US Environmental Protection Agency
Office of Pesticide Programs

APPENDIX A ECOLOGICAL EFFECTS DATA

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a. Toxicity to Terrestrial Animals

i. 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. 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.	5-Day LC50 (ppm a.i.)	Toxicity Category	MRID No. Author/Year	Study Classification
Northern Bobwhite quail ***(Colinus virginianus)***	92.9	> 5620 ppm	Practically nontoxic	158456 Grimes 1986	Acceptable
Mallard duck **(***Anas platyrhynchos***)**	92.9	> 5620 ppm	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 ppm. Since the LC_{50}, is greater than 5000 ppm, bensulide is practically nontoxic to birds on a subacute dietary basis. The guideline (71-2) is fulfilled (MRIDs 158456 and 158457).

ii. 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 Subacute Dietary Toxicity						
Species	% a.i.	NOAEL (ppm a.i.)	LOAEL (ppm a.i.)	Toxicity Category	MRID No. Author/Year	Study Classification
Mallard duck **(***Anas platyrhynchos* **)**	92.3	2.5	25	Eggshell thickness	44860901 (Mancell and Cameron 1998)	Acceptable
Northern Bobwhite quail ***(Colinus virginianus)***	92.4	250	600	Several endpoints concernin g the hatching and survival of chicks	43616001 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	4361002 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).

iii. Mammals, Acute and Chronic

Wild mammal testing is required on a case-by-case basis, depending on the results of lower tier laboratory mammalian studies, intended use pattern and pertinent environmental fate characteristics. In the case of bensulide, however, rat or mouse

toxicity values obtained from the Agency's Health Effects Division (HED) substitute for wild mammal testing. A single-dose oral LD_{50}, study (MRID 92005011) was performed in which technical bensulide (92.5 % pure) was administered to the laboratory rat (*Rattus norvegicus)***.** The LD_{50} was 360 mg/kg for males and 270 mg/kg for females. Since the rat LD_{50}, falls within the range of 51 to 500 mg/kg, bensulide is moderately toxic to small mammals on an acute oral basis. The acute toxicity of bensulide appears to be considerably greater for mammals than, for birds.

For the purpose of assessing the risk of chronic ecological effects in mammals, the NOEL is 150 ppm and the LOEL is 900 ppm (See table below). Rats fed 900 ppm of bensulide had decreased pup survival, whereas rats fed 150 ppm had no effects on reproduction: plasma cholinesterase activity was significantly reduced compared to control at dietary concentrations as low as 23 mg/kg/day. No developmental effects were observed in rats administered oral doses as great as 95 mg/kg/day, which is approximately equivalent to 1900 ppm in the diet **(**MRID 00146585).

Chronic Mammalian Toxicity							
Species	% a.i.	Test Type	NOAEL a.i. (ppm)	LOAEL a.i. (ppm)	Toxicity Endpoint	MRID No. Author/Year	Study Classification
Lab rat *(Rattus norvegicus)*	92.4	Multi. Gen Repro.	> 900	---	Systemic effects,	43948701	Acceptable
			150	900	F2 pup survival		
			---	25	Cholinesterase inhibition		

iv. Insects

Atkins *et al*. (1975) found that the honey bee acute contact LD is 1.6 micrograms bensulide per bee. This result indicates that bensulide is highly toxic to bees on an acute contact basis. The guideline (141-1) is fulfilled (MRID 00036935). Although the acute contact study indicated that honeybee LD_{50}**,** of bensulide is less than 11 micrograms per bee, the Agency is waiving the requirement for a toxicity of residues on foliage study with honeybees (GLN 141-2). Bensulide is applied as a spray only to bare ground (vegetables uses) or to turf. These uses are expected to result in little exposure to flowering plants, thus exposure to bees is expected to be minimal.

Toxicity to Freshwater Aquatic Animals -

i. Freshwater Fish, Acute

Two 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.

Avian Subacute Dietary Toxicity					
Species	% a.i.	5-Day LC50 (ppm a.i.)	Toxicity Category	MRID No. Author/Year	Study Classification
Rainbow trout (*Oncorrhynchus mykiss*)	92.9	1.1	Moderately to highly toxic	157315 McAllister et al. 1986	Acceptable
Rainbow trout (*Oncorrhynchus mykiss*)	95.0	0.72	Highly toxic	40098001 Mayer and Ellersieck 1986	Supplemental
Bluegill sunfish (*Lepomis macrochirus*)	95.0	0.81	Highly toxic	40098001 Mayer and Ellersieck 1986	Supplemental

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 very persistent in water (hydrolysis half-life is 220 days). The preferred test species is the rainbow trout.

There is one acceptable fish chronic toxicity study available to access the potential risk to the CRLF (See table below; MRID 447204). 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 ppm a.i. based on larval growth and survival.

Avian Subacute Dietary Toxicity						
Species	% a.i.	NOAEL	LOAEL	Effect	MRID No. Author/Year	Study Classification
Fathead minnow (*Pimephales promelas*)	93.4	374 ppb a.i.	789 ppb a.i.	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} (ppm a.i.)	Toxicity Category	MRID No. Author/Year	Study Classification
Waterflea (*Daphnia magna*)	92.9	0.58	Highly toxic	159322 Forbis, Burgess and Frazier, 1985	Supplemental[1]
Amphipod (*Gammarus fasciatus*)	95.0	3.3 (48-hr) 1.4 (96-hr)	Moderately toxic	40098001 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,'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 40098001, 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.

iv. Freshwater Invertebrate 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_{5O} 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 very persistent in water (hydrolysis half-life is 220 days). The preferred test species is *Daphnia magna.*

Currently, there are no valid freshwater invertebrate chronic toxicity studies available. The only registrant submitted invertebrate chronic toxicity study is deemed invalid because of significant guideline deviations. These deviations included 1) significant differences between the negative control and solvent control, and 2) differences between the concentrations of the solvent in the control and highest test concentrations. These deviations may have contributed to the effects observed in the study.

Toxicity to Terrestrial Plants

The registrant has submitted a vegetative vigor and seedling emergence study testing the bensulide formulation, 4LF. 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

The ECOTOX database list one terrestrial plant toxicity test evaluating the effect of bensulide on Bermuda grass (Study reference no. 2510). The endpoints produced in this study were lower than any of the endpoints produced by the registrant submitted terrestrial plant toxicity studies (MRID 447463-01). The lowest endpoint produced in this study was an effect on the population of growth of the Bermuda grass tested which resulted in an NOAEL of 12.5 lbs a.i./acre.

Toxicity to Freshwater Plants

The registrant has submitted two acceptable freshwater plant studies testing the technical grade active ingredient of bensulide. The species tested in these studies include the blue green algae, *Anabaena floss-aquae*, and the green algae, *Selenastrum capriconutum* (MRID 447204-03 and 447204-02 respectively). The endpoint values for the blue green algae study are an EC_{50} > 3.58 ppm a.i. and a NOAEC of 3.58 ppm a.i. The endpoint values for the green algae study are an EC_{50} of 1.8 ppm a.i. and an EC_{05} of 0.93 ppm a.i. The table below provides further details regarding these studies.

Aquatic Plant Toxicity				
Species/Test Type	% a.i.	EC_{50} (ppm a.i.)	MRID No. Author/Year	Study Classification
green algae (*Anabaena floss-aquae*)	93.4	> 3.58 ppm a.i.	447204-03	Acceptable
green algae (*Selenastrum capriconutum*)	93.4	1.8 ppm a.i.	447204-02	Acceptable

The ECOTOX database list 10 open literature aquatic plant studies testing bensulide toxicity to the green algae species, *Pseudokirchneriella subcapitata*. None these studies produced endpoints any more sensitive than the endpoints produced in the registrant submitted studies. The table below provides further details regarding these studies listed in ECOTOX. The EC_{50} of 1.8 ppm a.i. and an EC_{05} of 0.93 ppm a.i produced by the registrant submitted green algae study (MRID 447204-03) will be used to assess the risk of indirect effects to bensulide to the aquatic phase of the CRLF. This endpoint is selected because it is the most sensitive endpoint among all the available aquatic plant toxicity data.

Aquatic Plant toxicity data listed by ECOTOX as acceptable for use in an OPP risk assessment (ECOTOX Reference no. 2478 for all data provided below)

Species	Effect	Measurement	Endpoint	Endpoint (ppm)	Study Duration
Green algae (*Pseudokirchneriella subcapitata*)	POP	PGRT	EC100	2.8	10 days
Green algae	POP	PGRT	EC100	5.4	10 days

(*Pseudokirchneriella subcapitata*)					
Green algae (*Pseudokirchneriella subcapitata*)	POP	ABND	EC50	1.5	0 to 72 hrs
Green algae (*Pseudokirchneriella subcapitata*)	POP	PGRT	EC50	1.5	0 to 72 hrs
Green algae (*Pseudokirchneriella subcapitata*)	POP	PGRT	EC50	1.5	24 to 48 hrs
Green algae (*Pseudokirchneriella subcapitata*)	POP	PGRT	EC50	1.850	24 to 72 hrs
Green algae (*Pseudokirchneriella subcapitata*)	POP	ABND	EC50	1.862	0 to 72 hrs
Green algae (*Pseudokirchneriella subcapitata*)	POP	PGRT	EC50	2.352	0 to 72 hrs
Green algae (*Pseudokirchneriella subcapitata*)	POP	PGRT	EC50	2.842	24 to 48 hrs
Green algae (*Pseudokirchneriella subcapitata*)	POP	PGRT	EC50	2.842	24 to 72 hrs