ARPAE

Agenzia regionale per la prevenzione, l'ambiente e l'energia

dell'Emilia - Romagna

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Atti amministrativi

Determinazione dirigenziale	n. DET-2018-810 del 12/10/2018
Oggetto	Direzione Tecnica. Approvazione del Collaboration Agreement No. 35226 tra il Joint Research Centre della Commissione Europea e il Centro di Saggio Vitrox dell'Agenzia Regionale per la Prevenzione, l'Ambiente e l'Energia dell'Emilia-Romagna.
Proposta	n. PDTD-2018-811 del 10/10/2018
Struttura adottante	Direzione Tecnica
Dirigente adottante	Zinoni Franco
Struttura proponente	Ctr Tossicologia Ambientale
Dirigente proponente	Dott.ssa Colacci Annamaria
Responsabile del procedimento	Colacci Annamaria

Questo giorno 12 (dodici) ottobre 2018 presso la sede di Largo Caduti del Lavoro, 6 in Bologna, il Direttore Tecnico , Dott. Zinoni Franco, ai sensi del Regolamento Arpae sul Decentramento amministrativo, approvato con D.D.G. n. 87 del 01/09/2017 e dell'art. 4, comma 2 del D.Lgs. 30 marzo 2001, n. 165 determina quanto segue.

Oggetto: Direzione Tecnica. Approvazione del Collaboration Agreement No. 35226 tra il Joint Research Centre della Commissione Europea e il Centro di Saggio Vitrox dell'Agenzia Regionale per la Prevenzione, l'Ambiente e l'Energia dell'Emilia-Romagna.

VISTI:

- la L.R. 19 aprile 1995, n. 44 e s.m.i. che istituisce l'Agenzia Regionale per la Prevenzione e l'Ambiente (ARPA) e riorganizza le strutture preposte ai controlli ambientali ed alla prevenzione collettiva;
- l'art. 5 della legge citata L.R. 44/1995 che, al comma 2, prevede che "per l'adempimento delle proprie funzioni, attività e compiti, ARPA può definire accordi o convenzioni con Aziende ed Enti pubblici, operanti nei settori suolo, acque, aria, ambiente";
- la L.R. 30 luglio 2015 n. 13 "Riforma del sistema di governo regionale e locale e disposizioni su città metropolitana di Bologna, province, comuni e loro unioni" che, all'articolo 16 ridenomina questo ente "Agenzia Regionale per la Prevenzione, l'Ambiente e l'Energia dell'Emilia-Romagna" (acronimo Arpae) estendendone le competenze;
- l'art. 15 della L. 7 agosto 1990, n. 241, ai sensi del quale le Pubbliche Amministrazioni possono concludere tra loro accordi per disciplinare lo svolgimento in collaborazione di attività di interesse comune;

RICHIAMATA:

- la Direttiva 2010/63/EU sulla protezione degli animali usati per propositi scientifici, e in particolare l'articolo 47, che definisce:
  - gli obblighi della Commissione e degli Stati Membri in riferimento allo sviluppo e validazione di approcci alternativi;
  - l'obbligo per gli Stati Membri di assistere la Commissione nell'identificare ed indicare laboratori specializzati e qualificati per eseguire studi di validazione dei test alternativi;
  - l'obbligo per la Commissione di definire le priorità relativamente a tali studi e la distribuzione di tali studi tra i laboratori identificati a tale scopo;
  - l'obbligo per i Paesi Membri di assicurare a livello Nazionale la promozione e la diffusione dell'uso dei metodi alternativi;

RICHIAMATO ALTRESI':

- l'Allegato VII, della suddetta Direttiva 2010/63/EU riportante la lista dei doveri e delle azioni in capo ai laboratori della rete di riferimento europeo per i metodi alternativi, che includono fra gli altri:
  - coordinare e promuovere lo sviluppo e l'uso di alternative nelle procedure adottate nell'area della ricerca di base e applicata e dei test utilizzati in campo regolatorio;
  - coordinare le procedure di validazione di approcci alternativi a livello europeo;
  - agire come punto di riferimento per lo scambio di informazioni sullo sviluppo di approcci alternativi;
  - sviluppare, mantenere e gestire database pubblici e sistemi informativi sugli approcci alternativi e del loro stato di avanzamento;
  - promuovere il dialogo tra legislatori, regolatori e altri rilevanti portatori di interessi, specialmente dell'industria, della ricerca biomedica, delle organizzazioni dei consumatori e delle organizzazioni per il benessere degli animali, con particolare riferimento allo sviluppo, la validazione, l'accettazione in campo regolatorio, il riconoscimento internazionale e l'applicazione di approcci alternativi;
  - partecipare alla convalida di approcci alternativi;

PREMESSO:

- che il Joint Research Centre (JRC), in quanto Centro della Commissione Europea deputato ai servizi per il sapere scientifico e la conoscenza, fornisce supporto alle politiche comunitarie tramite la conduzione di ricerche che garantiscono consulenza scientifica indipendente lungo tutto il ciclo decisionale;
- che, mediante il laboratorio European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM), il Direttorato F "Health, Consumers and Reference Materials" del JRC conduce ricerche nel campo della validazione di metodi che permettano di ridurre, perfezionare o sostituire l'uso degli animali per gli studi di sicurezza e per gli studi di efficacia/potenza di sostanze chimiche, biologiche e vaccini;
- che, in base alla sopra citata Direttiva 2010/63/EU, è stata istituita la rete europea dei laboratori per la validazione dei metodi alternativi EU-NETVAL (European Union Network of Laboratories for the Validation of Alternative Methods) che ha lo scopo di fornire a EURL ECVAM supporto nella conduzione di studi di validazione miranti a valutare l'affidabilità e la rilevanza di metodi alternativi che hanno la potenzialità di sostituire, ridurre o perfezionare l'uso degli animali a propositi scientifici;
- che i rapporti della Commissione Europea con i laboratori della rete EU-NETVAL sono regolati dal EU-NETVAL Terms of Reference (ToR) del 26 novembre 2013, che costituisce l'allegato A) al presente atto quale parte integrante e sostanziale;

PREMESSO INOLTRE:

- che Arpae, in particolare il Centro Tematico Regionale (CTR) Tossicologia Ambientale della Direzione Tecnica, promuove ed esegue attività di ricerca sugli effetti tossicologici di inquinanti ambientali;
- che tale attività di ricerca comprende l'attività di sviluppo e l'implementazione di test alternativi per la predizione del rischio tossicologico da composti chimici e miscele ambientali;
- che, al fine di sviluppare e valutare test alternativi per la predizione del rischio tossicologico, Arpae ha allestito un laboratorio denominato Centro di Saggio Vitrox (da qui in avanti denominato Arpae - Vitrox) e collocato presso Arpae, Sezione di Bologna;
- che, dal dicembre 2015, il Centro di Saggio Arpae – Vitrox è entrato a fare parte della rete europea dei laboratori per la validazione dei metodi alternativi EU-NETVAL (European Union Network of Laboratories for the Validation of Alternative Methods);
- che Arpae-Vitrox ha risposto all'invito, lanciato da EURL ECVAM ai membri EU-NETVAL, per la partecipazione allo studio di validazione per metodi in vitro per l'identificazione di distruttori endocrini a bersaglio tiroideo "Call to EU-NETVAL Members for Participation in the validation study of in vitro methods for the detection of thyroid disruptors (Part1: Definition, and, Part2: Relevance)";
- che Arpae – Vitrox è stato tra i laboratori selezionati per la partecipazione al suddetto studio e, in particolare, alle attività riguardanti il metodo 6b) TR (ß)CALUX assay;
- che Arpae-Vitrox dovrà firmare con JRC il Collaboration Agreement, comprensivo dell'Annex 1, come risulta nell'allegato B) al presente atto quale parte integrante e sostanziale;

CONSIDERATO:

- che il Collaboration Agreement, allegato A,ha l'obiettivo generale di promuovere l'applicazione della Direttiva 2010/63/EU sulla protezione degli animali utilizzati a fini scientifici e di assicurare che le scoperte, le invenzioni e le creazioni prodotte nell'ambito del presente accordo siano utilizzate in modo coerente con l'interesse pubblico;
- che il Collaboration Agreement, allegato A, riporta in particolare i seguenti obiettivi:
    - la produzione di set di dati utilizzando il metodo assegnato per l'individuazione di distruttori endocrini tiroidei, secondo gli obiettivi (Tasks) definiti nell'EU-NETVAL (ToR):
        - ToR Task 4i Definizione e descrizione dei metodi in vitro;
        - ToR Task 4iii Valutazione della riproducibilità dei metodi in vitro;
        - ToR Task 4i Valutazione della capacità predittiva e del dominio di applicabilità dei metodi in vitro;

  - il miglioramento del coordinamento e dell'efficacia della cooperazione tra i laboratori della rete EU-NETVAL e la Commissione nel campo degli studi di validazione;
  - la promozione di interessi reciproci e di collaborazione nel comprendere e risolvere le problematiche tecniche insorte durante la sperimentazione;
  - di approfondire la comprensione delle problematiche scientifiche correlate alla realizzazione di studi di validazione;
- che, per Arpae, che partecipa in qualità di laboratorio aderente alla rete EU-NETVAL, la sottoscrizione del presente Collaboration Agreement rappresenta un'opportunità perché permette un confronto con importanti realtà a livello europeo in merito alle migliori azioni e ai più appropriati approcci metodologici per la realizzazione di studi volti allo sviluppo di test per inquinanti ambientali con azione di distruttori endocrini;
- che, con l'approvazione del Collaboration Agreement, sono definiti gli impegni e le responsabilità di ciascun partecipante;
- che, per il pieno raggiungimento degli obiettivi del Collaboration Agreement, le Parti dovranno agire così come definito dal Collaboration Agreement – Annex1 e, in particolare, Arpae-Vitrox si impegna ad eseguire entrambe le parti dello studio di validazione (Part 1 and Part 2) così come descritte nell'Annex1;
- che l'esecuzione della Part 2 dello studio di validazione inizierà solo dopo che EURL-ECVAM avrà dichiarato che la Part 1 dello studio si è conclusa con successo;
- che il JRC si impegna a mettere a disposizione di Arpae-Vitrox le sostanze in esame, di riferimento e i rispettivi controlli, il sistema di saggio e la procedura generale e/o le procedure operative standard per l'esecuzione del metodo 6b) TR (ß)CALUX assay, così come dettagliato nell'articolo 2 del Collaboration Agreement;
- che Arpae si impegna a utilizzare quanto fornito da JRC secondo le condizioni specificate nell'articolo 2 del Collaboration Agreement e a mettere a disposizione tutte le risorse necessarie per la realizzazione dello studio e, in particolare, il personale e la strumentazione, i reagenti e il materiale di consumo non sopra specificati;
- che, come indicato nell'articolo 7 del Collaboration Agreeement, e in accordo a quanto definito in EU-NETVAL ToR, non ci sarà trasferimento di denaro tra le parti in correlazione allo studio richiesto;

DATO ATTO:

- che il Collaboration Agreement entrerà in vigore quando tutte le seguenti condizioni saranno soddisfatte, così come specificato nell'Articolo 11:
  - entrambe le Parti avranno sottoscritto il Collaboration Agreement;
  - il laboratorio Arpae – Vitrox avrà ricevuto dal JRC il sistema di saggio, le necessarie sostanze di riferimento e i relativi controlli e le procedure generali per l'esecuzione del metodo assegnato;
  - il laboratorio Arpae – Vitrox avrà ricevuto dal JRC una lettera di conferma, che autorizza l'inizio dello studio di validazione;la data di tale lettera di conferma costituirà la data di piena entrata in vigore del Collaboration Agreement;
- che il progetto avrà una durata di n. 24 mesi, a partire dalla data riportata nella lettera di conferma dell'avvio dello studio di cui al punto precedente;
- che, nel caso in cui lo studio di validazione non sia completato nell'arco di 24 mesi, il Collaboration Agreement si rinnoverà automaticamente per un periodo addizionale di 12 mesi;
- che il Collaboration Agreement tra Arpae-Vitrox e JRC potrà essere esteso o modificato solo mediante un accordo scritto firmato dai rappresentati debitamente autorizzati di entrambe le parti;
- che per Arpae il soggetto competente all'attuazione e alla gestione del presente Collaboration Agreement è la Direzione Tecnica con il Centro di Saggio Arpae- Vitrox;
- che per Arpae il coordinatore dello studio di validazione di cui al presente Collaboration Agreement sarà la Dottoressa Annamaria Colacci, nella funzione di Direttore del Centro di Saggio Arpae-Vitrox della Direzione Tecnica;
- che per JRC il punto di contatto e coordinamento per lo studio di validazione è rappresentato dalla Dottoressa Sandra Coecke;
- che tutte le notifiche, comunicazioni e documenti che ricadono nell'ambito del presente Collaboration Agreement saranno inviati ai coordinatori attraverso i canali di comunicazione definiti nell'Articolo 4 del Collaboration Agreement;

RITENUTO OPPORTUNO:

- per quanto esposto in precedenza, che Arpae partecipi allo studio di validazione del metodo assegnato 6b) TR (ß)CALUX assay secondo quanto dettagliato nel Collaboration Agreement;
- pertanto, sottoscrivere il Collaboration Agreement nr. 35226, ed il relativo Annex 1, allegato B);
- nominare coordinatore dello studio di validazione di cui al presente Collaboration Agreement il Responsabile del Centro Tematico Regionale Tossicologia Ambientale, Dott.ssa Annamaria Colacci, nella funzione di Direttore del Centro di Saggio Arpae-Vitrox della Direzione Tecnica;

SU PROPOSTA:

- della Dottoressa Annamaria Colacci, Responsabile del Centro Tematico Regionale Tossicologia Ambientale, la quale ha espresso, ai sensi del Regolamento per il Decentramento amministrativo, il proprio parere favorevole in ordine alla regolarità amministrativa del presente provvedimento;

DATO ATTO:

- che il responsabile del procedimento è la stessa Dott.ssa Annamaria Colacci, Responsabile del Centro Tematico Regionale Tossicologia Ambientale;

DETERMINA

1. di approvare, e pertanto sottoscrivere, sulla base dei EU-NETVAL Terms od Reference (allegato A), il Collaboration Agreement No. 35226 tra il Joint Research Centre (JRC) della Commissione Europea e il Centro di Saggio Vitrox dell'Agenzia Regionale per la Prevenzione, l'Ambiente e l'Energia dell'Emilia-Romagna, allegato B) al presente atto quale parte integrante e sostanziale;
2. di dare atto che le attività da svolgere sono dettagliate nell'Annex 1, allegato al Collaboration Agreement nr. 35226;
3. di dare atto che lo studio di validazione ha una durata di 24 mesi, a decorrere dalla data della lettera di conferma avvio attività trasmessa dal JRC eche, qualora lo studio di validazione non sia completato nell'arco di 24 mesi, il Collaboration Agreement si rinnoverà automaticamente per un periodo addizionale di 12 mesi ;
4. di dare atto che, come indicato nell'articolo 7 del Collaboration Agreeement (allegato B), e in accordo a quanto definito in EU-NETVAL Terms od Reference (Allegato A), non ci sarà trasferimento di denaro tra le parti in correlazione allo studio di validazione;
5. di nominare coordinatore dello studio di validazione di cui al presente Collaboration Agreement il Responsabile del Centro Tematico Regionale Tossicologia Ambientale, Dott.ssa Annamaria Colacci, nella funzione di Direttore del Centro di Saggio Arpae-Vitrox della Direzione Tecnica;

IL DIRETTORE TECNICO

(F.to Dott. Franco Zinoni)

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

EURL ECVAM's

European Union Network of Laboratories for the Validation of Alternative Methods

- EU-NETVAL -

Terms of Reference

26 November 2013

European Commission Joint Research Centre · I-21027 Ispra (VA), Italy · TP 580
Institute for Health and Consumer Protection
European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)
http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eu-netval
E-mail: JRC-ECVAM-NETVAL@ec.europa.eu

1. Introduction

EU-NETVAL's mission is to provide support for EURL ECVAM validation studies that serve to assess the reliability and relevance of alternative methods that have a potential to replace, reduce or refine the use of animals for scientific purposes.

This document outlines the Terms of Reference for EU-NETVAL including the legislative anchor, the establishment of the network and the maintenance of its membership, tasks of network members and of EURL ECVAM in support of validation studies, the allocation of tasks to members, and the financing of network activities.

2. Background

2.1. Legislative anchor

The European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) was established by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of the European Commission's Joint Research Centre to address some of the provisions of Directive 2010/63/EU on the protection of animals used for scientific purposes. Article 47 of the Directive provides that;

> *"1. **The Commission and the Member States shall contribute to the development and validation of alternative approaches** which could provide the same or higher levels of information as those obtained in procedures using animals, but which do not involve the use of animals or use fewer animals or which entail less painful procedures, and they shall take such other steps as they consider appropriate to encourage research in this field.*
>
> *2. **Member States shall assist the Commission in identifying and nominating suitable specialised and qualified laboratories to carry out such validation studies**.*
>
> *3. After consulting the Member States, the Commission shall set the priorities for those validation studies and allocate the tasks between the laboratories for carrying out those studies.*
>
> *4. Member States shall, at national level, ensure the promotion of alternative approaches and the dissemination of information thereon."*

In line with Article 48 of the Directive, Annex VII lists the duties and tasks of the EU Reference Laboratory, covering *inter alia*:

> *"(a) coordinating and promoting the development and use of alternatives to procedures including in the areas of basic and applied research and regulatory testing;*
>
> *(b) coordinating the validation of alternative approaches at Union level;*
>
> *(c) acting as a focal point for the exchange of information on the development of alternative approaches;*
>
> *(d) setting up, maintaining and managing public databases and information systems on alternative approaches and their state of development;*
>
> *(e) promoting dialogue between legislators, regulators, and all relevant stakeholders, in particular, industry, biomedical scientists, consumer organisations and animal-welfare groups, with a view to the development, validation, regulatory acceptance, international recognition, and application of alternative approaches."*

EU-NETVAL will facilitate the Union Reference Laboratory meeting its objectives under Article 48. Furthermore, membership in EU NETVAL provides one channel, among others, for both the Commission and the Member States to actively contribute to the development and validation of alternative approaches as required by the Directive.

2.2. Validation of alternative methods

Validation is essential to ensure the acceptance and use of alternative (non-animal) approaches for a range of scientific purposes by a variety of end-users. It is also a prerequisite for the development of international standards and test guidelines that underpin regulatory decision making and global trade. Internationally accepted validation principles for *in vitro* methods are described in the OECD (2005) "Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment"[1] (no. 34) which sets out the essential considerations and steps to assess the *reliability* and *relevance* of an *in vitro* method.

The information required to demonstrate the reliability and relevance of an *in vitro* method in a systematic and comprehensive manner can be captured in seven independent 'validation modules' [2]. These modules address; 1) Definition and description of the method, 2) Within-laboratory reproducibility, 3) Transferability between laboratories, 4) Between-laboratory reproducibility, 5) Predictive capacity, 6) Applicability domain, and 7) Performance standards. Approaching validation in this modular way allows for consistency, flexibility and efficiency in the overall process.

Information addressing these validation modules may be gathered retrospectively from existing data sources. However, for novel *in vitro* methods much of the required data is usually missing and therefore needs to be generated within a prospective validation study, ideally under the Good Laboratory Practice (GLP) quality system. A typical prospective validation study commences with the analysis and optimisation of the *in vitro* method procedure to ensure that it is sufficiently well defined. Subsequently, data are generated on selected reference chemicals to demonstrate its reproducibility within an experienced laboratory. Thereafter the *in vitro* method is transferred to three or more test facilities and a between-laboratory ring trial carried out to demonstrate the between-laboratory reproducibility, thus completing the reliability assessment. Subsequent data generation in one or more laboratories provides the datasets and information required to determine how predictive the method is in relation to its intended purpose, its applicability domain (e.g. biological, physicochemical) and to establish performance standards for the class of assay that the *in vitro* method represents. The results of a validation study are compiled in a validation report and typically undergo review by the EURL ECVAM Scientific Advisory Committee (ESAC) before being released in the form of a EURL ECVAM Recommendation. A description of the EURL ECVAM validation process can be found at http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvams-validation-process.

[1] OECD 2005. OECD Series on Testing and Assessment. Number 34. Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. ENV/JM/MONO(2005)14.

[2] Hartung et. al, "A modular approach to the ECVAM principles on test validity", ATLA 32, 467–472, 2004

3. Appointment of Members

3.1 Establishment of EU-NETVAL

In order to establish EU-NETVAL through the appointment of its first members, the National Contact Points (NCPs) for Directive 2010/63/EU were requested by the Commission to provide a list of candidate test facilities (laboratories) that had shown interest in their respective Member State. These candidates were subsequently contacted by EURL ECVAM and invited to provide information on their test facilities by completing an on-line questionnaire. Each test facility was then evaluated against predefined eligibility criteria that had been previously agreed with NCPs. Out of the initial list of candidates received, 13 were deemed to be eligible. During July 2013 and after consultation with NCPs, these facilities were formally appointed by EURL ECVAM as EU-NETVAL members. The names of these first members were published on the EURL ECVAM website.

3.2 Expanding EU-NETVAL membership

EURL ECVAM intends to publish open calls for selection of new EU-NETVAL members on an ad hoc basis, depending on anticipated tasks, required member profiles, and the overall capacity of the network. The first call for new members was opened in July 2013 and published on the EURL ECVAM website. The closing date for applications is on the 25th October 2013. Currently application for membership of EU-NETVAL is restricted to test facilities based in the EU, EFTA countries and EU candidate countries. Similar to the process adopted for the initial establishment of the network, candidate facilities that apply to EURL ECVAM for membership will be evaluated against the eligibility criteria. Eligible facilities will be appointed by EURL ECVAM after consultation with the NCPs. The names of newly appointed members will be published on the EURL ECVAM website.

3.3 Duration of membership of EU-NETVAL

The duration of EU-NETVAL membership is indefinite as long as a member facility continues to satisfy the eligibility criteria. Members are, however, free to resign from the network at any time by simply sending a formal letter of notification to EURL ECVAM. Members will be expected to resign from the network if their circumstances change resulting in their facility failing to meet the eligibility criteria. The most recent list of EU-NETVAL members will be available from the EURL ECVAM website.

4. Tasks of Members

EU-NETVAL members are expected to support validation studies through the execution of one or more specific tasks. The support sought from members will vary in scope depending on the task and the capacities and the areas of expertise of the members. Tasks constituting this support will address the particular data and information requirements of one or more validation modules applicable to the study. Tasks include;

i. **Definition and description of *in vitro* methods**
Support the definition of *in vitro* method procedures including the technical assessment (non-experimental or experimental) of Standard Operating Procedures

(SOPs) in terms of their scientific basis, completeness, clarity, robustness and suitability for implementation within a GLP environment. This includes reflecting the definition of an *in vitro* method in a suitably elaborated method description, prepared in a format fit for public dissemination through EURL ECVAM's database on alternative methods, DB-ALM (see http://ecvam-dbalm.jrc.ec.europa.eu/).

ii. **Transfer of *in vitro* methods between laboratories**
Support the demonstration and assessment of the transferability of *in vitro* methods between one laboratory and another. This includes the preparation of technical training courses and related training materials on the method undergoing validation to aid in the transfer process.

iii. **Assessment of the reproducibility of *in vitro* methods**
Support the generation of datasets on selected reference chemicals for the assessment of within-laboratory and between-laboratory reproducibility of *in vitro* methods. This may include participation in a multi-laboratory ring-trial and acting as a lead laboratory for such trials, if appropriate.

iv. **Assessment of the predictive capacity and applicability domain of *in vitro* methods**
Support the generation of datasets on selected reference chemicals for the performance assessment of an *in vitro* method in relation to its predictive capacity in relation to its intended purpose and/or its contribution to an integrated testing strategy or testing battery. This will also include the assessment of the mechanistic, chemical, physico-chemical, sectorial and regulatory applicability domains of *in vitro* methods, and the generation of datasets suitable for the establishment of performance standards for particular classes of *in vitro* method.

v. **Guidance documents and training materials supporting validation**
Support the development of guidance documents and training materials covering various technical aspects of good *in vitro* method development and practices in order to sustain a high level of efficiency and effectiveness of the network in supporting validation studies, and to expand its capacity and expertise in order to keep pace with technological and methodological developments that are reflected in methods submitted for validation.

vi. **Surveillance of uptake and use of validated *in vitro* methods**
Support the surveillance of the uptake and use of *in vitro* methods that have undergone validation to assess in-field post-validation performance against the originally intended purpose and to exploit data generated by end-users to further refine the method description and review the applicability domain.

5. Tasks of EURL ECVAM

The primary tasks of EURL ECVAM in the context of its participation within EU-NETVAL are the following;

i. **Coordination of EU-NETVAL**
EURL ECVAM will coordinate the EU-NETVAL network.

ii. **Definition and description of *in vitro* methods**
Finalise the definition of *in vitro* methods including the technical assessment (non-experimental or experimental) of Standard Operating Procedures (SOPs) in terms of their scientific basis, completeness, clarity, robustness and suitability for implementation within a GLP environment. This includes ensuring that the definition of *in vitro* methods are suitably elaborated as method descriptions and prepared in a format fit for public dissemination through EURL ECVAM's database on alternative methods, DB-ALM[3]

iii. **Assessment of the reproducibility of *in vitro* methods**
Generate GLP compliant test data to determine within-laboratory reproducibility of an *in vitro* method subject to validation. This will also serve as a preparatory step towards the design and execution of validation ring-trials carried out by EU-NETVAL members.

iv. **Management of validation studies**
Manage EURL ECVAM validation studies according to project plans which includes aspects such as finalisation of test definition and SOPs, training and support of facilities participating in validation studies, provision of materials, collection and statistical analysis of test data, and the preparation of validation reports.

v. **Selection of test facilities for membership of EU-NETVAL**
Select EU-NETVAL members as the need arises through the publication of calls and the assessment of applicant test facilities against eligibility criteria.

vi. **Selection of test facilities to support validation studies**
Select EU-NETVAL members for the execution of tasks in support of validation studies based on specific allocation criteria as detailed in each task proposal presented to EU-NETVAL members.

vii. **Guidance documents and training materials supporting validation**
Take the lead in the development of guidance documents and training materials covering various technical aspects of good *in vitro* method development and practices in order to sustain a high level of efficiency and effectiveness of the network in its support of validation studies, and to expand its capacity and expertise in order to keep pace with technological and methodological developments that are reflected in methods submitted for validation.

viii. **Technical training courses and materials on validated *in vitro* methods**
Development and running of training courses and preparation of related training materials aimed primarily at supporting validation studies but which will also facilitate the dissemination and uptake of *in vitro* methods within the EU and the use of GLP as a quality system for *in vitro* test facilities.

ix. **Harmonisation and standardisation of *in vitro* methods**
Facilitate the international harmonisation and standardisation of validated *in vitro* methods to aid their translation into internationally recognised standards and test

[3] http://ecvam-dbalm.jrc.ec.europa.eu/

guidelines and to ensure their acceptance for regulatory use.

x. **Information exchange on best practice regarding *in vitro* methods**
Facilitate information exchange within EU-NETVAL on best practice concerning *in vitro* method development, validation and application, including exploitation of new technologies.

xi. **Promotion of EU-NETVAL and informing on activities**
Keep NCPs, collaboration partners (e.g. the European Partnership on Alternative Approaches to Animal Testing, the International cooperation on Alternative Testing Methods), stakeholders and the general public informed on EU-NETVAL activities and take a leading role in promoting the work of EU-NETVAL and ensuring its visibility both within the EU and world-wide.

xii. **Enabling collaboration**
Provide opportunities, processes, tools and coordination for efficient and effective collaboration between both network members and between EU-NETVAL and cooperation partners.

6. Allocation of tasks and consultation of Member States

EU-NETVAL members will be invited to submit a proposal to EURL ECVAM to undertake a specific task that has been proposed to the network. After receiving these proposals, EURL ECVAM will evaluate them against the allocation criteria defined for that task. Based on this evaluation, EURL ECVAM will select the members to undertake the task. Before final allocation of tasks to selected EU-NETVAL members, Member States will be consulted via the NCPs.

7. Financing of EU-NETVAL activities

The financing model employed to support EU-NETVAL activities will vary in nature depending on the specific task(s). In general however, the costs of EU-NETVAL activities are covered by a combination of direct and indirect (e.g. in-kind) financing from Member States, EU-NETVAL members, and the Commission.

EU-NETVAL members and Member States are typically expected to provide the necessary human resources and to cover related costs when undertaking the execution of a task. Support given by the Commission could include task coordination and reporting of task and study outcomes, the training of participating facilities on an *in vitro* method associated with a validation study, the supply of some *in vitro* method materials such as test systems (e.g. cells or tissues), chemicals and other consumables, the provision of data-reporting templates and bio-statistical support, and the organisation of virtual and physical meetings when necessary.

The model does not impose obligations on the Member States beyond those imposed by Directive 2010/63/EU.

On occasion, the GLP facility of EURL ECVAM will also carry out a laboratory evaluation of *in vitro* methods prior to the launch of a EU-NETVAL validation study to ensure, for example, that the *in vitro* method procedure is optimised and sufficiently described.

8. Abbreviations

EFTA: The European Free Trade Association
EU: European Union
EU-NETVAL: The European Union Network of Laboratories for the Validation of Alternative Methods
EURL ECVAM: European Union Reference Laboratory for Alternatives to Animal Testing
GLP: Good Laboratory Practice
JRC: [European Commission] Joint Research Centre
NCPs: National Contact Points of the Member States as provided under Article 59 of Directive 2010/63/EU on the protection of animals used for scientific purposes
SOP: Standard Operating Procedure

COLLABORATION AGREEMENT

The **Joint Research Centre of the European Commission**, located at Ispra, Italy, represented for the purpose of signing this Agreement by Dr. Elke Anklam, Director of Directorate F. Health, Consumers and Reference Materials of the Joint Research Centre, duly entitled to sign,

(hereinafter referred to as '**the JRC**'),

and

The Agenzia Regionale per la Prevenzione, l'Ambiente e l'Energia - Emilia Romagna (**Arpae**)– **Arpae-Vitrox Test Facility** with the registered address at Bologna, Via Po 5, I-40139 Italy, represented for the purpose of signing this agreement by Dr. Franco Zinoni, Arpae Technical Director, duly entitled to sign,

(hereinafter referred to as 'Arpae-**Arpae-Vitrox**').

Hereinafter referred to individually as '**the Party**' or collectively as '**the Parties**'.

PREAMBLE

WHEREAS:

Arpae-Vitrox is a member of the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL).

As the science and knowledge service of the European Commission, the Joint Research Centre's mission is to support EU policies with independent evidence throughout the whole policy cycle.

Through its European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) the Directorate F Health, Consumers and Reference Materials of the Commission's Joint Research Centre conducts research in the field validation of methods which reduce, refine or replace the use of animals for safety testing and efficacy/potency testing of chemicals, biologicals and vaccines.

The European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) was set up on the basis of Directive 2010/63/EU on the protection of animals used for scientific purposes and its mission is to provide support for EURL ECVAM validation studies that serve to assess the reliability and relevance of alternative methods that have a potential to replace, reduce, or refine the use of animals for scientific purposes.

Following the invitation to EU-NETVAL Members for **Participation in the validation study of *in vitro* methods for the detection of thyroid disruptors** (Part 1: Definition, and, Part 2: Relevance) launched by EURL ECVAM, Arpae-Vitrox has been selected to participate in the above validation study and more specifically for the activities regarding the allocated method(s) **(6b) TR (β) CALUX assay.**

The JRC and Arpae-Vitrox are for that purpose signing this Collaboration Agreement.

THE PARTIES HAVE AGREED AS FOLLOWS:

ARTICLE 1 – OBJECTIVES OF THIS COLLABORATION AGREEMENT

1.1 The general objective of this Collaboration Agreement is to contribute more effectively to the implementation of the Directive 2010/63/EU on the protection of animals used for scientific purposes and to ensure that discoveries, inventions and creations generated under this Collaboration Agreement are utilized in ways most likely to benefit the public.

1.2 This Collaboration Agreement will, in particular, have the following objectives:

a) To generate data sets using the allocated method(s) for detection of thyroid disruption, corresponding to the Tasks as defined in the EU-NETVAL Terms of Reference (ToR):
   - ToR Task 4 i. Definition and description of *in vitro* methods
   - ToR Task 4 iii. Assessment of the reproducibility of *in vitro* methods
   - ToR Task 4 iv. Assessment of the predictive capacity and applicability domain of *in vitro* methods

b) To improve the co-ordination and effectiveness of co-operation efforts between the EU-NETVAL Test Facility and the Commission in the field of validation studies.
c) To promote mutual interest and co-operation in understanding and resolving technical experimental issues.
d) To deepen the understanding of the scientific issues relating to validation studies.

1.3 In order to fully achieve the objectives of this Collaboration Agreement, the Parties will take the actions as defined in the Annex 1 to this Collaboration Agreement. Arpae-Vitrox commits to the execution of both parts of the validation study (Part 1 and Part 2) as described in the Annex 1.

1.4 In order to achieve a successful implementation of the method(s) allocated, Arpae-Vitrox is advised to contact the test developer for discussing technical issues, input and trouble-shooting. The JRC shall be kept informed in case of contacts between Arpae-Vitrox and the method developer of the allocated method(s).

1.5 Execution of Part 2 of the validation study shall begin following the successful completion of Part 1 declared by EURL ECVAM. The exact number of chemicals to be tested in Part 2 will be communicated after completion of Part 1.

1.6 Following successful completion of Part 2 Arpae-Vitrox may be invited to participate in a follow-up study.

ARTICLE 2 – RESPONSIBILITIES OF PARTIES

2.1 Each Party will be responsible for its personnel in relation to activities undertaken pursuant to this Collaboration Agreement. For the purposes of this Collaboration Agreement, '**personnel**' shall mean all persons associated with one Party, including (i) employees, (ii) guest researchers, (iii) persons under contracts similar to employment contracts and (iv) any other persons whose actions can be reasonably attributed to that Party.

2.2 When it is necessary for personnel from one Party to participate for brief periods in carrying out activities implemented by the other Party in accordance with the provisions of Article 1.3, the Parties shall conclude a separate agreement as regards the invitation of their personnel to perform work at the other Party's facilities. The agreement shall regulate their mutual rights and obligations, the conditions of co-operation to be provided

by the personnel, and the terms under which the Parties authorise their respective personnel to participate. Invited personnel shall comply with the rules and working conditions of the host Party. Invitation of persons not directly associated with one Party, for example, persons associated with subcontractors, is not permitted

2.3 The host Party will reasonably assist in meeting the personal and professional needs of the visitor, including access to institutional facilities within the context of the regulations in force at the host site.

2.4 For the purpose of the implementation of this Collaboration Agreement, each Party shall put in place policy that assigns to the Party all rights in any intellectual property generated by the Party's personnel (or – in case of subcontracting – by the subcontractor or its personnel), allowing the Party to efficiently assert ownership as required under Article 8 of this Collaboration Agreement. If the foregoing is not possible under the applicable law, the policy must ensure that the Party acquires other legal title to the intellectual property as close as possible to ownership; in that case, other provisions of this Collaboration Agreement shall be interpreted in a way to accommodate the changed legal title to the intellectual property. Upon a specific request of the other Party, the Party concerned shall provide in writing clarifications of its policy to assert the ownership or other legal title to the intellectual property (where required).

2.5 The JRC will provide to Arpae-Vitrox the necessary reference, control and test items for the activities described under Article 1. The JRC reserves the right to amend the number of test items for Part 2 of the validation study.

2.6 The JRC will provide to Arpae-Vitrox the test system(s) for the allocated method(s). Dedicated agreements (e.g. material transfer agreement) for each specific test system will be put in place by the JRC with the relevant parties. In view of consistency Arpae-Vitrox shall align with those terms, namely it shall not transfer to third parties the test system(s), nor disclose to third parties the technical information on the genetic modification and handling of the material, except where required by governmental entities with respect to safety level.

2.7 The JRC will provide to Arpae-Vitrox an outline procedure/sets of standard operating procedures for the allocated method(s). A dedicated agreement for the use of the method will be put in place by the JRC with the relevant party. Arpae-Vitrox shall not transfer nor disclose to third parties the outline procedure/sets of standard operating procedures.

2.8 Arpae-Vitrox agrees not to use the provided test system(s) for any other purposes than described in the Annex. JRC will communicate when the validation study is officially completed and when test system(s) need to be destroyed. Arpae-Vitrox agrees to destroy the remaining test system(s) (frozen or in culture) not later than 4 weeks after the completion of the validation study. In case of use of the material beyond the scope of the validation described in this Collaboration Agreement, Arpae-Vitrox shall address the test system supplier in view of an agreement.

2.9 If JRC so requests, Arpae-Vitrox agrees to return to JRC the remaining test system(s) and test items for analysis.

2.10 Arpae-Vitrox will perform the activities, described in the Annex 1, according to its in-house quality system.

ARTICLE 3 – LIABILITY

3.1 Any loss, damage or injury of non-nuclear origin suffered by one Party in connection with the performance of this Collaboration Agreement or the specific agreement shall be borne exclusively by it. If the loss, damage or injury is caused by a person invited by one Party, as described in Article 2.2, the sending Party will be liable for it.

3.2 Each Party shall be exclusively liable for any loss, damage or injury of non-nuclear origin caused by its personnel to third parties, arising out of the performance of this Collaboration Agreement or the specific agreement.

3.3 Each Party shall indemnify the other Party for all liability in respect of any action for damages brought by third parties and caused by their respective personnel in the course of the performance of this Collaboration Agreement or the specific agreement.

3.4 Any liability for loss, damage or injury of nuclear origin will be determined by the legislation of the state in which the installation, which is at the origin of the loss, damage or injury, is located.

ARTICLE 4 – COORDINATION

4.1 The Parties shall designate one person to serve as its co-ordinator for any matter concerning the implementation of this Collaboration Agreement. The co-ordinators may nominate other suitable persons to represent them or to attend meetings related to the execution of this Collaboration Agreement

4.2 The contact-point and coordinator in the JRC for this validation study shall be Dr. Sandra Coecke with contact details sandra.coecke@ec.europa.eu, telephone number +39-332789806 and functional mailbox JRC-ECVAM-NETVAL@ec.europa.eu.

The coordinator of Arpae-Vitrox shall be Dr. Annamaria Colacci with contact details annamaria.colacci@unibo.it, acolacci@arpae.it, telephone number +39-0512094789 and functional mail box cdsArpae-Vitrox@cert.arpa.emr.it.

4.3 All notifications, correspondence and documents under this Collaboration Agreement shall be sent to the co-ordinators via the indicated communication tools, e.g. functional mailbox or CIRCABC.

4.4 The Parties shall communicate to each other in writing any changes with regard to the above-mentioned co-ordinators.

ARTICLE 5 – PROGRESS OF THE WORK

5.1 The JRC shall maintain the right to check on the spot the progress of the work forming the subject matter of this Collaboration Agreement or the specific agreement and to make any observation or suggestion, which they may deem appropriate.

5.2 Arpae-Vitrox shall draw up and make available to the JRC upon request any documents necessary to establish the progress of the work forming the subject matter of this Collaboration Agreement.

5.3 Arpae-Vitrox shall attend any meeting/phone call or teleconference convened by JRC and by mutual agreement in order to establish the state of progress of work already completed and, where appropriate, to change the subsequent course of the work in the light of the results achieved.

ARTICLE 6 – REPORTS

6.1 Arpae-Vitrox shall report to the JRC as stipulated in the Annex 1.

6.2 When JRC receives a Draft Study Report from Arpae-Vitrox, it will provide comments in due time. The Final Reports shall be prepared by Arpae-Vitrox taking JRC's comments into consideration and delivered in the format of an electronic copy.

ARTICLE 7 – FUNDS

7.1 There will be no transfer of money between the Parties in connection with this Collaboration Agreement.

ARTICLE 8 – PROTECTION OF THE RESULTS OF THE COOPERATION

8.1 The JRC shall own the Intellectual Property (IP) and all rights pertaining thereto, created in and for the performance of this Collaboration Agreement that allows to identify, modify, develop, adapt and validate alternative methods that can be used to reduce or replace animal experiments. The JRC shall have the right to use, exploit, assign or dispose of such IP at its own will and discretion and, in particular, the possibility to use, distribute, disseminate, disclose or publish those Results from validation studies in collaboration with the Parties and to include on the list of methods approved at international level such as, but not limited to, OECD and/or the EU Test Methods Regulation.

8.2 For the purposes of execution of the Collaboration Agreement Arpae-Vitrox assigns free of charge to the JRC all raw and analysed data, intermediary results or intermediary analysis that are made available at the test facility and may/shall become part of the IP described in Article 8.1. As the sole owner the JRC may grant a licence to use the raw and analysed data, intermediary results or intermediary analysis free of charge for the purpose of execution of the validation study.

8.3 IP and all rights pertaining thereto, created in and for the performance of this Collaboration Agreement that is outside the scope of Article 8(1) shall belong to the Party whose Personnel created it. The owning Party shall have the right to use, exploit, assign or dispose of such IP at its own will and discretion, unless otherwise provided for in this Collaboration Agreement.

8.4 Upon termination or expiry of this Collaboration Agreement, Parties shall send each other a declaration including the list of IP which they have created in and for the performance of this Collaboration Agreement. Parties agree to grant each other rights of access and use for such IP on non-exclusive, royalty-free and non-transferable basis for internal and non-commercial purposes only.

8.5 In case the IP created in and for the performance of this Collaboration Agreement cannot be clearly or reasonably separated between the Parties, or if the Parties have mutually contributed to the creation of the IP, or if it is evident that the IP created by the Parties have merged to such an extent that different parts cannot exist independently of the other, then such shall be considered as a jointly-owned IP. This is without prejudice to Article 8.1.

8.6 Neither Party can dispose of, license, assign, or transfer such jointly-owned IP to third-parties without the prior written consent of the other Party in the absence of a particular joint-ownership agreement. Following the coming into existence of a jointly-owned IP, the Parties undertake to conclude a particular Joint-Ownership Agreement to govern the terms and conditions pertaining to rights, duties and obligations of the Parties concerning the jointly-owned IP.

8.7 In case the collaboration performed under this Collaboration Agreement leads to the creation of results in the form of scientific, technical or academic publications, conference proceedings, reports, and similar written work authored through the involvement of the Personnel of both Parties, the Parties undertake to respect each other's rights, moral or economic, and to duly acknowledge and reference the authors and contributors.

8.8 Without prejudice to Article 8.1, neither Party can publish, disseminate, make publicly available, or disclose to a third party any result of the cooperation without prior written consent of the other Party on the manner, timing and contents of such disclosure. Consent for the foregoing may not be unreasonably withheld. Any breach of this provision shall be considered not only a breach of this Article but also a breach of confidentiality.

8.9 The provisions of this Article shall remain valid and legally enforceable for a period of five years from the date of termination or expiry of this Collaboration Agreement. After the five-year period, the provisions of this Article shall remain valid and legally enforceable for as long as a valid intellectual property right protects the results of the cooperation or if the period has been extended by a separate agreement.

ARTICLE 9 – CONFIDENTIALITY

9.1 Arpae-Vitrox undertakes to keep any information, documentation, data, or any other material communicated to them by the JRC as confidential and shall not disclose it to third parties. This confidentiality obligation applies also to information communicated orally when such information shall be kept confidential, for instance in the context of information exchange through seminars and workshops.

9.2 The JRC reserves the right to share any information related to the execution of this Collaboration Agreement with any third party if it considers it necessary for the purpose of the validation study.

9.3 The obligations of this Article shall remain in full force for the duration of the present Collaboration Agreement and shall survive its expiration or termination, whatever is the reason.

ARTICLE 10 – APPLICABLE LAW AND SETTLEMENT OF DISPUTES

10.1 This Collaboration Agreement and the Specific Agreement shall be governed by the law of the European Union, complemented, where necessary, by the substantive law of Italy.

10.2 Parties shall seek to settle any dispute, controversy or claim arising out of or in connection with this Collaboration Agreement through amicable negotiations. Such effort shall be deemed to have failed when one of the Parties so notifies the other in writing.

10.3 If the Parties fail to settle their differences through amicable negotiations, each Party may initiate proceedings before the Court of Justice of the European Union in Luxembourg.

ARTICLE 11 – ENTRY INTO FORCE AND DURATION

11.1 This Collaboration Agreement shall enter into force where all the following conditions are met:

- signature by both Parties
- receipt by the EU-NETVAL laboratory of the test system(s), the necessary control and/or reference item(s) and outline procedure(s) for the allocated method(s)
- receipt by the EU-NETVAL laboratory of a confirmation letter issued by the JRC indicating that the validation study can start. The confirmation letter date is deemed the date of entry into force of this Collaboration Agreement.

It will be concluded within a period of 24 months from said date. In case Part 1 and Part 2 are not completed within 24 months, this Collaboration Agreement shall renew automatically for an additional period of 12 months.

11.2 This Collaboration Agreement may be extended or amended only by written agreement signed by the duly authorised representatives of both Parties.

11.3 Either Party may terminate this Collaboration Agreement upon three months prior written notice to the other Party only in exceptional circumstances and giving justified reasons for doing so. This may inter alia be the case where research programmes and budget allocations are no longer compatible with the continuation of the working relationship, procedure or work programme. Any decision to unilaterally terminate the Collaboration Agreement should be consulted a priori with the Party's Member State National Contact Point of Directive 2010/63/EU on the protection of animals used for scientific purposes.

11.4 The Parties shall evaluate the implementation of this Collaboration Agreement after it has been in force for 12 months. On the basis of this evaluation, the Parties may agree to make modifications to the invitation to EU-NETVAL Members for "Participation in the validation study of *in vitro* methods for the detection of thyroid disruptors Part 1 – Definition and Part 2 – Relevance" described in the Annex 1 without the need to formally amend the Agreement.

ARTICLE 12 – MISCELLANEOUS AND ANNEXES

12.1 All provisions of this Collaboration Agreement apply without prejudice to the applicable law, including without limitation the law governing the right of public access to documents. Neither Party can claim any damages or breach of this Collaboration Agreement in cases where the other Party acts according to its obligations resulting from the applicable law.

12.2 Any personal data included in or relating to this Collaboration Agreement or the specific agreement, including its execution shall be processed by the Commission pursuant to Regulation (EC) No 45/2001 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data. It shall be processed solely for the purposes of the performance, management and monitoring of this Collaboration Agreement by the Director General of the JRC without prejudice to possible transmission to the bodies charged with a monitoring or inspection task in conformity with Union law. The data subject shall have the right of access to her/his personal data and the right to rectify any such data. Should the data subject have any queries concerning the processing of her/his personal data, the data subject shall address them to the Director General of the JRC. The data subject shall have right of recourse at any time to the European Data Protection Supervisor.

12.3 The following shall form an integral part of this Collaboration Agreement:

- Annex 1: Invitation to EU-NETVAL Members for "Participation in the validation study of *in vitro* methods for the detection of thyroid disruptors Part 1 – Definition and Part 2 – Relevance".

Signed in two originals in the English language.

The **Joint Research Centre of the European Commission**

Done in Brussels on _____

Signature: _____

Dr. Elke Anklam
Director of Directorate F. Health, Consumers and Reference Materials
Joint Research Centre

The Agenzia Regionale per la Prevenzione, l'Ambiente e l'Energia - Emilia Romagna (**Arpae**)– **Arpae-Vitrox Test Facility**

Done in Bologna on _____

Signature: _____

Dr. Franco Zinoni
Arpae Technical Director
Arpae-Vitrox Test Facility

ANNEX 1

CALL TO EU-NETVAL MEMBERS FOR

PARTICIPATION IN THE VALIDATION STUDY OF *IN VITRO* METHODS FOR THE DETECTION OF THYROID DISRUPTORS

PART 1 – DEFINITION

PART 2 – RELEVANCE OF THE METHODS

Call to EU-NETVAL members

For participation in the validation study of *in vitro* methods for the detection of thyroid disruptors

PART 1– Definition

and

PART 2 – Relevance of the methods

Purpose:

In the frame of the detection of chemicals with thyroid disruptor (TD) potential, EURL ECVAM will coordinate a validation study using a set of selected, mechanistically informative *in vitro* methods. The validation study consists of 2 parts (I) defining the methods and assessing their robustness and reliability (PART 1 of the validation study hereafter called Definition), (II) assessing the overall relevance based on the underlying mechanisms of the selected *in vitro* methods using a common set of test items (PART 2 of the validation study hereafter called Relevance).

In this project EU-NETVAL test facilities are requested to support this activity, namely in tasks i, iii and iv described below, to facilitate the translation of *in vitro* methods for the detection of thyroid disruptors from the developers community to the *in vitro* method users community.

This activity corresponds to the following tasks as stipulated in the EU-NETVAL Terms of Reference (ToR[1]):

i. Definition and description of *in vitro* methods

iii Assessment of the (within lab) reproducibility of *in vitro* methods

iv. Assessment of the predictive capacity and applicability domain of *in vitro* methods.

[1] ToR: EURL ECVAM's European Union Network of Laboratories for the Validation of Alternative Methods – EU-NETVAL -Terms of Reference, 26 November 2013.

In the context of this validation study, the generated experimental data will be used to confirm if the selected *in vitro* methods for detecting thyroid disruptors cover the main blocks of interaction with the thyroid signalling pathway, *i.e.*

1. Central regulation (synthesis/release of hypothalamic thyroid releasing hormone (TRH), its delivery and action on pituitary thyrotrophs, and the synthesis/release of thyroid stimulating hormone (TSH))
2. Thyroid Hormone (TH) synthesis (thyroperoxidase (TPO) activity, activation of the sodium iodide symporter (NIS), and functional assessment of thyrocytes)
3. Secretion and transport in serum
4. Metabolism and excretion
5. Local cellular concentrations (thyroid hormone membrane transporters and the deiodinase activity in the peripheral tissues)
6. Cellular responses (activation of the TH nuclear receptors)

Furthermore, the validation study will also consider:

7. Relevant short term *in vitro* methods integrating multiple Modes Of Action (MOA)
8. Integrative cellular *in vitro* methods (cellular proliferation and differentiation regulated by the activation of the TH nuclear receptors)

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1 SUMMARY

EURL ECVAM intends to carry out a validation study of a number of *in vitro* methods for the detection of thyroid disruptors. Currently, EURL ECVAM has compiled information on several *in vitro* methods which may become candidates for future Test Guideline development.

The validation study will be organised in 2 parts, starting with PART 1: In-house Definition of a set of *in vitro* methods. This will involve immediate testing of the *in vitro* method for its performance or doing additional optimisation of the *in vitro* method, in order to attain an *in vitro* method which meets performance characteristics based on the mechanistic basis of the method. Each method shall be described in a defined set of Standard Operating Procedures (SOPs), such as for test system cultivation, etc., that should be complete, clear and experimentally feasible. PART 2 includes further assessment of the relevance (mechanistic relevance) of a number of *in vitro* methods with a common set of test items (up to 30).

PART 1 includes the following activities:

- Technical assessment of the methods (non-experimental and experimental)
- Drafting and/or editing of a set of SOPs (including the necessary forms)
- Assessing within laboratory reproducibility (WLR) by performing a study with the set of SOPs, consisting of 5 valid runs (experiments), with the reference and control items

PART 2 includes the following activity:

- Assessing the mechanistic relevance of the method by performing 3 independent and valid runs, with a set of test items (up to 30).

EU-NETVAL test facilities, by replying to this call agree to their participation in both PART 1 and PART 2 of the validation study.

The overall content of the tasks for PART 1 and PART 2 are described in detail in this document.

2 OBJECTIVE

Due to concerns about the potential of chemicals to interfere with the endocrine system in humans and animals, a number of OECD Test Guidelines (TGs) have been developed for the screening and testing of potential endocrine disrupting chemicals, as listed in the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disruptors[2]. TGs for the screening of chemicals that disturb the thyroid hormone signalling are however lacking, largely due to the complexity of the thyroid system.

EURL ECVAM intends to carry out a validation study with a selected number of *in vitro* methods in order to attain a set of methods that will cover the known targets of thyroid disruption. The validation study will be carried out in partnership with the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL)[3] to (1) respond to some of the provisions of Directive 2010/63/EU, (2) generate *in vitro* method information that is reliable, relevant and based on current best quality and scientific practices and (3) contribute to the Community Strategy on Endocrine disruptors (COM (1999) 706).

EURL ECVAM has compiled a list of *in vitro* methods with validation potential, based on a review paper published by the OECD (OECD, 2014) and with input from meetings and workshops. This set of methods will be the starting point for assessing the Definition of selected *in vitro* methods. Subsequently, a selection will be based on those that perform well for further assessment of relevance and gradually lead to a set of *in vitro* methods which can be proposed for regulatory use.

The validation study of *in vitro* methods, coordinated by EURL ECVAM, will consist of 2 parts:

- PART 1 will focus on the definition of the *in vitro* method and the related set of SOPs, and on the assessment of the performance of the *in vitro* methods. The Definition phase is expected to be finalised within a time span of one year.
- PART 2 will start only when the Definition of the *in vitro* methods (PART 1) has proven to be successful. This part aims at assessing the overall relevance based on the underlying mechanisms of the selected *in vitro* methods using a common set of test items. It is expected to be finalised within a time span of one additional year.

EURL ECVAM will carry out this validation study according to the EURL ECVAM modular approach (Hartung et al, 2004, OECD, 2005).

With this call for participation, EURL ECVAM aims to identify and select one EU-NETVAL test facility per *in vitro* method. Hereto, each test facility is requested to indicate up to five methods of preference. ECVAM will aim to cover all 17 *in vitro* methods by assigning the EU-NETVAL facilities to an *in vitro* method taking the test facilities' preferences into account and/or the test facilities' competences.

[2] https://www.oecd.org/env/ehs/testing/OECD%20Conceptual%20Framework%20for%20Testing%20and%20Assessment%20of%20Endocrine%20Disrupters%20for%20the%20public%20website.pdf

[3] https://eurl-ecvam.jrc.ec.europa.eu/eu-netval

3 IN VITRO METHODS FOR DETECTING THYROID HORMONE DISRUPTORS

There is a wide-variety of environmental contaminants that have the potential to cause thyroid hormone disruption[4]. Exposure to specific environmental toxins, including polychlorinated biphenyls, dioxins, phthalates, polybrominated diphenyl ethers (PBDEs), and other halogenated compounds, has been shown to interfere with the production, transportation, and/or metabolism of thyroid hormones by a variety of mechanisms. Some chemicals, with structural similarity to thyroid hormones, have been shown to bind to thyroid receptors with both agonist and antagonist effects on thyroid hormone signalling. Thyroid hormone disruption can therefore cause severe adverse effects on *e.g.* brain development, growth and metabolism.

Validated and internationally recognised tests methods are essential in assessing the potential of chemicals to interact with the hormonal system and cause adverse effects. Non-animal test methods are needed for efficient testing and screening of substances. However, until today no standardised OECD test guideline for non-animal tests for thyroid screening has been developed.

In 2014, OECD published a scoping document on *in vitro* and *ex vivo* assays for the identification of modulators of thyroid hormone signalling (OECD, 2014). Several key biological mechanisms of thyroid system disruption were reviewed and the corresponding methods evaluated for their state of readiness as candidates to enter the validation process. Relevant *in vitro* and *ex vivo* methods were identified and recommendations were given for their development/use. Eighteen methods were reported that cover the possible sites of action in the hypothalamic-pituitary-thyroid (HPT) axis (see figure below). These methods were categorised according to their 'level of readiness' for validation:

- Level A corresponds to *in vitro/ex vivo* methods that will be ready for validation in the short term, *i.e.* could be proposed for OECD Test Guideline development;
- Level B corresponds to *in vitro/ex vivo* methods that could be developed further for potential validation in the long term, *i.e.* after an optimisation step. In addition, methods which meet criteria for Level A but which screen for modalities of thyroid disruption that can be indirectly detected through other, high priority *in vitro* methods, should be treated as B level of readiness;
- Level C corresponds to assay gaps (no *in vitro/ex vivo* methods identified to cover a specific mode of action or disrupting pathways).

In addition, the methods were further evaluated for the toxicological relevance of the target site and the chemical space.

[4] http://www.oecd.org/chemicalsafety/oecd-encourages-development-of-non-animal-test-methods-for-detection-of-thyroid-disruptors.htm

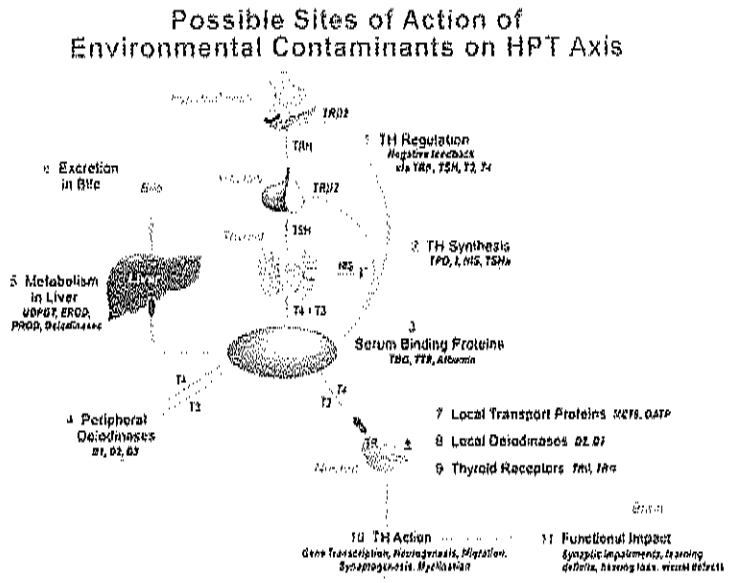

Figure1: The basic elements of the HPT axis showing feedback loops (centre) and potential target sites of chemicals that may alter thyroid function. Chemical sites of action are depicted in numbered circles. Image source: Gilbert ME et al. 2012.

The OECD review concluded that three assays (measuring thyroid hormone synthesis enzyme thyroperoxidase (TPO) activity, and the transthyretin (TTR) and thyroxine binding globulin (TBG) binding assays) are ready for validation in the short term (level A) and seven assays (TRH receptor - pituitary, TSH receptor – thyroid, NIS activation, deiodinase up-/down- regulation, MCT8/TR transactivation, thyroid gland explant, zebrafish Eleutheroembryo Thyroid Assay) could be ready for validation in the medium term (level B).

EURL ECVAM has collected information about the 18 methods reported in the OECD review. In addition, some other promising methods have also been included from the OECD Detailed Review Paper (1997) and as a follow-up from feedback received at various meetings (*e.g.* the EU NETVAL meeting of 2016, OECD Validation Management Group-Non-Animal meeting 2016 and the DG ENV/ANSES Thyroid Disruptor workshop in 2017). Further information has been retrieved for each of these *in vitro* methods, and on the basis of this information, a set of *in vitro* methods, covering all of the 8 blocks described in the OECD review (OECD, 2014), have been selected for the EURL ECVAM coordinated validation study.

4 INVITATION TO PARTICIPATE

EURL ECVAM invites the EU-NETVAL test facilities to present their candidacy for participation in PART 1 and PART 2 of the validation study of *in vitro* methods for detecting thyroid hormone disruptors.

EU-NETVAL members are hereto kindly requested to

1) Indicate a ranking for the preferred methods to work on (up to 5) by adding the numbers 1 (first method of choice), number 2, number 3, number 4 and number 5 (second, third, fourth, fifth method of choice). To this end, EURL ECVAM has compiled an Overview Table, describing 17 selected *in vitro* methods and their most important features. EU-NETVAL members can find the Overview Table of all selected methods, the corresponding Selection Criteria and the supporting information per *in vitro* method on CIRCABC. Furthermore, the OECD draft GIVIMP at its present status is made available for reference on CIRCABC.
2) Briefly indicate the motivation for implementing the selected methods.

A form for the EU-NETVAL members to complete with the above requested information is attached to this Call (Annex 1).

Please note that the time span for PART 1 of the validation study will be one year. This study will commence as soon as the appropriate collaboration agreements and material transfer agreements will have been put in place. It is envisioned that the start of PART 1 work will be in early 2018. PART 2 will start when PART I has been successfully concluded and the *in vitro* methods are ready for assessment of relevance (mechanistic relevance).

A scan of the completed form shall be sent *via* email to JRC-ECVAM-NETVAL@ec.europa.eu latest by 30 July, 2017

On the basis of the *in vitro* methods preferences selected by the EU NETVAL members, EURL ECVAM will allocate all 17 *in vitro* methods listed in the Overview Table to selected EU-NETVAL facilities. Once this allocation exercise is complete, the test facilities will be asked if they agree with their allocated method(s). All test facilities that presented their candidature will be informed on the outcome of the process.

5 DESCRIPTION OF THE WORK TO BE CARRIED OUT

5.1 Overall purpose

The overall purpose of PART 1 (Definition) is to arrive to a set of SOPs for a particular *in vitro* method, that are complete, clear and experimentally feasible. Therefore the allocated *in vitro* method(s) shall be assessed by the selected EU-NETVAL member(s) non-experimentally (addressing the completeness of the outline procedure provided by EURL ECVAM and related information supporting it) and experimentally (addressing the functionality of the procedure and, potentially, its optimization).

PART 2 (Relevance) aims at assessing the overall relevance based on the underlying mechanisms of the selected *in vitro* methods using a common set of test items.

EURL ECVAM will provide the test system, the reference and control items, and the coded test items for each *in vitro* method.

5.2 PART 1 – Definition of the *in vitro* method for detecting thyroid hormone disruptors

5.2.1 Technical assessment of the in vitro method

EU-NETVAL test facilities will receive documentation regarding the *in vitro* method(s) allocated per test facility including an outline procedure and related supporting information. This outline procedure will be compiled by EURL ECVAM on the basis of available information (*e.g.* publications) and, if possible, in collaboration with the test developer (see section 6 i). Depending on the method, this procedure may have varying degrees of completeness.

Each EU-NETVAL test facility is requested to assess the allocated *in vitro* method(s) by;

1) Reviewing the content of the outline procedure for completeness and clarity. During this non-experimental evaluation, it will become clear if and which parts of the procedure are missing, incomplete or are not clear. Further completion and modification may be needed.

2) Experimentally testing in-house and assessing the method(s) feasibility and performance. It is expected that when further development or optimisations are needed the test facility will allocate the adequate time and resources required to perform this work. The test facility is asked to record the data generated with the reference and control items and communicate them to EURL ECVAM, which can be used as historical database.

During the technical assessment, attention will be given to a number of key aspects, such as

- Are the elements of the *in vitro* method design sufficiently detailed including aspects of the statistical methods used, how to perform the data analysis, the presence and nature of acceptance criteria, signal intensity, the assessment of signal variability and

plate uniformity assessment and the reliability of endpoint calculations (OECD draft GIVIMP, 2017).

- Are the reference/control items adequate or should other controls be proposed; what is the variability between replicates.
- The expertise required for performing the method.
- The time estimates for testing one item.
- The technical limitations and drawbacks of the method.

The test facility is requested to use its in-house forms and procedures for raw data recording. In most cases, data recording and processing forms, both hard and soft copies, will not be available for the allocated *in vitro* method. The EU-NETVAL test facility is requested to put these in place with assistance from EURL ECVAM.

5.2.2 Drafting and/or editing of a set of SOPs (including forms for data recording and analysis)

Each *in vitro* method will be supported and documented by a number of different SOPs and forms for raw data recording as well as forms for analysis. Besides the SOP for the description of the main test procedure, SOPs for supporting procedures (*e.g.* the handling of the test system) critical to the *in vitro* method, need also to be available and referred to.

As a result of the technical assessment of the *in vitro* method, the test facility will have a number of observations which will be used to revise and/or complete the content of the outline procedure that was used at the initiation of the Definition part. The aim is to achieve well-defined and described, complete and clear SOPs that can readily be implemented in another laboratory. The test facility is asked to share these SOPs with EURL ECVAM for review and feedback.

5.2.3 Study to assess the in vitro method and within lab reproducibility (WLR)

After approval by EURL ECVAM of the generated set of SOPs for the *in vitro* method assessed, the test facility is requested to carry out one study with this set of SOPs, using the reference and control items. **This study shall aim to obtain 5 valid runs.** A study plan and a report will be drafted by the test facility. EURL ECVAM will carefully evaluate the outcomes of the study and decide on continuation of the study for assessing relevance (PART 2), or, to stop the study.

5.3 PART 2 – Relevance of the *in vitro* method for detecting thyroid hormone disruptors

After review and approval by EURL ECVAM of the assessment carried out for the *in vitro* method, the test facility will perform a study with the set of SOPs established in PART 1, using a set of coded test chemicals. **The aim of this study is to achieve 3 valid runs per test item.** The number of test items to be tested will be up to 30. The exact number of test items shall be communicated by EURL ECVAM before the start of PART 2 of the study. A study plan and final report will be required from the EU-NETVAL test facility.

5.4 Time frames, Output and Reporting

Each test facility is required to assess the allocated *in vitro* method(s) (PART 1) within a time frame of one year.
The start of the work outlined in this call depends on the fulfilment of the administrative requirements but is envisioned to be early 2018.

Each test facility is required to assess the *in vitro* method(s) (PART 2) within a time frame of one year. PART 2 will only start once EURL ECVAM will have decided on a successful Definition of the *in vitro* method.

The test facility will provide the following documentation per *in vitro* method assessed:

PART 1
- Set of well-defined and described SOPs
- Forms for data recording
- Forms for data analysis
- A report summarising what has been tested, what was modified and/or added to the SOP, the observations and conclusions on the performance of the *in vitro* method from the technical assessment.
- Study plan for the generation of 5 valid runs
- Study report

PART 2
- Study plan for the generation of 3 valid runs for up to 30 test items
- Study report

All documents shall be delivered in the format of an electronic copy, and, in English.

Exchange of documents shall occur via CIRCABC.

5.5 Modus operandi per *in vitro* method

EURL ECVAM aims to allocate one test facility for the assessment of each *in vitro* method in the Overview Table.

EURL ECVAM will act as coordinator and will provide assistance and/or training where appropriate.

Progress on the work will be monitored on a regular basis.

If the assessment of a particular *in vitro* method proves to be challenging, EURL ECVAM and the test facility will discuss in detail the best way forward. Depending on the issues encountered, this may result in dropping a particular method from further assessment.

6 EURL ECVAM ROLE AND RESPONSIBILITIES

i) Definition and description of *in vitro* methods (ToR EURL ECVAM task ii)

Prior to the start of PART 1 of the validation study, EURL ECVAM will collect existing procedures from the test developers if available. In those cases where they are not available, EURL ECVAM will create an "outline" procedure on the basis of available information (*e.g.* publications) and where possible in collaboration with the test developer.

ii) Management of validation studies (ToR EURL ECVAM task iv)

EURL ECVAM will conduct the validation study adhering to the principles described in the OECD guidance document number 34 on the Validation and International Acceptance of new or updated test methods for Hazard Assessment (OECD, 2005).

The following will be provided by EURL ECVAM (free of charge):

- the *in vitro* method (in the format of an outline procedure),
- the test system (*e.g.* cells) for the sole purpose of this validation,
- the reference/control items per method
- the coded test items (Part 2)

EURL ECVAM will be responsible for putting in place collaboration agreements and material transfer agreements with all parties involved; verifying the authenticity of the cells and cell lines and the distribution of all test systems; assessing the performance of all the methods; preparation of an assessment report concerning PART 1 Definition, and, of PART 2 Relevance. EURL ECVAM will provide assistance by involving where possible the test developers during the optimization of the *in vitro* method. Assistance when needed will also be provided for the development of the set of well-defined and described SOPs and the related forms.

It is foreseeable that, as a result of the assessment in PART 1, some *in vitro* methods will drop out from further assessment in PART 2 of the validation study. The test facilities will be informed about the outcome of the assessment of the *in vitro* method(s). Only methods that have proven to perform well will be retained to commence PART 2 of the validation study.

vi) Selection of test facilities to support validation studies (ToR EURL ECVAM task vi)

EURL ECVAM, in consultation with Member States, will assign EU-NETVAL test facilities for the assessment of the *in vitro* methods taking into account the provided information about equipment, experiences and motivation for a specific *in vitro* method. Depending on the feedback received, EURL ECVAM will try to respect the five choices made by the test facilities. The test facilities will be asked for agreement on the allocated *in vitro* method(s).

7 GENERAL REQUIREMENTS

7.1 Performance of work and Quality Assurance

The test facility will make all efforts to ensure that the experimental work for the Definition of the *in vitro* method is performed using the same equipment, the same reagents (*i.e.* of the same supplier and the same batch) and the same staff for all the test, reference/control items during the entire duration of PART 1 and PART 2 of the validation study. Change of equipment, reagents or/and staff has to be communicated immediately to EURL ECVAM and EURL ECVAM will evaluate any possible impact on the performance of the method. The change(s) shall be documented in the report of the *in vitro* method concerned.

Only adequately trained staff shall perform the experimental work.

7.2 Mycoplasma testing

In the case of testing *in vitro* methods that involve cultivation of the test system, the EU-NETVAL test facility will test at the beginning and at the end of PART 1 and PART 2 of the validation study the cells for mycoplasma contamination. Results of this testing will be communicated to EURL ECVAM.

7.3 Return of the test system(s) to EURL ECVAM for authentication

When the EU-NETVAL test facility uses cells and/or cell lines, it agrees to freeze down 4 million cells from the highest passage number of the cells used in the studies, and to send these to EURL ECVAM at the end of PART 1 and PART 2 of the validation study. EURL ECVAM will use these cells for a final test system authentication.

The test facility agrees not to use the test system for any other purposes than described in the validation project plan and to destroy the remaining test system (frozen or in culture) not later than 4 weeks after the completion of the entire validation study. EURL ECVAM will communicate when the validation study is officially completed.

7.4 Intellectual Property

The test facility agrees that the experimental work described in the present document cannot be published or presented, even in part, without the official approval by the European Commission. In case the approval is granted, the European Commission's Joint Research Centre and EURL ECVAM as well as the test developer must be acknowledged on all publications resulting from this work. All raw and analysed data, intermediary results, or intermediary analysis made available to the European Commission by the test facility will be treated as property of the European Commission. The latter may grant a licence to use such data, results and analysis under conditions to be specified in the collaboration agreement.

8 LIST OF RELEVANT DOCUMENTS

OECD (2014): New Scoping Document on *In Vitro* and *Ex Vivo* Assays for the Identification of Modulators Of Thyroid Hormone Signalling, Series on Testing and Assessment, No. 207, ENV/JM/MONO(2014)23.

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OECD (2005) Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Environmental Health and Safety Monograph Series on Testing and Assessment No.34.

OECD (2017). Draft OECD Guidance Document on Good *In Vitro* Method Practices (GIVIMP) for the Development and Implementation of *In Vitro* Methods for Regulatory use in Human Safety Assessment.

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Coecke, S., Bernasconi, C., Bowe, G., Bostroem, A.-C., Burton, J., Cole, T., Fortaner, S., Gouliarmou, V., Gray, A., Griesinger, C., Louhimies, S., Gyves, E.M., Joossens, E., Prinz, M.-J., Milcamps, A., Parissis, N., Wilk-Zasadna, I., Barroso, J., Desprez, B., Langezaal, I., Liska, R., Morath, S., Reina, V., Zorzoli, C., Zuang, V., 2016. Practical Aspects of Designing and Conducting Validation Studies Involving Multi-study Trials. Adv. Exp. Med. Biol. 856, 133–163.

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OECD, 2005. Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. ENV/JM/MONO(2005)14.

OECD, 2004a. OECD Series on Testing and Assessment. Number 14. The Application of the Principles of GLP to *in vitro* Studies. OECD Publishing.

Whelan, M. Eskes C. 2016. Evolving the Principles and Practice of Validation for New Alternative Approaches to Toxicity Testing, in: Eskes C, Whelan, M. (Eds.). Advances in Experimental Medicine and Biology Volume 856: Validation of Alternative Methods for Toxicity Testing. Springer International Publishing AG Switzerland.