DATED the 11th day of September 2017

BETWEEN

BIOINFORMATICS INSTITUTE

AND

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR COMPUTATIONAL TOXICOLOGY

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AGREEMENT FOR RESEARCH COLLABORATION

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Ref: AH/OCL/667/0615/IBN

AGREEMENT FOR RESEARCH COLLABORATION

THIS AGREEMENT is made on the 11th day of September 2017

BETWEEN

BIOINFORMATICS INSTITUTE , BIOMEDICAL SCIENCES INSTITUTES, (Co. Reg. No. 199702109N) having its principal office at 30 Biopolis Street, Matrix #07-01 Singapore 138671 ("**BII**");

AND

The NATIONAL CENTER FOR COMPUTATIONAL TOXICOLOGY of the UNITED STATES ENVIRONMENT PROTECTION AGENCY, located in Research Triangle Park, NC, the UNITED STATES of AMERICA (hereinafter referred to as **"EPA" and "NCCT"**).

(Each of BII and EPA are hereinafter referred to individually as "a Party" and collectively as "the Parties.")

RECITALS

(A) BII is a national research institution based in Singapore and funded by the Agency for Science, Technology and Research ("A*STAR"). BII has considerable knowledge, expertise and experience in, inter alia, the field of computational biology.

(B) EPA is an Agency of the United States of America. NCCT is a Laboratory under the EPA. NCCT has an interest and expertise in, and proprietary technologies and know-how in the fields of computational toxicology.

(C) BII and NCCT wish to collaborate in research and development in the areas of interest referred to above by undertaking the Project (as defined below) on the terms and conditions set out below.

NOW IT IS HEREBY AGREED as follows: -

1. DEFINITIONS

In this Agreement, unless otherwise expressly provided, the following terms shall have meanings ascribed to them below.

"**Affiliates**" means: (i) an organisation, which directly or indirectly controls either Party; or (ii) an organisation which is directly or indirectly controlled by either Party; or (iii) an organisation, which is controlled, directly or indirectly, by the ultimate parent company of either Party. The term "control" as used herein means the possession of the power to direct or cause the direction of the management and the policies of an entity, whether through the ownership of a majority of the outstanding voting security or by contract or otherwise. The term 'Affiliate' with regards to BII shall include A*STAR, ETPL and all research institutes and centres funded and managed by A*STAR.

"**A*STAR**" means the Agency for Science, Technology and Research.

"**BII Project IP**" means all IP which was discovered, developed, conceived or reduced to practice solely by BII or its employees, servants, invitees or agents in the course of the Project.

"**Confidential Information**" means the terms of this Agreement and any and all information, data, designs, memoranda, models, prototypes, and/or other material whether of scientific, technical, commercial, financial or other nature, furnished to or obtained by a Party from the other Party under this Agreement in written, oral or other tangible form clearly marked or designated as "Confidential" or by words of similar import. Information communicated by a disclosing Party orally or visually shall be summarized in writing, marked "Confidential" and delivered to the receiving Party within fourteen (14) days of such communication, failing which such information shall not constitute Confidential Information.

"**Effective Date**" means the date of the last signature of the parties on this agreement.

"**EPA Project IP**" means all IP which was discovered, developed, conceived or reduced to practice solely by EPA or its employees, servants, invitees or agents in the course of the Project.

"**ETPL**" means Exploit Technologies Pte Ltd, the commercialization and marketing arm of A*STAR and BII.

"**Intellectual Property (IP)**" means all patents, Inventions, copyright, registered designs, and semiconductor layout designs in all countries of the world arising under statutory or common law, and whether or not perfected, and any pending applications of the foregoing.

"**Invention**" means any invention or discovery which is or may (or may not) be patentable or otherwise protectable under the intellectual property laws of this or any foreign country.

"**Joint Project IP**" means all IP which was discovered, developed, conceived or reduced to practice jointly by one or more A*STAR RIs and EPA or their employees, servants, invitees or agents in the course of the Project.

"**Pre-Collaboration IP**" means all IP owned or controlled by each Party and which was conceived or reduced to practice either (a) prior to commencement of the work performed pursuant to this Agreement or (b) outside the scope of the work performed pursuant to this Agreement and which is introduced to or disclosed for the Project or otherwise supplied by each Party; and for BII shall mean the Pre-Collaboration IP which is expressly documented by it and made available to EPA.

"**Project**" means the research and development activities specified in the Project Plan.

"**Project IP**" means all IP including Inventions, Works, and Subject Data which was discovered, developed, conceived or reduced to practice by a Party or their employees, servants, invitees or agents in the course of the Project.

"**Project Plan**" means the statement of work set out in Schedule 1 annexed hereto.

"Subject Data" means all recorded information first produced in the performance of this Agreement,

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

"**Term**" means the period as specified in Clause 4.

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

2. STATEMENT OF WORK

2.1 The Parties hereby agree to collaborate in the Project.

2.2 The Parties recognize that the Project is research in nature and hence completion within the period of performance, or within the limits of financial support allocated, or the achievement of the deliverables and/or milestones specified in the Project within or outside the time schedule specified therein cannot be guaranteed. The Parties shall exercise reasonable efforts in the performance of the Agreement in accordance with the agreed scope of work.

2.3 The Parties agree and declare that the obligations of the Parties shall cease (except as otherwise set forth in Clause 13.2) upon the end of the Term.

2.4 Each Party shall obtain all relevant ethics and other approvals as may be relevant for its participation in the Project.

3. CO-ORDINATORS

3.1 The Project shall be supervised and coordinated by Loo Lit Hsin from BII, (hereinafter referred to as "BII Co-ordinator") and Katie Paul-Friedman from NCCT (hereinafter referred to as "NCCT Co-ordinator").

3.2 If for any reason the BII Co-ordinator is unable to continue to serve under the Project, BII agrees to appoint a successor within thirty (30) days of the unavailability of the Co-ordinator, failing which the provisions of Clause 12.2 shall apply.

4. PERIOD OF PERFORMANCE

This Agreement shall come into force on the Effective Date and shall continue for a period of three (3) years unless earlier terminated in accordance with the terms of this Agreement or extended by the Parties' agreement in writing.

5. PROJECT CONTRIBUTIONS

Each Party will make such contributions in terms of manpower deployment, equipment, facilities, cash funding and other contributions as specified in Schedule 1, or as agreed from time to time by the Parties in writing.

6. PUBLICATIONS

6.1 Each Party may publish at any symposia, national, international or regional professional meeting or in any journal, thesis, dissertation, newspaper or otherwise of its own choosing, the findings, methods and results derived from the Project, but always subject to due observance of this Clause 6.

6.2 The Party intending to make the publication ("the Publishing Party") shall furnish the other Parties ("the Other Parties") copies of such proposed publication or presentation in advance of the submission of such proposed publication or presentation to a journal, editor, or other third party. The Other Parties shall within fourteen (14) days of receipt of the proposed publication or presentation forward its written objections to the same either because there is patentable subject matter that needs protection and/or there is Confidential Information (as defined in Clause 1 herein) or patentable information of the Other Parties contained in the proposed publication or presentation. If no objection is made to the proposed publication or presentation within the stipulated time, the Publishing Party shall be free to proceed with the publication or presentation.

6.3 Confidential Information identified by the Other Parties, which is governed by Clause 7, shall be deleted from the proposed publication or presentation unless the Other Parties considers the Confidential Information to be patentable information, in which case it will be treated as set forth in the following sub-Clause.

6.4 In the event that a Party objects to any such publication or presentation on the basis that the same would disclose patentable information belonging to that Party, the Publishing Party shall refrain from making such publication or presentation for a further period of sixty (60) days from date of receipt of such objection in order for the relevant patent application(s) to be filed.

6.5 Each Party shall, in any publications it makes in relation to the methods, results and findings of the Project, acknowledge the other Party's contributions to the Project.

7. CONFIDENTIALITY

7.1 Each Party agrees, for the Term and for a period of thirty-six (36) months after the termination or expiration of the Agreement, to treat the Confidential Information of the other Parties as strictly confidential and not to disclose it to any third party for any purpose whatsoever and not make use of the Confidential Information or any part thereof other than for the Project and to treat it with at least the same care and in the same manner as its own secret and valuable information. The receiving Party shall ensure that its employees to whom Confidential Information is disclosed covenant to keep such information confidential to the extent that the receiving Party is bound by this Agreement and that such covenants on the part of employees are strictly observed.

7.2 The provisions of Clause 7.1 above shall not apply to any:

7.2.1 information which is or was already known to the receiving Party at time of disclosure to it, or

7.2.2 information which after disclosure to the receiving Party under this Agreement is published or otherwise generally available to the public otherwise than through any act, default or omission by the receiving Party of its obligations hereunder, or

7.2.3 information which can be established by tangible evidence was independently developed by the receiving Party without the use of or reference to the disclosing Party's Confidential Information; or

7.2.4 information which is required to be disclosed to governmental or regulatory bodies or to a court of competent jurisdiction pursuant to any written law, provided, however, that such disclosure is limited to that required to be disclosed; or

7.2.5 information which, pursuant to a court order, is required to be disclosed as evidence in a court of law, provided however that such disclosure is limited to that required to be disclosed; or

7.2.6 information which is disclosed to the receiving Party by a third party without restriction and without breach of the confidentiality obligations under this Agreement by the receiving Party.

7.3 It is agreed that BII may disclose all or any part of the Confidential Information to their Affiliates on the basis that BII shall procure that such Affiliates shall also agree to treat the information as confidential.

7.4 The receiving Party acknowledges that unauthorized disclosure or use of Confidential Information could cause great or irreparable injury to disclosing Party and that pecuniary compensation would not afford adequate relief or it would be extremely difficult to ascertain the amount of compensation which would afford adequate relief. Therefore, the receiving Party agrees that, in the event of such unauthorized disclosure or use of Confidential Information, the disclosing Party will have the right to seek and obtain injunctive relief in addition to any other rights and remedies it may have.

7.5 Except for the disclosure of the existence of this Agreement, including the title and identification of the Parties, which information shall not be deemed confidential, no Party shall disclose the specific terms and conditions of this Agreement without the express permission of the other Parties or as required by applicable laws.

8. INTELLECTUAL PROPERTY, DATA AND RESULTS

8.1 The Parties do not anticipate the necessity for the use of any third party licences ("Third Party Licence(s)") for the conduct of their scope of work under this Agreement.

8.2 All rights, title and interests to Pre-Collaboration IP shall remain with the Party introducing or disclosing the same and shall remain unfettered by this Agreement. Each Party grants to the other Parties the right to use its Pre-Collaboration IP for the purposes of the Project during the Term and for no other purposes except as provided in this Agreement.

8.3 All rights, title and interests to BII Project IP shall be solely owned by BII. For the avoidance of doubt, all source codes developed by BII under this Agreement shall be considered BII Project IP. It is agreed that BII shall be entitled to assign all their rights, titles and interests in the BII Project IP to A*STAR or its nominee, and that

A*STAR or its nominee shall be entitled to appoint ETPL to undertake all patenting, commercial and/or licensing activities relating to the same on its behalf.

All rights, title and interests to all Subject Inventions created solely by EPA shall be solely owned by EPA. Subject Data and copyrightable Works, created solely by EPA, will be shared with BII.

8.4 All rights, interests and title to Joint Project IP shall be governed by the following provisions:

8.4.1 The Parties shall own the Joint Project IP (except for copyright) as joint tenants. EPA will retain a free, irrevocable license to use any BII copyrightable Works arising under this agreement for its non-commercial purposes. EPA-created Works will not be copyrighted and may be posted on EPA's public website. EPA agrees and accepts that BII may, at their absolute discretion, assign or otherwise transfer to A*STAR, their share of the legal and beneficial ownership in the Joint Project IP (including any copyright) without any reference to EPA or any obligation to obtain EPA's consent.

8.4.2 The Parties agree that all arrangements relating to the filing, prosecution and maintenance of all applications for the registration of patents, trademarks, designs and copyrights (where applicable) for the protection of the Joint Project IP including any patentable joint Subject Inventions, and the commercialisation of the same, shall be discussed and agreed separately in writing if and when the same arises.

8.4.3 All rights, title, and interest in Subject Data and the results arising from the Project shall reside with the creator of the Subject Data. The parties are free to use all Subject Data and results arising from the Project for their non-commercial purposes, on the basis that all of the foregoing is not published without the prior written consent of the parties, as the case may be.

9. COLLABORATION

For the avoidance of doubt, it is agreed that notwithstanding the terms and conditions of this Agreement, each Party will have the following rights:

(a) to conduct any research or development work in any field (including work relating to the research contemplated under this Agreement) independently of the other Party, whether by itself or in collaboration with any other party subject to each Party observing the provisions of Clause 7 hereof;

(b) to continue existing commitments or to make new ones; and

(c) to use, exploit (including sub-licensing) or otherwise take advantage of its own Intellectual Property, other than the Joint Project IP which shall be subject to the terms and conditions of this Agreement.

10. WARRANTIES AND LIABILITIES

10.1 Each Party represents and warrants that it has the right to enter into this Agreement and provide the materials and services described herein. Except for the foregoing, the Parties do not make any representations, conditions or warranties, either express or implied with respect to any information, its Pre-Collaboration IP, the work performed pursuant to the terms of this Agreement, or the Project IP developed under this Agreement. Without limiting the generality of the foregoing, each Party expressly disclaims any implied warranty, condition or representation that the said information, its Pre-Collaboration IP and/or the Project IP developed under this Agreement:

10.1.1 shall correspond with a particular description;

10.1.2 is of a merchantable satisfactory quality;

10.1.3 is fit for a particular purpose; or

10.1.4 is durable for a reasonable period of time.

10.2 Nothing in this Agreement shall be construed as:

10.2.1 a warranty by any Party that anything made, used, sold or otherwise disposed of in connection with its Pre-Collaboration IP disclosed or introduced hereunder or that the Project IP or Project IP developed is or will be free from infringement of patents, copyrights, trademarks, industrial designs or other intellectual property rights of any third party; or

10.2.2 an obligation on RI or EPA to bring or prosecute or defend actions or suits against or by third parties for infringement of patents, copyrights, trademarks, industrial designs or other intellectual property or contractual rights, whether in connection with its Pre-Collaboration IP or the Project IP developed under this Agreement or otherwise.

10.3 No action whether in contract or tort (including negligence) or otherwise arising out of or in connection with this Agreement may be brought by a Party against the other more than three (3) years after the course of action has accrued.

10.4 Save for death or personal injuries caused by negligence, in no event shall BII, whether as a breach of contract, tort or otherwise, have any liability to EPA or to a third party for any indirect, special, incidental, consequential damages, loss of profits or pure economic loss.

10.5 EPA's responsibility for the payment of claims to BII or its employees for 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, shall be in accordance with applicable laws.

10.6 Notwithstanding anything to the contrary, BII's total and cumulative liability under this Agreement, however arising, shall not exceed Singapore Dollars Ten Thousand (S$10,000).

11. USE OF NAMES

No Party shall issue any press release relating to this Agreement without

obtaining the prior written consent of the other Parties. Prior to being released or made, a copy of all press releases which a Party intends to issue or make regarding this Agreement shall be provided to the other Parties for approval, which approval shall not be unreasonably withheld.

12. TERMINATION

12.1 Any of the Parties shall be entitled to terminate this Agreement immediately by notice in writing to the other Parties (but without prejudice to any rights any Party may have against the other arising prior to such termination) if any of the events set out below shall occur. The said events are:

12.1.1 if a Party shall commit any material breach of any of its obligations under this Agreement and shall fail to remedy such breach (if capable of remedy) within thirty (30) days after being given notice by the first Party so to do; or

12.1.2 if a Party (being a company) shall go into liquidation whether compulsory or voluntary (except for the purposes of a bona fide reconstruction or amalgamation with the consent of the first Party, such consent not to be unreasonably withheld) or if a Party shall have an administrator appointed or if a receiver, administrative receiver or manager shall be appointed over any part of the assets or undertaking of that other Party.

12.2 Pursuant to Clause 3.2, BII shall be entitled to terminate this Agreement if the events specified in Clause 3.2 hereof occur, in which case BII shall be relieved of its obligations herein (except for the obligations described in Clause 13.2 and any other obligations that are expressed to survive termination of this Agreement) and shall have no liability whatsoever to EPA in respect of such termination.

13. CONSEQUENCE OF TERMINATION

13.1 The provisions of Clauses 6, 7, 8, 9, 10, 11, 12.2, 13, 14, 18, 19, 20 and 21 shall continue in full force and in accordance with their terms, notwithstanding the expiration or termination of this Agreement for any reason.

13.2 Without prejudice to any claims for damages that either Party may be entitled to, upon termination or expiration of this Agreement, each Party shall promptly return all materials of the other Parties in its possession, including, without limitation, Confidential Information of the other Parties, upon the request of the other Parties.

14. ASSIGNMENT

14.1 Save as expressly provided in this Agreement, no Party shall assign this Agreement or otherwise transfer its rights or obligations, or any part thereof, under this Agreement without the prior written consent of the other Parties.

14.2 It is agreed that if at any time after the date of this Agreement the functions and operations of BII are assigned, merged, transferred into or otherwise forms part of another organization of A*STAR ("the New Entity"), such that the New Entity takes over the whole or substantially the whole of BII's operations, then it is agreed that that BII may:

14.2.1 at its option, assign this Agreement in its entirety to the New Entity which will then assume all of BII's rights and obligations hereunder; or

14.2.2 assign all or any part of its rights hereunder to the New Entity.

15. FORCE MAJEURE

15.1 No Party shall be liable for delays in delivery or performance when caused by any of the following which are beyond the actual control of the delayed Party: (i) acts of God, (ii) acts of the public enemy, (iii) acts or failure to act by the other Party, (iv) acts of civil or military authority, (v) governmental priorities, (vi) hurricanes, (vii) earthquakes, (viii) fires, (ix) floods, (x) epidemics or pandemics, (xi) embargoes, (xii) war, and (xiii) riots (hereinafter referred to as the "Force Majeure Event").

15.2 The respective obligations of the Parties hereunder shall be suspended during the time and to the extent that such Party is prevented from complying therewith by a Force Majeure Event provided that such Party shall have given written notice thereof, specifying the nature and details of such event and the probable extent of the delay to the other Parties.

15.3 In case of a Force Majeure Event, the time for performance required by each Party under this Agreement shall be extended for any period during which the performance is prevented by the event. However, the other Parties may terminate this Agreement by notice if such an event prevents performance continuously for more than thirty (30) days.

16. DISPUTE RESOLUTION

The Parties agree to attempt in good faith to settle any claim or controversy arising out of this Agreement through consultation and negotiation in good faith and spirit of mutual cooperation. Any dispute between the Parties relating to this Agreement will first be submitted in writing to one senior executive from each Party, who will promptly meet and confer in an effort to resolve such dispute. Each Party's senior executive will be identified by notice to the other Party, and may be changed at any time thereafter by notice to the other Party.

17. NOT IN USE

18. NOTICE

18.1 Any notice to be given by any Party to this Agreement shall be in writing and shall be deemed duly served if delivered personally or sent by facsimile transmission or by prepaid registered post to the addressee at the address as stated above or (as the case may be) the facsimile number of that Party or at such other address (or facsimile number) as the Party to be served may have notified the other Parties for the purposes of this Agreement.

18.2 Any notice sent by facsimile shall be deemed served when despatched and any notice served by prepaid registered post shall be deemed served forty-eight (48) hours after despatch thereof. In proving the service of any notice it will be sufficient to prove in the case of a letter that such letter was properly stamped addressed and place in the post or delivered or left at the current address if delivered

personally and in the case of a facsimile transmission was duly despatched to the facsimile number of the addressee given above or subsequently notified for the purposes of this Agreement.

19. EXPORT CONTROL

EPA shall ensure that it and its end-users of any IP licensed or otherwise made available to EPA shall comply with all applicable laws, rules and regulations governing the use, export and disposal of the IP, including those related to export control.

20. ENTIRE AGREEMENT

Unless otherwise expressly specified, this Agreement embodies the entire understanding between the Parties in respect of the Project and any prior or contemporaneous representations, either oral or written, are hereby superseded. No amendments or changes to this Agreement shall be effective unless made in writing and signed by authorized representatives of the Parties.

21. GENERAL

21.1 No exercise or failure to exercise or delay in exercising any right power or remedy vested in any Party under or pursuant to this Agreement shall constitute a waiver by that Party of that or any other right power or remedy.

21.2 The Parties shall co-operate with each other and execute and deliver to the other Party such instruments and documents and take such other action as may be reasonably requested from time to time in order to carry out and confirm the rights and the intended purpose of this Agreement.

21.3 In the event that any term, condition or provision of this Agreement is held to be a violation of any applicable law, statute or regulation the same shall be deemed to be deleted from this Agreement and shall be of no force and effect and this Agreement shall remain in full force and effect as if such term condition or provision had not originally been contained in this Agreement. Notwithstanding the above in the event of any such deletion the Parties shall negotiate in good faith in order to agree the terms of a mutually acceptable and satisfactory alternative provision in place of the provision so deleted.

21.4 This Agreement may be executed in any number of counterparts or duplicates each of which shall be an original but such counterparts or duplicates shall together constitute but one and the same agreement.

21.5 The recitals and schedules of this Agreement shall form an integral part of this Agreement.

21.6 It is agreed that for the purposes of this Agreement, "BII" shall refer to the Bioinformatics Institute only. Reference to BII herein shall not extend to any other research institute, center or division of the Biomedical Sciences Institutes. For avoidance of doubt, no research institute, center or division within the Biomedical Sciences Institutes (other than BII, as the case may be) shall have any obligation under this Agreement to the EPA or to disclose to or receive from the EPA any

information unless expressly agreed in writing.

22. THIRD PARTY CONTRACTS ACT

Save for the parties identified in Clauses 7, 8 and 14, the Parties do not intend that any term of this Agreement should be enforceable by any person or entity who is not a party to this Agreement.

AS WITNESS the hands of the Parties hereto the day and year first above written.

SIGNED by [redacted]

for and on behalf of

Bioinformatics Institute

in the presence of: [redacted]

[Name/title of signatory]

Dr. Frank Eisenhaber
Executive Director
BioInformatics Institute, BMSI

[redacted]

[Name/title of Witness]

Lit-Hsin Loo

SIGNED by [redacted]

for and on behalf of

United States Environmental Protection Agency, in the presence of: [redacted]

Russell Thomas
Director, NCCT

[redacted]

Monica Linnenbrink
Director, Communications

SCHEDULE 1

PROJECT PLAN

In vitro bioactivity based on phenotypic profiles as a conservative estimate of point-of-departure for chemical risk assessment

1. BACKGROUND

In September 2016, a workshop to accelerate the pace of chemical risk assessment based on animal-free, New Alternative Methods (NAMs) was held in Washington DC. Participants of the workshop included several key international chemical safety regulatory and research agencies, such as the US Environmental Protection Agency (EPA), Health Canada, European Chemicals Agency (ECHA), European Food Safety Authority (EFSA), and A*STAR. To help inform future applications of NAMs in the regulatory decision-making process, several international case studies have been convened to evaluate how NAMs can or cannot be used under specific decision-making contexts.

One of these case studies is to examine the utility of *in vitro* bioactivity data in chemical risk assessments. This inter-agency collaboration has resulted in the sharing and compilation of a unique and large list of >400 chemicals with available high-throughput bioactivity and toxicokinetic (HTTK) data based on *in vitro* measurements, and existing human health risk assessments based on traditional animal studies. This comprehensive dataset allows us to derive "administered dose equivalents" (ADEs) for these chemicals based on the *in vitro* data. ADE is a dose estimated to produce steady-state in vivo blood concentration equivalent to an *in vitro* bioactive concentration of a chemical. The dataset would also allow us to systematically compare ADE and Point-of-Departure (POD) derived from traditional risk assessments, such as no-observed-adverse-effect level (NOAEL). The main question is whether ADEs provide conservative estimates of PODs.

Dr. Lit-Hsin Loo's group at BII, A*STAR has developed a High-throughput *In vitro* Phenotypic Profiling for Toxicity Prediction (HIPPTox) platform, which can be used to automatically identify predictive toxicity endpoints based on microscopy images of human cells treated with reference toxic chemicals. One of the key advantages of HIPPTox over existing *in vitro* assays is that no assumption about toxicity mechanisms is required, and thus the platform can be used to discover new *in vitro* endpoints that may reflect previously unknown toxicity modes of action or mechanisms. The platform has led to the first predictive model for nephrotoxicity, which has ~90% prediction accuracy. Recently, the platform has also been applied to human liver and lung cells (unpublished). Phenotypic profiles generated using HIPPTox may be used to derive ADEs, in conjunction with the HTTK and reverse dosimetry models developed by EPA.

2. SCOPE OF WORK

The overall objective of this collaboration is to compare ADEs derived from phenotypic profiles and PODs derived from traditional animal studies. We hypothesize that phenotypic-profile-based ADEs are correlate to the PODs of chemicals with organ-specific effects.

A*STAR have selected 64 chemicals from the list of >400 chemicals compiled by EPA. These chemicals are chosen to represent 13 chemical classes with similar/related chemical structures and are relevant to human exposures and food and/or personal care products. Within each class, chemicals with diverse POD values were selected. This

design will allow us to compare both the inter- and intra-chemical-class performances of ADEs.

Some of the selected categories have low numbers of members (<5 chemicals), mostly due to the lack of POD data during the time of selection. The compilation of the master chemical list is still on-going, as more data and information will be made available by Health Canada, ECHA, and other agencies. Therefore, we expect more chemicals for these categories will be available, and thus may include additional ~10-15 chemicals for these categories in the middle of the project. This may increase the number of selected chemicals to ~80. However, some of these chemicals may be auto-fluorescent and not suitable for fluorescence imaging, thus we expect the final number of testable chemicals to be ~60-70.

Specific Aim 1. Quantify the phenotypic responses of human liver, kidney, and lung cell lines to a list of selected chemicals. Liver, kidneys, and lungs are three key internal organs responsible for the metabolism and/or excretion of many xenobiotics. Not surprisingly, they are also the main targets of many toxic chemicals. We will apply the HIPPTox platform to measure the phenotypic responses of three different human cell lines to the selected chemical. These cell lines will be HepG2 (hepatocytes), HK-2 (proximal tubule cells), and BEAS-2B (bronchial epithelial cells). The responses will be based on immunofluorescence markers that stain the nuclear and cellular morphologies, actin cytoskeleton structures, and DNA damage responses. The cells will be exposed to the chemicals in 7 concentrations (from 4 to 500μM) for 16 hours in at least two biological replicates. Then, the cells will be fixed, stained with the immunofluorescence markers, and imaged using an automated epifluorescence microscope at 20x. The images will be processed to identify individual cells and quantify phenotypic responses of the cells. We expect to capture the responses of ~2000-3000 cells per condition. Dr. Lit-Hsin Loo's lab from A*STAR has established the complete experimental and data-processing workflow, which is part of the HIPPTox platform. The generated dataset will provide a comprehensive characterization of the *in vitro* activities of the selected chemicals in these three important cell types, and allow us to predict potential organ-specific effects of these chemicals.

Specific Aim 2. Derive ADEs based on the phenotypic profiles and HTTK data. Intermediate changes in cellular phenotypes, such as change in cell size, re-organization of cytoskeletons, or activation of DNA damage responses often occur at lower concentrations than toxic concentrations that induce adverse cellular effects, such as cell death. Phenotypic profiling is a powerful method that can be used to characterize these intermediate changes. We will use the HIPPTox platform to compare the phenotypes of treated and untreated cells, and detect the lowest concentrations in which statistically significant changes start to occur. Once the *in vitro* activity levels for all the chemicals in three different cell lines have been determined, we will compare different analysis procedures to consolidate these values and the HTTK data, and derive a final ADE for each chemical. We will use the HTTK package developed by EPA to model and derive ADEs. Performance will be measured based on the correlation between the derived ADEs and PODs. The final outcome will be an optimized analysis procedure to determine ADEs from the obtained phenotypic profiles.

Specific Aim 3. Compare ADEs and PODs for different chemical classes. Can ADEs derived from *in vitro* phenotypic profiles provide conservative estimates of PODs? We will systematically compare ADEs and PODs for the 13 chemical classes, and identify specific classes in which median phenotypic-profile-derived ADEs correlate with median PODs. Chemical classes that do not follow this general trend or with median ADEs that are higher than median PODs may not be properly modelled by our models, and thus will be excluded from further analysis. For each of the remaining chemical classes, we

will determine the correlation between the ADEs and PODs of all the chemicals within the class.

For chemical classes with low intraclass ADE-POD correlations or with ADEs that are higher than PODs, we will determine if the insufficiencies are due to the toxicokinetic (HTTK data) or toxicodynamics (phenotypic profiles) models. We will also compare ADEs based on phenotypic profiles and existing Toxcast *in vitro* assays, and determine if the later may provide complementary information that can further improve the intraclass ADE-POD correlations. Based on all the results, we will derive a strategy to use data from both the HIPPTox platform and existing Toxcast assays to model *in vitro* bioactivity.

For chemical classes with high intraclass ADE-POD correlations, *in vitro* phenotypic profiles may be used to replace animal tests for assessing their safety. We will also study the structures of the chemicals within each of these classes to determine if their structures (such as the lengths of their side chains, etc.) may be associated to the differences in their ADE or POD values. The results will help us to develop structure-activity relationships that may be used to evaluate novel chemicals specifically for these classes of chemicals.

3. PROJECT SCHEDULE / TIME FRAME

	Milestones	Who	Year 1			
			Q1	Q2	Q3	Q4
1	Receive the first batch of chemicals (64 chemicals) from NCCT/EPA	NCCT/EPA	X			
2	Test the auto-fluorescence and solubility of the first batch of chemicals	BII/A*STAR	X			
3	Perform treatment and imaging experiments for the first batch of chemicals in human liver, kidney, and lung cell lines	BII/A*STAR	X	X	X	
4	Quantify the phenotypic responses of to the first batch of chemicals.	BII/A*STAR	X	X	X	
5	Request the second batch of chemicals (~10-15 chemicals) from NCCT/EPA	BII/A*STAR			X	
6	Receive the second batch of chemicals from NCCT/EPA	NCCT/EPA			X	
7	Test the auto-fluorescence and solubility of the second batch of chemicals	BII/A*STAR			X	
8	Perform treatment and imaging experiments for the second batch of chemicals in human liver, kidney, and lung cell lines	BII/A*STAR			X	X
9	Quantify the phenotypic responses of to the second batch of chemicals.	BII/A*STAR			X	X
10	Obtain HTTK data of all the chemicals from NCCT/EPA	NCCT/EPA		X	X	X
11	Derive ADEs based on the phenotypic profiles and HTTK data	BII/A*STAR			X	X
12	Compare ADEs and PODs for different chemical classes	NCCT/EPA and BII/A*STAR			X	X
13	Manuscript preparation	NCCT/EPA and BII/A*STAR				X

14	Disseminate the results to our collaborators and setup further collaborations to expand the technologies based on more chemicals	NCCT/EPA and BII/A*STAR				X

4. DELIVERABLES

- (EPA) Up to 80 selected chemicals in DMSO stocks
- (EPA) HTTK data for the selected chemicals
- (A*STAR) A list of ADE values derived from the phenotypic profiles and/or existing Toxcast *in vitro* assays for the finally selected chemicals,
- (A*STAR) A list of chemical classes for which ADEs derived from phenotypic profiles provide conservative estimates of PODs, and
- (A*STAR and EPA) One journal publication.

5. INPUTS TO THE PROJECT / RESOURCES

All the experiments and computational analyses for phenotypic profiling will be performed by BII, *ASTAR.

The HTTK data and additional *in vitro* bioactivity values from the Toxcast database for these chemicals, and the toxicokinetic modeling methods to derive ADE values are either publicly available or will be provided by NCCT, EPA.

The POD values for the master list of chemicals are public available from EPA, Health Canada, ECHA, and/or EFSA based on the databases in the respective agencies.

Finally, BII, A*STAR and NCCT, EPA will participate in the analysis, interpretation, and report of the final datasets.

6. BUDGET

6.1 A*STAR Budget

Contribution by A*STAR	**Amount (SGD)**
EOM	
Research officer	$65,000
OOE	
Reagents and materials for cell culture, antibodies and dyes, assay plates, plasticware, tips, and other consumables	$45,000
TRAVEL	
Oversea travel (meeting with collaborators)	$5,000
Grand total	**$115,000**

6.2 EPA Budget

Tasks – Send chemicals to A*STAR, compare chemical list to determine data available for chemicals of interest.

	1st year (S$)	2nd year (S$)	3rd year (S$)	Total (S$)
Personnel Resources w/benefits	$98,361	$5,410	$243	$104,015
Chemical prep/shipment	$3,000	$00	$00	$3,000
Research services	$5,000	$5,000	$00	$10,000
Total	**$106,361**	**$10,410**	**$243**	**$117,015**

7. BACKGROUND INTELLECTUAL PROPERTY

(Please list down each Party's Background IP to be utilized in this project, if applicable.)

7.1 BII, A*STAR:

- Phenotypic profiling analysis methods and markers/endpoints for specific cell types (patent-pending)
- Image processing and analysis software (copy-righted)
- Cell culture, treatment, and imaging procedures (know-now)

7.2 NCCT, EPA:

- ToxCast/inVitroDB

8. FOREGROUND INTELLECTUAL PROPERTY

Likelihood of protectable IP from this Project?

- **Patentable invention**

  Possible

- **Other form of IP (e.g.: Proprietary Know-How/Copyright)**

  Unlikely

Likelihood of commercialising/licensing the discovery/invention from this Project?

- **Commercialisation**

  Possible