COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT WITH THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

This Cooperative Research and Development Agreement (CRADA or "Agreement") 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 Congress of the United States, in enacting the Federal Technology Transfer Act of 1986 (the "FTTA"), has found that Federal laboratories' developments should be made accessible to private industry, state and local governments, and has declared that one of the purposes of such Act is to improve the economic, environmental and social well-being of the United States by stimulating the utilization of Federally-funded technology developments by such parties;

B. **WHEREAS**, the FTTA provides each Federal agency with the authority to permit the Directors of Government-operated laboratories to enter into cooperative research and development agreements with Federal or non-Federal entities, domestic or foreign, including private firms and organizations for the purpose of providing to, or obtaining from, collaborating parties, personnel, services, property, facilities, equipment, intellectual property or other resources toward the conduct of specified research and development efforts, which may include the disposition of patent or other intellectual property rights in the inventions resulting from such collaboration;

C. **WHEREAS**, the Center has performed and has sponsored substantial research and development with respect to computational and predictive toxicology;

D. **WHEREAS**, the Center possesses certain advanced scientific skills, facilities, special equipment, information, computer software, and know-how pertaining to computational and predictive toxicology relative to the ToxCast™ research program;

E. **WHEREAS**, the Cooperator possesses certain expertise in chemical risk assessment of consumer products, in vitro assays, metabolism, and exposure.

F. **WHEREAS**, the Center and the Cooperator are interested in the further research and development of the ToxCast™ project, especially in the areas of metabolism, high-

throughput transcriptomics, and translation of the results into risk assessment, and its utilization by private and public entities;

G. **WHEREAS**, the Cooperator desires to provide resources for the Center's development and/or evaluation of the ToxCast™ project; and

H. **WHEREAS**, the Center views its collaboration with the Cooperator to develop and evaluate the ToxCast™ predictive toxicology technology and translation to chemical risk assessment to be in the furtherance of the public interest; and

I **WHEREAS**, the Center and the Cooperator are interested in certain technology of the Center be used by the Cooperator and its affiliates and, to facilitate such use, scientists of the Cooperator may spend time in the Center's laboratories to gain expertise therewith as may be agreed.

NOW, THEREFORE, the parties hereto agree as follows:

Article 1. Definitions

As used in this CRADA, the following terms shall have the following meanings and such meanings should be equally applicable to both the singular and plural forms of the terms defined:

1.1 "Affiliates" means, in the case of the Cooperator the companies of the Unilever group controlled, direct or indirect, by Unilever PLC in London and Unilever NV in Rotterdam.

1.2 **"CRADA" or "Agreement"** means this Cooperative Research and Development Agreement entered into by the Center pursuant to 15 U.S.C. § 3710a.

1.3 **"Computer Software"** means any computer software, computer programs, computer data bases, and documentation thereof developed, in whole or in part, under this Agreement.

1.4 **"Government"** means the Government of the United States of America. "US" means the United States of America. "Federal," as used in this CRADA, shall mean of the United States of America.

1.5 **"Invention"** means any invention or discovery which is or may be patentable or otherwise protectable under the intellectual property laws of the US or any foreign country.

1.6 **"Made"** in relation to any Invention means the conception and/or first actual reduction to practice of such Invention.

1.7 "Proprietary Information" means information which embodies trade secrets developed at private expense, or which is confidential scientific, business or financial information, provided that such information:

(a) Is not known or available from other sources without obligation concerning its confidentiality;

(b) Has not been made available by the owners to others without obligation concerning its confidentiality; and

(c) Is not already available to the Government without obligation concerning its confidentiality.

1.8 "Subject Data" means all recorded information first produced in the performance of this Agreement. This term includes Computer Software.

1.9 "Subject Invention" means any Invention conceived or first actually reduced to practice in the performance of work under this Agreement.

1.10 "Technology" means ToxCast™ , its Computer Software, programs, data bases and documentation.

1.11 "Works" means any Computer Software or subject matter that is copyrightable.

Article 2. Cooperative Research

2.1 Statement of Work. Cooperative research and development work performed under this Agreement shall be performed in accordance with the Statements of Work ("SOW") attached hereto as Attachment A and the terms hereof. Both SOWs set forth a "period of performance." The Center and the Cooperator agree to perform the cooperative research and development work as set forth for each such party in the SOW and to utilize such personnel, resources, facilities, equipment, skills, know-how and information as are reasonably necessary to complete such provided such are agreed in writing by the Cooperator signed by its officer having authority therefor.

2.2 Review of Work. Periodic conferences shall be held between Center and Cooperator personnel for the purpose of reviewing the progress of the work to be accomplished under this Agreement. The Center shall have exclusive responsibility, control and supervision over the conduct of all cooperative research and development work conducted at the Center facilities. The Cooperator shall have exclusive responsibility, control and supervision over the conduct of all cooperative research and development work conducted at Cooperator facilities. It is understood that the nature of this cooperative research and development work is such that completion within the period of performance specified in the SOWs or within the limits of

financial support allocated, cannot necessarily be guaranteed. Accordingly, it is agreed that all cooperative research is to be performed on a best efforts basis.

2.3 Assigned Personnel. Each party to this Agreement shall perform its respective obligations under this Agreement under the direction of a "Project Manager" and a "Principal Investigator." Each party agrees that its Project Managers shall be responsible for overseeing the overall direction of its work, establishing budgets and providing such approvals and consents as are required hereunder save where indicated otherwise. Principal Investigators shall be responsible for the scientific and technical conduct of the work, including the exchange of Subject Data and other information. The parties designate the following individuals as their respective representatives:

	Center	Cooperator
Project Manager	Russell Thomas	Paul Carmichael/Paul Russell
Principal Investigator	Richard Judson	Richard Stark

Said representatives may be replaced by written notice to the other party provided such replacement shall have equivalent expertise.

2.4 Scope Change. If at any time the Project Managers determine that the research data justify a substantial change in the direction of the work, the parties shall make a good faith effort to agree on any necessary changes to the SOWs. Any change shall be effective only if agreed in writing by the Cooperator signed by its officer having authority therefor.

Article 3. Reports

3.1 Reports. The Center shall submit yearly progress reports to update the Cooperator on progress in Tasks I and II. A final report to the Cooperator of the Center's results within 90 calendar days after (a) completing the SOWs or (b) the termination of this Agreement. The Cooperator shall submit a final report to the Center to give full details of the Cooperator's results within 90 calendar days after (a) completing the SOWs or (b) the termination of this Agreement.

Article 4. Financial Obligations

4.1 Payments. The Cooperator agrees to pay the amount as set forth in Annex B Part 1. The Parties acknowledge and agree that all payments to be made pursuant to this clause 4.1 shall, unless otherwise agreed in writing, be paid only as and to the extent as set out in Annex B Part 2.. Payments to the Center shall be made to the USEPA via check to a central box or electronic transfer, details as provided in writing to Cooperator by the Center. Payments made by the Cooperator under this clause 4.1 will be accompanied by a copy of the first page of this Agreement, the signature page of this Agreement, and the Statement of Work in Attachment A.

Cincinnati Finance Center
P.O. Box 979078
St. Louis, MO 63197-9000

The check shall be accompanied by a copy of the first page of this Agreement, the signature page of this Agreement, and the SOW in Attachment A.

Alternatively, wire transfers may be directed to the Federal Reserve Bank of New York:

Federal Reserve Bank of New York
ABA = 021030004
Account = 68010727
SWIFT address = FRNYUS33
33 Liberty Street
New York NY 10045
Field Tag 4200 of the Fedwire message should read, "D 68010727 Environmental Protection Agency"

4.2 Assignment of Personnel. In addition to the funding by the Cooperator provided for in paragraph 4.1 above, the Cooperator shall provide the services of a qualified research associate who will assist in the efforts under the SOW as detailed in Attachment A. The associate shall be an employee of the Cooperator and shall be stationed at Unilever, SEAC, Sharnbrook, United Kingdom. The parties acknowledge their intention that any persons provided by the Cooperator are also to receive training in the use of the Technology.

4.3 Use of Funds. EPA shall ensure that all amounts paid by or on behalf of the Cooperator pursuant to this CRADA shall be used properly, prudently and exclusively for the conduct of the SOWs by the Center under and in accordance with the terms of this CRADA.

4.4 Accounting Records. The Center shall maintain separate and distinct current accounts, records, and other evidence supporting all its expenditures of the Cooperator's cash contributions under and properly in accordance with the terms of this Agreement. The accounts and records shall be available for reasonable inspection and copying by the Cooperator or its authorized representative.

4.5 No Further Payments by the Cooperator. The Cooperator shall have no obligation to make any payment hereunder, save as expressly provided by the terms of this Agreement. For clarity, SOWs shall impose no obligation upon the Cooperator to make any payment hereunder.

Article 5. Invention, Computer Software, and Patent Rights

5.1 The Center and the Cooperator acknowledge that it is not their intention that Subject Inventions or Computer Software will be created during the work specified in this Agreement. Notwithstanding the foregoing, where any activity of this Agreement results in the creation of Subject Inventions or Computer Software, the parties agree that all right, title, and interest in and to all Subject Inventions or Computer Software shall, regardless of inventorship, vest in and be the sole property of inventing party, subject to the terms hereof.

5.2 The Parties agree that:

for any Subject Inventions or Computer Software that are jointly owned pursuant to clause 5.1, the Center and the Cooperator will negotiate in good faith an amendment to this Agreement that shall include assignment of responsibilities for obtaining patents or other intellectual property rights pertaining to the Subject Inventions or Computer Software.

5.3 Subject Inventions. The Center, on behalf of the Government, hereby grants to the Cooperator a first option to an exclusive license of the Government's interest in each Subject Invention and in any resulting patents issued on such Subject Invention. This option may be exercised not later than six (6) months following the filing of a patent application on the Subject Invention in the U.S. Patent and Trademark Office pursuant to paragraph 5.6, above. Any exclusive license will be subject to the reservation by the Government of a non-exclusive, irrevocable, paid-up license to practice or have practiced on its behalf the Subject Invention throughout the world.

5.4 Center and Cooperator Jointly Developed Works. If the Cooperator wishes to retain ownership of its copyright interest in the Works, and wishes to rely on copyright protection, it may do so, subject to the Government license in 5.3 above, and subject to the provisions of Title 17, U.S. Code, Section 105. If the Cooperator asserts copyright to said Works, it hereby grants to the U.S. Government and others acting on its behalf a nonexclusive, irrevocable, paid-up worldwide license in such copyrighted Works to use, reproduce, distribute, prepare derivative works, perform publicly and display publicly the Work.

Article 6. Data and Publication

6.1 Proprietary Information. The Cooperator shall place a proprietary notice on all information it delivers to the Center under this Agreement which it asserts is Proprietary Information of the Cooperator. The Center agrees that: 1) any information designated as Proprietary Information which is furnished by the Cooperator to the Center or its staff by or on behalf of the Cooperator and its Affiliates under this Agreement; 2) any information obtained by either party during the performance of this CRADA that would be claimed as Proprietary Information had it been submitted by the Cooperator; or 3) any information furnished by the Cooperator in contemplation of this Agreement; shall be treated as Proprietary Information and will be used by the Center only for the purpose of carrying out this Agreement. Information designated as Proprietary Information shall not be, in whole or in part, disclosed, copied, reproduced or otherwise made available in any form whatsoever to any other person, firm, corporation, partnership, association or other entity without consent of the Cooperator; except as such information may be subject to disclosure under the Freedom of Information Act (5 U.S.C. § 552), and EPA's regulations at 40 C.F.R. Part 2, or as required to be disclosed by other statutes. The Center agrees to protect the information designated as Proprietary Information from unauthorized disclosure. The Cooperator agrees that the Laboratory is not liable for the disclosure of Proprietary Information which, after notice to and consultation with the Cooperator, EPA determines may not lawfully be withheld or which a court of competent jurisdiction requires to be disclosed.

Prompt written notice of such requirement of disclosure shall be given to the Cooperator so that it may endeavor to obtain appropriate relief to prevent or limit such disclosure. The Center shall disclose information only as and to the extent required by said administrative requirement or court order, and such disclosure shall not of itself be prejudicial to any of the other confidentiality obligations hereunder.

6.2 Release Restrictions. The Parties shall not release Subject Data publicly or provide such Subject Data to any Government regulatory body or agency other than the EPA except:

(a) the Center in reporting the results of cooperative research may publish Subject Data in accordance with the provisions of paragraph 6.3 below; and

(b) the Center may release such Subject Data only insofar as such release is required pursuant to a request under the Freedom of Information Act (5 U.S.C. § 552) and the EPA regulations at 40 C.F.R. Part 2.

(c) The Cooperator agrees to not release to third parties (excluding Affiliates) any Subject Data generated by the Center without obtaining prior written consent from the Center, not to be unreasonably refused or delayed.

(d) Neither the Center nor the Cooperator shall not release to the public any Subject Data or other data that discloses or enables an invention if a patent application is to be filed, until the party having the right to file a patent application or provisional patent application has had a reasonable time to file; and

(e) The Center may release any Subject Data that it can establish by written records that such Subject Data:

(i) Is known or available from other sources without obligation concerning its confidentiality;

(ii) Has been made available by the owners to others without obligation concerning its confidentiality; and

(iii) Is already available to the Government without obligation concerning its confidentiality.

6.3 Publication. The Center and the Cooperator have an objective to publish and disseminate research results. Where the Center or the Cooperator ("Publishing Party") intend to publish Subject Data, the Publishing Party shall ensure that the other of the Center or the Cooperator ("Reviewing Party") shall have been furnished with copies of any proposed publication or presentation of Subject Data or other information arising from the SOWs in a timely manner, being at least forty five (45) days, in advance of their submission to any third party. The Reviewing Party shall have the power to delay such proposed publication or presentation if in its reasonable opinion such delay is necessary to protect its business interests or its commercial use to the Reviewing Party, whether actual or potential, for not more than six (6) months from the later of: (i) notification of a request to publish, or (ii) termination of this Agreement. Whilst the Reviewing Party shall endeavor to keep any delay to a

minimum, before consent is obtained from the Reviewing Party or such time period for delay shall have elapsed, the Publishing Party shall ensure that Results arising from the Project shall be maintained in confidence. For clarity, the parties may agree to an earlier publication provided such shall have received the Reviewing Party's written consent. The Center and the Cooperator agree to confer and consult prior to the publication of Subject Data or other information arising from the SOWs and to ensure that no Proprietary Information is released and that patent rights are not jeopardized.

Article 7. Representations and Warranties

7.1 Representation and Warranties of the Center. The Center hereby represents and warrants to the Cooperator as follows:

7.1.1 Organization. The Center is a Federal center of the EPA and is wholly owned by the Government. The Center's substantial purpose is the performance of research or development.

7.1.2 Mission. The performance of the activities specified by this Agreement is consistent with the mission of the Center.

7.1.3 Authority. All prior reviews and approvals required by Federal regulations and laws have been obtained by the Center prior to the execution of this Agreement. The Center official executing this Agreement has the requisite authority to do so.

7.2 Representations and Warranties of the Cooperator. The Cooperator hereby represents and warrants to the Center as follows:

7.2.1 Corporate Organization. The Cooperator, as of the date hereof, is a corporation duly organized, validly existing and in good standing under the laws of the United Kingdom.

7.2.2 Power and Authority. The Cooperator has the requisite power and authority to enter into this Agreement and to perform according to the terms thereof.

Article 8. Termination

8.1 Termination by Mutual Consent. The Center and the Cooperator may elect to terminate this Agreement, or portions thereof, at any time by mutual consent. In such event the parties shall specify the disposition of all property, patents, unexpended or unobligated funds, and the results arising from the work completed or in progress under this Agreement. Upon termination by mutual consent, the Center, as of the termination date, shall make no new commitments, and as soon after the termination date as feasible, shall cancel all outstanding commitments that relate to those portions of this Agreement that have been mutually terminated.

8.2 Termination by Unilateral Action. Either party may unilaterally terminate this entire Agreement at the end of each step insofar as expressly described in the SOWs in Attachment A by giving the other party written notice not more than thirty (30) calendar days after the end of each step. The Center shall make no new commitments after receipt of a written termination notice from the Cooperator and shall to the extent possible, by the termination date, cancel all outstanding

commitments and contracts that were entered into as a consequence of the requirements of the SOWs in Attachment A. However, the Center may, at its own expense, continue said commitments beyond said termination date without liability on the part of the Cooperator.

8.3 Termination by the Cooperator. The Cooperator may unilaterally terminate this entire Agreement upon sixty (60) days written notice to the Center that the Agreement is to terminate pursuant to this clause 8.3. The Center shall make no new commitments after receipt of such a written termination notice from the Cooperator and shall to the extent possible, by the termination date, cancel all outstanding commitments and contracts that were entered into as a consequence of the requirements of the SOWs in Attachment A. However, the Center may, at its own expense, continue said commitments beyond said termination date without liability on the part of the Cooperator.

8.4 Termination Costs. Each party shall pay its own termination costs out of its own funds. Any funds furnished by the Cooperator which are unexpended or unobligated as of the date of termination will be returned to the Cooperator. In no event shall either party be liable for the direct and indirect termination costs of the other party or said other party's expenses caused by or related to the termination.

8.5 Survival. To the extent rights and obligations hereunder have accrued as of the date of expiration or termination, the following Articles of this Agreement shall survive any expiration or termination hereof: 5, 6, and 10, and any expiration or termination hereof shall not affect any license granted hereunder.

Article 9. Disputes

9.1 Settlement and Governing Law. Any dispute arising under this Agreement which cannot be readily resolved shall be submitted jointly to the signatories of this Agreement. A joint decision of the signatories or their designees shall be the disposition of such dispute. If the signatories are unable to jointly resolve a dispute within a reasonable period of time after submission of the dispute for resolution, the matter shall be submitted to the Administrator of EPA or the Administrator's designee for resolution. This Agreement shall be construed in accordance with and governed by the laws of the United States as interpreted and applied by the Federal courts in the District of Columbia, United States of America.

9.2 Continuation of Work. Pending the resolution of any dispute or claim pursuant to this Article, the parties agree that performance of all obligations shall be pursued diligently in accordance with the direction of the Center signatory.

Article 10. Liability

10.1 EPA. EPA's responsibility for the payment of claims to the Cooperator or its employees for loss of property, personal injury or death caused by the negligence or the wrongful act or omission of employees of EPA, while acting within the scope of their employment, is in accordance with the provisions of the Federal Tort Claims Act, 28 U.S.C. §§ 2671-80 and 40 C.F.R. Part 10.

10.2 No Warranty. Except as specifically stated in Article 7, neither party makes any express or implied warranty as to any matter whatsoever, including the conditions of the research or as to any

Invention made or product developed, or the ownership, merchantability, or fitness for a particular purpose, of the research or any such Invention or product.

10.3 Indemnification. The Cooperator agrees to hold the Government harmless and to defend and indemnify the Government for all liabilities, demands, damages, expenses and losses arising out of the use by the Cooperator, its employees or any party acting on the Cooperator's behalf or with its authorization, of the Center's research and technical developments, the Center's facilities or equipment, or out of any use, sale or other disposition by the Cooperator, its employees or others acting on its behalf or with its authorization, of products made by the use of the Center's technical developments. This provision shall survive the termination of this Agreement.

10.4 Force Majeure. Neither party shall be liable for any event or circumstance beyond its reasonable control not caused by the fault or negligence of such party, which causes such party to be unable to perform its obligations under this Agreement (and which it has been unable to overcome by the exercise of due diligence), including but not limited to flood, drought, earthquake, storm, fire, pestilence, lightning and other natural catastrophes, epidemic, war, riot, civil disturbance or disobedience, strikes, labor dispute, sabotage of the Center facilities, or any order or injunction made by a court or public agency. In the event of the occurrence or cessation of such a force majeure event, the party unable to perform shall promptly notify the other party in writing. It shall further use its best efforts to resume performance as quickly as possible and shall suspend performance only for such period of time as is necessary as a result of the force majeure event.

10.5 Cooperator. The Cooperator agrees that during the term of this Agreement it will carry appropriate liability insurance to cover any liability to the EPA (including the Center) that may arise as a result of negligent acts or omissions of any of the Cooperator's employees or agents while they are performing work under this Agreement including any work which such employee or agent may be performing at the Center. For clarity, said liability insurance cover includes any policy of self insurance.

10.6. Liability. The parties agree that:

(a) In no event shall any party be liable for any pure economic loss, special, exemplary, incidental or consequential damages arising under or pursuant to this Agreement, even if said party, their Affiliates or their employees have been advised of the possibility of, should have known of, or could reasonably have prevented, such damages.

(b) Nothing in this Agreement limits or excludes any party's liability for: (a) death or personal injury resulting from negligence; or (b) any fraud or for any sort of other liability which, by law, cannot be limited or excluded.

Article 11. Miscellaneous

11.1 No Benefits. No member of, or delegate to the United States Congress, or resident commissioner, shall be admitted to any share or part of this Agreement, nor to any benefit that may arise therefrom. This provision shall not be construed to extend to this Agreement if the Agreement is made with the Cooperator for the Cooperator's general benefit.

11.2 Governing Law. The construction, interpretation, validity, performance and effect of this Agreement for all purposes shall be governed by the laws applicable to the federal government.

11.3 Headings. Titles and headings of the Sections and Subsections of this Agreement are for the convenience of references only and do not form a part of this Agreement and shall in no way affect the interpretation thereof.

11.4 Waivers. None of the provisions of this Agreement shall be considered waived by any party hereto unless such waiver is given in writing to all other parties. The failure of any party to insist upon strict performance of any of the terms and conditions hereof, or failure or delay to exercise any rights provided herein or by law, shall not be deemed a waiver of any rights of any party hereto.

11.5 Severability. The illegality or invalidity of any provisions of this Agreement shall not impair, affect or invalidate the other provisions of this Agreement.

11.6 Amendments. If either party desires a modification to this Agreement, the parties shall, upon reasonable notice of the proposed modification by the party desiring the change, confer in good faith to determine the desirability of such modification. Such modification shall not be effective until a written amendment is signed by all the parties hereto by their representatives duly authorized to execute such amendments.

11.7 Assignment. Except as otherwise permitted herein, neither this Agreement nor any rights or obligations of any party hereunder shall be assigned or otherwise transferred by either party without the prior written consent of the other party. However, the Cooperator may assign this Agreement to the successors or assignees of a substantial portion of the Cooperator's business interests to which this Agreement directly pertains.

11.8 Notices. All notices pertaining to or required by this Agreement shall be in writing and shall be signed by an authorized representative and shall be delivered by hand or sent by certified mail, return receipt requested, with postage prepaid, addressed as follows:

If to the Cooperator:
Fiona Reynolds
Unilever, SEAC
Colworth Science Park
Sharnbrook
Bedfordshire MK44 1LQ
Fiona.Reynolds@Unilever.com
UNITED KINGDOM

If to the Center:

Russell Thomas
National Center for Computational Toxicology (NCCT)
US EPA
109 TW Alexander (MD-B-205-01)
Research Triangle Park, NC 27711
Tel: 919-541-5776
thomas.russell@epa.gov

With a copy to:
Sandra Roberts
National Center for Computational Toxicology (NCCT)
US EPA
109 TW Alexander (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-3850
roberts.sandra@epa.gov

and

Monica Linnenbrink
National Center for Computational Toxicology
Mail Drop B205-01
U.S. EPA
Research Triangle Park, NC 27711
USA

For commercial courier address use:
4930 Old Page Rd.
Durham, NC 27703

Any party may change such address by notice given to the other party in the manner set forth above.

11.9 Independent Parties. The relationship of the Center and the Cooperator is that of independent parties and not as agents of each other or as joint venturers or partners. The Center shall maintain sole and exclusive control over its personnel and operations. The Cooperator shall maintain sole and exclusive control over its personnel and operations.

11.10 Use of Name or Endorsements. The Cooperator shall not use the name of the Center or EPA, on any product or service which is directly or indirectly related to either this Agreement or any patent license or assignment agreement which implements this Agreement, without the prior approval of the Center. By entering into this Agreement the Center does not directly or indirectly endorse any product or service provided, or to be provided, by the Cooperator, its successors, assignees, or licensees. The Cooperator shall not in any way imply

Statement of Work ("SOW") Annex A
Cooperative Research and Development Agreement ("CRADA")
between U.S Environmental Protection Agency ("EPA")
and the Cooperator

I. Goal

EPA's National Center for Computational Toxicology (NCCT) and the Cooperator are interested in the further research and development of the ToxCast™ technology, high-throughput transcriptomics, integration of metabolic competence in 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 provide resources for NCCT's development, evaluation, and translation of these technologies and approaches using chemicals of scientific interest to the Cooperator.

II. Research Plan

The research performed under this SOW will be performed over the course of three years and is broken up into two tasks:

Task I –

Year 1 – EPA and the Cooperator will select a consensus set of 5 chemicals for Phase III of ToxCast™. The chemicals will be procured by the Cooperator and ~500 mg of each chemical will be shipped to the EPA or designee to be incorporated into the ToxCast library. Chemicals will be run through all or a subset of all ToxCast assays as agreed to by the Cooperator and the Center 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. The same 5 chemicals will be analyzed *in vitro* for metabolic stability in primary human hepatocytes and binding to human plasma protein by the EPA.

Year 2 –The five chemicals will also be used to treat at least 5 cell lines or cell types in multiple concentration-response format. The treated cell lines will be analyzed using, a yet to be chosen, high-throughput transcriptomic platform by the EPA. The Cooperator will perform appropriate bioinformatics analyses on the transcriptomic data using multiple tiered tools and will share all data interpretations with the EPA.

Year 3 – Results for the five chemicals will be combined with the hundreds of other Phase III chemicals being run through the ToxCast assays, and analyzed relative to the results from the ToxCast™ Phase I and II datasets. The results from the metabolic stability and plasma protein binding assay will be used to translate the results from the ToxCast™ assays into an administered dose value. Working collaboratively the EPA and the Cooperator will integrate the results from the ToxCast™ assays, *in vitro* metabolic stability assays, plasma protein binding assays, and high-throughput transcriptomic assays to perform a proof-of-concept human health risk assessment on the 5 chemicals. The Cooperator will provide exposure estimates for the 5 chemicals based on knowledge

of their use and standard assumptions of human activity. These multiple exposure scenario determinations will require the Cooperator to perform skin penetration experiments, physicochemical measurements and the construction of PBPK models for accurate calculation of ingredient exposure. The risk assessments will generally follow the biological pathway altering dose (BPAD) approach outlined by Judson *et al.* (*Chem Res Toxicol.* 24(4):451-6, 2011), but the Cooperator and the EPA will work together to craft new safety assessments that will challenge traditional approaches for consumer product safety.

Task II –

Year 1 – The S9 and microsomal cell fractions have 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. The EPA will attempt to adapt or develop workaround solution to the S9 toxicity problem in an HTS-amenable format (e.g., Yamamoto *et al., J Biosci Bioeng* 111:454, 2011; Sakai-Kato et al., *Anal Biochem* 308:278, 2002). The solution involves the encapsulation of human S9 or microsomal fractions in a compatible solid matrix (e.g., alginate, sol-gel) that allows for passive diffusion of low molecular weight chemicals, but retains molecules larger than the polymer network pores such as S9 lipid peroxides. The encapsulated S9 or microsomal 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.

In parallel with the encapsulation approach, the EPA will attempt to develop an intracellular biotransformation method. In this approach, transfection of pooled mRNA will be performed to transiently establish cellular biotransformation. mRNA transfection provides a flexible and versatile format to co-transfect multiple enzyme-encoding genes simultaneously. Since the relative mRNA expression profiles of human tissues is known, mRNAs of representative genes will be pooled in a ratio that reflects the expression profile of a particular target tissue such a liver or lung and transfect the pool into cells prior to or even after plating. To overcome activation of interferon- and NFkB-directed antiviral responses, modified RNA bases may be used such as 5-methylcytidine (5mC; substituted for cytidine) and pseudouracil (Psi; substituted for uracil) (e.g., Warren *et al.,Cell Stem Cell*, 7:618, 2010). Further modifications of mRNAs including 5' capping and phosphatase treatment to remove residual 5' phosphates may also be attempted if deemed necessary. The mRNA from the different enzymes will be pooled based on known expression profiles and transfected by the EPA into cell types used in the ToxCast assays high-throughput toxicology such as HepG2, HEK293, HeLa and MCF7.

Year 2 – Assuming successful development of the encapsulation method in Year 1, the metabolic activity of the encapsulated S9 or microsomes will be evaluated by the EPA. One attempted approach may utilize luciferase-based assays for various biotransformation enzymes (e.g., P450-Glo, UGT-Glo; Promega) to confirm that the encapsulation of the enzyme-enriched fractions was successful and that enzymatic

activity was retained. Enzymes that could be tested in this manner include CYP1A1, 1A2, 1B1, 2C8, 2C9, 2C19, 2D6, 3A4, 3A7 and UGT1A1 and 2B7. Batch-to-batch variability will also be assessed. Following characterization, the encapsulated enzymes may be tested in different assay formats using a suite of positive control chemicals. For example, the encapsulated S9 or microsomes may be incubated with cells exposed to 2,3-dichloro-1,4-naphthoquinone (cytotoxic control) or medium only and cytotoxicity measured.

For the intracellular biotransformation method, expression of a subset of transgenes may be confirmed by the EPA on a protein basis (e.g., Western blot). Functional characterization may also be performed by the EPA using luciferase assays for the biotransformation enzymes (e.g., P450-Glo and UGT-Glo; Promega). Following characterization, the transfected cells may be tested by the EPA in different assay formats using a suite of positive control chemicals. For example, the encapsulated S9 or microsomes may be incubated with cells exposed to 2,3-dichloro-1,4-naphthoquinone (cytotoxic control) or medium only and cytotoxicity measured.

Year 3 – If the experimental development is successful in Year 2, the EPA may couple the extracellular and/or intracellular methods to one or more existing cell-based HTS assays for distinct pathways. The pathways whose assays would most likely benefit from in-well biotransformation are DNA damage response and ER/AR transactivation/antagonism. If the experimental development is not successful, the EPA and the Cooperator will decide on the best path forward.

III. Milestones

Task I –

Year 1

1) Select a consensus set of 5 chemicals.
2) Chemicals procured by the Cooperator and shipped to EPA or designee.
3) EPA or designee performs ToxCast™ assays on the 5 chemicals.
4) EPA or designee performs *in vitro* metabolic stability and plasma protein binding assays on the 5 chemicals.

Year 2

1) EPA or designee continues performing ToxCast™ assays on the 5 chemicals with appropriate data transfers.
2) EPA or designee performs high-throughput transcriptomic measurements on the 5 chemicals across at least 5 cell lines or cell types.
3) The Cooperator will perform bioinformatics analyses on the transcriptomic data.

Year 3

1) EPA compiles results for the five chemicals across the ToxCast™ assays and compare to existing ToxCast™ Phase I, II, and III results.

2) EPA converts the *in vitro* potency results from the ToxCast™ assays into administered dose equivalents using the metabolic stability and plasma protein binding assays.
3) EPA integrates the results from the ToxCast™ assays, *in vitro* metabolic stability assay, plasma protein binding assay, and high-throughput transcriptomic assays.
4) EPA and the Cooperator performs a proof-of-concept human health risk assessment on the 5 chemicals using the above data and the exposure estimates generated by the Cooperator.
5) Delivery of high throughput risk assessment for the 5 chemicals, including calculation of Biological Pathway Altering Concentration (BPAC) and Dose (BPAD). These data to be provided in a format consistent with exising ToxCast™ data, such as mySQL database allowing comparison to other chemicals in the ToxCast™ data through the ToxCast™ package.

Task II –

Year 1

1) EPA develops method to encapsulate S9 or microsomes into a matrix.
2) EPA develops method to *in vitro* transcribe and transfect mRNAs for multiple CYPs, UGTs, GSTs, and SULTs into cells.

Year 2

1) EPA characterizes metabolic activity of the encapsulated S9 or microsomes.
2) EPA establishes the technical feasibility of the encapsulated S9 or microsomes to metabolize positive control chemicals with known biotransformation profiles in a cell viability study with appropriate data transfer.
3) EPA characterizes the metabolic activity of the cells transfected with pooled mRNAs.
4) EPA establishes the ability of the cells transfected with pooled mRNAs to metabolize a training set of chemicals with known biotransformation profiles in a cell viability study.
5) EPA and cooperator finalize plan for transfer of knowledge and experimental capability build at the Cooperators facility, to include lab visits by relevant experts at respective sites as required at this point and at the end of year 3.

Year 3

1) EPA performs one or more high-throughput screens using encapsulated S9 or microsome method or the intracellular biotransformation method

Throughout the programme of work regular discussions (ideally 3 monthly but minimum 6 monthly 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. Visits by EPA personnel to the Cooperator and vice versa shall be undertaken. All data and results from Tasks I, II, and III of ToxCast™ and the additional studies described above will be made wholly available to the Cooperator on completion of the work and before publication, as well as the analysis of these result by the scientists of the NCCT.

IV. Estimated Value and Benefits

A. Value of Contributions

1. Estimated value of EPA contributions (in-kind): $26,000,000
   ToxCast™ Phase I data- $6,000,000
   ToxCast™ Phase II data- $14,000,000
   ToxCast™ Phase III data- $6,000,000

2. Value of the Cooperator's estimated contribution (in-kind): $500,000
   [Exposure estimates, bioinformatics on the selected chemicals]
   The Cooperator's cash contribution to EPA: $873,660
   The Cooperator's total contribution (cash and in-kind): $1,375,000

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 fund 5 or more additional compounds to be run in the EPA ToxCast™ assays for Phase III, 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, the Cooperator will fund the development of 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. Finally, the Cooperator will fund the additional compounds to be run through high-throughput transcriptomic screening assays. Additional chemicals will strengthen this dataset and enhance its ability to identify important modes-of-action for toxicity.

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:

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

- 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

- Knowledge transfer on pathways-based QIVIVE and safety assessment decisions for the Cooperator; utilizing EPA methodologies and thought leadership for biodynamic/biokinetic-based non-animal derived risk assessments

- 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

- Addressing the lack of metabolic capacity in existing HT screening tools (ToxCast) and transfer of learnings to the developing the Cooperator's metabolism experimental capability; increasing awareness of relevant approaches and adopting practice in-house

- Development and implementation of LINCS streamlined omics appropriate for the Cooperator's needs and application to ingredient assessment/characterization

- 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.

- 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.

Paul Carmichael (Strategic Lead), Paul Russell (Project Leader) and Richard Stark (Principal Investigator) will be the primary representatives for the Cooperator in order to manage/effect the collaboration with the EPA.

V. General Provisions for the Conduct of the SOW

The Center shall:

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

(b) commence the SOW on the dates as indicated in this Annex A 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 the Center for an extension to said period to permit a later commencement date, such request not to be unreasonably refused. For clarity, any extensions under this clause shall be agreed in writing by the Cooperator signed by its officer having authority therefor;

(c) keep, or procure such shall be kept by its staff carrying out the SOW, 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 (that 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 the Center's address first above written (or such other address(es) as the Center shall notify the Cooperator from time to time) for the Term and five (5) years thereafter. At the Cooperator's request and reasonable cost, the Center shall provide copies of the said records to the Cooperator as soon as practicable;

(d) 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;

(e) in its conduct of SOWs, use all reasonable endeavours to not infringe third party intellectual property rights;

(f) not, and shall procure that it and its staff shall not, use any animals classed within the superphylum Deuterostomia or any cells or tissue therefrom in the conduct of the SOWs. For clarity, the superphylum Deuterostomia includes all insects, echinoderms, arachnids, molluscs, crustaceans, fish, amphibians, reptiles, birds and mammals. On written request, the Center shall provide to the Cooperator written evidence of its compliance with the foregoing. Notwithstanding the foregoing, the Center may use animal tissue as and to the extent set forth in the SOWs on the condition that such use shall be strictly in accordance with all applicable legislation for the regulation thereof.

The Cooperator shall:

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

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

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

(d) in its conduct of SOWs, use all reasonable endeavours to not infringe third party intellectual property rights;

(e) not, and shall procure that it and its staff shall not, use any animals classed within the superphylum Deuterostomia or any cells or tissue therefrom in the conduct of the SOWs. For clarity, the superphylum Deuterostomia includes all insects, echinoderms, arachnids, molluscs, crustaceans, fish, amphibians, reptiles, birds and mammals. On written request, the Center shall provide to the Cooperator written evidence of its compliance with the foregoing. Notwithstanding the foregoing, the Center may use animal tissue as and to the extent set forth in the SOWs on the condition that such use shall be strictly in accordance with all applicable legislation for the regulation thereof.

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	$873,660

Said amount shall be allocated to the following cost headings:

Item	**Funding**
Task I	$453,250
Task II	$390,570
Travelling and other expenses (excluding VAT)	$29,840
Total (excluding VAT)	$873,660

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 Coordinator 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**
Start of Project	Start of Project	$291,220
End of Year 1	Progress report for Tasks I and II	$291,220
End of Year 2	Final report for Tasks I and II	$291,220
Total		$873,660