APPENDIX C: ZIRAM TOXICITY STUDIES

Toxicity studies used to assess the risk to aquatic organisms include the effects of Ziram on fish, aquatic invertebrates and aquatic plants.

Freshwater Fish: Acute Exposure (Mortality) Studies

1) (MRID 423863-03). An acute toxicity test for fish was completed by Douglas, M.T., R.O. Stonehewer and I.A. Macdonald (1991). The 96-hour acute flow-through toxicity test using Bluegill (*Lepomis macrochirus*) resulted in an LC_{50}=9.7 µg/l. Sub-lethal effects including lethargy, and lying on bottom of test vessel, surfacing and moribund fish were also observed. The results from this study classify Ziram as very highly toxic to fish.

2) (MRID 473079-01). An unpublished flow-through acute toxicity study using the Bluegill (*Lepomis macrochirus*) was completed September 27, 2007 by Palmer, Susan J., T.Z. Kendall and H.O. Krueger. The endpoints affected included mortality and sub-lethal effects (e.g., loss of equilibrium, lethargy, and lying on bottom of test vessel).

In a 96-h acute toxicity study, bluegill sunfish (*Lepomis macrochirus*) were exposed to Ziram at nominal concentrations of 0 (negative and solvent controls), 0.19, 0.32, 0.54, 0.90 and 1.50 mg ai/L under flow through conditions; the reviewer-calculated TWA concentrations were <0.0295 (<LOQ; controls), 0.19, 0.32, 0.55, 0.94 and 1.5 mg ai/L. The 96-h LC_{50} (and 95% C.I.) was 0.57 (0.32-0.94) mg ai/L. The NOAEC value, based on mortality and sub-lethal effects, was 0.32 mg ai/L; the reviewer was unable to determine an EC_{50} value, but assumed that it was greater than the NOAEC, based on sublethal effects data. Sub-lethal effects (e.g., loss of equilibrium, lethargy, and lying on bottom of test vessel) were observed in the groups exposed to 0.55, 0.94 and 1.5 mg ai/L of Ziram. Based on the results of this study, Ziram would be classified as highly toxic to *Lepomis macrochirus* in accordance with the classification system of the U.S. EPA.

3)MRID 423863-04. An acute toxicity test for fish was completed by Douglas, M.T., G. Bell and I.A. Macdonald (1991). Mean measured concentrations (0.17, 0.36, 0.75, 1.4, and 2.7mg/L) were used in the statistical analysis. The 96-hour acute flow-through toxicity test using Rainbow trout (*Oncorhynchus mykiss*) resulted in an LC_{50}=1700µg/l. Sub-lethal effects including increased pigmentation, lethargy, and lying on bottom of test vessel, and loss of equilibrium were also observed. The results from this study classify Ziram as practically non-toxic to fish.

The toxicity values classify Ziram as practically non-toxic (MRID 423863-04) to very highly toxic (MRID 423863-03). An acceptable acute freshwater fish study was submitted (MRID 423863-03) which resulted in an LC50=0.0097 mg/L. The results from an ecotox study classified as supplemental (ID5003523) resulted in an LC_{50}=0.008 mg/L for the fathead minnow. The most sensitive toxicity value for the fathead minnow (LC50=8µg/L) will be used in the assessment.

Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

An early-life-stage (MRID468931-04) unpublished study using the fathead minnow (*Pimephales promelas*) was performed by Wildlife International., Ltd., Easton, MD. This study was submitted July 18, 2006. Endpoint(s) affected include hatching success, post-hatch clinical signs of toxicity, and post-hatch survival. The most sensitive endpoint was post-hatch survival

The 33-day chronic toxicity of ziram to the early life stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs/embryos (80/level, <24 hours old) of fathead minnow were exposed to a mixture of radiolabeled plus unlabeled ziram at nominal concentrations of 0 (negative and solvent controls), 47, 94, 188, 375, and 750 µg ai/L. TWA concentrations were <7.26 (<LOQ, controls), 49, 101, 196, 395, and 750 µg total residues/L, respectively. The test system was maintained at 24.0-25.8 °C and a pH of 8.0-8.1. The 33-day EC_{50} was 218 µg ai/L for post-hatch survival and 590 µg total residues/L for hatching success. NOAEC and LOAEC values were 101 and 196 µg total residues/L, respectively, based on post-hatch larval survival.

Hatching occurred during days 3 to 5 at all levels, with no treatment-related effect observed. Hatching success, however, was statistically-reduced at the 750 µg total residues/L level compared to the negative control (10 versus 98%, respectively). All hatched larvae from the 750 µg total residues/L level died within 1 day post-hatch, and all hatched larvae from the 395 µg total residues/L level died within 21 days post-hatch. Organisms from these two levels were weak and/or small prior to death. Post-hatch larval survival was the most sensitive endpoint, with statistically-significant reductions from the negative control observed at the ≥195 µg total residues/L levels. On Day 33 (28 days post-hatch), larvae survival averaged 94% in the negative control group, compared to 88, 95, 97, and 72% in the solvent control and mean-measured 48, 101, and 195 µg total residues/L groups, respectively. No treatment-related effect on terminal growth was observed at up to 195 µg total residues/L.

Aquatic-phase Amphibian: Acute and Chronic Studies

Registrant submitted studies as well as open literature is reviewed to provide data to assess the effect of Ziram on aquatic-phase amphibians. No data is available for amphibians.

Freshwater Invertebrates: Acute Exposure Studies

An acute toxicity test for invertebrates was completed by Douglas, M.T., R.O. Stonehewer and I.A. Macdonald (1991). The 48-hour acute static toxicity test (MRID 423863-05) using *Daphnia magna* resulted in an EC_{50}=48 µg/l. The EC50 is based on concentrations above the detection limit for Ziram in water (3.4 µg/L).This classifies Ziram as very highly toxic to aquatic invertebrates. This toxicity value will be described in the risk estimation section.

Freshwater Invertebrates: Chronic Exposure Studies

A chronic freshwater invertebrate study was completed by Palmer, S.J., T.Z. Kendall, and H.O. Krueger (2006). The unpublished study performed by Wildlife International., Ltd., Easton, MD was submitted April 24, 2006.

The 21-day-chronic toxicity of ziram to *Daphnia magna* was studied under flow-through conditions. Daphnids were exposed to a mixture of radiolabelled and unlabelled ziram at nominal concentrations of 0 (negative and solvent controls), 9.4, 19, 38, 75, and 150 µg ai/L. TWA concentrations were <1.37 (<LOQ, controls), 9.2, 22, 39, 77, and 154 µg total residues/L, respectively. Length was the most sensitive endpoint, with significant reductions at the 77 and 154 µg total residues/L treatment levels. Based on this effect, the 21-day NOAEC and LOAEC were 39 and 77 µg total residues/L, respectively.

Endpoint(s) affected include first generation survival, reproduction, and terminal total lengths. Mortality was significantly-reduced at the 154 µg total residues/L test level compared to the negative control (45 versus 100% survival, respectively). In addition, the number of offspring produced per reproductive day was also significantly-reduced at the 154 µg total residues/L level compared to the negative control (4.29 versus 11.24 young per day, respectively), although there was no treatment-related effect on the time of first brood release (visually- determined). The 21-day LC_{50} for survival was 144 (115-261) µg total residues/L. The 21-day EC_{50} (with 95% C.I.) for reproduction was 130 (110-160) µg total residues/L.

Total length was the most sensitive endpoint, with statistically-significant reductions compared to the negative control at the 77 and 154 µg total residue/L levels (5.13 versus 4.80 and 4.77 mm, respectively). A similar effect on dry weight was not observed, with no statistically-significant differences at any treatment level.

Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether Ziram may affect primary production and the availability of aquatic plants as food for CRLF tadpoles. Primary productivity is essential for indirectly supporting the growth and abundance of the CRLF.

Aquatic Plants: Vascular Plants

To estimate the effect of Ziram on the aquatic habitat for the CRLF, aquatic vascular studies were reviewed. In a 7-day acute toxicity study, the freshwater floating aquatic vascular plants Duckweed (*Lemna gibba* G3) were exposed to Ziram Technical at nominal concentrations of 0 (negative and solvent controls), 0.019, 0.041, 0.090, 0.20, 0.44, 0.96, 2.1 and 4.6 mg/L under static renewal conditions.. The NOAEC and EC_{50} values based on biomass, the most sensitive endpoint, were 0.0351 and 0.37 mg/L, respectively. The percent growth inhibition, based on frond number, in the treated culture as compared to the control ranged from -1.7 to 72.5%. The percent growth

inhibition, based on biomass, in the treated culture as compared to the control ranged from -7.1 to 90.6%. Observed effects included breaking up of colonies, root destruction, small fronds, chlorosis and necrosis. Effects were isolated to the measured 0.178-4.52 mg/L treatment groups. The results from this toxicity study will be described in the risk estimation section of the assessment.

Aquatic Plants: NonVascular Plants

The toxicity value for Ziram for the non-vascular aquatic plant *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was EC50=67 µg/L (MRID438339-01). The toxicity value for Ziram will be described in the risk description section.

In addition to reviewing aquatic toxicity studies, terrestrial studies for Ziram are also evaluated. Representative species used to evaluate risk from pesticides include birds, mammals, terrestrial invertebrates and terrestrial plants.

Birds: Acute Exposure (Mortality) Studies

Results from the acute avian oral toxicity study with an LD50=97 mg/kg for the (*Colinus virginianus*) quail (MRID 417257-01) indicated that Ziram is moderately toxic to birds. This study was classified as acceptable and will be used in the CRLF assessment.

Results from an acute Ziram dietary toxicity study for (*Anas platyrhynchus*) mallard ducks (MRID 423863-002) resulted in an LC50=5156 mg/kg. This study was classified as acceptable. However, the acute oral toxicity value is more sensitive and will used in the assessment.

Birds: Chronic Exposure (Growth, Reproduction) Studies

A Ziram reproductive study using the mallard duck conducted by Temple, D.L., *et al.(*2007) was performed by Wildlife International Ltd., Easton, MD. The study was submitted September 25, 2007. Endpoint(s) affected included eggs set and percentage of eggs set to eggs laid, embryo viability (percentage of live 3-week embryos to viable embryos), hatchability (number of hatchlings, percentage of hatchlings to eggs laid, percentage of hatchlings to eggs set, and percentage of hatchlings to live 3-week embryos), and survival (14-day survivors and 14-day survivors to eggs set).

The one-generation reproductive toxicity of Ziram Phyto (ziram) to 16 pairs per level of 23-week old mallard duck (*Anas platyrhynchos*) was assessed over approximately 20 weeks. Ziram was administered to the birds in the diet at nominal concentrations of 0 (negative control), 7.5, 15, 30, and 60 mg ai/kg dw diet. Mean-measured concentrations were <0.01 (<LOQ, control), 5.9, 12, 29, and 64 mg ai/kg diet, respectively. The estimated daily dietary dose was 0.0, 1.0, 1.9, 4.1, and 8.1 mg ai/kg bw/day, respectively.

There were no treatment-related effects upon any adult parameter at any treatment level, or upon any offspring parameter at the 5.9, 12, or 29 mg ai/kg diet treatment levels. At

the 64 mg ai/kg diet level, there were statistically-significant reductions in parameters related to fecundity, embryo viability, hatchability, and offspring survival. Specifically, reductions were noted for eggs set (37% of control, $p<0.05$), eggs set to eggs laid (34% of control, $p<0.001$), live 3-week embryos of viable embryos (6% of control, $p<0.05$), number hatched (56% of control, $p=0.01$), number hatched of eggs laid (57% of control, $p<0.001$), number hatched of eggs set (39% of control, $p<0.05$), number hatched of live 3-week embryos (40% of control, $p<0.001$), 14-day survivors (57% of control, $p=0.01$), and 14-day survivors of eggs set (40% of control, $p<0.05$). Based upon these treatment-related effects on reproduction at the 64 mg ai/kg diet treatment level, the NOAEC was 29 mg ai/kg diet.

This toxicity study is classified as scientifically sound and does satisfy the guideline requirement for a mallard duck (*Anas platyrhynchos*) reproductive toxicity study.

Terrestrial-phase Amphibian Acute and Chronic Studies

Registrant submitted studies as well as open literature were reviewed for amphibian data. No toxicity data is available for terrestrial-phase amphibians.

Toxicity to Mammals

Mammalian toxicity data are used to assess potential indirect effects of Ziram to the terrestrial-phase CRLF. Effects to small mammals resulting from exposure to Ziram may also indirectly affect the CRLF via reduction in available food. As discussed in Section 2.5.3, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant, 1985).

Toxicity to Terrestrial Invertebrates

Terrestrial invertebrate toxicity data are used to assess potential indirect effects of ZIRAM to the terrestrial-phase CRLF. Effects to terrestrial invertebrates resulting from exposure to ZIRAM may also indirectly affect the CRLF via reduction in available food.

Toxicity studies submitted to be used to assess the risk to terrestrial invertebrates include studies for Ziram . A Ziram toxicity study for the honey bee (MRID 416679-01) was classified as acceptable. This study indicates that Ziram is practically nontoxic to bees based on the LD50>100 μg/bee. The toxicity value for Ziram will be used in the risk estimation section.

Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for Ziram to affect riparian zone and upland vegetation within the action area for the CRLF. Impacts to riparian and upland (i.e., grassland, woodland) vegetation may result in indirect effects to both aquatic- and terrestrial-phase CRLFs, as well as modification to designated critical habitat PCEs via increased sedimentation, alteration in water quality, and reduction in of

upland and riparian habitat that provides shelter, foraging, predator avoidance and dispersal for juvenile and adult CRLFs.

Plant toxicity data from both registrant-submitted studies and studies in the scientific literature were reviewed for this assessment. Registrant-submitted studies are conducted under conditions and with species defined in EPA toxicity test guidelines. Sub-lethal endpoints such as plant growth, dry weight, and biomass are evaluated for both monocots and dicots, and effects are evaluated at both seedling emergence and vegetative life stages. Guideline studies generally evaluate toxicity to ten crop species.

A Seedling Emergence toxicity test (MRID 468931-01) for monocots resulted in an EC25 >6lbs/A NOAEC=6 lbs/A. No treatment effects were observed for the highest concentration. This study was classified as acceptable.

A Seedling Emergence toxicity test (MRID 468931-01) for dicots resulted in an EC25 >6lbs/A NOAEC=6 lbs/A. No treatment effects were observed for the highest concentration. Most sensitive dicot was soybean based on 16% reduction relative to negative control. This study was classified as acceptable.

A vegetative vigor toxicity test (MRID 468931-02) for monocots resulted in an EC25 >6lbs/A NOAEC<6 lbs/A. Most sensitive monocot was rye grass with a 13% reduction in comparison to the control. No treatment effects were observed for the highest concentration. This study was classified as acceptable.

A vegetative vigor toxicity test (MRID 468931-02) for dicots resulted in an EC25 >6lbs/A NOAEC=6 lbs/A. No treatment effects were observed for the highest concentration. Most sensitive dicot was tomato with a 12% reduction in comparison to the control. This study was classified as acceptable.

The results of the Tier II seedling emergence and vegetative vigor toxicity tests on non-target plants are summarized in 5.1.2.3**.**

Although toxicity data for terrestrial plants is available for the parent Ziram, no terrestrial plant studies were requested for Thiram. Therefore risk to terrestrial plants for this assessment are based on Ziram toxicity values.