Appendix A
Registrant Submitted Ecotoxicity Data

I. Fish Toxicity Data

A. Fish Acute Toxicity Data

The following provides a summary of the registrant submitted fish toxicity data for the technical grade active ingredient, the formulated products and some degradates of diflubenzuron.

Freshwater Fish Acute Toxicity Data for the TGAI

There are 12 reported registrant submitted freshwater fish acute toxicity studies testing the technical grade active ingredient of diflubenzuron (Table 1). These studies tested 7 different species of freshwater fish. These studies demonstrate that the technical grade active ingredient of diflubenzuron is slightly to practically non-toxic to freshwater fish. The LC_{50} from these studies range from 129 ppm to > 500 ppm. Two of the studies were deemed acceptable upon Agency review and the remainder studies were deemed supplemental. The most sensitive, acceptable, freshwater fish LC_{50} of 129 ppm (MRID 00056150) will be use to assess the acute risk of the technical grade active ingredient to the aquatic-phase CRLF and to freshwater fish prey of the CRLF.

Table 1. Fish Acute Toxicity for the Technical Grade Active Ingredient Diflubenzuron						
Species	**%A.I.**	**Study Duration**	**LC50 mg/L a.i.**	**Toxicity classification**	**MRID**	**Classification**
Bluegill sunfish (*Lepomis macrochirus*)	99.4	96 hr	129 (CI: 116 to 142) Slope: 4.7 (3.5 to 5.9)	Practically non-toxic	00056150	Acceptable [Note 1]
Rainbow trout (*Oncorhynchus mykiss*)	99.4	96 hr	136 95% CI: 122 to 151) **Slope**: 4.2 (3.0 to 5.4)	Practically non-toxic	00056150	Acceptable [Note 1]
Bluegill sunfish (*Lepomis macrochirus*)	100	96 hr	> 100	Practically non-toxic	TN 0699, 56035	Supplemental
Fathead minnow (*Pimephales promelas*)	Technical	96 hr	> 500	Practically non-toxic	00060376	Supplemental
Fathead minnow (*Pimephales promelas*)	Technical	96 hr	< 100	Practically non-toxic	00041709	Supplemental
Rainbow trout (*Oncorhynchus mykiss)*	95	96 hr.	> 100	Practically non-toxic	40098001	Supplemental
Atlantic salmon (*Salmo salar*)	95	96 hr.	> 50	At least slightly toxic	40098001	Supplemental
Brook trout (*Salvelinus fontinalis*)	95	96 hr.	>50	At least slightly toxic	40098001	Supplemental
Channel catfish (*Ictalurus punctatus*)	95	96 hr.	>100	Practically non-toxic	40098001	Supplemental
Bluegill sunfish (*Lepomis macrochirus*)	95	96 hr.	>50	At least slightly toxic	40098001	Supplemental
Yellow perch (*Perca flavescens*)	95	96 hr.	>25	At least slightly toxic	40098001	Supplemental

Note 1: As a means of verification, the data in this study was re-evaluated for this assessment using probit analysis.

Freshwater Fish Acute Toxicity Data for Formulated Products of Diflubenzuron

There are 16 registrant submitted studies testing several formulated products of diflubenzuron (Table 2). The toxicity results of these studies range from slightly to practically nontoxic (LC_{50}= 57 ppm to >1000 ppm), which suggests that the toxicity of formulated products are not dramatically greater than the toxicity of the technical grade material.

Table 2. Freshwater Fish Acute Toxicity for Formulated Products of Diflubenzuron

Species	Formulated product	LC_{50} mg/L	MRID	Classification
Bluegill sunfish(*Lepomis macrochirus*)	1GR	> 1000	00060380	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	1GR	> 1000	00060380	Acceptable
Bluegill sunfish (*Lepomis macrochirus*)	25WP	> 100	40098001	Supplemental
Bluegill sunfish (*Lepomis macrochirus*)	25WP	230	40228401	Acceptable
Channel catfish (*ctalurus punctatus*)	25WP	> 100	40098001	Supplemental
Cutthroat trout (*Oncorhynchus clarki*)	25WP	57	40098001	Supplemental
Fathead minnow (*Pimephales promelas*)	25WP	> 100	40098001	Supplemental
Rainbow trout (*Oncorhynchus mykiss*)	25WP	240	00041709	Supplemental
Rainbow trout (*Oncorhynchus mykiss*)	25WP	342	00060384	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	25WP	195	00056150	Acceptable
Bluegill sunfish (*Lepomis macrochirus*)	Dimilin 1.0% Granular	> 1000	00060380	Acceptable (for formulated product)
Rainbow Trout (*Oncorhynchus mykiss*)	Granular Dimilin 1.0%	> 1000	00060380	Acceptable (for formulated product)
Common Carp (*Cyprinus carpio*)	25% a. i .	389.52	00060380	Acceptable
Rainbow Trout (*Oncorhynchus mykiss)*	25% a. i.	341.75	00060380	Acceptable
Rainbow Trout (*Oncorhynchus mykiss*)	79.4% a.i.	>129	45252203	Supplemental
Rainbow Trout (*Oncorhynchus mykiss*)	25WP	190	2018264	Acceptable

Freshwater Fish Acute Toxicity for the Degradates of Diflubenzuron

There are 10 registrant submitted freshwater fish acute toxicity studies testing the degradates of diflubenzuron (Table 3). The data suggest that the degradate, PCA, is more toxic than diflubenzuron to fish with LC_{50} values ranging from 2 mg/L to 23 mg/L. DFBA and PCPU appear to have similar toxicity relative to parent diflubenzuron.

Table 3 Freshwater Diflubenzuron degradate toxicity data

Degradate	Test Type	Species	Result
PCA	96-hr LC50	Bluegill	2.4 mg/L (95% CI: 1.8 to 3.2)
PCA	96-hr LC50	Rainbow trout	12 mg/L (95% CI: 12 to 17)
PCA	96-hr LC50	Fathead minnow	23 mg/L (95% CI: 18 to 29)
DFBA	96-hr LC50	Fathead minnow	69 mg/L (95% CI: 55 to 87)
DFBA	96-hr LC50	Rainbow trout	>100 mg/L
DFBA	96-hr LC50	Bluegill sunfish	>100 mg/L
DFBA	96-hr LC50	Channel Catfish	>100 mg/L
PCPU	96-hr LC50	Rainbow trout	72 mg/L (95% CI: 57 to 90)
PCPU	96-hr LC50	Channel catfish	>100 mg/L
PCPU	96-hr LC50	Fathead minnow	>100 mg/L

Marine/Estuarine Fish Acute Toxicity of the TGAI and Formulated Product

There were 3 registrant submitted acute toxicity studies for estuarine/marine fish. Two of the studies tested the TGAI and one of the studies tested the formulated product of diflubenzuron (Tables 4 and 5). The studies that tested the TGAI demonstrated that the LC_{50} was higher than the highest doses tested which were 0.013 mg/L and 0.13 mg/L. The study testing the formulated product demonstrated that the formulated product, 25 WP, was practically nontoxic to marine/estuarine fish.

Table 4. Estuarine/Marine Fish Toxicity for the Technical Grade Active Ingredient Diflubenzuron

Species	%A.I.	LC_{50} mg/L a.i.	MRID	Classification
Sheepshead minnow (*Cyprinodon variegatus*)	97.6	> 0.013	42940101	Acceptable
Sheepshead minnow (*Cyprinodon variegatus*)	96.5	> 0.13	40262701	Acceptable

Table 5. Estuarine/Marine Fish Toxicity for the Formulated Product of Diflubenzuron

Species	%A.I.	LC_{50} mg/L a.i.	MRID	Classification
Mummichog (*Fundulis heteroclitus*)	25WP	255	56150	Acceptable

B. Fish Chronic Toxicity Data

Freshwater Fish Chronic Toxicity Data of the TGAI

There is one registrant submitted freshwater fish chronic toxicity study testing the TGAI, diflubenzuron (Table 6). This study demonstrated that that diflubenzuron caused no significant chronic toxic effects to fish at any of the doses tested. The NOAEC of 0.1 mg/L will be used to assess the chronic risk of diflubenzuron to the aquatic-phase CRLF and to freshwater fish prey of the CRLF since this endpoint is the most sensitive chronic toxic endpoint available for freshwater fish.

Table 6. Freshwater Fish Chronic Toxicity for the Technical Grade Active Ingredient Diflubenzuron

Species	% A.I.	LOEAL (ppm)	NOEAL (ppm)	MRID/Study Id	Classific
Fathead minnow (*Pimephales promelas*)	99.4	>0.100	0.100	00099755	Acceptal

Marine/Estuarine Fish Chronic Toxicity Data of the TGAI

There is also one registrant submitted marine/estuarine fish chronic toxicity study testing the TGAI, diflubenzuron (Table 7). This study also demonstrated that that diflubenzuron caused no significant chronic toxic effects to fish at any of the doses tested.

Table 7. Marine/Estuarine Fish Chronic Toxicity Data for the Technical Grade Active Ingredient Diflubenzuron

Mummichog (*Fundulis heteroclitus*)	Diflubenzuron Technical Grade	>. 020	> 0.020	00099722	Supplem

II. Aquatic Invertebrate Toxicity Data

A. Freshwater Invertebrate Toxicity Data.

Freshwater Invertebrate Acute Toxicity Data of the TGAI

There are 8 registrant submitted freshwater invertebrate aquatic acute toxicity studies testing the technical grade of diflubenzuron. This included 4 studies that tested the water flea, 1 study that tested the scud, 1 study that tested the stonefly, and 1 study that tested the midge (Table 8).

Based on these studies the TGAI, diflubenzuron, is very highly toxic to slightly toxic to freshwater invertebrates (EC_{50} ranges from 0.0026 ppm to > 100 ppm).

Table 8. Freshwater Invertebrate Acute Toxicity for the Technical Grade Active Ingredient Diflubenzuron

Species	Percent Active Ingredient	Study Duration	LOEAL	NOEAL %A.I.	Toxic endpoint mg/L	MRID	Classification
Scud (*Gammarus pseudolimnaeus*)	95	96 hr	Not reported	Not reported	EC_{50} = 45	40098001	Supplemental
Stonefly (*Skwala sp.*)	95	96 hr	Not reported	Not reported	EC_{50} > 100	40098001	Supplemental
Water flea (*Daphnia magna*)	99	48 hr	Not reported	Not reported	EC_{50} = 0.0026	45252204	Supplemental Nc
midge, (*Chironomus*	43	48 hr	Not reported	Not reported	EC_{50} = 43	2018144	Supplemental
midge, (*Chironomus tentans*)	96.7%	10 day sediment toxicity test	>0.105 ppm a.i. Note 3 0.030 ppm a.i. Note 4	0.105 ppm a.i. Note 3 0.014 ppm a.i. Note 4	LC_{50} > 0.000105 ppm a.i. Note 3 EC_{50} = 0.068 ppm Note 4	463693-01	Supplemental Nc
Water flea (*Daphnia magna*)	Not reported	< 24 hr	Not reported	Not reported	EC_{50} = 0.002	00010856	Supplemental

Water flea (*Daphnia magna*)	Tech	< 24 hr	Not reported	Not reported	EC_{50} = 0.0037	43665801	Acceptable
Water flea (*Daphnia magna*)	79.4	48 hr	Not reported	0.032	0.0032	45252204	Supplemental

Note 1: The study was deemed supplemental because there was missing information regarding water quality (i.e. water hardness and analytical determination of all treatment levels) and the analytical limits of detection and quantification.
Note 2: The study was deemed supplemental for the following reasons: a. Initial measurements of length and weight should have been provided for a sub-set of the test midges, b. The pre-test health, including mortality, of the midge culture was not reported, c. Sediments were not analyzed for total volatile sulfides,water holding capacity, BOD, COD, Eh, total inorganic carbon, total volatile solids, acid volatile sulfides,metals, oil and grease, and petroleum hydrocarbons; these analyses are suggested in the guidance documents, d. The water solubility of the test material was not provided, e. The ratio of sediment: overlying water ratio in the test systems (100 mL:150 mL) differed from recommendations (100 mL:175 mL), f. The overlying water flow rate (renewal rate) was only sufficient for approximately two volume additions (exchanges) per replicate test compartment rather than the recommended 5-10 volume additions per 24-hour period, g. Dimethyformamide (DMF) was used in the preparation of the spiked sediment and a solvent control was tested in parallel with the negative control and treatment groups, however, the actual maximum concentration of solvent used to prepare the treatment sediments and the solvent control was not reported.
Note 3: This endpoint is based on survival and mean-measured pore-water (0.4. and 10 day) concentrations.
Note 4: This endpoint is based on ash-free dry weight.
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Freshwater Invertebrate Acute Toxicity Data of the Formulated Product of Diflubenzuron

There are also several freshwater invertebrate acute toxicity studies testing the diflubenzuron formulated product, 25 WP (Table 9). The toxicity values of these studies range from 0.015 mg/L to >1,000 mg/L. These data suggest that the toxicity of the formulated product,25 WP, is not greater than the toxicity of the technical grade material.

Table 9. Freshwater Invertebrate Acute Toxicity for the Formulated Product of Diflubenzuron

Species	Tech.	Study Duration	LOEAL	NOEAL %A.I.	Toxic endpoint mg/L	MRID	Classification
Stonefly (*Skwala sp.*)	25WP	96 hr.	Not reported	Not reported	96 hr EC_{50} =57.5	40098001	Supplemental
Scud (*Gammarus pseudolimnaeus*)	25WP	96 hr.	Not reported	Not reported	96 hr EC_{50} =25	40098001	Supplemental
Water flea (*Daphnia magna*)	25WP	48 hr.	Not reported	Not reported	48 hr EC_{50}: 5.5	40098001	Supp
Blackfly (Simulium vittatum)	25WP	24 hr	Not reported	Not reported	LC_{50}: 1.3	Not reported (Documented in EFED TOXDATA BASE)	Supplemental
Freshwater mussel (*Anodonta sp.*)	25WP	96 hr.	Not reported	Not reported	EC_{50} > 1000	00039088	Supplemental
Water flea (*Daphnia magna*)	25WP	48	Not reported	Not reported	EC_{50} = 0.015	40098001	Supplemental

Comment [FJ1]: Unable to locate on toxdata base.

Freshwater Invertebrate Acute Toxicity Data of the Degradates of Diflubenzuron

The freshwater invertebrate degradate acute toxicity data are also summarized below in table 10. The degradates were orders of magnitude less toxic to invertebrates than parent diflubenzuron. For example, LC_{50}s in midge for PCA and DFBA are 43 mg/L and >100 mg/L, respectively, compared with an LC_{50} of 0.07 mg/L for parent chemical in midge.

Table 10. Freshwater Invertebrate Acute Toxicity for the Degradates of Diflubenzuron

Degradate	Test Type	Species	Result	Comment
PCA	48-hr LC50	Midge; *Chironomus plumosus*	LC50: 43 mg/L 95% CI: 36 – 51	Mortality was 0% to 10% at 32 mg/L and less; Data from Mayer and Ellersieck raw data files.
DFBA	48-hr LC50	Midge; *Chironomus plumosus*	>100 mg/L	20% immobilization occurred at 10 mg/L; 4th instar organisms were used; Data from Mayer and Ellersieck raw data files.

Freshwater Invertebrate Chronic Toxicity Data

The registrant-submitted chronic toxicity studies in aquatic invertebrates are summarized below in Table 11. The most sensitive endpoints produced in these studies were a NOAEL of 0.05 ug/L and a LOAEL of 0.075 ug/L. In the registrant submitted studies, no sublethal effects were observed at levels lower than the NOAEC used to calculate RQs. There is uncertainty in the most sensitive NOAEC because diflubenzuron was not detected in 4 of the 7 test samples analyzed. Therefore, the mean measured values assumed a test concentration of the detection limit when measured values were below the detection limit.

Table 11. Freshwater Invertebrate Chronic Toxicity for the Technical Grade Active Ingredient Diflubenzuron

Species	**% A.I.**	**LOEAL (ppb)**	**NOEAL (ppb)**	**MRID/Study Id**	**Classific**
Water flea (*Daphnia magna*)	99	0.12	0.06	Beltsville TN2424	Supplem
Water flea (*Daphnia magna*), 2-generation study	98	0.075	0.05 [Note 1]	00010856	Supplem
Freshwater snail (*Lymnaea sp.*)	Technical	6.7	< 6.7	00099723	Supplem

[Note 1] Mean measured values assumed that concentrations below the detection limit (4/7 samples at the NOAEL and 2/8 samples at the LOAEL) were equal to the detection limit of 0.05 ug/L.

Species	% A.I.	LOEAL (ppb)	NOEAL (ppb)	MRID/Study Id	Classific
Water flea (*Daphnia magna*)	99	0.12	0.06	Beltsville TN2424	Supplem
Water flea (*Daphnia magna*), 2-generation study	98	0.075	0.05[a]	00010856	Supplem
Freshwater snail (*Lymnaea sp.*)	Technical	6.7	< 6.7	00099723	Supplem

[a] Mean measured values assumed that concentrations below the detection limit (4/7 samples at the NOAEL and 2/8 samples at the LOAEL) were equal to the detection limit of 0.05 ug/L.

Species	% A.I.	LOEAL (ppb)	NOEAL (ppb)	MRID/Study Id	Classific
Water flea (*Daphnia magna*)	99	0.12	0.06	Beltsville TN2424	Supplem
Water flea (*Daphnia magna*), 2-generation study	98	0.075	0.05[a]	00010856	Supplem
Freshwater snail (*Lymnaea sp.*)	Technical	6.7	< 6.7	00099723	Supplem

[a] Mean measured values assumed that concentrations below the detection limit (4/7 samples at the NOAEL and 2/8 samples at the LOAEL) were equal to the detection limit of 0.05 ug/L.

Table XXX. Freshwater Invertebrate Chronic Toxicity for the Technical Grade Active Ingredient Diflubenzuron

Species	% A.I.	LOEAL (ppb)	NOEAL (ppb)	MRID/Study Id	Classific
Water flea (*Daphnia magna*)	99	0.12	0.06	Beltsville TN2424	Supplem
Water flea (*Daphnia magna*), 2-generation study	98	0.075	0.05[a]	00010856	Supplem
Freshwater snail (*Lymnaea sp.*)	Technical	6.7	< 6.7	00099723	Supplem

[a] Mean measured values assumed that concentrations below the detection limit (4/7 samples at the NOAEL and 2/8 samples at the LOAEL) were equal to the detection limit of 0.05 ug/L.

B. Marine/Estuarine Invertebrate Toxicity Data

Marine/Estuarine Invertebrate Acute Toxicity Data of the TGAI

There were 4 marine/estuarine acute invertebrate toxicity studies testing the TGAI, diflubenzuron. Based on the results of these studies diflubenzuron is highly toxic to marine/estuarine invertebrates (Table 12).

Table 12. Acute Estuarine/Marine Invertebrate Toxicity for Technical Grade Active Ingredient Diflubenzuron

Species	% A.I.	Study Duration	EC_{50} mg/L a.i.	LOAEL	NOAEL	MRID	Classification
Quahog clam (*Mercenaria mercenaria*)	97.6	48 hr.	> .320	Not reported	Not reported	41392001	Acceptable
Mysid (*Americamysis bahia*)	95	96 hr.	0.002	Not reported	Not reported	40228401	Acceptable
Marine aphipod (*Leptocheirus plumulosus*	97	10 day sediment toxicity study	0.00204 a.i. Note 1	LOAEC: 0.0018 ppm a.i. Note 1 (survival)	NOAEC: 0.00087 ppm a.i. Note 1 (survival)	46369302	Supplemental Note 2
Grass shrimp (*Palaemonetes pugio*)	98	96 hr.	0.00064	NR	0.010	00038612	Supplemental

Note 1: This endpoint is based on survival and mean-measured pore-water (Day 0 and 10) concentrations.
Note 2: The study was deemed supplemental because it was not designed to fulfill any current U.S. EPA FIFRA guideline, however, the study does provide information that may be useful for risk assessment purposes. The major deviation involved the definitive exposure period, where the amphipods were observed on the surface of the sediment and the frequency of these observations increased with the increasing treatment concentrations. OPPTS guideline 850.1 740 recommends that the test photoperiod be continuous (24 hours light) rather than 163 (1ight:dark) so that the test organism remain buried during the entire duration of the exposure period. Based on the observed increase in the frequency of amphipods on the sediment surface at the higher treatment levels and the shorter than recommended photoperiod it is plausible that sediment avoidance may have occurred during this study.

Marine/Estuarine Invertebrate Acute Toxicity Data of the Formulated Product

There are 5 marine/estuarine acute toxicity studies testing the formulated product, 25 WP, of diflubenzuron. These studies demonstrated that the formulated product, 25 WP, is practically nontoxic to diflubenzuron (Table 13). Thus, the formulated product, 25 WP is much less toxic than the TGAI.

Table 13. Acute Estuarine/Marine Invertebrate Toxicity for the Formulated Product of Diflubenzuron

Species	% A.I.	Study Duration	EC_{50} mg/L	LOAEL	NOAEL	MRID	Classification
Eastern Oyster (*Crassostrea virginica*)	25 WP	96 hr	> 130	Not reported	Not reported	00038611	Supplemental
Eastern Oyster (*Crassostrea virginica*)	25 WP	96 hr	> 250	Not reported	Not reported	00038611	Supplemental
Quahog clam (*Mercenaria mercenaria*)	25 WP	96 hr	> 1000	Not reported	Not reported	00099752	Supplemental
Green crab (*Carcinus maenas*)	25 WP	96 hr	> 100	Not reported	Not reported	00099752	Supplemental
Fiddler (*Uca pugilator*)	25 WP	96 hr	> 0.001	Not reported	Not reported	00099752	Supplemental

Note 1: This endpoint is based on survival and mean-measured pore-water (Day 0 and 10) concentrations.

Chronic Marine/Estuarine Invertebrate Toxicity Data of the TGAI

There are 6 registrant submitted marine/estuarine chronic toxicity studies testing the TGAI, diflubenzuron. Based on the results of these studies the NOAECs were demonstrated at concentrations as low as 0.0067 ppm (Table 14).

Table 14. Estuarine/marine Invertebrate Chronic Toxicity for the Technical Grade Active Ingredient Diflubenzuron

Species	% A.I.	LOEAL (ppm) a.i.	NOEAL (ppm) a.i.	MRID/Study Id	Classific
Mysid shrimp (*Mysidopsis bahia*)	Not reported	0.093 Note 1	0.048	40237501	Acceptal
Mysid shrimp (*Americamysis bahia*)	97.6	0.000086	0.000045	40197001	Acceptal
Mysid shrimp (*Americamysis bahia*)	99	< 0.0075	Not reported	2018289	Supplem
Brine shrimp (*Artemia salina*)	Technical	< 0.0001	< 0.0001	00073933	Supplem
Blue Crab (*Callinectes sapidus*)	Technical	0.0067	0.1	2018255	Supplem
Stone crab (*Menippe mercenaria*)	Technical	< 0.0005	Not reported	2018255	Supplem
Stone crab (*Menippe mercenaria*)	Technical	< 0.0001	Not reported	Not reported	Supplem
Grass shrimp (*Palaemonetes pugio*)	99	< 0.0007	Not reported	00038612	Supplem

Note 1: This effect entailed reduced reproduction in F1 generation and reduced survival.

III. Aquatic Plant Toxicity Data

There are four available aquatic plant toxicity studies. In all of these studies the EC_{50} values were greater than the highest diflubenzuron concentrations tested (124 mg/L; Table 15). The most sensitive endpoint found was an EC_{50} and NOAEL of >0.20 ppm, and 0.20, respectively (MRID 42487101). Since these are the most sensitive endpoints, these values will be used to assess the risk of diflubenzuron to aquatic plants.

Comment [c2]: Is there a table?

Table 15. Nontarget Aquatic Plant Toxicity for Diflubenzuron

Species	Tech.	Study Duration	EC50 (mg/L)	NOAEL (mg/L)	MRID	Classification
Selanustrum capricornutum	79.4	72 hr.	> 124	124	45252205	Supplemental
Selanustrum capricornutum	Technical	72 hr.	> 0.20	0.20	42487101	Supplemental[1]
Skeletonema costatum	95.6	120 hr.	> .270	> .270	42940106	Supplemental
Lemna Gibba	95.6	14 day	> .190	.190	42940103	Supplemental[2]

[1] The study was deemed scientifically sound but was classified as supplemental because it was only conducted for 72 hours (3 days).
[2] The terminal mean measured concentration was only 20% of the initial measured concentration.

IV. Terrestrial Toxicity Plant data

Currently, there is no registrant submitted plant toxicity data available for the Agency to review.

V. Avian Toxicity Data

Avian Acute Toxicity Data of the TGAI

There are 3 registrant submitted avian acute oral toxicity studies testing diflubenzuron. Based on the results of these studies diflubenzuron is categorized as practically non-toxic to avian species on an acute oral toxicity basis. Studies in bobwhite quail and mallard duck did not produce toxic effects at any dose tested. All three studies used the technical grade material (Table 16). The most sensitive study available produced an LD_{50} value of 3763 mg/kg-bw in the Red-Winged Blackbird (MRID 00038614).

Table 16. Avian Acute Dose Base Toxicity for Diflubenzuron

Species	Tech.	Study Duration	LD_{50} (mg/kg)	MRID	Classification
Bobwhite quail (*Colinus virginianus*)	99.4	14 Day	> 5000	00073935	Acceptable
Mallard duck (*Anas platyrhynchos*)	99.4	14 Day	> 5000	00073936	Acceptable
Red-winged blackbird (*Agelaius*	99	14 Day	3763	00060384	Supplemental [Note 1]

Note 1: Need to determine why this study is supplemental.

There are 3 registrant submitted acceptable avian acute dietary toxicity studies testing the TGAI, diflubenzuron. Based on the results of these studies, diflubenzuron is categorized as practically non-toxic to avian species on a subacute dietary toxicity basis based on an LC50 value of >4640 ppm for the bobwhite quail and mallard duck (MRID 00039080). A 1 % Granular formulation was also categorized as practically non-toxic to bobwhite quail and mallard ducks based on an LC50 value of >20,000 ppm (MRID 00060381). Table 17 provides further details regarding these studies.

Table 17. Avian Acute Dietary Toxicity for Diflubenzuron

Species	% A.I.	Study Duration	LC_{50} (mg/kg) a.i.	MRID	Classification
Bobwhite quail (*Colinus virginianus*)	Diflubenzuron Technical	8 Days	> 4640	00039080	Acceptable
Bobwhite quail (*Colinus virginianus*)	Diflubenzuron 1G ???	8 Days	> 20000	00060381	Acceptable

Mallard duck (*Anas platyrhynchos*)	Diflubenzuron 1G ???	8 Days	> 20000	00060381	Acceptable
Mallard duck (*Anas platyrhynchos*)	Diflubenzuron (100%)	8 Days	> 4640	00038613	Acceptable

Avian Chronic Toxicity Data of the TGAI

There are nine reported registrant submitted avian chronic toxicity studies. Among all these studies only one of them produces results that were acceptable, reliable, and verifiable. This was the study identified by MRID 4166800102. This study produce a NOAEC of 500 mg/kg-diet (MRID 4166800102). Other studies are available that produced lower NOAECs. However, these studies either did not produce a LOAEC (no effects were observed at any test concentration) or the NOAECs were considered unreliable. In particular, a NOAEC of <10 mg/kg-diet has been reported in a non-guideline study in mallard ducks (MRID 99862, Reinert et. al., 1975) based on a reduction in the number of eggs embryonated at 10 mg/kg-diet. However, this effect was not observed at higher test concentrations in the same study and has not been observed in more recent guideline studies. Therefore, a NOAEC of <10 mg/kg-diet was not considered reliable and was not chosen for use in risk estimation.

The NOAEC of 500 mg/kg-food was based on effects on eggshell thickness in mallard ducks and reduced egg production in bobwhite quail at 1000 mg/kg-diet. Further details about the avian reproduction studies are summarized below in table 18.

Table 18. Avian Chronic Toxicity for Diflubenzuron

Species	% A.I.	LOEAL (ppm) a.i.	N0EAL (ppm) a.i.	MRID	Classification
Mallard duck (*Anas platyrhynchos*)	97.6	1000	500	41668001	Acceptable
Mallard duck (*Anas platyrhynchos*)	99.4	> 40	40	00099862	Supplemental
Mallard duck (*Anas platyrhynchos*)	99.4	> 250	250	00039085	Supplemental
Bobwhite quail (*Colinus virginianus*)	99.4	> 250	250	00099719	Supplemental
Bobwhite quail (*Colinus virginianus*)	97.6	1000	500	41668002	Acceptable

Bobwhite quail (*Colinus virginianus*)	99.4	< 250	Not reported	00099730	Supplemental
Bobwhite quail (*Colinus virginianus*)	99.4	10	< 10	00099862	Supplemental
Leghorn cockerel (*Gallus gallus*)	99.4	250	250	00039085	Supplemental
Ring-necked pheasant (*Phasianus colchicus*)	99.4	> 250	250	00039085	Supplemental

VI. Mammalian Toxicity Data

Mammalian Acute Toxicity Data of the TGAI

The available registrant submitted mammalian acute toxicity data demonstrate that diflubenzuron is practically nontoxic to mammals (LD50 > 5000 mg/kg; MRID 00157103; Table 19).

Table 19. Mammalian Acute Toxicity for Diflubenzuron

Species	**% A.I.**	**LC_{50} (mg/kg a.i.)**	**MRID**	**Classification**
Rat (*Rattus sp.*)	Technical	>5,000 mg/kg	00157103	Acceptable

Mammalian Chronic Toxicity Data of the TGAI

In the registrant submitted 2-generation reproduction study technical grade diflubenzuron was administered in the diet to rats at dose levels of 0 (control), 500, 5000 or 50000 ppm (equivalent to about 0, 25, 250 or 2500 mg/kg/day).

No effects on reproductive performance were observed at any dose level in F0 or F1 males or females. Litter and mean pup weights decreased slightly from birth to 21 days postpartum in F1 offspring at 2500 mg/kg/day. The NOEL for reproductive performance in parental adults is 2500 mg/kg/day. The NOEL for developmental toxicity in progeny is 250 mg/kg/day and the LEL is 2500 mg/kg/day, based on decreased body weights in F1 pups from birth to 21 days postpartum. (MRID 43578301). A NOAEL of 250 mg/kg-bw is used in this assessment (Table 20).

Table 20. Mammalian Chronic/Reproductive Toxicity for Diflubenzuron				
Species	**% A.I.**	**NOAEL (mg/kg/ day a.i.)**	**MRID**	**Classification**
Rat (*Rattus sp.*)	Technical	250	43578301	Acceptable

VII. Terrestrial Invertebrate Toxicity Data

Guideline studies in honeybees have been submitted and accepted by the Agency (Table 21). These tests produced acute oral and contact LD50s of >30 ug/bee (MRID 05001991), resulting in a practically non-toxic classification. However, given the short duration of these tests (typically 48-72 hours), and the typical age of organisms used in the tests, it is unlikely that the contact / ingestion coincided with a molting stage, and, thus, the results may not accurately reflect the toxicity of diflubenzuron to terrestrial invertebrates.

A number of field studies regarding effects on terrestrial invertebrates were submitted to the Agency and reviewed in 1984 to 1985 (MRIDs 00099743, 000171816, 00071207, 00095416, 00071215, 00070179, 00071212, 00095407, and 00071210). The following information is based on the reviews of these studies.

Studies evaluated a number of different organisms and exposure routes. Three honey bee studies resulted in no effects on colonies near fields treated with application rates of up to 0.5 oz Dimilin W-25/A or 0.06 lbs a.i./A (MRIDs 0071816, 00099743, 00071212). Another study that exposed honey bees to 100 ppm a.i. in their water source determined that the exposure "almost eliminated production of a sealed brood" (MRID 0095407). A study on a stream receiving runoff from a treated field indicated that amphipod and aquatic beetle larvae populations were reduced and copepods and ostracods may also have been impacted" at an application rate of 0.08 lb a.i./A (MRID 00071210). A study evaluating the effects of diflubenzuron (2.24 kg a.i./ha) on soil arthropods when the chemical was incorporated into the soil showed some decrease in numbers of springtails at 2 months post-treatment and on soil mites at 6 weeks, but little effect on other soil organisms (MRID 0007121 5). Differences between control and treatment plots were not distinguishable at 4 months post-treatment. The Agency reviewer concluded diflubenzuron had "little adverse effect on the soil fauna." A study on beneficial

arthropods showed some species were severely affected, while other species were not affected at all (MRID 00070179). Aerial application of granular Dimilin to salt marsh mosquito habitat significantly reduced numbers and diversity of aquatic invertebrates (MRID 00095416). Measured water concentrations of Dimilin (diflubenzuron) ranged from 0.24-1.8 ug/L. Based on a study on soil arthropods, dimilin appears to have "little or no effect on soil microarthropods, but may adversely affect insect parasites" (MRID 007 1207).

Overall, studies on terrestrial invertebrates illustrate that the effect of diflubenzuron is highly dependent on the life stage of the organism when it is exposed. Diflubenzuron is very highly toxic to invertebrates that rely on chitin as an exoskeleton at the critical life stage (i.e., a molting event). Available data are not sufficiently robust to estimate an assessment endpoint for this taxon, but adverse effects on non-target arthropods should be anticipated following use of diflubenzuron.

Table 21. Acute Insect Toxicity for Diflubenzuron

Species	% A.I.	Study Type	Toxic endpoint	MRID	Classification
Honey bee (*Apis mellifera*)	Technical	Acute oral and acute contact	LD_{50}: > 30 µg/bee	05001991	Acceptable
Honey bee (*Apis mellifera*)	Technical	Acute contact	NOEAL: > 114.8 µg/bee	00099890	Acceptable
Honey bee (*Apis mellifera*)	Not reported	Field testing for pollinators	NOEAL: > 2 oz. a.i./acre	00099743	Acceptable
Honey bee (*Apis mellifera*)	25%	Toxicity to nontarget insects	NOEAL: > 5 oz. a.i./acre.	00071816	Acceptable
Parasitic wasp (*Aphytis lingnanensis*)	25%	Toxicity to nontarget insects	NOEAL: > 4 oz. a.i./100 gal. water.	00071816	Acceptable