## Appendix G. Ecological Effects Data

### a. Toxicity to Terrestrial Animals (see Appendix J for mammalian data)

### Birds, Acute and Subacute

An acute oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the toxicity of bensulide to birds. The preferred test species is either mallard duck (a waterfowl) or bobwhite quail (an upland gamebird). A single dose oral test with the bobwhite quail found the $LD_{50}$**,** of bensulide (92.9 % purity) was 1386 mg a.i./kg-bw. This classifies bensulide as slightly toxic on an acute oral basis. This study is classified as core and fulfills guideline 71-1 (Grimes 1986, MRID 158455). Two subacute dietary studies using the TGAI are required to establish the toxicity of bensulide to birds. The preferred test species are mallard duck and bobwhite quail. Results of these tests are tabulated below.

| Avian Subacute Dietary Toxicity | | | | | |
|---|---|---|---|---|---|
| Species | % a.i. | Toxicity Value | Toxicity Category | MRID No. Author/Year | Study Classification |
| Northern Bobwhite quail *(Colinus virginianus)* | 92.9 | 5-Day LC50 > 5620 mg a.i./kg-diet | Practically nontoxic | 158456 Grimes, 1986 | Acceptable |
| Mallard duck **(***Anas platyrhynchos***)** | 92.9 | 5-Day LC50 > 5620 mg a.i./kg-diet | Practically nontoxic | 158457 Grimes, 1986 | Acceptable |

In both subacute dietary tests, there was no mortality or overt signs of toxicity at the highest test concentration of 5620 mg a.i./kg-diet. Since the $LC_{50}$ is greater than 5000 mg a.i./kg-diet, bensulide is practically nontoxic to birds on a subacute dietary basis. The guideline (71-2) is fulfilled (MRIDs 158456 and 158457).

### Birds, Chronic

Avian reproduction studies using the TGAI are required for bensulide because the following conditions are met: (1) birds may be subject to repeated or continuous exposure to the pesticide, especially preceding or during the breeding season, (2) the pesticide is stable in the environment to the extent that potentially toxic amounts may persist in animal feed, (3**)** the pesticide is stored or accumulated in plant or animal tissues, and, (4) information derived from mammalian reproduction studies indicates reproduction in terrestrial vertebrates may be adversely affected by the anticipated use of the product, The preferred test species are mallard, duck, and bobwhite quail. Results of these tests are tabulated below.

| Avian Chronic Reproduction Toxicity | | | | | | |
|---|---|---|---|---|---|---|
| Species | % a.i. | NOAEC mg a.i./kg-diet | LOAEC mg a.i./kg-diet | Toxicity Category | MRID No. Author/Year | Study Classification |
| Mallard duck **(***Anas platyrhynchos***)** | 92.3 | 2.5 | 25 | Eggshell thickness | 444869-01 Mancell and Cameron, 1998 | Acceptable |
| Northern Bobwhite quail *(Colinus virginianus)* | 92.4 | 250 | 600 | Several endpoints concernin g the hatching and survival of chicks | 436160-01 Beavers *et al.*, 1995 | Acceptable |
| Mallard duck **(***Anas platyrhynchos***)** | 92.4 | Not determined (< 250) | 250 | Eggshell thickness, percent of eggs laid that are cracked percent of hatchlings that survived to 14 days | 436160-02 Beavers *et. al.*, 1995 | Supplemental |

These studies show that bensulide can impair avian reproduction at relatively low dietary concentrations. The most serious effect appears to be a reduction of eggshell thickness, which begins to occur at dietary concentrations between 2.5 and 25 ppm a.i. At 250 ppm a.i., the reduction in eggshell thickness was severe **(**11% reduction in MRID 44486901 and 15 % in MRID 43616002**)** which resulted in a significant increase in the number of cracked eggs (MRID 44486901 and 43616002**).** Compared to the control, the percentage of eggs cracked at 250 ppm a.i. represented a 2. 1x increase in MRID 44486901 and a 24.2X increase MRID 43616002**.** Cracking of eggs usually causes the embryo to die before hatching. Additionally, a dietary concentration of 250 ppm a.i. fed to mallards reduced the percentage of eggs hatched and the percent survival to the 3-week embryo and 14-day-old chick stages (MRID 44486901 and 43616002).
The guidelines for avian reproduction testing with an upland gamebird (71-4a) and with a waterfowl (71-4b) have been fulfilled (MRIDs 44486901 and 43616001).

**Insects**

Atkins *et al*. (1975) found that the honey bee acute contact $LD_{50}$ is >24.17 micrograms bensulide per bee. This result indicates that bensulide is practically non-toxic to bees on an acute contact basis. The guideline (141-1) is fulfilled (MRID 00018842).

**b. Toxicity to Aquatic Animals**

**Freshwater Fish, Acute**

Three freshwater fish acute toxicity studies using the TGAI are required to establish the toxicity of bensulide to fish. The preferred test species are rainbow trout (a coldwater fish) and bluegill sunfish (a warmwater fish). Results of these tests are tabulated below.

In addition, one study testing the effects of bensulide on rainbow trout was classified as invalid due to the presence of precipitates in some test solutions (MRID 157316).

| Freshwater Fish Acute Toxicity | | | | | |
|---|---|---|---|---|---|
| Species | % a.i. | 96-hr LC50 | Toxicity Category | MRID No. Author/Year | Study Classification |
| Rainbow trout (*Oncorrhynchus mykiss*) | 92.9 | 1.1 mg a.i./L | Moderately to highly toxic | 157315 McAllister et al. 1986 | Acceptable |
| Rainbow trout (*Oncorrhynchus mykiss*) | 95.0 | 0.72 mg a.i./L | Highly toxic | 400980-01 Mayer and Ellersieck 1986 | Supplemental. Raw data not available. |
| Bluegill sunfish (*Lepomis macrochirus*) | 95.0 | 0.81 mg a.i./L | Highly toxic | 400980-01 Mayer and Ellersieck 1986 | Supplemental. Raw data not available. |

**Freshwater Fish, Chronic**

A freshwater fish early life-stage test using the TGAI is required for bensulide because (1) the end-use product is expected to be transported to water from the intended use site, (2) aquatic acute fish $LC_{50}$s and the Waterflea $EC_{50}$ are less than 1 mg/l, and (3) the EEC in water is equal to or greater than 0.01 of acute $LC_{50}$, and $EC_{5O}$ values. A further factor that triggers this test is that bensulide is persistent in water. The preferred test species is the rainbow trout.

There is one acceptable fish chronic toxicity study available (See table below; MRID 447204-08). This study is an "Early Life-Stage Toxicity Test of the Fathead, Pimephales promelas, Under Flow-through Conditions". The results of the study demonstrate a NOAEC of 0.374 mg a.i./L based on larval growth and survival.

| Freshwater Fish Early Life-stage Toxicity | | | | | | |
|---|---|---|---|---|---|---|
| Species | % a.i. | NOAEC | LOAEC | Effect | MRID No. Author/Year | Study Classification |
| Fathead minnow (*Pimephales promelas*) | 93.4 | 0.374 mg a.i./L | 0.789 mg a.i./L. | Larval growth and survival | 447204-08 | Acceptable |

**Freshwater Invertebrates, Acute**

A freshwater aquatic invertebrate toxicity test using the TGAI is required to establish the toxicity of bensulide to aquatic invertebrates. The preferred test species is *Daphnia magna*. Results of this test are tabulated below.

| Freshwater Invertebrate Acute Toxicity | | | | | |
|---|---|---|---|---|---|
| Species/Test Type | % a.i. | $LC_{50}$ | Toxicity Category | MRID No. Author/Year | Study Classification |
| Waterflea (*Daphnia magna*) | 92.9 | 0.58 mg a.i./L | Highly toxic | 159322 Forbis, Burgess and Frazier, 1985 | Supplemental[1] |
| Amphipod (*Gammarus fasciatus*) | 95.0 | 3.3 mg a.i./L (48-hr) 1.4 mg a.i./L(96-hr) | Moderately toxic | 400980-01 Mayer and Ellersiek 1996, also 05001497 Sanders, 1970 | Supplemental[2] |

**Note: 1 The dissolved oxygen at the four highest test concentrations were unacceptably low (27.2-48.9%).**
**Note: 2 These $LC_{50}$s are for mature organisms. The test procedure deviated significantly from the guidelines.**

Acceptable toxicity data on the effects of bensulide to freshwater invertebrates are lacking. The data from MRID 05001497, which were also reported in MRID 400980-01, are from a study that is scientifically sound but was not conducted according to EPA's test guidelines. Also, the study was a test of adult organisms, whereas the EPA test guidelines require testing with immature organisms that are usually more sensitive to toxicants. Results from the study with the Waterflea **(**MRID 159322) are uncertain because the low dissolved oxygen at the higher dose levels could have contributed to the observed mortality. However, since the, dissolved oxygen problem probably reduced the observed $LC_{50}$ this value can be used to give a conservative (*i.e.*, possibly overprotective) assessment of risk. Based on supplemental data, the $LC_{50}$, falls in the range of 0.1 to 1.0 ppm, classifying bensulide as highly toxic to freshwater invertebrates on an acute basis. The guideline, 72-2 is not fulfilled.

**Freshwater Invertebrates, Chronic**

A freshwater aquatic invertebrate life-cycle test using the TGAI is required for bensulide because the end-use product is expected to be transported to water from the intended use site, aquatic acute fish $LC_{50}$s and the Waterflea $EC_{50}$ are less than 1 mg/l, and the EEC in water is equal to or greater than 0.01 of acute $LC_{50}$ and $EC_{50}$ values. A further factor that triggers this test is that bensulide is persistent in water. The preferred test species is *Daphnia magna.*

A chronic daphnid study was submitted and classified as supplemental because the NOAEC was non-definitive (<4.2 ppb a.i.), as effects occurred at the lowest test concentration (MRID 450634-01). The study was also evaluated under MRID 447204-07.

Another chronic daphnid study was submitted in 2011 and classified as invalid due to potential solvent interference (there was a significant difference (p=0.015) between the

negative control (9.6 neonates per day) and the solvent control (7.0 neonates per day)) (MRID 485043-01).

**Estuarine/marine Fish, Acute**

There are two registrant-submitted study testing bensulide, TGAI, toxicity to marine estuarine fish. One species tested was the spot, *Leiostomus xanthurus* (MRID 402284-01). The Agency's review of the study deemed it as supplemental. Based on the results of the study, bensulide is classified as highly toxic to marine estuarine fish ($LC_{50}$ = 0.32 mg a.i./L). The sheepshead minnow, *Cyprinodon variegatus*, was also tested (MRID 427502-01) with bensulide (92% a.i.). The study was classified as acceptable and yielded an $LC_{50}$ of 0.56 mg a.i./L (NOAEC = 0.282 mg a.i./L).

| Estuarine/marine Fish Acute Toxicity | | | | |
|---|---|---|---|---|
| Species | Toxicity Value | Toxicity Category | MRID No. Author/Year | Study Classification |
| Spot (*Leiostomus xanthurus* ) | 96-hr $LC_{50}$ = 0.32 mg a.i./L | Highly toxic | 402284-01 Mayer, 1986 | Supplemental |
| Sheepshead minnow (*Cyprinodon variegatus*) | 96-hr $LC_{50}$ = 0.56 mg a.i./L | Highly toxic | 427502-01 Morrow and Ward, 1993 | Acceptable |

**Estuarine/marine Invertebrates, Acute**

An acute toxicity study evaluating the effects of bensulide to mysid shrimp (92% a.i.) determined that bensulide is very highly toxic to estuarine/marine invertebrates with an $LC_{50}$ of 0.0624 mg a.i./L (MRID 427502-03). This study was classified as acceptable. An acute toxicity study evaluating the effects of bensulide on the shell deposition of eastern oysters produced an $EC_{50}$ of 0.25 mg a.i./L and a NOAEC of <0.178 mg a.i./L (MRID 427502-02). The study was classified as acceptable.

| Estuarine/marine Invertebrate Acute Toxicity | | | | |
|---|---|---|---|---|
| Species | Toxicity Value | Toxicity Category | MRID No. Author/Year | Study Classification |
| Mysid (*Americamysis bahia*) | 96-hr $LC_{50}$ = 0.0624 mg a.i./L NOAEC = 0.0373 mg a.i./L | Very highly toxic | 427502-03 (Morrow and Ward, 1993) | Acceptable |
| Eastern oyster (*Crassostrea virginica*) | 96-hr $EC_{50}$ = 0.25 mg a.i./L NOAEC < 0.178 mg a.i./L | Highly toxic | 427502-02 (Morrow and Ward, 1993) | Acceptable |

**Estuarine/marine Invertebrates, Chronic**

A chronic life cycle toxicity study (MRID 484245-01) that evaluated the effects of bensulide on the mysid (*Americamysis bahia*) was submitted in 2011. The study was determined to be supplemental based on potential solvent interference at 14 days.

However, the solvent effect was not observed at 28 days, and no effects were observed at 28 days. Therefore, the NOAEC for the study was 0.0485 mg a.i./L and the LOAEC was >0.0485 mg a.i./L. The NOAEC determined from this study will be used for quantitative risk assessment.

| Estuarine/marine Invertebrate Chronic Toxicity | | | | |
|---|---|---|---|---|
| Species | Toxicity Value | Toxicity Category | MRID No. Author/Year | Study Classification |
| Mysid (*Americamysis bahia*) | 48-hr NOAEC = 0.0485 mg a.i./L LOAEC > 0.0485 mg a.i./L (no effects) | Not applicable | 484245-01 (Gerke, 2011) | Supplemental. There was a significant difference between the solvent control and the negative control for the 14-day male length endpoints. However, at 28 days, this difference was not observed. |

**c. Toxicity to Plants**

**Toxicity to Terrestrial Plants**

The registrant has submitted a vegetative vigor and seedling emergence study testing the bensulide formulation 4LF (MRID 447463-01). The results of the vegetative vigor study demonstrated that cucumber is the most sensitive species tested of all the species tested. The cucumber yielded an $EC_{25}$ of 1.3 lb a.i. /A and a NOAEC of 0.38 lb a.i. /A. The endpoints of all the species tested in this study are listed below.

| Terrestrial Plant Toxicity Based on the Registrant Submitted Vegetative Vigor Study MRID 447463-01 | | | |
|---|---|---|---|
| Species | Parameter | $EC_{25}$ (lb a.i./A) | NOAEL (lb a.i./A) |
| Carrot | All parameters similar | > 6.0 | 6.0 |
| Cucumber | Phytotoxicity | 1.3 | 0.38 |
| Lettuce | Phytotoxicity = Shoot fresh weight | > 6.0 | 1.5 |
| Radish | Phytotoxicity | > 6.0 | 1.5 |
| Soybean | Phytotoxicity | 1.5 | 0.38 |
| Tomato | Phytotoxicity | > 6.0 | 3.0 |
| Corn | Shoot Fresh Weight | > 6.0 | 0.75 |
| Oat | All Parameters Similar | > 6.0 | 6.0 |
| Onion | All Parameters similar | > 6.0 | 6.0 |
| Ryegrass | All Parameters Similar | > 6.0 | 6.0 |

The results of the seedling emergence study demonstrate that the most sensitive species tested was ryegrass. Ryegrass yielded an $EC_{25}$ of 1.9 lb a.i. /A. and a NOAEC of 0.38 lb a.i. /A. The endpoints of all the species tested in this study are demonstrated in the table below.

| Terrestrial Plant Toxicity Based on the Registrant Submitted Seedling Emergence Study MRID 447463-01 | | | |
|---|---|---|---|
| Species | Parameter | $EC_{25}$ (lb a.i./A) | NOAEL (lb a.i./A) |
| Carrot | All parameters similar | > 6.0 | 6.0 |
| Cucumber | Shoot fresh weight | > 6.0 | 1.5 |
| Lettuce | All Parameters similar | > 6.0 | 6.0 |
| Radish | All Parameters similar | > 6.0 | 6.0 |
| Soybean | All Parameters Similar | > 6.0 | 6.0 |
| Tomato | All Parameters Similar | > 6.0 | 6.0 |
| Corn | Phytotoxicity | 5.0 | 1.5 |
| Oat | All Parameters Similar | > 6.0 | 6.0 |
| Onion | Shoot fresh weight | 3.2 | 0.75 |
| Ryegrass | Phytotoxicity | 2.1 | 0.38 |

**Toxicity to Aquatic Plants**

The registrant has submitted three acceptable aquatic nonvascular plant studies and one supplemental vascular plant study testing bensulide, TGAI. The three acceptable studies tested three species of nonvascular plants including green algae, (*Pseudokirchneriella subcapitata*), marine diatom (*Skeletonema costatum*), blue-green algae (*Anabaena flos-aquae*) and duckweed (*Lemna gibba*) (MRID 447204-02, MRID 447204-05, MRID 447204-03, and MRID 447204-06, respectively). The endpoints of these studies were $EC_{50s}$ of 1.8 mg a.i./L (NOAEC = 0.93 mg a.i./L), 0.78 mg a.i./L (NOAEC = 0.635 mg a.i./L), >3.58 mg a.i./L, and 0.14 mg a.i./L (NOAEC < 0.0421 mg a.i./L; $EC_{05}$ = 0.017 mg a.i./L), respectively.

In addition, the registrant submitted a study evaluating the effects of bensulide on the freshwater diatom (*Navicula pelliculosa*), but the study was classified as invalid because the test concentrations were not properly chosen (MRID 447204-04).

| Aquatic Plant Toxicity | | | |
|---|---|---|---|
| Species | $EC_{50}$ | MRID No. Author/Year | Study Classification |
| blue-green algae (*Anabaena floss-aquae*) | > 3.58 mg a.i./L | 447204-03 | Acceptable |
| green algae (*Pseudokirchneriella subcapitata*) | 1.8 mg a.i./L | 447204-02 | Acceptable |
| marine diatom (*Skeletonema costatum*) | 0.78 mg a.i./L | 447204-05 | Acceptable |
| duckweed (*Lemna gibba*) | 0.14 mg a.i./L | 447204-06 | Supplemental |