AMENDMENT NO 2.
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. 2" 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 15th, 2015 ("Agreement");

B. **WHEREAS**, the Cooperator and the Center executed an Amendment 838-A-18, effective November 28th, 2017 ("Amendment No. 1");

C. **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;

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.

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 Evaluating the Use of High Content Data Streams for Measuring Bioactivity as a Conservative Point-of-Departure for Use in Screening Level Risk Assessments.

I. Goal

The USEPA National Center for Computational Toxicology (NCCT) has begun exploring the use of high content, non-targeted in vitro assays as an approach to efficiently and cost effectively evaluate the potential effects of a chemical across a broad range of biological space. These include a high-throughput transcriptomics (HTTr) assay (i.e. TempO-Seq human whole transcriptome assay, Yeakley et al. 2017) as well as a high-throughput phenotypic profiling (HTPP) assay (i.e. Cell Painting, Bray et al. 2016). Both assays are amenable for use in many different cell types and culture models and provide information on the integrated biological response of cells following treatment with a chemical stressor. The HTTr assay was developed as part of the original CRADA and used to evaluate the 5 consensus chemicals across 5 different cell types/lines. The SOW in the first amendment then ran a large number of reference chemicals in multiple cell types. The data from the reference chemicals are being combined with data from various public sources (e.g., Broad Connectivity Map database, DrugMatrix). The combined reference profiles are being used to help identify potential modes-of-action for the 5 consensus chemicals and other chemicals being run as part of the ToxCast library.

The USEPA National Center for Computational Toxicology (NCCT) and Unilever, respectively, are interested in evaluating the use of these high-content data streams to generate in vitro bioactivity estimates and evaluate the use of the data in non-animal, screening level risk assessments. NCCT has previously developed a list of >300 case study chemicals that: 1) have been run through the ToxCast assay battery; 2) have high-throughput toxicokinetic (HTTK) data for reverse dosimetry analysis using the NCCT httk R package; and 3) have legacy NOAEL/LOAEL values from traditional in vivo repeat dose toxicity studies.

II. Description of statement of work's steps

This scope of work (SOW) leverages the results of previous studies performed as part of the NCCT / Unilever Cooperative Research and Development Agreement (CRADA). The SOW includes the following objectives:

1. Four cell lines will be selected for use in this study using the data-driven biological diversity modeling data generated under the previous CRADA amendment (838-A-18).
2. The entire set of chemicals (n=~380) included as part of the EPA case study and the 5 Unilever reference chemicals will be screened in each cell type (8 point conc.-resp., 1 time point) using the HTPP assay.
3. In vitro-to-in vivo extrapolation (IVIVE) analysis will be performed on the HTPP assay data and the results converted into an oral equivalent dose (OED).
4. A subset of bioactivity case study chemicals (n=154) and the 5 Unilever reference chemicals will be selected jointly by NCCT and Unilever. The chemicals will be screened in two of the four cell types (8 point conc.-resp., 1 time point) using the HTTr assay.
5. IVIVE analysis will be performed on the HTTr data and the results converted into OEDs.
6. OEDs derived from the HTPP, HTTr and ToxCast assays (from the original CRADA) will be compared with the *in vivo* NOAEL/LOAEL values.

NCCT will perform the laboratory screening associated with the HTPP and HTTr assays. NCCT and Unilever scientists will collaborate with regards to data analysis including derivation of *in vitro* points-of-departure, OED calculations, and data comparisons.

Anticipated timeline: 12-24 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 to continue enhancing the EPA ToxCast™ assays, by strengthening this dataset and demonstrating the application of the assays to predict toxicity of environmental chemicals for use by EPA Program Offices. 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 and first amendment, the project was extended to evaluate whether the high-content assay developed as part of the original CRADA could be used to quantitatively predict NOAEL/LOAEL values from traditional *in vivo* repeat-dose toxicity studies.

This additional research was developed to supplement the initial project and aims to extend the application of the high-content assays (HTTr and HTPP) for quantitative chemical risk assessment.

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 cost-effectively and comprehensively evaluate the biological processes disrupted by chemicals and the dose at which this occurs is a priority research area in the FY16-19 StRAP. The development and application of high-throughput transcriptomic technology is part of the FY16-19 CSS Project Plans.

2. For the Cooperator: The use of the high-content technologies (HTTr and HTPP) will generate quantitative estimates of NOAEL/LOAEL values from traditional toxicity studies without the use of animals. Furthermore, the Cooperator 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 reference chemicals

4. Enhanced access to the ToxCast™ chemical database and bioinformatic tools 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

5. 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.

6. 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): $526,987:

General Lab Supplies	$25,000
HTTP Screening	$25,000
HTTr Screening	$396,987
Post-doctoral trainee (1 year)	$80,000
GRAND TOTAL:	$526,987

Date: 10/07/2018

Annex B

Financial

Part 1: Level of Funding

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

The Cooperator's Total Funding Commitment pursuant to this Agreement	$526,987

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

Part 2: Payment Details

Subject to the conditions set forth in the Agreement, the Cooperator shall make payment pursuant to clause 4 as and to the extent set forth hereafter provided the relevant deliverables as set forth hereafter shall have been fully attained:

Expected date	**Phase to be funded**	**Funding**
On commencement	Supplemental SoW (Attachment A)	$526,987
Total		$526,987

Invoicing Details

Payment pursuant to this Agreement shall be made in response to invoices sent by Centre to the Cooperator via the Unilever e-invoicing tool http://www.tungsten-network.com/unilever/

All invoices shall quote the (i) reference number MA-2018-01023N and the (ii) purchase order number DO12697518 and addressed to:

Unilever R&D Colworth
Accounts Payable
Mail-Point 13598
Omega House
Emerald Way
Stone Business Park
Stone
Staffordshire
ST15 0SR