UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

AMENDMENT NO. 1

to

MATERIALS COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT

BETWEEN

CORTEVA AGRISCIENCE LLC

AND

THE CENTER FOR COMPUTATIONAL TOXICOLOGY AND EXPOSURE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

This "Amendment No. 1" is entered into by and between Corteva Agriscience LLC, a Indiana Corporation, which has its principal place of business at 9330 Zionsville Road, Indianapolis, IN 46268 ("the Cooperator"), and the Center for Computation Toxicology and Exposure ("the Center") on behalf of the U.S. Environmental Protection Agency ("EPA" or the "Agency") under the authority of Title 15, United States Code § 3710a, et seq. (commonly known as the Federal Technology Transfer Act of 1986).

WITNESSETH:

A. WHEREAS, the Cooperator and the Center executed a Cooperative Research and Development Agreement No. 1374-21 effective December 9, 2021 ("Agreement");

B. WHEREAS, the Cooperator and the Center want to amend and supplement the Agreement;

C. WHEREAS, the Cooperator and the Center want to amend the Agreement to update the Research Plan to include the RTgill (rainbow trout derived) cell line into the image analysis pipeline development;

D. WHEREAS, the Cooperator and the Center want to extend the duration of the Agreement an additional 12 months from the original termination date of December 9, 2023, to a new termination date of December 9, 2024;

E. WHEREAS, the Center views its continued cooperation with the Cooperator to be in furtherance of the public interest.

NOW, THEREFORE, the parties amend the Agreement as follows:

1. The description of the Research Material provided by Cooperator in Article 1, Determination of Provider and Recipient, is amended to read as follows: "Fixed cell culture plates (i.e., 384-well cell culture plates) with treated and stained HepaRG, U-2 OS, and RTgill-W1 cells for imaging and image analysis."

2. Article 17 is amended to read "This Materials CRADA shall be effective upon execution by the Parties when the last signatory has signed the document. The term of this Materials CRADA is 36 months from execution."

3. Appendix A, Research Plan, is removed entirely and replaced with Appendix 1, Research Plan, attached to this Amendment.

4. All other provisions of the Agreement shall remain in force and effect.

IN WITNESS WHEREOF, the Parties have caused this Amendment No. 1 to be executed by their duly authorized representatives as follows:

FOR THE EPA

[redacted]

FOR CORTEVA AGRISCIENCE LLC

[redacted]

Signed Agreements sent to:
Kathleen Graham
FTTA Program Coordinator
graham.kathleen@epa.gov
(303) 312-6137
FTTA@epa.gov

Appendix 1

EPA-Corteva MCRADA #1374-21

Research Plan

Cell Painting, a morphological profiling assay that multiplexes six fluorescent dyes, is a cutting-edge tool that is currently being leveraged for high-throughput toxicology screening. To date, cell painting has been applied to a number of different cell lines, such as U-2 OS, to examine the effects of chemicals on cellular morphology. A current gap in cell painting is that the cell lines that are being leveraged have minimal to no chemical metabolizing capacity, which therefore limits testing to individual parent compounds and neglects relevant metabolism processes. The HepaRG cell line is an immortalized human hepatic cell line that exhibits several key characteristics of primary hepatocytes, including expression of nuclear receptors and high P450 activity. Therefore, adapting the cell painting technique to HepaRGs will enable high-throughput and high-content toxicology screening capabilities to capture bioactivation of toxic metabolites and detoxification processes that are currently being missed.

An additional gap in cell painting is that the cell lines that are commonly being leveraged are human-derived. The ability to use data from human cell lines to inform hazard to ecological species is unknown. The RTgill-W1 cell line is a gill cell line derived from rainbow trout (*Oncorhynchus mykiss*) and is currently used in an OECD test guideline (OECD TG 249) for acute in vitro toxicity testing. Preliminary results at EPA indicate that the cell painting approach can be adapted for use in the RTgill-W1 cell line. The resulting data have to the potential to inform ecological hazards.

The objective of this collaboration is to amend cell painting techniques to the HepaRG and RTgill-W1 cell lines and develop image analysis pipelines for each. This will include characterization of the HepaRG and RTgill-W1 cell culture conditions, staining protocols, and imaging techniques. The Provider shall conduct in vitro experiments to optimize cell painting staining techniques, and characterization of the HepaRG and RTgill-W1 cell culture conditions (e.g., bile acid staining, urea, gene expression, metabolizing capacity in HepaRG and seeding density, mode of action morphological characterization, endpoint concordance with in vivo fish toxicity, and gene expression in RTgill-W1). Provider shall conduct in vitro toxicological experiments with the optimized cell painting protocol on known positive and negative control compounds from peer reviewed literature (e.g., Berberine). The provider shall also conduct in vitro cell painting experiments using the U-2 OS cell line as a comparator to both the HepaRG and RTgill-W1 models. The Recipient shall conduct imaging on the Opera Phenix and develop a data analysis pipelines in Harmony software. Provider shall ship fixed plates with treated and stained HepaRG, RTgill-W1 and U-2 OS cells to Recipient for imaging. Recipient shall share data from the image analysis with Provider. HepaRG and RTgill-W1 cell culture condition characterization shall be completed during the Term. HepaRG and RTgill-W1 cell painting optimization experiments and imaging on the Recipient Opera Phenix shall be conducted during the Term. Expected outcomes include optimized cell painting protocol developed for the HepaRG and RTgill-W1 cell lines, data analysis pipelines on Harmony software, and a peer-reviewed publication(s) describing the results of the Research Project. This is an in-kind collaboration and therefore no budget is necessary.