APPENDIX D: THIRAM TOXICITY STUDIES

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

Freshwater Fish: Acute Exposure (Mortality) Studies

There are several registrant submitted freshwater fish studies. One study (MRID070801) using Bluegill (*Lepomis macrochirus*) resulted in a 96-h LC50=42 µg/l. This study was classified as acceptable. This classifies Thiram as very highly toxic.

Another study (BAOTH102) using Bluegill (*Lepomis macrochirus*) resulted in a 96-h LC50=280 µg/l This study was classified as supplemental. This classifies Thiram as highly toxic.

A freshwater fish study (MRID 0090293) using Rainbow Trout (*Oncorhynchus mykiss*) resulted in a 96-h LC50=500 µg/l. This study was classified as supplemental. This classifies Thiram as highly toxic.

A freshwater fish study (MRID BAOTH102) using Rainbow Trout (*Oncorhynchus mykiss*) resulted in a 96-h LC50=130 µg/l This study was classified as acceptable. This classifies Thiram as highly toxic.

One study (MRID 00034713) using Harlequinn Fish (*Rasbar heteromorpha*) resulted in a 96-h LC50=7 µg/l. This study was classified as acceptable. This classifies Thiram as very highly toxic.

Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

Due to the absence of chronic data for Thiram, the acute-to-chronic (ACR) method will be used to estimate a chronic value. The chronic Thiram NOAEC is based on [the Ziram chronic fish NOAEC=101 µg/L divided by the fish LC50=8 µg/L] multiplied by the Thiram acute fish LC50=42 µg/L. Therefore, the Thiram chronic fish NOAEC is 530.25 µg/l. This calculation using this value will be described in the risk estimation section of the assessment.

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 Thiram on aquatic-phase amphibians. No data is available for amphibians.

Freshwater Invertebrates: Acute Exposure Studies

Thiram's effect on aquatic invertebrates was used as an indirect measure effecting survival, growth and reproduction. A 48-h toxicity study (MRID 164662) was classified

as acceptable. The *Daphnia* EC50=210 µg/L classifies the chemical as highly toxic to aquatic invertebrates.

Freshwater Invertebrates: Chronic Exposure Studies

Due to the absence of chronic data for Thiram, the acute-to-chronic (ACR) method will be used to estimate a chronic value for aquatic invertebrates. The chronic Thiram NOAEC is based on [the Ziram chronic *Daphnia* NOAEC=39 µg/L divided by the *Daphnia* LC50=48 µg/L] multiplied by the Thiram acute *Daphnia* EC50=210 µg/L. Therefore, the Thiram chronic *Daphnia* NOAEC is 170.6 µg/l. This calculation using this value will be described in the risk estimation section of the assessment.

Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether Thiram 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

In a 7-day acute toxicity study, freshwater floating aquatic vascular plants Duckweed, *Lemna gibba* G3, was exposed to Thiram Technical under static conditions. Measured concentrations at test initiation were 0.057, 0.179, 0.391, 0.798, 2.05, and 5.22 mg a.i/L under static conditions. The EC_{50} value based on frond number was 1.6 mg a.i./L and the NOAEC was lower than the lowest concentration tested, 0.057 mg a.i./L, because frond number was significantly reduced at all test concentrations. The % growth inhibition in the treated culture ranged from 9.5 to 89%, compared to the pooled control. Abnormalities occurred in all treatment groups and included chlorosis and necrosis. A large increase in frond death was observed in the highest treatment group (5.22 mg a.i./L).

This toxicity study is scientifically sound and it satisfies the guideline requirements for an acute toxicity study with aquatic vascular plants. This study is classified as Core.

Aquatic Plants: NonVascular Plants

A 120-hour static Algal growth inhibition toxicity study using *Selenastrum capricornutum* (MRID 440861-01) was completed on July, 1992 by Huntingdon Research Centre, Cambridgeshire, England An EC_{50}: 0.14 ppm ai (CI= 0.07 - 0.26 ppm ai) and an EC_{05}: 0.003 ppm ai (CI= 0007 -0.0127 ppm ai) using initial measured concentrations. This study was classified as acceptable.

In addition to reviewing aquatic studies, terrestrial toxicity studies are also reviewed. Toxicity studies for birds, mammals, terrestrial invertebrates and terrestrial plants for Thiram are described below.

Toxicity to Birds

Birds: Acute Exposure (Mortality) Studies

Toxicity Studies for Thiram are also used to support lines of evidence.
Four acute toxicity studies are reviewed for Thiram. The first study (MRID BAOTH103) using the Ring-neck pheasant (*Phasianus colchicus*) resulted in an LD50=673 mg/kg. Based on the LD50, this supplemental study is classified as slightly toxic.

The second acute avian study (MRID BAOTH103) using the Mallard Duck (*Anas platyrhynchos*) resulted in an LD50>2800mg/kg. Based on the LD50 this study is classified as practically non-toxic to birds.

The third acute avian study (MRID 075683) using the Red-wing Blackbird (*Agelaius phoeniceus*) resulted in an LD50>100 mg/kg. Based on the LD50, this study is classified as moderately toxic to birds.

The fourth acute avian study (075683) using the Starling (*Sturnus vulgatis*) resulted in an LD50>100 mg/kg. This study was classified as supplemental due to using an alternate species. Based on the LD50, this study is classified as moderately toxic to birds.

Acute avian LD50s range from >100 mg/kg for the Red-wing Blackbird and Starling (MRID 075683) to >2800 mg/kg for the mallard (MRID BAOTH103).

Birds: Chronic Exposure (Growth, Reproduction) Studies

Toxicity Studies for the Major Degradate Thiram will be used in this assessment due to the persistence of the degradate. The one-generation reproductive toxicity of Thiram Technical to groups (16 pens/treatment level) of 1 male and 1 female of 17-week-old mallard ducks was assessed over approximately 23 weeks. Thiram was administered to the birds in the diet at measured concentrations of 0, 2.43, 9.61, and 39.7 mg ai/kg bw diet. There were significant reductions in several reproductive parameters at the highest treatment level, including eggs set, viable embryos, live 3-week embryos, normal hatchlings, 14-day old survivors, eggs set/eggs laid, normal hatchlings/live 3-week embryos, and normal hatchlings/eggs laid. As a result, the NOAEC was determined to be 9.6 mg/kg a.i. This study (MRID 45441202) is classified as Acceptable and will be used in the assessment.

Terrestrial-phase Amphibian Acute and Chronic Studies

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

Terrestrial Invertebrates: Acute Exposure (Mortality) Studies

Toxicity studies submitted to be used to assess the risk to terrestrial invertebrates include studies for the degradate Thiram. An acceptable toxicity study (MRID 0003635) resulted in an LD50=73.72 µg/bee. The Thiram toxicity value (LD50=73.72 µg/bee) will be reported in the risk characterization section as an additional line of evidence.

Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for Thiram 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.

No terrestrial plant studies were requested for Thiram. Therefore risk to terrestrial plants for this assessment is based on Ziram toxicity values.