EPA & UNILEVER Amendment 838-A-18
UL PRN: MA-2017-01854N

AMENDMENT NO 1.
COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT
BETWEEN
UNILEVER U.K. CENTRAL RESOURCES LIMITED
AND
THE NATIONAL CENTER FOR COMPUTATIONAL TOXICOLOGY
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

This "Amendment No. 1" is entered into by and between Unilever U.K. Central Resources Limited a company incorporated in England and Wales (registered under number 00029140) and whose registered office is at Unilever House, 100 Victoria Embankment, London EC4Y 0DY, UK ("the Cooperator"), and the National Center for Computational Toxicology ("the Center"), of the U.S. Environmental Protection Agency ("EPA") under the authority of Title 15, United States Code §§3710a-3710d (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, effective July 15, 2015 ("Agreement");

B. **WHEREAS**, the Cooperator and the Center want to amend and supplement the Agreement to add additional research and funding to the project, which is outlined in the Statement of Work provided in Attachment A;

C. **WHEREAS**, the Cooperator and the Center want to amend the agreement to extend the duration an additional two (2) years from its original expiration date of July 15, 2018 to July 15, 2020;

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

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

1. Paragraph 2.1, Statement of Work is supplemented by adding: “The SOW shall be expanded to include the additional research project as described in the Supplemental Statement of Work (see Attachment A).
2. Paragraph 2.3, Assigned Personnel, is amended to add Principal Investigator Joshua Harrill for the Center. Amend to remove Richard Stark and add Sophie Malcomber and Andrew White for the Cooperator.

3. Paragraph 4.1, Transfer of Funds, is amended by adding the following: "The Cooperator agrees to pay the amount set forth in the Supplemental Statement of Work (see Attachment A).
4. Paragraph 12.2, Duration, is amended by changing the language to read, “This project shall now remain in effect until July 15, 2020, which is five years from the date of last signature of the original Agreement (July 15, 2015).”

5. 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:

U.S. ENVIRONMENTAL PROTECTION AGENCY

ATTATCHMENT A: Supplemental SOW

Added Statement of Work ("SOW")
Cooperative Research Development Agreement ("CRADA)
between U.S. Environmental Protection Agency ("EPA")
and UNILEVER U.K. CENTRAL RESOURCES LIMITED

Project Proposal:
UNILEVER U.K. CENTRAL RESOURCES LIMITED - U.S. EPA Development of Characterization of Biological Diversity in Cell-Based *In Vitro* Test Systems and High-Throughput Transcriptomics (HTTr) Screening of a Reference Chemical Set

I. Goal

The USEPA National Center for Computational Toxicology (NCCT) and Unilever, respectively, are interested in evaluating the use of the high-throughput targeted RNA-Seq as a tool for chemical bioactivity screening in cultured cells and the application of such data for next generation risk assessment. Pilot experiments have demonstrated that HTTr technology, specifically the TempO-Seq human whole transcriptome assay, can be used to generate whole transcriptome profiles from lysates of MCF7 cells cultured in 384-well format and identify concentration-responsive genes as well as *in vitro* points of departure (i.e. no observable transcriptional effect levels, NOTELs) at the gene and pathway level.

The original SOW in the CRADA specified the analysis of the 5 consensus chemicals across 5 different cell types/lines in the HTTr platform. The Cooperator (i.e., Unilever) was going to perform bioinformatics analysis. Progress is being made on completing this deliverable. To help put this data into context, a large number of reference chemicals are being run as part of the initial screening activity within NCCT. The initial screening activity in NCCT is being performed in a single cell type, MCF7 cells. The data from the reference chemicals being run by NCCT will be combined with data from various public sources (e.g., Broad Connectivity Map database, DrugMatrix). The combined reference profiles will be used to help identify potential modes-of-action for the 5 consensus chemicals and unknown chemicals to be run in the future.

II. Description of statement of work's steps

This Statement of Work was developed to supplement the initial project and aims to augment the database of reference profiles with an additional cell type. Increasing the number of cell types screened across the reference chemicals provides additional functional pathways/biological targets. In other words, not all pathways/biological targets are expressed in a single cell type and in order to develop robust signatures for the entire complement of reference chemicals, additional cell types are needed.

This Statement of Work includes the following objectives:

1) Characterize basal gene expression in a set of cell culture models using TempO-Seq whole transcriptome profiling,

2) Model biological diversity within this cell line panel using computational tools.
3) Select a cell line which maximizes the difference in biological space as compared to MCF7 cells. 4) Screen a set of reference chemicals with well characterized molecular/pathway targets
5) Use the combined set of reference profiles to identify potential molecular/pathway targets for the 5 consensus chemicals

The work will be divided into two phases:

Phase 1:
NCCT will procure a set of human cancer cell lines (n ~ 10) with diverse basal gene expression profiles (as determined from an Affymetrix database, the CCLE) as well as a set of hTERT-immortalized human primary cells (n ~ 14) of various tissue origins. Cells will be expanded a limited number of passages (n = 3) according to the suppliers' recommended protocol. Cell lysates will be created from cells grown in microtiter plate format and whole transcriptome profiles will be generated using the TempO-Seq human whole transcriptome assay. The resultant baseline gene expression data will be used to map biological diversity in comparison with the reference cell line (i.e. MCF7 cells). The cell line with the maximal distance in biological space as compared to MCF7 cells will be selected for reference chemical screening as detailed in Phase 2.

Anticipated time line: 6 – 9 months

Phase 2:
NCCT will expand and create a large cryostock of the cell line selected from Phase 1. The cell line will then be screened with a set of approximately 350 reference chemicals which were previously tested in the MCF7 cell model. The chemicals will be screened in 8-point concentration-response in triplicate cultures. Cell lysates from this screening campaign will be analyzed using the BioSpyder human whole transcriptome assay. The resultant data will be used to compare transcriptional responses across the two cell lines in terms of the identity and point-of-departure of gene and pathway level effects.

Anticipated time line: 6 – 9 months

III. Governance

Close collaboration between EPA and UNILEVER U.K. CENTRAL RESOURCES LIMITED will be maintained through regular interactions by scientific staff and management. Tele- or Video-conferences will be held as necessary.

IV. Benefits of cooperative effort:

As set forth in the original Agreement, and in accordance with the terms hereof, the Cooperator will fund continue to enhance the EPA ToxCast^{TM} assays for Phase III, by strengthening this dataset and enhancing the ability to predict toxicity for use by EPA Program Offices in

environmental chemical prioritization. ToxCast™ is providing an innovative solution to a persistent and pervasive issue facing EPA regulatory programs: there are too many environmental chemicals for current testing guidelines to even start characterizing hazard. Second, due to the progress made in completing the original Agreement the Cooperator will help put this data into context, a large number of reference chemicals are being run as part of the initial screening activity within NCCT. This additional research was developed to supplement the initial project and aims to augment the database of reference profiles with an additional cell type. Increasing the number of cell types screened across the reference chemicals provides additional functional pathways/biological targets.

1. Each of these activities are closely aligned with current or planned activities in the Chemical Safety for Sustainability (CSS) National Program. The development and application of ToxCast is contained within the current Strategic Research Action Plan (StRAP) and the fiscal year 2016 – 2019 (FY16-19) StRAP. The development of technology to incorporate metabolic competence into high-throughput *in vitro* assays is a priority research area in the FY16-19 StRAP and there are plans to screen a subset of the ToxCast chemical library in the FY16-19 StRAP.

2. For the Cooperator: EPA's ToxCast™ program will generate toxicity predictions for chemicals of interest to the Cooperator's goals. Furthermore, will gain access to the complete ToxCast™ datasets and EPA experience in using these for predictive toxicology. ToxCast™ will help the Cooperator meet the challenging deadlines for moving to non-animal alternative toxicity testing for cosmetics under European legislation and will enable the following Transfer of Knowledge/Capabilities:

3. Integrated program and collaboration with the EPA that for the Cooperator cross-straps projects using common case study chemicals

4. In depth assessment of >700 HT in vitro assays; tapping into the large EPA experience in this space, bringing opportunities to build capability in people in line with the Cooperator's Lab Evolution strategy

5. Enhanced access to the ToxCast™ chemical database for use in progressing the Cooperator's informatics strategy – big and diverse datasets for risk assessment purposes; strengthening and applying bioinformatics. Opportunity for informaticians to interact closer with EPA experts to refine and develop new approaches

6. Visits to the Cooperator for knowledge transfer to wider teams by EPA key opinion formers. Opportunities available for the Cooperator's people to visit the EPA.

7. The opportunity to build a truly collaborative relationship to be built with a highly influential world leading organization (EPA) on multiple levels (theoretical, experimental, thought leadership) to positively impact the development of a large number of the Cooperator's scientists.

V. Estimated Value of Change:

The Cooperator's new project contribution (cash): $599,694:

Phase 1:	
Cell Procurement	$17,500
Tissue Culture Supplies and Consumables	$8,000
Sequencing	$20,060
Phase 2:	
Tissue Culture Supplies and Consumables	$8,000
Sequencing	$546,134
GRAND TOTAL:	$599,694

Annex B

Financial

Part 1: Level of Funding

The Coordinator's total funding commitment under this Agreement shall not exceed the following amount:

The Coordinator's Total Funding Commitment pursuant to this Agreement	$599,694

Said amount shall be allocated to the following cost headings:

Item	**Funding**
Phase 1	$45,560
Phase 2	$554,134
Total (excluding VAT)	$599,694

The foregoing shall include all travelling and other expenses as may be incurred by the Center and/or its staff connected with the SOWs.