AMENDMENT NO. 2

COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT

BETWEEN

PROCTER AND GAMBLE CORPORATION

AND

THE CENTER FOR COMPUTATIONAL TOXICOLOGY AND EXPOSURE
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

This "Amendment No. 1" is entered into by and between Procter and Gamble Corporation (the "Cooperator"), which has its principal place of business at 1 P&G Plaza, Cincinnati, Ohio 45202, and the Center for Computational Toxicology and Exposure (the "Center") of the U.S. Environmental Protection Agency ("EPA") 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, effective February 4, 2019 ("Agreement");

B. **WHEREAS**, the Cooperator and the Center executed an amendment to the Agreement (#1055-A-21) on March 23, 2021, to extend the term of the Agreement an additional two years from the original termination date and to amend the original Statement of Work ("SOW");

C. **WHEREAS**, the Cooperator and the Center want to amend the Agreement to extend the duration an additional one year from the amended expiration date, until February 4, 2025;

D. **WHEREAS**, the Cooperator and the Center want to replace the amended SOW with the amended SOW attached hereto as Attachment A and to the Agreement as Attachment C; and

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:

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1. Paragraph 2.1, Statement of Work is amended to read:

> Cooperative Research and development work performed under this Agreement shall be performed in accordance with the amended Statement of Work ("SOW") attached hereto as Attachment C. The SOW sets forth a "period of performance." The Center and the Cooperator agree to perform the cooperative research and development work and to utilize such personnel, resource, facilities, equipment, skills, know-how, and information as is reasonably necessary.

2. Paragraph 12.2, Duration, is amended to read:

> This Agreement shall remain in effect for a period of six years from the effective date.

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

IN WITNESS WHEREOF, the Parties have caused this Amendment No. 2 to be executed by their duly authorized representatives as follows:

U.S. ENVIRONMENTAL PROTECTION AGENCY

By: [redacted]

THE COOPERATOR

By: [redacted]

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Signed Agreements sent to:

Kathleen Graham
FTTA Program Coordinator
Graham.kathleen@epa.gov
(303) 312-6137
FTTA@epa.gov

Statement of Work (SOW) Annex A

Amendment 2

Cooperative Research and Development Agreement (CRADA)

between U.S Environmental Protection Agency (EPA)

and the Procter and Gamble Corporation (P&G)

I. Goal

EPA's Center for Computational Toxicology and Exposure ("EPA" or "CCTE") and the Procter and Gamble Corporation ("P&G" or "Cooperator") are interested in the further development and application of the Alginate Immobilization of Metabolic Enzymes technology for integration of metabolic competence into high-throughput *in vitro* assays, and its translation of the results into risk assessment for use by private and public entities. The Cooperator desires to evaluate, optimize, and implement the technology developed by CCTE using chemicals (including botanical substances) of scientific interest to the Cooperator.

II. Research Plan

The research performed under this SOW will be performed over the course of six years:

Phase 1 – The S9 cell fraction has been used historically to recapitulate metabolism from liver for *in vitro* assays such as the Ames mutagenicity assay. However, S9 cannot readily be used in many cell-based assays due the cytotoxic lipid peroxides formed by CYP metabolism of microsomal lipids, or in biochemical assays where S9 may interfere with protein binding dynamics. To circumvent the technical limitations associated with direct S9 addition to biochemical and cell-based assays, CCTE has developed a method called Alginate Immobilization of Metabolic Enzymes ("AIME"). The method involves the encapsulation of rat S9 fractions in an alginate solid matrix that allows for passive diffusion of low molecular weight chemicals but retains molecules larger than the polymer network pores that may contribute to assay interference. The encapsulated S9 fraction can be incubated with chemically treated cells or proteins in multi-well format or used to pre-treat chemical-stock solutions that would be subsequently added to cells or proteins in multi-well plates. The method has been characterized for functional activity across a panel of CYP-dependent substrates and deployed in an estrogen receptor (ER) transactivation assay (VM7Luc4E2) using a reference compound with verified estrogenic metabolites.

To expand the adoption of the AIME method, EPA will provide the Cooperator with the established standard operating protocols ("SOPs") and customized microtiter plate lids necessary to perform the method. The Cooperator will conduct an initial method transfer

study using the provided SOPs and materials to evaluate the performance of the assay. Performance will be benchmarked by analytical liquid chromatography-mass spectrometry (LC-MS) using a defined set of reference chemical substrates to 4-6 CYPs previously used for AIME method development by EPA.

Assuming successful transfer of the AIME method, the Cooperator will initiate appropriate modifications to the method to align with previous SOPs using S9 fractions in suspension. Modifications may include substitution of reaction buffer, timing of metabolic reactions, lyophilization of reaction components, and reconstitution of reaction components in assay buffer or medium suitable for downstream applications. In exchange, the Cooperator will provide EPA with all optimized SOPs to conduct the method using the alternative approach, as well as any generated LC-MS results used in method validation and modification.

Phase 2 – If the method transfer study is successful, EPA and the Cooperator will proceed with a proof of concept study based on two reference substances for which interference was seen previously using S9 fraction in suspension (inclusive of appropriate assay positive and negative controls). The test samples pre- and post-incubation will first be analyzed using a LC-ESI combined with charged-aerosol detection (CAD), full-scan MS in positive and negative ionization mode and diode array (UV) detection. This approach is an effective way to observe the variety of compounds in the extracts and can be used to qualitatively compare the complexity or "cleanliness" provided by up to three metabolism strategies being pursued. As a follow-on, the generated reaction components with the chosen metabolism strategy, containing primary and/or secondary metabolite products, will be run in a suite of targeted assays with emphasis on assays where interference or differences due to metabolism has been observed previously. The assays that would most likely benefit from biotransformation will be focused on molecular targets for developmental and reproductive toxicity where interference has been observed previously as well those where differences due to biotransformation have previously been detected. Assays will be conducted in screening mode using single concentration formats, at a multiple of the predicted/measured serum exposure or in the case of reference substrates as appropriate for the specific assay platform. The Cooperator will provide EPA with all assay data and LC-MS results generated from the proof of concept study.

Phase 3 – If the AIME approach is successful, the Cooperator and EPA may deploy the assay in a larger set of assays and/or using an expanded set of chemicals. Assays will be conducted by the Cooperator in either single-concentration screening format or multiple concentration-response format as appropriate for the specific assay platform. For single-concentration format, all positive compound-assay combinations will be followed up in multiple concentration-response format. If the experimental development is not successful, EPA and the Cooperator will decide on the best path forward.

III. Milestones

Phase 1

1) EPA provides training, SOPs, and microtiter plate lids necessary to conduct the AIME assay.
2) Cooperator performs method transfer study of the AIME method with EPA SOPs.
3) Cooperator provides resultant LC-MS results of method transfer study to EPA to evaluate assay performance.

Phase 2

1) EPA and the Cooperator select at least two reference substrates for the AIME assay.
2) EPA provides plated reference chemicals and provides blinded samples to designee.
3) EPA and the Cooperator select appropriate assay suite to perform the alternative AIME method.
4) The Cooperator performs analytical characterization of the reference substrates in metabolically active and inactive modes (starting with 3 different metabolism strategies: i) protein precipitation with organic solvent; ii) solid-phase extraction; and iii) Liver S9 encapsulation).
5) The Cooperator selects appropriate metabolism strategy based on a qualitative analytical comparison of the "cleanliness" provided by each strategy.
6) If the AIME method is deemed a suitable strategy, the Cooperator performs screening in a targeted set of assays using AIME in both metabolically active and inactive modes.
7) Cooperator provides to EPA SOPs for the modified AIME method and all LC-MS results and assay data.
8) Cooperator publishes results with support from EPA.

Phase 3

1) EPA and the Cooperator evaluate expansion of reference chemical set and/or targeted assay suite.
2) Cooperator performs screening in a targeted set of assays using AIME in both metabolically active and inactive modes.
3) Cooperator provides to EPA all assay data on the expanded test substance set and/or assay suite using the modified method.

Throughout the program of work, regular discussions (ideally every 3 months by video- or tele-conference) will be held between scientists from both parties to review progress and build productive connections to facilitate scientific discussion and knowledge sharing. The Cooperator and EPA may undertake visits to each other. All data and SOPs from the studies described above will be made wholly available by the Cooperator to EPA upon completion of the work and before publication.

IV. Estimated Value and Benefits

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A. Value of Contributions
1. Estimated value of EPA contributions (in-kind): Personnel in-kind over the period of the agreement is approximately $79, 201. Plated reference chemicals and supplies for project $109,201 over the period of this CRADA.

2. Value of the Cooperator's estimated contribution (in-kind):
SOPs, LC-MS data, non-proprietary assay data is approximately $50,000

B. Benefits of Cooperative Effort
1. For EPA: To the extent set forth in this Agreement and in accordance with the terms hereof, the Cooperator will evaluate, optimize, and deploy the AIME technology to incorporate metabolic activity into high-throughput *in vitro* screening assays. The lack of metabolic activity has been a significant limitation for using the *in vitro* assays to predict toxicity of bioactivated toxicants. The Cooperator will provide EPA with modified methods for running the AIME assay, and all non-proprietary LC-MS and assay data generated from the work. This will allow EPA to perform metabolism of higher volumes of chemicals for screening in the future.

2. For the Cooperator: The work will address the lack of metabolic capacity in existing high throughput screening tools and the transfer of AIME technology to the Cooperator's metabolism experimental capability to address current technical limitations. In addition, the Cooperator's representative will visit EPA, or attend remote learning sessions, to receive training on the method and to have the opportunity to speak with EPA scientists developing the method to further enhance optimization and application efforts. There will be opportunity to build a truly collaborative relationship with a world leading organization (EPA) on multiple levels (theoretical, experimental, thought leadership) to positively impact the development of the Cooperator's scientists and contracting partners.

Catherine Mahony (Strategic Lead), Cindy Obringer (Project Leader) and Julia Przibilla, (Principal Investigator) will be the primary representatives for the Cooperator in order to manage/effect the collaboration with EPA.1

V. General Provisions for the Conduct of the SOW

CCTE shall:

(a) use best efforts to complete the SOW (including providing all agreed-upon deliverables) under and in accordance with the terms of the CRADA;

(b) commence the SOW on the dates as indicated in this CRADA or, if no date is prescribed, not later than two (2) months after the effective date of the CRADA, unless the Cooperator shall have agreed in writing to a request from CCTE for an extension of said period to permit a later commencement date. Such request shall not be unreasonably

1 For clarity, EPA will not be directing the Cooperator's contractors. No actions taken by EPA will be construed as creating privity between EPA and the Cooperator's contractors.

refused. For clarity, any extensions under this clause shall be agreed upon in writing by the Cooperator and signed by its officer having authority therefore;

(c) keep the Cooperator fully informed of all Subject Data, Subject Inventions, Technology, and other information arising from the SOW by means of reports or otherwise as requested by the Project Manager of the Cooperator;

(d) in conducting SOWs, use all reasonable endeavors to not infringe third-party intellectual property rights;

The Cooperator shall:

(a) use best efforts to complete the SOW (including providing all agreed-upon deliverables) under and in accordance with the terms of the CRADA;

(b) commence the SOW on the dates as indicated in this CRADA or, if no date is prescribed, not later than two (2) months after the effective date of the CRADA;

(c) keep EPA fully informed of all Subject Data, Subject Inventions, Technology, and other information arising from the SOW as requested by the CCTE Project Manager;

(d) in conducting SOWs, use all reasonable endeavors to not infringe third-party intellectual property rights;

All parties are responsible for carrying out the SOW and for keeping good administration in the form of complete records of all work carried out as part of any SOW (including all activities undertaken, and the results thereof). The foregoing shall include the making of contemporaneous records in notebooks (which may include electronic notebooks) which shall in all instances be in accordance with good research procedures. Furthermore, all of said records shall be maintained confidentially and securely at CCTE's address above written (or such other address(es) as CCTE shall notify the Cooperator from time to time) for five (5) years after the Term of this Agreement and any extensions expire. At the Cooperator's request and reasonable cost, CCTE shall provide copies of the said records to the Cooperator as soon as practicable.