

EXHIBIT 32



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

January 29, 2016

PC Code: 027602
DP Barcode: 431377

MEMORANDUM

SUBJECT: **Flubendiamide:** Addendum to Clarify Invertebrate Terminology in January 28, 2016 Ecological Risk Assessment Addendum Summarizing all Submissions and Discussions to Date

FROM: Edward Odenkirchen, Ph.D., Senior Advisor 
Divisional Front Office Staff
Environmental Fate and Effects Division (7507P)

THRU: Sujatha Sankula, Ph.D., Branch Chief
Environmental Risk Branch 1
Environmental Fate and Effects Division (7507P)

**SUJATHA
SANKULA**

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DN: c=US, o=U.S. Government,
ou=USEPA, ou=Staff, cn=SUJATHA
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Date: 2016.01.29 14:13:09 -0500'

TO: Carmen Rodia, Risk Manager Reviewer
Richard Gebken, Risk Manager, PM Team 10
Debbie McCall, Branch Chief
Invertebrate-Vertebrate Branch 2
Registration Division (7505P)

The Registration Division (RD) requested that the Environmental Fate and Effects Division (EFED) provide additional explanation regarding the terminology used to describe invertebrates of concern in freshwater systems in the January 28, 2016 Ecological Risk Assessment Addendum Summarizing all Submissions and Discussions to Date (DP Barcode 431037) and to explain more fully how, conceptually, the risk findings are best related to aquatic invertebrates.

A variety of terms of art can be used to describe invertebrate species within freshwater aquatic systems and this document will source terms from the Aquatic Biodiversity Glossary (USEPA 2010) and the Glossary of Aquatic Ecological Terms (USEPA 1972).

The term invertebrate in Office of Pesticide Programs (OPP) EFED ecological risk assessments refers to animals without back bones. Aquatic invertebrates would be those invertebrates that are associated with aquatic systems. Commonly, the OPP/EFED suite of effects testing requirements, for practical reasons, involve toxicity testing with macroinvertebrates: "animals without backbones of a size large enough to be seen by the unaided eye and which can be retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings)", but it is possible that effects endpoints derived from such organisms could be extrapolated to similar



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effects in other complex multicellular organisms that fall within the microinvertebrates: “animals without backbones that are not large enough to be seen by the unaided eye; they will not be retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings). For the purposes of this document, focus will be placed on macroinvertebrates.

Aquatic macroinvertebrates in freshwater systems may occupy different habitats within an aquatic system. Some may be part of the zooplankton “*tiny, sometimes microscopic, floating aquatic animals*” or free swimming animals “*actively moving about in water or capable of moving about in the water*” within the water column. Others may be part of the benthos “*Organisms growing on or associated principally with the bottom of waterways. These include: (1) sessile animals such as sponges, barnacles, mussels, oysters, worms, and attached algae; (2) creeping forms such as snails, worms and insects; (3) burrowing forms, which include clams, worms, and some insects; and (4) fish whose habits are more closely associated with the benthic region than other zones; e.g. flounders.*”

In the case of aquatic effects testing with flubendiamide and the des-iodo degradate, effects endpoints are available for aquatic macroinvertebrates that are free swimming in the water column (e.g., *Daphnia magna*) as well as macroinvertebrates that are associated with the benthos (e.g., *Lumbriculus variegatus*, *Hyaella azteca*, *Centroptilum triangulifer*, *Chironomus tentans* and *Chironomus riparius*). Acute short term lethality or motility studies are available for all the above species using water-only exposures. For chronic exposure effects, data are available for *D. magna* in a water-only test, which is achievable because the organism can thrive in a water-only environment. However, for longer term exposures with *C. riparius*, the testing systems must employ a sediment phase because the organisms cannot thrive in a water-only testing environment. In the case of the *C. riparius* long-term studies, initial chemical exposure was conducted either as a water column spike or a sediment spike, and effects endpoints were expressed in terms of both water column concentrations and sediment pore water concentrations of the test materials. These later endpoints figure prominently in the EFED risk assessments and the endpoints are frequently referred to as benthic invertebrate effects endpoints because the test organism is indeed an invertebrate of the benthos.

EFED consulted two guiding documents for determining policy to describe a consistent and reasonable approach for relating the available toxicity information to the various aquatic invertebrates in aquatic systems. The Overview of Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency (USEPA 2004) describes the use of limited effects testing in a surrogate approach where testing of a few species within a taxa group is used to represent a variety of organisms within that group. The document also indicates that effects endpoints for risk assessment reasonably come from the most sensitive species tested within that taxa group. Of all the aquatic invertebrate species tested for flubendiamide, the chronic endpoints from *C. riparius* indicate that this species is the most sensitive tested aquatic invertebrates. As a second policy check, EFED consulted guidance entitled “Toxicity Testing and Ecological Risk Assessment Guidance for Benthic Invertebrates” (USEPA 2014), which suggests that endpoints from water-only toxicity tests with invertebrates are important risk evaluation tools to ascertain potential risk to sediment organisms because

bioavailability in benthic organisms is largely mediated by dissolved concentrations of the toxicant in sediment pore waters or overlying water. It then follows that risk estimates based on water column environmental exposures compared with overlying water expressed endpoints from sediment toxicity tests with invertebrates would have reasonable applicability as a surrogate for risks to aquatic invertebrates existing in the water column because the dissolved water concentration of the toxicant remains the important source of exposure.

Conclusion

The risk assessment results for flubendiamide, conducted using water column and pore water estimates of exposure and compared with effects endpoints from the benthic macroinvertebrate *C. riparius* are appropriate sensitive indicators of risks to invertebrates occupying the benthos including sessile and mobile invertebrate organisms growing on or associated principally with the bottom of waterways. The risk findings are also reasonably applied to invertebrates existing within the water column. In both cases, the standard issue of the use of toxicological surrogates to represent effects in a given taxa is discussed in USEPA 2004.

The most appropriate description of invertebrates of concern in the context of the flubendiamide risk assessment would best be termed risks to **invertebrates of aquatic systems** as this would be inclusive of invertebrates (macro and potentially micro) in a variety of water column and benthic associated habitats within a given aquatic system where exposure to either overlying water or benthic pore water could occur.

References

United States Environmental Protection Agency (USEPA). 1972. Glossary of Aquatic Ecological Terms, Office of Water Programs.

(<http://nepis.epa.gov/Exe/ZyNET.exe/9100ULPU.TXT?ZyActionD=ZyDocument&Client=EPA&Index=Prior+to+1976&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C70thru75%5Ctxt%5C00000015%5C9100ULPU.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=p%7Cf&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL>)

USEPA. 2004. Overview of Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, January 23, 2004.

USEPA. 2010. Aquatic Biodiversity Glossary, Office of Environmental Information, USEPA: updated December 8, 2010.

(http://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details&glossaryName=Aquatic+Biodiversity+Glossary)

USEPA. 2014. Toxicity Testing and Ecological Risk Assessment Guidance for Benthic Invertebrates. Memorandum from Donald Brady, Director of the Environmental Fate and Effects Division. April 10, 2014.

EXHIBIT 33

DATA EVALUATION RECORD
FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST

1. **CHEMICAL:** Flubendiamide PC Code: 027602
2. **TEST MATERIAL:** NNI-0001-des-iodo Purity: 99.3%
3. **CITATION:**

Authors: Dorgerloh, M.

Title: *Chironomus riparius* 28-day Chronic Toxicity Test with NNI-0001-des-iodo in a Water-Sediment System using Spiked Water

Study Completion Date: November 22, 2004

Laboratory: Bayer CropScience AG
Development-Ecotoxicology
40789 Monheim, Germany

Sponsor: Bayer CropScience AG
Portfolio Management, Project Management/Project Planning
40789 Monheim am Rhein, Germany

Laboratory Report ID: DOM 23069; Project ID E 416 2518-7

MRID No.: 468170-23

4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature:



Date: 07/30/07

- APPROVED BY:** Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature:



Date: 08/02/07

5. **APPROVED BY:** Holly Galavotti, Biologist, ERB1

Signature:



Date: 5/21/08

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Chironomus riparius*

Age of Test Organism: 1st instar (L1) larvae, 2 to 3 days post-hatch

Definitive Test Duration: 28 days

Study Method: Static with aeration

Type of Concentrations: Initial nominal overlying water and TWA (pore and overlying water)



M-310710-02-1

7. CONCLUSIONS:

Results Synopsis:

Percent Emergence:

28-day NOAEC: 4.00 µg ai/L

28-day LOAEC: 8.00 µg ai/L

EC₅₀: 20 µg ai/L

Probit slope: 4.07±0.664

95% C.I.: 18-24 µg ai/L

Male Development Rate:

28-day NOAEC: 16.00 µg ai/L

28-day LOAEC: 32.00 µg ai/L

EC₅₀: >32.00 µg ai/L

Female Development Rate:

28-day NOAEC: 16.00 µg ai/L

28-day LOAEC: 32.00 µg ai/L

EC₅₀: >32.00 µg ai/L

Assessment endpoints: percent emergence and development rate

Endpoints affected: percent emergence and development rate

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This study followed methods described in the proposal for a new OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (December 2002), and was not submitted to fulfill any current U.S. EPA data requirement.

C. Reparability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

1. Overlying water was spiked, prefer that the sediment is spiked.
2. Sediment was not analyzed for degradate NNI-0001 des-iodo levels.

10. SUBMISSION PURPOSE: To assess the toxicity of the degradate NNI-0001 des-iodo to the chironomid in a water spiked water-sediment system for the purpose of new chemical

registration (PRIA).

11. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: Overlying water and pore water samples from the surrogate vessels prepared at 0.25, 4.00 and 32.0 $\mu\text{g ai/L}$ (one vessel per interval) were analyzed at 0 (1 hour), 7, and 28 Days and analyzed for NNI-0001-des-iodo. Residues associated with the overlying water were decreased from 82% of nominal concentrations at Day 0 to 32% of nominal concentrations by Day 28. Concentrations in the pore water did not exceed 0.7% of nominal concentrations. No test material was detected in the negative or solvent controls.

Nominal Initial Conc. Applied to Water ($\mu\text{g ai/L}$)	Day	Analytical Results of NNI-0001-des-iodo In Overlying Water and Pore Water Sample			
		Pore Water		Overlying Water	
		$\mu\text{g ai/L}$	% of Nominal	$\mu\text{g ai/L}$	% of Nominal
Control	0, 7, 28	<0.05	N/A	<0.05	N/A
Solvent Control	0, 7, 28	<0.05	N/A	<0.05	N/A
0.25	0	<0.05	N/A	0.199	80
	7	<0.05	N/A	0.156	62
	28	<0.05	N/A	0.119	48
4.00	0	0.135	0.2	3.20	80
	7	0.363	0.7	2.31	58
	28	0.217	0.4	0.914	23
32.0	0	1.18	0.3	27.6	86
	7	5.98	1.4	18.7	58
	28	2.07	0.5	8.52	27

Storage conditions of test chemical: 5°C, dark

Physicochemical properties of NNI-0001-des-iodo.

Parameter	Values	Comments
Water solubility at 20°C	~0.42 mg/L	
Vapour pressure	Not reported	
UV adsorption	Not reported	
pKa	Not reported	
Kow	Not reported	

OECD requires water solubility, stability in water and light, pK_a , P_{ow} , and vapor pressure of the test compound.

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
<p><u>Species</u> <i>Chironomus riparius</i></p>	<p><i>Chironomus riparius</i></p>
<p><u>Source</u></p>	<p>In-house laboratory culture originally obtained from the University of Sheffield (UK) in autumn 1991.</p>
<p><u>Culture Conditions</u> A reproduction and oviposit chamber should consist of an adult area, sufficiently large to allow swarming (minimum 30 x 30 x 30 cm), and an oviposit area. Crystallizing dishes or larger containers with a thin layer of quartz sand (5 to 10 mm) or Kieselgur (thin layer to a few mm) spread over the bottom and containing suitable water to a depth of several cm are suitable as an oviposit area. Environmental conditions: temperature 20±2°C; 16:8 hours light:dark (intensity ca. 1000 lux); air humidity ca. 60%</p>	<p>For breeding, the midges were kept in cages (60 x 60 x 55 cm) with gauze on each side of the cage. A glass basin (45 cm x 55 cm x 10 cm), made of inert plastic, was set on the bottom of each cage, and the bottom of the basin was covered with a thin layer of silica and 2- to 3-cm of reconstituted water (Elendt M7). The water was gently aerated. To begin each culture, two to four egg masses are placed into the prepared basin. The cultures were maintained at 20 ± 2°C and a 16:8 hour light:dark photoperiod.</p>
<p><u>Egg Mass Acclimation Period</u> Four to five days before test initiation freshly laid egg masses should be taken from cultures and maintained separately in culture medium, temperature change should not exceed 2°C per day.</p>	<p>Fresh egg masses were incubated in small dishes with test medium. The temperature was not reported.</p>
<p><u>Age of Test Larvae</u> First instar (1 to 4 days post-hatch with confirmation)</p>	<p>1st instar (L1), 2-3 days post-hatch</p>
<p><u>Food</u> Green algae (e.g., <i>Scenedesmus subspicatus</i>, <i>Chlorella vulgaris</i>) or flaked fish food as a ground powder, suspension, or filtrate</p>	<p>Hatched chironomus larvae were fed green algae and an aqueous suspension of a vegetable fish food (Tetra Phyll®).</p>

Guideline Criteria	Reported Information
<p><u>Health of parent culture stock</u> Were parent chironomids in good health during the culture period?</p>	Not reported.

B. Test System

Guideline Criteria	Reported Information
<p><u>Type of Test System</u> Static (static-renewal or flow-through of overlying water is evaluated on a chemical-specific basis). Distilled or deionized water may be added to overlying water once daily as needed to maintain volume.</p>	<p>Static with aeration. Once a week, test beakers were refilled with deionized water up to the mark (indicating 380 mL of overlying water) to replicate water lost by evaporation.</p> <p>Additional test vessels (with chironomids) were used for chemical analysis of the test item on days 0 and 7 (single additional replicate for the negative and solvent controls, and two additional replicates for the 0.25, 4.00 and 32.0 µg ai/L levels). For chemical analysis on day 28, one beaker of the four beakers for biological evaluations was used. Therefore, the method for analytical sampling did not affect volume, biological load, or test concentration.</p> <p>A further replicate of each test concentration was prepared (with chironomids) to measure the temperature, pH, and oxygen content in the overlying water during the study.</p>

Guideline Criteria	Reported Information
<p><u>Test Water</u> Soft reconstituted water or water from a natural source is preferred. Dechlorinated tap water may be used if the test organism will survive in it for the duration of the culturing and testing without showing signs of stress.</p>	<p>Elendt M7 medium was prepared with deionized water 7 days prior to test initiation; a detailed chemical composition was provided. Alkalinity, hardness and ammonium were measured in the negative control and 32.0 µg ai/L treatment levels on Days 0 and 28. Alkalinity ranged from 213.6-284.8 mg CaCO₃/L, total hardness ranged from 302.6-338.2 mg CaCO₃/L and ammonium ranged from 1.3-16.2 mg/L.</p>
<p><u>Test Sediment</u> Formulated (reconstituted, artificial, or synthetic) sediment is recommended. Content of sediment by dry weight: 5% peat (dry) (pH 5.5-6.0) or alpha-cellulose, 75% quartz sand (>50% in size range of 50-200 microns), 20% kaolinite clay (kaolinite content ca. 30%), CaCO₃ 0.05-0.1%). Moisture content 30-50%, TOC 2% (±0.5%) and pH 6.5 - 7.5. Natural sediment can be used if it is fully characterized, unpolluted, and free of organisms that might compete with or consume chironomids. (If solvent other than water will be used, sand content of artificial sediment is adjusted accordingly.)</p>	<p>Formulated (artificial) sediment was prepared on a dry weight basis 7 days before the start of the exposure period: 74% fine quartz sand (68.2% with a particle size of 0.05-0.2 mm) 5.0% dried, finely-ground peat (sphagnum peat, pH 2-4) 20% kaolin (kaolinite content of about 36%, pH 7) ca. 1% calcium carbonate to adjust the pH value to 7 ± 0.5.</p> <p><u>Sediment characterization:</u> TOC: 2.5% Moisture content: 31.4% pH: 6.6 CEC (meq/100 g sediment): 7.4</p>
<p><u>Sediment Conditioning</u> <u>Artificial sediment:</u> 7 days in flowing dilution water prior to test initiation, chambers may be aerated</p>	<p>Prepared sediment was equilibrated for 7 days prior to test initiation.</p>

Guideline Criteria	Reported Information
<p><u>Introduction of Test Organisms</u> Twenty-four hours prior to test initiation aeration of chambers is stopped and organisms are added to the chambers. Aeration should not resume for at least 24 hours. At test initiation, the test substance is spiked into the overlying water column.</p>	<p>On day -1, test organisms were introduced into the equilibrated test vessels five at a time, until each replicate test vessel contained 20 larvae. On Day 0 the test substance was applied just below the water surface with a pipette. The bottom of the test vessels were covered with a 1.5-cm layer of sediment. Gentle mixing of the water ensured homogenous distribution without disturbing the sediment.</p> <p>Dilution water (0.38 L) was added over the sediment layer with the aid of a sheet to avoid disturbance of the sediment. The sheet was removed following flooding. The final water height was 6.0 cm. Vessels were gently aerated throughout the study.</p>
<p><u>Solvents</u> If used, minimal (i.e., #0.1 ml/l) and same concentration in all treatments. Suitable solvents are acetone, ethanol, methanol, ethylene glycol monoethyl ether, ethylene glycol dimethyl ether, dimethylformamide or triethylene glycol. (OECD guidelines also allows use of dispersants: Cremophor RH40, Tween 80, methycellulose 0.01%, and HCO-40)</p>	<p>DMF, 16.3 mg test substance was dissolved in 50 mL DMF to obtain the stock solution. The stock solution was stirred on a magnetic stirrer for 2 minutes. To obtain the application solution, 1.180 mL of the stock solution was made up to 1 L with M7 medium and was stirred on a magnetic stirrer for 2 minutes.</p>
<p><u>Water Temperature</u> 20°C ± 2°C (Should not deviate between vessels by more than 1°C.)</p>	<p>19.2-19.5°C</p>
<p><u>pH</u> <u>Sediment:</u> 7.0 ± 0.5 <u>Interstitial Water:</u> <u>Overlying Water:</u> 6.0 to 9.0 (Should not vary by more than 1 unit during test)</p>	<p><u>Sediment:</u> Not determined <u>Interstitial Water:</u> Not determined <u>Overlying Water:</u> 8.4-8.7</p>

Guideline Criteria	Reported Information
<p><u>TOC</u> <u>Sediment</u>: 2 ± 0.5% <u>Overlying Water</u>: 2 mg/L</p>	<p><u>Sediment</u>: 2.5% (determined prior to introduction into vessels) <u>Overlying Water</u>: Not determined</p>
<p><u>Ammonia</u> <u>Interstitial Water</u>: <u>Overlying Water</u>:</p>	<p><u>Interstitial Water</u>: Not determined <u>Overlying Water</u>: 1.3-1.7 mg/L on day 0 and 15.7-16.2 mg/L on day 28 (as measured in the control and highest treatment level)</p>
<p><u>Total Water Hardness</u> 200 mg/L as CaCO₃ (prefer 160 to 180 mg/L as CaCO₃)</p>	<p>302.6-338.2 mg/L as CaCO₃ on days 0 and 28 (as measured in the control and highest treatment level)</p>
<p><u>Dissolved Oxygen</u> 60% air saturation value throughout test</p>	<p>≥8.2 mg/L (≥91% saturation)</p>
<p><u>Aeration</u> Aeration (ca. one bubble/sec) is allowed except for when larvae are being added and for at least 24 hours after introduction of test organisms to a test chamber. If one test chamber is aerated all test chambers must be treated the same.</p>	<p>Continuously at a rate of ca. 2 bubbles/sec through Pasteur pipettes.</p>
<p><u>Test Vessels or Compartments</u> 1. <u>Material</u>: Glass, No. 316 stainless steel, teflon or perfluorocarbon plastics 2. <u>Size</u>: Sediment depth of 1.5- 3 cm and the depth ratio of sediment to water should be ca. 1:4, must not be >1:4; 600 ml beaker with 8 cm diameter</p>	<p><u>Material</u>: glass <u>Size</u>: 600 mL; 1.5-cm layer of sediment and 6-cm laboratory dilution water depth (380 mL). The height ratio was 1:4 sediment to overlying water.</p>
<p><u>Covers</u> Test vessels should be covered with a glass plate.</p>	<p>Test vessels were covered with clear plastic plates to prevent evaporation</p>
<p><u>Photoperiod</u> 16 hours light, 8 hours dark (Light intensity 500 to 1000 lux)</p>	<p>16 hours light, 8 hours dark Light intensity ~800 lux</p>

Guideline Criteria	Reported Information
<p><u>Food</u> Green algae (e.g., <i>Scenedesmus subspicatus</i>, <i>Chlorella vulgaris</i>) or flaked fish food as a ground powder, suspension, or filtrate</p>	Tetra Phyll® ornamental fish food suspension (1 g Tetra Phyll® per 20 mL water)
<p><u>Food Concentration and Frequency</u> Preferably feed daily but at least 3 times per week. <u>day 1 to 10</u>: 0.25-0.5 mg per larvae per day <u>remainder of test</u>: 0.5-1 mg per larvae per day (keep to a minimum, should not accumulate on sediment surface, cause overlying water to be cloudy or cause drop in DO)</p>	<p>At least 3 times per week</p> <p>1 mg Tetra Phyll® per larvae day every 1 to 3 days</p>

C. Test Design

Guideline Criteria	Reported Information
<p><u>Duration</u> <i>Chironomus riparius</i>: 28 days (if midges emerge early the test can be terminated after a minimum of 5 days after emergence of the last adult in the control).</p>	28 days

Guideline Criteria	Reported Information
<p><u>Nominal Concentrations</u> Negative control, solvent control (if a solvent was used) and at least 5 test concentrations. (Note exception to dilution factors described below can be made for shallow slope responses but minimum number of test concentrations may need to be increased)</p> <p><u>ECx endpoint:</u> test concentrations should bracket ECx and span the environmental concentration range. Dilution factor should not be greater than two between exposure concentrations.</p> <p><u>NOAEC/LOAEC endpoint:</u> factor between concentrations must not be greater than 3.</p>	<p>Negative control, solvent control, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.0 and 32.0 µg ai/L</p> <p><u>ECx endpoint:</u> N/A.</p> <p><u>NOAEC/LOAEC endpoint:</u> A nominal factor rate of 2.0 was used.</p>
<p><u>Number of Test Organisms**</u> <u>ECx endpoint:</u> 60 larvae per treatment level; 3 replicates per treatment level</p> <p><u>NOAEC/LOAEC endpoint:</u> at least 80 larvae per treatment level with at least 4 replicates per treatment level (adequate power to detect a 20% difference, Type I error rate 5%)</p> <p>** (Optional) If data on 10-day growth and survival are needed additional replicates (number based on ECx or NOEC/LOEC endpoint determination) should be included at test initiation..</p>	<p><u>ECx endpoint:</u> N/A</p> <p><u>NOAEC/LOAEC endpoint:</u> 80 larvae per treatment level with 4 replicates per treatment level.</p> <p>** (Optional) 10-day growth data were not collected.</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Organisms were randomly assigned to test containers.</p>

Guideline Criteria	Reported Information
<p><u>Overlying Water Parameter Measurements</u></p> <p>1. Dissolved oxygen should be measured daily in all test chambers.</p> <p>2. Temperature and pH should be measured in all test chambers at the start and end of the test and at least once a week during the test.</p> <p>3. Temperature should be monitored at least hourly throughout the test in one test chamber.</p> <p>4. Hardness and ammonia should be measured in the controls and one test chamber at the highest concentration at the start and end of the test.</p>	<p>1. Dissolved oxygen was measured twice weekly in the supplemental replicate vessels prepared for each treatment level.</p> <p>2. Temperature and pH were measured once per week in the supplemental replicate vessels prepared for each treatment level.</p> <p>3. Criteria not required in OECD 219 guidance.</p> <p>4. Hardness and ammonia levels were measured in one control and one 32.0-mg ai/kg vessel at study initiation and termination.</p>
<p><u>Chemical Analysis-Overlying Water</u></p> <p>At a minimum must be analyzed at test initiation (i.e., one hour after introduction of test substance into the test chamber) and at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>The overlying water of the three surrogate vessels prepared at the control, solvent control 0.25, 4.00 and 32.0 µg ai/L levels were analyzed at 0 (before addition of larvae), 7, and 28 days.</p>
<p><u>Interstitial Water and Sediment Isolation Method</u></p> <p>Centrifugation (e.g., 10,000 g and 4 EC for 30 min) is recommended. If test substance is demonstrated not to adsorb to filters, filtration may be acceptable.</p>	<p>Overlying water was decanted carefully. The wet sediment of each beaker was filtered by vacuum (glass micro fiber filter, mesh size 1.0 µm) and the filtrate (pore water) was analyzed.</p>
<p><u>Chemical Analysis-Interstitial Water</u></p> <p>At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>The isolated pore water of the three surrogate vessels prepared at the control, solvent control, 0.25, 4.00 and 32.0 levels were analyzed at 0 (before addition of larvae), 7, and 28 days.</p>

Guideline Criteria	Reported Information
<p><u>Chemical Analysis-Bulk Sediment</u> At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>Analysis of the sediment was not conducted. OECD guidance states that sediment analysis may not be necessary if the partitioning of the test substance between water and sediment has been demonstrated in a separate water/sediment study under similar conditions. No such study was reported.</p>

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>Yes. This study was conducted in compliance with the GLP standards of the OECD and German Chemical Law (ChemG). It also meets the USEPA-FIFRA Good Laboratory Standards (40 CFR Part 160) as well as the GLP standards of the Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF, 11 Nohsan No. 6283 from Oct. 1999) with the exception that recognized differences exist between the GLP principles/standards of OECD and the GLP principles/standards of FIFRA and JMAFF.</p>
<p><u>Control Mortality</u> <30%</p>	<p>Yes</p>
<p>Did chironomids emerge in controls between day 12 and 23?</p>	<p>Yes. Emergence was first observed on Day 14 for both controls and was done by Days 22 and 23 in the negative and solvent controls, respectively.</p>
<p><u>Control Emergence</u> Mean emergence between 50-70%</p>	<p>Negative control – 82.5% emergence (66/80) Solvent control – 83.8% emergence (67/80)</p>

Guideline Criteria	Reported Information
<p><u>Data Endpoints</u></p> <p><u>Emergence Test (28 day)</u></p> <ul style="list-style-type: none"> - Number alive - Time to emergence - Number of emerged male and female midges - Number of visible pupae that have failed to emerge - Number of egg masses deposited - Observations of other effects, abnormal behavior, or appearance or clinical signs (e.g., leaving sediment, unusual swimming) <p><u>Growth and Survival (10-day) (Optional)</u></p> <ul style="list-style-type: none"> - Number alive - Instar level of surviving larvae - Dry weight (ash free) per test chamber of surviving larvae by instar level 	<p><u>Emergence Test (28 day)</u></p> <ul style="list-style-type: none"> - Number emerged; differentiated by sex - Development rate - Time to emergence - Number of dead larvae, pupae and midges which failed to emerge (visible) - Observations of other effects (i.e., sediment avoidance) <p><u>Growth and Survival (10-day) (Optional)</u></p> <p>N/A</p>
Raw data included?	Yes

Effects DataTable 1. Summary of NNI-0001-des-iodo effects on *Chironomus riparius* emergence success and sex ratio

Toxicant Concentration				Initial No.	Total Number Emerged ^(c)			Mean Sex Ratio ^(d) (%)		% Inhibition in Emergence ^(e)
Nominal Overlying Water ($\mu\text{g ai/L}$)	TWA Concentrations ^(a)				σ	ϕ	Total	σ	ϕ	
	Overlying Water ($\mu\text{g ai/L}$)	Sediment ^(b)	Pore Water ($\mu\text{g ai/L}$)							
Negative control	<LOQ	N/A	<LOQ	80	38	28	66	57.6	42.4	N/A
Solvent control	<LOQ	N/A	<LOQ	80	35	32	67	52.2	47.8	-1.5
0.25	<LOQ	N/A	<LOQ	80	31	31	62	50.0	50.0	6.1
0.50	Not analyzed			80	33	34	67	49.3	50.7	-1.5
1.00	Not analyzed			80	27	33	60	45.0	55.0	9.1
2.00	Not analyzed			80	29	34	62	46.0	54.0	4.5
4.00	1.90	N/A	0.280	80	30	35	65	46.2	53.8	1.5
8.00	Not analyzed			80	29	26	55	52.7	47.3	16.7*
16.00	Not analyzed				25	19	44	56.8	43.2	33.3*
32.00	16.0	N/A	3.91	80	5	8	13	38.5	61.5	80.3*

^(a) Reviewer-calculated time-weighted average for NNI-0001-des-iodo residues (from both overlying and pore water samples; refer to associated Excel spreadsheet). The LOQ for aqueous samples was 0.05 $\mu\text{g/L}$; when test material was <LOQ, $\frac{1}{2}$ of the LOQ (0.0250 $\mu\text{g ai/L}$) was used in the TWA calculations.

^(b) Samples were not collected from the sediment for analytical verification.

^(c) Reviewer-calculated from the raw data

^(d) ER_{σ} = number of emerged males/number of emerged larvae; ER_{ϕ} = number of emerged females/number of emerged larvae; reviewer-calculated.

^(e) Reviewer-calculated relative to the negative control

N/A- Not Applicable

* Significant difference in percent emergence (% not emerged as calculated by the study author) from the pooled control ($\alpha=0.05$).

Table 2. Summary of NNI-0001-des-iodo effects on *Chironomus riparius* development time and rate.

Toxicant Concentration				Days to First Emergence ^(b)	Mean Development Rate ^(c) (1/day)	% Inhibition in Mean Development Rate
Nominal Overlying Water (µg ai/L)	TWA Concentrations ^(a)					
	Overlying Water (µg ai/L)	Sediment ^(b)	Pore Water (µg ai/L)			
Negative control	<LOQ	N/A	<LOQ	14	0.057	N/A
Solvent control	<LOQ	N/A	<LOQ	14		
0.25	<LOQ	N/A	<LOQ	14	0.057	0.0
0.50	Not analyzed			14	0.056	1.8
1.00	Not analyzed			15	0.058	-1.8
2.00	Not analyzed			15	0.058	-1.8
4.00	1.90	N/A	0.280	15	0.057	0.0
8.00	Not analyzed			14	0.058	-1.8
16.00	Not analyzed			15	0.059	-3.5
32.00	16.0	N/A	3.91	16	0.053	7.0

^(a) Reviewer-calculated time-weighted average for NNI-0001-des-iodo residues (from both overlying and pore water samples; refer to associated Excel spreadsheet). The LOQ for aqueous samples was 0.05 µg/L; when test material was <LOQ, ½ of the LOQ (0.0250 µg ai/L) was used in the TWA calculations.

^(b) Reviewer-determined from summarized data tables. Does not represent mean days to first emergence.

^(c) Mean development rate =
$$\sum_{i=1}^m \frac{f_i x_i}{n_e}$$

where: i = index of inspection interval; m = maximum number of inspection intervals; f_i = number of midges emerged in the inspection interval i ; n_e = total number of midges emerged; and

$x_i = \frac{1}{\left(\text{day}_i - \frac{l_i}{2}\right)}$ which is the development rate of the midges emerged in interval i ; day_i = inspection day (days since application); and l_i = length of inspection interval i (days, 1 day in this study)

N/A- Not Applicable

Toxicity Observations: The Chi-square test indicated no statistically-different distribution (in number emerged) between sexes compared to the assumption of 50% females and 50%

males. Therefore, males and females were pooled for all further endpoint calculations to increase statistical power. Statistically-significant reductions in emergence were observed compared to the pooled controls at the 8.00, 16.00 and 32.00 $\mu\text{g ai/L}$ treatment levels. No statistically-significant effects were observed in development rate.

No abnormal observations (dead larvae, pupae or midges) were observed in the controls or in the 0.25-16.00 $\mu\text{g ai/L}$ treatment levels. At the highest treatment level, 32.00 $\mu\text{g ai/L}$, dead midges were observed on Days 17, 20, 21 and 23 and dead larvae/pupae were observed on Days 18, 19, 20 and 22. As these findings were observed in test concentrations clearly above the NOAEC (for emergence rates), they did not affect the outcome of the study.

B. Statistical Results (From Study Report)

Midge emergence, sex ratio, and development rate were statistically analyzed.

Midge emergence was evaluated as the percentage of midges that failed to emerge for each test level. Negative and solvent control emergence and development rate data were compared using a two-sided Chi-square 2 x 2 Table test ($\alpha = 0.05$); no significant differences were observed, and the data were pooled for subsequent comparisons. Threshold concentrations (NOAEC) for emergence were determined using the Williams Multiple Sequential t-test Procedure ($\alpha = 0.05$, one-sided).

The statistical distribution between sexes compared to the assumption of 50% males and 50% females were judged by a Chi-square 2 x 2 Contingency Table test. No significant effects were observed in sex distribution, and therefore, development rate data were reported using combined sexes.

For both endpoints, a range-to-standard-deviation-ratio test on Normal Distribution was tested ($\alpha=0.05$) to test correspondence with normal distribution and Cochran's Test was conducted to test homogeneity of variance. Both the normality and homogeneity tests were passed, hence the use of a parametric multiple test.

For all endpoints, effective concentrations (EC_x) were calculated using probit analysis.

Results were reported in terms of nominal initial overlying water concentrations.

Most sensitive endpoint: percent emergence

Endpoint	Methods	EC ₅₀ (95% CI) (µg ai/L)	NOAEC (µg ai/L)	LOAEC (µg ai/L)
28-d Percent Emergence (Pooled Sex)	Williams	18.6 (15.7-22.1)	4.00	8.00
28-d Development Rate (Pooled Sex)	Williams	>32.0	16.0	32.0
10-d Survival (Optional)	---	---	---	---
10-d Growth (Optional)	---	---	---	---

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method(s): Analyzed endpoints included percent emergence of the combined sexes, male development rate and female development rate. First, data from the negative and solvent control groups for all endpoints were compared using a Student's t-test to determine if a significant difference existed between the two controls; no differences were detected between the controls for any of the analyzed endpoints. Next, treatment data were tested for normality using Chi-square and Shapiro-Wilks tests and for homogeneity of variance using Hartley and Bartlett tests. As all data sets met these assumptions of ANOVA, NOAEC and LOAEC values were determined using the parametric Dunnetts' t-test (or Bonferroni's t-test for unequal replicates) and Williams' test via Toxstat Statistical software. The EC_x values (with 95% C.I.) and probit slopes were determined using the probit analysis via Nuthatch Statistical software. All analyses were conducted using the nominal overlying water concentrations (µg ai/L). The mean replicate growth rate values for both males and females were multiplied by 10 by the reviewer in order to avoid treatment means of 0 within Toxstat.

Summary of Statistical Methods used for NOAEC/LOAEC Analyses.

Endpoint	Solvent vs Dilution Control		NOAEC/LOAEC	
	Method	Diff ⁽¹⁾ (%)	Method	Diff ⁽²⁾ (%)
28-d Percent Emergence (Pooled sexes)	Student's t-test	-1.5%	Williams	1.5
28-d Development Rate- Male	Student's t-test	1.6%	Bonferroni	-0.8
28-d Development Rate- Female	Student's t-test	3.3%	Bonferroni	-2.8
10-d Survival (Optional)	N.D.	N.D.	N.D.	N.D.
10-day Dry Weight (Optional)	N.D.	N.D.	N.D.	N.D.

⁽¹⁾ Difference between the mean dilution water and solvent control responses.

⁽²⁾ Difference between the dilution water and NOAEC concentration treatment.

N.D.- Not Determined

Most sensitive endpoint: Percent Emergence (combined sexes)

Verification Statistical Endpoint Values^(a).

Statistical Endpoint	28-Day Emergence	28-Day Dev. Rate- Male	28-Day Dev. Rate- Female	10-D Survival	10-D Dry Weight
NOAEC	4.00	16.00	16.00	ND	ND
LOAEC	8.00	32.00	32.00	ND	ND
IC ₅₀ (95% C.I.)	20 (18-24)	>32.00	>32.00	ND	ND
Slope (Standard Error)	4.07±0.664	N/A	N/A	ND	ND

^(a) Results are based on nominal initial overlying water concentrations (µg ai/L).

The 10-Day Survival and Dry Weight were not assessed

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were more conservative than those of the study author as the reviewer detected significant inhibitions in male and female development rate at the highest treatment level relative to the negative control. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

Overlying water and pore water samples from the surrogate vessels (one vessel per interval) were analyzed at 0, 7, and 28 days and analyzed for residues of NNI-0001-des-iodo for TWA calculations. However, as actual concentrations were not determined for each treatment level, results were reported in terms of initial nominal overlying water levels.

A detailed statistical report was provided in the study. Development rate data were assessed not only for combined sexes, but also for individual sexes. As assessment of the sex ratio percentages were not statistically different, only combined-sex data were reported within the study. However, the reviewer analyzed both data sets separately as both were readily available.

Overlying and pore water samples were analyzed by direct injection of the samples into an HPLC-MS/MS instrument. The mass spectrometric detector showed linear response in the concentration range of 0.042 µg/L to 12.5 µg/L for NNI-0001 in surface water with a correlation coefficient of 0.9995 and in the concentration range of 0.041 µg/L to 12.3 µg/L for NNI-0001-des-iodo in surface water with a correlation coefficient of 0.9997.

The MS/MS detection of NNI-0001 and NNI-0001-des-iodo were slightly affected by the matrix. The peak area of NNI-0001 in a surface water sample containing 0.5 µg/L was reduced to approximately 82% of the corresponding peak area in milli-Q-water. The peak area of NNI-0001-des-iodo in a surface water sample containing 0.5 µg/L was reduced to approximately 83% of the corresponding peak area in milli-Q-water.

The reviewer calculated the time-weighted average concentrations for the nominal 0.25, 4.00 and 32.00 µg ai/L treatment levels using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C_{TWA} is the time-weighted average concentration,

C_j is the concentration measured at time interval j ($j = 0, 1, 2, \dots, n$)

t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j

(e.g., $t_0 = 0$ hours (test initiation), $t_1 = 24$ hours, $t_2 = 96$ hours)

The experimental work began on March 5, 2004. The biological and analytical portions of the study were completed on April 9, 2004 and April 26, 2004. An initial definitive test was initiated on October 31, 2003; however, the chosen test concentrations did not show enough dose-related effects. The results from this test were not included in the study report.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	82.5000	CALCULATED t VALUE =	-0.3612
GRP2 (BLANK CTRL) MEAN =	83.7500	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-1.2500		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.412	8.712	13.752	8.712	2.412
OBSERVED	0	13	12	11	0

Calculated Chi-Square goodness of fit test statistic = 7.7586
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 2693.750

W = 0.964

Critical W (P = 0.05) (n = 36) = 0.935
 Critical W (P = 0.01) (n = 36) = 0.912

Data PASS normality test at P=0.01 level. Continue analysis.

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 22.75
 Closest, conservative, Table H statistic = 281.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 9, df (# reps-1) = 3
 Actual values ==> R (# groups) = 9, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

 Calculated B statistic = 8.58
 Table Chi-square value = 20.09 (alpha = 0.01)
 Table Chi-square value = 15.51 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
 Used for Chi-square table value ==> df (#groups-1) = 8

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	14925.000	1865.625	18.699
Within (Error)	27	2693.750	99.769	
Total	35	17618.750		

Critical F value = 2.31 (0.05,8,27)
 Since F > Critical F REJECT Ho:All groups equal

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	82.500	82.500		
2	0.25	77.500	77.500	0.708	
3	0.50	83.750	83.750	-0.177	
4	1.00	75.000	75.000	1.062	
5	2.00	78.750	78.750	0.531	
6	4.00	81.250	81.250	0.177	
7	8.00	68.750	68.750	1.947	
8	16.00	55.000	55.000	3.894	*
9	32.00	16.250	16.250	9.380	*

DP Barcode: 77777777

MRID No.: 468170-23

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=24,8)

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.25	4	17.869	21.7	5.000
3	0.50	4	17.869	21.7	-1.250
4	1.00	4	17.869	21.7	7.500
5	2.00	4	17.869	21.7	3.750
6	4.00	4	17.869	21.7	1.250
7	8.00	4	17.869	21.7	13.750
8	16.00	4	17.869	21.7	27.500
9	32.00	4	17.869	21.7	66.250

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	82.500	82.500	82.500
2	0.25	4	77.500	77.500	80.625
3	0.50	4	83.750	83.750	80.625
4	1.00	4	75.000	75.000	78.333
5	2.00	4	78.750	78.750	78.333
6	4.00	4	81.250	81.250	78.333
7	8.00	4	68.750	68.750	68.750
8	16.00	4	55.000	55.000	55.000
9	32.00	4	16.250	16.250	16.250

% Emergence (pooled sex), Day 28; ug ai/L
 File: 7023pe Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	82.500				
0.25	80.625	0.265		1.71	k= 1, v=27
0.50	80.625	0.265		1.79	k= 2, v=27
1.00	78.333	0.590		1.81	k= 3, v=27
2.00	78.333	0.590		1.82	k= 4, v=27
4.00	78.333	0.590		1.83	k= 5, v=27
8.00	68.750	1.947	*	1.84	k= 6, v=27
16.00	55.000	3.894	*	1.84	k= 7, v=27
32.00	16.250	9.380	*	1.84	k= 8, v=27

s = 9.988

Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	8.0	5.4	12.	0.087	0.67
EC10	9.9	7.0	14.	0.073	0.71
EC25	14.	11.	18.	0.051	0.79
EC50	20.	18.	24.	0.031	0.87

Slope = 4.07 Std.Err. = 0.664

Goodness of fit: p = 0.84 based on DF= 6.0 27.

7023PE : % Emergence (pooled sex), Day 28; ug ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	82.5	79.1	3.43	100.	0.00
0.250	4.00	77.5	79.1	-1.57	100.	3.59e-13
0.500	4.00	83.8	79.1	4.68	100.	2.73e-09
1.00	4.00	75.0	79.1	-4.07	100.	4.88e-06
2.00	4.00	78.8	79.1	-0.321	100.	0.00202
4.00	4.00	81.3	78.9	2.34	99.8	0.200
8.00	4.00	68.8	75.2	-6.43	95.1	4.93
16.0	4.00	55.0	52.6	2.43	66.5	33.5
32.0	4.00	16.3	16.7	-0.482	21.2	78.8

Male development rate, Day 28; ug ai/L

File: 7023md Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	0.6150	CALCULATED t VALUE =	0.7746
GRP2 (BLANK CRTL) MEAN =	0.6050	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0100		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Male development rate, Day 28; ug ai/L

File: 7023md Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.345	8.470	13.370	8.470	2.345
OBSERVED	0	12	13	10	0

Calculated Chi-Square goodness of fit test statistic = 6.4478
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Male development rate, Day 28; ug ai/L

File: 7023md Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.019

W = 0.949

Critical W (P = 0.05) (n = 35) = 0.934

Critical W (P = 0.01) (n = 35) = 0.910

Data PASS normality test at P=0.01 level. Continue analysis.

Male development rate, Day 28; ug ai/L
File: 7023md Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 73.50
Closest, conservative, Table H statistic = 281.0 (alpha = 0.01)Used for Table H ==> R (# groups) = 9, df (# reps-1) = 3
Actual values ==> R (# groups) = 9, df (# avg reps-1) = 2.89
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).Male development rate, Day 28; ug ai/L
File: 7023md Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 15.82
Table Chi-square value = 20.09 (alpha = 0.01)
Table Chi-square value = 15.51 (alpha = 0.05)Average df used in calculation ==> df (avg n - 1) = 2.89
Used for Chi-square table value ==> df (#groups-1) = 8

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).Male development rate, Day 28; ug ai/L
File: 7023md Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
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DP Barcode: 7777777

MRID No.: 468170-23

Between	8	0.0068	0.0008	1.143
Within (Error)	26	0.0193	0.0007	
Total	34	0.0261		

Critical F value = 2.32 (0.05,8,26)
 Since F < Critical F FAIL TO REJECT Ho:All groups equal

Male development rate, Day 28; ug ai/L
 File: 7023md Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.615	0.615		
2	0.25	0.600	0.600	0.802	
3	0.50	0.608	0.608	0.401	
4	1.00	0.620	0.620	-0.267	
5	2.00	0.623	0.623	-0.401	
6	4.00	0.610	0.610	0.267	
7	8.00	0.613	0.613	0.134	
8	16.00	0.620	0.620	-0.267	
9	32.00	0.570	0.570	2.227	

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=26,8)

Male development rate, Day 28; ug ai/L
 File: 7023md Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.25	4	0.050	8.2	0.015
3	0.50	4	0.050	8.2	0.007
4	1.00	4	0.050	8.2	-0.005
5	2.00	4	0.050	8.2	-0.007
6	4.00	4	0.050	8.2	0.005
7	8.00	4	0.050	8.2	0.003
8	16.00	4	0.050	8.2	-0.005
9	32.00	3	0.054	8.8	0.045

Male development rate, Day 28; ug ai/L
 File: 7023md Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	0.615	0.615	0.615
2	0.25	4	0.600	0.600	0.613
3	0.50	4	0.608	0.608	0.613

4	1.00	4	0.620	0.620	0.613
5	2.00	4	0.623	0.623	0.613
6	4.00	4	0.610	0.610	0.613
7	8.00	4	0.613	0.613	0.613
8	16.00	4	0.620	0.620	0.613
9	32.00	3	0.570	0.570	0.570

Male development rate, Day 28; ug ai/L
 File: 7023md Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.615				
0.25	0.613	0.093		1.71	k= 1, v=26
0.50	0.613	0.093		1.79	k= 2, v=26
1.00	0.613	0.093		1.81	k= 3, v=26
2.00	0.613	0.093		1.82	k= 4, v=26
4.00	0.613	0.093		1.83	k= 5, v=26
8.00	0.613	0.093		1.84	k= 6, v=26
16.00	0.613	0.093		1.84	k= 7, v=26
32.00	0.570	2.166	*	1.84	k= 8, v=26

s = 0.027

Note: df used for table values are approximate when v > 20.

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	0.5350	CALCULATED t VALUE =	1.5275
GRP2 (BLANK CRTL) MEAN =	0.5175	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0175		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.345	8.470	13.370	8.470	2.345
OBSERVED	0	11	13	11	0

Calculated Chi-Square goodness of fit test statistic = 6.2117
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.006

W = 0.969

Critical W (P = 0.05) (n = 35) = 0.934

Critical W (P = 0.01) (n = 35) = 0.910

Data PASS normality test at P=0.01 level. Continue analysis.

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 14.75
 Closest, conservative, Table H statistic = 281.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 9, df (# reps-1) = 3
 Actual values ==> R (# groups) = 9, df (# avg reps-1) = 2.89
 (average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 6.40
 Table Chi-square value = 20.09 (alpha = 0.01)
 Table Chi-square value = 15.51 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.89
 Used for Chi-square table value ==> df (#groups-1) = 8

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	0.0043	0.0005	2.500

Within (Error)	26	0.0060	0.0002
Total	34	0.0103	

Critical F value = 2.32 (0.05,8,26)
 Since F > Critical F REJECT Ho:All groups equal

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.535	0.535		
2	0.25	0.528	0.528	0.750	
3	0.50	0.525	0.525	1.000	
4	1.00	0.540	0.540	-0.500	
5	2.00	0.540	0.540	-0.500	
6	4.00	0.543	0.543	-0.750	
7	8.00	0.545	0.545	-1.000	
8	16.00	0.545	0.545	-1.000	
9	32.00	0.507	0.507	2.623	

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=26,8)

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.25	4	0.027	5.0	0.007
3	0.50	4	0.027	5.0	0.010
4	1.00	4	0.027	5.0	-0.005
5	2.00	4	0.027	5.0	-0.005
6	4.00	4	0.027	5.0	-0.008
7	8.00	4	0.027	5.0	-0.010
8	16.00	4	0.027	5.0	-0.010
9	32.00	3	0.029	5.4	0.028

Female development rate, Day 28; ug ai/L
 File: 7023fd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	0.535	0.535	0.538
2	0.25	4	0.528	0.528	0.538
3	0.50	4	0.525	0.525	0.538
4	1.00	4	0.540	0.540	0.538

DP Barcode: 7777777

MRID No.: 468170-23

5	2.00	4	0.540	0.540	0.538
6	4.00	4	0.543	0.543	0.538
7	8.00	4	0.545	0.545	0.538
8	16.00	4	0.545	0.545	0.538
9	32.00	3	0.507	0.507	0.507

Female development rate, Day 28; ug ai/L
File: 7023fd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.538				
0.25	0.538	0.233		1.71	k= 1, v=26
0.50	0.538	0.233		1.79	k= 2, v=26
1.00	0.538	0.233		1.81	k= 3, v=26
2.00	0.538	0.233		1.82	k= 4, v=26
4.00	0.538	0.233		1.83	k= 5, v=26
8.00	0.538	0.233		1.84	k= 6, v=26
16.00	0.538	0.233		1.84	k= 7, v=26
32.00	0.507	2.446	*	1.84	k= 8, v=26

s = 0.015

Note: df used for table values are approximate when v > 20.

EXHIBIT 34

DATA EVALUATION RECORD
FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST

1. **CHEMICAL:** Flubendiamide **PC Code:** 027602

2. **TEST MATERIAL:** [¹⁴C]Flubendiamide-desiido **Purity:** 99%

3. **CITATION:**

Authors: Thomas, S., *et al.*

Title: [¹⁴C]NNI-0001-desiido: A Prolonged Sediment Toxicity Test with *Chironomus riparius* Using Spiked Sediment.

Study Completion Date: July 28, 2010

Laboratory: Wildlife International Ltd.
8598 Commerce Drive
Easton, MD 21601

Sponsor: Bayer CropScience
P.O. Box 12014, 2T.W. Alexander Drive
Research Triangle Park, NC 27709

Laboratory Report ID: 149A-235

MRID No.: 48175605

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: *Christie E. Padova*

Date: 01/31/11

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: *Teri S. Myers*

Date: 02/16/11

5. **APPROVED BY:** Robin Stenberg, EPA

Signature: *Robin Stenberg*

Date: 7/19/11

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Chironomus riparius*

Age of Test Organism: 1st instar larvae, 1 to 4 days post-hatch

Definitive Test Duration: 28 days

Study Method: Static, with aeration

Type of Concentrations: TWA sediment, pore water, and overlying water



M-425247-01-1

7. CONCLUSIONS:

Results Synopsis:

Time-Weighted Average (TWA) Sediment Concentrations:

28-day LC₅₀: >52.6 µg TRR/kg
28-day NOAEC: 52.6 µg TRR/kg
28-day LOAEC: >52.6 µg TRR/kg

Time-Weighted Average (TWA) Pore Water Concentrations:

28-day LC₅₀: >19.5 µg TRR/L
28-day NOAEC: 19.5 µg TRR/L
28-day LOAEC: >19.5 µg TRR/L

Time-Weighted Average (TWA) Overlying Water Concentrations:

28-day LC₅₀: >7.18 µg TRR/L
28-day NOAEC: 7.18 µg TRR/L
28-day LOAEC: >7.18 µg TRR/L

Assessment endpoints: percent emergence (survival), emergence ratio, development rate, and development time

Most sensitive endpoints: none

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This study was conducted according to OECD Guideline 218: *Sediment-Water Chironomid Toxicity Test Using Spiked Sediment* (April 2004), and does not fulfill any current U.S. EPA data requirement.

C. Reparability: N/A

9. MAJOR GUIDELINE DEVIATIONS (from OECD Guideline 218):

It was not reported if aeration of the overlying water was stopped for a 24-hour period during and immediately following the insertion of the larvae.

10. SUBMISSION PURPOSE: RS Non-PRIA 575 data

11. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: The stability of flubendiamide-desiido was not specifically assessed. However, overlying water, pore water, and sediment samples were analyzed for total radioactive residues (TRR) of the test substance using LSC analyses on Days 0, 7, and 28. In general, the concentrations of TRR were variable but showed an overall decrease in sediment, while concentrations of TRR decreased in pore water and increased in overlying water. The majority of radioactivity remained associated with the sediment.

In the treated sediment, recoveries of TRR ranged from 55.2 to 71.3% of nominal concentrations at 0 Days, 40.5 to 83.2% of nominal at 7 Days, and 43.4 to 57.7% of nominal at 28 Days. In overlying water samples, concentrations of TRR increased 71 to 146% of initial measured levels from Days 0 to 28 at all levels (reviewer-calculated). In pore water, concentrations of TRR decreased 43 to 52% of initial measured levels from Days 0 to 28 at all levels. For all matrices, time-weighted averaged (TWA) concentrations were reviewer-calculated (using Excel software; copy provided in Appendix II).

Mass balance approximations were provided by the study authors. The TRR recovered ranged from 71.7 to 112% of the applied for all levels and intervals.

Physicochemical properties of flubendiamide-desiido.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV adsorption	Not reported	
pKa	Not reported	
Kow	Not reported	

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound.

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
<p><u>Species</u> <i>Chironomus riparius</i></p>	<p><i>Chironomus riparius</i>, identity verified by supplier</p>
<p><u>Source</u></p>	<p>Egg masses were supplied by Environmental Consulting and Testing, Superior, Wisconsin</p>
<p><u>Culture Conditions</u> A reproduction and oviposit chamber should consist of an adult area, sufficiently large to allow swarming (minimum 30 x 30 x 30 cm), and an oviposit area. Crystallizing dishes or larger containers with a thin layer of quartz sand (5 to 10 mm) or Kieselgur (thin layer to a few mm) spread over the bottom and containing suitable water to a depth of several cm are suitable as an oviposit area. Environmental conditions: temperature 20±2°C; 16:8 hours light:dark (intensity ca. 1000 lux); air humidity ca. 60%</p>	<p>N/A</p>
<p><u>Egg Mass Acclimation Period</u> Four to five days before test initiation freshly laid egg masses should be taken from cultures and maintained separately in culture medium, temperature change should not exceed 2°C per day.</p>	<p>The organisms were held for 5 days prior to the start of the test at approximately the same temperature used during testing and in water from the same source as used during testing.</p> <p>During the 5-day holding period preceding the test, water temperatures ranged from 19.9 to 20.4°C, the pH ranged from 8.3 to 8.5, and the dissolved oxygen ranged from 7.8 to 8.9 mg/L (≥86% saturation).</p>
<p><u>Age of Test Larvae</u> First instar (1 to 4 days post-hatch with confirmation)</p>	<p>1st instar, 1 to 4 days post-hatch</p> <p>The hatched midges from at least three separate egg masses were used to initiate the test.</p>

Guideline Criteria	Reported Information
<p>Food Green algae (e.g., <i>Scenedesmus subspicatus</i>, <i>Chlorella vulgaris</i>) or flaked fish food as a ground powder, suspension, or filtrate</p>	Ground Hartz® pet rabbit food
<p>Health of parent culture stock Were parent chironomids in good health during the culture period?</p>	N/A

B. Test System

Guideline Criteria	Reported Information
<p>Type of Test System Static (static-renewal or flow-through of overlying water is evaluated on a chemical-specific basis). Distilled or deionized water may be added to overlying water once daily as needed to maintain volume.</p>	<p>Static with aeration. Additional vessels were prepared at each level for analytical sampling; thus, the method for analytical sampling did not affect volume, biological load, or test concentration.</p>
<p>Test Material</p>	<p>Identity: [¹⁴C]flubendiamide-desiido Batch No.: Not reported Description: solid Radiochemical purity: 99% Specific activity: 79.26 mCi/mmol Label position: uniformly on the phthalic acid ring Storage: frozen conditions</p>

Guideline Criteria	Reported Information
<p><u>Test Water</u> Soft reconstituted water or water from a natural source is preferred. Dechlorinated tap water may be used if the test organism will survive in it for the duration of the culturing and testing without showing signs of stress.</p>	<p>Moderately-hard freshwater obtained from an on-site well <i>ca.</i> 40-m deep was sand-filtered, aerated, and filtered again (0.45 μm) and UV-sterilized prior to use.</p> <p>During the 4-week period immediately preceding the study, the specific conductance of the well water ranged from 338 to 366 μS/cm, the hardness ranged from 140 to 144 mg/L as CaCO₃, the alkalinity ranged from 180 to 182 mg/L as CaCO₃, and the pH ranged from 8.1 to 8.2.</p>
<p><u>Test Sediment</u> Formulated (reconstituted, artificial, or synthetic) sediment is recommended. Content of sediment by dry weight: 5% peat (dry) (pH 5.5-6.0) or alpha-cellulose, 75% quartz sand (>50% in size range of 50-200 microns), 20% kaolinite clay (kaolinite content <i>ca.</i> 30%), CaCO₃ 0.05-0.1%. Moisture content 30-50%, TOC 2% (\pm0.5%) and pH 6.5 - 7.5. Natural sediment can be used if it is fully characterized, unpolluted, and free of organisms that might compete with or consume chironomids. (If solvent other than water will be used, sand content of artificial sediment is adjusted accordingly.)</p>	<p>Formulated (artificial) sediment consisted of 75% industrial quartz sand, 20% kaolin clay, and 5% sphagnum peat moss. The dry ingredients were mixed in a PK Twinshell® mixer for 40 minutes and stored under ambient conditions until use. The amount of peat added to the batch sediment was adjusted for the moisture content in the peat suspension (70%). The laboratory-determined pH of the sediment was 7.2.</p> <p>The soil was characterized by Agvise Laboratories (Northwood, ND). The following characteristics were provided:</p> <p>Composition: 77% sand, 9% silt, and 14% clay USDA textural class: sandy loam Bulk density: 1.24 g/cm³ CEC: 9.3 meq/100 g Moisture at 1/3 bar: 11.5% Organic carbon: 1.9% Organic matter: 3.2% pH (1:1 soil:water ratio): 7.5</p>

Guideline Criteria	Reported Information
<p><u>Sediment Spiking</u></p>	<p>A 0.140 mg/mL primary stock solution was prepared by dissolving the radio-labeled test material in acetone. Secondary stocks (10.0, 5.00, 2.50, 1.30, 0.63, and 0.31 $\mu\text{g/mL}$) were prepared by proportional dilution and mixed by inversion. The primary and secondary stock solutions appeared clear and colorless. A 15-mL aliquot of the appropriate stock solution was added to 150 g of formulated sediment and mixed by hand, and the acetone was allowed to partially evaporate. The 150-g premix was added to 600 g of untreated sediment and mixed for an unspecified period of time, and then 750 g of untreated sediment was added and the final batches (1500 g final weight) mixed using a rotary mixer for <i>ca.</i> 40 hours.</p> <p>Batches of negative and solvent control sediment were also prepared. No adjustments were made for the purity of the test material.</p>
<p><u>Sediment Conditioning</u> <u>Artificial sediment:</u> 7 days in flowing dilution water prior to test initiation, chambers may be aerated</p>	<p>Test systems (spiked-sediment:overlying water) were prepared and acclimated for <i>ca.</i> 50 hours prior to the introduction of the test organisms. The systems were gently aerated and maintained in an environmental chamber.</p>
<p><u>Introduction of Test Organisms</u> Twenty-four hours prior to test initiation aeration of chambers is stopped and organisms are added to the chambers. Aeration should not resume for at least 24 hours. At test initiation, the test substance is spiked into the overlying water column.</p>	<p>At test initiation, midge larvae were impartially added one and two at a time to the test chambers. It was not reported if aeration was discontinued during and 24 hour immediately following the insertion of larvae.</p>

Guideline Criteria	Reported Information
<p><u>Solvents</u> If used, minimal (i.e., ≤ 0.1 mL/l) and same concentration in all treatments. Suitable solvents are acetone, ethanol, methanol, ethylene glycol monoethyl ether, ethylene glycol dimethyl ether, dimethylformamide or triethylene glycol. (OECD guidelines also allows use of dispersants: Cremophor RH40, Tween 80, methylcellulose 0.01%, and HCO-40)</p>	<p>Acetone, 15 mL/1500 g sediment</p> <p>The reviewer-calculated maximum possible concentration of acetone in the sediment (assuming no evaporation occurred) was equivalent to 0.8% (where ρ of acetone = 0.79 g/mL).</p>
<p><u>Water Temperature</u> $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ (Should not deviate between vessels by more than 1°C.)</p>	<p>Daily: 19.8 to 20.8°C Continuous: 19 to 20°C</p>
<p><u>pH</u> <u>Sediment</u>: 7.0 ± 0.5 <u>Interstitial Water</u>: <u>Overlying Water</u>: 6.0 to 9.0 (Should not vary by more than 1 unit during test)</p>	<p><u>Sediment</u>: 7.2 to 7.5 (initial analysis) <u>Interstitial Water</u>: Not determined <u>Overlying Water</u>: 8.0 to 8.6</p>
<p><u>TOC</u> <u>Sediment</u>: $2 \pm 0.5\%$ <u>Overlying Water</u>: 2 mg/L</p>	<p><u>Sediment</u>: 1.9% (initial analysis) <u>Overlying Water</u>: Not determined</p>
<p><u>Ammonia</u> <u>Interstitial Water</u>: <u>Overlying Water</u>:</p>	<p><u>Interstitial Water</u>: Not determined <u>Overlying Water</u>: Day 0: <0.17 mg/L Day 28: ≤ 1.57 mg/L</p>
<p><u>Total Water Hardness</u> 200 mg/L as CaCO_3 (prefer 160 to 180 mg/L as CaCO_3)</p>	<p>156 to 164 mg/L as CaCO_3</p>
<p><u>Dissolved Oxygen</u> 60% air saturation value throughout test</p>	<p>≥ 5.6 mg/L ($\geq 62\%$ of saturation)</p>

Guideline Criteria	Reported Information
<p>Aeration (ca. one bubble/sec) is allowed except for when larvae are being added and for at least 24 hours after introduction of test organisms to a test chamber. If one test chamber is aerated all test chambers must be treated the same.</p>	<p>Gentle aeration (>1 bubble/sec) was provided to each vessel through a glass pipette that did not extend to a depth closer than 2 cm from the sediment's surface.</p> <p>It was not reported if aeration was stopped during the addition of larvae.</p>
<p><u>Test Vessels or Compartments</u> 1. <u>Material</u>: Glass, No. 316 stainless steel, teflon or perfluorocarbon plastics 2. <u>Size</u>: Sediment depth of 1.5- 3 cm and the depth ratio of sediment to water should be ca. 1:4, must not be >1:4; 600 ml beaker with 8 cm diameter</p>	<p>Test vessels were 1-quart glass jars containing 2 cm of sediment and 600 mL of overlying water. The measured depth in sediment and overlying water from one representative chamber was 2.1 and 8.3 cm, respectively. Thus, the sediment:water ratio was \approx1:4.</p>
<p><u>Covers</u> Test vessels should be covered with a glass plate.</p>	<p>Vessels were loosely covered with plastic dishes.</p>
<p><u>Photoperiod</u> 16 hours light, 8 hours dark (Light intensity 500 to 1000 lux)</p>	<p>16 hours light:8 hours dark, with 30-minute low light transition periods</p> <p>Light intensity was 446 lux at the surface of one representative test chamber.</p>
<p><u>Food</u> Green algae (e.g., <i>Scenedesmus subspicatus</i>, <i>Chlorella vulgaris</i>) or flaked fish food as a ground powder, suspension, or filtrate</p>	<p>Ground Hartz® pet rabbit food</p>
<p><u>Food Concentration and Frequency</u> Preferably feed daily but at least 3 times per week. <u>day 1 to 10</u>: 0.25-0.5 mg per larvae per day <u>remainder of test</u>: 0.5-1 mg per larvae per day (keep to a minimum, should not accumulate on sediment surface, cause overlying water to be cloudy or cause drop in DO)</p>	<p>Three times per week 10 to 30 mg per vessel per feeding</p>

C. Test Design

Guideline Criteria	Reported Information
<p><u>Duration</u> <i>Chironomus riparius</i>: 28 days (if midges emerge early the test can be terminated after a minimum of 5 days after emergence of the last adult in the control).</p>	<p>28 days</p>
<p><u>Nominal Concentrations</u> Negative control, solvent control (if a solvent was used) and at least 5 test concentrations. (Note exception to dilution factors described below can be made for shallow slope responses but minimum number of test concentrations may need to be increased)</p> <p><u>ECx endpoint</u>: test concentrations should bracket ECx and span the environmental concentration range. Dilution factor should not be greater than two between exposure concentrations.</p> <p><u>NOEC/LOEC endpoint</u>: factor between concentrations must not be greater than 3.</p>	<p>Negative control, solvent control, 3.1, 6.3, 13, 25, 50, and 100 µg/kg dw sediment (not corrected for purity)</p> <p><u>ECx endpoint</u>: N/A</p> <p><u>NOAEC/LOAEC endpoint</u>: A nominal factor rate of 2 was used.</p>
<p><u>Number of Test Organisms**</u> <u>ECx endpoint</u>: 60 larvae per treatment level; 3 replicates per treatment level</p> <p><u>NOAEC/LOAEC endpoint</u>: at least 80 larvae per treatment level with at least 4 replicates per treatment level (adequate power to detect a 20% difference, Type I error rate 5%)</p> <p>*(Optional) If data on 10-day growth and survival are needed additional replicates (number based on ECx or NOEC/LOEC endpoint determination) should be included at test initiation..</p>	<p><u>ECx endpoint</u>: N/A</p> <p><u>NOAEC/LOAEC endpoint</u>: 80 larvae per treatment level divided evenly into four replicates (each containing 20 organisms).</p> <p>** (Optional) 10-day growth data were not collected.</p>

Guideline Criteria	Reported Information
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Yes</p>
<p><u>Overlying Water Parameter Measurements</u></p> <p>1. Dissolved oxygen should be measured daily in all test chambers.</p> <p>2. Temperature and pH should be measured in all test chambers at the start and end of the test and at least once a week during the test.</p> <p>3. Temperature should be monitored at least hourly throughout the test in one test chamber.</p> <p>4. Hardness and ammonia should be measured in the controls and one test chamber at the highest concentration at the start and end of the test.</p>	<p>1. – 3. DO and temperature were measured daily in one alternating replicate chamber for each level. Temperature was also continuously monitored in a beaker of water adjacent to the test chambers. The pH was measured at test initiation, weekly during the test, and at test termination in one alternating replicate chamber for each level.</p> <p>4. Hardness, ammonia, specific conductance, and alkalinity were measured in a composite sample of overlying water from the control groups and from the highest treatment level (i.e., 100 µg/kg) at study initiation and termination.</p>
<p><u>Chemical Analysis-Overlying Water</u></p> <p>At a minimum must be analyzed at test initiation (i.e., one hour after introduction of test substance into the test chamber) and at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>Surrogate samples (three per level) were collected for analysis on Days 0, 7, and 28. Overlying water was decanted and 10-mL aliquots analyzed for total radioactive residues of [¹⁴C]flubendiamide-desiodo using LSC. The limit of quantitation (LOQ) was 0.0133 µg/L.</p>
<p><u>Interstitial Water and Sediment Isolation Method</u></p> <p>Centrifugation (e.g., 10,000 g and 4 EC for 30 min) is recommended. If test substance is demonstrated not to adsorb to filters, filtration may be acceptable.</p>	<p>Not reported</p>

Guideline Criteria	Reported Information
<p><u>Chemical Analysis-Interstitial Water</u> At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>Surrogate samples (three per level) were collected for analysis on Days 0, 7, and 28; 10-mL aliquots were analyzed for total radioactive residues of [¹⁴C]flubendiamide-desiido using LSC. The limit of quantitation (LOQ) was 0.0133 µg/L.</p>
<p><u>Chemical Analysis-Bulk Sediment</u> At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>Surrogate samples (three per level) were collected for analysis on Days 0, 7, and 28. Isolated sediment was dried overnight and analyzed for total radioactive residues of [¹⁴C]flubendiamide-desiido using LSC following combustion. The limit of quantitation (LOQ) was 0.293 µg/kg.</p>

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>Yes. This study was conducted in compliance with U.S. EPA 40 CFR, Parts 160 and 792, with the following exceptions: periodic analysis of well water and sediment for potential contaminants, and the stability of the test substance under conditions of storage at the testing facility. It was reported that the periodic analysis (of water and sediment) was performed using a certified laboratory and standard U.S. EPA analytical methods.</p>
<p><u>Control Mortality</u> <30%</p>	<p>Negative control – 29% Solvent control – 30%</p>
<p>Did chironomids emerge in controls between day 12 and 23?</p>	<p>Negative controls – Days 15 to 28 Solvent control – Days 15 to 28</p>

Guideline Criteria	Reported Information
<p><u>Control Emergence</u> Mean emergence between 50-70%</p>	<p>Negative control – 71% emergence Solvent control – 73% emergence</p>
<p><u>Data Endpoints</u> <u>Emergence Test (28 day)</u> - Number alive - Time to emergence - Number of emerged male and female midges - Number of visible pupae that have failed to emerge - Number of egg masses deposited - Observations of other effects, abnormal behavior, or appearance or clinical signs (e.g., leaving sediment, unusual swimming) <u>Growth and Survival (10-day) (Optional)</u> - Number alive - Instar level of surviving larvae - Dry weight (ash free) per test chamber of surviving larvae by instar level</p>	<p><u>Emergence Test (28 days)</u> - Mortality - Time to emergence - Number of emerged male and female midges - Emergence rate - Development rate - Development time <u>Growth and Survival (10-day) (Optional)</u> N/A</p>
<p>Raw data included?</p>	<p>Yes</p>

Effects DataTable 1. Summary of [¹⁴C]Flubendiamide-desiido effects on *Chironomus riparius* emergence success and sex ratio

Toxicant Concentration				Initial No.	Mean Number Emerged			Mean Sex Ratio ^(b) (%)		% Emergence (Day 28)
Mean-measured (and Nominal) Sediment (µg/kg dw)	TWA Measured ^(a)				♂	♀	Total	♂	♀	
	Sediment (µg TRR/kg dw)	Overlying Water (µg TRR/L)	Pore Water (µg TRR/L)							
Negative control	<LOQ	<LOQ	<LOQ	80	34	23	57	60	40	71
Solvent control	<LOQ	<LOQ	<LOQ	80	36	22	58	62	38	73
1.7 (3.1)	1.75	0.195	0.551	80	27	22	49	55	45	61
4.3 (6.3)	4.44	0.453	1.10	80	27	24	51	53	47	64
7.8 (13)	7.31	0.895	2.44	80	25	32	57	44	56	71
13 (25)	12.2	1.72	4.28	80	32	27	59	54	46	74
30 (50)	28.5	3.50	9.06	80	23	32	55	42	58	69
55 (100)	52.6	7.18	19.5	80	35	29	64	55	45	80

^(a) TWA concentrations were determined by the reviewer using Excel software (copy of worksheet in Appendix II). The limit of quantitation (LOQ) was 0.293 µg TRR/kg for sediment and 0.0133 µg TRR/L for overlying and pore water. TRR = Total Radioactive Residues of [¹⁴C]flubendiamide-desiido.

^(b) Equivalent to the number of emerged males (or females)/number of emerged larvae x 100; reviewer-calculated.

Table 2. Summary of [¹⁴C]Flubendiamide-desido effects on *Chironomus riparius* development time and rate.

Mean-measured (and Nominal) Sediment (µg/kg dw)	Toxicant Concentration			Mean Emergence Ratio	Mean Development Rate ^(b) (1/days)	Mean Development Time (days)
	TWA Measured ^(a)					
	Sediment (µg TRR/ kg dw)	Overlying Water (µg TRR/L)	Pore Water (µg TRR/L)			
Negative control	<LOQ	<LOQ	<LOQ	0.71	0.0471	22.5
Solvent control	<LOQ	<LOQ	<LOQ	0.73	0.0482	21.9
1.7 (3.1)	1.75	0.195	0.551	0.61	0.0466	22.6
4.3 (6.3)	4.44	0.453	1.10	0.64	0.0490	21.7
7.8 (13)	7.31	0.895	2.44	0.71	0.0494	21.1
13 (25)	12.2	1.72	4.28	0.74	0.0495	21.4
30 (50)	28.5	3.50	9.06	0.69	0.0469	22.4
55 (100)	52.6	7.18	19.5	0.80	0.0480	21.9

^(a) TWA concentrations were determined by the reviewer using Excel software (copy of worksheet in Appendix II). The limit of quantitation (LOQ) was 0.293 µg TRR/kg for sediment and 0.0133 µg TRR/L for overlying and pore water. TRR = Total Radioactive Residues of [¹⁴C]flubendiamide-desido.

$$^{(b)} \text{Mean development rate} = \sum_{i=1}^m \frac{f_i x_i}{n_e}$$

where: i = index of inspection interval; m = maximum number of inspection intervals; f_i = number of midges emerged in the inspection interval i ; n_e = total number of midges emerged; and $x_i = \frac{1}{\left(\text{day}_i - \frac{l_i}{2}\right)}$ which is the development rate of the midges emerged in interval i ; day_i = inspection day (days since application); and l_i = length of inspection interval i (days, 1 day in this study).

Toxicity Observations: Emergence was first noted on Day 15, and adults that emerged appeared normal. There were a few observations of organisms climbing the walls of the test chamber, on the surface of the sediment, and/or swimming in the water column prior to adult maturation; occasional partial emergence; and adults that emerged and subsequently died during the maturation period. The observations were few in incidence and occurred in the controls as well as the treatment levels, and were thus not considered to be related to treatment.

Mean mortality at Day 28 was 29, 30, 39, 39, 29, 29, 31, and 20% for the negative control, solvent control, and mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 $\mu\text{g TRR/kg}$ test levels, respectively (TRR = total radioactive residues of [^{14}C]flubendiamide-desido). The observed EC_{50} for mortality of midges was $>55 \mu\text{g TRR/kg}$ based on mean-measured sediment concentrations. Conversely, percent emergence averaged 71, 73, 61, 64, 71, 74, 69, and 80% for the negative control, solvent control, and mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 $\mu\text{g TRR/kg}$ test levels, respectively. No statistically-significant differences were indicated at any treatment level compared to the pooled control, and the NOAEC for percent emergence was 55 $\mu\text{g TRR/kg}$.

Mean development time was 22.5 and 21.9 days in the negative and solvent control groups, respectively, compared to 22.6, 21.7, 21.1, 21.4, 22.4, and 21.9 days for the mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 $\mu\text{g TRR/kg}$ test levels, respectively. There were no statistically-significant differences indicated for any treatment level compared to the pooled control. Thus, the NOAEC for development time was 55 $\mu\text{g TRR/kg}$, based on mean-measured sediment concentrations.

Based upon an ANOVA procedure looking at the interaction between sexes, no significant interaction was found between sex and treatments for development rates, and therefore the data for each sex were pooled for this endpoint. Mean development rates were 0.0471, 0.0482, 0.0466, 0.0490, 0.0494, 0.0495, 0.0469, and 0.0480 days^{-1} for the negative control, solvent control, and mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 $\mu\text{g TRR/kg}$ test levels, respectively; no statistically-significant differences were indicated for any treatment level compared to the pooled control. Thus, the NOAEC for development rate was 55 $\mu\text{g TRR/kg}$ based on mean-measured sediment concentrations.

As previously described for development rates, the interaction between sexes was evaluated for emergence ratios (although it was noted that evaluations of the sensitivity for this endpoint are not meaningful as it is impossible to know the initial number of male and female 1- to 4-day old larvae). No significant interaction was found between sex and treatment. Emergence ratios averaged 0.71, 0.73, 0.61, 0.64, 0.71, 0.74, 0.69, and 0.80 for the negative control, solvent control, and mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 $\mu\text{g TRR/kg}$ test levels, respectively. No statistically-significant differences were indicated for any treatment

level compared to the pooled control. Thus, the NOAEC for emergence ratio was 55 μg TRR/kg based on mean-measured sediment concentrations.

B. Statistical Results (From Study Report)

Endpoints that were statistically evaluated included percent emergence (i.e., survival data), development time, emergence ratio, and development rate. The emergence ratio data were arcsine transformed prior to analysis. NOAEC and LOAEC values were determined by visual interpretation of the dose-response pattern and statistical significance of the data.

The data were analyzed using an appropriate t-test to determine any statistical differences between the negative and solvent control groups. No significant differences were indicated for any endpoint, and the control data were pooled for all subsequent comparisons. Data were analyzed using Dunnett's test, at the $p < 0.05$ level of sensitivity. ANOVA was used to evaluate sensitivity between sexes.

The 28-day EC_{50} was determined by visual interpretation of the mortality data collected at study termination.

All statistical procedures were performed using SAS statistical software and were reported in terms of mean-measured sediment concentrations.

Most sensitive endpoint: none

Endpoint	Methods	LC_{50}/EC_{50} (95% CI) (μg TRR/kg)	NOAEC (μg TRR/kg)	LOAEC (μg TRR/kg)
Percent Emergence	Dunnett's t-test	>55	55	>55
Emergence Ratio	Dunnett's t-test	---	55	>55
Development Rate	Dunnett's t-test	---	55	>55
Development Time	Dunnett's t-test	---	55	>55

13. VERIFICATION OF STATISTICAL RESULTS**Summary of Statistical Methods used for NOAEC/LOAEC Analyses.**

Endpoint	Solvent vs Dilution Control		NOAEC/LOAEC	
	Method	Diff ⁽¹⁾ (%)	Method	Diff ⁽²⁾ (%)
28-d Emergence Rate	Student's t-test	-1.8	ANOVA, Dunnett's test	-12.7
28-d Survival	Student's t-test	1.4	ANOVA, Dunnett's test	-12.7
Development time	Student's t-test	3.1	ANOVA, Dunnett's test	2.9
28-d Development Rate	Student's t-test	-2.3	ANOVA, Dunnett's test	-2.0
10-d Survival (Optional)	---	---	---	---
10-day Dry Weight (Optional)	---	---	---	---

⁽¹⁾ Difference between the mean dilution water and solvent control responses; a negative number indicates a promoted response in the solvent control, relative to the negative control.

⁽²⁾ Difference between the dilution water and NOAEC concentration treatment; a negative number indicates a promoted response in the NOAEC, relative to the negative control.

Most sensitive endpoint: none

Verification Statistical Endpoint Values^(a).

Statistical Endpoint	28-day Emergence	28-day Survival	Development time	28-day Development Rate
NOAEC				
Sediment:	52.6 µg TRR/kg	52.6 µg TRR/kg	52.6 µg TRR/kg	52.6 µg TRR/kg
Overlying Water:	7.18 µg TRR/L	7.18 µg TRR/L	7.18 µg TRR/L	7.18 µg TRR/L
Pore Water:	19.5 µg TRR/L	19.5 µg TRR/L	19.5 µg TRR/L	19.5 µg TRR/L
LOAEC				
Sediment:	>52.6 µg TRR/kg	>52.6 µg TRR/kg	>52.6 µg TRR/kg	>52.6 µg TRR/kg
Overlying Water:	>7.18 µg TRR/L	>7.18 µg TRR/L	>7.18 µg TRR/L	>7.18 µg TRR/L
Pore Water:	>19.5 µg TRR/L	>19.5 µg TRR/L	>19.5 µg TRR/L	>19.5 µg TRR/L
IC ₅₀ (95% C.I.)				
Sediment:	>52.6 µg TRR/kg	>52.6 µg TRR/kg	>52.6 µg TRR/kg	>52.6 µg TRR/kg
Overlying Water:	>7.18 µg TRR/L	>7.18 µg TRR/L	>7.18 µg TRR/L	>7.18 µg TRR/L
Pore Water:	>19.5 µg TRR/L	>19.5 µg TRR/L	>19.5 µg TRR/L	>19.5 µg TRR/L
Slope (Standard Error)	N/A	N/A	N/A	N/A

^(a) Results are based on TWA test concentrations.

14. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study authors'. There was no treatment-related toxicity in this study.

The study was designed to fulfill OECD Guideline 218 *Sediment-Water Chironomid Toxicity Test Using Spiked Sediment* (2004). Although this study does not fulfill any current U.S. EPA guideline requirement, there were no significant deviations from OECD Guideline 218 that would affect the scientific soundness of this study.

In order for the test to be valid, OECD 218 Guidance requires the following conditions: emergence in the controls must be at least 70% at the end of the test; *C. riparius* emergence to adults should occur between 12 and 23 days after their insertion into the vessels; at the end of the test, pH and dissolved oxygen should be measured in each vessel (the oxygen concentration should be at least 60% of the air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels); and the water temperature should not differ by more than $\pm 1.0^\circ\text{C}$. In this study, all validity requirements were considered to be fulfilled. Although emergence in controls occurred between Days 15 and 28 (both groups), this deviation did not have any effect on the scientific soundness of this study.

Although OECD 218 prefers that results are provided in terms of (initial) nominal sediment concentrations, TWA concentrations were reviewer-calculated (refer to associated Excel worksheet in Appendix II). As TWA concentrations are more indicative of exposure levels throughout the study, they were reported in the Statistical Verification and Conclusions sections of the DER. TWA concentrations were calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_n}{2}\right)(t_{n-1} - t_n) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C_{TWA} is the time-weighted average concentration,

C_j is the concentration measured at time interval j ($j = 0, 1, 2, \dots, n$)

t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., $t_0 = 0$ hours (test initiation), $t_1 = 24$ hours, $t_2 = 96$ hours).

At test initiation, the overlying water appeared slightly cloudy and light tan in all test chambers. At termination, it appeared cloudy and tan in all test chambers.

The mean recovery from LSC analysis of the primary stock solution (nominal 150 $\mu\text{g/mL}$) was 93.3% of nominal. Recoveries from LSC analyses of the working stock solutions (nominal 0.31, 0.63, 1.30, 2.50, 5.00, and 10.0 $\mu\text{g/mL}$) ranged from 106 to 108% of nominal concentrations.

Experimental test dates were November 17 to December 16, 2009.

15. REFERENCES:

OECD Guideline 218. 2004. *Sediment-Water Chironomid Toxicity Test Using Spiked Sediment*. Adopted April 2004.

APHA, AWWA, WPCF. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. American Public Health Association. American Waterworks Association. Water Pollution Control Federation, New York.

The SAS Sytem for Windows. 1999-2001. Release 8.2 (TS2M0). SAS Institute, Inc., Cary, North Carolina.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

Title: Percent Emergence
 File: 5605e Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean
 =====
 GRP1 (Solvent cntl) Mean = 0.7125 Calculated t value = -0.1777
 GRP2 (Blank cntl) Mean = 0.7250 Degrees of freedom = 6
 Difference in means = -0.0125
 =====
 2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
 2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!
 Title: Percent Emergence
 File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

 D = 0.4953
 W = 0.9269

Critical W = 0.8960 (alpha = 0.01 , N = 28)
 W = 0.9240 (alpha = 0.05 , N = 28)

 Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Percent Emergence
 File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	0.0451	0.0075	0.6245
Within (Error)	21	0.2528	0.0120	
Total	27	0.2979		

(p-value = 0.7088)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
 = 2.5727 (alpha = 0.05, df = 6,21)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Percent Emergence
 File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	0.1251	0.0208	0.8841
Within (Error)	21	0.4953	0.0236	
Total	27	0.6204		

(p-value = 0.5237)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
 = 2.5727 (alpha = 0.05, df = 6,21)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Percent Emergence
 File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

Dunnnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	TRANS T STAT	SIG
0.05					
1	Neg Control	1.0110	0.7125		
2	1.75	0.9079	0.6125	0.9498	
3	4.44	0.9266	0.6375	0.7773	
4	7.31	1.0068	0.7125	0.0389	
5	12.2	1.0684	0.7375	-0.5286	
6	28.5	0.9844	0.6875	0.2455	
7	52.6	1.1111	0.8000	-0.9218	

Dunnnett critical value = 2.4600 (1 Tailed, alpha = 0.05, df [used] = 6,20)
 (Actual df = 6,21)

Title: Percent Emergence
 File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

Dunnnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.75	4	0.2595	36.1	0.1000
3	4.44	4	0.2595	36.1	0.0750

DP Barcode: 38201C

MRID No.: 48175605

4	7.31	4	0.2595	36.1	0.0000
5	12.2	4	0.2595	36.1	-0.0250
6	28.5	4	0.2595	36.1	0.0250
7	52.6	4	0.2595	36.1	-0.0875

Title: Percent Emergence
 File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL	TRANSFORMED	ISOTONIZED
			MEAN	MEAN	MEAN
1	Neg Control	4	0.7125	1.0110	1.0110
2	1.75	4	0.6125	0.9079	1.0009
3	4.44	4	0.6375	0.9266	1.0009
4	7.31	4	0.7125	1.0068	1.0009
5	12.2	4	0.7375	1.0684	1.0009
6	28.5	4	0.6875	0.9844	1.0009
7	52.6	4	0.8000	1.1111	1.0009

Title: Percent Emergence
 File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	1.0110				
1.75	1.0009	0.0935		1.7200	k= 1, v=21
4.44	1.0009	0.0935		1.8000	k= 2, v=21
7.31	1.0009	0.0935		1.8300	k= 3, v=21
12.2	1.0009	0.0935		1.8400	k= 4, v=21
28.5	1.0009	0.0935		1.8500	k= 5, v=21
52.6	1.0009	0.0935		1.8500	k= 6, v=21

s = 0.1536

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Title: Percent Survival
 File: 5605s Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean = 0.7125 Calculated t value = 0.1901

GRP2 (Blank cntl) Mean = 0.7000 Degrees of freedom = 6
 Difference in means = 0.0125

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
 2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Percent Survival
 File: 5605s Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.4496
 W = 0.9478

Critical W = 0.8960 (alpha = 0.01 , N = 28)
 W = 0.9240 (alpha = 0.05 , N = 28)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Percent Survival
 File: 5605s Transform: ARC SINE(SQUARE ROOT(Y))

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	0.0349	0.0058	0.6688
Within (Error)	21	0.1827	0.0087	
Total	27	0.2176		

(p-value = 0.6758)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
 = 2.5727 (alpha = 0.05, df = 6,21)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Percent Survival
 File: 5605s Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA Table

SOURCE	DF	SS	MS	F
--------	----	----	----	---

DP Barcode: 382010

MRID No.: 48175605

Between	6	0.1254	0.0209	0.9763
Within (Error)	21	0.4496	0.0214	
Total	27	0.5750		

(p-value = 0.4654)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
 = 2.5727 (alpha = 0.05, df = 6,21)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal (alpha = 0.05)

Title: Percent Survival
 File: 5605s

Transform: ARC SINE(SQUARE ROOT(Y))

Dunnnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	TRANS T STAT	SIG
0.05					
1	Neg Control	1.0110	0.7125		
2	1.75	0.9079	0.6125	0.9969	
3	4.44	0.9015	0.6125	1.0590	
4	7.31	1.0068	0.7125	0.0409	
5	12.2	1.0274	0.7125	-0.1584	
6	28.5	0.9844	0.6875	0.2577	
7	52.6	1.1111	0.8000	-0.9674	

Dunnnett critical value = 2.4600 (1 Tailed, alpha = 0.05, df [used] = 6,20)
 (Actual df = 6,21)

Title: Percent Survival
 File: 5605s

Transform: ARC SINE(SQUARE ROOT(Y))

Dunnnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.75	4	0.2469	34.4	0.1000
3	4.44	4	0.2469	34.4	0.1000
4	7.31	4	0.2469	34.4	0.0000
5	12.2	4	0.2469	34.4	0.0000
6	28.5	4	0.2469	34.4	0.0250
7	52.6	4	0.2469	34.4	-0.0875

Title: Percent Survival
File: 5605s

Transform: ARC SINE(SQUARE ROOT(Y))

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL	TRANSFORMED	ISOTONIZED
			MEAN	MEAN	MEAN
1	Neg Control	4	0.7125	1.0110	1.0110
2	1.75	4	0.6125	0.9079	0.9898
3	4.44	4	0.6125	0.9015	0.9898
4	7.31	4	0.7125	1.0063	0.9898
5	12.2	4	0.7125	1.0274	0.9898
6	28.5	4	0.6875	0.9844	0.9898
7	52.6	4	0.8000	1.1111	0.9898

Title: Percent Survival
File: 5605s

Transform: ARC SINE(SQUARE ROOT(Y))

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	1.0110				
1.75	0.9898	0.2048		1.7200	k= 1, v=21
4.44	0.9898	0.2048		1.8000	k= 2, v=21
7.31	0.9898	0.2048		1.8300	k= 3, v=21
12.2	0.9898	0.2048		1.8400	k= 4, v=21
28.5	0.9898	0.2048		1.8500	k= 5, v=21
52.6	0.9898	0.2048		1.8500	k= 6, v=21

s = 0.1463

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Title: Development time
File: 5605t

Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

```

=====
GRP1 (Solvent cntl) Mean = 22.5000 Calculated t value = 0.5932
GRP2 (Blank cntl) Mean = 21.8500 Degrees of freedom = 6
Difference in means = 0.6500
=====
2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01
    
```

WARNING: This procedure assumes normality and equal variances!

Title: Development time
 File: 5605t Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 36.8450
 W = 0.9852

Critical W = 0.8960 (alpha = 0.01 , N = 28)
 W = 0.9240 (alpha = 0.05 , N = 28)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Development time
 File: 5605t Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	6.2521	1.0420	2.7268
Within (Error)	21	8.0250	0.3821	
Total	27	14.2771		

(p-value = 0.0405)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
 = 2.5727 (alpha = 0.05, df = 6,21)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal (alpha = 0.01)

Title: Development time
 File: 5605t Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	8.6821	1.4470	0.8247
Within (Error)	21	36.8450	1.7545	
Total	27	45.5271		

DP Barcode: 382010

MRID No.: 48175605

(p-value = 0.5636)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
= 2.5727 (alpha = 0.05, df = 6,21)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Development time
File: 5605t

Transform: NO TRANSFORMATION

Dunnnett's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
0.05					
1	Neg Control	22.5000	22.5000		
2	1.75	22.6000	22.6000	-0.1068	
3	4.44	21.6500	21.6500	0.9075	
4	7.31	21.0750	21.0750	1.5214	
5	12.2	21.3500	21.3500	1.2278	
6	28.5	22.4250	22.4250	0.0801	
7	52.6	21.8500	21.8500	0.6940	

Dunnnett critical value = 2.4600 (1 Tailed, alpha = 0.05, df [used] = 6,20)
(Actual df = 6,21)

Title: Development time
File: 5605t

Transform: NO TRANSFORMATION

Dunnnett's Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.75	4	2.3041	10.2	-0.1000
3	4.44	4	2.3041	10.2	0.8500
4	7.31	4	2.3041	10.2	1.4250
5	12.2	4	2.3041	10.2	1.1500
6	28.5	4	2.3041	10.2	0.0750
7	52.6	4	2.3041	10.2	0.6500

Title: Development time
File: 5605t

Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL	TRANSFORMED	ISOTONIZED
			MEAN	MEAN	MEAN
1	Neg Control	4	22.5000	22.5000	22.5500
2	1.75	4	22.6000	22.6000	22.5500
3	4.44	4	21.6500	21.6500	21.6700
4	7.31	4	21.0750	21.0750	21.6700
5	12.2	4	21.3500	21.3500	21.6700
6	28.5	4	22.4250	22.4250	21.6700
7	52.6	4	21.8500	21.8500	21.6700

Title: Development time
 File: 5605t Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	22.5000				
1.75	22.5500	-0.0534		1.7200	k= 1, v=21
4.44	21.6700	0.8862		1.8000	k= 2, v=21
7.31	21.6700	0.8862		1.8300	k= 3, v=21
12.2	21.6700	0.8862		1.8400	k= 4, v=21
28.5	21.6700	0.8862		1.8500	k= 5, v=21
52.6	21.6700	0.8862		1.8500	k= 6, v=21

s = 1.3246

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Title: Development rate
 File: 5605d Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

```

=====
GRP1 (Solvent cntl) Mean = 4.7075 Calculated t value = -0.4155
GRP2 (Blank cntl) Mean = 4.8200 Degrees of freedom = 6
Difference in means = -0.1125
=====
2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01
  
```

WARNING: This procedure assumes normality and equal variances!

Title: Development rate
 File: 5605d Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 2.0659
 W = 0.9843

Critical W = 0.8960 (alpha = 0.01 , N = 28)
 W = 0.9240 (alpha = 0.05 , N = 28)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Development rate
 File: 5605d Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	0.3531	0.0588	3.2532
Within (Error)	21	0.3799	0.0181	
Total	27	0.7330		

(p-value = 0.0202)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
 = 2.5727 (alpha = 0.05, df = 6,21)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal (alpha = 0.01)

Title: Development rate
 File: 5605d Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	0.3711	0.0619	0.6288
Within (Error)	21	2.0659	0.0984	
Total	27	2.4370		

(p-value = 0.7056)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
 = 2.5727 (alpha = 0.05, df = 6,21)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal ($\alpha = 0.05$)

Title: Development rate
 File: 5605d Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
0.05					
1	Neg Control	4.7075	4.7075		
2	1.75	4.6600	4.6600	0.2142	
3	4.44	4.8975	4.8975	-0.8567	
4	7.31	4.9450	4.9450	-1.0709	
5	12.2	4.9475	4.9475	-1.0821	
6	28.5	4.6875	4.6875	0.0902	
7	52.6	4.8025	4.8025	-0.4283	

Dunnett critical value = 2.4600 (1 Tailed, $\alpha = 0.05$, df [used] = 6,20)
 (Actual df = 6,21)

Title: Development rate
 File: 5605d Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.75	4	0.5456	11.6	0.0475
3	4.44	4	0.5456	11.6	-0.1900
4	7.31	4	0.5456	11.6	-0.2375
5	12.2	4	0.5456	11.6	-0.2400
6	28.5	4	0.5456	11.6	0.0200
7	52.6	4	0.5456	11.6	-0.0950

Title: Development rate
 File: 5605d Transform: NO TRANSFORMATION

Williar's Test - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	4	4.7075	4.7075	4.8315

DP Barcode: 382010

MRID No.: 48175605

2	1.75	4	4.6600	4.6600	4.8315
3	4.44	4	4.8975	4.8975	4.8315
4	7.31	4	4.9450	4.9450	4.8315
5	12.2	4	4.9475	4.9475	4.8315
6	28.5	4	4.6875	4.6875	4.7450
7	52.6	4	4.8025	4.8025	4.7450

Title: Development rate
File: 5605d

Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	4.7075				
1.75	4.8315	-0.5591		1.7200	k= 1, v=21
4.44	4.8315	-0.5591		1.8000	k= 2, v=21
7.31	4.8315	-0.5591		1.8300	k= 3, v=21
12.2	4.8315	-0.5591		1.8400	k= 4, v=21
28.5	4.7450	-0.1691		1.8500	k= 5, v=21
52.6	4.7450	-0.1691		1.8500	k= 6, v=21

s = 0.3136

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

**APPENDIX II. COPY OF REVIEWER'S TIME-WEIGHTED AVERAGE (TWA)
CALCULATIONS USING EXCEL SOFTWARE:**

SEDIMENT				
Nominal Concentration (ug/kg)	Time (Day)	14C-Novaluron Equivalents		TWA (ug/kg)
		Measured Concentration (ug/kg)		
3.1	0	1.73		1.75
	7	2.06		
	28	1.35		
6.3	0	3.98		4.44
	7	5.24		
	28	3.53		
13	0	9.26		7.31
	7	6.9		
	28	7.21		
25	0	13.8		12.2
	7	10.1		
	28	14.4		
50	0	35.2		28.5
	7	29.3		
	28	25.2		
100	0	60.1		52.6
	7	50.6		
	28	52.8		

EXHIBIT 35



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

PC Code: 027602
DP Barcodes: 412791 and 425073
Date: February 20, 2015

MEMORANDUM

SUBJECT: Review of Three Reports Related to a 3-year Flubendiamide Water Monitoring Project in Support of the Conditional Registration of Flubendiamide

FROM: Stephen Wentz, Ph.D., Biologist
Environmental Risk Branch 1
Environmental Fate and Effects Division (7507P)

Stephen Wentz 2/20/15

THROUGH: Sujatha Sankula, Ph.D., Branch Chief
Environmental Risk Branch 1
Environmental Fate and Effects Division (7507P)

Sujatha Sankula 2/20/15

Edward Odenkirchen, Ph.D., Senior Advisor
Immediate Office
Environmental Fate and Effects Division (7507P)

Edward Odenkirchen

TO: Carmen Rodia, Risk Manager Reviewer
Richard Gebken, Risk Manager
Debbie McCall, Branch Chief
Invertebrate & Vertebrate Branch 2
Registration Division (7504P)

Introduction

Flubendiamide, an insecticide, was conditionally registered in 2008 for aerial and/or ground application to corn, cotton, tobacco, pome fruit, stone fruit, tree nuts, grapes, cucurbit vegetables, fruiting vegetables, leafy vegetables, and brassica leafy vegetables. Registrant-submitted effects studies indicate that both the parent (flubendiamide) and degradate (des-iodo) exhibit chronic toxicity to aquatic invertebrates¹. Submitted fate data indicate flubendiamide slowly converts to its des-iodo degradate, which does not breakdown. EFED modeling (D329613+) predicts that

¹ Flubendiamide's mode of action is taxa-specific to an unknown degree (targets lepidopteran ryanodine receptors). EFED does not have endpoints specific to lepidopterans. There are numerous species of aquatic lepidopterans of which four are listed species.

flubendiamide and its degradate (des-iodo) will accumulate in aquatic systems eventually exceeding Agency levels of concern (LOCs).

The registrant has argued that: 1) vegetative filter strips (VFSs) would prevent accumulation from exceeding Agency LOCs (flubendiamide labels require a 15 ft VFS buffer around aquatic areas); and 2) the Agency overestimates aquatic exposure because EFED modeling cannot account for the effect of VFSs. According to the flubendiamide preliminary acceptance letter, the Registration Division stated, the “Agency believes that the efficacy of vegetative buffers for flubendiamide use is uncertain.” The conditions of registration required a VFS study and, if the VFS study did not allay the Agency’s concerns, a pond monitoring study. EFED identified a major modeling error in the VFS study (MRIDs 48175602, 48175604, and 48175606) and asked the registrant to correct it and re-submit (D382010). The VFS study was never re-submitted, therefore, the monitoring study was required. The 3-year monitoring study of water column, sediments, and pore water in 3 ponds (2 in Georgia and 1 in North Carolina) was submitted in December of 2014.

EFED has reviewed the monitoring data and associated studies and has identified several issues with this monitoring data. Despite these issues, EFED believes the monitoring data shows clear evidence that both flubendiamide and des-iodo accumulate in the ponds monitored. The accumulation measured in the first three years of the pond data least impacted by the identified issues largely matches the initial 3 years of concentration predictions of EFED’s aquatic exposure modeling. Because EFED’s modeling does not account for the effect of VFSs, but still largely matches the monitoring data, EFED believes the effect of VFSs is not large enough to mitigate the ecological risks posed by flubendiamide applications. Therefore, EFED concludes the original and subsequent ecological risk assessments performed by the Agency adequately reflect the risks posed by flubendiamide applications and rejects the registrant’s argument that the label-required 15 ft VFSs would prevent accumulation from exceeding Agency LOCs.

The registrant submitted three reports related to this monitoring study: 1) “Monitoring for Flubendiamide and its Metabolite Des-iodo Flubendiamide in Sediment and Surface Water” (MRID 49415303), “Flubendiamide Aquatic Risk – Summary of Surface Water Monitoring and Toxicity Testing” (MRID 49415302), and “Aquatic Exposure Assessment for Flubendiamide and its Metabolite Des-iodo Flubendiamide based on a 3-Year Monitoring Study” (MRID 49415301). This memo provides EFED’s analysis of the monitoring data provided in the 3-year monitoring study, summarizes the individual registrant reports, and responds to the major issues raised in these reports.

EFED’s Analysis of the Monitoring Data

The residues of flubendiamide and its metabolite Des-iodo were monitored in three ponds in two locations: one pond in Louisburg, NC, and two adjacent ponds (attached by a culvert) in Omega, GA (MRID 49415303). The monitoring study ponds in North Carolina (NC) (Negley et al. 2011; MRID 48535201) and Georgia (GA) (Hanzas et al. 2011; MRID 48644901) were approved by the Agency (D394006 and D398132, respectively). The ponds were selected from areas with high flubendiamide use based on confidential 2009 U.S. sales data. Ponds were selected based on

the similarity of their surface area and watershed area to the standard pond that EFED uses in exposure modeling and the requirement that the entire watershed be planted to one crop. Additionally, an attempt was made to select ponds with watersheds that had similar characteristics to EFED standard scenarios for the crop planted in that watershed.

Although not requested by the Agency, the registrant also sampled intermittent and perennial streams near the monitored ponds. The *intermittent* stream sites were up and downstream of where the discharge of the pond(s) flowed into the intermittent stream, while the *perennial* stream sites were up and downstream of where the discharge of the intermittent stream flowed into the perennial stream. (Both Georgia ponds flowed into the same intermittent and perennial streams, so the total number of monitoring sites included 3 ponds, 4 intermittent stream sites (2 in GA and 2 in NC), and 4 perennial streams sites (2 in GA and 2 in NC).) Monthly water and sediment samples, with a few exceptions, were taken from each monitoring site for three years. Water quality parameters including pH, temperature, conductivity, dissolved oxygen, and oxidation/reduction potential (ORP) were measured on-site during each sampling event. Composite water and sediment (top 2 inches) samples were collected during each monthly sampling event. Applications of the flubendiamide product Belt™ were made to the watershed of the pond(s) at each location every year during the study period.

Pore water was separated from sediment samples by vacuum filtration at about 10 PSI to quantify the benthic water residue. Flubendiamide and des-iodo in the water column, pore water and sediment extracts were analyzed by LC/MS/MS, using isotopically-labelled internal standards for quantitation. The method detection limits were 0.004 µg/L for flubendiamide and des-iodo in water and pore water samples, and 0.02 µg/kg for flubendiamide and des-iodo in sediment samples.

Experimental Design and Data Quality Issues

EFED identified six major issues with the monitoring study that affect the interpretability of the study. The first four issues concern the experimental treatment of the watershed: 1) the variability in crops grown on the pond watersheds; 2) the variability in the date of application(s); 3) the variability in the application rates; and 4) the magnitude of the study application rates compared to the maximum annual label application rates (Table 1). Because the participation of the growers was voluntary, the registrant did not have much control over the treatment of the watersheds. The crops rotated in both watersheds – from tobacco (2011) to soybean (2012 and 2013) to tobacco (2014) in the NC pond watershed and from cotton (2011 and 2012) to peanut (2013) in the watershed of the GA ponds. The application dates were quite variable in the NC pond watershed with 15 months between the 1st and 2nd application, 12.5 months between the 2nd and 3rd, and 6.5 months between the 3rd and 4th application with a second application in 2014 occurring a month later. The application rates also varied in the NC pond watershed from 0.06 to 0.09 lb/A. Both the application dates (all in August) and rates (all 0.09 lb/A) in the watershed of the GA ponds were much more consistent.

Table 1. Timeline of applications within the watersheds of the monitoring study ponds and comparison to the maximum annual application rates allowed by flubendiamide labels.

Year	Crop	Application Date	Rate Applied (lb/A)	Label Maximum Annual Rate (lb/A)	Percent of Maximum Annual Rate (%)
North Carolina Pond Watershed					
2011	Tobacco	May 26	0.06	0.375	16
2012	Soybean	Aug 27	0.075	0.188	40
2013	Soybean	Nov 12	0.09	0.188	48
2014	Tobacco	May 31	0.0675	0.375	34
		June 28	0.06		
Georgia Ponds Watershed					
2011	Cotton	August 18 (25% of area) August 23, (75% of area)	0.09	0.282	32
2012	Cotton	August 13	0.09	0.282	32
2013	Peanut	August 30	0.09	0.375	24

The first three issues (variation in crops grown, application dates, and application rates) would be expected to add variability to the monitoring data; making it harder to detect trends in the data. The fourth issue (low application rates) reduces the magnitude of the trends, which makes it harder to detect trends from the noise in the data.

The fifth issue concerns the installation of maintained grass swales (grass waterways) in the watershed of the GA ponds. On page 15 of the GA Site selection Report (Hanzas, et al. 2011; MRID 48644901), it is stated “Primary entry points of runoff into the ponds originate from the southeast via two distinct, un-cropped (but not vegetated) drainage pathways.” However the Interim Report 1 (MRID 48892501; after the first year of monitoring data) p. 13, the Interim Report 2 (MRID 49139801; after the second year of monitoring data) p. 13, and the Final Monitoring Report (MRID 49415303) p. 15, all state the same sentence “Primary entry points of runoff into the ponds originated from the southeast via three maintained grass swales.” The Agency obtained aerial photography of the GA ponds and watershed from September 16, 2010 (Figure 5a) and September 13, 2013 (Figure 5b) from the National Agriculture Imagery Program (NAIP)².

² <http://fsa.usda.gov/FSA/apfoapp?area=home&subject=prog&topic=nai>

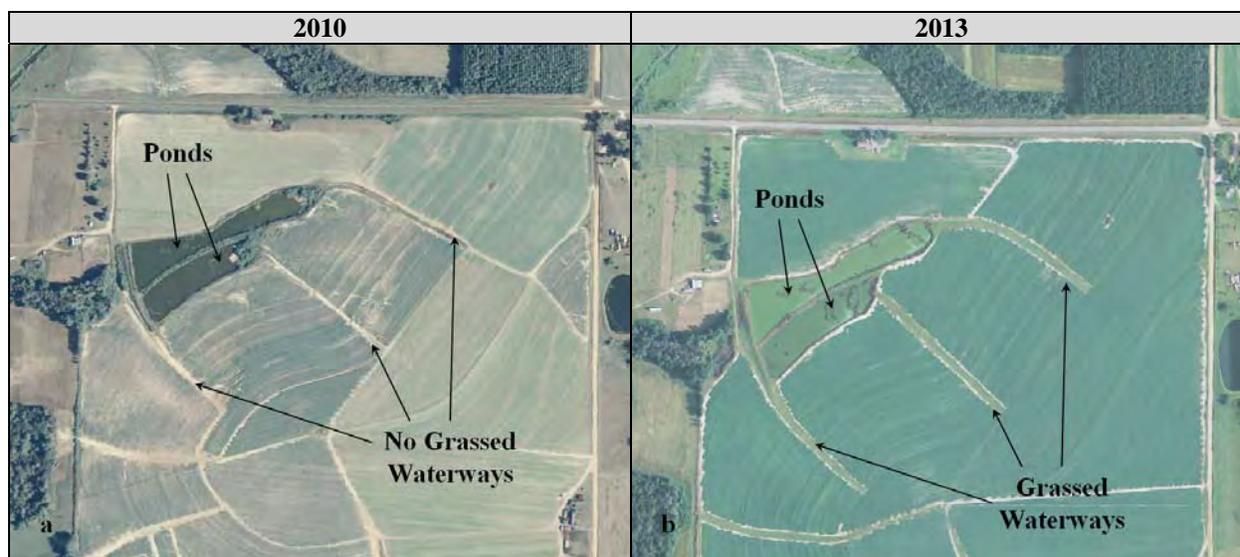


Figure 1. Aerial photography of the Georgia pond watershed taken before (a) and after (b) installation of grass waterways.

The purpose of grassed waterways is to reduce soil and chemical loadings to waterbodies. They occupy the main drainage pathways through which the majority of the pesticide in runoff and attached to eroded soil would travel. Grassed waterways are designed to trap eroded soil and allow runoff and the chemicals in the runoff to infiltrate into the ground. The flubendiamide labels require a 15 ft VFS between the treated field and waterbodies, but do *not* require grassed waterways. The presence of the grassed waterways would be expected to reduce the accumulation of flubendiamide and des-iodo in the GA ponds and therefore, make it more difficult to identify accumulation trends in the GA ponds. Additionally, the trends measured from water column, sediment, and pore water in the GA ponds would be diminished [*i.e.*, the magnitude (steepness) of those trends would be diminished relative to what would be expected in the absence of the grassed waterways].

The final issue with the submitted monitoring data concerns the magnitude of the pore water concentrations compared to the water column concentrations from samples collected from the same pond and at the same time. In the ponds, EFED expects the pore water and water column concentrations to equilibrate over time for both flubendiamide and des-iodo with only short-term excursions from nearly equal concentrations after drift and storm events. However, the observed pond pore water concentrations were typically much lower than the observed water column concentrations from samples collected from the same pond and at the same time.

To show the magnitude and pervasiveness of this discrepancy, the ratio of the pore water to water column concentration was plotted over time for all pond samples that had measured concentrations that were above the detection limit for both pore water and water column samples. If the pore water to water column concentration were equal, this ratio should equal 1. In the NC and GA pond samples (Figure 2a and c, respectively), almost all of the observed ratios plot below 1 (equilibrium) with many equal to, or less than, 0.1 indicating the pore water concentration is 10 times lower than the corresponding water column concentration for many of these samples. For comparison, similar ratios are plotted for samples from the up and downstream perennial stream sites (Figure 2b and d). The perennial stream site ratios tend to

straddle a ratio value of 1 indicating much more equality between pore water and water column concentrations.

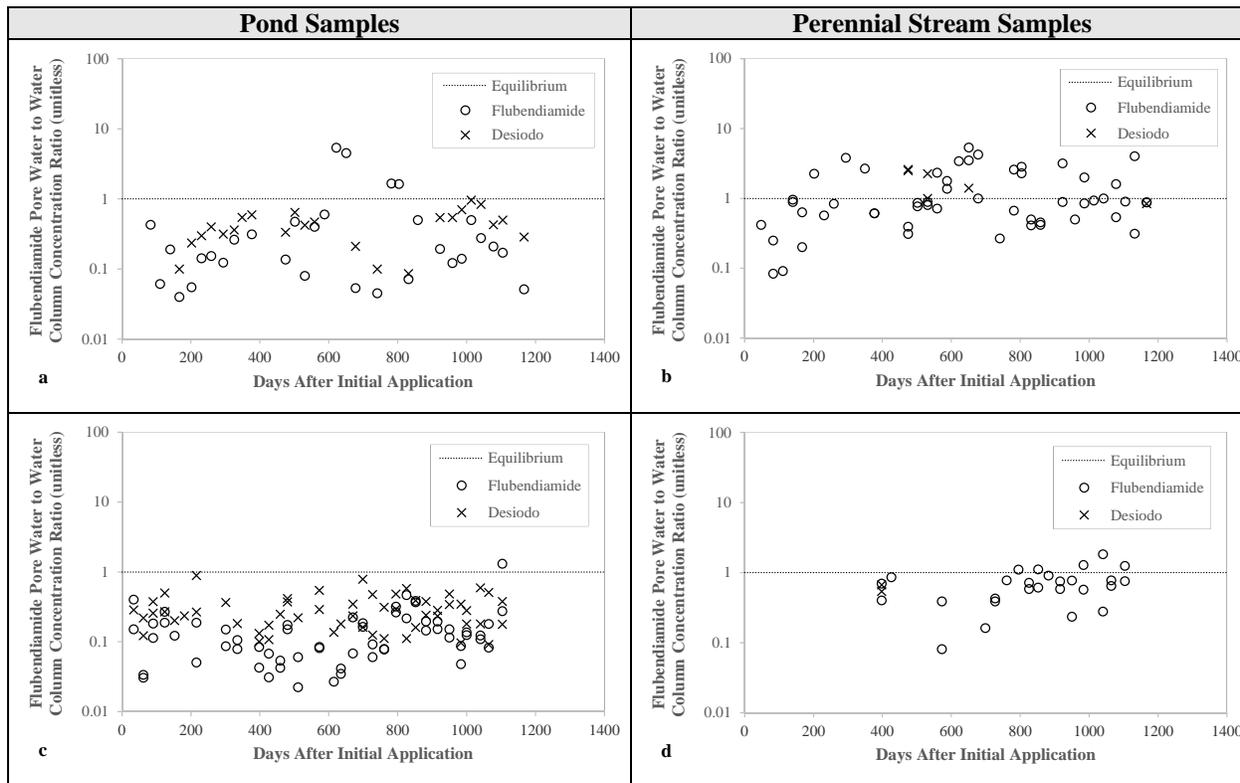


Figure 2. Comparison of observed pore water to water column concentration ratios for flubendiamide and des-iodo from the NC pond (a) the NC perennial stream (b) and GA ponds (c) the GA perennial stream (d) samples.

A potential explanation for why the pond pore water concentrations are so much lower relative to the pond water column concentrations may be that the depth of sediment and pore water contaminated with flubendiamide and des-iodo may be very shallow relative to the total depth of sediment and pore water extracted (~2 inches) during sample collection. Consequently, the pond sediment and pore water samples would constitute a mixture of flubendiamide/des-iodo and uncontaminated sediment and pore water, thus diluting the concentration flubendiamide and des-iodo in the sample.

The NC perennial stream exhibits pore water to water column concentration ratios that are much closer to 1 (Figure 2b). This stream (the Tar River) is a large river at the sites sampled. Sediment depths are likely deeper and better mixed due to turbulent flow in the river, which may make it easier to sample sediment and pore water sample from a surficial layer with less dilution from deeper uncontaminated sediment and pore water. The GA perennial stream water column and pore water concentrations were relatively low, so that early in the monitoring time frame, ratios could not be calculated because one or both concentrations fell below the detection limit. However, the later ratios from the GA perennial stream sites (Figure 2d) were distributed closer to 1 than the pond ratios, but further from 1 than the Tar River (NC perennial stream) ratios (the GA perennial stream is much smaller at the GA sample sites than the NC perennial stream is at

the NC sample sites). (Observed pore water to water column concentration ratios for flubendiamide and des-iodo for all stream sites are depicted in Appendix B.)

Assuming the measured sediment and pore water concentrations from the pond samples are biased low, it would be harder to detect trends in the sediment and pore water data because the observed rate of accumulation will have been diminished due to dilution with the uncontaminated layers. Additionally, these ‘diluted’ samples would be much lower than model predicted ‘non-diluted’ pore water concentrations.

Accumulation

EFED used the “LifeReg” regression procedure in SAS statistical software to fit trend lines to the pond concentration data because some of the data are only known to be less than the method detection limit (left-censored). This procedure better accounts for the presence of this left-censored data without biasing the fitted trend estimates. The fitted trends increase with time (accumulate) in all of the 18 time-series data sets collected from these ponds [3 ponds \times 3 media (water column, sediments, and pore water) \times 2 chemicals = 18 time series data sets]. Fitting these trends as exponential trends (*i.e.*, fitting a linear trend to the natural log of the concentration observations) indicated that 13 of these 18 trends were statistically significant at the $p = 0.05$ level of confidence (Figures 3, 4, and 5) despite the issues with this data described in the previous section. (The exponential trends appear as linear trends in these figures because the y-axis is presented as a log scale).

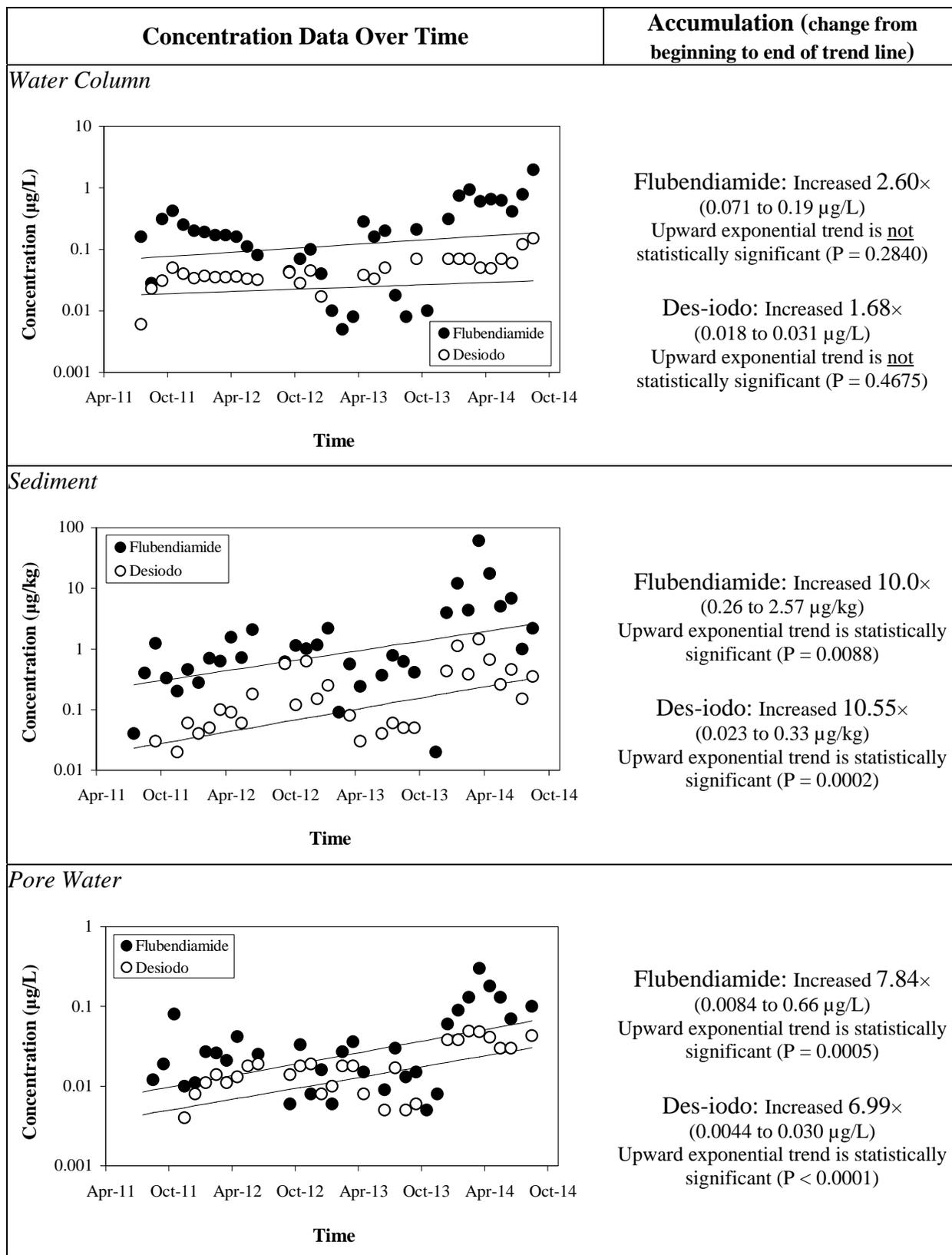


Figure 3. Accumulation of flubendiamide and des-iodo in the water column (a), sediment (b), and pore water (c) of North Carolina pond.

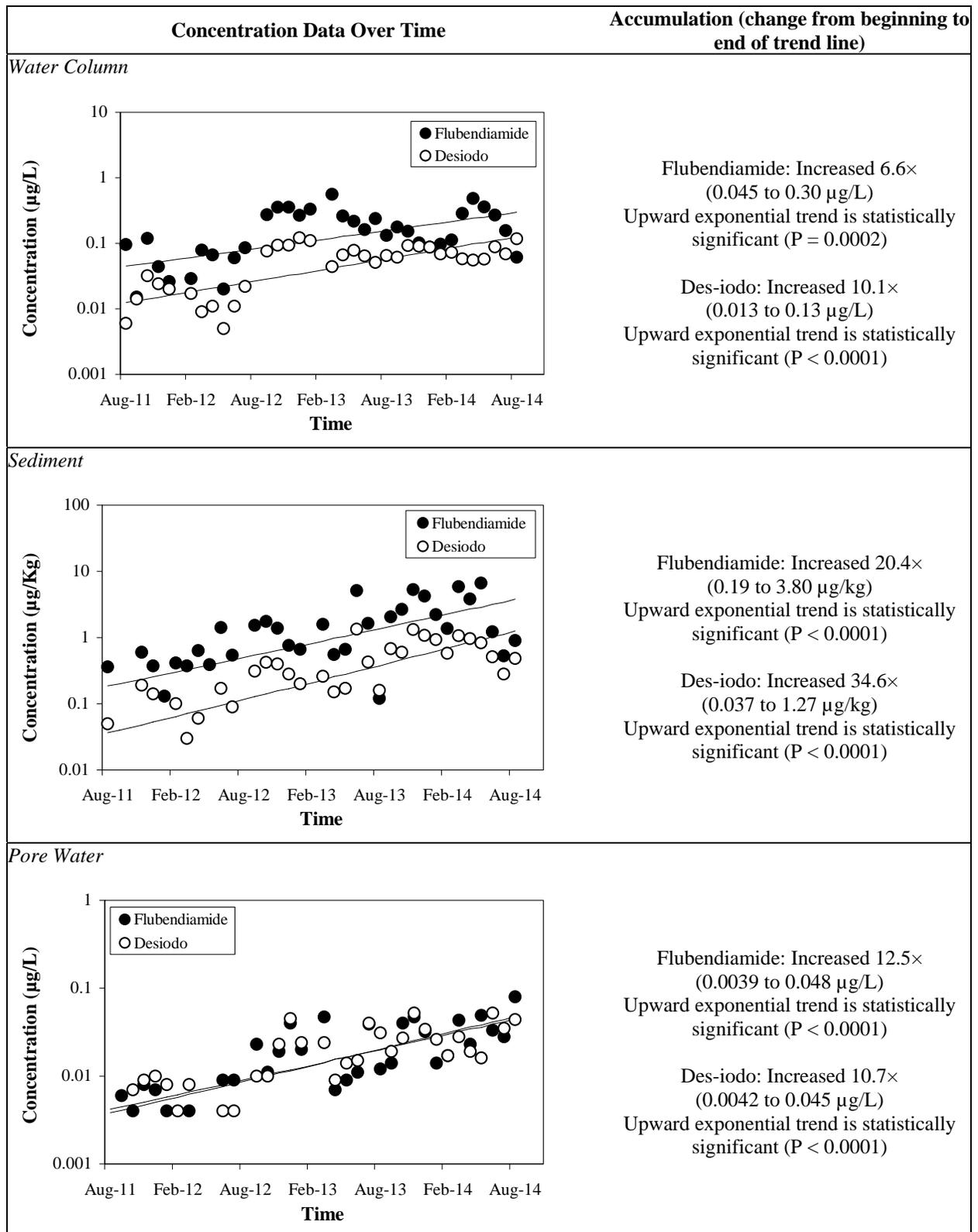


Figure 4. Accumulation of flubendiamide and des-iodo in the water column (a), sediment (b), and pore water (c) of Georgia pond #1.

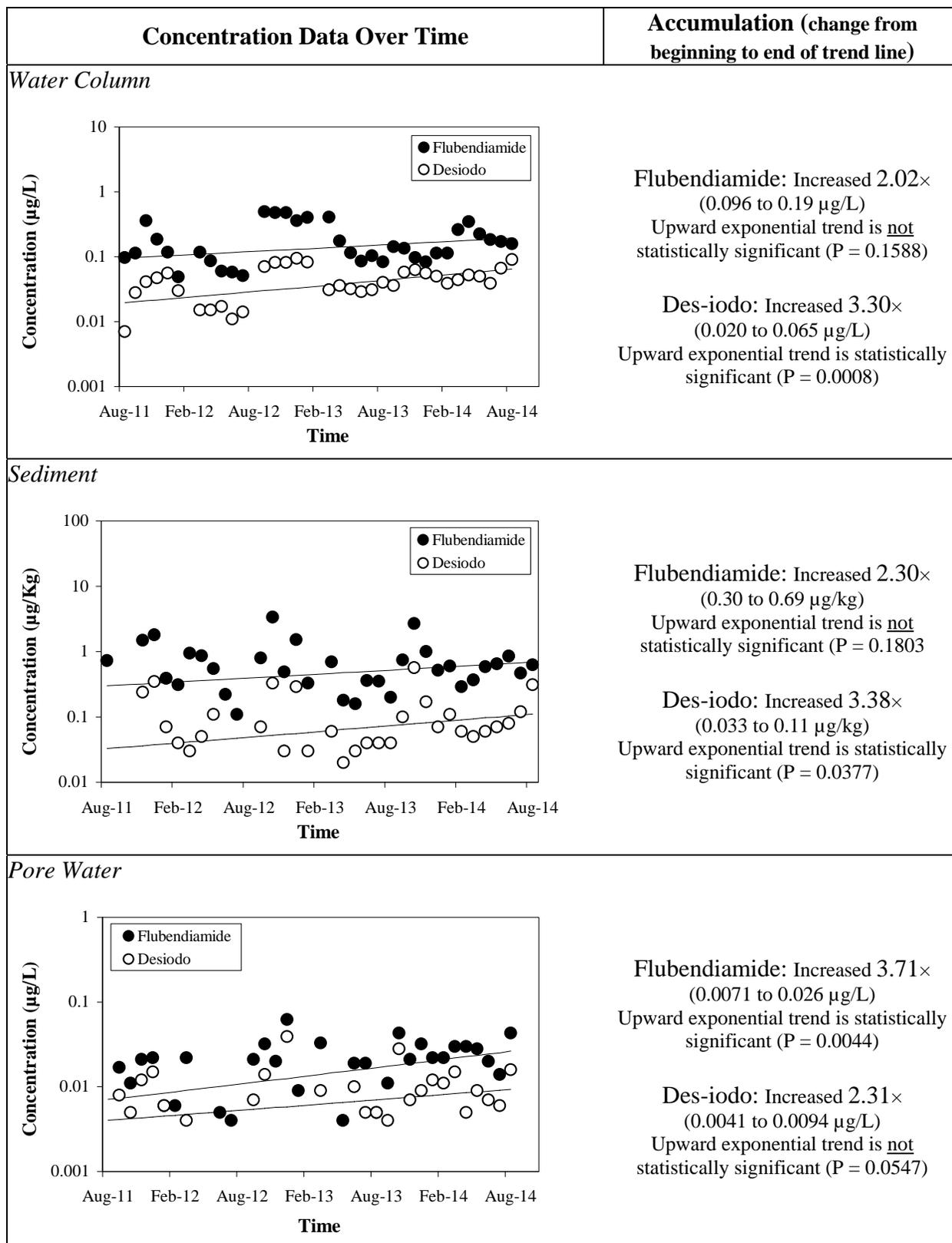


Figure 5. Accumulation of flubendiamide and des-iodo in the water column (a), sediment (b), and pore water (c) of Georgia pond #2.

Comparison of Observed Concentrations in the Monitoring Study to Exposure Model Predictions

During the site selection phase of the monitoring study, the registrant made an attempt to select combinations of crops to be planted and pond watershed characteristics that were similar to EFED standard scenarios. However, EFED exposure scenarios are designed to represent high-end exposures and have many parameters embedded within the standard scenarios that would likely need adjustment to make a valid comparison between exposure model predictions to observed concentrations in a strict sense (field slopes, etc). Additionally, the SWCC cannot be parameterized for crop rotations, cannot account for VFSs (or the grassed waterways in the watershed of the GA ponds), assumes similar application timing and rates of pesticides applied each year, and assumes wind direction always deposits drift into the pond(s). Therefore the comparisons presented should be considered very “rough”.

Figure 6 provides the comparison between exposure model predictions and observed concentrations for the NC pond. The SWCC modeling used the NC tobacco scenario³ with the same input values as appear in Table 1 of the aquatic exposure report (MRID 49415301) with the exceptions that the benthic metabolism half-life value of 855 days was used (rather than the registrant modified value of 7300 days), the soil half-life of 0 (stable) was used (rather than the 10,000 day value in the aquatic exposure report), the efficiency (0.95) and drift (0.05) fractions were changed to 0.99 and 0.0082⁴ because Table 1 indicates that these were ground applications under the application method section of this table, and standard pond dimensions were used. (EFED did not use the registrant modified weather files, files because they only provided to the agency in a pdf format as part of the report.)

The monitoring report does not contain sufficient information to identify a unique set of SWCC parameters for comparison with the NC pond data. For example, the report does not indicate whether the wind direction on the application date would have blown drift toward the pond. Therefore, three SWCC scenarios were run with different combinations of application rates and spray drift assumptions to bound reasonable SWCC parameterizations for the NC pond. The highest rates applied to the NC pond watershed (0.09 lb/A) with the EFED’s current spray drift fraction (0.0082) is shown solid lines in Figures 6a to f). The lowest rates applied to the NC pond watershed (0.06 lb/A) with the EFED’s current spray drift fraction (0.0082) is shown as dashed lines in Figures 6a to f). The third SWCC scenario used the lowest rates applied to the NC pond watershed (0.06 lb/A) with no drift (to simulate the lowest reasonable exposure scenario) and is show as dotted lines in Figures 6a to f). (Note: Figures 6a through f are presented with the y-axis as a log scale.)

The observed water column flubendiamide concentrations display a lot of scatter in Figure 6a, but contain concentrations that plot both above and below the SWCC predictions. Similarly, the observed water column des-iodo concentrations plot both above and below the SWCC predictions, but the concentrations that plot above the SWCC predictions occur toward the

³ The crop in the NC pond watershed rotated from tobacco to soybean for two years and back to tobacco. EFED does not have mixed crop scenarios, but does have soybean scenarios from states other than NC. However, EFED simply used the scenario modeled by the registrant (MRID 49415301) without further exploration of alternative scenarios.

⁴ Calculated with AgDrift based on a high boom ground application with a droplet size of ASAE fine to medium coarse (DV50 of 341um).

beginning of the monitoring. Additionally, the observed water column des-iodo concentrations display a lot less scatter (Figure 6b) than the flubendiamide concentrations (Figure 6a) and follow the trend much better in the latter half of the monitoring. The respective sediment concentrations (Figure 6c and d) and pore water concentrations (Figure 6e and f) all plot somewhat low compared to the SWCC predictions, consistent with the hypothesis that these samples are diluted with the underlying uncontaminated sediment and pore water lying below the higher surficial sediment and pore water concentrations (see previous discussion). Overall, the Agency believes the monitoring data tracks reasonably well with the modeled data and therefore, supports the previous predictions of aquatic exposure modeling and the prior flubendiamide risk assessments despite the fact that EFED's modeling cannot account any effect of the VFSs.

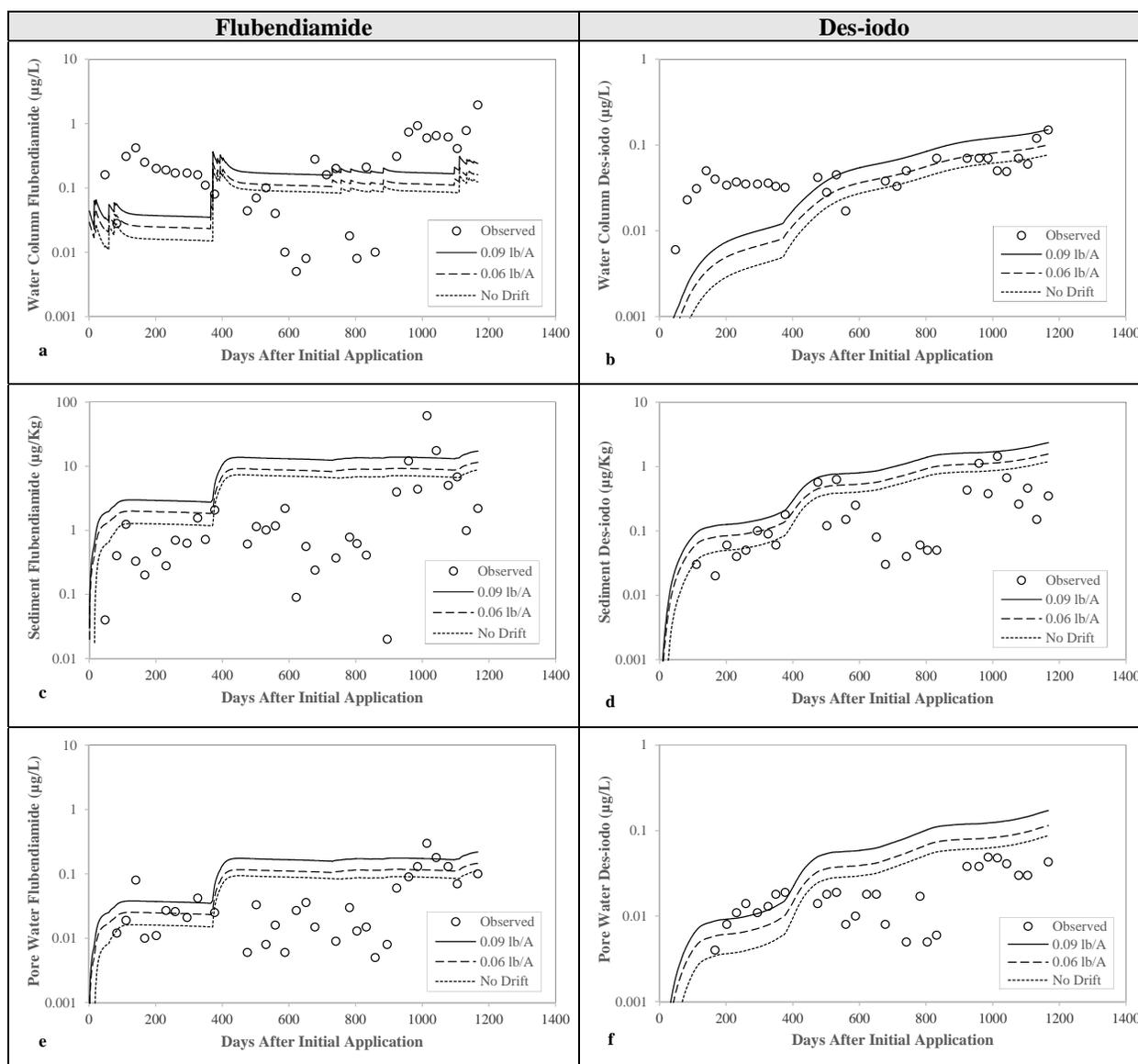


Figure 6. Comparison of Surface Water Concentration Calculator (SWCC) daily predictions from the North Carolina tobacco scenario to monitoring data from the North Carolina pond for water column flubendiamide (a) and des-iodo (b), sediment flubendiamide (c) and des-iodo (d), and pore water flubendiamide (e) and des-iodo (f) based on the range of application rates (0.06 to 0.09 lbs/A) used in the pond watershed during monitoring.

EFED assumes that any reduction in pond chemical concentrations in water column, sediment, and pore water concentrations from VFSs would be greatest when the chemical is first used and would diminish with time as the VFS became saturated with flubendiamide and des-iodo. Once saturated, the VFS might become a net source of the contaminants to the pond rather than a net sink. (EFED believes that VFSs would be more efficacious for pesticides that would rapidly breakdown into non-toxic degradates within the VFS.) From this rough comparison, the impact of the VFS does not appear to be large in the NC pond data.

Similar to the NC analysis, Figure 7 compares exposure model predictions and observed concentrations for the GA ponds. The SWCC modeling used the MS cotton⁵ (solid lines in Figures 7a to f) and NC cotton⁶ (dashed lines in Figures 6a to f) scenarios with the same input values as described for the NC scenario (only one application rate 0.09 lb/A was used since this did not vary in the GA pond watershed). A no drift scenario does not appear in the in Figure 7 because drift only accounts for ~2% of the flubendiamide reaching the pond in the MS and NC cotton scenarios and would have been indistinguishable from the predictions including drift. (Note: Figures 7a through f are presented with the y-axis as a log scale.)

Almost all of the GA ponds concentration data plots below the SWCC predictions. The interpretation of the GA ponds data is confounded by the presence of grassed waterways in the watershed. The combination of grassed waterways and VFSs (only VFSs are required by flubendiamide labels) would be expected to diminish transport of both flubendiamide and des-iodo to the ponds. The GA ponds data does appear to show the same pattern of sediment and pore water dilution in that the water column observations are much closer to the SWCC predictions (Figures 7a and b) than the sediment and pore water observations are (Figures 7c through d).

⁵ The MS cotton scenario was modeled by the registrant in MRID 49415301.

⁶ The NC cotton scenario was added by EFED because it is located in the same general region and to provide comparison with the MS cotton scenario.

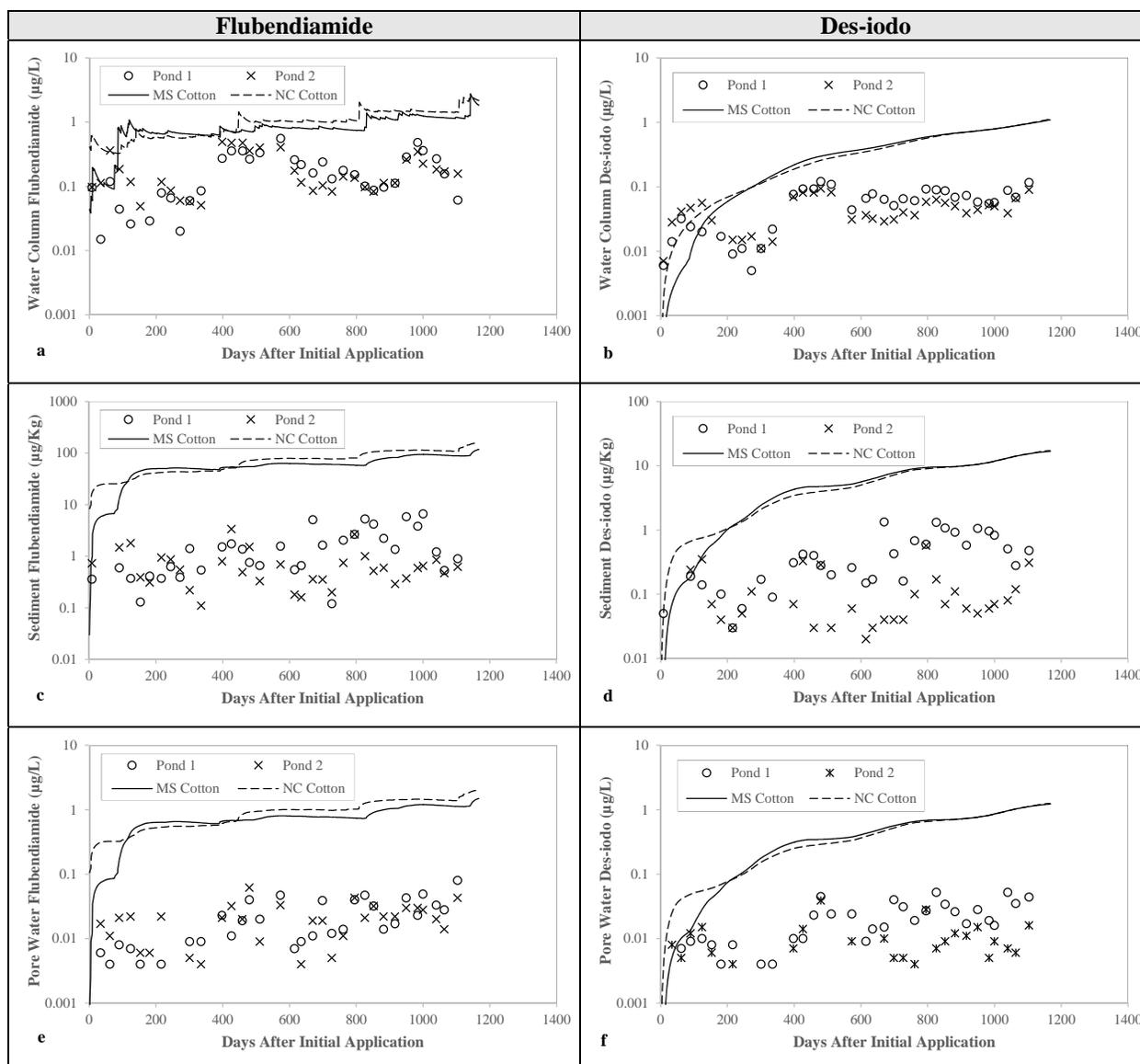


Figure 7. Comparison of Surface Water Concentration Calculator (SWCC) daily predictions from the Mississippi cotton and North Carolina cotton scenarios to monitoring data from the Georgia pond for water column flubendiamide (a) and des-iodo (b), sediment flubendiamide (c) and des-iodo (d), and pore water flubendiamide (e) and des-iodo (f).

Ecological Risk

Ecological risk is determined by comparing exposure estimates to Agency levels of concern (LOCs). Aquatic exposure is predicted over 30 years in Figure 8 for the NC tobacco scenario. These model results are based on the same parameters as the predictions that fit the NC pond data well, but use the maximum label rates instead (4 applications of 0.09 lb/A for an annual maximum of 0.375 lb/A assuming it is continuously planted to tobacco). Chronic aquatic invertebrate endpoints are also included in Figure 8. Because these chronic endpoints have an LOC of 1, an exposure exceeding an endpoint also exceeds the Agency LOC (*i.e.*, the LOC and the endpoint are the same number). Drawing a vertical line down from where the exposure crosses the appropriate endpoint indicates the time required for flubendiamide or des-iodo accumulation to exceed Agency LOCs. The water column des-iodo NOEC is exceeded after 8

years in Figure 8a and the pore water des-iodo NOEC is exceeded after 23 years in Figure 8b, while the pore water flubendiamide NOEC is exceeded after 7 years (also in Figure 8b). [Note: flubendiamide has already been on the market for 5 years (2009 to 2014). Also, at the lower application rates used in the monitoring study, it would take ~4 times as long to exceed all of these LOCs.] The NC tobacco scenario is not the worst case use (other scenarios exceed LOCs in shorter time periods).

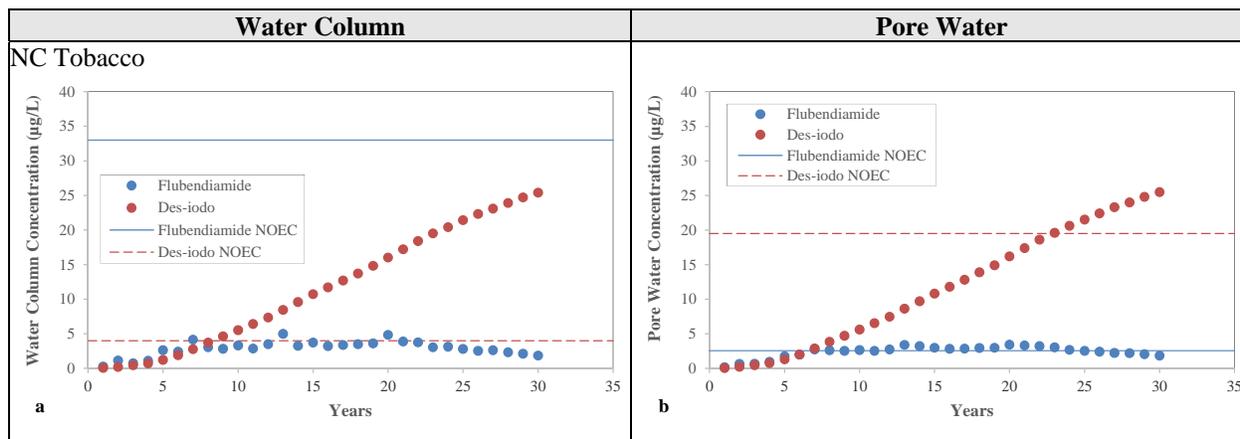


Figure 8. Accumulations of flubendiamide and its des-iodo degradate exceeding chronic risk endpoints in the standard pond water column (a) and pore water (b) based on ground applications to North Carolina tobacco at the maximum allowed application rate.

Additional monitoring at stream sites near these ponds found both flubendiamide and des-iodo in water column, sediment and pore water samples at all eight stream sites monitored (Appendix A). This stream data indicates low-level contamination in streams is currently pervasive in regions where flubendiamide is used.

The next section addresses each of the three submitted studies individually. For each study, a brief summary is provided with a list of issues raised in the study along with EFED comments on those issues.

Monitoring for Flubendiamide and its Metabolite Des-iodo Flubendiamide in Sediment and Surface Water (MRID 49415303)

Summary:

The objective of this study was to assess the potential for flubendiamide and its des-iodo metabolite to accumulate in aquatic environments (water and sediment) following drift and runoff of flubendiamide into surface water with multiple years of applications.

EFED Issue:

Much of the report only discusses measurements above the limit of quantitation (LOQ) rather than the method detection limits (MDL). For example, “(d)es-iodo flubendiamide was not detected above the LOQ in pore water in the farm pond in North Carolina” (p. 24). Yet, the flubendiamide data from the NC farm pond show a statistically significant ($P < 0.0001$) exponentially increasing trend according to the Agency’s modeling from values below the LOQ. The Agency has discussed this issue with the registrant and has indicated that the registrant

should use all values down to the MDL. If the values between the MDL and LOQ are as randomly distributed as the registrant claims, including these values should make it more difficult to detect trends in accumulation over time. Use of these data should not spontaneously create trends where none actually occur.

Limits of quantitation are typically set between 3 and 10 times the MDL. The registrant has chosen 10 times the MDL for an accumulation study that modeling suggests will not accumulate to much more than the LOQ by the end of the monitoring study. Had the registrant applied the pesticide at the maximum application rate (and brought the grassed waterways to the attention of the Agency so that a different site could be monitored), using only the values above the LOQ may have been an option.

Registrant Comment:

“Overall, the results show negligible concentrations of des-iodo flubendiamide in water, pore-water or sediment, and no indication of formation of des-iodo flubendiamide in the water or sediment (*i.e.*, a decline in flubendiamide in sediment or water did not result in increases in des-iodo flubendiamide in sediment or water). Year-to-year variations in concentrations were observed, with highest residues occurring a few months after application, and then declining. There is no indication of accumulation of flubendiamide or des-iodo flubendiamide in pore-water, water or sediment in the pond, intermittent streams or permanent streams.” (p. 27)

“These results indicate that low levels of flubendiamide residues can occur due to runoff from fields with recent applications of flubendiamide products. These residues are not significantly accumulating after three years of applications. This is expected due to the turnover of water and sediment in the moving water bodies, and water from the ponds. The sediment in the ponds, which might be expected to have accumulating residues, only showed year-to-year variations, and no indication of significant accumulation.” (p. 30)

EFED Comments:

The report purports to look for accumulation over time, but there is no trend analysis presented. The Agency found that fitting trend lines to the data indicated that all 18 of the time series data sets from the ponds [3 ponds × 3 media [water column, sediments, and pore water] × 2 chemicals = 18 time series data sets] increased over time with 13 of the 18 identified as statistically significant. Considering just the sediment data discussed in the second quote above, five of the six sediment concentration trends were statistically significant. The Agency strongly disagrees with the registrant’s assessment of no significant accumulation.

Flubendiamide Aquatic Risk – Summary of Surface Water Monitoring and Toxicity Testing (MRID 49415302)

Summary:

The registrant summarized the toxicity studies submitted to date for flubendiamide and des-iodo as well as a midge (*Chironomus riparius*) 28-d spiked sediment flubendiamide study that is yet to be submitted to the Agency.

Registrant Comment:

The appropriate chronic risk assessment endpoints to use for a flubendiamide and des-iodo flubendiamide sediment risk assessment are:

- Flubendiamide overlying water – NOEC 33 µg/L
- Flubendiamide pore water – NOEC 2.56 µg/L
- Des-iodo flubendiamide overlying water – NOEC 4 µg/L
- Des-iodo flubendiamide pore water – NOEC 19.5 µg/L

EFED Response:

EFED has evaluated all of these studies and provided a Data Evaluation Record (DER) for each with the exception of the aforementioned midge study that has yet to be submitted to the Agency. Some of these registrant-calculated endpoints differ slightly from the Agency determined endpoints. If the registrant believes the Agency-calculated endpoints are in error, the appropriate course of action would be to rebut the individual DERs. This report (MRID 49415302) does not contain sufficient explanation and analysis for the Agency to reconsider the endpoints. However for purposes of evaluating the studies submitted with the monitoring study (MRIDs 49415301 to 49415303), the Agency will use the registrant-calculated endpoints to avoid diverting focus from the issues the Agency has with the submitted monitoring and aquatic exposure reports.

Aquatic Exposure Assessment for Flubendiamide and its Metabolite Des-iodo Flubendiamide based on a 3-Year Monitoring Study (MRID 49415301)

Summary:

The overall objective of this report was to compare the results from a 3-year monitoring study at two locations with the potential aquatic estimated environmental concentrations (EECs) produced by the SWCC model. Both standard and modified scenarios were used as a means to better simulate field observations and to achieve insights into the factors governing the fate of flubendiamide and des-iodo at the field sites.

Registrant Comment:

“For GA, the SWCC overestimated peak flubendiamide concentrations in water and pore water by a factor of 3 and 17, respectively. Peak des-iodo concentrations were over-predicted by a factor of 11 and 26 in water and pore water, respectively.” (p. 7)

EFED Response:

The Agency agrees the SWCC concentration predictions based on the MS cotton and NC cotton scenarios are higher than the concentrations observed in the GA pond. However, the Agency ascribes these discrepancies to problems with the registrant’s data. The Agency believes the presence of the grassed waterways in the watershed of the GA ponds render these data unusable for comparison with the SWCC predictions. The pore water data discrepancy, which is larger than the water column data, is impacted by both the presence of the grassed waterways and potentially, the sample dilution issue. Additionally, there are other parameters such as field slope that would need adjustment before a direct comparisons could be made.

Registrant Comment:

“For NC, the SWCC under-predicted flubendiamide in water and pore water by a factor of 5 and 3 respectively. However, the NC site received an off-season, bare ground application in November of 2013 which led to greater runoff than would be expected in a typical growing season⁷. However, for des-iodo, the SWCC over-predicted water and pore water concentrations by a factor of 2 and 7, respectively.” (p. 7)

EFED Response:

The Agency believes the SWCC predictions fit the water column data quite well (Figure 6a and b) and believes the differences in pore water concentrations (Figures 6e and f) are better ascribed to the previously discussed sample dilution issue.

Registrant Comment:

“The model also predicted exponential accumulation of both flubendiamide and des-iodo in the water and pore water, which was not observed in the field study.” (p. 7)

EFED Response:

The Agency believes exponential accumulation was observed in the field study.

EFED Issue:

The registrant developed a series of increasingly complex model adjustments in order to get the SWCC predictions to align with the water column and pore water observations. The justification for making these adjustments was based almost entirely on the GA pond data and pore water data from both the GA and NC ponds, which the Agency believes to be inaccurate due to the presence of the grassed waterways (GA data) and the sample dilution issue (pore water data).

Conclusions

The monitoring study shows accumulation in all of the ponds monitored for both flubendiamide and des-iodo in water column, sediments, and pore water with 13 of the 18 pond accumulation trends identified as statistically significant. The VFS study (MRIDs 48175602, 48175604, and 48175606) and monitoring studies (MRIDs 49415301 to 49415303) did not provide evidence that VFSs provided significant reductions in flubendiamide and des-iodo transport to aquatic environments. The NC pond data provide a good match to the SWCC modeling (Figures 6a and b). This same model parameterization (after adjusting to maximum label application rates) produces exposure estimates that exceed Agency chronic LOCs (Figures 8a and b) for aquatic invertebrates in as little as 7 years. The NC tobacco scenario is not the worst case use (other scenarios exceed LOCs in shorter time periods). Flubendiamide and des-iodo are expected to accumulate in the environment and pose chronic risk concerns for aquatic invertebrates. Therefore, EFED concludes the original (D329613+) and subsequent ecological risk assessments performed by the Agency adequately reflect the risks posed by flubendiamide applications and

⁷ According to the monitoring report, “The concentrations of flubendiamide and des-iodo flubendiamide were higher in 2013 and first part of 2014 which was mainly caused by the off-season application of Belt™ on bareground after soybean harvesting in 2013. Although application of Belt™ on bareground in November was not a good agricultural practice, the application was made to compensate for the grower not making a summertime application as expected.”

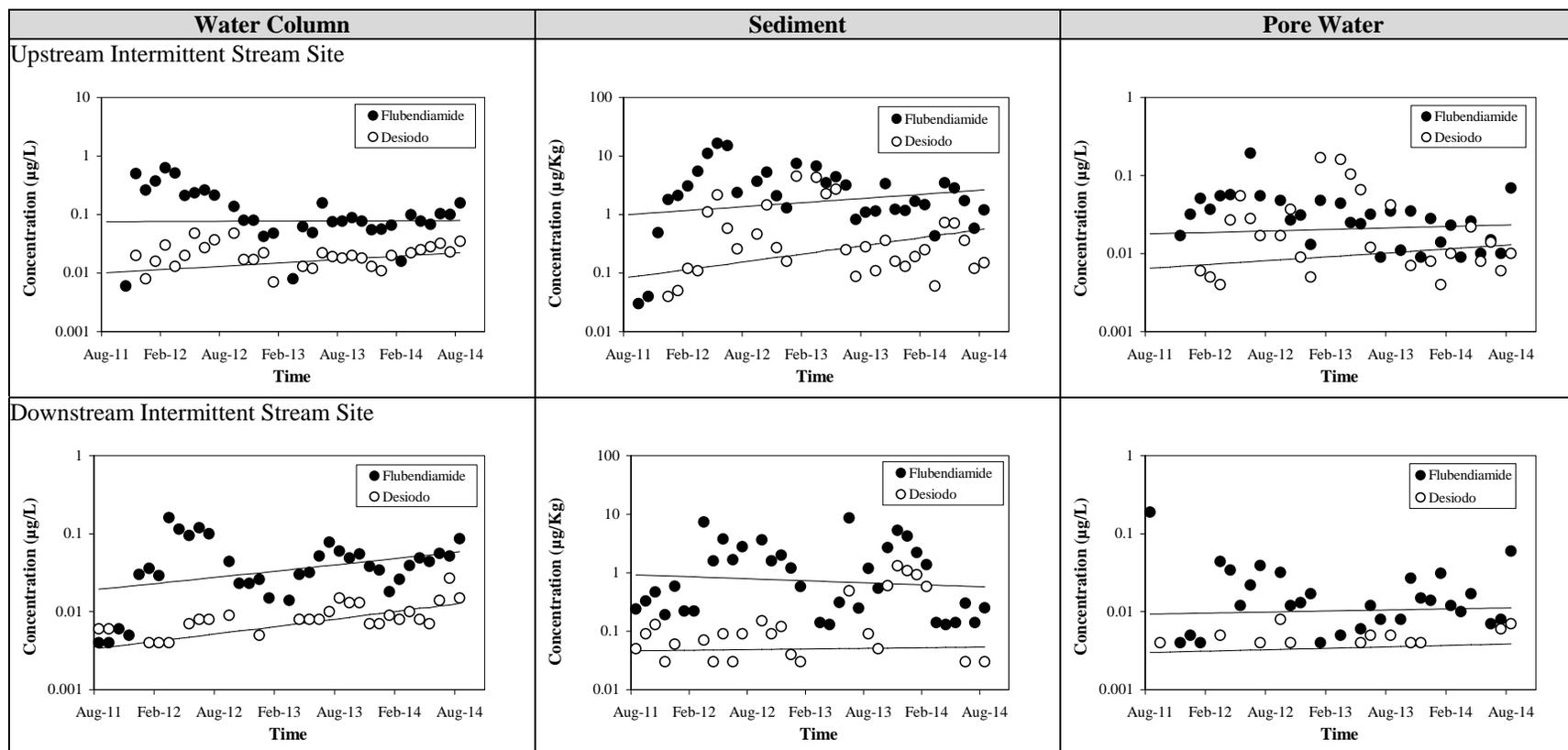
rejects the registrant's argument that the label-required 15 ft VFSs would prevent accumulation from exceeding Agency LOCs.

Literature Cited

- Dyer, D.G., and A.T. Hall. 2014. Flubendiamide Aquatic Risk – Summary of Surface Water Monitoring and Toxicity Testing. Bayer CropScience. Report Number: US0453. 16 pp. (MRID 49415302)
- Hanzas, J.P., B. Toth, and J. White. 2011. Georgia Site Selection Report for “Monitoring for Flubendiamide and its Metabolite des-iodo Flubendiamide in Sediment and Surface Water”. Bayer CropScience. Study Number: MEAMP011. 37 pp. (MRID 48644901)
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- Xu, T. 2013. Monitoring for Flubendiamide and its Metabolite Des-iodo Flubendiamide in Sediment and Surface Water: Interim Report 2. Bayer CropScience. Study Number: MEAMP011. 74 pp. (MRID 49139801)
- Xu, T. 2014. Monitoring for Flubendiamide and its Metabolite Des-iodo Flubendiamide in Sediment and Surface Water: Final Report. Bayer CropScience. Study Number: MEAMP011. 518 pp. (MRID 49415303)

Appendix A. Additional Monitoring Data from Flowing-Water Sites

EFED does not anticipate continuous accumulation at these flowing-water sites because any accumulation is continuously (water) or periodically (sediment) flushed downstream. Data from the Georgia and North Carolina flowing-water sites (located at different points in the larger watersheds that contain the GA and NC ponds) with trend lines (solid for flubendiamide and dashed for des-iodo) are presented in Figure A1 and A2, respectively. Because some of the data time-series from stream sites have few concentrations measured above the detection limit, the trend lines appear counter-intuitive.



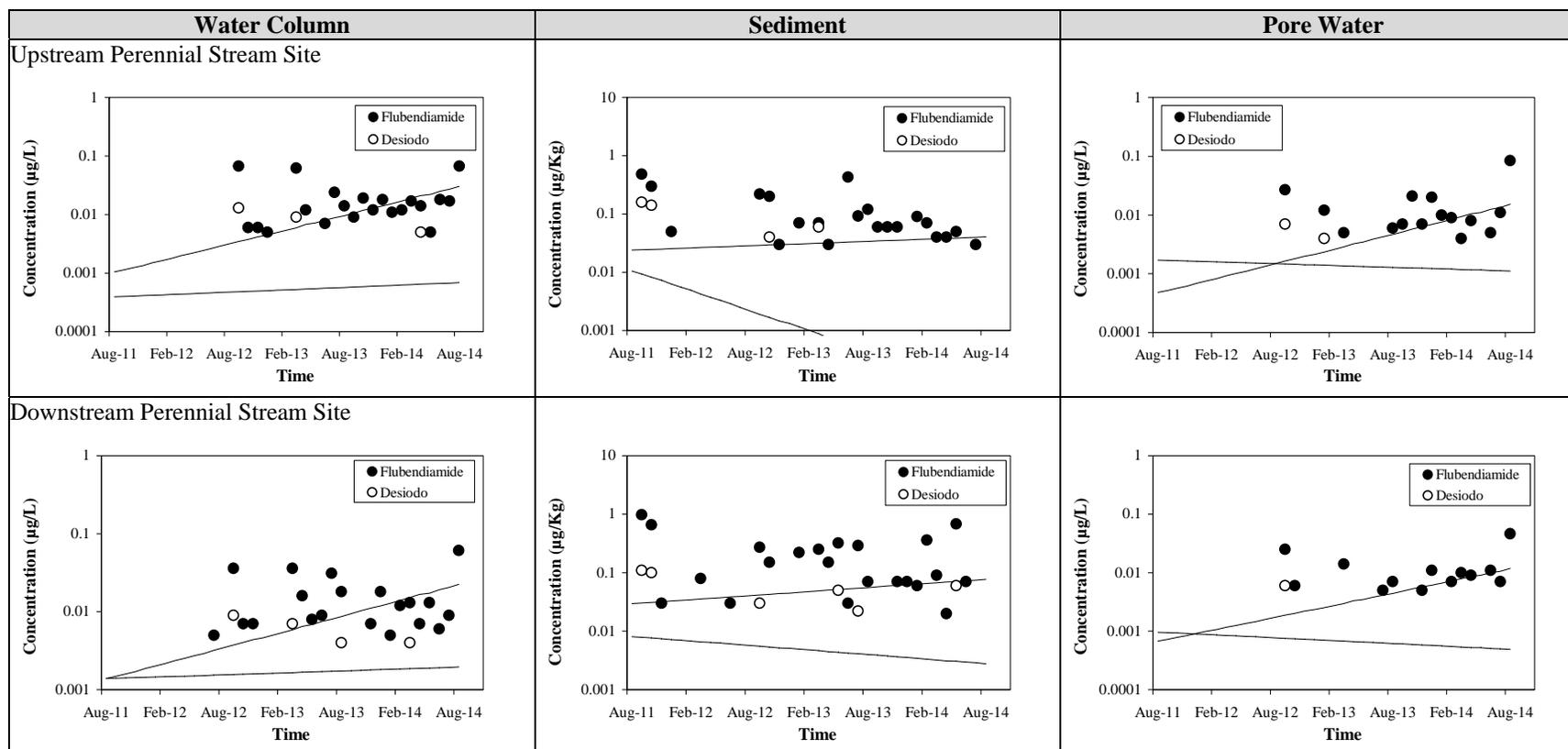
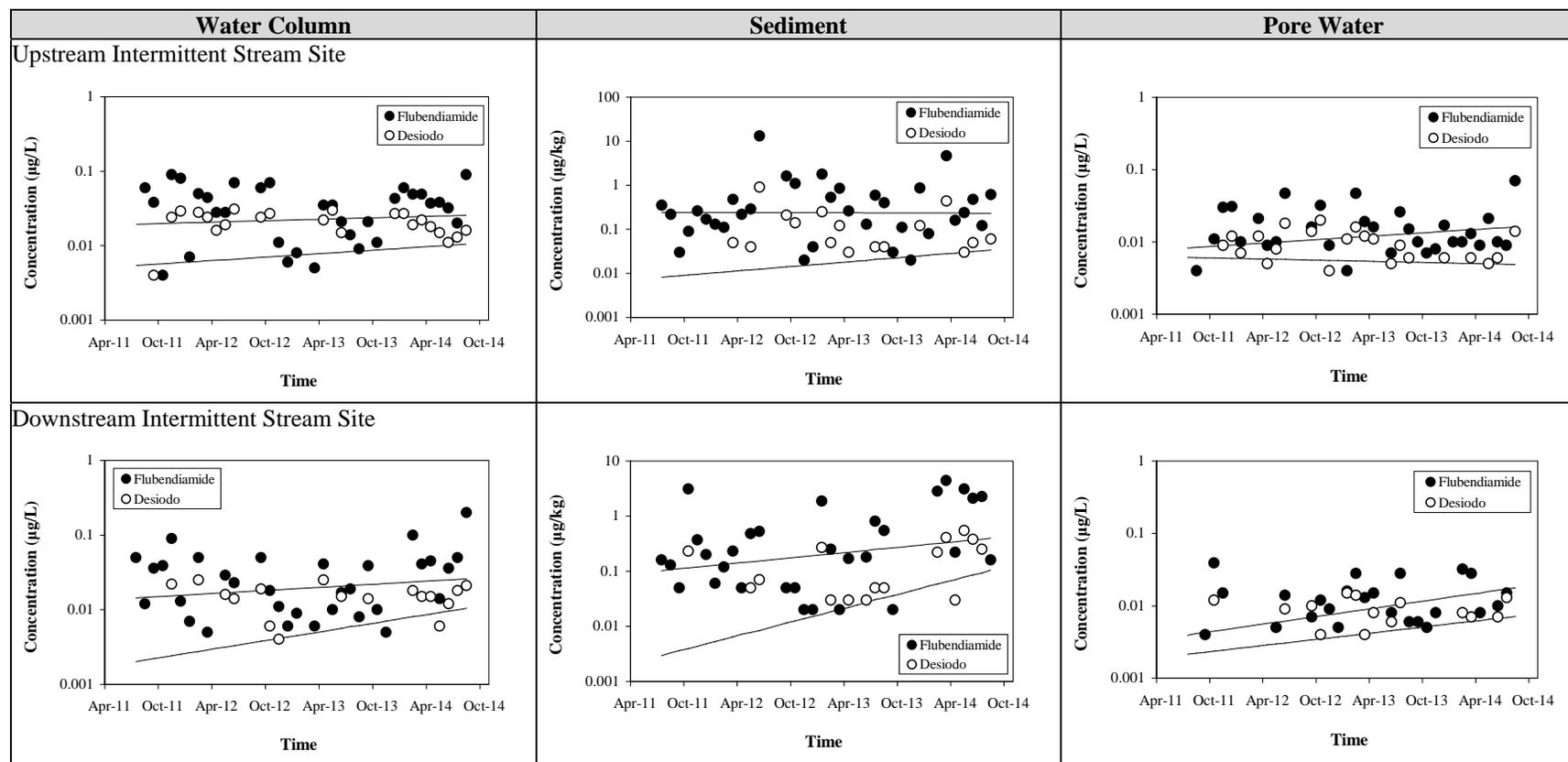


Figure A1. Georgia monitoring data from stream sites.

North Carolina Flowing-water Sites (located at different points in a larger watershed that contains the North Carolina Pond)



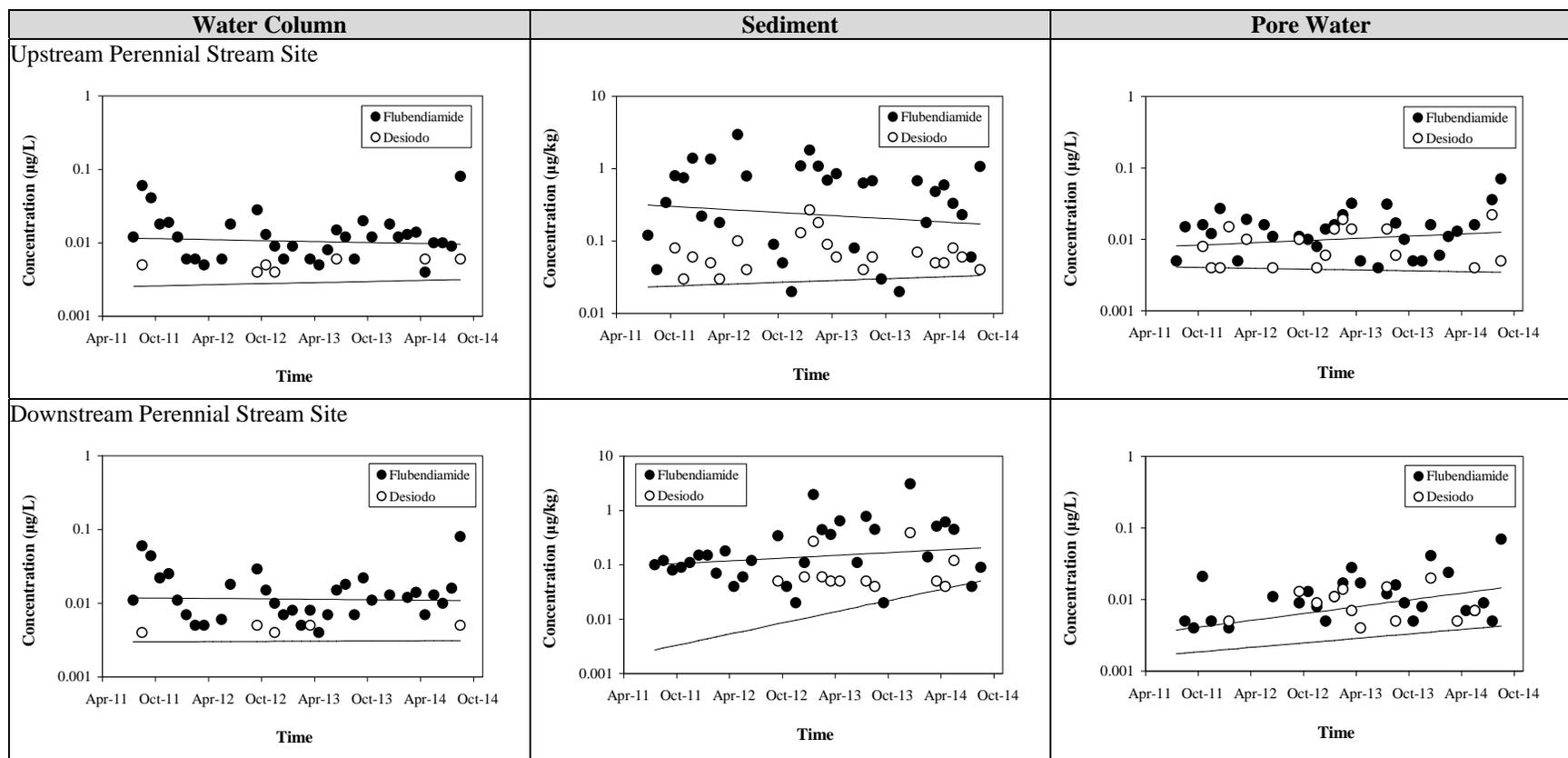


Figure A1. North Carolina monitoring data from stream sites.

Appendix B. Pore Water to Water Column Concentrations Ratios for Flowing Water Sites

The NC perennial stream exhibits pore water to water column concentration ratios that are much closer to 1 (Figure B1c and d) than the intermittent sites (Figure B1a and b) or the pond samples (see Figure 2a in the text). The NC perennial stream (the Tar River) is a large river at the sites sampled. Sediment depths are likely deeper and better mixed due to turbulent flow in the river, which may make it easier to sample sediment and pore water sample from a surficial layer with less dilution from deeper uncontaminated sediment and pore water. The intermittent stream samples had ratios that were intermediate in that they fell closer to 1 than the pond ratios, but further from 1 than the perennial stream samples.

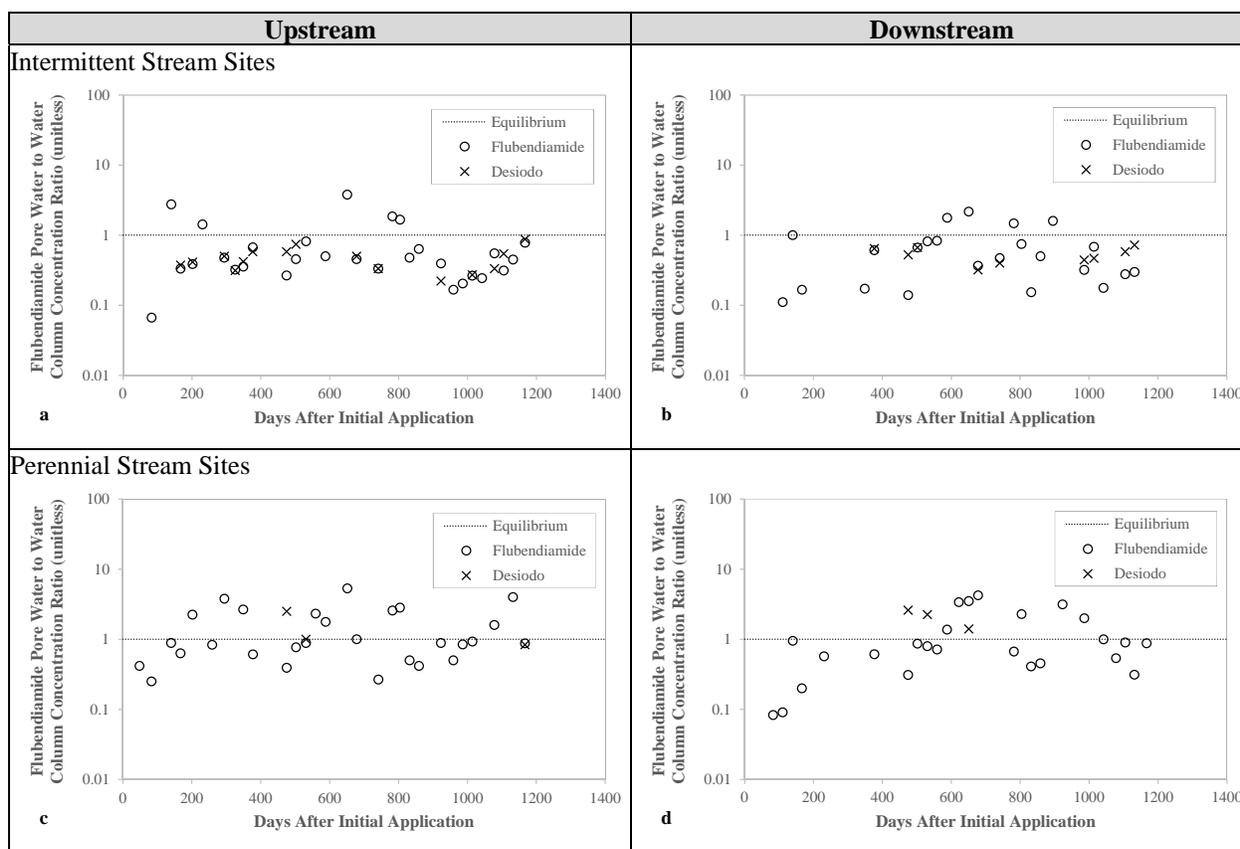


Figure B1. Comparison of pore water to water column concentration ratios for flubendiamide and des-iodo from intermittent (a and b) and perennial (c and d) near the North Carolina pond.

The GA perennial stream water column and pore water concentrations were relatively low. Therefore, early in the monitoring time frame, ratios could not be calculated because one or both concentrations fell below the detection limit. The later ratios from the GA perennial stream sites (Figure B2c and d) were distributed more like the GA (Figure B2a and b) and NC (Figure B1a and b) intermittent streams (the GA perennial stream is much smaller at the GA sample sites than the NC perennial stream is at the NC sample sites). Similar to the NC streams, the GA intermittent and perennial streams were much closer to a ratio of 1 than the GA pond ratios (Figure 2c in the text).

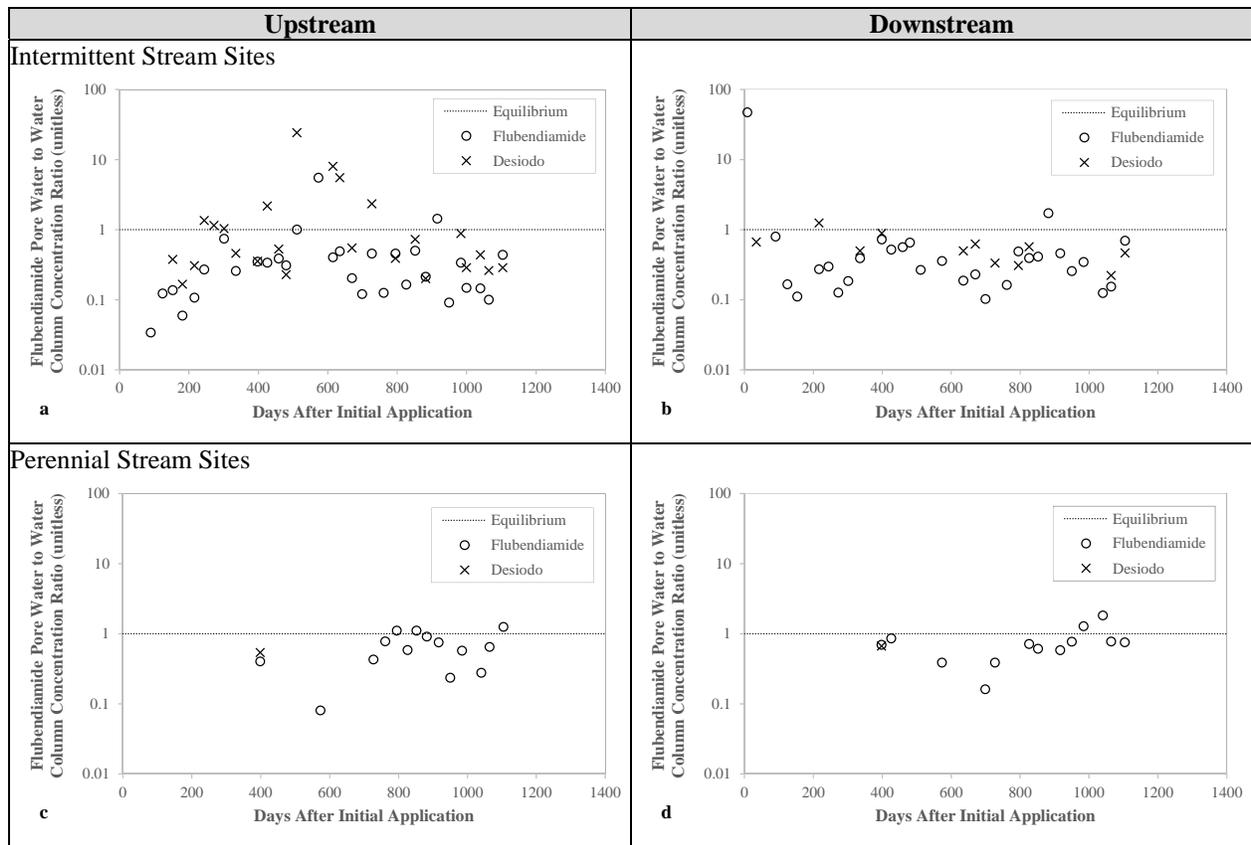


Figure B2. Comparison of pore water to water column concentration ratios for flubendiamide and des-iodo from intermittent (a and b) and perennial (c and d) near the Georgia ponds.

EXHIBIT 36



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

PC Code: 027602
DP Barcode: 427901
Date: July 8, 2015

MEMORANDUM

SUBJECT: Response to Bayer CropScience's "Flubendiamide Aquatic Risk: Evaluations of (1) USGS Stream Monitoring and (2) Proximity of Farm Ponds to Crop Areas with Flubendiamide Use" (no MRID number) submitted through email dated June 22nd, 2015

FROM: Stephen Wentz, Ph.D., Biologist
Environmental Risk Branch 1
Environmental Fate and Effects Division (7507P)

THROUGH: Sujatha Sankula, Ph.D., Branch Chief
Environmental Risk Branch 1
Environmental Fate and Effects Division (7507P)

Edward Odenkirchen, Ph.D., Senior Advisor
Immediate Office
Environmental Fate and Effects Division (7507P)

TO: Carmen Rodia, Risk Manager Reviewer
Richard Gebken, Risk Manager
Debbie McCall, Branch Chief
Invertebrate & Vertebrate Branch 2
Registration Division (7504P)

Introduction

Bayer CropScience (BCS) submitted comments in a document entitled "Flubendiamide Aquatic Risk: Evaluations of (1) USGS Stream Monitoring and (2) Proximity of Farm Ponds to Crop Areas with Flubendiamide Use". This submission follows a series of back-and-forth comments and responses following the Flubendiamide farm pond monitoring study reports submitted by BCS (MRIDs 49415301 to 49415303) and addresses three topics: 1) the USGS water monitoring data; 2) "water bodies and farm ponds in flubendiamide use areas"; and 3) proposes aquatic photolysis as an explanation for the 66-day mesocosm half-life. **After consideration of this information, EFED concludes that the information contained in this submission would not**

change the conclusions of previous EFED responses subsequent to the pond studies or previous EFED risk assessments.

Discussion

USGS Stream Monitoring

BCS's comments on USGS monitoring data compare the USGS sampling sites to flubendiamide sales data and make additional comments on expectations of flubendiamide concentrations in unfiltered samples vs. USGS filtered samples. The comparison of USGS sampling data to flubendiamide sales data showed that the USGS had sampled in some of the high sales areas. No description was found in this document of how the zip code-level sales data were calculated. Assuming the mapped sales data are standardized to the area of the zip code, this sales data could be useful for interpreting any future monitoring data.

In the filtered vs. unfiltered discussion, the registrant concludes that unfiltered samples should have less than 2× higher flubendiamide concentrations than filtered samples, which would still not result in exceedances of levels of concern (LOCs) in streams and rivers (flowing water bodies). In summary, the registrant's overall conclusion in this section is: 1) considering the USGS data captures the high sales areas; 2) the unfiltered samples should not exceed twice the filtered samples; and 3) mathematically converting the USGS filtered to unfiltered samples did not result in LOC exceedances; therefore, it is unlikely that unfiltered samples exceed LOCs in flowing water anywhere in the U.S.

The Agency does not agree or disagree with the registrant's argument, but rather feels the point concerning the USGS samples being filtered was missed by the registrant. EFED is interpreting the registrant pond monitoring study data (MRID 49415303), which found accumulation in ponds and detections in unfiltered samples from streams/rivers in the pond watersheds monitored, as providing evidence that detections in the USGS streams/rivers likely indicates accumulation in lentic waterbodies (wetlands, ponds, lakes and estuaries) within those USGS monitored watersheds. EFED's point was not that EPA expected exceedances in flowing water bodies, but rather that the widespread detections in the USGS filtered flowing water samples indicate that accumulation in lentic waterbodies across the U.S. is likely even more widespread than indicated by the filtered USGS water column samples. (Note that USGS does not have a sediment method for flubendiamide and/or des-iodo at this time and typically samples flowing waterbodies.)

Proximity of Farm Ponds to Crop Areas with Flubendiamide Use

In the registrant's comments on water bodies and farm ponds in flubendiamide use areas, the registrant seems to conclude based on GIS (Geographic Information System) data that relatively few farm ponds are in arid flubendiamide use areas and farm ponds are more common in wetter climates where ponds would be expected to overflow. This line of discussion seems to be predicated on the idea that the Agency is only concerned about farm ponds; therefore, any flubendiamide- and/or des-iodo-laden runoff not captured by a farm pond is of no concern to EPA. As previously discussed relative to farm pond overflow, any flubendiamide and des-iodo in runoff *not* accumulated in a farm pond will simply accumulate in the depositional zone of some other higher-value aquatic environment (reservoirs, lakes, or estuaries) causing more problems.

EFED models farm ponds because they are relatively easy to model and serve as surrogates for other aquatic environments, not because farm ponds are the only aquatic resource of concern.

Aquatic Photolysis as an Explanation for the 66-day Mesocosm Study Half-life

BCS proposed aquatic photolysis as an explanation for the 66-day mesocosm half-life. In the flubendiamide aerobic and anaerobic aquatic metabolism studies (MRIDS 46816913 and 46816914) as well as the mesocosm study (MRID 46817002), flubendiamide is introduced similarly into the water layer and then partitions into the sediment. In the aerobic and anaerobic aquatic metabolism study, the flubendiamide concentration in sediment exceeds the concentration in water within 4 days (*i.e.*, the majority of flubendiamide has partitioned or moved from water into sediment within 4 days). However in the mesocosm study the concentration in sediment never even approaches the concentration in water within the 112 day duration of the mesocosm study.

The amount of material measured in the mesocosm study water samples appears to be relatively similar to the aerobic and anaerobic aquatic metabolism studies (*i.e.*, appears to be slowly partitioning to sediment in a dynamic equilibrium at similar rates across all three studies). It is the mesocosm sediment data that does not make sense when compared to the aerobic and anaerobic aquatic metabolism studies' sediment data. There simply does not appear to be enough material in the mesocosm sediment to maintain the dynamic equilibrium between the sediment and water concentrations in the mesocosm study.

Aquatic photolysis which occurs in the upper layers of water would not explain the lack of flubendiamide in the sediment. As stated previously, it is far more likely that the mesocosm half-life is problematic rather than the aerobic and anaerobic aquatic metabolism studies since the mesocosm study is not designed to measure half-lives whereas the aerobic and anaerobic aquatic metabolism studies are designed to measure half-lives.

Additionally, the aquatic photolysis study produced two additional identified degradates (and other unidentified degradates) that would probably be of concern to the Agency because the identified degradates are structurally very similar to flubendiamide and des-iodo. Therefore even if aquatic photolysis were a suitable explanation for the mesocosm half-life (which it is not), EFED still would not use the mesocosm half-life because the additional degradates of concern in the aquatic photolysis study were not measured in the mesocosm study (*i.e.*, we would need the data for the additional photolysis identified and unidentified degradates to calculate the total half-life for all of the degradates of concern).

EXHIBIT 37

Curriculum Vitae

Name, Rank, and Contact Information:

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Tidewater Agricultural Research and Extension Center
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Education:

Ph.D., Entomology, Auburn University, Auburn, AL, 1985.
M.S., Entomology, Auburn University, Auburn, AL, 1975.
B. S., Biology, Johnson State College, Johnson, VT, 1971.

Professional Experience:

Professor, Dept. of Entomology, Tidewater Agric. Res. and Ext. Center, Virginia Tech, Suffolk, VA, 2002-present.
Associate Professor, Dept. of Entomology, Tidewater Agric. Res. and Ext. Center, Virginia Tech Suffolk, VA, 1994-2002.
Adjunct Associate Professor, Dept. of Entomology, North Carolina State University, Raleigh, NC, 1995-present.
Assistant Professor, Dept. of Entomology, Tidewater Agric. Res. and Ext. Center, Virginia Tech, Suffolk, VA, 1988-1994.
Postdoctoral Fellow, Dept. of Plant Pathology, Auburn Univ., Auburn, AL, 1986-1988.
Research Associate, Dept. of Entomology, Auburn, Univ., Auburn, AL, 1979-1986.

Honors and Awards:

Research awards and recognition

Insects Research and Control Conference Recognition Award for Excellence in Cotton Integrated Pest Management, Beltwide Cotton Conferences, New Orleans, LA, 2016.
Lifetime Achievement Award, 2012 Friends of Southern IPM, Southern IPM Center, Blacksburg, VA.
Andy Swiger Land-Grant Award, Virginia Tech College of Agriculture and Life Sciences, Blacksburg, VA, 2008.
Award for Excellence in Integrated Pest Management, Entomological Society of America, Eastern Branch, Ocean City, MD, 2002.
Research Award for “Outstanding contributions by developing and implementing information necessary to manage wheat insects economically and environmentally in Virginia”, Virginia Small Grains Growers Association, 1998.
Nominated for the Bailey Award recognizing outstanding research, American Peanut Research and Education Society for the paper titled “A Risk Index for Determining Insecticide Treatment for Southern Corn Rootworm in Peanut”, Orlando, FL, 1996.
Nominated for the Bailey Award recognizing outstanding research, American Peanut Research and Education Society for the paper entitled “Effects of soil texture and drainage on peanut pod damage by southern corn rootworm”, Tulsa, OK, 1994.
Meritorious Research Award, Virginia Soybean Growers Association, Williamsburg, VA, 1993.

Bailey Award for outstanding research, American Peanut Research and Education Society for the paper entitled “Impact of chemical use restrictions on disease, weed, and insect management in peanuts”, Atlanta, GA, 1990.

Extension and public service awards and recognition

Extension Service Award for Outstanding Service to Virginia’s Agribusiness Industry, Virginia Agribusiness Council, Warsaw, VA, 2010.

Extension Award of Merit, Gamma Sigma Delta, Virginia Tech Chapter, Blacksburg, VA, 2006.

Alumni Association Award for Excellence in Extension, Virginia Tech Alumni Association, Blacksburg, VA, 2006.

Outstanding Extension Display Award, Entomological Society of America for the presentation titled “Research leading to new management strategies for cereal leaf beetle in wheat”, Nashville, TN, 1997.

Outstanding Extension Display Award, Entomological Society of America for the presentation titled “Biology and New Management Strategies for Cereal Leaf Beetle in Virginia and North Carolina”, Louisville, KY, 1996.

Recognition of outstanding services rendered as advisory member of the Board of Directors for the years 1993-1996, Virginia Crop Production Association, Inc., Richmond, VA, 1996.

Certificate of Excellence in recognition for the development of outstanding agronomic educational material in the category of publication manuals for “Intensive Soft Red Winter Wheat Production — A Management Guide”, M. Alley, D. Brann, E. Stromberg, S. Hagood, A. Herbert, E. Jones and W. Griffith, American Society of Agronomy, Indianapolis, IN, 1996.

Nominated for the Entomological Society of America Distinguished Achievement Award in Extension, Las Vegas, NV, 1995.

Distinguished Achievement Award in Extension Entomology, Entomological Society of America, Eastern Branch, Harrisburg, PA, 1995.

Outstanding Extension Display Award, Entomological Society of American for presentation titled “Development and verification of a new scouting tool for Virginia small grains”, Dallas, TX, 1994.

National Recognition Award, American Society of Agronomy Educational Material Contest for the development of a satellite teleconference video on “Successful Soybean Technology and Management Considerations for 1993”, 1994.

State IPM Coordinator—1997-present

Extension Project Leader—Department of Entomology, College of Agriculture and Life Sciences, 1994-2014.

Refereed Scientific Journal Articles or Book Chapter—70 total

Extension Publications—133 total

Graduate Student Committees—PhD, 4 Chair, 2 Co-Chair; MS, 7 Chair, 1 Co-Chair, 15 Member

David Ames Herbert, Jr. -- List of Publications (selected, last 5 years)

Papers in Refereed Journals (*denotes student or postdoctoral scientist)

Lorenz, G., A. Herbert, and R. Leonard. 2015. Arthropod Pests-Caterpillars. *In* Compendium of Soybean Diseases and Pests, 5th Edition, The American Phytopathological Society, St. Paul, MN. Pp. 139-141.

Philips, Christopher R.; Kuhar, Thomas P.; Hoffmann, Michael P.; Zalom, Frank G.; Hallberg, Rosemary; HERBERT, D. Ames; Gonzales, Christopher; and Elliott, Steve (October 2014) Integrated Pest Management. In: eLS. John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0003248.pub2

Kamminga, K., D.A. HERBERT, M.D. Toews, S. Malone, and T. Kuhar. 2014. *Halyomorpha halys* (Hemiptera: Pentatomidae) feeding injury on cotton bolls. *J. Cotton Sci.* 18:68-74, <http://www.cotton.org/journal/2014-18/1/>.

Philips, C.R.*, T.P. Kuhar, and D.A. HERBERT. 2014. Effect of buckwheat farmscapes on abundance and parasitism of *P. rapae* (L.) in Virginia collards. *Journal of Entomological Science.* 49: 1-12.

Stewart, S.D., D.S. Akin, J. Reed, J. Bacheler, A. Catchot, D. Cook, J. Gore, J. Greene, A. HERBERT, R.E. Jackson, D.L. Kerns, B.R. Leonard, G.M. Lorenz, S. Micinski, D. Reising, P. Roberts, G. Studebaker, K. Tindall, and M. Toews. 2013. Survey of thrips species infesting cotton across the southern U.S. cotton belt. *J. Cotton Sci.* 17:1-7, <http://journal.cotton.org>

Owens, D.R.*, D.A. HERBERT, Jr., G. Dively, D.D. Reising, T.P. Kuhar. 2013. Does feeding by *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) reduce soybean seed quality and yield? *J. Econ. Entomol.* 106:1317-1323, ISSN 0022-0493, Online ISSN: 1938-291X

Owens, D.R.*, D.A. HERBERT, Jr., T.P. Kuhar, and D.D. Reising. 2013. Effects of temperature and relative humidity on the vertical distribution of stink bugs (Hemiptera: Pentatomidae) within soybean canopies and implications for field sampling. *J. Entomol. Sci.* 48(2): 90-98.

Owens, D.R.*, D.A. HERBERT, Jr., T.P. Kuhar, and D.D. Reising. 2012. Effects of temperature and relative humidity on the vertical distribution of stink bugs (Hemiptera: Pentatomidae) within soybean canopies and implications for field sampling. *J. Entomol. Sci.* (accepted, Aug. 30, 2012).

Kamminga, K.L.*, A.L. Koppel*, D.A. HERBERT, Jr., and T.P. Kuhar. 2012. Biology and management of the green stink bug. *J. IPM.* 3(3): C1-C8(8). <http://esa.publisher.ingentaconnect.com/content/esa/jipm/2012/00000003/00000003/art00006;jsessionid=2h3vms6ebliuo.victoria>

Reising, D.D., D.A. HERBERT, and S. Malone. 2012. Impact of neonicotinoid seed treatments on thrips (Thysanoptera: Thripidae) and soybean yield in Virginia and North Carolina. *J. Econ. Entomol.* 105(3): 884-889; DOI: <http://dx.doi.org/10.1603/EC11429>.

Philips, C.R.*, D.A. HERBERT, T.P. Kuhar, D.D. Reising, and E.A. Roberts. 2012. Using degree days to predict cereal leaf beetle (Coleoptera: Chrysomelidae) egg and larval population peaks. *Environ. Entomol.* 41(4): 761-767.

Samler, J.A.*, D.A. HERBERT, S. Malone, D. Owens*, T.P. Kuhar, and C. Brewster. 2012. Location of thrips (Thysanoptera: Thripidae) on soybean seedlings and implications for sampling. *Virginia J. of Sci.* 62 (1 & 2): 17 (abstract).

Cook, D., D.A. HERBERT, Jr., S.D. Akin, and J. Reed. 2011. Biology, crop injury, and management of thrips (Thysanoptera: Thripidae) infesting cotton seedlings in the United States. 2(2): <http://dx.doi.org/10.1603/IPM10024>.

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Koppel, A.L.*, D.A. HERBERT, Jr., T. P. Kuhar, S. Malone, and M. Arrington. 2011. Efficacy of selected insecticides against eggs of *Euschistus servus* and *Acrosternum hilare* (Hemiptera: Pentatomidae) and the egg parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae). *J. Econ. Entomol.* 104: 137-142.

Numbered Extension Publications (*denotes student or postdoctoral scientist)

Reising, D. and D.A. HERBERT, Jr. 2013. Soybean Insect Guide. United Soybean Board, Chesterfield, MO. http://unitedsoybean.org/wp-content/uploads/47574_Insect-Guide1.pdf

Holshouser, D., D.A. HERBERT, P.M. Phipps, and M. Reiter. 2013. Troubleshooting the Soybean Crop. VCE Publ. AREC-25NP.

Flanders, K., D. Reising, D. Buntin, M. Winslow, D.A. HERBERT, and D. Johnson. 2013. Biology and Management of Hessian Fly in the Southeast. VCE Publ. AREC-39. <http://www.pubs.ext.vt.edu/AREC/AREC-39/AREC-39.html>.

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EXHIBIT 38

Second Edition

Mid-Atlantic Guide to the

Insect Pests and Beneficials of Corn, Soybean,

and

Small Grains



VIRGINIA
IPM
Integrated Pest
Management

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

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Don Steinkraus	7
Richard G. Weber.....	12
Roger Youngman.....	4

Seedcorn maggot

corn, soybean



M. Spellman

Larvae

Feed on seed contents, leaving only empty shells; occasionally feed on seedling stems; damage pattern generally field-wide.

corn

Wireworm

Larva

Pale yellow to reddish-brown body; hard bodied; feed on corn seed and below-ground seedling stems and roots.



M. Spellman

Annual white grub

corn



Alton N. Sparks, Jr., The University of Georgia,
www.insectimages.org

Larva

There are several scarab species (Japanese beetle, June beetle, oriental beetle) that have similar-looking larvae called white grubs. They are typically cream-colored with a brown head and hold their body in a C-shape. They feed on germinating corn seed and newly developing roots. Damage is usually localized within fields.

Oriental beetle larva



M. Spellman

corn

Western corn rootworm

Larva

Cream-colored with dark brown head and rear end; feed on corn roots.

Adult

Yellow in color with three black stripes running down the length of the wing covers; feed on silks and tassels.



M. Spellman



M. Spellman

CORN

Seedcorn maggot • Wireworm
• Annual white grub • Western corn rootworm 2

Billbug

corn



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Adult

Ash-gray or brown in color, usually covered with soil. Often attached upside-down on corn seedling near the ground. Chew into side of corn seedling and feed on inner plant tissue which can result in excessive plant suckering.

corn, soybean Common stalk borer



Larva

Small larvae are cream-colored; first four abdominal segments of larger larvae are dark brown/purple; several dark lengthwise stripes may be present. They tunnel inside corn stalks in lower portion of plant.

M. Spellman

European corn borer

corn



R. Youngman

Larva

Flesh-colored, ranging from creamy-white to faint pink in color with a dark brown head; has several small dark spots on top of each body segment. They initially feed on the leaf surface, generally in the whorl, and later bore into stems and stalks.



M. Spellman

They initially feed on the leaf surface, generally in the whorl, and later bore into stems and stalks.

corn, soybean

Fall armyworm

Larva

In corn, they frequently feed on leaf whorls, causing ragged holes when blades unfurl. Later, larvae may feed on tassels and bore into ears and stalks. In soybean, they primarily feed on leaves.



M. Spellman

See key on page 30 for more details.

CORN

Billbug • Common stalk borer
• European corn borer • Fall armyworm 4

Black cutworm

corn



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Larva

Have grainy skin like sandpaper; curls into C-shape when disturbed. Young larvae feed on leaves. Older larvae found near base of plants and cut plants near base or below ground. Larvae feed at night and hide in soil during the day.

corn, soybean

Dingy cutworm

Larva

Smooth-skinned; eats leaves on young corn plants but rarely cuts corn.

To distinguish black from dingy, use a hand lens to look at the four tubercles (warts) along the top center of each body segment. On the black, the inside pair of tubercles is about half the diameter of the outside pair. On the dingy, these tubercles are about the same diameter.



Marlin E. Rice

Black cutworm



Marlin E. Rice



Marlin E. Rice

Dingy cutworm

Western bean cutworm

corn



Krista Hamilton, Wisconsin Department of Agriculture, Trade & Consumer Protection

Larva

Larvae feed on reproductive tissue of corn plants, primarily on tassels and inside husks on developing kernels. Larval coloration ranges from gray to tan to pink. Unlike many larvae, they do not have stripes extending down the sides of the body. Immediately behind the head they have a dark brown to black collar that is interrupted by light brown lines.



Marlin E. Rice



Marlin E. Rice

Head patterns (left to right)

Western bean cutworm (dark collar just behind the head with light brown lines, no stripes on the body)

Corn earworm (no dark collar, has distinct stripes down the sides of the body)

Thrips

soybean



Larva

Less active than adults; usually pale to yellowish in color.

Adult

Active crawlers; slender and cigar-shaped; feed on soybean leaves, causing faint striping and silvery appearance. Injury usually occurs to seedlings and because of plant regrowth is rarely of economic importance.

D. Steinkraus



D. Steinkraus

soybean

Twospotted spider mite

Eggs, nymphs, adults (non-insect)

Especially common during periods of hot, dry weather. Usually first seen along field edges. Initial damage appears as stippling at the base of leaves. Extreme webbing and defoliation can occur if populations are large.



Whitney Cranshaw, Colorado State University, www.insectimages.org

Soybean aphid

soybean



M. Spellman

Wingless aphids

Pale yellow to light green with black tailpipes; can be found on leaves, stems, petioles, and pods; generally the only aphid that reproduces on soybean. Feeding causes leaf spotting, leaf loss, and pod shed if populations are large.

soybean

Threecornered alfalfa hopper

Nymph

Bright green with spines along top of back.

Adult

Bright green wedge-shaped hoppers; girdle soybean stems and petioles, causing lodging and breakage.

Charles Lewallen



*Clemson University
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SOYBEAN

Thrips • Twospotted spider mite
• Soybean aphid • Threecornered alfalfa hopper 8

Potato leafhopper

soybean



Marlin E. Rice



Marlin E. Rice

Nymph

Nymphs resemble adults but are smaller and wingless. They feed by sucking plant juice and injecting toxic substances which causes stippling, leaf curling, and yellowing then desiccation of leaf margins.

Adult

Spindle-shaped, yellow-green, elongate hoppers.

corn, soybean

Green stink bug

Adult

Pierce soybean pods destroying young seed resulting in flat pods or dark spots and shriveling of older seed. Feeding on seedling corn may kill plants or cause stunting or suckering; attacked ears may be misshapen.

See key on page 33 for more details.

Nymph

Damage, especially by older nymphs, is similar to that caused by adults.

See key on page 34 for more details.



M. Spellman



Lynette Schimming

Brown stink bug

soybean



Russ Ottens,
The University of Georgia,
www.insectimages.org

Adult

Damage is similar to green stink bug.

See key on page 33 for more details.



Jerry Leonard

Nymph

See key on page 34 for more details.

soybean, corn Brown marmorated stink bug

Adult

A newly introduced species; speckled brownish-gray in color; a white stripe on the next to last antennal segment; several white spots on outside edges of rear abdominal segments; small round coppery patches appear on or near head.



David R. Lance, USDA APHIS PPQ, www.insectimages.org

Kudzu bug

soybean

The species has a preference for leguminous hosts, such as kudzu, wisteria, soybeans, and others, but it has been reported on fruit trees and various other hosts also. Loss of soybean yield can result from extended exposure to these insects.

Jeremy K. Greene,
Clemson University



Eggs



Nymphs

Young nymphs are small and orange, and older nymphs are very hairy but resemble adults in body shape. When disturbed, the insects produce a foul odor similar to that produced by stink bugs.

Jeremy K. Greene, Clemson University

Adult

Adults are about 5-mm long, olive-green colored with dark brown speckles, and are almost square-shaped but taper near the head region.

Jeremy K. Greene, Clemson University

Jeremy K. Greene,
Clemson University



Heavily infested
soybean plants



Grasshopper

soybean, corn



Richard C. Weber

Adult

There are approximately 600 grasshopper species in the U.S. The redlegged (pictured here) is commonly found feeding on foliage, especially seedlings on field edges during dry periods.

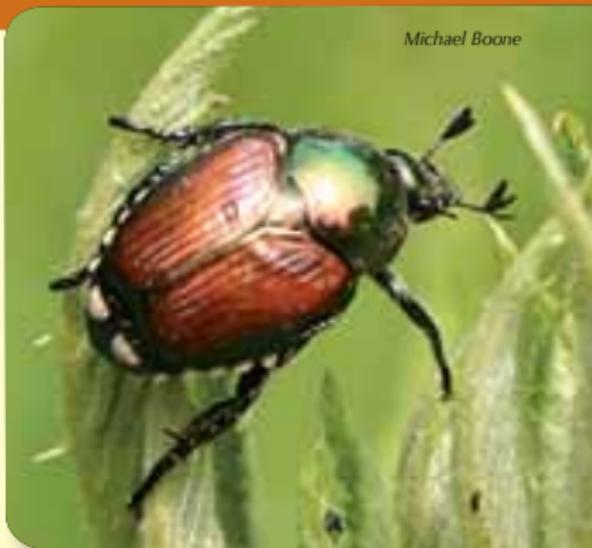
soybean

Japanese beetle

Adult

Metallic green body and coppery wing covers with 12 tufts of white hairs bordering the margin of wing covers. Adults skeletonize leaves, leaving large veins intact.

Michael Boone



SOYBEAN

Kudzu bug • Grasshopper
• Japanese beetle 12

Bean leaf beetle

soybean



M. Spellman



M. Spellman



M. Spellman

Adult

Green, yellow, tan, or red with a darkened triangular-shaped marking behind head; the number of black spots varies. They chew characteristic round holes in soybean leaves and scar outer pod walls later in the season.

See key on page 35 for more details.

Blister beetle

soybean

Clemson University - USDA Cooperative Extension Slide Series, www.insectimages.org



Margined blister beetle

Adult

Strictly foliage feeders; feed in clusters and skeletonize leaves similar to Japanese beetle.



Striped blister beetle

Adult

Orange with dark brown/black stripes.

Clemson University - USDA Cooperative Extension Slide Series, www.insectimages.org

soybean

Mexican bean beetle

Larva

Larvae and adults feed between the veins on the surface of leaves, leaving a lacy network of the tougher leaf tissues and veins. Damaged leaves turn brown and heavily damaged fields have a brown or burnt cast.

Adult

See key on page 35 for more details.

S. Malone



Michael Boone

SOYBEAN

Bean leaf beetle • Blister beetle
• Mexican bean beetle 14

Dectes stem borer

soybean



Mark Graustein

Larva

Creamy-white color with a head wider than body and an amber head capsule; found in soybean stems. Older larvae girdle stems causing plants to lodge.



M. Kogan

Adult

Dark gray elongate beetles, about 1.5 cm long, with banded antennae longer than the body.

soybean, corn

Corn earworm

M. Spellman



Larva

In corn, larvae will feed on foliage but most typically feed on developing kernels in the ear tip. In soybean, young larvae feed on flowers and tender foliage. Older larvae feed on seed within the pods.

See key on page 30 for more details.

Beet armyworm

soybean



M. Spellman

distinctive dark spot on each side just above the second pair of true legs. They are foliage feeders and may cause severe levels of leaf damage when populations are high.

See key on page 32 for more details.

Larva

Light-green to black; green forms with many fine, white wavy lines along the back and a broader stripe along each side; usually a

soybean

Yellowstriped armyworm

M. Spellman

Larva

Range from almost black to light brown; feed on leaves but rarely in large enough numbers to cause economic damage.

See key on page 31 for more details.



M. Spellman



Soybean looper

soybean



M. Spellman

Larva

Light green, body usually thicker towards the rear. Leaf feeding gives plants a ragged appearance and large populations are capable of causing heavy leaf loss.

See key on page 28 for more details.

soybean

Green cloverworm

Larva

Pale green, often with 2 white longitudinal stripes on each side; thrash violently when disturbed. Feed exclusively on leaves but rarely cause economic damage.

See key on page 28 for more details.



M. Spellman

Saltmarsh and yellow woollybear caterpillars

soybean



Alton N. Sparks, Jr., *The University of Georgia*,
www.insectimages.org

Saltmarsh caterpillar

Fuzzy looking; pale yellow to red to nearly black. Look very similar to the yellow woollybear caterpillar. Both feed on leaves, causing damage similar to other caterpillars.



Marlin E. Rice

Yellow woollybear caterpillar

Color varies from pale yellow to red to black.

soybean

Silverspotted skipper

Larva

Greenish-yellow with dark brownish-red head and large, round, bright orange eye spots; young larvae construct a characteristic “folded leaf” nest. They feed on leaves at night.



M. Spellman

Adult

Large silver spot on undersides of hind wings.



Richard Leung

SOYBEAN

Soybean looper • Green cloverworm
• Saltmarsh and yellow woollybear caterpillars
• Silverspotted skipper 18

Small grains aphids

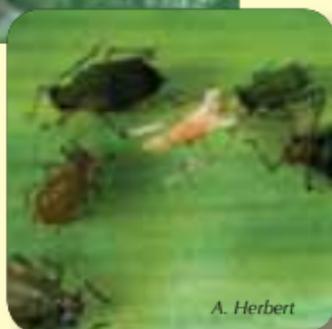
small grains



Alton N. Sparks, Jr., The University of Georgia, www.insectimages.org

◀ Greenbug

All grain aphids feed by removing plant sap, which can introduce disease and cause leaf mottling and discoloration. Greenbug is more damaging, as it releases a toxin when it feeds, causing yellow spots and plant death. Greenbug is light green with a dark green stripe down middle of back; antennae and tailpipes not all black.



A. Herbert

M. Spellman

▲ Bird cherry-oat aphid

Dark green with distinctive reddish color around base of tailpipes.

▶ English grain aphid

Solid green with long black antennae and black tailpipes.



▶ Corn leaf aphid

Appear pale blue-green to dark blue; black antennae and tailpipes; dark blue area at base of tailpipes. Body often seems to have a powdery coating. More common on sorghum but also found on wheat.



Jack Kelly Clark, courtesy University of California Statewide IPM Program

Cereal leaf beetle

small grains



A. Herbert

Eggs

Orange colored; laid in groups of 1-3 end-to-end on tops of leaves often along midveins.



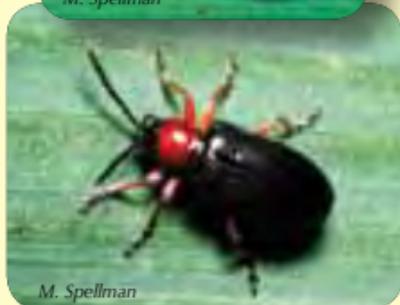
M. Spellman

Larva

Most damage is done by larvae feeding on the leaf surface, causing a frosted appearance to heavily damaged fields. They are yellow but usually covered with a brown or black coating of fecal material.

Adult

Metallic blue-black head and wing covers; area behind head is red.



M. Spellman

small grains

Hessian fly

Larvae

Red upon hatching but turn white after 4-5 days. Larvae extract juices from between leaf sheaths and stems. Fall feeding causes plant yellowing and death; spring feeding causes stunting and lodging of new tillers.

M. Spellman

Pupae

Red to dark brown spindle-shaped 'flax seed.' Usually found below the soil (singly or in clusters) near or burrowed into plant crown.



M. Spellman

True armyworm

small grains, corn



Larva

M. Spellman

Typically a spring or early summer pest. In grain, they feed on leaves and later cut through stems just below heads. In corn, they feed on lower leaves, progressing upwards, leaving midribs of mature leaves. They migrate as an 'army' to new hosts. *See key on page 29 for more details.*

small grains

Grass sawfly

Larva

Solid green color, amber head with a brown band, and a pair of prolegs on every body segment. They prefer to feed on stems and clipping often occurs before grain reaches maturity.

*M. Spellman*

Syrphid fly



Larva

Maggot-like larva with a body that tapers to the head end. No legs but moves well.



Adult

The adult looks like a small bee with a bright yellow and black striped body. They fly quickly and hover, hence the common name 'hover fly.'

Jack Kelly Clark, courtesy University of California Statewide IPM Program.

Lady beetle



M. Spellman

Larva

Look like tiny alligators with blue to black bodies and distinct yellow to orange markings.

Convergent lady beetle larva



Jack Kelly Clark, courtesy University of California Statewide IPM Program.

Scott Bauer, USDA



Multicolored Asian lady beetle adult

See key on page 36 for more details.



Pink spotted lady beetle adult

Body elongated (not round like other lady beetles); pink to orange with black spots.

See key on page 36 for more details.

M. Spellman

Convergent lady beetle adult

See key on page 36 for more details.

Jack Kelly Clark, courtesy University of California Statewide IPM Program.



Lacewings



Jack Dykinga, USDA

Larva

Similar to lady beetle larva but with prominent forward-extending mandibles.

Green lacewing adult

Yellowish green with four delicate transparent wings with many veins; has long hair-like antennae and red-gold eyes.



Alton N. Sparks, Jr., The University of Georgia, www.insectimages.org

Brown lacewing adult

Similar to green lacewing but brown and about half the size.



Jack Kelly Clark, courtesy University of California Statewide IPM Program.

Orius species



John Ruberson, The University of Georgia,
www.insectimages.org

Insidious flower bug

No V-shaped mark on back; have light yellow/tan wings.

Nymph

(not pictured) Shiny yellow-orange and do not have wings.

Minute pirate bug nymph

Yellow to amber pear-shaped body with red eyes and no wings.



Jack Kelly Clark, courtesy
University of California
Statewide IPM Program.



Jack Kelly Clark, courtesy University of California Statewide IPM Program.

Minute pirate bug adult

Has a black V-shaped mark on back and a faint gray spot on the hind wing membrane.

Bigeyed bug



Jack Kelly Clark,
courtesy University of
California Statewide
IPM Program.



Jack Kelly Clark,
courtesy University of
California Statewide
IPM Program.

Nymph

Slightly smaller than adults; predominately silver-gray with black markings.

Adult

Oval, somewhat flattened, about 4 mm long, usually brownish or yellowish, with a wide head and prominent bulging, widely-spaced eyes.

Parasitized aphids

Aphids can be parasitized by small wasps that develop inside the aphid body and exit leaving a hollow brown outer shell called a mummy.



M. Spellman

Fungal infected insects



M. Spellman

Fungal diseases infect several insect species, leaving powdery-looking cadavers.



M. Spellman



M. Spellman

Spined soldier bug



This stink bug resembles brown stink bug but is a predator that feeds on caterpillars and other small insects.

See key on page 33 for more details.

M. Spellman

Nabids

Adult

Slender mostly yellowish, gray, or dull brown with elongated heads, with long pointed beak-like mouthparts and long elbowed antennae.

M. Spellman



Lepidoptera larvae with 2 or 3 pair of prolegs



M. Spellman

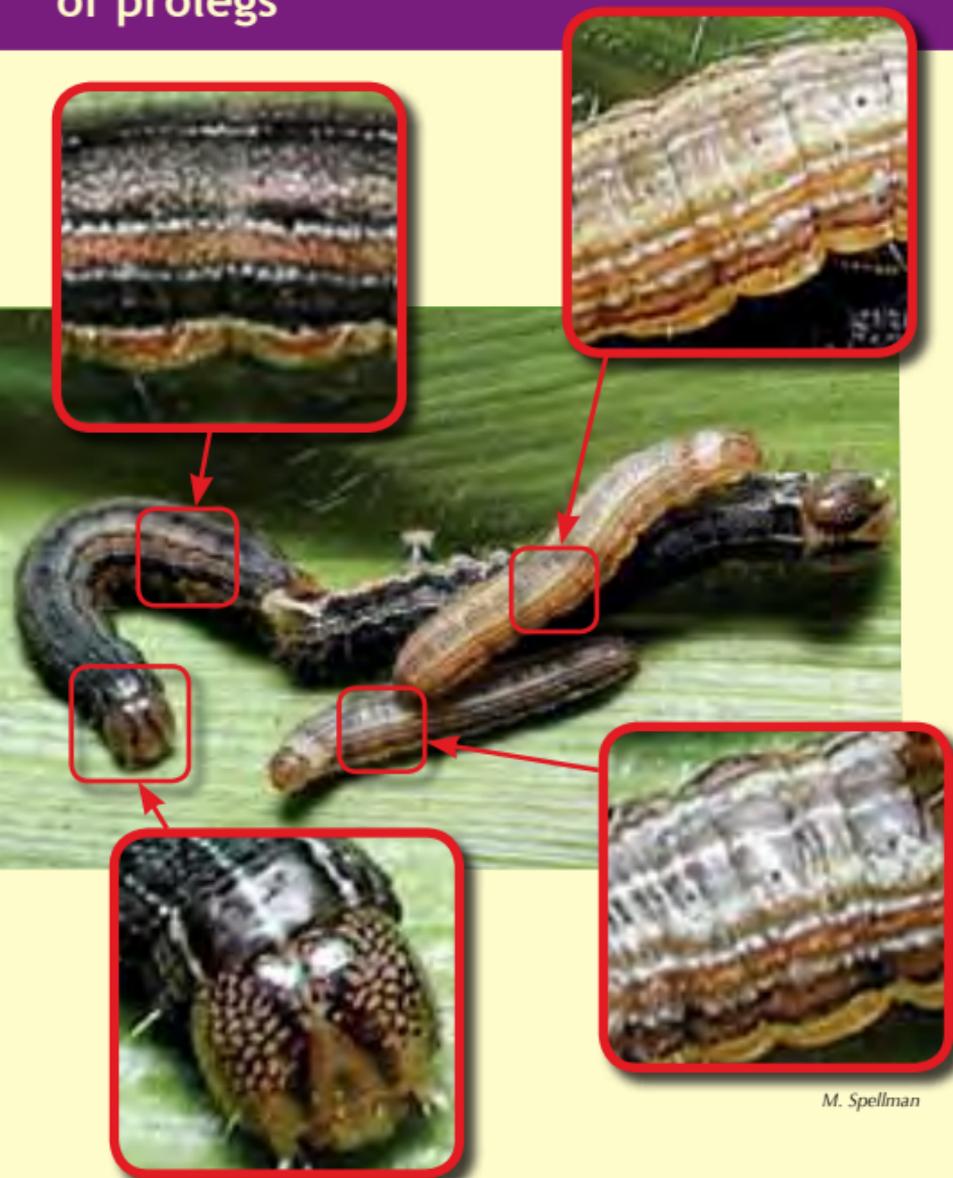
Green cloverworm - 3 pair of prolegs.

Soybean looper - 2 pair of prolegs.



M. Spellman

Lepidoptera larvae with 4 pair of prolegs



M. Spellman

True armyworm

Orange or brown stripe edged with white along sides with dark diagonal bands at the top of each abdominal proleg; head mottled with two dark stripes; commonly found in spring/early summer attacking grasses or grains.



M. Spellman

Corn earworm

Tan to amber head color and conspicuous black hairs on body.



M. Spellman

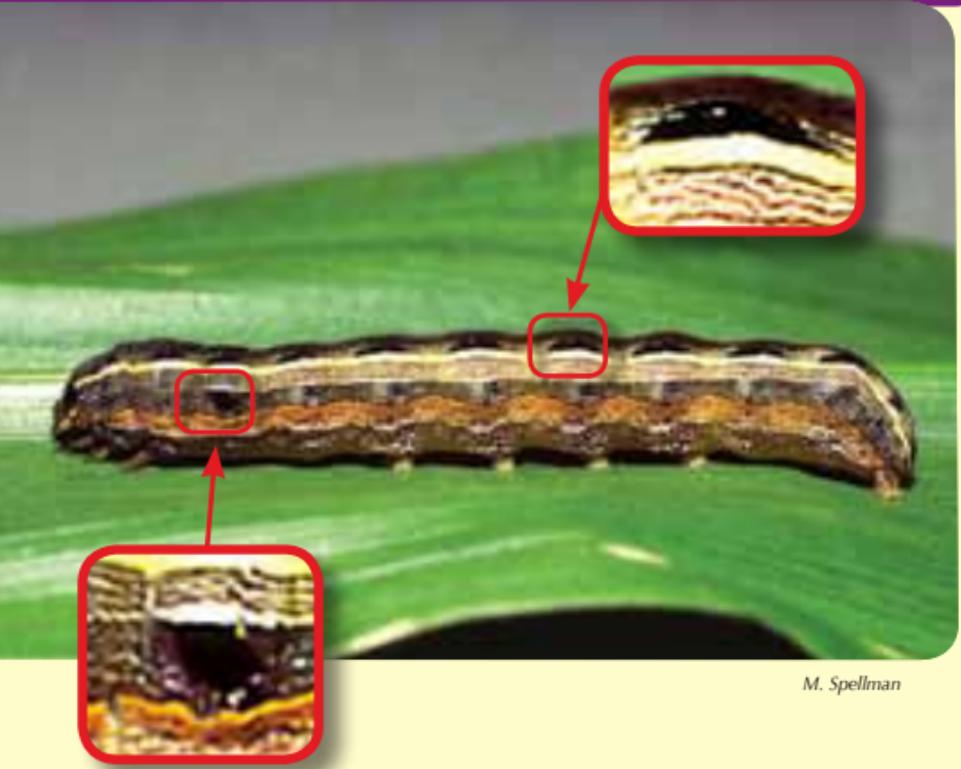
Fall armyworm

Dark brown head color with conspicuous cream-colored inverted "Y".

Black dots form a square on top of rear end.



Lepidoptera larvae with 4 pair of prolegs



M. Spellman

Yellowstriped armyworm

Pairs of black triangular markings on each segment of the back with bright yellow stripe just below; dark spot above first abdominal segment.

Beet armyworm

Light green to black with many fine white wavy lines along back and a broader stripe along each side; small black spot on each side of body above second true leg.



M. Spellman



Marlin E. Rice

Head patterns (left to right)

True armyworm (head mottled with 2 dark stripes)

Fall armyworm (dark brown color with conspicuous cream-colored inverted "Y")

Corn earworm (tan to amber head color)

Stink bugs



Green stink bug adult

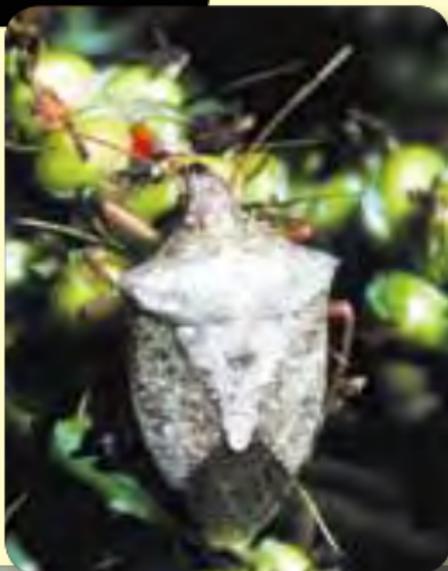
Bright green with black bands on antennae.

M. Spellman

M. Spellman

Brown stink bug adult

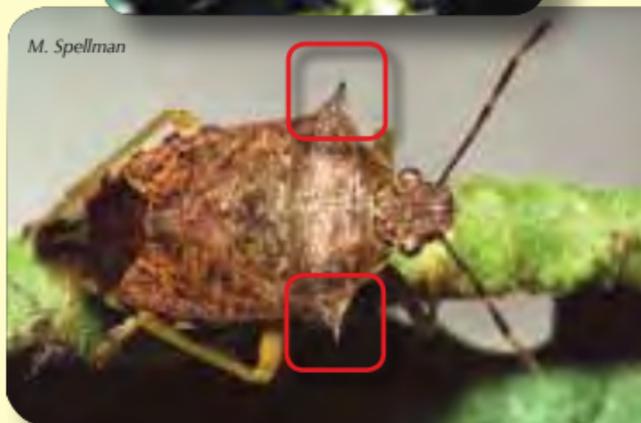
Brown with either a yellow or light green underside; has rounded shoulders.



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Spined soldier bug (beneficial)

Brown with a white to light cream-colored underside; has sharp-pointed shoulders.





Lynette Schimming

Green stink bug nymph

Predominately black when small, but become green with orange and black markings as they mature.

Brown stink bug nymph

Yellow to tan with brown spots down the middle of the back.

*Russ Ottens, The University of Georgia,
www.insectimages.org*



Beetle adults



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Bean leaf beetle

Body color and number of black spots variable but always has black triangle behind head.

Mexican bean beetle

Copper-orange color with 3 rows of black spots (16 spots total).



Michael Boone



Jack Kelly Clark, courtesy University of California Statewide IPM Program.

Convergent lady beetle

Has two distinct white lines behind the head that converge towards the back.



Scott Bauer, USDA

Multicolored Asian lady beetle

The 19 spots may be faint or missing; ranges from yellow to red-orange in color; has W-shaped mark behind head.

Pink spotted lady beetle

Lacks the black triangle behind head that helps distinguish it from bean leaf beetle.



M. Spellman

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