Appendix G. Summary Ecotoxicity Data for Chlorothalonil

G.1 Toxicity to Birds, Reptiles, and Terrestrial Phase Amphibians

G.1.1 Birds: Acute and Subacute Studies

Chlorothalonil

An acute oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the toxicity of chlorothalonil to birds. The preferred test species is either mallard duck (*Anas platyrhynchos*; a waterfowl) or bobwhite quail (*Colinus virginianus*; an upland gamebird). Results of these studies are summarized below in Table G.1-1 and G.1-2. These studies suggest that chlorothalonil is practically non-toxic to birds on an acute basis.

Table G.1-1. Avian Acute Oral Toxicity Findings for Chlorothalonil					
Test Species	**% a.i.**	**LD_{50} mg/kg**	**Citation (MRID)**	**Toxicity Category**	**Fulfills Guideline?**
Mallard	96%	> 4640	00068753	Practically non-toxic	Yes
Japanese quail	Tech.	> approx. 2000	40964105	Practically non-toxic	Supplemental

Table G.1-2. Avian Subacute Dietary Toxicity Findings for Chlorothalonil					
Test Species	**% a.i.**	**LC_{50} ppm**	**Citation (MRID)**	**Toxicity Category**	**Fulfills Guideline?**
Northern Bobwhite	96%	> 10,000	00030388	Practically non-toxic	Yes
Mallard	93.6%	> 21,500	00039146	Practically non-toxic	Yes
Mallard	96%	> 10,000	00030389	Practically non-toxic	Yes

SDS-3701

Acute avian LD_{50} data for the chlorothalonil degradate, SDS-3701, are summarized in Table G.1-3. These studies show that SDS-3701 is "moderately toxic" on an acute oral basis and "slightly toxic" on a dietary basis to the test birds on an acute basis. Sublethal effects were seen in some birds at the lowest test level with the mallard, including lethargy, depression, lost reaction to stimuli, lost coordination, and wing droop.

In an acute oral study (MRID 00030395), fourteen-day old mallard ducks were dosed with 46, 100, 215, 464, and 1000 mg/kg of technical SDS-3701. No deaths occurred in the control pens. The acute oral LD50 for SDS-3701 was 158 mg/kg, with 95% confidence limits of 125 to 201 mg/kg. The highest dose at which no deaths occurred was 46 mg/kg. All the birds died in the two highest dosing groups.

Table G.1-3. Avian Acute Oral and Subacute Dietary Toxicity Findings for SDS-3701					
Test Species	**% a.i.**	**Results**	**Citation (MRID #)**	**Toxicity Category**	**Fulfills Guideline?**
Mallard	SDS-3701 (87%)	LD_{50} = 158 mg/kg	00030395	Moderately toxic	Yes
Northern Bobwhite	SDS-3701 (87%)	LC_{50} = 1746mg/kg NOEL = 562 mg/kg	00115109	Slightly toxic	Yes
Mallard	SDS-3701 (87%)	LC_{50} = 2000 mg/kg	00115108	Slightly toxic	Yes

G.1.3 Birds: Reproduction Studies

Chlorothalonil

Avian reproduction studies using the TGAI are required because chlorothalonil is persistent (i.e., half-life exceeds 4 days in aerobic soils) and has multiple applications per growing season. The preferred test species are mallard duck and bobwhite quail. Results of these tests are summarized in Table G.1-4.

Table G.1-4. Avian Reproduction Findings of Chlorothalonil Exposure						
Test Species	**% a.i.**	**NOEL PPM**	**LOEL PPM**	**Endpoints affected**	**Citation (MRID #)**	**Fulfills Guideline ?**
Bobwhite	Tech.	1000 (reprod.)	5000 (reprod.)	"Overt signs of toxicity and reduced reproduction" cited at 5000 ppm; "overt signs of toxicity, mortalities, and profound effects upon several reproductive parameters related to egg production, hatching success, and survival of hatchlings" cited at 10,000 ppm.	40964104	Yes
Bobwhite	Tech.	153	624	18% reduction in	45710218	Yes

Table G.1-4. Avian Reproduction Findings of Chlorothalonil Exposure						
Test Species	**% a.i.**	**NOEL PPM**	**LOEL PPM**	**Endpoints affected**	**Citation (MRID #)**	**Fulfills Guideline ?**
				no. of eggs laid per hen.		
Bobwhite	99.6	50 ppm	Not established. Highest level of 50 ppm did not cause impairment.	None	00041440	Supplemental
Mallard	Tech.	>10,000 (reprod.)	>10,000 (reprod.)	No reproductive effects cited at any test level (1000, 5000, 10,000 ppm)	40964102	Yes
Mallard	99.6	50 ppm	Not established. Highest level of 50 ppm did not cause impairment.	None	00041441	Supplemental

In the most sensitive avian reproduction study (MRID 45710218) chlorothalonil was administered to bobwhite quail in the diet at nominal concentrations of 0 (negative control), 40, 160, or 640 ppm. Mean-measured concentrations were <1.5 (<LOD, control), 41, 153, and 624 ppm a.i., respectively. A treatment-related reduction in the number of eggs laid/hen and thus in the number of 14-day old survivors/hen were observed at the 624 ppm a.i. level. The number of eggs laid/hen was 62.0 for the control group, and 62.4, 68.9, and 51.0 for the 41, 153, and 624 ppm a.i. test groups, respectively. The number of 14-day old survivors/hen was 37.2 for the control group, and 42.8, 42.2, and 30.4 for the 41, 153, and 624 ppm a.i. test groups, respectively. Although not statistically significant, these findings were considered to be biological significance by the study authors and the study reviewers.

SDS-3701

Avian reproduction studies have also been required for SDS-3701. These studies are summarized in the following table. The most sensitive NOAEC was 50 ppm based on reduction in eggshell thickness.

Table G.1-5. Avian Reproduction Findings (SDS-3701)

Test Species	% SDS-3701	NOEL PPM	LOEL PPM	Endpoints affected	Citation (MRID)	Fulfills Guideline?
Mallard	99.6	50	100	Reduction in eggshell thickness seen at 100 ppm; at 250 ppm adult body weight, food consumption, and gonad development affected, as well as effects on numbers of eggs laid, embryonic development, eggshell thickness, hatchability, and hatching survival.	40729402	Yes
Bobwhite	99.6	100	250	Reduction in numbers of eggs laid	40729404	Yes

G.1.4. Birds – Open Literature

Based on a review of the open literature, no additional information on the acute or chronic toxicity of SDS-3701 to birds was located that produced more sensitive endpoints relevant to ecological risk assessment. For chlorothalonil, a study in the open literature examined feeding behavior/avoidance of chlorothalonil-treated seeds in house sparrows (Passer domesticus) (Babu 1988). Feeding behavior was observed for three days in which the birds were exposed to chlorothalonil-treated seeds at a limit dose of 2000 mg/kg for 24 hours. Feed consumption and body weights were recorded and a significant antifeeding percentage of 30% was reported for chlorothalonil. However, chlorothalonil was not used in the definitive testing in which the authors claimed were chemicals that were found to be effective in producing avoidance were tested during the preliminary trial.

G.2 Toxicity to Mammals

Wild mammal testing is required on a case-by-case basis, depending on the results of lower tier laboratory mammalian studies, intended use patterns, and pertinent environmental fate characteristics. For this assessment, registrant-submitted reproduction toxicity data obtained from the Agency's Health Effects Division (HED) was used.

Acute and chronic toxicity data for mammals is presented in Sections B.2.1 and B.2.2, respectively.

G.2.1 Mammals, Acute

Chlorothalonil

Acute mammalian toxicity studies for chlorothalonil are summarized in Table G.2-1. The available mammalian data indicate that chlorothalonil is "practically non-toxic" to small mammals on an acute oral basis, based on the rat oral LD_{50}.

Table G.2-1 Mammalian Acute Toxicity Findings--Chlorothalonil

Test Species	% a.i.	LD50	Citation (MRID #)	Toxicity Category	Fulfills guidelines?
Rat (small mammal surrogate)	96%	Oral >10,000	MRID 00094941	practically non-toxic	Yes

SDS-3701

Data on the toxicity of SDS-3701 to mammals are tabulated below in Table G.2-2. These data indicate that the degradate SDS-3701 is more toxic to mammals than the parent chlorothalonil, and is moderately toxic on an acute oral basis.

Table G.2-2 Mammalian Acute Toxicity Findings--SDS-3701

Test Species	LD_{50} mg/kg	Comments	Citation (MRID #)	Toxicity Category
Rat (small mammal surrogate)	242 (females)	The LD50 for males was 422 mg/kg-bw and was 332 for the combined sexes	MRIDs MRID 00047938, 00047939, and 00095783	moderately toxic

G.2.2 Mammals, Reproduction Studies

Chlorothalonil

When available, 2-generation reproduction toxicity studies are used to estimate chronic risk to mammals. In a two-generation study, Sprague Dawley rats were administered chlorothalonil (98%) in the diet at levels of 0, 500, 1500 or 3000 ppm (0, 38, 115 and 234 mg/kg/day) (Table G.2-3). For parental/systemic toxicity, the NOAEL was less than 500 ppm (<38 mg/kg/day). The LOEL was 500 ppm (38 mg/kg/day) based on hyperplasia of renal and forestomach tissues. For offspring toxicity, the NOEL was 1500 ppm (115 mg/kg/day) and the LOEL was 3000 ppm (234 mg/kg/day) based on lower neonatal body weights by day 21 (MRID 41706201).

Table G.2-3 Mammalian Chronic Toxicity Findings--Chlorothalonil

Test Species	Offspring NOAEL	Offspring LOAEL	Citation (MRID)	Fulfills Guidelines?
Rat (2 generation reproduction)	1500 ppm	3000 ppm decrease in pup weight	41706201C	Yes

SDS-3701

Data on the toxicity of the SDS-3701 degradate to mammalian reproduction are tabulated below. In a 1-generation reproduction study in Sprague-Dawley rats, SDS-3701 was administered at 0, 10, 20, 30, 60, or 120 ppm (approximately 0, 0.5, 1.0, 1.5, 3.0 or 6.0 mg/kg/day) (Table G.2-4). For parental systemic toxicity, the NOEL was 1.5 mg/kg/day and the LOEL was 3.0 mg/kg/day. No ecologically relevant reproductive or offspring toxicity occurred at up to the highest level tested (MRID 00127845).

In a 3-generation reproduction study in Sprague-Dawley rats, SDS-3701 was administered at 0, 10, 60 or 125 ppm (approximately 0, 0.5, 3.0 or 6.25 mg/kg/day). No ecologically relevant reproductive or offspring toxicity occurred at up to the highest level tested, 6.25 mg/kg/day (MRID 00127844).

Table G.2-4 Mammalian Chronic Toxicity Findings--SDS-3701

Test Species	Reproduction NOAEL	Reproduction LOAEL	Citation (MRID #)

Table G.2-4 Mammalian Chronic Toxicity Findings--SDS-3701			
Test Species	**Reproduction NOAEL**	**Reproduction LOAEL**	**Citation (MRID #)**
Rat (3-generation reproduction)	120 ppm	None	00127844
Rat (1-generation reproduction)	125 ppm	None	00127845

G.2.3 Mammals: Open Literature

Based on a review of the open literature, no additional information on the acute or chronic toxicity of chlorothalonil or SDS-3701 to mammals was located that produced more sensitive endpoints relevant to ecological risk assessment.

G.3.1 Reptiles: Open Literature

A study examined the potential for snapping turtle (*Chelydra serpentina*) eggs to adsorb chlorothalonil after exposure to chemically-treated soil (de Solla and Martin 2011). The soil was treated with a mixture of pesticides of which one was chlorothalonil. Two concentrations of a chlorothalonil formulation (1.92 and 19.2 kg a.i./ha; Bravo) were added to the soil; the same concentrations were used for all chemicals. Snapping turtle eggs were field collected and placed into two replicates for each treatment (4 eggs/treatment). Eggs were collected one and eight days after exposure and soil was collected on Day 1. Measurements of chlorothalonil in the eggs were below detection. Eggs were also directly exposed to aqueous solutions of the pesticides, and measured concentrations of chlorothalonil were 0.002 µg/g.

G.3 Toxicity to Non-Target Terrestrial Invertebrates

G.3.1 Honey Bee Acute Contact Study

Honey bee acute contact LD_{50} study is required if the proposed use will likely result in exposure to honey bees. The available acute contact toxicity findings for chlorothalonil are summarized in Table G.3-1. The available data suggests that chlorothalonil is practically non-toxic to honey bees.

Table G.3-1: Non-target Insect Acute Contact Toxicity Findings					
Test Species	**% a.i.**	**Results**	**Citation (MRID)**	**Toxicity Category**	**Fulfills Guideline?**
Honey bee	Tech.	at 181 ug/bee, 14.28% mortality	00036935	Practically non-toxic	Yes
Honey bee	Tech.	non-toxic at 181 ug/bee	00077759	Practically non-toxic	Yes

In addition, a number of studies were located in the open literature that evaluated the toxicity of chlorothalonil to terrestrial invertebrates (Table G.3-2). The available data suggest that sensitive terrestrial invertebrates exist, but many of the species tested were not sensitive to chlorothalonil at the levels tested, which typically approximated maximum labeled application rates.

Table G.3-2: Nontarget Invertebrate Acute Contact Toxicity Findings from the Open Literature

Test Species	Material Tested	Results Summary	Citation ECOTOX#	Comment
Aphid endoparasitoid wasp *(Aphidius rhopalosiphi*)	Daconil 500 Flowable (50% a.i.)	NOEL =1250 g a.i./ hectare (1.1 lbs a.i./Acre;	64665 (Jansen, 1999)	Chlorothalonil was considered "slightly harmful" to *A. rhopalosiphi.* Endpoints evaluated included mortality and reproduction
Earthworms and arthropods	Daconil 2787	NOAEL = 12.6 kg (AI)/ha (11.25 lbs a.i./acre)	71484 (Potter et. al. 1990)	Endpoint studied was abundance and biomass
Carabidae	Daconil (54% a.i.)	NOAEL = 8.2 kg a.i./ha (7.3 lbs a.i./Acre)	89639 (Smitley and Rothwell, 2003)	Transient reduction in abundance occurred after the first week of treatment.
Onion Thrips *Thrips tobaci*	Bravo 500	No reduction in abundance occurred at an application rate of 4.5 pints/acre (approx. 2.3 lbs a.i./Acre).	90255 (Al-Dosari et.al 1996)	--
Corn earworm; fall armyworm	Bravo 720	Dietary exposure to the fall armyworm and corn earworm at 800 ppm and higher resulted in high mortality rates.	90193 (Lynch, 1996)	--

Test Species	Material Tested	Results Summary	Citation ECOTOX#	Comment
Mustard beetle (*Phaedon cochleariae*)	Jupital	No significant effects on survival.	90531 (Cherry et. al. 1992)	--
Rove Beetle (*Aleochara bilineata*)	Daconil 500 (50%)	No effects occurred at levels reportedly equivalent to maximum application rate.	63488 (Samsoe-Petersen, 1995)	Effects evaluated included reproduction, egg production and viability.
Predatory mite (*Amblyseius victoriensis*)	Bravo	<10% mortality at up to 10-times the reported maximum field rate	67984 (James et.al. 1995)	--
Aphid Predatory Midge (*Aphidoletes aphidimyza*)	Repulse	Mortality was approximately 10% in larvae after 72 hours at 1100 mg a.i./L.	89884 (Helyer, 1991)	Test level was reportedly equivalent to the maximum labeled application rate.
Aphareta pallipes E. muscae Delia antique D. platura *Coenosio tigrina*	Bravo 6 F	LC50 = 7.83% 100% mortality at 0.0054% No effects to these species were observed at any test level	71029 (Carruthers et. al. 1985)	Cups sprayed with various concentrations of chlorothalonil from 0.0054% to 10.8%.

G.4 Toxicity to Freshwater Animals

G.4.1 Freshwater Fish, Acute Submitted Data

In order to establish the toxicity of a pesticide to freshwater fish, the minimum data required on the technical grade of the active ingredient are two (one cold water and one warm water) freshwater fish toxicity studies. The freshwater fish acute toxicity findings for the technical grade of the active ingredient are summarized in the Table G.4-1. The registrant submitted studies are consistent with each other and indicate that chlorothalonil is very highly toxic to freshwater fish.

Table G.4-1: Freshwater Fish Acute Toxicity Findings					
Test Species	**% a.i.**	**LC_{50} ppb a.i.**	**Citation (MRID #)**	**Toxicity Category**	**Fulfills Guideline?**
Rainbow trout	96	42.3	00056486	very highly toxic	Supplemental
Rainbow Trout	96	18	45710219	very highly toxic	Yes
Bluegill	96	60	00041439	very highly toxic	Yes
Bluegill	99	84	00029410	very highly toxic	Yes
Bluegill	98	51	RIOCHL01 Pitcher (1976)	very highly toxic	Yes
Channel catfish	96	48	00030390	very highly toxic	Yes
Fathead minnow	96	23	00030391	very highly toxic	Yes

The most sensitive LC50 value for registrant-submitted freshwater fish studies was from MRID 45710219. In this study Rainbow Trout (*Oncorhynchus mykiss*) were exposed under flow-through conditions to chlorothalonil at nominal concentrations of 0 (negative and solvent controls), 0.010, 0.018, 0.032, 0.056, and 0.10 ppm. Mean-measured concentrations were ≤0.0004 (≤LOD, solvent control), 0.0085, 0.0177, 0.0357, 0.0491, and 0.0739 ppm a.i., respectively. Following 96 hours of exposure, cumulative mortality was 0% in control groups and 0, 60, 90, 100, and 100% in the 0.0085, 0.0177, 0.0357, 0.0491, and 0.0739 ppm a.i. treatment groups, respectively. The 96-hour LC_{50} (with 95% C.I.) was 0.018 (0.013-0.023) ppm a.i., which classifies chlorothalonil as very highly toxic to Rainbow Trout (*Oncorhynchus mykiss*) on an acute toxicity basis. Swimming and/or hanging at the surface, lethargy, loss of equilibrium, and/or morbidity were observed in surviving fish from the ≥0.0177 ppm a.i. groups; effects were first observed within 6 hours of exposure and continued through 96 hours in groups with surviving fish. The NOEC (for mortality and sub-lethal effects) was 0.0085 ppm a.i.

G.4.2 Freshwater Fish, Open Literature Data

Acute LC50s reported in the open literature are summarized in Table G.4-2. One study (Davies and White, 1985) located in the open literature reported an LC50 that was more sensitive than the most sensitive registrant submitted studies. Davies and White (1985) reported an LC50 of 10.5 ppb in rainbow trout. The most sensitive rainbow trout LC50 from registrant submitted studies was 18 ppb (MRID 45710219), which is similar to the LC50 reported by Davies and White (1985) of 10.5 ppb which was conducted under low oxygen levels.

Table G.4-2: Freshwater Fish Lethal Effect Studies from the Open Literature

Test Species	LC_{50} ppb a.i.	Citation (MRID or Ecotox No.)	Toxicity Category	Comment
Jollytail	16	Ecotox No. 87454	very highly toxic	--
Spotted mountain galaxias	19 – 29	Ecotox No. 87454	very highly toxic	Tested species has not been evaluated in registrant submitted studies.
Threespine stickleback	69	Ecotox No. 7055	very highly toxic	Tested species has not been evaluated in registrant submitted studies.
Rainbow trout	10.5 - 76	Ecotox No. 87454; 7055	very highly toxic	Study used an oxygen level of 50%.
Tilapia	100 - 120	Ecotox No. 229772	highly toxic	--

A number of studies were also located in the open literature that evaluated sublethal endpoints such as biochemical endpoints. These studies did not report more sensitive toxicity values on endpoints that are correlated with assessment endpoints (survival and reproduction).

B.4.3. Toxicity of Formulated Products:

Formulated product testing is specified for products with direct application to aquatic habitats and for typical end-use products where the EEC for the active ingredient is $\geq$ LC_{50}. The previous Phase IV Review (1/12/93) specified further testing of a 54% ai flowable concentrate due to a cranberry use. The freshwater fish acute toxicity findings for the 54%, 75%, and Bravo W-75 formulations are summarized in Table G.4-3. The data suggest that studies using chlorothalonil products were of similar toxicity to studies using technical grade chlorothalonil.

In a study by Teather *et al.* (2003), the activity level in Japanese medaka (Oryzias latipes) fry, measured by the distance swam in two minutes were reported to be significantly less ($p<0.05$) when compared to the control at a chlorothalonil concentration of 0.06 µg/L (the only concentration tested) using a formulation (purity and brand not reported, and the reviewer assumed that the reported concentration was for technical chlorothalonil). However, based on the information presented in the study, there is a discrepancy in the text and figure representing activity level whereas in the figure the activity level for chlorothalonil is not significantly different than the control, therefore, there is uncertainty in this effect. In addition, after five months (it appears that the fish were only exposed to chlorothalonil for 7-days post-hatch), the sex ratio in fish exposed to 0.06µg/L was reported to be altered and biased toward females by departing significantly from an even sex ratio, although the mechanism for this change is not known. Chlorothalonil was reported to not affect survival, hatching time, or foraging ability. Based on the study,

survival rates by 7 days post-hatch in this study ranged from 61-69%, and appear to range from approximately 33-50% after five months.

Table G.4-3: Freshwater Fish Acute Toxicity Findings—Formulated Products

Test Species	% a.i.	LC_{50} ppb formulation	Citation (MRID #)	Toxicity Category (FP)
Rainbow trout	54 (Bravo 720)	61 (33.2 ppb ai)	43302101	very highly toxic
Bluegill	54 (Bravo 720)	49 (26.3 ppb ai)	42433804	very highly toxic
Rainbow trout	75	152 (114 ppb ai; 48-hr study)	00087304	highly toxic
Rainbow trout	75	103 (77.2 ppb ai)	00087303	highly toxic
Bluegill	Bravo W-75	167 (125 ppb ai)	00087258	highly toxic

G.4.4. Toxicity of SDS-3701

Testing using the degradate SDS-3701 has been previously specified due to its persistence in water. Freshwater fish acute toxicity findings for the degradate SDS-3701 are summarized in Table G.4-3. These studies show that SDS-3701 is "slightly toxic" to the bluegill and therefore is significantly less toxic than parent chlorothalonil.

Table G.4-4: Freshwater Fish Acute Toxicity Findings—SDS-3701

Test Species	% SDS-3701	LC_{50} (ppb)	Citation (MRID #)	Toxicity Category
Bluegill	99	45,000	00029415	slightly toxic
Bluegill	99	15,000	00030393	slightly toxic

G.4.5. Freshwater Fish, Chronic Submitted Data

Data from fish early life-stage testing is required for chlorothalonil since it can be expected to be transported to water from the intended use site, acute LC_{50} values are less than 1 mg/L, and aquatic EECs are $\geq$ 0.01 of LC_{50}s. The fish early life stage data are summarized in Table G.4-5.

In an early life-stage study in fathead minnows (MRID 00030391), fish were exposed to chlorothalonil at 0, 0.6, 1.4, 3.0, 6.5, and 16 ppb under flow-through conditions (uncertain whether concentrations adjusted for purity). The study authors performed statistics on number of eggs/spawn; however, a decrease in the total number of spawns and eggs was observed in the 3.0 µg/L compared to the control. The number of eggs per female in the negative control was 494 whereas it was 103 in the 3.0 µg/L group; however, because there is inherent variability in egg production and the use of only two replicates, this decrease (79% difference) was not significant. However, the decrease is thought to be biologically significant and treatment-related and therefore, the NOAEC is 1.4 µg/L where the number of eggs per female was 459. The report did not indicate if the test solutions were adjusted for purity (96%), therefore, the 1.4 µg/L value was adjusted for purity and the NOAEC is 1.3 µg a.i./L.

Table G.4-5. Chronic Fish Toxicity Findings

Test Species	% a.i.	NOAEL (ppb)	LOAEL (ppb)	Citation (MRID #)	Endpoints Affected
Fathead minnow	96	1.3	2.9	00030391	Reproduction

G.4.7. Effects to Amphibians

A study that examined lethality for three species of amphibians (for 10 days exposed to chlorothalonil under semi-static conditions was available (McMahon *et al.,* 2012). Amphibian species, *Rana sphenocephala*, *Osteopilus septentrionalis*, and *Hyla cinerea* (Gosner stage 25) were exposed to nominal technical chlorothalonil concentrations ranging from 0.0164, 0.164, 1.64, 16.4, 82.0, or 164µg/L in addition to negative and solvent (acetone) controls for 10-days in which the test solutions were changed on day 7. The concentration of the stock used to prepare the test solutions was measured in a previous study, however, the test solutions were not measured in this study. At the highest treatment group, 164 µg/L, 100% of the tadpoles were dead by Day 10. The mortality response in *R. sphenocephala* and *H. cinerea* was not monotonic, and for *R. sphenocephala* and *H. cinerea*, 0.164 or 0.0164 µg/L exhibited significantly more mortality than either adjacent concentration, respectively. Based on the figures provided in the study, after 10 days of exposure using 50% mortality as a benchmark, it appears that control survival was 80% or greater, and that the survival in the treatment groups for *R. sphenocephala*, *O. septentrionalis*, and *H. cinerea* ranged from 0-55%, 0-90%, and 0-70%, respectively. For *O. septentrionalis*, and *H. cinerea*, the use of the acute 96-hours toxicity value for rainbow trout appear to be protective, however, this may not be true for *R. sphenocephala*, but given the reported variation in response in this study around the 100 hour timepoint, there is uncertainty in this comparison. The study also examined amphibian mortality under a mesocosm scenario at chlorothalonil levels of 164 and 328 µg/L and mortality was significantly greater at both concentrations compared to the

control. As this study evaluated mortality over a 10-day exposure, and did not evaluate other sublethal endpoints (*i.e*., growth) and the study was not conducted over chronic exposure duration (*i.e.*, such as the early-life stage toxicity test with fish), there is uncertainty in whether the chronic toxicity value for fish is conservative or not for aquatic-phase amphibians.

G.4.9. Estuarine/marine Fish, Acute Submitted Data and Open Literature Data

For estuarine/marine fish, a sheepshead minnow study was submitted with a reported LC_{50} of 33 µg/L (MRID 00127863; reviewer calculated). This study was conducted using 3-7 day old larvae. Sublethal effects were not reported but there was no mortality reported at 30 µg/L. The study was conducted using 'whole material' and the purity of the material was not reported. This study was conducted using a static system without measuring the test concentrations and as such there is uncertainty in the actual exposure chlorothalonil concentrations. This uncertainty is significant given that for several other studies conducted with chlorothalonil, maintaining nominal concentrations was difficult even under flow-through conditions (MRID 45710219; 00030391). This study has been classified as Supplemental and for qualitative use only.

One study was reported in the open literature (Bao *et al.*, 2011). In this study marine medaka (*Oryzias melastigma*) were exposed as larvae (<24 hrs old) to chlorothalonil under static-renewal conditions for 96 hours (48-hr renewal). The fish were not fed during the study. The reported 96-hr LC50 was 110 µg/L (100-110 95% CI) based on nominal concentrations. In this study, DMSO was used as a co-solvent carrier (<1%), and there is uncertainty in the actual test concentrations. Control mortality may have been as high as 20% (mortality not specified but not greater than 20%) and it is also unknown what the implications were of not feeding newly-hatched larvae. As such this study is for qualitative use only.

G.4.10. Estuarine/marine Fish, Chronic Data.

No chronic toxicity data were submitted or reported in the open literature.

G.4.11. Freshwater Invertebrates, Acute Submitted Data

Registrant submitted freshwater invertebrate toxicity studies for technical grade chlorothalonil are summarized in Table G.4-6. Two studies in daphnids were submitted that reported consistent results. The lowest EC50 for daphnia was 54 ppb (MRID 45710221). Another study which evaluated many species of freshwater invertebrates reported acute (48 hrs for all but the rotifer test which was 24 hr) LC/EC50 values of 19.5 - >1,600 µg/L (MRID 4341601).

Table G.4-6: Freshwater Invertebrate Acute Submitted Data					
Test Species	**% a.i.**	**LC_{50} (ppb)**	**Citation (MRID #)**	**Toxicity Category**	**Fulfills Guideline?**
Daphnia magna	Tech.	68	00068754	very highly toxic	Yes
Daphnia magna	Tech.	54	45710221	very highly toxic	Supplemental
Multiple spp. (crustacea, insecta, gastropoda spp., *Planaria* sp., *Brachionus calyciflorus*, and *Erpobdella* sp.)	Tech	19.5 - >1600 (*Leptocer us* sp. most sensitive)	4341601	Very highly toxic – at most moderately toxic	Supplemental[1]
[1] Supplemental (qualitative) due to: static conditions (concs unstable); the following occurred in some tests: negative control not used, unacceptable control mortality; prior exposure to field collected organisms unknown (acclimation unknown); potential cannibalism					

G.4.12. Freshwater Invertebrates, Open Literature Data

Table G.4-7 summarizes the information available from the open literature. Acute studies in several additional species were located in the open literature, which are summarized in Table G.4-7.

Table G.4-7: Freshwater Invertebrate Acute Effect Studies from the Open Literature					
Study type / Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment**
Acute (48 hr) Bravo 500 (40% a.i.)	Water flea (Daphnia magna) adult egg-bearing	Static Renewal; 4 treatment levels; 2 replicates, one fed and one not fed; algal food source	LC50 = 129 (95% CI 84-174) EC50 = 97 (95% CI 81-113)	Ernst et. al. 1991 (7055)	Qualitative. EC50 is not more sensitive than the lowest registrant-submitted study. Results based on nominal chlorothalonil levels.
Acute: Lethal and Sublethal 4 and 7 day values	Giant Tasmanian Freshwater Crayfish (*Astacopsis gouldi*)	Flow through; 4 and 7 day values reported; 5 treatment	LC50 = 12.0 (7.9-18.1) 4 Day LC50 = 3.6 (2.1-6.0) 7-Day	Davies et. al 1994 (64835)	Qualitative (acute lethal studies only). No data on previous exposure (field-collected organisms), control performance,

reported ≥ 98% a.i.		levels	value		concentration levels not reported, fed during study (may have reduced bioavailability)
Acute: Lethal and Sublethal 4 and 7 day values reported ≥ 98% a.i.	Amphipod (*Neoniphargus sp. A*)	Flow through; 4 and 7 day values reported; 5 treatment levels	LC50 = >40 (4 and 7 Day)	Davies et. al 1994 (64835)	QUAL The LC50 value was above the highest test concentration. See deficiencies above.
Acute: Lethal and Sublethal 4 and 7 day values reported ≥ 98% a.i.	Isopod (*Colubotelson chiltoni minor*)	Flow through; 4 and 7 day values reported; 5 treatment levels	LC50 = >40 (4 and 7 Day)	Davies et. al 1994 (64835)	QUAL The LC50 value was above the highest test concentration. See deficiencies above
Acute: Lethal and Sublethal 4 and 7 day values reported ≥ 98% a.i.	Freshwater aytid shrimp *Parataya australiensis*	Flow through; 4 and 7 day values reported; 5 treatment levels	LC50 = 16 (14.4-17.9) 4-Day LC50 = 10.9 (9.1-13.1) 7-Day	Davies et. al 1994 (64835)	QUAL (acute lethal data). The LC50 value is definitive and bounded by a 95% confidence interval. See deficiencies above.
Acute: embryonic development – 48hr values reported 99% a.i.	Zebra mussel *Dreissena polymorpha*	Static; 48-hr values reported; 6-8 treatment levels	48-hr EC50 = 0.97	Faria et al. 2010 (156417)	QUAL. Raw data not provided; unclear about sample size used to calculate EC50
Acute: embryonic development – 48 hr and 96-hr values reported 98% a.i.	Freshwater mussel *Lampsilis siliquoidea*	Static for 48 – hr and static-renewal 96-hr values reported; 5-6 treatment levels	48-hr EC50 = 40 (20-110) – glochidia study 96-hr EC50 = 280 (190-410) – juvenile study	Bringolf 2007 (100597)	QUAL. Raw data not provided, solvent conc. not specified; 95% confidence intervals overlap or EC50 value is greater than Daphnia endpoint

Toxicity of Formulated Product: The freshwater invertebrate toxicity findings for formulated product testing are summarized in the following table.

Table G.4-8: Freshwater Invertebrate Toxicity Findings—Formulated Product					
Test Species	**% a.i.**	**LC_{50} (ppb) formulation**	**Citation (MRID #)**	**Toxicity Category (FP)**	**Fulfills Guideline? (for FP tested)**
Daphnia magna	54 (Bravo 720)	180 (97; ai) Probit slope = 7.9	42433806	highly toxic	Yes

Toxicity of SDS-3701: The freshwater invertebrate toxicity findings for the degradate, SDS-3701, are summarized in the following table. The data indicate that SDS-3701 is slightly toxic to aquatic invertebrates.

Table G.4-8: Freshwater Invertebrate Toxicity Findings—SDS-3701					
Test Species	**% SDS-3701**	**EC_{50} (ppb)**	**Citation (MRID #)**	**Toxicity Category**	**Fulfills Guideline?**
Daphnia magna	99	26,000 Probit slope = 5.75	00030394	slightly toxic	Yes (for SDS-3701)

In MRID 00030394, the acute toxicity of SDS-3701 was evaluated in daphnids. Procedures used in this acute toxicity test were based on EPA protocols specified in "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and amphibians (U.S.EPA, 1975). Acetone was used as a solvent and in a solvent control. Nominal concentrations were 10, 15. 22. 32,46, 68 and 100 mg/L (ppm) and were tested in triplicate. The 48-hour EC50 for Daphnids exposed to SDS-3701 was calculated to be 26 ppm with 95% confidence limits of 21 to 31 ppm. The lowest concentration at which 100% mortality occurred was 68 ppm, while highest concentration in which there were no deaths was 10 ppm.

G.4.13 Freshwater Invertebrate, Chronic Submitted Data

The aquatic invertebrate life-cycle toxicity findings are summarized in the following table.

Table G.4-9: Aquatic Invertebrate Life-Cycle Toxicity Findings

Test Species	NOEL (ppb)	LOEL (ppb)	Citation (MRID #)	Endpoints Affected	Fulfills Guideline?
Daphnia magna	39	79	00115107	survival, cumulative numbers of offspring/ female	Yes
	0.6	1.8	45710222	Survival	

In the most sensitive chronic study in aquatic invertebrates (MRID 45710222), the 21-day-chronic toxicity of chlorothalonil to *Daphnia magna* was studied under static renewal conditions. Nominal concentrations were 0 (negative and solvent controls), 0.0010, 0.0032, 0.010, 0.032, and 0.10 ppm. Mean-measured concentrations were <0.0001 (<LOD, solvent control), 0.00060, 0.0018, 0.0058, 0.019, and 0.075 ppm a.i., respectively. However, chlorothalonil was unstable under the static renewal conditions employed in this test, declining to less than the level of detection to 62% of nominal concentrations in expired test media. Because chlorothalonil concentrations declined to less than the level of detection at the lower concentrations, there is uncertainty in the chlorothalonil levels associated with toxic effects in this study. Immobilization of the parental generation was statistically significantly ($p<0.05$, using Dunnett's and William's test) at concentrations ≥ 1.8 µg/L (75% survival); therefore, the NOAEC value is 0.6 µg/L based on immobilization. Significant effects ($p<0.05$) on reproduction (number of live young per adult) were also reported for the 75 µg/L treatment group. No dead young were observed during the study in any test group, and the number of unhatched eggs per adult was <1 for all control and test groups. Terminal growth measurements were not performed. The NOAEC for survival was 0.6 µg/L (0.0006 mg/L).

G.4.14 Estuarine/marine Invertebrates, Acute Submitted Data

Registrant submitted estuarine/marine invertebrate toxicity studies for technical grade chlorothalonil are summarized in Table G.4-6. The study with the lowest toxicity value is for the Eastern oyster with a 96-hr EC50 value of 3.6 µg a.i./L based on shell deposition (MRID 00138143).

Table G.4-6: Estuarine/marine Invertebrate Acute Submitted Data					
Test Species	**% a.i.**	**LC_{50} (ppb)**	**Citation (MRID #)**	**Toxicity Category**	**Fulfills Guideline?**
Eastern oyster *Crassostrea virginica*	Tech	96-hr EC50 = 3.6	00138143	Very highly toxic	Yes
Northern pink shrimp *Penaeus duorarum*	Tech	96-hr LC50 = 154	00127864	Highly toxic	Supplemental[1]
Northern pink shrimp *Penaeus duorarum*	Tech	48-hr EC50 = 320	40228401	Highly toxic	Supplemental[2]
[1] Supplemental due to: uncertainty in exposure concentrations [2] Supplemental due to : uncertainty in exposure concentrations; uncertainty in effect					

G.4.14 Estuarine/marine Invertebrates, Open Literature Data

Several open literature studies with invertebrate toxicity studies for technical grade chlorothalonil were available and are summarized in Table G.4-6. The study with the lowest toxicity value is for the Eastern oyster with a 96-hr EC_{50} value of 3.6 µg a.i./L based on shell deposition (MRID 00138143).

Table G.4-6: Estuarine/marine Invertebrate Open Literature Data					
Study type / Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment**

Table G.4-6: Estuarine/marine Invertebrate Open Literature Data					
Study type / Test material	**Test Organism (Common and Scientific Name) and Age and/or Size**	**Test Design**	**Endpoint Concentration in ppb**	**Citation (ECOTOX #)**	**Rationale for Use in Risk Assessment**
Acute mortality / 98%	Grass shrimp *Palaemonetes pugio*	96-h static-renewal (24hrs)	96-hr LC50=153 (adult) 96-hr LC50= 49.5 (larvae) 96-hr LC50=396 (embryo)	101032	QUAL. raw data were not provided; no negative control group only solvent (acetone,0.1%); control performance difficult to interpret
Acute mortality / tech (assumed)	Grass shrimp *Palaemonetes pugio*	96-h static-renewal (24hrs); conducted under standard conditions and higher temp. and salinity	96-hr LC50=156 (adult, standard conditions) 96-hr LC50= 32.6-116 (adult, higher salinity, temp or both) 96-hr LC50=49 (larvae, standard) 96-hr LC50= 36.4 (larvae, higher salinity)	120220	QUAL. raw data were not provided; no negative control group only solvent (acetone,0.1%);
Life-cycle growth / 98%	Grass shrimp *Palaemonetes pugio*	50-d pulsed exposure (6-hr pulses)	NOAEC <31.3 (↑ # of molts to postlarvae) NOAEC > 125 (dry wt., days to post-larvae)	101032	QUAL. Raw data not provided, high control mortality after day 25; no negative control

G.4.14 Estuarine/marine Invertebrates, Chronic Data

No acceptable chronic estuarine/marine invertebrate data are available.

G.5 Toxicity to Non-target Terrestrial Plants

G.5.1 Non-Target Terrestrial Plants: Submitted Data

The required tier 1 plant toxicity data are summarized in Table G.5-1.

Table G.5-1: Nontarget Terrestrial Plant Toxicity Findings				
Study	**% a.i.**	**Results (lb ai/A)**	**Citation (MRID #)**	**Fulfills Guideline?**
Seed germination/seedling emergence--Tier 1 (122-1A); 10 species	97.9	EC_{25} = >16 lb a.i./A NOAEL < 16 lb a.i./acre (for onion and soybean)	42433808	No; 26% inhibition (growth) for onion compared to control, NOAEC for soybean < limit dose
Vegetative vigor--Tier 1 (122-1B); 10 species	97.9	EC_{25} = >16 lb a.i./A NOAEL < 16 lb a.i./acre (for cucumber and oats)	42433809	No; 26% inhibition (growth) for cucumber compared to control; NOAEC for oats < limit dose

G.5.2 Non-Target Terrestrial Plants: Open Literature Data

Based on a review of the open literature, no additional information was located that indicates greater non-target terrestrial plant sensitivity to chlorothalonil than the submitted data. Studies located in the open literature were predominantly efficacy studies (i.e., studies that evaluated effects of chlorothalonil on fungal diseases) or were studies that did not elicit adverse effects to plants (*e.g.*, Bordoloi *et al*. 1997).

G.5.3 Aquatic Plants: Registrant Submitted Data

Available aquatic plant toxicity data summarized in Table G.5-2. The most sensitive aquatic plant species from registrant studies was the diatom with an EC50 of 14 ppb.

Table G.5-2: Nontarget Aquatic Plant Toxicity Findings				
Test Species	**% a.i.**	**Results (ppb)**	**Citation (MRID #)**	**Meets Guideline Requirements**
Freshwater Vascular Plant Duckweed (*Lemna gibba*)	TGAI	Number of fronds EC50 = 730 ppb (670-800ppb Probit slope = 8.08	44908102	The only DER available is a contractor

Table G.5-2: Nontarget Aquatic Plant Toxicity Findings				
Test Species	**% a.i.**	**Results (ppb)**	**Citation (MRID #)**	**Meets Guideline Requirements**
		Biomass (dry weight) EC50 = 630 ppb (550-730ppb) Probit Slope = 5.3 NOEC 290ppb		version. The contractor classifies this study as core.
Freshwater non-vascular plant. *Selenastrum capricornutum*	97.9	EC_{50} = 190 NOEC = 50 LOEC = 100 Slope = 4.027 95% CI 1.34-6.71	42432801	Yes
Freshwater non-vascular plant. (Diatom) *Navicula pelliculosa*	TGAI	EC50 = 14 ppb (12-17 ppb) Probit slope = 4.49 NOEC = 3.9 ppb	44908105	The only DER available is a contractor version. The contractor classifies this study as core.

G.5.3 Aquatic Plants: Open Literature Data

Aquatic plant studies located in the open literature are summarized in Table G.5-3. The most sensitive aquatic plant study was from Mezcua et al. (2002), which reported a 72-hour EC50 of 6.8 ppb in *Selenastrum capricorotum*. This study utilized the Algaltoxkit which is reported to be a miniaturized assay that uses 'algal beads', which can be stored for long periods of time, as the initial source of the algal cells. The assay is performed in disposable spectophotometric cells of 10 cm path length, which are used as test vessels and analytical containers. This assay is reported to basically adhere to the ISO 8692 norm and the OECD 201 Test Guideline[1]. Since this study design seems to deviate substantially from the OPPTS 850.5400 guideline, this study, while reporting the most sensitive algal endpoint will only be used qualitatively.

[1] http://www.microlan.nl/uploads/files/qaqc/intercalibration_exercise_on-the_algal_toxicity_toxkits.pdf?phpMyAdmin=90a1592554ecf76ebe7c76e2d531280f (accessed December 13, 2011).

Several submerged macrophytes were tested for 21-day exposed to a chlorothalonil formulation and analyzed for several growth endpoints whereas several species reported EC50 values for relative growth lower than the 7-day Lemna value. The most sensitive species and endpoint is Elodea nuttallii with a 21-d EC50 value of 94 µg a.i./L based on length of new shoots. If comparing based on biomass (dry weight), all the test species 21-day EC50 were greater than Lemna. This study is classified as qualitative due to: 1) raw data not reported; 2) only initial measured concentrations available for one series of tests (which include the most sensitive endpoint); and 3) it is not known if formulation tested (study conducted in Europe) accurately reflects a U.S. formulation.

Table G.5-3: Aquatic Plant Toxicity Tests (Laboratory)

Test material	Test Organism (Common and Scientific Name)	Test Design	Endpoint Concentration / Results	Citation (ECOTOX)	Study Classification[(1)]
30% SC	Green Algae *Scenedesmus obliqnus*	Laboratory bioassay	EC50 = 100 ppb	65723 (Ma et. al., 2001)	QUAL
30% SC	Green Algae *Chlorella pyrenoidosa*	Laboratory bioassay	EC50 = 8069 ppb		QUAL
Tech.	Green Algae *Selenastrum capricorotum*	Laboratory bioassay using Toxkit Algaltoxkit™ (Creasel Belgium) a growth inhibition assay.	72-hr EC50 = 6.8 ppb. The EC50 @ 30 h = 42,400ppb	80747 (Fernandez-Alba et. al., 2002) 80359 (Mezcua et. al. 2002)	QUAL.; appears to deviate substantially from OPPT 850.4500
40.4% Bravo	*Elodea nuttallii*	Laboratory bioassay – 21-d	21-day EC50 = 94 µg a.i./L (based on length of new shoots)	E108046 (Belgers et al., 2009)	Qual.; no raw data, conc. not measured in all tests, unknown if formulation relevant to U.S.

[(1)] QUAL = The paper is not appropriate for quantitative use but is of good quality, addresses issues of concern to the risk assessment and is used in the risk characterization discussion.

G.6 References

ECOTOX or MRID	Citation
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