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Chlorothalonil

Review and Assessment of the Potential Impact of Chlorothalonil on Endangered and Threatened Salmonid Species in the Pacific Northwest Based on Reproductive, Physiological, and Behavioral Considerations and Recent Environmental Monitoring Data

Assessment

DATA REQUIREMENT(S): Supplemental to EPA OPPTS Guideline Series 850

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Since this volume contains an assessment using published or previously submitted studies, a Good Laboratory Practice Compliance Statement as defined by 40 CFR Part 160 is not appropriate.

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1.0 EXECUTIVE SUMMARY

The Environmental Protection Agency conducted an assessment of 26 listed salmonid species in the Pacific Northwest (EPA, 2003), which was subsequently submitted for consultation with the National Marine Fisheries Service (NMFS). The EPA assessment suggested that chlorothalonil use may affect 9 ESUs, may affect but is not likely to adversely affect 11 ESUs, and will have no effect on 6 ESUs. Syngenta is providing additional information in order to place these effects into context: this report provides a critical review and assessment of the currently available reproductive, physiological and behavioral effects data in fish for chlorothalonil, and details the current range of chlorothalonil measured environmental concentrations (MECs). New data available since 2003 are provided and discussed in this assessment as well as a preliminary spatial analysis. Based on the best available data for chlorothalonil, reproductive, physiological, and behavioral effects are not manifested at concentrations markedly different than those required to cause lethality, and therefore do not portend impacts beyond those identified for lethal effects. Moreover, concentrations required to cause mortality in fish are at least 40 times higher than the highest surveyed MEC for North America, and 200 to 300 times higher than the lower practical quantitation limit (LPQL) for regionally specific analyses in Washington State, where no detections above the LPQL were found. Consequently, models forecasting potential impacts on salmonid species should accurately reflect the parity between sub-lethal and lethal endpoint sensitivity and the comparative divide between effect concentrations and actual MECs. Moreover, given that chlorothalonil dissipates rapidly from the water column, and generates metabolites considerably less toxic than the parent compound, chlorothalonil is unlikely to present biologically consequential exposures in aquatic ecosystems. This is supported by a robust survey database, which consistently characterize residue levels of chlorothalonil in surface waters in the low ng/L range (0.065 – 433 ng/L). Based on the best available effects data in fish, prey (invertebrates and small fish) and supporting trophic species (algae and macrophytes), in conjunction with extensively surveyed MECs from monitoring studies, chlorothalonil is unlikely to pose unreasonable adverse effects to listed salmonid species under labelled uses. Beyond biology and chemical considerations, utilizing refined spatial analyses provides a powerful line of evidence to further refine the risk assessment. Therefore further mitigations are not warranted. Moreover, the analysis presented herein strongly indicates that the EPA determinations for the 9 ESUs identified as may affect should be classified as not likely to adversely affect, and further consideration should be given to potential no effect designations for 11 ESUs identified as not likely to adversely affect.

2.0 INTRODUCTION

Chlorothalonil is a broad-spectrum fungicide used in a variety of crop and non-crop applications and was first registered in 1966 for use on turf and for use on crops in 1970 (EPA, 1999). The most recent re-registration for chlorothalonil was issued in 1999 (EPA, 1999). EPA conducted an endangered species assessment (ESA) (EPA, 2003) and initiated consultation with the National Marine Fisheries Service (NMFS) (December 1, 2003). The exclusive focus of the ESA (EPA, 2003) concerned potential impacts of chlorothalonil to 26 Evolutionary Significant Units (ESUs) of threatened and endangered salmonid species in the Pacific Northwest. "May affect" and "may affect but not likely to adversely affect" (NLAA) determinations were made for 9 and 11 of the 26 ESUs, respectively, and EPA requested consultation with NMFS for the 9 "may affect" ESUs and concurrence from NMFS for the 11 NLAA.

There is currently no formal guidance or criteria for the assessment or incorporation of behaviorally-based sub-lethal endpoints, yet they are becoming more commonly integrated into the risk assessment process for endangered species assessments. For example, one recent NMFS biological opinion for organophosphates (NMFS, 2008) relied heavily on behavioral endpoints, which were used to extrapolate and forecast impacts on salmon populations. For example, feeding behavior was used to define model parameters for morphological endpoints, and ultimately survivability and population-level impacts. Extrapolation uncertainties across such broad levels of biological organization using this model have not been characterized or validated using actual data.

The goal of the present report is to provide a critical review of the reproductive, physiological, and behavioral effects data associated with chlorothalonil. In addition, an extensive review of all available exposure data has also been conducted in conjunction with the effects data to characterize potential risks. Chlorothalonil metabolites demonstrate considerably less toxicity to fish, invertebrates and aquatic plants (EPA, 1999 and 2003), and therefore only the parent compound was assessed.

3.0 MODE OF ACTION IN FISH

The mode of action (MOA) of chlorothalonil in fungi involves inhibition of glucose oxidation following depletion of cellular glutathione (GSH) reserves (Vincent and Sisler, 1968; Tillman *et al.,* 1973). A similar mode of action has subsequently been demonstrated in fish (Davies and White, 1985; Davies, 1985b), particularly exhibited by hepatic activity of glutathione-s-tranferase (GST) towards chlorothalonil (Davies, 1985b). Glutathione has also been suggested to play a protective preventative role in detoxification (Davies, 1985b) since chlorothalonil has a general affinity for thiol groups. Although the exact mode of action of chlorothalonil toxicity in fish is presently not known, limited evidence suggests that it may inactivate key respiratory proteins in the liver and gills at high doses if GSH reserves are depleted beyond critical levels necessary for residue sequestration/inactivation (Gallagher *et al.,* 1992; Davies, 1985a; Davies 1985b). However, the relationship between GSH stores and chlorothalonil exposure does not consistently support this contention (Gallagher et al. 1992; Davies and White 1985). Notwithstanding, the MOA in fish is likely via interaction with,

and potential inactivation of, thiol-containing proteins, though chlorothalonil is rapidly eliminated from fish (*Oncorhynchus mykiss*) at a rate of 1,500 µg chlorothalonil equivalents/100 g tissue within 96 hours after removal from exposure (Davies and White, 1985). A more detailed description of MOA is provided in Appendix 1.

4.0 OVERVIEW OF THE NMFS ORGANISMAL AND POPULATION MODELS

One recent approach for evaluating the potential effects of pesticides on listed salmonid species commonly relied on models which incorporate behavioral components as determinants for assessing and forecasting organismal and population viability (NMFS, 2008). The generic organismal model that was used by NMFS (2008) was based on extrapolation between various non-standard sub-lethal endpoints and somatic growth rate. Dose-response curves defining the response of sub-lethal endpoints to the pesticide of interest were related/extrapolated to feeding behavior (consumption) providing a purported linear relationship; this relationship was then used to define the impact on somatic growth rate specific to particular life-stages (NMFS, 2008). For the analysis of pesticide impact, exposure duration and concentration were modeled such that the time-to-effect half-life was set arbitrarily at 0.5 days, and time to recovery (recovery of the sub-lethal endpoint) was assumed at 30 days (NMFS, 2008), though no justification was provided. Given that depuration of chlorothalonil metabolites occurs exponentially in blood samples from trout within 96 hours after removal from exposure (Davies and White, 1985) recovery is expected to be considerably faster than 30 days. For impacts on prey items, empirical data from a single mesocosm experiment (Van den Brink *et al.*, 1996) was used to define taxa abundance of salmonid prey. Based on the distributions generated from the organismal model, size-dependent first-year survival estimates were calculated as inputs into the population level model comprised of a life-history matrix, assuming a conversion from size (ultimately defined by sub-lethal endpoints) to length distributions and population growth and abundance by applying species-specific "condition factors" (NMFS, 2008). Length is simply compared to competing sub-yearling salmon and is related to size-dependent-survival using two equations; Equation 1: Δlength = fish length (mm) – mean length(mm), and Equation 2: Survival $\varphi = (e^{(\alpha+(0.0329* \Delta\text{length}))})/(1+ e^{(\alpha+(0.0329* \Delta\text{length}))})$, where α is a species-specific parameter defined such that it produces the correct control survival φ when Δlength equals zero (NMFS, 2008). Utilizing the randomly selected output length values from the normal distributions in the organismal model according to the above equation, survival probabilities are generated and subsequently utilized as vital rates (NMFS, 2008). These rates are then incorporated into a transition matrix to explore the intrinsic population growth rate (λ), forming the basis of the biological opinion (NMFS, 2008). However, as noted in the biological opinion, no stochastic impacts were considered and all other influences were considered constant (NMFS, 2008).

Among the criticisms identified by the Agency in a transmittal letter (EPA, 2008), uncertainty was documented concerning the aforementioned models, which rely on methods, data, assumptions and calculations that are not transparent. There was also the acknowledgment that neither the Agency nor the public could reproduce the findings of the biological opinion based on the model and information provided, and no explanation was

provided to characterize the realism or rationale of the model assumptions (EPA, 2008). Particularly relevant was the lack of underlying mathematical description linking the aforementioned sub-lethal effects to food acquisition behavior (EPA, 2008), which ultimately dictated the model output and biological opinion conclusions. Furthermore, questions were raised concerning model exposure assumptions, periodicity, levels and durations of exposure, and generalized assumptions across life-stages (EPA, 2008). Collectively, considering the model and the Agencies concerns, it is critical to evaluate the actual relationship between sub-lethal endpoints, particularly behavioral, and species viability, as well as the actual profile of environmental exposure.

5.0 REPRODUCTIVE PHYSIOLOGICAL AND BEHAVIORAL EFFECTS IN FISH

Biological opinions (NMFS, 2008 and 2009) derived from the aforementioned models have been heavily influenced by sub-lethal (particularly behavioral) metrics employed as inputs without validated relevance and realism, as a matter of course, the published literature concerning these effects in fish species exposed to chlorothalonil needs to be evaluated. Detailed summaries of reproductive, physiological and behavioral effects studies are provided in Appendix 2. Effects critically assessed to be scientifically valid, based on scientific method, interpretation of the results, and satisfaction of the Hill criteria of causality (Hill, 1965) are outlined in Table 1.

According to the Ecological Effects Test Guidelines (OPPTS 850.1400) the early-life stage (ELS) test for fish is intended to define the lethal and sub-lethal effects of chemicals. For chlorothalonil, the ELS study with the fathead minnow indicated significantly reduced hatchability and survival in first generation (F_0) eggs at 6.5 µg/L, but no significant effects in either generation (F_0 or F_1) at ≤3 µg/L, yielding a no-observable effect concentration (NOEL) of 3.0 µg/L (Shults *et al.*, 1980a).

Davies (1987) found significant differences under chronic exposure to chlorothalonil for a variety of biochemical, histopathological, and physiological sub-lethal endpoints (i.e. haematocrit content, ventilatory frequency, gross body movements, and multiple gill function parameters; see Table 1 and Appendix 2) in salmon (*Salmo gairdneri* Rich.). However, these effects were observed at concentrations similar to those causing acute lethality, resulting in acute to chronic ratios (ACRs) of ~5-9, commensurate with the range of ACRs typically observed for chemicals acting via non-polar and polar narcosis (mean ACRs of 2.58 and 9.8 respectively; Roex *et al*., 2000); chemicals that have more specific modes of action typically have much higher ACRs (mean ACR of 17.31; Roex *et al*., 2000). Consequently, based on ACRs, these results do not portend chronic sub-lethal impacts beyond those realized under acutely lethal exposures. Similar results were obtained in channel catfish (*Ictalurus punctatus*) acutely exposed to chlorothalonil where a near parity in sensitivity between lethal and sub-lethal endpoints (i.e. hematocrit ratio, plasma chloride concentration, aspartate aminotransferase content; see Table 1 and Appendix 2) was observed under acute exposures (Gallagher *et al.*, 1992).

In previous Biological Opinions and in their own publications, NMFS has highlighted potential effects on olfactory-mediated behaviors following exposure to various pesticides. Chlorothalonil does not influence olfaction in salmonids, even at concentrations tested well beyond those causing lethality or greater than the solubility limit (1 mg/L) as evidenced in juvenile coho salmon (*Oncorhynchus kisutch*) subject to electro-olfactograms (EOG) (Tierney *et al.,* 2006).

Behavioral modification investigations with Japanese Medaka (*Oryzias latipes*) exposed to chlorothalonil revealed no significant effect on survival, fry length, hatching time, or on foraging ability (Teather *et al.,* 2005), another critical behavioral input in the NMFS models (NMFS, 2008). The reduced swimming activity reported by Teather et al. (2005) for Medaka exposed to chlorothalonil appears to be erroneous (mislabelled in the journal article) as discussed in Appendix 2. Although sex-ratio was reported to be significantly biased toward females, under chlorothalonil exposure in Medaka (Teather *et al.,* 2005), the number of males was similar to controls. Furthermore, there has been no evidence that chlorothalonil exhibits endocrine effects in mammals (EPA, 2003), which is also in agreement with the results of the full life-cycle test with fathead minnows (Shults *et al.,* 1980a; EPA, 2003).

There are currently a number of studies detailing immune system effects of chlorothalonil in fish; however the results are contradictory between *in vivo* and *in vitro* test systems. Significant reductions in reactive oxygen species (ROS) production in striped bass (*Morone saxatilus*) macrophages under *in vitro* exposures to chlorothalonil, mediated by a hypothesized mechanism of NADPH oxidase inhibition, have been reported (Baier-Anderson and Anderson, 1998; Baier-Anderson and Anderson, 2000). However, at lower and more environmentally relevant exposures (see Appendix 2) the opposite trend has been observed (Baier-Anderson and Anderson, 1998). Furthermore, under *in vivo* exposures of rainbow trout (*Oncorhynchus mykiss*), Shelley *et al.,* (2009) found an increased ROS burst in extracted kidney leukocytes, though there was no concentration-response trend (see Appendix 2). Furthermore, no significant effects or trends were identified for a host of other key immunotox endpoints, including pathogen challenge (Shelley *et al.,* 2009). Although research has suggested an affinity of chlorthalonil for sulfhydryl groups, commensurate with mode of action proposed by Davies (1985b), lipid peroxidation has not been detected in these systems at any tested concentration, and cell viability was found to be compromised only at concentrations orders of magnitude above those measured in the environment (Baier-Anderson and Anderson, 2000; see Appendix 2 and Table 2).

All studies satisfying evaluation criteria and demonstrating potential effects of chlorothalonil on reproductive, physiological and behavioral endpoints in fish clearly indicate overlap with the range of lethal concentrations determined for corresponding and closely related species (Table 2). Consequently, this phenomenon suggests a more general toxic mechanism and underscores the lack of justification concerning weighting and application of these metrics for this type of risk assessment. Collectively these data demonstrate that the sensitivity of measured behavioral and physiological endpoints, are not impacted at chlorothalonil exposure concentrations that are dissimilar from those which result in acute mortality and which are collectively higher than environmentally relevant concentrations by a considerable

margin. The use of standard endpoints is therefore, commensurate with protection goals and suitable for prediction of the potential impact of this compound on listed salmonid species.

Table 1. Scientifically valid reproductive, physiological and behavioral effects of chlorothalonil in fish.

Species	Endpoint	Type of response	Response	Duration	Conc.	Reference/ Notes
Pimephales promelas	Reproductive Success (hatchability)	Decrease	NOEL[1]	283-d	3 µg/L	Shults *et al.*, 1980a
Salmo Gairdneri Rich.	Haematocrit Content	Decrease	Significantly different than controls	6 to 12-hrs	20 µg/L	Davies, 1987
Salmo Gairdneri Rich.	Ventilatory frequency	Increase	Minimal response threshold	2-hr	30 µg/L	Davies, 1987
Salmo Gairdneri Rich.	Gross body movements	Increase	Minimal response threshold	30-min	30 µg/L	Davies, 1987
Salmo Gairdneri Rich.	Multiple gill function parameters	Variable, Endpoint-dependent	Significantly different than controls	24-d	2 µg/L	Davies, 1987
Ictalurus punctatus	Hematocrit ratio	Decrease	NOEL	144-hr	30 µg/L	Gallagher *et al.*, 1992
Ictalurus punctatus	Plasma chloride concentration	Increase	NOEL	144-hr	30 µg/L	Gallagher *et al.*, 1992
Ictalurus punctatus	Aspartate aminotransferase content	Increase	NOEL	144-hr	30 µg/L	Gallagher *et al.*, 1992
Oncorhynchus kisutch	Electro-olfactogram (EOG)	Amplitude reduction	NOEL	30-min	>1 mg/L	Tierney *et al.*, 2006

[1]NOEL is no-observable effect level

6.0 LETHALITY AND TOXICITY TO AQUATIC SPECIES

6.1 Fish

Toxicity data for fish obtained from the OPP database (EPA, 2010a) and the ECOTOX database (EPA, 2010b), as well as the primary literature are summarized in Table 2. The lowest acute toxicity value for fish, reviewed and accepted as "core" by the USEPA, was the 96-h LC_{50} for rainbow trout (18 µg/L; Douglas *et al.*, 1992a; MRID: 45710219). As indicated in the chlorothalonil ESA (EPA, 2003), the value of 10.5 µg/L, based on a portion of an open literature study by Davies and White (1985), was derived using fish that were intentionally stressed with low oxygen concentrations, and the stress was likely exacerbated by a temperature higher than allowed in the study guidelines for coldwater fish. Water quality was also questionable in that filtered tap water was used without any report of its characteristics. Control fish survived adequately in the water, but there may have been residual stressors in the water, e.g., chlorine not completely removed by filtration. In addition, a number of items required to be reported (EPA, 1996a) were not included. A second portion of this same study was conducted with normal oxygen levels and temperature within guideline limits. The 96-hour LC_{50} in this portion of the test was 17.1 µg/L consistent with the lowest rainbow trout toxicity data (18 µg/L; Douglas *et al.*, 1992a) which has met EPA guidelines (EPA 2007a) and consequently utilized in the present risk assessment.

Table 2. Aquatic Toxicity Data for Effects of Chlorothalonil in Fish					
Organism	**Life Stage**	**Endpoint**	**Effect Conc. (µg/L)**	**Formulation (% a.i.)**	**Reference**
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile; 1.1-2.5g	10-d LC_{50}	>8.2	Technical (>98%)	Davies *et al.*, 1994
Pseudaphritis urvillii	Juvenile; 6-30g	10-d LC_{50}	>8.2	Technical (>98%)	Davies *et al.*, 1994
Common jollytail (*Galaxias maculates*)	Adult; 2.5-11g	10-d LC_{50}	>8.2	Technical (>98%)	Davies *et al.*, 1994
Rainbow trout (*Oncorhynchus mykiss*)	6-11g	96-h LC_{50}	10.5	Technical (99%)	Davies and White, 1985
Rainbow trout (*Oncorhynchus mykiss*)	6-11g	96-h LC_{50}	10.5	Technical (99%)	Davies, 1987
Common jollytail (*Galaxias maculatus*)	7-10g	96-h LC_{50}	16.3	Technical (99%)	Davies and White, 1985
Rainbow trout (*Oncorhynchus mykiss*)	6-11g	96-h LC_{50}	17.1	Technical (99%)	Davies and White, 1985
Rainbow trout (*Oncorhynchus mykiss*)	6-11g	96-h LC_{50}	17.1	Technical (99%)	Davies, 1987
Rainbow trout (*Oncorhynchus mykiss*)	6-11g	96-h LC_{50}	18	Technical (99%)	Davies and White, 1985
Rainbow trout (*Oncorhynchus mykiss*)	-	96-h LC_{50}	18	Technical (NR)[1]	Douglas *et al.*, 1992a
Common jollytail (*Galaxias maculatus*)	7-10g	48-h LC_{50}	18.2	Technical (99%)	Davies and White, 1985
Rainbow trout (*Oncorhynchus mykiss*)	6-11g	48-h LC_{50}	18.8	Technical (99%)	Davies and White, 1985
Spotted galaxias (*G. truttaceus*)	8-20g	96-h LC_{50}	18.9	Technical (99%)	Davies and White, 1985
Rainbow trout (*Oncorhynchus mykiss*)	6-11g	48-h LC_{50}	19	Technical (99%)	Davies and White, 1985
Fathead Minnow (*Pimephales promelas*)	fry	96-h LC_{50}	23	Technical (96%)	Shults *et al.*, 1980a
Spotted galaxias (*G. truttaceus*)	8-20g	48-h LC_{50}	25.8	Technical (99%)	Davies and White, 1985
Golden galaxias (*G. auratus*)	7-11g	96-h LC_{50}	29.2	Technical (99%)	Davies and White, 1985
Rainbow trout (*Oncorhynchus mykiss*)	1.7 ± 0.16 SD	96-h LC_{50}	39	Technical (97.9%)	Peither, 2003
Rainbow trout (*Oncorhynchus mykiss*)	0.64 - 1.54 g	96-h LC_{50}	42.3 (according to the RED)	Technical (96%)	Shults *et al.*, 1980b
Golden galaxias (*G. auratus*)	7-11g	48-h LC_{50}	46.6	Technical (99%)	Davies and White, 1985
Channel catfish (*Ictalurus punctatus*)	1.77 g (1.19 - 2.75)	96-h LC_{50}	48 (according to the RED)	Technical (96%)	Shults *et al.*, 1980c
Bluegill (*Lepomis macrochirus*)	-	96-h LC_{50}	51	Technical (98%)	Hutchinson *et al.*, 1982
Channel catfish (*Ictalurus punctatus*)	Juvenile; 40-80g	96-h LC_{50}	52	Technical (99%)	Gallagher *et al.*, 1992
Rainbow trout (*Oncorhynchus mykiss*)	Fry	28-d LC_{50}	54	Technical (96%)	Ernst *et al.*, 1993
Rainbow trout (*Oncorhynchus mykiss*)	Fry	96-h LC_{50}	57	Technical (96%)	Ernst *et al.*, 1993
Bluegill sunfish (*Lepomis macrochirus*)	1.03 g (0.60 - 1.84)	96-h LC_{50}	59.5 (according to the RED)	Technical (96%)	Shults *et al.*, 1980d
Channel catfish (*Ictalurus punctatus*)	Juvenile; 40-80g	48-h LC_{50}	62	Technical (99%)	Gallagher *et al.*, 1992
Rainbow trout (*Oncorhyncus mykiss)*	Juvenile; 2.7 ± 7g	96-h LC_{50}	72	Technical (98.1%)	Forster, 1998a[2]

Rainbow trout (*Oncorhynchus mykiss*)	Fingerling; 3.5-4.0 g	96-h LC_{50}	76	Technical (98%)	Ernst *et al.*, 1991
Bluegill sunfish (*Lepomis macrochirus*)	0.92 g	96-h LC_{50}	84	Technical (99%)	Szalkowski *et al.*, 1979a
Japanese medaka (*Oryzias latipes*)	NR	48-h LC_{50}	88	Technical (NR)	Hashimoto and Nishiuchi,1981
Rainbow trout (*Oncorhyncus mykiss)*	Juvenile; 2.7 ± 7g	24-h LC_{50}	89	Technical (98.1%)	Forster, 1998a[2]
Channel catfish (*Ictalurus punctatus*)	Juvenile; 40-80g	24-h LC_{50}	90	Technical (99%)	Gallagher *et al.*, 1992
Carp	NR	48-h LC_{50}	110	Technical (NR)	Hashimoto and Nishiuchi, 1981
Oriental weather fish	NR	48-h LC_{50}	150	Technical (NR)	Hashimoto and Nishiuchi, 1981
Goldfish	NR	48-h LC_{50}	170	Technical (NR)	Hashimoto and Nishiuchi, 1981

[1]NR is not reported
[2]Experimental result obtained from a test system containing sediment

As mentioned previously, the most sensitive chronic endpoint reported in the literature was for the hatching success and survival of fathead minnow eggs exposed to chlorothalonil (Shults *et al.*, 1980a). This regulatory ecotoxicology study reported a NOEC of 3 µg/L and a LOEC of 6.5 µg/L based on results of a full life-cycle test.

6.2 Invertebrates

Toxicity data for aquatic invertebrates obtained from the OPP database (EPA, 2010a) and the ECOTOX database (EPA, 2010b), as well as the primary literature are summarized in Table 3. In a recent assessment of chlorothalonil (EPA, 2007b) the EPA cited a study by Davies et al (1994) on 4 species of Tasmanian aquatic invertebrates, however, these species are not native to North America and the applicability of these data for risk assessment in the United States is uncertain; the use of a foreign, relatively unstudied species and the limited reporting of required information (EPA, 1996b) in that study make it less reliable than the guideline *D. magna* study (LeBlanc, 1977). Even if the *A. gouldi* study were found acceptable, the 96-hour LC_{50} would be more closely consistent with standard acute toxicity endpoints (48-h acute toxicity to *D. magna*) than the 7-day LC_{50} reported for the Tasmanian giant crayfish (*A. gouldi*) study. Moreover, tests with estuarine shrimp (Key *et al.*, 2003) have demonstrated considerably less sensitivity to chlorothalonil, thus as a reasonable compromise only the 96-h results from Davies et al. (1994) were included in the risk assessment.

Table 2. Aquatic Toxicity Data for Effects of Chlorothalonil Aquatic Invertebrates					
Organism	**Life Stage**	**Endpoint**	**Effect Conc. (µg/L)**	**Formulation (% a.i.)**	**Reference**
Tasmanian giant crayfish (*Astacopsis gouldi*)	0.13g	7d-LC_{50}	3.6	Technical (>98%)	Davies *et al.*, 1994
Shrimp *(Paratya australiensis)*	0.05-0.15g	7d-LC_{50}	10.9	Technical (>98%)	Davies *et al.*, 1994
Tasmanian giant crayfish *Astacopsis gouldi*	0.13g	4d-LC_{50}	12	Technical (>98%)	Davies *et al.*, 1994
Shrimp *(Paratya australiensis)*	0.05-0.15g	4d-LC_{50}	16	Technical (>98%)	Davies *et al.*, 1994
Rotifer (*Brachionus calyciflorus*)	<24h	24h-EC_{50}	24	Technical (98.1%)	Hamer and Gentle, 1999
Caddisfly (*Leptocerus*)	Larva	48h-EC_{50}	38	Technical (98.1%)	Hamer and Gentle, 1999
Phreatoicid crustacean *(Colubotelson chilton minor)*	NR	4d-LC_{50}	>40	Technical (>98%)	Davies *et al.*, 1994
Phreatoicid crustacean *(Colubotelson chilton minor)*	NR	7d-LC_{50}	>40	Technical (>98%)	Davies *et al.*, 1994
Amphipod *(Neoniphargus sp. A.)*	NR	4d-LC_{50}	>40	Technical (>98%)	Davies *et al.*, 1994
Amphipod *(Neoniphargus sp. A.)*	NR	7d-LC_{50}	>40	Technical (>98%)	Davies *et al.*, 1994
Grass shrimp (*Palaemonetes pugio*)	Larvae	96h-LC_{50}	49.5	Technical (98%)	Key *et al.*, 2003
Daphnia magna	NR	48h-LC_{50}	52[3]	40%	Ernst *et al.* 1991
Freshwater shrimp (*Crangonyx pseudogracilis*)	Juvenile	48h-EC_{50}	64	Technical (98.1%)	Hamer and Gentle, 1999
Water flea (*Daphnia magna*)	<24 hours old	48h-LC_{50}	68 (according to the RED)	Technical (96%)	LeBlanc, 1977
Cladoceran (*Chydorus*)	Adult	48h-EC_{50}	74	Technical (98.1%)	Hamer and Gentle, 1999
Midge (*Chironomus riparius*)	Larva	48h-EC_{50}	110	Technical (98.1%)	Hamer and Gentle, 1999
Ramshorm snail (*Planorbis*)	NR	48h-EC_{50}	120	Technical (98.1%)	Hamer and Gentle, 1999
Water flea (*Daphnia magna*)	<24h	48h-LC_{50}	120	Technical (99.8%)	Shults *et al.*, 1982
Grass shrimp (*Palaemonetes pugio*)	Adult	96h-LC_{50}	152.9	Technical (98%)	Key *et al.*, 2003
Leech (*Erpobdella*)	NR	48h-EC_{50}	160	Technical (98.1%)	Hamer and Gentle, 1999
Water flea (*Daphnia magna*)	24-48h	48h-EC_{50}	170	Technical (98.1%)	Hamer and Gentle, 1999
Flatworm (*Planaria*)	NR	48h-EC_{50}	200	Technical (98.1%)	Hamer and Gentle, 1999
Freshwater shrimp (*Gammarus pulex*)	Juvenile	48h-EC_{50}	240	Technical (98.1%)	Hamer and Gentle, 1999
Amphipod (*Hyalella azteca*)	Adult	48h-EC_{50}	250	Technical (98.1%)	Hamer and Gentle, 1999
Water flea (*Daphnia magna*)	<24h	48h-EC_{50}	250	Technical (98.1%)	Forster, 1998b[1]
Greater pond snail	NR	48h-EC_{50}	260	Technical	Hamer and Gentle,

(*Lymnaea stagnalis*)				(98.1%)	1999
Macrocyclops fuscus	Adult	48h-EC_{50}	260	Technical (98.1%)	Hamer and Gentle, 1999
Water flea (*Daphnia magna*)	<24h	24h-EC_{50}	315	Technical (98.1%)	Forster, 1998b[1]
Midge (*Chironomus riparius*)	<24h	28d-EC_{50}, (emergence)	370	Technical (98.1%)	Forster, 1998c[1]
Seed shrimp (*Ostracoda*)	NR	48h-EC_{50}	390	Technical (98.1%)	Hamer and Gentle, 1999
Grass shrimp (*Palaemonetes pugio*)	Embryos	96h-LC_{50}	396	Technical (98%)	Key *et al.*, 2003
Water louse (*Asellus aquaticus*)	Juvenile	48h-EC_{50}	450	Technical (98.1%)	Hamer and Gentle, 1999
Water flea (*Daphnia magna*)	<24h	1-6h-EC_{50}	>500	Technical (98.1%)	Forster, 1998b[1]
Mayfly (*Cloeon dipterum*)	Nymph	48h-EC_{50}	600	Technical (98.1%)	Hamer and Gentle, 1999
Phantom midge (*Chaoborus crystallinus*)	Larva	48h-EC_{50}	>800	Technical (98.1%)	Hamer and Gentle, 1999
Diving beetle (*Dytiscus*)	Adult	48h-EC_{50}	>1,600	Technical (98.1%)	Hamer and Gentle, 1999
Damselfly (*Ischnura elegans*)	Nymph	48h-EC_{50}	>1,600	Technical (98.1%)	Hamer and Gentle, 1999
Water boatman (*Corixa*)	Juvenile/adult	48h-EC_{50}	>1,600	Technical (98.1%)	Hamer and Gentle, 1999
Water flea (*D. pulex*)	NR	3h	7,800	NR[2]	Hashimoto and Nishiuchi, 1981
Water flea (*Moina macrocopa*)	NR	3h	>10,000	NR[2]	Hashimoto and Nishiuchi, 1981

[1]Experimental result obtained from a test system containing sediment
[2]NR is not reported
[3] From geometric mean of two EC_{50} values reported as 172 µg formulation/L and 97 µg formulation/L

According to the chlorothalonil ESA (EPA, 2003) the chronic NOEC and LOEC for *Daphnia magna* were 39 and 79 µg/L, respectively (MRID: 00115107; Suprenant *et al.*, 1981); this study was considered core and fulfilling the chronic life-cycle guideline requirement (EPA, 2010b). A second *D. magna* study has subsequently been submitted (MRID: 45710222; Douglas *et al.*, 1992b) and cited in more recent assessments (EPA 2007b), but is not included in the OPP Pesticide Toxicity Database (EPA, 2010b) or the chlorothalonil RED (EPA 1999), and its category and details are not known. The reported NOEC and LOEC were 0.6 and 1.8 µg/L, respectively. Some discussion of the second test (MRID: 45710222), but no review of results has been provided (EPA 2007b); it was noted as being a static-renewal test with mean measured concentrations, on which the presented results were based, of only 62% of the nominal concentrations. Furthermore, chlorothalonil test concentrations "declined to < the level of detection at the lower concentrations" (EPA, 2007b) and were thus uncertain. Although most of the submitted data (described in Appendix B in EPA, 2007b) were categorized as to whether they fulfilled guidelines requirements, the category of this study was not listed. Without further information on this test, a thorough evaluation cannot be made, though the 44-fold disparity between the two tests is notably large compared to most situations where there are two similar tests on the same species. Therefore, the chronic NOEC = 39 µg/L and LOEC = 79 µg/L for *Daphnia magna* (MRID: 00115107) are the best available data for the risk assessment.

6.3 Aquatic Plants

Toxicity data for aquatic plants obtained from the OPP database (EPA, 2010a) and the ECOTOX database (EPA, 2010b), as well as the primary literature are summarized in Table 4. Two studies reported in ECOTOX with *S. capricornutum* were not considered for inclusion. One study (Kikuchi et al. 1993) is in Japanese with an English abstract, and was not directly examined for the current assessment; it indicated a 72-h EC_{50} of 170 µg/L, similar to the guideline study with this species (Hughes and Williams, 1992). The other study (Fernandez-Alba et al. 2002) used a miniaturized, non-standard test system, the Algaltoxkit[1], and reported a 72-h EC50 of 6.8 µg/L. In a recent assessment EPA (2007b) selected this latter value, rather than the core guideline study, to represent the toxicity of chlorothalonil to *S. capricornutum*. However, in light of the non-standard test system and the extreme result, the study by Fernandez-Alba et al. (2002) was not considered valid or appropriate and should not be used as the basis for a regulatory decision.

Table 4. Aquatic Toxicological Data for Effects of Chlorothalonil Aquatic Plants

Organism	Life Stage	Endpoint	Effect Conc. (µg/L)	Formulation (% a.i.)	Reference
Green Algae *(Selenastrum capricornutum)*[a]	Logarithmic growth	7-d LC_{50}	8,500	Technical (96%)	Ernst *et al.*, 1993
Green Algae (*Scenedesmus subspicata*)	NA	96-h EC_{50}	450	NA	Douglas et al 1992c
Green Algae *(Selenastrum capricornutum)*	Logarithmic growth; 3000 cells/mL	5-d EC_{50} (growth and reproduction)	190 (according to the RED)	Technical (97.9%)	Hughes and Williams, 1992
Blue-green algae (*Anabaena flos-aquae*)	Exponential growth	96-h EbC_{50}, (biomass)	65	Technical (98.1%)	Smyth *et al.*, 1998a
Diatom (*Skeletonema costatum*)	Exponential growth	120-h EbC_{50}, (biomass)	11	Technical (98.1%)	Smyth *et al.*, 1998b
Diatom (*Navicula pelliculosa*)[b]	Exponential growth)	72-h EbC_{50}, (biomass)	14	Technical (98.1%)	Smyth *et al.*, 1998c
Duckweed (*Lemna gibba*)	Strain G3 - From 14 day old cultures	14-d EC_{50} (growth; based on dry weight)	510	Technical (98.1%)	Smyth *et al.*, 1998d

[a]*Selenastrum capricornutum = Pseudokirchneriella subcapitata*
NA is Not Available

[1] "A miniaturised algal assay, the Algaltoxkit, has been developed (Algaltoxkit, 1996), which basically adheres to the ISO 8692 method (ISO, 2004) and the OECD 201 Test Guideline for 72h algal growth inhibition tests (OECD, 2006). This microbiotest uses 'algal beads' which can be stored for long periods of time, as the initial source of the algal cells. This micro-scale algal assay further uses disposable spectrophotometric cells of 10 cm path length as test containers which allow rapid direct scoring of the algal densities in a colorimeter or spectrophotometer without any additional manipulations." (International Intercalibration Exercise On The Algaltoxkit Microbiotest – Comparison With Conventional Algal Assays; G. *Persoone and C. Janssen, Laboratory for Environmental Toxicology and Aquatic Ecology Ghent University, Ghent, Belgium*)

7.0 MEASURED CONCENTRATIONS IN THE ENVIRONMENT

In order to place the lethal, reproductive, physiological and behavioral effects of chlorothalonil into the appropriate environmental context it is critical to assess exposure data. In-depth summaries of cited studies, including descriptions analytical sensitivity, are provided in Appendix 3, and the results are outlined in Table 5. Chlorothalonil has been the subject of multiple analytical surveys in a variety of matrices targeted to coincide spatially with areas of intense agricultural activity, areas associated with salmon habitat, and temporally during planting/application. Residues have been found in surface waters receiving inputs from a variety of land-use types with detection frequency dependent on the sensitivity of the employed analytical methods. However, the actual measured environmental concentrations are typically quite low (<0.5 µg/L) with infrequent exceptions, even in circumstances where targeted analyses focus on periods and areas of intensive use. Moreover, given its affinity for sediments, with an estimated Soil Partition Coefficient (K_{oc}) of 3840 L/kg (MRIDs 00115105 and 00029406 submitted in support of the IR-4 new use registration of chlorothalonil on fruiting vegetables, cucurbit vegetables, okra, persimmon, horseradish, rhubarb, ginseng, yam, lupin, lentils and brassica head and stem vegetables; EFED, 4/2/2008, DP Barcode 346321), chlorothalonil will quickly partition to suspended organic matter and sediments, where measured concentrations are still generally low (<5 ppb).

In a multi-year monitoring study of select salmon-bearing streams sampled during typical pesticide-use periods between 2006 and 2008 chlorothalonil was not detected above the lower practical quantitation limit (LPQL) of 33 ng/L in any sample analyzed in any of the three years of monitoring at any site for either urban or agricultural land use categories in Washington state (Sargeant *et al.,* 2010). Similarly, during the course of a previous three year monitoring effort, in a subset of the same salmon-bearing waters, no chlorothalonil residues were detected above LPQLs ranging from 75 and 79 ng/L between 2003 and 2005 (Burke *et al.,* 2006).

In the agriculturally intensive Lower Fraser Valley of British Columbia, Canada chlorothalonil residues were detected at sites in agricultural, urban, and reference land use characterizations at mean concentrations of 0.498 ng/L, 0.407 ng/L, and 0.082 ng/L, respectively, and all residues were below 4.01ng/L; the highest detection occurring in agricultural sites (Woudneh *et al.,* 2009).

As part of its ongoing sampling efforts, the U.S. Geological Survey (USGS) has analyzed chlorothalonil residues in a number of different regions and matrices throughout the United States. In samples collected from several peanut growing areas in Alabama, Florida, Georgia, Oklahoma, and Texas, chlorothalonil was detected in just 4 of 113 samples at a mean concentration of 150 ng/L for the 4 samples which were detected (Scribner *et al.,* 2006). Based on a more sensitive method, chlorothalonil was detected in 5 of 68 samples at a mean concentration of 10 ng/L (Scribner *et al.,* 2006). In a similar survey of streams in Texas and Oklahoma, chlorothalonil residues were not detected in any of the 20 analyzed samples, (Battaglin *et al.,* 2008). Comparatively greater residues for chlorothalonil's degradates (4-hydroxy-chlorothalonil, 1-amide-4-hydroxy-chorothaloni,l and 1,3-diamide-

chlorothalonil) have been detected in both of these regions at higher frequencies (Scribner *et al.*, 2006; Battaglin *et al.*, 2008), however, as indicated by the EPA (1999), the major metabolite "SDS-3701 does not represent a significant risk to aquatic organisms" . In soil samples from Georgia, Florida and Alabama, residues were not found of either the parent compound or the major metabolites in any sample (Hladik and Kuivila, 2008).

Chlorothalonil has been measured at a single maximum concentration of 48.1 µg/L directly at the outflow of a stream flowing through a golf course (King and Balogh, 2008) though no further definitive analytical data are provided (See Appendix 3 for details). Based on visual inspection of the presented data the median chlorothalonil concentration was approximately <1 µg/L with an upper 95th centile of approximately <5 µg/L, suggesting that the maximum reported concentration was likely a unique extreme circumstance or an analytical anomaly (see Appendix 3).

In California chlorothalonil has been detected in sediment and fish tissue samples taken from the Salton Sea at low concentrations of up to 8.9 and 4.4 ng/g, respectively (Sapozhnikova *et al.*, 2004). From a total of 24 samples taken from the Alamo and New Rivers in the Imperial Valley/Salton Sea Basin, chlorothalonil was only detected in 1 spring sample from both rivers at 1.4 and 5.6 ng/L, respectively (Orlando *et al.*, 2008). Sediment samples revealed no concentrations above the method detection limit (see Appendix 3), indicating that the residue levels detected in sediment samples from supplying tributaries (Orlando *et al.*, 2008) are comparatively lower than those identified in the Salton Sea receiving body (Sapozhnikova *et al.*, 2004); no water sample analyses from the receiving body were available for comparison.

Other sampling efforts did not detect chlorothalonil in any suspended sediment sample taken from four predominately agricultural locations in northern California during high-flow events (Smalling and Kuivila, 2008). Residues were detected in bed sediments at a 62.2 µg/kg dry weight in a single sample from the San Joaquin River, though two other samples from the same region failed to detect residues above the MDL. Chlorothalonil has also been found, albeit at trace levels, in winter-spring precipitation (rain and snow) from the Sequoia National Park in the Sierra Nevada Mountains at multiple elevations at concentrations up to 85 ng/L and additionally in surface water samples collected from the Lake Tahoe basin up to 3.2 ng/L (McConnell *et al.*, 1998).

As indicated in the ESA (EPA, 2003), at the current time data were available for 6439 chlorothalonil samples in the USGS National Water-Quality Assessment Program (NAWQA) database, residues were not detected above 1 µg/L in any sample based on revised analyses (see Appendix 3) by the EPA (2003). No residues have been detected above 1 µg/L (maximum of 433 ng/L) in subsequent USGS surveys (Scribner *et al.*, 2006; Battaglin *et al.*, 2008).

Monitoring studies focused specifically in the Pacific Northwest region discussed above (Sargeant *et al.*, 2010; Burke *et al.*, 2006; McConnell *et al.*, 1998; Woudneh *et al.*, 2009) provide the most appropriate data to characterize potential exposure of chlorothalonil to the 26 identified ESUs. Moreover, USGS survey data (Scribner *et al.*, 2006; Battaglin *et al.*, 2008; Orlando et al., 2008) targeted to areas with intensive chlorothalonil use and during the

growing season of specific crops is also considered to accurately describe potential worst-case peak chlorothalonil exposures, albeit geographically distinct from the region of interest. Therefore the relevant high-quality monitoring studies in conjunction with USGS survey data were used in the present evaluation to conservatively and realistically encompass potential exposure.

Table 5. Recent survey results for measured environmental concentrations (MECs) of chlorothalonil in surface waters.

Study Region	Land Use Type	Concentration (ng/L)	Analytical Sensitivity (ng/L)[2]	Frequency (Detects Per # of Samples)	Reference/Notes
Salmon-Bearing Streams, Washington State	Urban and Agricultural	<LPQL of 33	LPQL: 33	0% (0 of 453)	Sargeant *et al*., 2010
Salmon-Bearing Streams, Washington State	Urban and Agricultural	<LPQL of 75 - 79	LPQL: 75 - 79	0% (0 of 1087)	Burke et al., 2006
Fraser Valley, British Columbia, Canada	Agricultural	0.064 – 4.01	DL: 0.055 – 0.064	82% (18 of 22)	Woudneh *et al*., 2009
Fraser Valley, British Columbia, Canada	Urban	0.074 - 2.40	DL: 0.001 - 0.064	78% (7 of 9)	Woudneh *et al*., 2009
Fraser Valley, British Columbia, Canada	Reference	0.065 - 0.122	DL: 0.001 - 0.064	33% (3 of 9)	Woudneh *et al*., 2009
Southern U.S.-Alabama, Florida, Georgia, Oklahoma, and Texas	Agricultural	89 - 433	LRL: 50	3.5% (4 of 113)	Scribner *et al*., 2006 Analyzed via LC/MS
Southern U.S.-Alabama, Florida, Georgia, Oklahoma, and Texas	Agricultural	4 - 35	LRL: 10	7% (5 of 68)	Scribner *et al*., 2006 Analyzed via GC/MS
Oklahoma and Texas	Agricultural	ND[1]	LRL: 50*	0% (0 of 20)	Battaglin *et al*., 2008
Imperial Valley/Salton Sea Basin, CA	Agricultural	<MDL of 12.1	MDL: 12.1	0% (0 of 24)	Orlando *et al*., 2008
Lake Tahoe, California	Reference	0.47 - 3.2	DL: 0.14 - 2.3	100% (NR[3])	McConnell *et al*., 1998

[1]ND is not detected
[2]DL is detection limit, LRL is lower reporting limit, MDL is method detection limit, LPQL is the lower practical quantitation limit
[3]NR is not reported
*Value assumed based on cited method, see Appendix 3 for details

8.0 ENVIRONMENTAL FATE

Investigations concerning the environmental fate and transport of chlorothalonil subsequent to the issuance of the RED (EPA, 1999), and comparisons between the ESA (EPA, 2003) and other reviews (e.g. Hamer, 2003) have revealed a number of inconsistencies, which are discussed in detail in Appendix 4. As indicated in the ESA (EPA, 2003) aquatic photolysis was not considered to be an important dissipation pathway relative to microbial metabolism pathways. However, Kwon and Armbrust (2006) demonstrated that under simulated sunlight 87-88% of chlorothalonil residues dissipated from both creek and pond sediment systems within 1 hr whereas 60-68% remained in the water under dark conditions highlighting the importance of photodegradation to the dissipation of chlorothalonil in the environment. Half-life was identified in the ESA (EPA, 2003) as a point of "debate"; the agency used a 44-hr half-life (EPA, 2003), whereas other authors have reported considerably shorter half-lives (Gentle, 1999; Gentle and Tattersfield, 1999). The dissipation of chlorothalonil under more realistic aquatic systems has been assessed in both indoor and outdoor microcosms, applied as a 720 g/L soluble concentrate formulation at nominally 25 µg a.i./L (Gentle, 1999; Gentle

and Tattersfield, 1999). In an indoor microcosm containing water, sediment and aquatic plants, at approximately 18°C, chlorothalonil disappeared from the water with a half-life of 4 hours (Gentle, 1999). In two singular outdoor microcosms (evaluated individually not as replicates) containing water, sediment, aquatic plants and invertebrates at 10°C subject to two applications of chlorothalonil, residues were found to dissipate from the water column with DT_{50}'s ranging from 7.1 to 8.4 hours after the first application and 7.4 to 8.4 hours after a second application 7 days later (Gentle and Tattersfield, 2000). Kwon and Armbrust (2006) found that in water sediment systems, the half-life under light conditions was <1 day, and 2.1 to 3.0 days under dark conditions. Therefore, recent investigations indicate that the aquatic half-life of chlorothalonil is more appropriately assessed at <24 hrs, and is substantially decreased under light. Considering the environmental fate characteristics of chlorothalonil, particularly the short half-life in aquatic systems, biologically consequential chronic exposures are unlikely.

9.0 MODELED EXPECTED ENVIRONMENTAL CONCENTRATIONS

Expected Environmental Concentrations (EECs) for chlorothalonil uses other than cranberries were estimated using PRZM/EXAMS version 5 (PE5), EPA's Tier 2 model for pesticide aquatic exposure assessment (EPA 2007b). PE5 is a graphical interface for PRZM (Pesticide Root Zone Model) Version 3.12.2 and EXAMS (Exposure Analysis Modeling System) version 2.98.04.06. The model was executed using the PE5 input parameters defined in Table 6 and specific crop scenarios outlined in Table 7. Crop scenarios were initially based on those outlined in the ESA (EPA, 2003) and updated using the most recent and appropriate scenario versions and label information (e.g. rates, application intervals). For each of the crop scenarios, EECs were derived for the water column of a standard pond (1 hectare, 2 m deep, receiving spray drift and runoff from a 10-hectare field). The 1-year-in-10 annual maximum instantaneous (peak) EEC and appropriate time-weighted average EECs were used in the risk characterization. Chlorothalonil may be applied by ground (e.g., boom sprayers, hand wand and backpack sprayers, etc.), air, and chemigation. Aerial application, ground application, or both application methods were modeled as appropriate for each exposure scenario.

Cranberry EECs were based on the first tier GENEEC2 model, which was used to simulate discharge to a bog flooded at harvest after 3 applications at a rate of 5.0 lb ai/A, with a 10-day interval, and 50 day pre-harvest interval (PHI). Model inputs included the following: ground application, fine to medium droplet application, not wetted-in, 20 yard (60 ft) spray buffer; K_{oc}, soil $t_{½}$, water $t_{½}$, hydrolysis, and photolysis values were identical to those outlined for PRZM/EXAMS (Table 6). The GENEEC-based bog discharge EECs should be regarded with caution since these concentrations would decrease by dilution when added to water in the receiving water as indicated in the ESA (EPA, 2003).

Table 6. Chemical-specific input parameters used to estimate aquatic exposure of chlorothalonil with PE5.

Parameter	Input Value	Reference
Molecular Weight	265.91 g/mol	EPA, 1999
Vapor Pressure	5.72 x 10-7 torr @ 25°C	MRID 00153732
Henry's Law Constant	2.47 x 10-7 atm-m3/mol	EPA, 1999
Aqueous Solubility	8.1 mg/L	(10x) EPA, 1999

Soil Partition Coefficient (K_{oc})	3840 L/Kg	MRIDs 00115105 and 00029406
Aqueous Photolysis Half-life	0.4 days	MRIDs 45710223 and 40183418
Aerobic Aquatic Metabolism Half-life	2.5 days	MRIDs 45908001 and 47207701
Anaerobic Aquatic Metabolism Half-life	15 days	MRID 00147975
Aerobic Soil metabolism Half-life	23 days	MRIDs 00087351, 47207703, and 47207704
Hydrolysis Half-life at pH 5	Stable	MRID 0040539
Hydrolysis Half-life at pH 7	Stable	MRID 0040539
Hydrolysis Half-life at pH 9	Stable	MRID 0040539

The exposure modeling results using PRZM/EXAMS are summarized in Table 7. Special considerations apply for modelling aquatic exposure from golf course uses. For most agricultural and non-agricultural crops, it is assumed that the entire field is treated. This is not usually the case for golf courses which have different use areas that are classified as greens, tees, and fairways, and other (e.g., the rough). Depending on the area being treated, management practices and intensity can vary. For example, tees and greens are typically the most intensely managed areas. These areas tend to receive higher inputs than fairways and rough, yet they represent, on average, only 5% of the total acreage of a golf course. Golf Course Adjustment Factors (GCAFs; EPA, 2006) are used to account for the percent acreage of a golf course that is treated. EECs for chlorothalonil use on greens, fairways, and tees were adjusted using GCAF values of 0.026, 0.29, and 0.024, respectively (EPA, 2006). Unadjusted and adjusted golf course EECs are presented in Table 8.

Table 7. Estimated Exposure Concentrations (EECs) for chlorothalonil use scenarios modeled using PE5.

Crop Use	App Rate (lb a.i. /A)	Number of Apps	App Interval (days)	PE5 Scenario	First App Date	App Method	1-in-10-Year Annual Exceedence Probability		
							Peak EEC (ppb)	21-Day EEC (ppb)	60-Day EEC (ppb)
Cucurbits	2.25	7	7	CA Melons RLF	May 15	Ground	2.3201	0.78135	0.53758
						Air	7.935	3.3741	2.572
Tomatoes	2.2	6	7	CA Tomato	July 24	Ground	4.118	0.70889	0.48426
						Air	6.7667	2.5841	1.7894
Potatoes (CA)	1.125	10	5	CA Potato RLF	Feb 17	Ground	4.2806	1.525	0.86716
						Air	7.5874	4.1113	3.1121
Potatoes (ID)	1.125	10	5	ID Potato	Jun 21	Ground	3.5632	1.9744	1.4227
						Air	6.6373	3.6235	2.8421
Stone Fruit and Cherries	3.1	5	10	CA Fruit	Mar 1	Ground	5.5733	2.0037	1.2042
						Air	11.897	5.8402	4.3916
Cranberries[1]	5	3	10	GENEEC2	-	Ground	85.5	25.4	9.2
Turf General[2]	11.3 + 7.3	1 (11.3) + 2 (7.3)	14 (11.3) + 7 (7.3)	CA Turf RLF	Nov 1	Ground	7.5993	3.3493	2.0481
Total Golf Course Turf[2]	11.3 + 7.3	Mixed[2]	14 (11.3) + 7 (7.3)	CA Turf RLF	Jan 3	Ground	8.2760	3.6068	2.2200
Sod Farms[3]	11.3 + 1.7	1 (11.3) + 1 (1.7)	14	CA Turf RLF	Jan 3	Ground	6.8054	3.3275	1.6225
Conifers	4.1	4	7	OR Xmas trees	Oct 1	Ground	9.3695	3.4716	1.89
						Air	17.677	9.7732	5.8847

[1]Cranberry EECs modeled using GENEEC2 assuming a flooded bog scenario.
[2]For total adjusted EECs for golf courses see Table 8
[3]All Daconil labels explicitly state not to use for sod farms at application rates greater than 13 pounds of active ingredient, per acre, per year, thus a rate of 11.3 lb a.i./A followed by a second application rate of the difference (1.7 lb a.i./A) was used instead of a single application at the highest allowable rate (11.3 lb a.i./A) to be more conservative.

Table 8. Unadjusted and adjusted EECs for golf course uses of chlorothalonil.										
Golf Course Turf Type	Application Specification			Unadjusted 1-in-10-Year Annual Exceedance Probability			GCAF[1]	Adjusted 1-in-10-Year Annual Exceedance Probability		
	App Rate(s) lbs a.i./A	Number of Apps	App Interval	Peek EEC (ppb)	21-day EEC (ppb)	60-day EEC (ppb)		Peek EEC (ppb)	21-day EEC (ppb)	60-day EEC (ppb)
Tees	11.3 + 7.3	2 (11.3) + 4 (7.3)	14 (11.3) + 7 (7.3)	21.134	8.1072	4.9958	2.4%	0.5072	0.1946	0.1199
Greens	11.3 + 7.3	2 (11.3) + 7 (7.3)	14 (11.3) + 7 (7.3)	21.134	8.8616	5.9377	2.6%	0.5495	0.2304	0.1544
Fairways	11.3 + 7.3	1 (11.3) + 2 (7.3)	14 (11.3) + 7 (7.3)	7.5993	3.3493	2.0481	29%	2.2038	0.9713	0.5939
Roughs	11.3 + 7.3	1 (11.3) + 2 (7.3)	14 (11.3) + 7 (7.3)	7.5993	3.3493	2.0481	66%	5.0155	2.2105	1.3517
Total Golf Course Turf	11.3 + 7.3	Mixed	7	-	-	-	-	8.2760	3.6068	2.2200

[1] EECs for chlorothalonil use on golf greens, fairways, and tees were adjusted using EPA golf course adjustment factor (GCAF) values of 0.026, 0.29, and 0.024, respectively (EPA, 2006)

If the field dissipation half-life of 8.4 hours (Gentle and Tattersfield, 1999) was utilized in EXAMS as a more realistic surrogate for aerobic aquatic metabolism (2.5 days MRID: 00147975) the predicted EECs would decrease by 20, 76, and 78% for peak, 21-, and 60-day periods, respectively. Consequently, utilizing the same effects inputs, RQs would correspondingly decrease by these respective proportions for acute and chronic exposures, respectively.

10.0 SPATIAL ANALAYSIS OF LAND USE PATTERN WITHIN SALMON EVOLUTIONARY SIGNIFICANT UNITS

Spatial analysis of land use pattern is essential to ensure the use of best available data relative to the assessment of salmon ESUs in relation to chlorothalonil use. A spatial analysis was conducted to evaluate land use patterns within watersheds designated as salmon evolutionary significant units (ESUs) in the Pacific Northwest and California in order to identify potential use areas of chlorothalonil within ESUs. In this assessment, as an example, the results from a comprehensive spatial analysis for the Central California Coast Coho Salmon ESU were presented. This ESU is listed as "endangered" and is one ESU where the EPA assessment concluded a "may affect, but not likely to adversely affect" determination. The spatial approach highlighted as an example in this document should be used for all of the ESUs under consideration. Syngenta will evaluate additional ESUs and submit them at a later date.

For spatial analysis, ESU boundaries were obtained from the National Marine Fisheries Services (NMFS; http://webapps.nwfsc.noaa.gov/ and http://www.nwr.noaa.gov/ESA-Salmon-Listings/Salmon-Populations/Maps/). These boundaries, developed by NMFS, depict watershed boundaries by which distinct populations are tracked and managed (Figure 1 shows an example map of Coho salmon ESUs). The 2009 Cropland Data Layer (USDA, 2010; http://www.nass.usda.gov/research/Cropland/SARS1a.htm) was used to identify specific crop and other land use areas. California pesticide use reporting (PUR) data were downloaded from California Pesticide Information Portal (http://calpip.cdpr.ca.gov/main.cfm) to provide site- specific use records of chlorothalonil.

The latest information for California pesticide use, which is for the year 2008, was used to understand actual use patterns and areas of chlorothalonil use within the Central California Coast Coho Salmon ESU. Spatial analysis was used to map and identify a site-specific use area of chlorothalonil within the ESU. All spatial analyses were conducted using ArcGIS 9.3.1 software with Spatial Analyst extension (ESRI, 2009).

Land use pattern analyses showed very limited agricultural lands (< 0.3%) within the Central California Coast Coho Salmon ESU (Table 9 and Figure 2). The PUR data analysis also showed very limited agricultural uses of chlorothalonil (total of ~ 560 ai lbs) within the Central California Coast Coho Salmon ESU in 2008 (Table 10 and Figure 3). The data also indicated that only a total of 449 acres were treated with chlorothalonil within the Central California Coast Coho Salmon ESU (Table 10). Interestingly, Santa Cruz County showed relatively high use records of chlorothalonil for brussels sprouts, but spatial analysis of PUR data indicated that all uses occurred outside of the Central California Coast Coho Salmon ESU boundary. Also, all agricultural use records in Humboldt County occurred outside of the boundary of the Central California Coast Coho Salmon ESU. No agricultural uses in Mendocino and Marin Counties were reported in 2008. Overall results indicated that only minimal agricultural acreage could potentially be treated with chlorothalonil within the Central California Coast Coho Salmon ESU, consequently, the likelihood for effects from agricultural uses will be very low.

The total amount of non-agricultural uses of chlorothalonil within counties intersecting the Central California Coast Coho Salmon ESU are reported in Table 10, since no site-specific information of use records is available for non-agricultural use data from the PUR database. According to 2008 PUR data, a total of 4981 ai lbs were applied to non-agricultural areas. However, further spatial analysis showed that the urban area intensity is lower in the Central California Coast Coho Salmon ESU than the county areas outside of the watersheds of the Central California Coast Coho Salmon ESU. As an example, although San Mateo County showed the highest non-agricultural use of chlorothalonil, only ~ 21% of the total urban areas in San Mateo County are located within the Central California Coast Coho Salmon ESU boundary (Figure 4). This result indicates that the large amount of non-agricultural use of chlorothalonil is likely not in the watersheds of the Central California Coast Coho Salmon ESU; consequently, low chlorothalonil use on non-agricultural sites within the Central California Coast Coho Salmon ESU is expected.

In summary, this example of a spatial analysis conducted for the salmon ESUs confirm that either a no effect or not likely to adversely affect determination can be made for the use of chlorothalonil on crops or non-agricultural sites within the Central California Coast Coho Salmon ESU. This type of analysis should be conducted for all salmon ESUs prior to proceeding with any Biological Opinion. Syngenta will continue to evaluate additional ESUs and provide this information.

Table 9. Crop and land use patterns within the Central California Coast Coho Salmon ESU				
Land Cover Class Group	**CDL Land Cover Class**	**Acreage**	**% land cover class**	**% land cover class group**
Cultivated Cropland	Corn	31	0.0010	0.23
	Rice	153	0.0048	
	Sunflowers	66	0.0020	
	Barley	16	0.0005	
	Durum Wheat	4	0.0001	
	Winter Wheat	274	0.0085	
	Rye	3	0.0001	
	Oats	88	0.0027	
	Safflower	5	0.0001	
	Alfalfa	429	0.0134	
	Other Hays	1481	0.0462	
	Dry Beans	12	0.0004	
	Other Crops	9	0.0003	
	Misc. Vegs. & Fruits	868	0.0271	
	Herbs	1	0.00003	
	Clover/Wildflowers	3	0.0001	
	Fallow/Idle Cropland	749	0.0234	
	Cherry Orchard	12	0.0004	
	Grapes	508	0.0158	
	Other Tree Fruits	20	0.0006	
	Pecans	1	0.00003	
	Almonds	876	0.0273	
	Walnuts	497	0.0155	
	Aquaculture	22	0.0007	
	Pistachios	4	0.0001	
	Triticale	3	0.0001	
	Prunes	184	0.0057	
	Olives	1004	0.0313	
	Apricots	1	0.00003	
Pasture/Grass	Pasture/Grass	106148	3.3099	3.31
Water	NLCD - Open Water	15873	0.4949	0.49
Urban	NLCD - Developed/Open Space	222461	6.9366	9.72
	NLCD - Developed/Low Intensity	48862	1.5236	
	NLCD - Developed/Medium Intensit	35082	1.0939	
	NLCD - Developed/High Intensity	5434	0.1694	
Barren	NLCD - Barren	3982	0.1242	0.12
Forest	NLCD - Deciduous Forest	32117	1.0014	55.43
	NLCD - Evergreen Forest	1510826	47.1097	
	NLCD - Mixed Forest	234727	7.3191	
Shrubland	NLCD - Shrubland	453040	14.1264	14.13
Grassland Herbaceous	NLCD - Grassland Herbaceous	522526	16.2931	16.29
Wetlands	NLCD - Woody Wetlands	5978	0.1864	0.27
	NLCD - Herbaceous Wetlands	2654	0.0827	
	Wetlands	8	0.0002	

Table 10. Summary of chlorothalonil use within the Central California Coast Coho Salmon ESU reported in 2008 PUR database.			
County	**Use Crop**	**Al Lbs Applied**	**Treated acres**
Humboldt	No crop use reported within the ESU boundary		
Marin	No crop use reported within the ESU boundary		
Mendocino	No crop use reported within the ESU boundary		
San Mateo	Beans (All Or Unspec)	43	31
	Brussels Sprouts	448	350
	N-Outdr Grwn Cut Flwrs Or Greens	44	50
	Swiss Chard (Spinach Beet)	2	2
Santa Cruz	No crop use reported within the ESU boundary		
Sonoma	N-Grnhs Grwn Trnsplnt/Prpgtv Mtrl	7	8.7
	N-Outdr Container/Fld Grwn Plants	20	6.7
Total Ag Use		**563**	**448.4**

Non-agricultural use within the intersecting counties with the Central California Coast Coho Salmon ESU	
County	**Al Lbs Applied**
Humboldt	98
Marin	980
Mendocino	0
San Mateo	2257
Santa Cruz	497
Sonoma	1149
Total Non Ag Use	**4981**

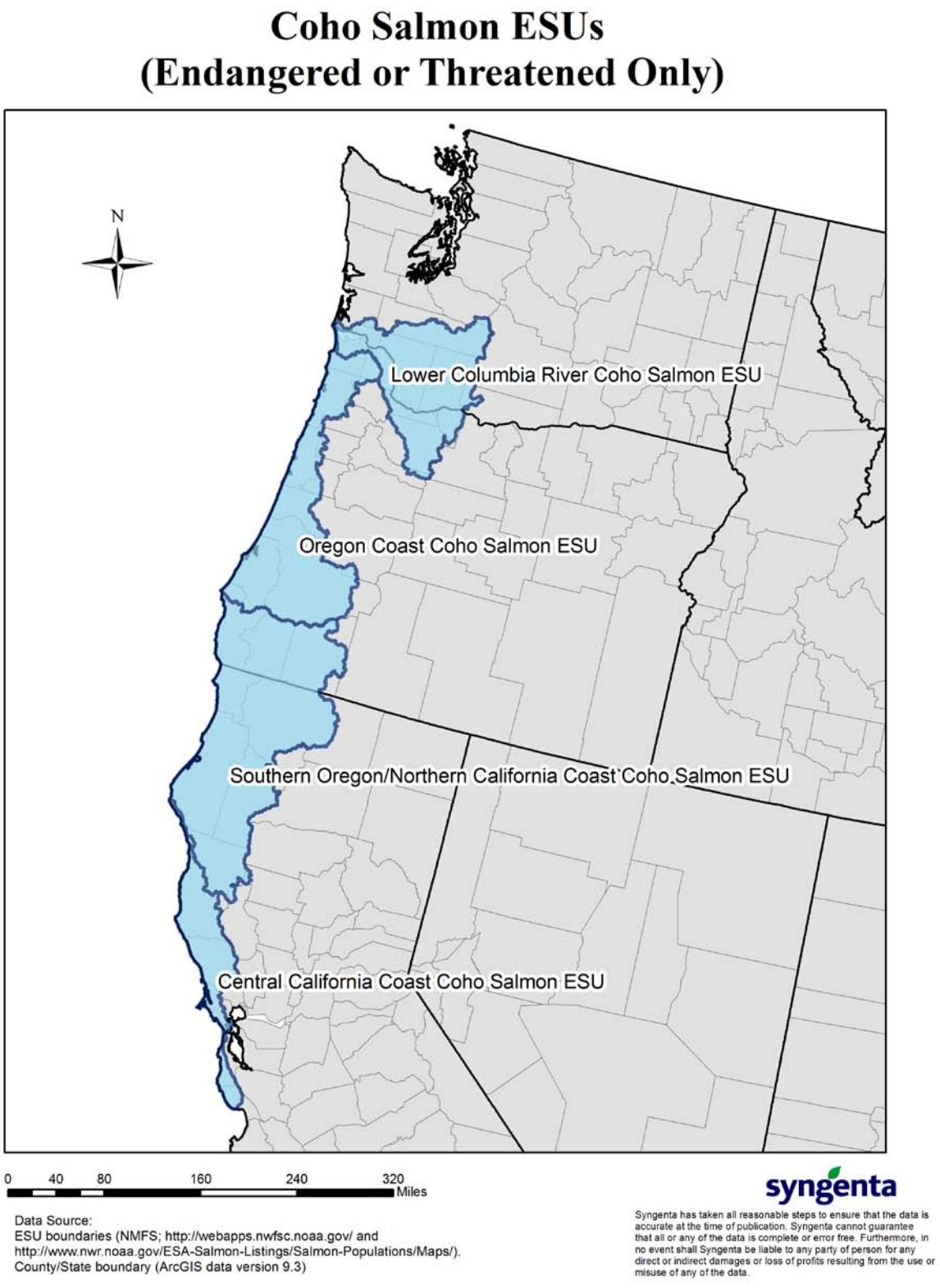

Figure 1. Coho salmon evolutionary significant units (ESUs) in the Pacific Northwest and California

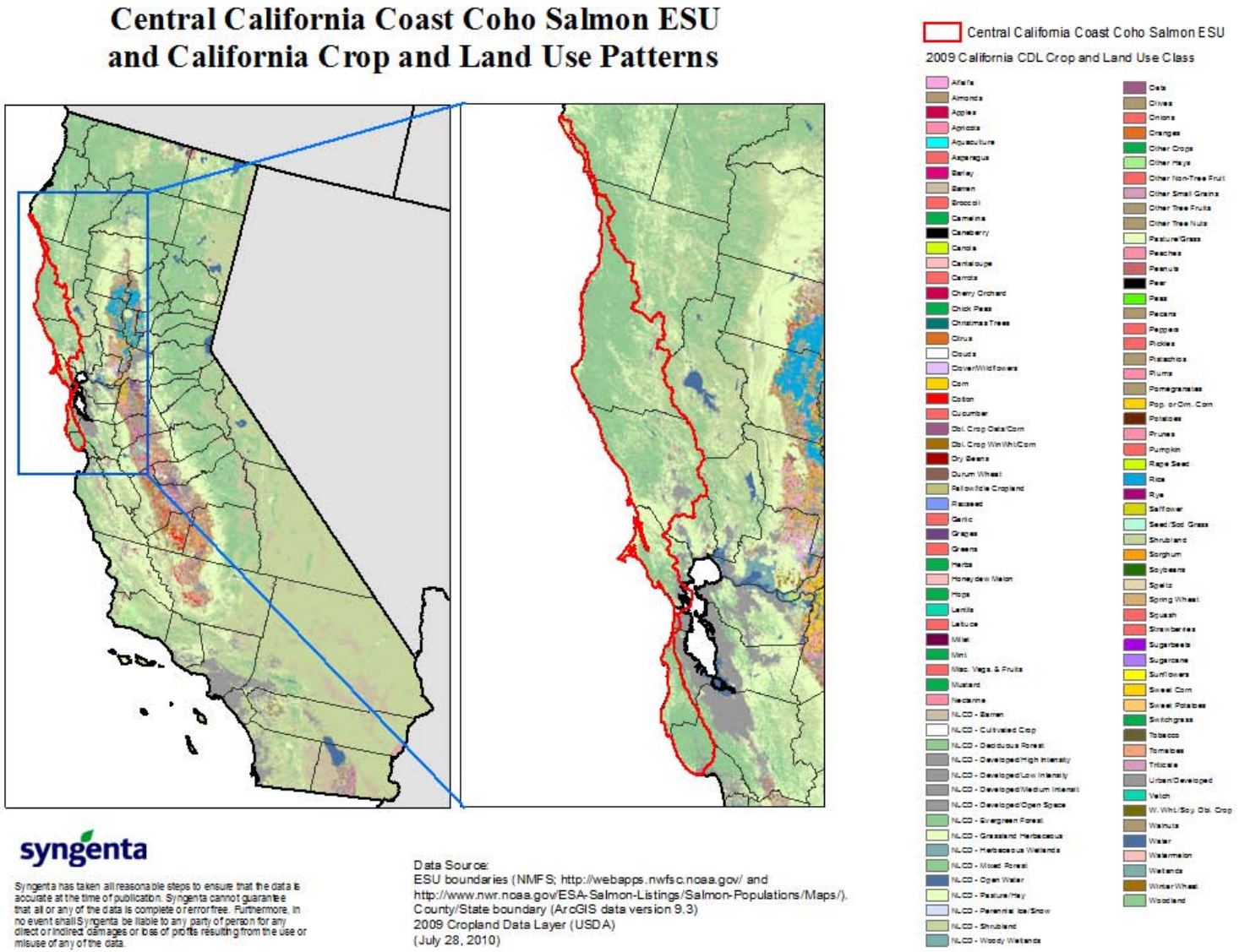

Figure 2. An example map of land use patterns within the Central California Coast Coho Salmon ESU

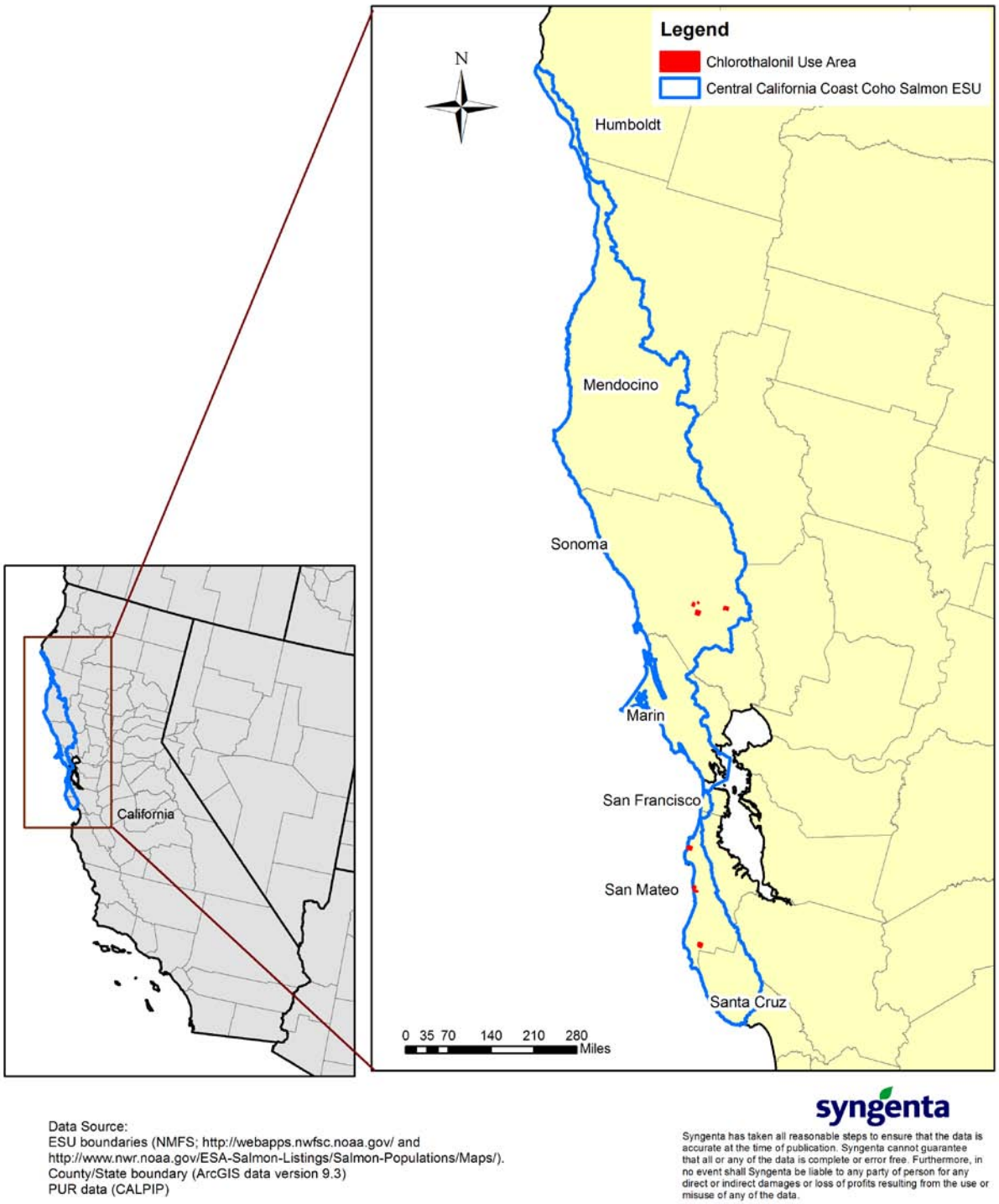

Figure 3. 2008 Agricultural use areas of Chlorothalonil within the Central California Coast Coho Salmon ESU reported in PUR database (CALPIP).

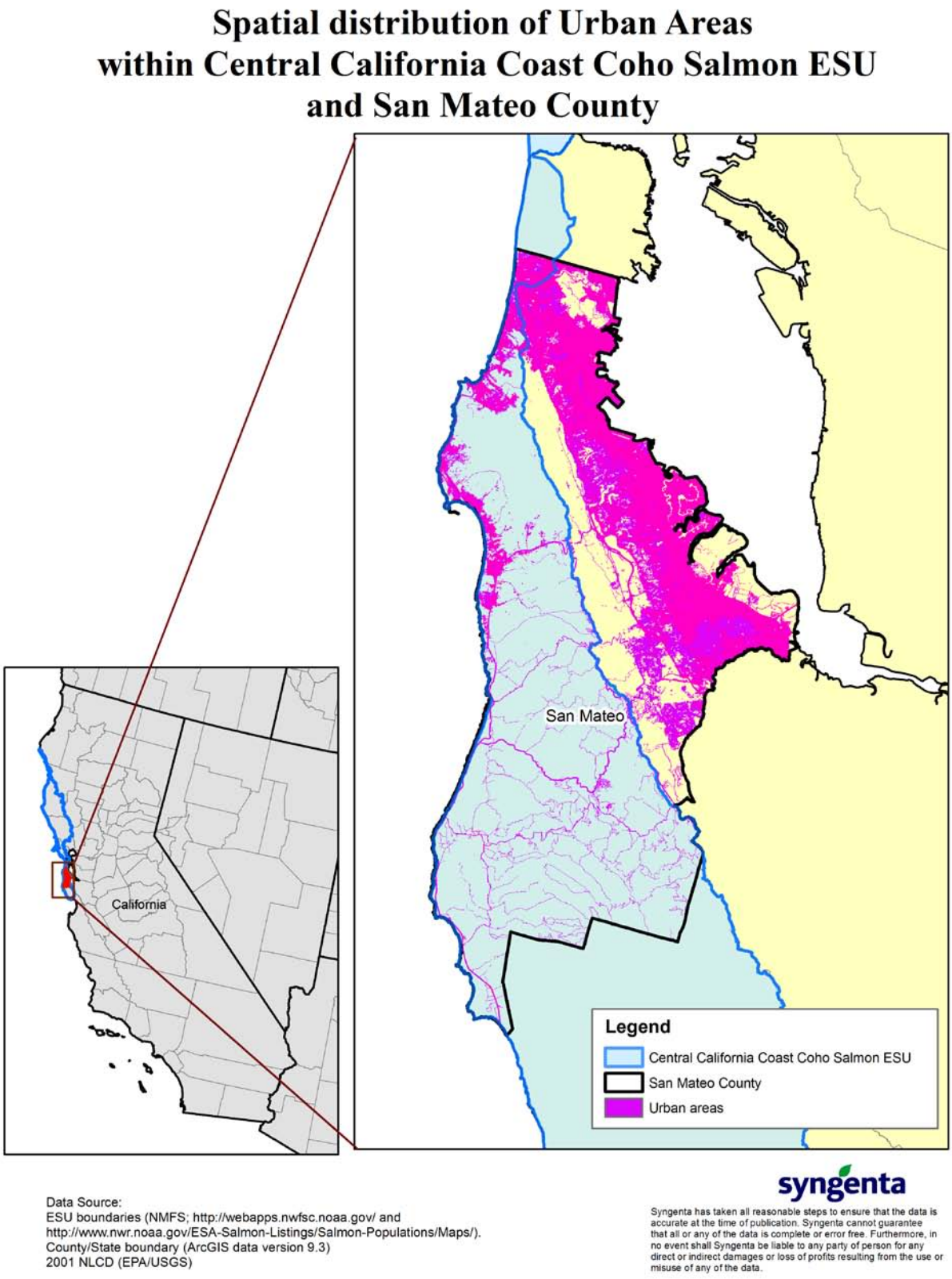

Figure 4. Urban areas in San Mateo County

11.0 RISK ASSESSMENT

Traditional risk assessments concerning endangered species are defined and driven by risk quotients (RQs) derived from guideline studies (e.g. acute toxicity and early life-stage assessments in fish; 850.1075 and 850.1400, respectively) and modeled expected environmental concentrations (EECs; e.g. PRZM-EXAMS). These RQs are compared to conservative levels of concern (LOCs). However, as mentioned previously, attempts have been made to incorporate sub-lethal endpoints (e.g. physiological and behavioral) into endangered species assessments as demonstrated in the recent NMFS Biological Opinions (NMFS, 2008). For purposes of comparison with standard endpoints, these effect measures have been considered in this risk assessment also. Only those sub-lethal endpoints which satisfied the evaluation criteria were incorporated (Table 1). In addition to sub-lethal effects specific to fish, mortality data were also considered (Table 2). Finally, potential indirect effects on prey items and organisms which provide cover, refugia, and support the trophic hierarchy in aquatic ecosystems were considered by incorporating toxicity data for invertebrates, algae, and macrophytes (Tables 3 and 4). In conjunction with these effects data, the MECs tabulated in Table 5 and reviewed above were used to define potential exposures. Modeled EECs were also considered in order to characterize and contrast these values with actual measured data (Table 7). The collated data are summarized and presented in Figure 5. With the exception of a few notable data-points explicitly outlined in the corresponding previous section, all currently available fate and effects were considered.

As shown in Figure 5, the lowest chronic behavioral endpoint (2 µg/L; Table 1) is 5X higher than the highest MEC (0.433 µg/L) and the lowest acute behavioral endpoint (20 µg/L; Table 1) is 50X higher. For fish mortality, the lowest appropriate lethal endpoint (LC_{50} of 18 µg/L for rainbow trout; Douglas *et al.*, 1992a; MRID: 45710219) is ~40X higher. The highest MEC in this case represents a worst-case exposure, which was not measured in the geographic region of interest. Compared to LPQLs for surveys focused on salmon-bearing streams in Washington State (33 to 79 ng/L; Sargeant *et al.*, 2010; Burke *et al.*, 2006), which did not detect chlorothalonil above these levels, concentrations potentially required to cause sub-lethal and lethal effects are 25 to 60 and 200 to 300 times higher, respectively by comparison. Comparative differences in magnitude between exposure concentrations and effects thresholds are similar when considering the lowest LC- and EC_{50}s for invertebrates and aquatic plants (Appendix 3 and 4). The hatched line represents a worst-case threshold of the highest MEC, and as evidenced by the distribution of effects data, this threshold is below all reproductive, physiological, behavioral, and lethal thresholds in fish, and below all toxicity thresholds for invertebrates and aquatic plants. Furthermore, the single highest MEC (0.433 ug/L) is greater than an order of magnitude higher than values from ranges identified in other surveys (see Table 5), particularly those focused in the Pacific Northwest (Sargeant *et al.*, 2010; Burke *et al.*, 2006; Woudneh *et al.*, 2009). This indicates that actual realistic exposure concentrations of chlorthalonil in the environment are likely well below effects thresholds, sub-lethal or lethal. It is also evident from Figure 5 that modeled exposures (peak, 21-day, and 60-day) are considerably higher than actual measured values, highlighting disparity between EECs and MECs. Given that MECs were sampled in salmon-bearing waters, as well as targeted surveys during periods of chlorothalonil use, and that the behavioral effects summarized in this report were realized at concentrations similar to those required to cause lethality, it is considered highly unlikely that chlorothalonil would cause

adverse behavior-induced feeding or reproductive modification in salmonids in the Pacific Northwest.

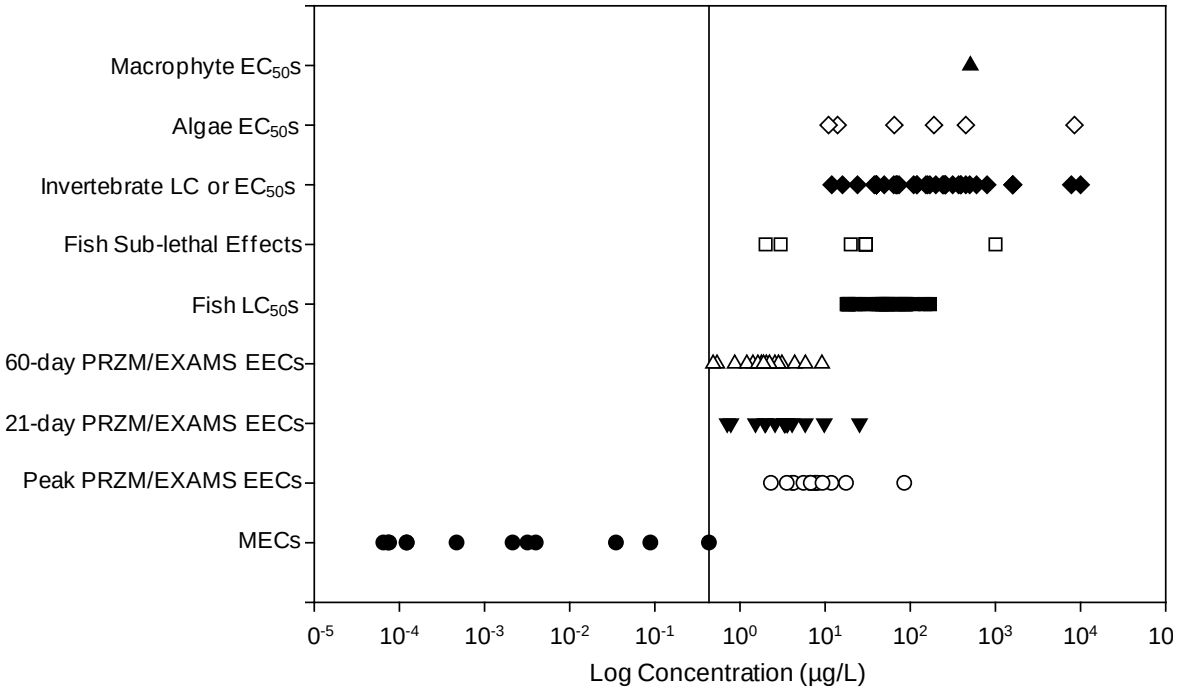

Figure 5. Distribution of measured environmental concentrations (MECs), peak PRZM-EXAMS expected environmental concentrations (EECs), lethal (LC_{50}s) and reproductive, physiological , and behavioral effects in fish, and lethal or toxic effects (LC or EC_{50}s) in invertebrates, algae and macrophytes. MECs and reproductive, physiological, and behavioral effects concentrations were obtained from the literature, peak, 21-day, and 60-day EECs were modeled using PRZM-EXAMS, and LC_{50} and EC_{50} values were obtained from OPP (EPA, 2010a) and ECOTOX (EPA, 2010b) databases as well as the primary literature. The hatched line represents the threshold of the highest MEC (0.433 µg/L).

Given the extensive literature demonstrating rapid dissipation of residues in the environment, and the considerably lower toxicity of major metabolites to fish, invertebrates and aquatic plants, relative to parent chlorothalonil (EPA, 1999 and 2003), the metabolites are not expected to contribute significantly to potential aggregate risks. Infrequently sediment exposures have been measured which exceed some reported behavioral and lethal effects thresholds (Smalling and Kuivilia, 2008). However, given the partitioning behavior of chlorothalonil (Kwon and Armbrust, 2006) and the low potential to bioaccumulate (EPA, 1999), residues in sediment matrices are not considered to be a significant route of exposure. As further evidence, the presence of sediment in test systems substantially reduces the toxicity of chlorothalonil up to 4 times in Rainbow trout and *Daphnia*, and up to 7 times in algae (*Navicula pelliculosa*) (Forster, 1998a; Forster, 1998b; Smyth and Shillabeer 2000). This phenomenon would be expected for a material with the physicochemical properties of chlorothalonil, and has also been demonstrated in field studies with fish, invertebrates and plants (Ashwell *et al*, 2002; Ernst *et al.*, 1991).

An outdoor aquatic mesocosm study looked at the effect of chlorothalonil on aquatic invertebrates, algae and aquatic plants (Ashwell *et al*, 2002 as cited in Hamer, 2003). The

test material was a 720 g/L SC formulation, applied at 3, 10, 30 100 and 300 µg ai/L to replicated systems at weekly intervals. The NOEC was at 10 µg/L, short-term effects on the phytoplankton community were apparent at 30 µg/L and above, however all concentrations showed recovery. At 100 and 300 µg/L there were effects on the zooplankton populations and although recovery was apparent, significant differences in the communities remained at the end of the study. Thus it can be concluded that concentrations up to 30 µg/l will have no significant impact on aquatic invertebrate and algal/plant communities.

Fish were studied in a field study using a small freshwater pond (2000 m2 x 0.5 m mean depth) on Prince Edward Island, Canada (Ernst *et al.*, 1991 as cited in Hamer, 2003). Three direct applications of formulated chlorothalonil at a rate of 875 g ai/ha were made at weekly intervals to the surface of the pond. Measured deposit on collectors after each spray event indicated mean deposition of 67 - 88 % of the application rate and measured concentrations sampled just below the water surface immediately after each treatment ranged from 150 - 2900 µg/l. One year-old rainbow trout were present in the pond prior to and during the three applications. Despite the nominal concentration being 10x the laboratory LC50s there were no mortalities.

Therefore, given the collective exposure and effects data detailed in the present assessment, under current labelled uses, chlorothalonil is not likely to cause unreasonable adverse effects on the viability of listed salmonid species. Moreover, it is imperative to consider spatial analysis of land use pattern in order provide the best available data relative to the assessment of salmon ESUs in relation to chlorothalonil use. Utilizing refined spatial analysis, as demonstrated for the example of chlorothalonil use on crops or non-agricultural sites within the Central California Coast Coho Salmon ESU, provides evidence beyond biological and chemical considerations to further refine the risk assessment.

12.0 CONCLUSIONS

Based on the best available exposure and effects data chlorothalonil is not considered to pose unreasonable risks to listed salmonid species which would jeopardize viability. Based on critical review of all available data, reproductive, physiological, and behavioral effects do not manifest at concentrations markedly different than those required to cause mortality, and therefore do not portend impacts beyond those identified for lethal effects. Moreover, exposures measured in the environment are considerably lower than modelled values. Consequently, models forecasting potential impacts on salmonid species should accurately reflect this reality. Moreover, given that chlorothalonil dissipates rapidly from the water column, partitions readily to sediment, and generates metabolites considerably less toxic to aquatic species than the parent compound, bioavailable chlorothalonil and metabolites will be below adverse effect concentrations. This position is supported by a wealth of monitoring data, which characterize residue levels of chlorothalonil in surface waters as consistently in the low ng/L range. Therefore, further mitigation measures beyond those already in place are not warranted based on the best available data. Moreover, the analysis presented herein strongly indicates that the EPA determinations for the 9 ESUs identified as may affect should be classified as not likely to adversely affect, and further consideration should be given to potential no effect designations for 11 ESUs identified as not likely to adversely affect.

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APPENDICES SECTION

APPENDIX 1 Mode of action of Chlorothalonil

The mode of action of chlorothalonil in fungi is thought to be via inhibition of glucose oxidation following depletion of cellular glutathione (GSH) reserves (Vincent and Sisler, 1968; Tillman *et al.*, 1973). Similar chemical behavior was first demonstrated in fish (*Salmo gairdneri*) by Davies and White (1985), who characterized the rapid build-up of chlorothalonil in bile, where subsequently excreted residues showed properties of polar water soluble compounds. These compounds were later identified as mono- and di-glutathione conjugates of chlorothalonil (Davies, 1985a). The hepatic activity of glutathione-s-tranferase (GST) towards chlorothalonil was subsequently demonstrated in *S. gairdneri* and *Salmo trutta*, and additionally in several Galaxiid species (Davies, 1985b). Evident phylogenetic differences in GST activity were reported, and speculated to be an underlying determinant in differing species sensitivity to chlorothalonil based on correlations with respective species-specific lethality values (Davies, 1985b). Further experimentation confirmed that a GST catalyzed-GSH dependent reaction was the primary mode of chlorothalonil metabolism in fish, and induction of GST activity appeared to be concentration-dependent (Davies, 1985b). Davies (1985b) also showed that GSH plays a protective preventative role since depletion of GSH could result in chlorothalonil covalently binding to the enzyme (GST) and irreversibly resulting in inactivation. The general affinity of chlorothalonil for thiol groups was also verified via assay with glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a thiol rich enzyme (Davies, 1985b).

Although the exact mode of action of chlorthalonil toxicity in fish is presently not known, limited evidence suggests that it may inactivate key respiratory proteins in the liver and gills if GSH reserves are depleted beyond critical levels necessary for residue sequestration/inactivation (Gallagher *et al.*, 1992; Davies, 1985a; Davies 1985b). However, experimental evidence is not conclusive as exposure of *S. gairdneri* to 10 µg/L chlorothalonil increased GSH levels, and results from exposure to 30 µg/L were not significantly different than controls (Davies, 1985b). In channel catfish (*Ictalurus punctatus*) kidney GSH levels were found to increase 180% compared to that of controls at 6 hrs after exposure to 13µg/L chlorothalonil, though this effect was not significant by 24 hrs (Gallagher *et al.*, 1992). Moreover, gill GSH concentrations were significantly higher than controls at 24 and 72 hrs, and hepatic GSH concentrations were significantly higher at 72 hrs by an approximate 150% (Gallagher *et al.*, 1992).

In terms of kinetics, although chlorothalonil uptake is rapid, accumulating by a factor of ~840 in muscle tissue of exposed rainbow trout (*Oncorhynchus mykiss*), blood levels were found to drop exponentially at a rate of 1,500 µg chlorothalonil equivalents/100 g tissue within 96 hours upon removal of fish to fresh water indicating a rapid depuration phase (Davies and White, 1985). Greater than 99% of the excreted metabolites were identified in the water-phase indicating polar conjugates; <0.5% was parent chlorothalonil (Davies and White, 1985). This is in agreement with EPA (1999), which indicated that the bioaccumulation potential of chlorothalonil is low given a total residue BCF of about 500X for fish (EPA, 1999). Thus a mechanism of rapid GST catalyzed-GSH dependent metabolism of chlorothalonil in fish is likely, and has been corroborated by the EPA (1999).

APPENDIX 2 Reproductive Physiology and Behavioral Effects in Fish

Reproductive success, egg hatchability, survival, and growth of first- (F_0) and second- (F_1) generation fish under chronic exposure to chlorothalonil was assessed in fathead minnows (*Pimephales promelas*) (Shults *et al.*, 1980). This study serves as the only early life-stage study (ELS) for this compound, fulfilling this requirement (EPA, 2010a). Over the course of the 283-day exposure to analytically verified concentrations of 16, 6.5, 3.0, 1.4, and 0.60 µg/L, no significant effects were observed in either generation at ≤3 µg/L (Shults *et al.*, 1980). Significantly reduced hatchability and survival of fry in first generation (F_0) eggs were observed at 16 µg/L after 35 days of exposure, and the reproductive success (reduced number of eggs per spawn) of F_0 fish was affected at ≥6.5 µg/L (Shults *et al.*, 1980). For second generation fish (F_1) hatchability was significantly reduced at 6.5 µg/L, though survival was unaffected at this concentration (Shults *et al.*, 1980). Based on these results, the ELS no observable effect level (NOEL) was 3.0 µg/L, with a lowest observable effect level (LOEL) of 6.5 µg/L (Shults *et al.*, 1980). Based on these results, and given that the LC_{50} for fathead minnows is within the range of sensitivities demonstrated in laboratory studies with salmonids (Table 2), the NOEL of 3 µg/L is considered representative and protective of these species.

Davies (1987) evaluated physiological, anatomical and behavioral changes associated with respiratory system impairment in salmon (*Salmo gairdneri* Rich.) co-exposed to chlorothalonil and oxygen stress. Concomitant exposure of chlorothalonil with low oxygen (5 mg/L) was found to enhance toxicity and lower the LC_{50} by 40%, indicating potentially greater sensitivity for salmonid species in matrices experiencing low dissolved oxygen content. Although hematocrit content was found to decrease in concert with chlorothalonil concentration, only exposures greater than 20 µg/L deviated significantly from controls (Davies, 1987). Distinct haemolysis was identified in exposed fish, with intensity noted as varying in a dose-response manner, however no quantitative estimates are provided rendering exposure comparisons incalculable. Tissue hypoxia was not related to chlorothalonil exposure, however ventilatory frequency was found to increase as did gross-body-movements, indicating acute stress with a maximal response at 200-310 µg/L, though for a 2 hr exposure the minimum response was quantified at 30 µg/L (Davies, 1987). In terms of chronic exposure, although trends were identified at lower concentrations, significant differences were only found at the 2.0 µg/L exposure level (Davies, 1987). These differences were comprised of reduced diffusive capacity, increased thickening of the blood-water barrier, increased volume of secondary lamellae, an increase in the tissue volume outside the pillar cell system, and decreased erythrocyte to secondary lamellar volume ratio in accordance with the reduction of haematocrit (Davies, 1987). Given the proposed mode of action of chlorothalonil these effects are plausible, however manifestation of significant impacts on salmonid species are not likely given that the sub-lethal impacts are realized at concentrations similar to those required to cause acute lethality (Table 1).

Sub-lethal effects associated with acute chlorothalonil exposure have also been investigated in channel catfish (*Ictalurus punctatus*) via biochemical and histopathological evaluations (Gallagher *et al.*, 1992). Subsequent to a 96 hr acute exposure at 10, 30, 90, 270, and 540 µg/L chlorothalonil, surviving fish were evaluated for hematocrit ratios, plasma chloride concentrations (an indicator of gill damage), and aspartate aminotransferase content, however

no significant differences in these metrics were found between the 10 and 30 µg/L treatments and the controls (Gallagher *et al.,* 1992). These results demonstrate that, given the corresponding calculated 96 hr LC_{50} of 52 µg/L (Gallagher *et al.,* 1992), significant sublethal effects do not occur in surviving fish at concentrations lower than those inducing acute lethality. Furthermore, the results provide a NOEL of 30 µg/L based on evaluation of acute sub-lethal effects. In a separate 144 hr exposure ranging from 8 to 42 µg/L chlorothalonil, the right holobranch gill arch, liver, posterior kidney, brain, intestine, spleen, and heart were subsequently excised and evaluated for histopathological anomalies. Evidence of acute necrosis of the intestinal epithelium was identified in 57% of fish exposed to 42µg/L, though not causing morbidity, globular eosinophilic substance-containing vacuolated tubular epithelial cells were also observed, albeit rarely, in posterior kidneys in 29% of the exposed fish (Gallagher *et al.,* 1992). However, tissue from the lower treatment concentrations (8 and 21 µg/L) were not evaluated due to the absence of elicited behavioral signs in these groups (Gallagher *et al.,* 1992) rendering definitive no effect determinations based on histopathology impossible. A time-course gill pathology study was also conducted at a single exposure concentration of 42 µg/L over 144 hr with marked gill epithelial hyperplasia with lamellar fusion noted, however due to concomitant contamination with myxosporidian parasites, which induces similar inflammation responses, gill lesions could not be directly related to chlorothalonil exposure (Gallagher *et al.,* 1992). Based on the species and metrics evaluated, these results collectively demonstrate that sub-lethal histopathological effects associated with acute exposures to chlorothalonil are not consistently demonstrated even at exposure levels approaching those required to cause lethality.

Proper functioning of olfaction, and consequently olfactory-mediated behaviors, was purported by NMFS (NMFS, 2008) as being critical in terms of predator avoidance, prey detection and subsequent growth, imprinting of juvenile fish to natural waters, homing of adults returning from the ocean, and spawning/reproduction. In this context, data indicating potential impacts to olfaction were weighted heavily and utilized extensively in characterizing behavioral effects and driving model outputs generated by NMFS in assessing organophosphate insecticides (NMFS, 2008). Chlorothalonil however was not found to influence olfaction in salmonids, even at an exposure concentrations greater than solubility limit (1 mg/L) as evidenced in juvenile coho salmon (*Oncorhynchus kisutch*) subject to electro-olfactogram (EOG), a measure of odorant-evoked field potentials (Tierney *et al.,* 2006). Chlorothalonil did not appear to affect EOG within 30 min of exposure, even at concentrations well beyond those causing lethality (Tierney *et al.,* 2006).

Teather *et al.,* (2005) evaluated behavioral modification in fish, Japanese Medaka (*Oryzias latipes*), in addition to sex ratio modification upon exposure to select pesticides, including chlorothalonil, singly and in combination. In fish exposed from fertilization until 7 days post-hatching at a single concentration of 0.06 µg/L, chlorothalonil was found to have no significant effect on survival, fry length, hatching time, or foraging ability, although fry exposed to chlorothalonil singly and in combination with azinphos-methyl and endosulfan were claimed to have demonstrated reduced activity (Teather *et al.,* 2005). In addition, adult sex ratios were found to be significantly biased toward females in groups exposed to chlorothalonil (Teather *et al.,* 2005). The purported claim that activity level was significantly reduced in Medaka fry exposed to chlorothalonil at 0.06 µg/L compared to controls appears

to be erroneous considering the contradiction between the described results and the data presented in Figure 3A in Teather *et al.,* (2005). The publication text claims that Medaka fry exposed to chlorothalonil singly resulted in a swimming distance of 67.5 cm compared to 92 cm for the control group, however upon scrutiny of Figure 3A (Teather *et al.,* 2005) the bar corresponding to chlorothalonil (fourth from the left) indicates nearly 80 cm, whereas the bar corresponding to endosulfan clearly indicates ~67.5 cm. Therefore it is obvious that an erroneous and ultimately incorrect conclusion was derived from the actual data or a gross mislabelling of the figure in question occurred. However, regardless of the nature and source of the error, the conclusions concerning reduced activity in Medaka caused by chlorothalonil are invalid and should not be considered for assessment purposes. Moreover, chlorothalonil, exposed singly and in combination, was found to have no significant effect on foraging behavior compared to controls (Teather *et al.,* 2005). These results and identified errors indicate that chlorothalonil, tested at low and environmentally relevant concentrations (60 ng/L), does not cause behavioral modification in swimming activity or foraging activity. In terms of sex-ratios, Teather *et al.,* (2005) reported that chlorothalonil exposure caused significant changes in sex ratio with an increased proportion of females. Although the results do in fact support an increased number of females in groups exposed to chlorothalonil, it is interesting to note that the number of males is quite similar to the controls, 17 versus 20. Moreover, although there were 32 females in the chlorothalonil exposed group compared to 18 in the control, the total number of individuals in the chlorothalonil treatment was considerably higher than in the controls, 49 versus 38. This indicates that, of the 11 more fish in the chlorothalonil treatment a greater proportion were female, and consequently there is no evidence that chlorothalonil exposure in fact reduces the number of male fish as may be misinterpreted from the results, and which could be erroneously interpreted as convincing evidence of endocrine disrupting characteristics. In comparison with the controls, given that the comparative number of males was not considerably reduced, only that the number of females was significantly increased it is difficult and potentially misleading to extrapolate wider implications from these results, and as a consequence this reported effect was not considered in this assessment as convincingly meaningful. Furthermore, given the experimental design limitation of testing a single exposure concentration demonstration of causality was not possible or convincingly attempted.

According to the Agency (EPA, 1997), the current working definition for endocrine disruptor chemicals (EDCs) is: An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism. Commensurate with this definition it was concluded that, given the current data, there was no evidence that chlorothalonil exhibited endocrine effects in mammals (EPA, 2003). Moreover, based on the available full life cycle test with fathead minnows, which did not indicate sub-lethal effects at concentrations substantially lower than lethal levels, no evidence of EDC activity was found regarding other taxa (EPA, 2003). Subsequent to the publication of the ESA (EPA, 2003) there has been no convincing research suggesting that chlorothalonil affects the neuruendocrine or reproductive systems in fish, however there are currently a number of studies detailing immune system effects, though the results are highly contradictory between *in vivo* and *in vitro* test systems.

In early *in vitro* research with striped bass (*Morone saxatilus*) phagocytes Baier-Anderson and Anderson (1998) asserted that, as had been previously hypothesized in mammalian systems, chlorothalonil caused an immunotoxicological response at high doses. Subsequent to a 20 hr *in vitro* exposure of striped bass macrophages to chlorothalonil, reactive oxygen species (ROS) production was found to decrease significantly at exposures of 0.9 µM (~250 µg/L) or higher in cells previously stimulated with zymosan and phorbol 12-myristate 13-acetate (PMA) (Baier-Anderson and Anderson, 1998). Since ROS stimulation was found to be suppressed, via the hypothesized mechanism of NADPH oxidase inhibition, where NADPH oxidase is integral in mediating the ROS response, fish were considered to be vulnerable to potential pathogen challenge (Baier-Anderson and Anderson, 1998). However, at lower and more environmentally relevant exposures to chlorothalonil (0.04 – 0.4 µM; ~10 – 100 µg/L) the opposite trend was observed and ROS production was stimulated, mechanistically suggesting stimulation of NADPH oxidase. Moreover chlorthalonil was not found to significantly affect phagocyte uptake capacity (Baier-Anderson and Anderson, 1998).

In a similar study Baier-Anderson and Anderson (2000) expanded the scope of their previous work to evaluate the suppression of superoxide production by chlorothalonil in striped bass macrophages. Similar to previous results from the same research group indicating suppression of H_2O_2 and HOCl ROS species in macrophages exposed to chlorthalonil at 0.2 – 2 µM (50 – 500 µg/L) *in vitro*, superoxide was found to decrease in zymosan and PMA stimulated macrophages at 0.4 and 0.2 µM (50 and 100 µg/L), respectively (Baier-Anderson and Anderson, 2000). The superoxide-suppression EC_{50}s were estimated at 0.321 and 0.267 µM (85 and 71 µg/L), respectively (Baier-Anderson and Anderson, 2000). In contrast, superoxide production in unstimulated cells was found to be suppressed at all test concentrations, the lowest being 0.04 µM (10 ug/L) (Baier-Anderson and Anderson, 2000). However, given that cells were fully capable of ROS stimulation subsequent to chlorothalonil exposure indicates that the baseline inhibition results do not indicate inhibition of response potential. Pretreatment of cells with L-buthionine-(S,R)-sulfoximine (BSO), an inhibitor of g-glutamyl-cysteine synthetase and consequently GSH synthesis, was found to enhance chlorothalonil toxicity, whereas dithiothreitol (DTT), a thiol that reduces disulfide bonds within the cell maintaining sulfhydryls in their reduced state, alleviated toxicity (Baier-Anderson and Anderson, 2000). These results suggest an affinity of chlorthalonil for sulfydryl groups and provide support for depletion of GSH as the mode of immunotoxic action. However, given that cell viability was only compromised at the highest concentration (~500 µg/L), that lipid peroxidation was not detected at any concentration, and that macrophages experiencing baseline superoxide inhibition at low chlorothalonil were fully capable of stimulated ROS production, these results do not portend adverse immunotoxic effects at environmentally realistic concentrations.

Contrary to the ROS burst findings identified by Baier-Anderson and Anderson (1998 and 2000), Shelley *et al.*, (2009) found a conflicting trend (a ROS burst increase) in kidney leukocytes extracted from rainbow trout (*Oncorhynchus mykiss*) exposed to chlorothalonil *in vivo*. Fundamental differences between *in vitro* and *in vivo* exposures, differences in dose, exposure or incubation time, test species, and differences in methods were cited as potential

reasons for the discrepancy (Shelley *et al.,* 2009). Baier-Anderson and Anderson (1998 and 2000) utilized lucigenin-enhanced chemiluminescence, whereas Shelley *et al.,* (2009) utilized a flow cytometric technique measuring fluorescent probe activation. However, it is important to note that based on statistical significance, the aforementioned trend recognized by Shelley *et al.,* (2009) did not satisfy the conditions of causality since a definitive concentration-response of the measured effect was not actually demonstrated. Following a 14-d recovery period subsequent to a 28-d exposure of rainbow trout to chlorothalonil, ROS burst was found to be significantly elevated in phorbol myristate acetate (PMA) induced kidney leukocytes (Shelley *et al.,* 2009), though only at the lowest concentration (100 ng/L). In contrast a decreasing trend in ROS burst was evident at 200 and 500 ng/L, with a slight increase again at 1000 ng/L (Shelley *et al.,* 2009). Moreover, although respiratory burst appeared to be elevated during the 28-d exposure with increasing exposure concentration, the relationship was not significant (Shelley *et al.,* 2009). Percent of phagocytic cells was also significantly increased in the 100 ng/L, and additionally in the 200 ng/L chlorothalonil exposure treatments (Shelley *et al.,* 2009), though again the trend decreased at the higher exposure levels (500 and 1000 ng/L) indicating a lack of concentration-dependence. The percent of phagocytic cells in the 28 d exposure tended to demonstrate an increasing concentration-response trend, however as with the ROS burst results this effect was not significant. Furthermore, no significant effects or trends were identified concerning the phagocytic capacity, relative lymphocyte and granulocyte differential counts, and serum lysozyme activity of leukocytes for either the 28-d exposure period or the 14-d recovery period (Shelley *et al.,* 2009). In terms of immunological function, no significant relationship was established between chlorothalonil exposure and pathogen challenge of fish to the bacterium *Listonella anguillarum* in *ex vivo* cellular assays, which suggests that, in this case, qualitative changes in phagocytosis and respiratory burst may not be critical for whole organism defense (Shelley *et al.,* 2009). Given the collective lack of significant concentration-response and the inflecting trends in the ROS burst and phagocytic capacity assays, it is considered unlikely that leukocytes predisposed to chlorotahlonil would experience sustained ROS excess following antigen stimulation resulting in potential peripheral tissue damage as speculated by Shelley *et al.,* (2009). Furthermore, similar studies with striped bass failed to detect lipid peroxidation in macrophages (Baier-Anderson and Anderson 2000) further suggesting that tissue damage due to anthropogenic manipulation of immunological function via chlorothalonil exposure is not likely considering the best available data.

APPENDIX 3 Measured Concentrations in the Environment (Subsequent to the 2003 ESA)

As a cooperative effort, the Washington State Departments of Agriculture and Ecology conducted a multi-year monitoring study to characterize pesticide concentrations in selected salmon-bearing streams during a typical pesticide-use period between 2006 and 2008 (Sargeant *et al.*, 2010). Monitoring was conducted in five basins including an urban area in the Cedar-Sammamish basin and agricultural areas in the Yakima, Skagit-Samish, Wenatchee, and Entiat basins, representing both irrigated agriculture and tree fruit agriculture (Sargeant *et al.*, 2010). As indicated in Appendices B–J of the triennial report (Sargeant *et al.*, 2010), chlorothalonil was not detected above the lower practical quantitation limit (LPQL) of 33 ng/L in any sample analyzed in any of the three years of monitoring at any site for either land use category. As part of the same monitoring effort (Sargeant *et al.*, 2010), two of the basins (Cedar-Sammamish and lower Yakima) have been characterized previously (Burke *et al.*, 2006). During the course of the three year monitoring effort no chlorothalonil residues were detected above LPQLs of 79, 75, and 78 ng/L in 2003, 2004, and 2005, respectively (Burke *et al.*, 2006).

The Lower Fraser Valley of British Columbia has been identified as having some of the most intense agricultural activity in Canada, particularly fruits and vegetables, and surface waters of this region were recently subject to an extensive screening initiative for 78 pesticide residues, including chlorothalonil, in three reference (considered pristine), five agricultural, and two urban sites (Woudneh *et al.*, 2009). Chlorothalonil residues were identified in each of the three land use characterizations; however concentrations at all sites were ≤4.01ng/L, with the highest levels identified in agricultural sites (Table 2; Woudneh *et al.*, 2009). In the five agricultural sites, Frew Creek, Westham Island, Fishtrap Creek at Canadian/US Border, Abbotsford, McQuatt Ditch, Langley, Matsqui Slough, Abbotsford, Sumas Drainage Canal, Abbotsford, chlorothalonil concentrations ranged from 0.147 - 0.487, 0.083 - 1.33, 0.075 - 0.226, 0.072 - 0.381, and 0.064 - 4.01ng/L, respectively. In urban sites, chlorothalonil concentrations ranged from 0.074–2.14 and 0.076–0.240 ng/L at Scott Creek, Coquitlam and Mosquito Creek, North Vancouver, respectively. Although residues were found at two of the three reference sites, the levels were quite low at ≤0.122 ng/L (Woudneh *et al.*, 2009). The detection limit (DL) varied by site and ranged from as low as 0.001 ng/L at the reference sites to as high as 0.064 ng/L at the agricultural sites (Woudneh *et al.*, 2009).

Under the U.S. Geological Survey (USGS) Toxic Substances Hydrology Program, chlorothalonil residues have been analyzed from a number of different regions and matrices throughout the United States. Chlorothalonil was specifically investigated at 22 surface-water sites in five southern states (Alabama, Florida, Georgia, Oklahoma, and Texas) in 2003-2004 coinciding with the peanut-growing season (Table 2; Scribner *et al.*, 2006). Chlorothalonil was detected in just 4 of 113 samples analyzed by liquid chromatography/mass spectrometry (LC/MS) at a mean concentration of 150 ng/L, with a range of 89 to 433 ng/L and a lower reporting limit (LRL) of 50 ng/L (Scribner *et al.*, 2006). The primary degradation product of chlorothalonil, 4-hydroxy-chlorothalonil, was detected at a frequency of 23% with concentrations ranging from 2 to 930 ng/L; two other analyzed degradation products, 1-amide-4-hydroxy-chorothalonil and 1,3-diamide-chlorothalonil, were only detected in a

single water sample at 20 and 161 ng/L, respectively (Scribner *et al.*, 2006). In addition, as a separate analysis samples were also analyzed by the USGS National Water-Quality Laboratory (NWQL) schedules 2001 and 2060 via LC/MS and additionally via a gas chromatography/mass spectrometry (GC/MS) method developed specifically to increase sensitivity below the LC/MS method LRL of 35 ng/L down to 10 ng/L (Scribner *et al.*, 2006). Based on the more sensitive GC/MS method, chlorothalonil was detected in 5 of 68 samples at a mean concentration of 10 ng/L (Scribner *et al.*, 2006).

As part of the larger study previously detailed by Scribner *et al.*, (2006), the USGS also conducted a survey of chlorothalonil and select degradation product residues in four streams located in Texas and Oklahoma sampled at multiple sites in 2003-2004, again coinciding with the peanut crop growing season (Battaglin *et al.*, 2008). Although not equivocally stated, the LRL is assumed to be 50 ng/L, which is the LRL outlined in the referenced method and site-location of performed analysis (Scribner *et al.*, 2006). Chlorothalonil residues were not detected in any of the 20 analyzed samples, and although the 4-hydroxy of chlorothalonil degredation product was detected in three samples collected in 2004, the maximum concentration was only 18 ng/L; the other two transformation products (diamide chlorothalonil and 1-amide-4-hydroxy chlorothalonil) were not detected in any sample (Battaglin *et al.*, 2008).

The USGS also conducted an analysis on twenty depositional (top 2 cm) sediment samples from Georgia, Florida and Alabama collected in July of 2005, and as with the Scribner *et al.*, (2006) study these areas were known to have intense chlorthaolonil applications (approximately 4-7 applications per season) coinciding with the peanut crop growing season during the summer months (Hladik and Kuivila, 2008). Based on a GC-MS method with a detection limit ranging from 1-5 µg/kg dry weight sediment, no chlorothalonil residues were found for either the parent compound or the major metabolites in any sample (Hladik and Kuivila, 2008).

As part of a broader small watershed-scale hydrologic and surface water quality study (21.8 hectares of drainage area) supported by the U.S. Golf Association (USGA), chlorothalonil residues were analyzed at the inflow and outflow of a small unnamed stream flowing through the Northland Country Club (NCC), located in Duluth, MN (King and Balogh, 2008). During the course of the four year study period chlorothalonil was measured at a single maximum concentration of 48.1 µg/L directly at the outflow of the stream from the golf course (King and Balogh, 2008). However, this is the only definitive value provided from the study; the comprehensive data analyses are summarized in a single figure (Figure 6 in King and Balogh, 2008), rendering precise interpolation subject to error. Notwithstanding this issue, interpolating from the aforementioned figure indicates that the median chlorothalonil concentration was <1 µg/L and the upper 95th centile was <5 µg/L, suggesting that the maximum reported concentration was likely a unique circumstance or an analytical anomaly. Furthermore, no details concerning the employed enzyme-linked immunosorbent assay (ELISA) analytical method are provided, for example the detection and quantitation limits, the relative standard error, or any other metrics concerning method recovery efficiency, accuracy and precision. Accessing the ELISA kit manufacturer's specifications (Strategic Diagnostics Inc. RaPID assay) indicates reliable quantification between 0.1 and 5 µg/L with

a minimum detection level of 70 ng/L. Given that the maximum measured chlorothalonil concentration was outside the specified reliable range of the analytical method and no account for this is provided in the study the validity of this single value is questionable. Moreover, since residues were not appreciably detected at the inflow, the detections were attributed entirely to turf use on the golf course (King and Balogh, 2008).

Atmospheric transport and deposition of pesticides has been surveyed in more remote areas. A California based study concerning several pesticides, including chlorothalonil indicated residue presence in remote areas, albeit at quite low levels (McConnell *et al.,* 1998). Sampling was conducted via collection of winter-spring precipitation (rain and snow) from the Sequoia National Park in the Sierra Nevada Mountains and additionally in surface water samples collected from the Lake Tahoe basin, for pesticide residues originating from the California Central valley, which is a region of intensive agricultural production. Chlorothalonil was detected with levels ranging from <0.4 - 85 ng/L at a lower elevation (533 m) and levels ranging from <0.57 - 13 ng/L at a higher elevation (1,920 m) (McConnell *et al.,* 1998). In addition, chlorothalonil residues were detected in snow samples from the Lake Tahoe basin at an elevation of 2,200 m with residue levels ranging from 0.66 - 1.7 ng/L and in deep water samples at two locations in Lake Tahoe at concentrations ranging from 0.47 - 3.2 ng/L; water samples from the surface indicated chlorothalonil at concentrations ranging from 0.92 - 1.4 ng/L (McConnell *et al.,* 1998). The detection limits achieved in this study were quite low ranging from 0.14 to 2.3 ng/L for lake water and snow/rain samples, respectively (McConnell *et al.,* 1998). Although these results indicate that wet deposition may be an important source of pesticides to the lake water in more remote areas, in addition to other atmospheric processes such as dry particle and gas deposition, the levels of chlorothalonil detected are quite low.

Sediment and fish tissue samples taken from the Salton Sea, the largest man-made lake in California, which receives significant agricultural input from the Imperial Valley, have identified chlorothalonil at low concentrations in these matrices (Sapozhnikova *et al.,* 2004). Chlorothalonil was found to be commonly detected in sediments from the Salton Sea with concentrations ranging from <0.1 – 8.9 ng/g (parts per billion) at up to five locations within the lake (Sapozhnikova *et al.,* 2004). In muscle, liver, gonads, and gills of Orange mouth Corvina (*Cynoscion xanthulu*) chlorthalonil was detected at concentrations ranging from <0.12 – 0.7, <0.12 – 3.1, 0.3 – 1.6, and 0.2 – 1.0 ng/g respectively (Sapozhnikova *et al.,* 2004). Similarly, chlorothalonil was also detected in Tilapia (*Tilapia mossambique*) at concentrations ranging from <0.12 – 1.2, 0.2 – 4.4, <0.12 – 1.4, and <0.12 – 1.4 ng/g, respectively, in muscle, liver, gonad, and gill tissues (Sapozhnikova *et al.,* 2004). These fish tissue results are in agreement with the Agencies conclusion from the RED (EPA, 1999) that chlorothalonil does not appreciably bioconcentrate in fish.

Recently the USGS conducted a survey of water and suspended sediment samples from two rivers (Alamo and New rivers) located in the Imperial Valley/Salton Sea Basin, California, screening for pesticide residues, including chlorothalonil using GC/MS (Orlando *et al.,* 2008). Chlorothalonil was detected only in the spring in 1 sample from each of the Alamo and New rivers, respectively, out of a combined total of 24 samples collected at 8 sites indicating a detection frequency of ~8%, however, the residue concentrations from both

samples (1.4 and 5.6 ng/L) were below the MDL of 12.1 ng/L (Orlando *et al.*, 2008). In sediment samples chlorothalonil was detected with frequency and seasonal characteristics identical to that of the water samples at two concentrations, 1.5 and 1.4 µg/kg, both of which were below the MDL of 1.6 µg/kg (Orlando *et al.*, 2008). These results indicate that residue levels detected in sediment samples from supplying tributaries (Orlando *et al.*, 2008) are comparatively lower than those identified in the Salton Sea receiving body (Sapozhnikova *et al.*, 2004); no water sample analyses from the receiving body were available for comparison.

Current sampling efforts have also identified chlorothalonil in suspended sediments, and additionally in bed sediments, from a broader geographical area across the U.S. (Smalling and Kuivila, 2008). With a MDL of 1.6 µg/kg, GC/MS analysis did not detect chlorothalonil residues in any suspended sediment sample taken from four predominately agricultural locations in northern California (Coloussa Basin Drain, KL Ridge Cut, Willow Slough, and Mallard Island) during high-flow events (Smalling and Kuivila, 2008). In bed sediment samples chlorothalonil was detected at a relatively high level of 62.2 µg/kg dry weight in a single sample from San Joaquin, CA, however two other samples from the same region failed to detect residues above the MDL, with estimated concentrations of 0.4 and 0.6 µg/kg dry weight) (Smalling and Kuivila, 2008). Furthermore chlorothalonil was not detected in any bed sediment samples taken from Big Creek, AL, Little Abrams Creek, GA, Clark's Creek, WA, and Thorton Creek, which represent areas of agricultural and urban pesticide use (Smalling and Kuivila, 2008).

As indicated in the Endangered and Threatened Salmon and Steelhead evaluation conducted by the EPA (EPA, 2003) at the current time there were 6439 samples available for chlorothalonil contained in the USGS National Water-Quality Assessment Program (NAWQA) database. Up until 2001, 5762 samples were available, from which the highest measured chlorothalonil concentration was 0.71 µg/L and the detection frequency was 0.5%, or 28 in 5762 (Hamer, 2003). Subsequently, as identified in the ESA (EPA, 2003) a number of anomalous high detects were encountered in the 677 samples added to the database, however upon further investigation and sample re-analyzing the detects were attributed to interference, and the revised analyses revealed no chlorothalonil detects above 1 µg/L. The agency noted that although the NAWQA program produced high quality data the sampling regime may not encompass peak exposure periods. No residues have been detected above 1 µg/L (maximum of 433 ng/L) in subsequent USGS surveys (Scribner *et al.*, 2006; Battaglin *et al.*, 2008).

Collectively, the monitoring studies focused specifically in the Pacific Northwest region (Sargeant *et al.*, 2010; Burke *et al.*, 2006; McConnell *et al.*, 1998; Woudneh *et al.*, 2009) provide the most appropriate, relevant and realistic exposure data for assessing potential impacts of chlorothalonil to threatened and endangered salmon, which are considerably lower than concentrations resulting in sub-lethal and lethal effects. Although geographically distinct from the Pacific Northwest region, the USGS Toxic Substances Hydrology Program and NWQL survey data outlined previously, targeted to areas specific to intensive chlorothalonil use and during the growing season of specific crops (Scribner *et al.*, 2006; Battaglin *et al.*, 2008; Orlando et al., 2008), are considered to accurately encompass potential worst-case peak chlorothalonil exposures. Therefore the high-quality recent monitoring

studies in conjunction with the current USGS survey data were used in the present evaluation. It is worthwhile to note that the detection frequency of chlorothalonil in more recent USGS surveys is still low, however as evidenced by the high detection frequency calculated by Woudneh *et al.*, (2009) and McConnell *et al.*, (1998), chlorothalonil may be present in systems with varying proximity to use, albeit at extremely low concentrations. Moreover, it is important to point out that the detection limit reported by Woudneh *et al.*, (2009) and McConnell *et al.*, (1998) were 100-1000 times lower than the lower reporting limit associated with the USGS surveys (Scribner *et al.*, 2006; Battaglin *et al.*, 2008), likely accounting for the discrepancy in detection frequency. Given that there is considerable uncertainty surrounding the maximum chlorothalonil residue concentration reported from the previously summarized turf study (King and Balogh, 2008), in addition to lack of analytical methodology description and detail, these data were not included in characterizing the environmental exposure. Furthermore, given that the analyses were conducted from the stream outflow at the margin of the golf course, dilution and dissipation would reduce these exposures considerably in receiving drainages.

APPENDIX 4 Environmental Fate

The environmental fate and transport of chlorothalonil has been comprehensively detailed previously (EPA, 1999; Hamer, 2003). Consequently the following content focuses mainly on studies conducted subsequent to the publication of the chlorothalonil ESA (EPA, 2003).

In the context of environmental fate the Agency indicated in the ESA (EPA, 2003) that given chlorothalonil is considered stable to hydrolysis at pH 5 & 7, with a half life of 40-60 days at pH 9, and that the aquatic photolysis half-life is estimated at 65 days, these pathways are not important relative to the microbial metabolism pathways. However, a recent study (Kwon and Armbrust, 2006) investigating the partitioning behavior between waters and sediments, as well as the degradation of chlorothalonil tends to dispel this contention. When chlorothalonil was applied to both creek and pond sediment systems for 30 days, 87-88% dissipated from the water phase within 1 day when irradiated by simulated sunlight, whereas 60-68% remained in the water under dark conditions (Kwon and Armbrust, 2006). Moreover, only a relatively small proportion of chlorthalonil (3-6% under light conditions at day 1 and 10-16% in the dark at day 3, respectively) was found in sediments, representing the highest amounts observed in the study (Kwon and Armbrust, 2006). Therefore, since chlorothalonil behaved similarly in irradiated water/sediments and sediment-free aqueous solutions, photodegradation was considered to be important to the dissipation of chlorothalonil in aqueous solutions, whereas microbial degradation was considered to play an important role for residues ultimately partitioning to sediments (Kwon and Armbrust, 2006). The major metabolite 4-Hydroxychlorothalonil was detected only in water in the dark systems up to 3.4% of the applied chlorothalonil, and Trichloro-1,3-dicyanobenzene and 3-cyano-2,4,5,6-tetrachlorobenzamide were also detected, though only in trace amounts (Kwon and Armbrust, 2006).

Another issue of contrast has been consensus on half-life, with the Agency considering a 44 hr half-life more appropriate for chlorothalonil (EPA, 2003), whereas other reports have suggested half-lives as low as 4 and 8 hrs for indoor and outdoor microcosms, respectively (Gentle, 1999; Gentle and Tattersfield, 2000). Based on the water-sediment studies conducted by Kwon and Armbrust (2006), the estimated half-life was <1 day, under irradiated conditions for both pond and creek water. Under dark conditions the half-lives calculated in creek and pond water were 3.0 days and 2.1 days, respectively, showing faster dissipation from pond water (Kwon and Armbrust, 2006). In the same study, the half-lives by hydrolysis and photolysis (net) were 17.0 days and 1.2 days in distilled-deionized water and 1.9 days and 2.1 days in pH 9 buffer solution, respectively (Kwon and Armbrust, 2006). Moreover, degradation appeared to follow a pseudo-first-order kinetic model, and a faster dissipation (3.0 and 2.1 days) from the water phase in water/sediment systems was observed than in distilled-deionized water (17.0 days) (Kwon and Armbrust, 2006). Therefore it appears as though the half-life is shorter under simulated field conditions, particularly under irradiated conditions, and in the actual environmental context is more appropriately considered to be <24 hrs.