Dear Members of ICCVAM,

On behalf of the Institute for In Vitro Sciences (IIVS), we are pleased to submit our comments on the draft document titled "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies". We appreciate the opportunity to provide feedback on this important document.

First and foremost, we would like to express our overall satisfaction with the draft document and commend the ICCVAM Validation Workgroup for their diligent efforts in producing it. The document represents an important evolution in the framework for validation of alternative methods.

We are particularly pleased with your decision to include the Good In Vitro Method Practices document (GIVIMP) as a key reference. GIVIMP serves as a valuable resource for researchers, regulators, and stakeholders worldwide, offering a comprehensive compilation of best practices for *in vitro* method development, optimization, and routine performance. Furthermore, we would like to express our support for the content of the section on Communication and Training to Encourage Non-Animal Method (NAM) Use. Effective communication and training initiatives are integral to the widespread adoption of alternative methods once validations have been completed. Ensuring that stakeholders have access to accurate information and the necessary knowledge to implement these methods is critical. We commend ICCVAM for recognizing the importance of this aspect in the document.

Attached to this letter, you will find a table containing several specific points and recommendations for your consideration. These points reflect our experience in participating in and supporting many NAM validation activities over the past several decades. We believe that these suggestions can enhance the document and contribute to its overall effectiveness in supporting method developers and method assessors alike. We thank you in advance for your consideration.

Sincerely,

Amanda Ulrey, RQAP-GLP
President

Hans Raabe, MS
VP/COO

Enclosure: Comments Table on ICCVAM Draft Document

Full Name of Document:	**Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies**

Submitter's Name:	**Amanda Ulrey and Hans Raabe**
Position in Organisation:	**President and Vice-President/COO**
Organisation / Affiliation:	**Institute for In Vitro Sciences, Inc.**
Address	**30 W Watkins Mill Road Suite 100 Gaithersburg, MD 20878**
Email:	**aulrey@iivs.org; hraabe@iivs.org**

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Line No(s). / Para No. & Line No(s)	**Comment (justification for change)**	**Proposed change**
175-177	NAMs by the document's definition include small organism models. As is the case for all test methods, the relevance of these models for specific purposes need to be established to avoid the potential for assuming relevance of biological processes simply because of the full organism aspect.	Add the concept that the relevance of reduction and/or refinement methods and those methods in an alternate species (other than the species of interest) needs to be assessed as part of the method validation and cannot be assumed.
216-217	The document states, "Specifically, there should be evidence to support that the use of an alternative method will lead a regulatory review to the same or more protective decision as the reviewer would make based on existing methods." There are two reasons why this statement should be stricken. First, the statement suggests that the existing methods are providing sound, human-relevant predictions. In the absence of a fundamental evaluation of the relevance of the reference test method or any data demonstrating the human-relevance of the existing test method, we cannot require that alternative methods provide the same predictions. Indeed the existing method may readily provide an incorrect or irrelevant prediction with respect to human toxicology. Second, we strongly recommend that as we move forward in evaluating alternative test methods and defined approaches, we don't undermine our efforts by specifying that new candidate methods be "as protective or more protective" without statistical evaluation of **both the reference and candidate test method performance and reliability**. Otherwise, we risk endorsing progressively more over-predictive test methods in the future, and thus find ourselves routinely over-labeling. For example, because of the practice of taking the most conservative in vivo results as definitive finite reference values, promising test methods in the past may not have met validation criteria, while other candidate test methods were inherently calibrated to be excessively sensitive. This unfortunately has already been occurring within the eye and skin irritation hazard assessment arena, resulting in industry reluctance to utilize certain NAMs. In contrast to the aforementioned statement, the following statement in Lines 224 – 227 better meets the goals and needs for accepting NAMs: "Where appropriate and possible, building confidence in NAMs may include demonstrating that the NAM provides information of equivalent or better quality and relevance for regulatory decision-making as compared, either quantitatively or qualitatively, to the information provided by the traditional animal test method."	Strike the statement: "~~Specifically, there should be evidence to support that the use of an alternative method will lead a regulatory review to the same or more protective decision as the reviewer would make based on existing methods.~~" Edit the section to focus on and support the later statement "Where appropriate and possible, building confidence in NAMs may include demonstrating that the NAM provides information of equivalent or better quality and relevance for regulatory decision-making as compared, either quantitatively or qualitatively, to the information provided by the traditional animal test method."
225	Throughout the document, there is a common theme of assessing the quality and relevance of NAMs against the ultimate species of interest. To further support this, we suggest adding "to the species of interest" in this line.	"…demonstrating that the NAM provides information of equivalent or better quality and relevance **to the species of interest** for regulatory decision-making…"

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336-342	Please add that one must consider **the specific cell culture conditions and test system platform**. This is highly important since the culture conditions have significant impacts on the phenotypic characteristics of the cells utilized, and thus the test system and not just the sourced cells must recapitulate the human organ or tissue of interest. For example, a test system using primary human cells in 2D culture may not be particularly human-relevant when compared to the performance of test systems developed using human cells in 3D/organoid culture; the latter of which may allow cells to express a more-native phenotype, while the former may drive dedifferentiation of desired phenotypes.	Add the following sentence: "…Parish et al., 2020). **Lastly consider the effect that cell culture conditions and the test system platform may have on the test system during performance of the method.**"
367-371	The important concept of using defined approaches in NAMs is discussed here and the OECD guidance on defined approaches is referenced. To provide an example of what a defined approach looks like in practice, we recommend adding a reference to the OECD defined approach for skin sensitization, reference OECD 2021a in this document.	Add the following sentence to the end of the paragraph. **"An example of mapping NAMs to key events along an AOP resulting in a defined approach to address a regulatory endpoint is found in the OECD Guideline No. 497: Defined Approaches on Skin Sensitization (OECD, 2021a)."**
439-456	This section does not call into question the relevance of the animal test data, and essentially suggests that the new method should be evaluated for confidence against the reliability and reproducibility of the animal data. This perpetuates the notion that the existing/reference animal test and data are biologically "correct" and appropriate for human relevance. Indeed, there are limited data for most toxicological endpoints to determine human relevance of the reference method, and thus one cannot assume that the reference method provides acceptable relevance. Furthermore, the statement in Lines 452 – 456 only focuses on the NAMs' abilities or failures to align with the traditional animal test.	Add a comment in the beginning of this section indicating that the relevance of existing animal models to the species of interest cannot be assumed. Lines 452-456 should clarify that in the absence of an evaluation of the relevance of the traditional animal test, it is highly reasonable to suggest that the animal test may not align with the human outcome.
535-536	Bullet 5) states "…interlaboratory evaluation (if needed)…" We feel that test method transferability is needed in all cases where a test method may be utilized by more than one stakeholder (for example, some agencies may indeed consider evaluating a novel NAM that may be used for a single specific application). Whereas recent literature has questioned the need to test all of the reference chemicals in all laboratories participating in a validation or ring trial, some level of evaluating test method transferability to determine between laboratory reproducibility is required and can readily and economically be done with a select subset of reference chemicals (for example, by including specific reference chemicals falling near the prediction thresholds).	Please clarify under what circumstances (with examples) inter-laboratory studies are not needed.

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566 – Table 4	Throughout the document, there are recommendations to follow Good Laboratory Practices (GLPs) when conducting method validation work. GLPs are a strong quality system intended to support the integrity of the studies performed in compliance with them. However, regulatory authorities, and method development define the scope of work falling under GLPs and validation activities often are not included within this scope. Furthermore, GLPs require the existence of an independent Quality Assurance Unit and other laboratory management and quality structure elements that are not present in academic laboratories and biotech laboratories that do not routinely perform work for regulatory submissions. The key concepts of data integrity and reproducibility discussed in GLPs are also discussed in the OECD Good In Vitro Method Practice document (reference OECD, 2018 in this document). We recommend siting GIVIMP in addition to GLPs to assure that even labs that are not GLP compliant put in place key recommendations to support data integrity and reproducibility of the work.	Add GIVIMP to column 3 row 5 of the table. Fulfil requirements **for Good In Vitro Method Practices and** Good Laboratory Practices, monitor in-process control measurements and support troubleshooting when issues arise, support data analysis and reproducibility.
569-594	Include guidance from GIVIMP on **establishing and justifying** appropriate culture conditions, test method procedures, equipment specifications, operator proficiency and training and facility conditions as they influence quality. GLPs focus on the successful execution of procedures according to the documented methods. GIVIMP includes guidance on improving the quality of the method itself and assuring that procedures have been examined to assure they are relevant to the ultimate endpoint and the effects of the variables mentioned above have been considered.	Reference GIVIMP guidance as appropriate throughout this section.
586-594	Same comment as above about expanding beyond GLP work and including GIVIMP.	Add GIVIMP document as a reference for additional details on documentation to retain to support quality. “Developers should also maintain documentation of suppliers for materials, cells, and reagents if this information is relevant for evaluation of a NAM (discussed in detail in Section 3.3.3). **For further detail on quality practices, equipment procedures, and documentation to retain please reference GIVIMP (OECD 2021a).**”
592-594 and 839-840	It is unlikely that any laboratories that are not GLP compliant or are research laboratories functioning within a larger GMP compliant facility would have in place procedures to perform an IQ/OQ/PQ on equipment. A broader approach to requiring the reporting of the same information would be to focus on requesting documentation that the equipment, along with all associated software, have been installed properly and function according to the requirements of the method.	Add the following as the last sentence to the paragraph ending on line 594: “**At a minimum, instrumentation should have documentation of proper installation from the vendor or the laboratory and documented testing showing that it can reliably perform the functions required by the method.**” Add the following as the last sentence to the paragraph ending on line 840: “**For non-GLP studies, documentation of proper installation and testing to show equipment performs as intended should be retained**.”

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663-665	This section states that test methods have SOPs "to ensure a complete system of quality control and assurance is in place and functional." The existence of an SOP for a test method does not ensure that there is adequate quality control or quality assurance is in place. SOPs also do not ensure that they are being routinely followed, which is one key activity to standardize performance of the method. Well-written SOPs provide detailed instructions on the performance of the method to minimize variation in the performance of the method over time and between individuals.	Modify the text as follows: It is recommended that the proposed test method have a well-documented standard operating procedure (SOP) ~~to ensure a complete system of quality control and assurance is in place and functional~~ **to support uniform performance of the method and related laboratory activities such as test system handling and equipment calibration**~~.~~ SOPs should cover all aspects of testing and analysis.
683-685	GIVIMP includes a comprehensive listing of items requiring documentation and should be referenced here for additional information.	Please reference the guidance in GIVIMP for a more complete listing of items requiring documentation.
722-729	To support future independent review activities, endpoint measurements and metadata should be retained and stored securely (i.e. the raw data or first capture data should be available). Many systems and software save these data locally in a program specific format on the individual machine performing the reading. These data should be exported in a human readable format like a pdf document, and saved securely so they can be referenced during independent reviews as needed to confirm downstream calculations. This activity is critical for achieving data integrity as discussed in section 3.4.	Add the following sentence: "…that may contribute to assay variation or batch effects. **These data and metadata should be exported in a human readable (e.g. pdf) format and saved in a secure location so that they can be referenced during independent reviews of the validation.** This topic is…"
786-798	Ideally, validation studies and ring trials are conducted in full GLP compliance to ensure confidence in the resulting study data. In the absence of GLP compliance, it is recommended to require justification for not conducting validation studies per GLPs. Further, for validation studies that are not conducted per GLPs (i.e., those "…conducted to the extent possible according to principles of GLP…"), a listing of the exceptions to the GLPs should be required. It is not useful to simply state that a study was conducted according to the principles of GLP without addressing each of the specific GLP elements.	Add the following sentence to the end of the paragraph: "**Where studies have not been conducted in full GLP compliance, a listing of the exceptions to GLP should be included to provide reviewers with information on the level of data integrity present**."

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903-911	The statement “The performance of a NAM intended to replace an existing approach will generally need to be compared to the existing approach. There is often high confidence in existing approaches with which there is substantial experience. These existing approaches may not have undergone formal validation but repeated successful use of the existing approach along with assumed inherent validity of testing in animals often builds substantial confidence in the approaches,” erroneously suggests that extensive use of the existing animal test imparts the ability to provide “correct” human-relevant predictions. This concept must be changed, and in the absence of any validation or evaluation of predictions (prospective or retrospective), the confidence in the reference method/data must be considered to be low a priori. Although data on human-relevance of a reference method may be difficult to obtain, the evaluation of reliability retrospectively must be conducted, and not assumed. In the absence of such evaluations, it is not clear how any statistical evaluation of the performance of a NAM relative to an existing test method can be conducted.	Add language supporting a retrospective evaluation of an existing method’s reliability so that a statistical evaluation of the performance of the NAM relative to it can be conducted.