

May 3, 2019

Dr. Michiel Claessens
Regulatory Ecotoxicologist
Chemours Belgium BVBA
Ketenislaan 1 – Haven 1548
9130 Kallo
Belgium

Re: Eurofins EAG Agroscience, LLC - Experience and Testing Approach

Dear Michiel:

Thank you for the opportunity to share information with you concerning our testing capabilities and experience at Eurofins EAG Agroscience, LLC. I have attempted to answer your specific questions within the body of this letter and through a more detailed description of our test facilities and experience in the attachments. Following are responses to your questions:

- *Narrative descriptions of each lab's experience in the type of toxicity assays to be provided to Chemours;*

See a general overview of our laboratory experience in Attachment A.

- *List of references for similar contract work that generated toxicity study data and what chemicals or classes of chemicals were tested for these applications, including whether such contract work was provided for Federal or state regulatory or research agencies;*

This is difficult to provide, since all of our work is protected by confidentiality agreements and we do not share information about our clients with other clients. As indicated in Attachment A, we have conducted many studies over the years to fulfill EPA Task Order requests, and this information should be available online. Our scientists have also been involved in many working groups in both the U.S. and EU in review and development of testing guidelines. There should be DuPont and Chemours sponsor monitors that can provide references for previous work performed for these clients.

- *List of certifications for the specified bioassays;*

In the United States, the government does not issue "Certificates" of Good Laboratory Practice Compliance. However, periodic inspections by the United States Environmental Protection Agency (USEPA) Department of Compliance Monitoring verify that Eurofins Agroscience Services is conducting studies in a GLP compliant manner. I have attached information concerning the most recent facility audits conducted by the EPA in Attachment B.

- *Location of the facility at which the testing will be done, and experience of the staff who will conduct the testing;*

For Aquatic toxicology, we have testing laboratories in Easton Maryland, USA and Columbia, Missouri, USA; descriptions of these testing facilities are provided in Attachment A.

- *Source of the testing animals (i.e., in-house cultures or external suppliers);*

Information regarding our laboratory cultures and source of test animals is provided in Attachment A.

- *Source of the testing material and how will it be characterized chemically for dosing;*

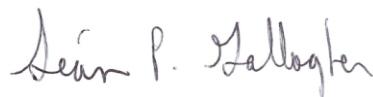
The Sponsor is responsible for providing the testing facility with verification that the test substance has been characterized according to GLPs prior to its use in the study. If the sponsor doesn't provide the test substance, it may be purchased from a commercial supplier (e.g. Sigma Aldrich) at the request of the sponsor. If verification of the GLP test substance characterization is not provided to the testing facility, it will be noted in the compliance statement of the final report. The concentration of the test substance in the test system will typically be based upon chromatographic methodology provided by the Sponsor and/or developed at Eurofins. The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report. The accuracy, limit of detection (LOD), limit of quantification (LOQ) and method detection limit (MDL) for the analytical method used to analyze the samples will be assessed during the first and last analysis conducted during the conduct of the study, when possible. Analytical methods are typically validated prior to use in a GLP study according to USEPA or OECD guidelines.

- *List any affiliation with DuPont, Chemours or any of their associated companies*

Eurofins EAG Agrosience Services is not affiliated with DuPont, Chemours or any of their associated companies except as a provider of contract research.

We hope this provides sufficient information to answer the questions above. Please let us know if additional detail is needed.

Best regards,



Sean P. Gallagher
Scientific Advisor - Ecotoxicology

ATTACHMENT A

Availability and Adequacy of Facilities and Technical Approach

Eurofins EAG Agrosience Services has environmental testing facilities at two of the most widely respected facilities in the world, located in Easton, MD and in Columbia, MO. Both laboratories have been conducting environmental tests for regulatory support worldwide for over 40 years. The facilities at both locations are designed to accommodate a wide range of acute and chronic aquatic tests and are capable of supporting a large number of concurrent studies. The overall technical approach is largely based upon our previous experience following and helping to develop regulatory test guidelines. We have performed thousands of tests for the agrichemical, industrial chemical and pharmaceutical industries according to EPA, OECD, JMAFF, TSCA, FDA, PMRA and IBAMA regulatory requirements.

Eurofins EAG Agrosience Services have highly trained and experienced staff including more than 20 Ph.D. scientists and dozens of M.S. and B.S. level scientists, and numerous individuals with 20 and 30+ years of experience in toxicology and ecotoxicity. We have supported EDSP development efforts in collaboration with Prime Contractors, USEPA, and others under multiple previous Task Orders and test guideline ring tests. Our scientists have acted as U.S. representatives to the OECD ecotoxicology validation management (VMG-ECO), participating in the development of the OECD guidance document “Current Approaches in the Statistical Analysis of Ecotoxicity Data”. Eurofins EAG Agrosience Services scientists have also served as members of the OECD expert groups developing the following test guidelines: avian acute and reproduction tests, amphibian and fish screening tests for endocrine disruptors, bioconcentration testing in fish. We have developed improved testing methods for chronic testing with sediment dwelling invertebrates.

The facilities that would be used for the required testing are described below:

Aquatic Facilities (Algae, Invertebrates, Fish, Amphibian) – Easton, MD (formerly Wildlife International)

Eurofins EAG Agrosience Services - Easton MD maintains one of the most modern aquatic toxicology laboratories in the world with over 50 test systems. A recent 10,000 ft² expansion of the aquatic testing facility was completed in 2017 and was added primarily to meet the growing demand for endocrine testing, expanding the laboratory to ~35,000 ft². The expansion includes a dedicated laboratory space for qPCR assays for determination of the genetic sex and vitellogenin endpoints in the endocrine test guidance and space for conducting *in vitro* assays according to OECD 319A & 319B. Eurofins EAG Agrosience Services - Easton has an active Institutional Animal Care and Use Committee (IACUC) that reviews all aquatic vertebrate study protocols and SOPs to ensure compliance with Customer, Corporate, and guideline expectations for the use of animals in research.

Testing is performed in walk-in environmental control chambers with built-in reconfigurable diluter systems (capable of holding up to 64 replicate test tanks), or in smaller test systems each consisting of an integrated temperature-controlled water bath and diluter, enclosed in an isolation chamber. The sixteen walk-in environmental control chambers are particularly suitable for one and two generation fish tests. Ten of the test systems are specifically designed to perform large-scale fish and amphibian endocrine disruptor tests, and construction materials that come into contact with water are plasticizer free wherever possible. Smaller self-contained test systems are suitable for studies such as FSTRA with Fathead Minnows, Medaka or Zebra Fish and are used for various vertebrate and invertebrate assays. All test systems designed for chronic testing can also be utilized for acute testing with both invertebrate and fish

species. Algae testing is conducted in a dedicated laboratory space with separate non-tox areas for maintenance of algal cultures and preparation of sterile media.

The Easton facility maintains extensive water treatment facilities in which we treat and control both freshwater and marine waters. Freshwater comes from a 120 foot on-site well and marine water is collected from Indian River Inlet on the Atlantic Ocean. Marine water is trucked via our 4,000 gallon tank truck on an as needed basis (usually once or twice per week). Water from both sources is analyzed annually and has historically been free of contaminants of concern.

We have extensive experience in rearing and holding a variety of fish species that include fathead minnows, sheepshead minnows, medaka, zebra fish, rainbow trout, common carp, and other species as needed. We have experienced staff and in-house capabilities to maintain breeding cultures of fathead minnows, *Xenopus laevis*, zebra fish and medaka. Our experienced staff routinely performs acute and chronic tests with fish and amphibians that involve both full life cycle and early life stage testing including FSTRA, FSDT, AMA, MEOGRT and LAGDA. Invertebrate cultures include *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, *Hyalella Azteca*, *Chironomus riparius*, *Chironomus dilutus*, *Leptocheirus plumulosus*, *Lumbriculus variegatus* and *Amercamysis bahia*. Algae cultures of *Anabaena flos-aquae*, *Anabaena cylindrica*, *Navicula pelliculosa*, *Raphidocelis subcapitata*, *Skeletonema costatum*, *Lemna gibba* G3, are maintained in our algal testing area.

Relevant aquatic toxicology equipment and instruments:

- Sixteen walk-in environmentally controlled rooms
- Ten environmental testing units specifically designed for ED testing
- Twelve self-contained diluter systems with temperature controlled water baths
- Five large Percival incubators
- Three 10,000 gallon and one 5000 gallon fiberglass holding tanks (fresh/marine)
- One 4,000 gallon tank truck (marine)
- Culture/holding units
- Extensive water filtration/treatment systems
- Coulter Counters
- Various peristaltic/syringe pumps/mixers
- Water quality instrumentation
- Fumehoods
- Dedicated effluent waste pits with filtration

All culture and exposure systems are equipped with a continuous temperature monitoring system and will alarm if a malfunction occurs, an alarm sequence will be initiated where laboratory personnel are notified first by audible alarms and if the problem persists, by an email and telephone call sequence. Exposure systems are provided with an oil-free supply of air and the facility is equipped with backup generators for emergency power in the unlikely event of a total power failure incident.

All studies are supported by the Eurofins EAG Agrosience Services - Easton chemistry group that validates analytical methods prior to test initiation then analyses test concentrations throughout each study. Our onsite qPCR laboratory provides dedicated support for endocrine testing requirements.

Aquatic Facilities (Algae, Invertebrates, Fish, Amphibian) – Columbia, MO (formerly ABC Labs)

Eurofins EAG Agrosience Services - Columbia MO maintains a 10,000 ft² aquatic toxicology laboratory which also has an extensive algae, fish, frog and invertebrate culture area. Fathead minnows, sheepshead minnow, *Medaka*, and *Xenopus* frogs are in continuous culture under the supervision of an experienced aquaculturist. With the exception of sediment dwelling invertebrates, our Columbia laboratory maintains cultures of the same invertebrate and algal species as our Easton laboratory. Eurofins EAG Agrosience Services - Columbia has an active Institutional Animal Care and Use Committee (IACUC) that reviews all aquatic vertebrate study protocols and SOPs to ensure compliance with Customer, Corporate, and guideline expectations for the use of animals in research.

Dilution water for ecotoxicology studies originates from an on-site deep well. Dilution water is prepared by blending naturally hard well water with well water that has been de-mineralized. The well water and demineralized water are blended together to yield an appropriate total hardness range for testing purposes and stored in 10,000-gallon in-ground holding tanks with an overall capacity of up to 30,000 gallons of blended freshwater. Marine water is created for culture and testing by addition of salt mix designed for bioassays to freshwater to achieve the appropriate salinity level. Dilution water is heated or chilled to near the appropriate test temperature and passed through a sediment filter and UV sterilizer before delivery to the exposure systems. The dilution water is monitored for potential contaminants. Eurofins EAG Agrosience Services - Columbia has developed pH adjustment systems that can be utilized for test chemicals that are more stable under certain pH conditions.

Testing can be conducted under static and intermittent-flow proportional diluter systems. The diluter systems, test chemical metering systems, water bath temperatures, and photoperiod are computer-controlled and continuously monitored. The exposure aquaria can be randomly distributed within the water bath or baths as needed. The water bath(s) are shielded from the surrounding laboratory area with curtains. The exposure systems and equipment are alarmed such that if a malfunction occurs, an alarm sequence will be initiated where laboratory personnel are notified first by audible alarms and if the problem persists, by a telephone call sequence. Exposure systems are provided with an oil-free supply of air and the facility is equipped with backup generators for emergency power in the unlikely event of a total power failure incident.

Analytical Chemistry Facilities and Capabilities

Eurofins EAG Agrosience Services has all the required analytical chemistry capabilities and equipment for this program. The newly expanded Easton MD facility comprises over 15,000 square feet of laboratory equipment and bench space and houses analytical instrumentation, method development, method validation and sample preparation laboratories. The Columbia MO facility has approximately 2000 square feet of laboratory space within the Aquatic testing facility dedicated to method development, method validation and sample preparation and analysis.

Histopathology Facilities and Capabilities

Eurofins EAG Agrosience Services, provides in-house histopathology services at its Easton, MD location to evaluate the potential of chemicals to affect endocrine-sensitive tissues in fish, amphibians, and birds.

- Dedicated facilities for the preservation, embedding, sectioning, staining, and histopathological assessment of tissues
- In-house evaluation of sections by highly experienced pathologist, Dr. Reinhardt Sahmel
- Evaluation of fathead minnows, *Medaka*, zebra fish, *Xenopus*, Japanese quail and other species
- Close integration of in-life test phase with histological processing and data handling
- Full compliance with Good Laboratory Practice (GLP) Standards
- Services performed in accordance with relevant OECD and EPA guidance
- Peer review of histopathological findings by external experts can be arranged, or we can provide peer review of findings from other laboratories

Technical Approach

Most *in vivo* invertebrate and fish studies are preceded by one or more range-finding preliminary exposures to determine the definitive (nominal) concentrations of the test substance in the given species. All studies require the drafting and approval of a GLP protocol that governs the conduct of each study. The protocols will include specifics for procuring test substances, procuring and maintaining test animals, preparing and administering test substances, analyzing samples for measured concentrations, observing animal behavior, collecting data on other endpoints, conducting histopathology, performing QA activities, analyzing data, managing deviations and unexpected events, and preparing and reviewing draft and final assay reports.

Animals are acquired and undergo a pre-exposure period. The chemical exposure system is set up, and treatment levels are verified through analytical sampling. Barring complications, definitive exposures will run for the duration specified in the guidelines and protocol, with in-life and post-mortem samples, specimens, and other data being collected for a prescribed set of toxicological and reproductive endpoints. Concentrations of test chemical in the tanks are verified by periodic sampling and analysis. Data will be collected into EPA-defined Data Entry Spreadsheet Templates (DESTs) if appropriate. Specimens such as blood/plasma, fin clips, and organs or tissues that are subjected to biochemical, histopathological, and other evaluations, and selected data are analyzed statistically in accordance with defined statistical analysis plans.

Any changes to the protocol known in advance are documented by formal, signed amendments, or, if the differences are discovered during or after the performance of the work, by deviations. Scientists and

technicians prepare draft reports for their respective disciplines, and the Study Director/Toxicologist reviews and synthesizes the information into a draft assay report that meets the sponsor-defined outline and content requirements. This report undergoes internal technical reviews, GLP/QA reviews, and sponsor reviews, until a final, fully signed GLP report is completed and delivered to the Sponsor.

Various guidelines, especially those promulgated by the EPA and OECD provide the objectives and outline the methods, performance criteria, and test validity goals for each assay, along with appendices that detail specific required or optional procedures such as biochemical specimen collection and analysis or histopathological evaluations. To these guidelines, the performing laboratories add their own expertise in having performed ecotoxicology studies as they develop GLP study protocols for each combination of assay and test substance.

Analytical Chemistry Instrumentation

Instrumentation	Brand	Columbia Site 1	Columbia Site 2	Easton
Analytical HPLC				
2690	Waters			2
2695 with duo view detector	Waters	8	0	0
2695 with photo diode array detector	Waters	2	2	0
1100/1200 with variable wavelength detector	Agilent	30	11	13
1100 with multiple wavelength detector	Agilent	1	0	0
1100/1200 with Diode array detector	Agilent	11	1	6
1260 with variable wavelength detector	Agilent			3
Ion Chromatograph HPLC ICS - ICS-2100 Series	Thermo Electron			1
Ion Chromatograph HPLC ICS	Dionex	3	0	
Waters breeze HPLC	Waters	0	2	
Total		55	16	25
Additional Detectors				
Sedex 75 ELSD	Sedere	2	0	
Model 432 conductivity detector	Waters	1	0	
Conductivity detector model 650	Alltech	1	0	
1100 fluorescence detector	Agilent	2	1	2
Charged aerosol detector (CAD)	ESA Magellan Biosciences	3	0	
Refractive index detector model 2410/2414	Waters	1	1	1
Wyatt MALS/Quals	Wyatt	1	0	
Wyatt Refractive index detector	Wyatt	1	0	1
Total		12	2	4
Preparative HPLC				
model 2545 with 2996 photo diode array detector	Waters	1	0	
1200 with MWD	Agilent	0	1	
600 with 486 detector	Waters	0	2	
Total		1	3	0

Analytical Chemistry Instrumentation

Instrumentation	Brand	Columbia	Site 1	Columbia Site 2	Easton
UPLC					
UPLC classic with Photo diode array detector	Waters	2		0	
UPLC classic with tunable absorbance detector for AAA	Waters	2		0	
UPLC H class with tunable absorbance detector	Waters	2		0	
UPLC H class with tunable absorbance detector and PDA	Waters	2		0	
UPLC H class with PDA	Waters	6		0	
UPLC for LC/MS/MS	Waters/Shimadzu	2		9	
Total		16		9	0
Gas Chromatography					
5890 Series II	Agilent				3
6890 with Flame Ionization Detector	Agilent	6		7	1
6890 with Flame Ionization Detector and NPD	Agilent	1		0	1
6890 with Flame Ionization Detector and FPD	Agilent	1		1	1
6890 with Flame Ionization Detector and TCD	Agilent	2		0	
6890/7890 with FID and ECD	Agilent	0		2	5
6890 with FPD and NPD	Agilent	0		1	
Total		10		11	11
Additional Autosampler					
Static Headspace Autosampler model 7694	Agilent	10		1	
Total		10		1	0
Capillary electrophoresis					
CE	Beckman	1		0	
Total		1		0	0
Atomic Absorption					
Analyst AA400	Perkin elmer	1		0	
Total		1		0	0

Analytical Chemistry Instrumentation

Instrumentation	Brand	Columbia	Site 1	Columbia Site 2	Easton
Compound Characteristics					
Ultra Violet/Visible Spectrophotometer					
UV/Vis	Agilent	1		0	
DU800 UV/Vis	Beckmann	1		0	
UV/Vis Model V-550	Jasco				1
Fourier Transform Infrared Spectrometer - Model 4200	Jasco				1
Total		2		0	0
FT Infra red detector					
FTIR	Thermo	1		0	
FTIR	perkin elmer	0		1	
Total		1		1	0
Optical Rotation					
Autopol III	Rudolph Research Analytical	2		0	
Total		2		0	0
TGA/DSC					
STAR System	Mettler Toledo	1		0	0
Total		1		0	0
Plate Reader					
plate reader and washer	Bio Tek	3		0	
Colorimeter	Hunter Labs	1		0	
Total		4		0	0
Imager					
Image Quant 350	GE	1		0	
IEF image analyzer	ICE 280	1		0	
Total		2		0	0

Analytical Chemistry Instrumentation

Instrumentation	Brand	Columbia Site 1	Columbia Site 2	Easton
Mass Spectroscopy				
LC/MS				
Single Quad Mass spec with HPLC with DAD	Agilent	1	0	0
ION Trap with diode array detector	Thermo	1	0	0
Time of flight with UPLC with PDA	Waters	1	0	0
Q TOF with UPLC	Waters	1	0	0
Q exactive MS	Thermo	1	0	0
sciex 3000 LC/MS/MS	sciex	0	0	2
sciex 5000 LC/MS/MS	sciex	0	3	2
sciex 4000 LC/MS/MS	sciex	1	0	2
sciex 6500 LC/MS/MS Qtrap	sciex	1	3	0
scies 5500 LC/MS/MS Qtrap	sciex	0	3	1
Impact UHR-TOF coupled with a Thermo (Dionex) UltiMate 3000	Bruker maXis	0	0	1
Total		7	9	7
GC/MS				
6890 GC with mass spec detector with EI/CI capability	Agilent	6	3	1
7890 GC with 5977C <i>inert</i> XL MSD with EI/CI capability	Agilent	0	0	3
7890 GC with 5975 <i>inert</i> XL MSD with EI/CI capability	Agilent	0	0	2
Total		6	3	1
Additional Autosampler				
Purge and trap	Tekmar	2	0	0
Purge and trap	??			1
Total		2	0	0
Inductive coupled Argon Plasma Mass Spec				
Model 7500 ICP/MS	Agilent	1	1	
Model 7700 ICP/MS	Agilent			1
iCAP QC	Thermo	1	0	
Optima 3000 DV - ICP/OES	Perkin Elmer			1
Total		2	1	2

Instrumentation for PCR Assays

Instrumentation	Brand	Easton
Airclean 600 PCR Workstation	Airclean Systems	1
Roto-Gene Q Thermocycler	Qiagen	2
QuantStudio 6 Flex Thermocycler	Thermo Fischer Scientific	1
Nanodrop 2000 Spectrophotometer	Thermo Fischer Scientific	1

ATTACHMENT B



LABORATORY GLP COMPLIANCE INSPECTIONS

The US EPA regularly inspects laboratories for compliance with Good Laboratory Practices (GLP) Regulations issued under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (40 CFR Part 160). The most recent list posted by the EPA may be found at:

<https://www.epa.gov/compliance/epa-good-laboratory-practices-inspections-0>

The laboratory that participated in the conduct of this inspection, with the latest inspection dates, is listed below:

Laboratory	Location (City, State)	Inspection Date	Inspection Status
Eurofins EAG Agrosience, LLC ¹	Easton, MD	June 09, 2008	Closed
Eurofins EAG Agrosience, LLC ¹	Easton, MD	Feb. 11, 2014	Closed
Eurofins EAG Agrosience, LLC ²	Easton, MD	June 05, 2018	Closed ³

Data produced by these laboratories are acceptable, *via* the OECD Mutual Acceptance of Data (MAD) agreement, in the countries listed below.

OECD Members -

Australia
Austria
Belgium
Canada
Chile
Czech Republic
Denmark
Estonia
Finland
France
Germany
Greece


Hungary
Iceland
Ireland
Israel
Italy
Japan
Korea
Latvia
Lithuania
Luxembourg
Mexico
Netherlands

New Zealand
Norway
Poland
Portugal
Slovak Republic
Slovenia
Spain
Sweden
Switzerland
Turkey
United Kingdom
United States

Non-Member Adherents -

Argentina[†]
Brazil
India
Malaysia
Singapore
South Africa
Thailand

Regulatory authorities in these countries may obtain further information by contacting the Good Laboratory Practices (GLP) Program in the US EPA Office of Compliance.


Eric Bodle, Ph.D.
General Manager, Ecotoxicology & Analytical
Chemistry
Eurofins EAG Agrosience, LLC

13 Feb 2019
Date

¹ Previously Wildlife International, Ltd.

² Previously EAG, Inc.

³ Includes inspection for compliance with GLP Regulations issued by the US EPA under the Toxic Substance Control Act (TSCA) (40 CFR Part 792).

⁴ Full adherence for Argentina only applies to industrial chemicals, pesticides and biocides.