The PHOS-CHEK acute mortality plus any delayed mortality in seawater represents the cumulative effect of PHOS-CHEK exposure in fish. This cumulative effect is graphically depicted with shaded lethal concentration curves in Fig. 6, with dark shading representing acute mortality and light shading representing additional delayed mortality. The dark-shaded boundaries are the logistic regression of the 96-hour smolt acute PHOS-CHEK exposures presented in Fig. 2. The light-shaded boundaries were constructed by performing logistic regressions of the total mortality observed (acute plus seawater) for each of the PHOS-CHEK formulations and Chinook life history strategies. Cumulative lethal concentration curves demonstrate that LC-95A exposure has acute plus delayed effects at almost all exposure concentrations. In contrast, delayed effects due to 259F exposure are only expected among OT Chinook salmon that survive 259F concentrations less than or equal to the acute LC50. The resulting cumulative lethal effects curves can be used to re-interpret the sensitivity of Chinook salmon to the PHOS-CHEK formulations. For example, minimal delayed effects in fish exposed to 259F implies minimal additional dilution is required to meet the cumulative LC50 for 259F; whereas, an additional 25 to 50% dilution is required for accidental LC-95A drops into ST and OT habitats, respectively. The amount of increased environmental dilution increases as the cumulative LC decreases. Returning to the scenario of a misapplication in a tributary of the South Fork John Day River in Buhl and Hamilton (1998), if ST Chinook salmon smolts were present in the South Fork John Day River and 259F had been misapplied, the dilution volume required to meet the acute and cumulative LC01 are the same. However, if LC-95A had been misapplied, 1.68 km was required to sufficiently dilute the fire retardant to the acute LC01, and an additional 1.63 km would be needed to dilute to the cumulative LC01. Consequently, the geographic range of the misapplication's adverse effects in salmon habitat has almost doubled in length.

5. Conclusions

PHOS-CHEK LC-95A and 259F formulations were acutely toxic to Chinook salmon at concentrations significantly diluted from manufacturer suggested mix ratios. OT and ST Chinook salmon as presmolts (subyearling and yearling) and smolts were most sensitive to 259F. ST Chinook salmon were the most sensitive to 259F at all tested life stages. Chinook salmon are most sensitive to LC-95A as ST subyearling presmolts and OT smolts.

OT and ST Chinook salmon smolts that survived previous exposure to PHOS-CHEK fire retardants all had significantly reduced seawater survival, with the exception of ST Chinook salmon smolts exposed to

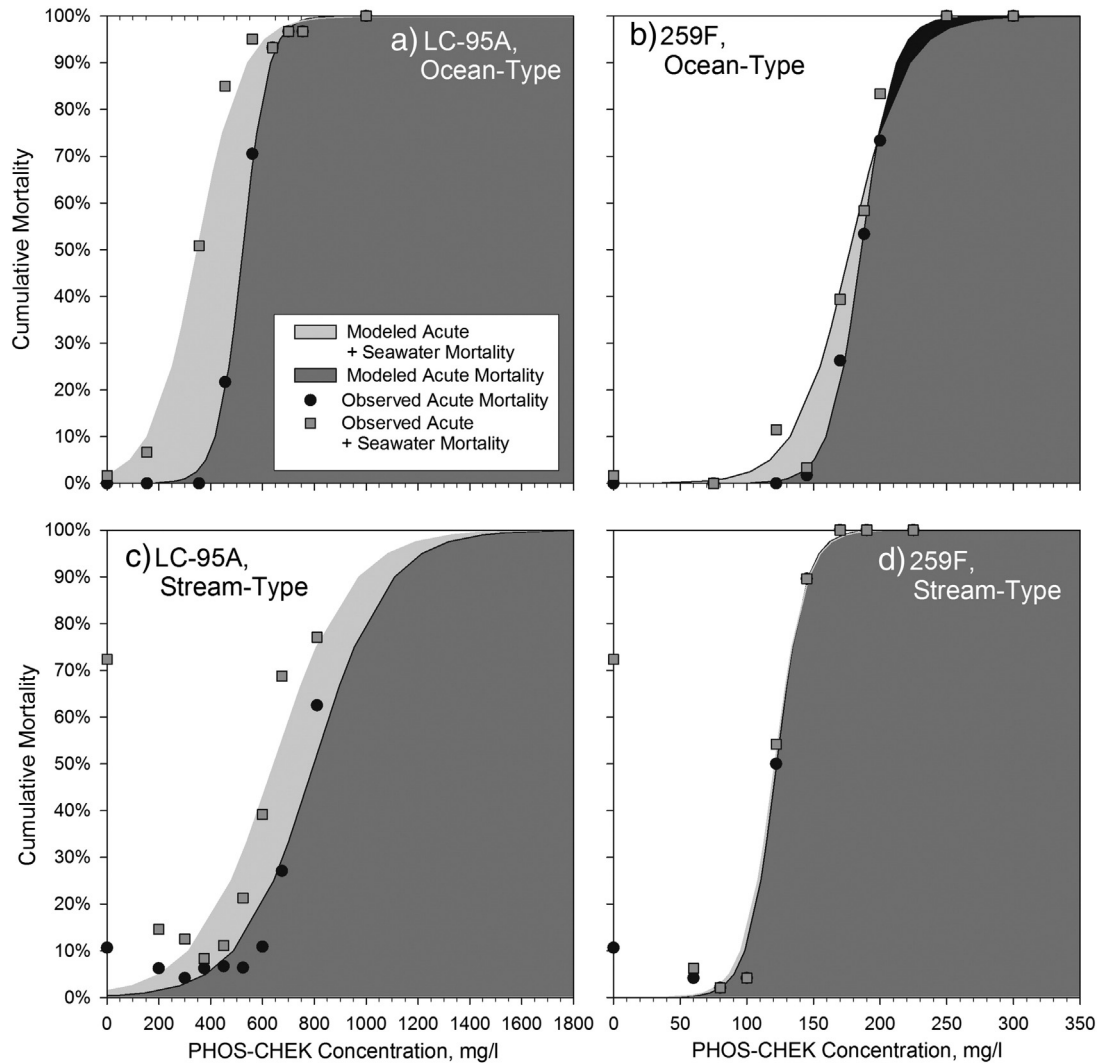


Fig. 6. Cumulative lethal concentration curves for ocean-type (a, b) and stream-type (c, d) Chinook salmon based on logistic regression models of observed fish survival during PHOS-CHEK fire retardant exposure and seawater challenge. The dark-shaded curves indicate mortality due to acute PHOS-CHEK exposure. The light-shaded curves indicate cumulative effect (acute plus seawater mortality) of PHOS-CHEK exposure.

259F. No disruption to physiological indicators of smoltification were found, but gill damage may have contributed to the inability of Chinook salmon smolts to acclimate to immediate seawater entry (Dietrich et al., 2013). The delayed effects increased the cumulative mortality associated with the fire retardants and, by extension, the geographic range of Chinook salmon habitat impacted during a misapplication. Consequently, PHOS-CHEK LC-95A is more toxic to Chinook salmon than the acute LC50s would suggest.

Differences in Chinook salmon life history strategies (ocean-type and stream-type) and durations of freshwater rearing offer various opportunities for PHOS-CHEK exposure during the fire season. Individual Chinook salmon were most sensitive to the cumulative effects of PHOS-CHEK exposure at smolt stage, with no delayed effects observed when PHOS-CHEK exposures occurred as psmolts and time to recover before seawater entry was available. Consequently, accidental drops of PHOS-CHEK LC-95A or 259F in salmon habitat at the time of Chinook salmon migration to the ocean would also affect salmon outside the estimated acute mortality area and may result in mortality upon seawater entry.

Conflict of interest statement

The authors have no conflicts of interest. Funding was provided by the USDA Forest Service Wildland Fire Chemical Systems, but the

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2014.05.038>.

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