APPENDIX F:

Ecological Effects Data Summaries

I. Freshwater Fish, Acute

Rainbow Trout *Oncorhynchus mykiss).* For the rainbow trout, acute toxicity test results indicate bifenthrin is highly toxic to cold water fish based on the 96-hr LC_{50} of 0.15 ppb and 120-hr LC_{50} of 0.10 ppb; the study was classified as acceptable, core (MRID 00163156). The study consisted of 5 treatment levels ranging from a concentration of 1.33ppb to 0.083 ppb with 20 exposed organisms (mean weight 1.0 g) exposed at each level. Due to the low solubility of bifenthrin, the study was conducted using a flow through system and co-solvent. The D.O., pH, loading, fish size, holding period and other parameters were all in compliance with EPA guidelines. Since the concentrations used in this study exceed the reported solubility of bifenthrin (0.014 ppb) and centrifugation of water samples was not indicated prior to analysis, there is uncertainty as to the concentration of bifenthrin that was actually bioavailable to test organisms

Blue gill *(Lepomis macrochirus)*. For the bluegill, acute toxicity test results show bifenthrin is very highly toxic to warm water fish species based on the 96-hr LC_{50} of 0.35 ppb and 144-hr LC_{50} of 0.30 ppb; the study was classified as acceptable/core (MRID 00132536). The study consisted of 5 treatment levels ranging from a concentration of 1 ppb to 0.15 ppb with 20 organisms (mean weight of 2.5 g) exposed at each level. The study was conducted using a basic flow-through and co-solvent. The pH, D.O. and temperature were within the acceptable range as outlined in EPA guidelines. Since the concentrations used in this study exceed the reported solubility of bifenthrin (0.014 ppb) and samples were not centrifuged prior to analysis, there is uncertainty as to the concentration of bifenthrin that was actually bioavailable to test organisms.

II. Freshwater Fish, Chronic

Fathead minnow *(Pimephales promelas).* The fish full life-cycle toxicity test was deemed unacceptable upon review in 2007 (MRID 40791301). The study was classified as unacceptable due to a low performance standard with regards to survival, potential solvent effect on test organisms and variability among the test concentrations.

III. Freshwater Invertebrates, Acute

Scud *(Hyalella azteca.)* The most sensitive acute endpoint for freshwater invertebrates was found in an open literature study conducted by Weston and Jackson in 2009; it reported a 96-hr acute LC_{50} of 2.7 ng/L and an EC_{50} of 1.9 ng/L based on static, water-only exposures. The study was classified as supplemental but considered acceptable for quantitative use in the assessment (Weston & Jackson, 2009). There were 5 to 8 treatment levels with 3 replicates at each concentration. Each replicate contained 10 organisms aged 7-14 days. Concentrations of bifenthrin were measured at test initiation, although only summary results were provided for the three pyrethroids studied. The measured concentrations ranged from 64-189% of nominal

concentrations with a median of 114% for the 3 pyrethroids evaluated. Acetone was used as the co-solvent and a solvent control was included. The median survival in negative and solvent controls was 95% and 98% respectively (range: 84%-100%) for all six tests conducted of the three pyrethroids. Correspondence with the study author confirmed that control survival for bifenthrin was $\geq$ 88%. Endpoints used to determine the EC_{50} were measured and reported in terms of swimming impairment and mortality. Correspondence with the study author indicates that swimming impairment was severe and included a complete immobility except for limited movement of appendages and swimming in circles on the bottom of the test vessel[1]. These effects are therefore considered ecologically relevant and likely to result in organism mortality in the environment.

Waterflea *(Daphnia magna)*. For the waterflea, acute toxicity test results show bifenthrin is very highly toxic to aquatic invertebrates (MRID 132537); it was not used for RQ derivation in this assessment because the Weston and Jackson open literature study reported a more sensitive endpoint. It reported a 48-hr LC_{50} of 1.6 ppb. The study consisted of 6 treatment levels ranging from 0.5 ppb to 10 ppb with 80 test organisms per level. There was no mortality seen in the water or solvent control (DMF). The D.O. was reported to be between 82% and 90% with a pH of 8.3. Since the concentrations used in this study exceed the reported solubility of bifenthrin (0.014 ppb) and samples were not centrifuged prior to analysis, there is uncertainty as to the concentration of bifenthrin that was actually bioavailable to test organisms.

IV. Freshwater Invertebrates, Chronic

Waterflea *(Daphnia magna)*. For the waterflea, chronic toxicity test results show bifenthrin to have a NOAEC of 1.3 ng/L and a LOAEC of 2.9 ng/L based on significant reductions in length, mean young/adult/reproduction day; the study was classified as acceptable/core (MRID 41156501). The test was conducted using a flow-through system and consisted of 5 concentrations, a dilution water control and a solvent control with four replicates at each level. Each concentration level contained 40 organisms (10 per replicate). Concentrations ranged from 0.6 ng/L to 10 ng/L. The mean measured concentration ranged from 50% to 76% of expected nominal concentrations. Daphnid survival in the test concentrations was not significantly different from the pooled controls. The average time to first brood for the controls was 8 days; the time to first brood was significantly affected in the highest dose, increasing from 8 days in the first 4 levels to 16 days at the highest dose (mean measured concentration 7.6 ng/L). The mean young/adult reproduction day after 21 days was significantly affected in the two highest concentrations (mean measured concentrations 2.9 ng/L and 7.6 ng/L). Reproduction day decreased from 4.7 at level 1 to 2.1 and 1.8 respectively for level 4 and 5. Statistical analysis shows growth in the two highest concentrations (mean measured concentrations 2.9 ng/L and 7.6 ng/L) were significantly reduced from 4.1 mm to 3.6 mm and 3.2 mm respectively. .

V. Freshwater Benthic Invertebrates, Subchronic

Scud *(Hyalella azteca)*. For the scud, a 10-d sediment toxicity test was identified in the open literature and shows bifenthrin to have a 10-d NOAEC and LOAEC of 0.17 ng a.i./L and 0.34 ng a.i./L, respectively, based on pore water concentrations (Amweg *et al.* 2005). On the basis

[1] personal communication between Keith Sappington and Donald Weston, 10 October 2012.

of sediment concentrations normalized to organic carbon, the 10-d NOAEC is 40 µg/kg_{oc} and the 10-d LOAEC is 80 µg/kg_{oc}. This study is classified as supplemental but acceptable for quantitative use in risk assessment. . The subchronic toxicity test was conducted using EPA Agency protocols in 400 mL beakers with 3 replicates per concentration. Recovery of bifenthrin from sediment was very low (mean = 35%) despite analytical recoveries of near 100% on freshly spiked (no aging) sediments. Recoveries were also low (< 70%) for other pyrethroids studied. The study authors indicate they could not determine if this low recovery is due to degradation during the aging period, chemical loss due to adsorption on container walls, or an incomplete chemical extraction from the sediments. Toxicities reported in the study are based on measured concentrations adjusted for the mean analytical spike recovery. Control survival averaged 94% in negative controls and 95% in solvent (acetone) controls. At the LOAEC, mean reduction in growth ranged from 22% to 49% across three separate tests. Survival data were not reported for each treatment, however, the mean 10-d LC50 across all 3 bifenthrin tests is reported as 180 µg/kg_{oc}, indicating 50% lethality occurring within a factor of 2 of the growth-based NOAEC of 80 µg/kg_{oc}.

VI. Estuarine/Marine Fish, Acute

Sheepshead Minnow *(Cyprinodon variegatus).* For the sheepshead minnow, acute toxicity test results show bifenthrin to be very highly toxic to estuarine fish based on a 96-hr LC_{50} of 17.5 ppb; the study is classified as acceptable, core (MRID 163101). The test design included five test concentrations, a control and a solvent control (acetone). Each treatment had 2 replicates. Each treatment contained 10 minnows for a total of 20 organisms per treatment. The mean test concentrations > 17.2 µg/L ranged from 106% to 115% compared to nominal concentrations; mean measured test concentration <10.9 µg/L were found to range from 138% to 145% (higher than nominal concentrations). The aquarium shows a decrease in temperature to 2ºC below the test limit but did not affect the organisms in the chamber. The confidence limits were calculated as 14.7 µg/L to 21.8 µg/L with an LC_{50} of 17.5 µg/L; no adverse effects were observed at 5.24 µg/L. Since the concentrations used in this study exceed the reported solubility of bifenthrin (0.014 ppb) and samples were not centrifuged prior to analysis, there is uncertainty as to the concentration of bifenthrin that was actually bioavailable to test organisms.

VII. Estuarine/Marine Fish, Chronic

No studies were submitted to the Agency on the chronic toxicity of bifenthrin to estuarine/marine fish.

VIII. Estuarine/Marine Invertebrates, Acute

Eastern Oyster *(Crassostrea virginica).* For the Eastern oyster, acute toxicity test results show bifenthrin to be highly toxic to estuarine invertebrates with a 48-hr EC_{50} of 285 ppb (MRID 40383501). This study is classified as acceptable/core. The study was conducted using 7 nominal concentrations ranging from 0.77 ppm to 17 ppm with each test concentration and control triplicated with 370 embryos per container. Seawater and acetone were used as the water and solvent controls. Measured concentrations, with the exception of one treatment, were found to be between 10 to 25% of nominal concentrations; low concentrations may have been low

because of the low solubility and high adsorption of bifenthrin. Bifenthrin was found to be acutely toxic to embryos and larvae at >0.448 ppm. Since the concentrations used in this study exceed the reported solubility of bifenthrin (0.014 ppb) and samples were not centrifuged prior to analysis, there is uncertainty as to the concentration of bifenthrin that was actually bioavailable to test organisms.

Mysid Shrimp *(Americamysis bahia).* For the mysid shrimp, acute toxicity test results show bifenthrin to be very highly toxic to estuarine invertebrates based on a 96-hr LC_{50} of 3.97 ng/L; study is classified as acceptable/core (MRID 00163102). The study was conducted using a flow-through system with 5 test concentration and 20 mysids per treatment; the test design also included a control and solvent control (acetone). The mean measured concentrations averaged 77% to 117% of nominal concentrations during the testing. An LC_{50} of 3.97 ng/L was calculated, with a 95% confidence interval of 3.09-4.97 ng/L.

IX. Estuarine/Marine Invertebrates, Chronic

No studies were submitted to the Agency on the chronic toxicity of bifenthrin to estuarine/marine invertebrates.

X. Estuarine Benthic Invertebrates, Subchronic

Leptocheirus plumulosus. For the amphipod, *Leptocheirus plumulosus*, a NOAEC of 0.2 ng/L was reported based on estimated pore concentrations and a NOAEC of 122.0 $\mu g/kg_{oc}$ was reported based on organic carbon-normalized sediment concentrations (MRID 46591501). The study assessed survival and growth, but not reproduction; therefore, this study is classified as supplemental because it did not include reproduction as an assessed endpoint. The study was conducted using 6 test concentrations and an acetone solvent and negative control. The 28-d NOAEC and LOAEC for survival are 50 and 130 µg/kg sediment, respectively, based on mean measured sediment concentrations. The two highest treatments were excluded from statistical analysis of growth due to 100% mortality. The pore water endpoints were estimated from measure concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment and the mean K_{oc} due to uncertainty in the measured pore water concentrations with respect to bioavailable chemical. Due to the variation in reported K_{oc} for bifenthrin and assumptions of equilibrium partitioning, the estimated pore water endpoints are subject to uncertainty.

XI. Avian Oral, Acute

Bobwhite quail *(Colinus Virginianus)*. For the bobwhite quail, acute oral toxicity test results show bifenthrin to be slightly toxic to upland game species based on a 21-d LD_{50} of 1,800 mg/kg (MRID 132532). This study is classified as acceptable/core. A total of 5 test concentrations were used ranging from 464 mg/kg to 2,150 mg/kg with 10 organisms per treatment. Organisms were dosed using a gavage with corn oil as a vehicle. Information on the occurrence of sublethal effects was not available.

Mallard Duck *(Anas platyrhynchos).* For the mallard duck, acute oral toxicity test results show bifenthrin to be practically nontoxic to waterfowl based on a 21-d LD_{50} of 2,150 mg/kg (MRID

132534). This study is classified as acceptable/core. Two test concentrations were used with 10 organisms per level. No other effects-related information was available for this study.

XII. Avian Dietary, Subacute

Mallard Duck (*Anas platyrhynchos)*. For the mallard duck, subacute dietary toxicity test results show bifenthrin to be slightly toxic to waterfowl based on an 8-d LC_{50} of 1,280 ppm (MRID 132535). This study is classified as core/supplemental. A total of 5 test concentrations were used ranging from 312 ppm to 5,000 ppm. Other sublethal effects included a decrease in food consumption, reduced body weight gain, increased incidence of anorexia and tremors relative to the controls.

Bobwhite Quail (*Colinus virginianus)*. For the bobwhite quail, subacute dietary toxicity test results show bifenthrin to be slightly toxic to upland game species based on an 8-d LC_{50} of 4,450 ppm (MRID 132533). This study is classified as core/acceptable. A total of 5 test concentrations were used ranging from 312 ppm to 5,000 ppm with 10 organisms per treatment. No other effects-related information was available for this study.

XIII. Avian Reproductive, Chronic

Mallard Duck (*Anas platyrhynchos).* For the mallard duck, chronic, oral toxicity test results show that concentrations of bifenthrin up to 75 ppm do not result in significant impairment to reproduction in waterfowl (MRID 163099). ; This study is classified as core/acceptable. A total of 3 test levels were used: 25, 50, and 75 ppm with 2 males and 5 females per replicate and 2 replicates per treatment (48 males, 120 females total). Endpoints measured included timing of egg laying, egg production, egg shell thickness, embryo and chick mortality, and hatching rate. All treatments showed an overall increase in body weight but were determined to be statistically insignificant relative to controls. Variability in food consumption across treatments was also determined to be insignificant. A total of 4 bird deaths were recorded among all treatments? but these were determined not to be treatment-related. No treatment-related effects were observed for egg production, number of cracked or broken eggs, shell thickness and chick survival. A dose-related (but not statistically significant) increase in # eggs laid, % eggs hatched and % 14-day survivors was indicated in this study. However, while these data suggest that bifenthrin has some activity in relation to the reproductive physiology of the waterfowl, the effects are not considered a biologically significant impairment of reproduction.

Bobwhite Quail *(Colinus virginianus).* For the bobwhite quail, chronic, oral toxicity test results show that concentrations of bifenthrin up to 75 ppm do not result in significant adverse impairment to reproduction in upland game bird (MRID163097). This study is classified as core/acceptable. A total of 3 test levels were used: 25, 50 and 75 ppm with 10 males and 10 females per treatment (80 males, 80 females total). Endpoints measured included timing of egg laying, egg production, egg shell thickness, embryo and chick mortality, and hatching rate. No treatment-related effects were observed for body weight, food consumption, chick mortality, egg production, # cracked or broken eggs, egg shell thickness, embryo mortality. The study reviewer concluded that there was a significant effect on egg shell integrity at the highest doses and a dose-related pattern of stimulation to eggs laid, eggs hatched and 14-day survivors which suggest

that bifenthrin has some activity in relation to reproductive physiology of game birds. However, while these data suggest that bifenthrin has some activity in relation to the reproductive physiology of the quail, the effects are not considered a biologically significant impairment of reproduction.

XIV. Mammalian Oral, Acute

Sprague-Dawley Rat *(Rattus norvegicus)*. For the rat, acute oral toxicity test results show bifenthrin to be acutely moderately toxic to mammals with a LD_{50} of 53.8 mg/kg (MRID 132519). This study is classified as acceptable. A total of 6 test concentrations were used with 10 males and 10 females dosed per treatment. Symptoms of toxicity included death, clonic convulsions, tremors, ataxia, loss of muscle control, decreased activity, chromorhinorrhea, chromodacryorrhea and oral discharge. Signs of toxicity were observed from hour 3 to day 5. Rats showed an increase in weight over the course of the study. Male LD_{50} was calculated to be 70.1 mg/kg (95% confidence interval $\pm$13.04) and the female LD_{50} was calculated to be 53.8 (95% confidence interval $\pm$ 4.92).

XV. Mammalian Reproductive, Chronic

Rat *(Rattus norvegicus)*. A 2-generation reproduction study with rats resulted in a a LOAEL of 60 ppm and a NOAEL of 30 ppm based on dose-related lower weights in females during first and second lactation periods and second gestation (MRID 00157225). Other effects at the LOAEL included a significant decrease in mean absolute ovarian weight; the study is classified as acceptable, core. The study was conducted using 3 treatment levels and one control ranging from 30 ppm to 100 ppm with 25 males and 25 females per treatment. Exposure to chemical was through diet, using acetone as a solvent and incorporated with laboratory chow. No significant differences in mortality occurred among groups; survival ranged from 96-100% for P_1. Males tended to have slightly higher body weights at higher test concentrations; this was found to not be statistically significant. There were no statistically significant differences found in female mean body weights up through week 8; at week 17 mean body weights were below the controls. No statistically significant differences in mean maternal weights. There was no evidence of correlation between tremors and weight gain or loss. There was a 96-100% rate of survival in the F_1 generation; surviving males had a decrease in lower mean body weights but none that were statistically significant. The reviewer found the test to show no indications of any dose-related trends with respect to reproduction. There were no significant dose-related trends with respect to total number of pups delivered, pups per litter, live birth index, number of stillborns, or number of pups cannibalized. A statistically significant increase in still born pups for F_2a litter is accounted for based on machine malfunction. There were no significant dose-related differences between groups with relation to progeny survival for either the first or second litters.

XVI. Terrestrial Invertebrate Oral, Acute

Honey bee *(Apis mellifera)*. For the honeybee, acute contact toxicity test results show bifenthrin to be highly toxic to honeybees with a 96-h LD_{50} of 0.015 µg/bee; the study was classified as core and considered to be scientifically sound (Atkins, 1981). All procedures considered to be in compliance with EPA standards. Study was conducted using a bell-jar vacuum duster; chemical

was mixed with a pyrolite dust diluent. Bees are aspirated into dusting cages for exposure and are then transferred into holding cages for observation.