Report of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Implementation Working Group (Draft)

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Submitted by:
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Background

At the June 16 – 17, 2011 Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) meeting in Arlington, Virginia, several SACATM members recommended establishing a working group (WG) to assess the status of implementation of Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)-recommended alternative methods. The suggestion was based on Committee discussion over the course of SACATM meetings in 2009 – 2011 in which general concerns were raised about lack of information on integration and lack of a process to measure (i.e., metrics) the impact on the volume of animals used following implementation of ICCVAM-recommended methods into product safety assessment. Other questions had been raised regarding how U.S. regulatory agencies viewed use of data from alternative approaches in filings and applications. The SACATM charter allows the Designated Federal Officer (DFO) to establish *ad hoc* WGs to provide recommendations, gather information, or provide assistance on specific, limited projects. The reports prepared by WGs are presented to the SACATM members at a public meeting and SACATM reviews, discusses, and votes on whether to approve the report.

SACATM chair Steven Niemi, ICCVAM Executive Director William Stokes, SACATM member Joy Cavagnaro, National Toxicology Program Deputy Director for Policy Mary Wolfe, and SACATM DFO Lori White met in February 2012 to discuss formation of the Implementation Working Group (IWG). They drafted a charge to the IWG: *Assess implementation of ICCVAM-recommended alternative methods,* agreed that Dr. Cavagnaro would chair the IWG, and agreed to invite Drs. Eugene Elmore, Steven Hansen, Michael Olson, and Daniel Wilson to the IWG. All accepted the invitation and agreed to the confidentiality and conflict of interest guidelines.

The IWG met eight times by teleconference between March and August 2012 and prepared this report. The report summarizes IWG deliberations and provides responses to a survey instrument developed by the IWG and distributed to stakeholders. The IWG early discussions focused on obtaining a better understanding of the status of implementation of ICCVAM-recommended methods by end-users, i.e., U.S. regulatory agencies and regulated industries that do life science research. After further discussion, it was determined that regulated industries would be the focus of the IWG. Importantly, a key objective was to identify whether there were any obstacles to implementation. If needed, the IWG would recommend a path forward to improve uptake of ICCVAM-recommended methods by stakeholders.

Scope and Viability of ICCVAM

The WG acknowledged that ICCVAM couldn't do everything. There was consensus that the interest in alternative testing has grown since the inception of ICCVAM and agencies are working and or partnering with other organizations independent of ICCVAM, e.g., the Society of Toxicology, Tox21, small business initiatives, etc. While it was acknowledged that ICCVAM validations, done one method at a time, take a great deal of time, it was also recognized that there was great value in the growing collection of ICCVAM-validated methods. ICCVAM is now one of many organizations contributing to the alternatives enterprise. Better alignment of priorities; however, is needed in nomination of tests that will be relevant to ICCVAM stakeholders. There also may be ways to make the current process of validation more efficient as agencies look to other ways to proceed more quickly in adopting alternatives.

Global Perspective

In addition to assessment of U.S. uptake of methods, there is a need to measure progress against the worldwide move to alternative methods for a more comparative view. Some other countries are moving ahead at a more rapid pace due, in part, to political pressures and the potential for working in groups with common economic and other interests. In any case, it will be necessary to integrate the issue of global markets and obstacles of international regulation into future ICCVAM considerations. It is therefore necessary to understand the relationship between ICCVAM and global acceptance, e.g., gaining a better understanding whether ICCVAM-recommended methods need Organisation for Economic Co-operation and Development (OECD)/International Conference on Harmonization (ICH) approval before they are adopted more globally. It was suggested that International Cooperation on Alternative Test Methods (ICATM) could potentially assist with international acceptance of alternative methods. It was also recognized that ICCVAM via ICATM should advocate for the broadest possible acceptance of alternative methods and recognition of the importance of such methods so that world-wide acceptance is achieved. This, along with global harmonization of tiered testing requirements may help limit animal-use intensive methods required to satisfy the regulatory requirements of a few countries.

Adoption of ICCVAM-recommended Methods

There is now a wide variety of validated ICCVAM-recommended alternative methods, many of which have moved on to full OECD method status. While some companies consider ICCVAM-recommended methods to be the gold standard, many of the methods are still not fully integrated for regulatory or product safety testing. The IWG discussed the need for more cross-talk regarding the applicability domain so that tests work hand-in-hand as practical for pharmaceuticals, consumer products, agrochemicals, etc. The IWG concurred that U.S. regulatory agencies need to provide guidelines as to when they will or will not accept OECD approved guidelines and provide on an annual basis metrics demonstrating progress toward reduction of animal use.

Current Status of Acceptance of ICCVAM-recommended Methods by U.S. Agencies

Adoption of alternative methods may be inextricably linked to acceptance. There is a perceived lack of interest in the success of alternative methods by U.S. regulatory agencies. Additionally, there is the perception that despite ICCVAM's efforts to validate alternative methods, the ultimate responsibility for acceptance is with the regulatory agencies; the lack of agency support or mandates for reducing animal use is limiting implementation. Currently there are no apparent metrics and no recognizable internal champions to popularize methods by and within agencies. While data may be collected institutionally by agencies, there does not appear to be generalized methods for tracking. Related to this lack of tracking is limited oversight and accountability for the money spent on validation and implementation efforts. Outcomes of implementation of ICCVAM-recommended method are not evaluated. Therefore no determinations can be made to either increase/continue funding of successful alternatives or reevaluate less successful alternatives and look for more promising approaches. The IWG recognizes that it is somewhat burdensome for U.S. regulatory agencies and industry to proactively track the implementation of ICCVAM-recommended methods. But without accurate tracking there can be no effective allocation of resources to fund alternative methods development.

Early in the process of developing an ICCVAM-recommended method, when ICCVAM agencies are weighing in on validation, it is important that they also work to try to figure out how the new

method would fit in a regulatory and risk assessment strategy. This can be done in parallel to the validation efforts. Once recommendations are made, there is a general concern by users that agencies initially agree to accept an assay and then once submitted they may request additional information without providing criteria for why a specific result may stand alone or require additional support. This is one of the reasons given for why companies do not submit data from alternative methods. It appears that there is a shared thought that results from alternative tests may not be accepted or viewed as definitive; therefore, they will continue to submit the assays that they know have been accepted in the past. The IWG recognized that results of single assays are just one aspect of the assessment of safety of a product. Alternative methods are commonly used in tiered approaches to compensate for lack of physiological integration as might be achieved with whole animal testing. But if tiered test paradigms frequently use whole animal testing as a final definitive and mandatory step, the reduction in animal number sought by 3Rs alternative approaches is constrained.

There is a critical need from ICCVAM members who are responsible for regulated products to provide input during the early phase of nomination as well as throughout the validation phase. Regulatory agency input should be used to define the validation "standards" to help to ensure acceptance and implementation once the assay is considered as valid. Also, industry needs to be more proactive in submitting methods for validation that would facilitate the study, validation and implementation of alternative methods.

Assessment Strategy

Successful implementation requires alignment of the end-users (regulated industry and U.S. regulatory agencies). To evaluate that alignment, the IWG decided that a survey mechanism could be used to compile responses from end-users to help determine what methods are being used and how data are being accepted. Getting feedback from end-users as to how they approach utilization of methods would also be useful. A strong rationale for a survey is that there is confusion within the government and industry regarding the status of acceptance and implementation of ICCVAM-recommended alternatives; a survey may ascertain whether certain assays are being more broadly used as alternatives. In previous reporting at SACATM meetings, the EPA had provided limited quantification on submissions (number that used animals, number that used alternatives, and number that used both). Similar information was not presented for other U.S. regulatory agencies.

Working within Office of Management and Budget (OMB) constraints regarding surveys and the IWG's time frame, initial surveys were designed to gather information from regulated industry only, with the understanding that complementary information should be obtained in the future from U.S. regulatory agencies. The IWG developed two different surveys to assess implementation of ICCVAM-recommended methods in the life sciences research and services industry, one for companies and one for contract research organizations (CROs). Each survey needed to be limited to nine or fewer respondents to adhere to OMB regulations. Therefore, there was no attempt to obtain a statistically relevant sampling of the entire industry; that was beyond the scope of the IWG. Rather the goal was to develop a survey instrument that could then serve as a starting template for future assessments of implementation of ICCVAM-recommended methods.

The IWG members suggested individuals within the life sciences industry working at companies and CROs knowledgeable about the application of *in vitro* and other techniques that further the 3Rs of laboratory animal use. There was an attempt to limit responders to companies

headquartered in the U.S. and to query on ICCCAM-recommended alternatives rather than the entire gamut of *in vitro* methods. The IWG requested timely completion of the surveys, noting that partial responses were of value and responders were not required to address each of the survey questions. The IWG also informed responders that no information would be tracked on the identity of specific responders and that comments may be used verbatim, but without attribution. The data, collected without identifiers, were analyzed and used by the IWG in preparing this report and may be used in future ICCVAM and SACATM efforts.

The surveys focused on whether ICCVAM-recommended assays are being used rather than attempting to quantify how many fewer animals may be used due to adoption of ICCVAM-recommended alternative methods. Questions were broken down into toxicity endpoint and relevance to a specific regulatory agency.

Survey Limitations

The survey size for both companies and CROs was small due to both time and regulatory constraints. Given the small number of respondents (seven companies and six CROs responded to the survey), there is not a statistically valid sampling of the industry, but rather only a snapshot of opinions of the state of implementation of alternative methods. The IWG considers the surveys a first pass and, in some respects, initial survey instruments, which can be used to identify flaws in the methodology and make future improvements in assessment of implementation of ICCVAM-recommended alternative methods.

Additionally, with the global reach of many international companies, much of the laboratory work is currently done in England or Germany. Results may be skewed because for companies that distribute internationally; they may use standard animal testing because some countries' regulatory agencies will not accept alternative methods. Additionally, many companies use a weight of evidence approach to studies, which could alter their adoption of ICCVAM-recommended methods.

Therefore, it is not possible to make any definitive conclusions on the status of implementation of ICCVAM-recommended methods based on the survey results. However, we will present the data collected and highlight some of the information.

Results – Company Survey

Q1. Is your company U.S. based or non-U.S. based?

Yes, it is headquartered in the U.S. 87%
No, it is headquartered outside the U.S. 14.3%

Q2. Does your company submit data from ICCVAM-recommended alternative methods to U.S. regulatory agencies?

Regularly 14.3%
Sometimes 71.4%
Never 14.3%
Comments:

- "We make few submissions"
- "Submitted when data accepted by US Regulatory Agency, and method applicable to chemistry being assessed. Also submissions for REACH."

Q3. Have U.S. regulatory agencies accepted your data from ICCVAM-recommended alternative methods?

Yes 83.3%
No 16.7%
Comments:

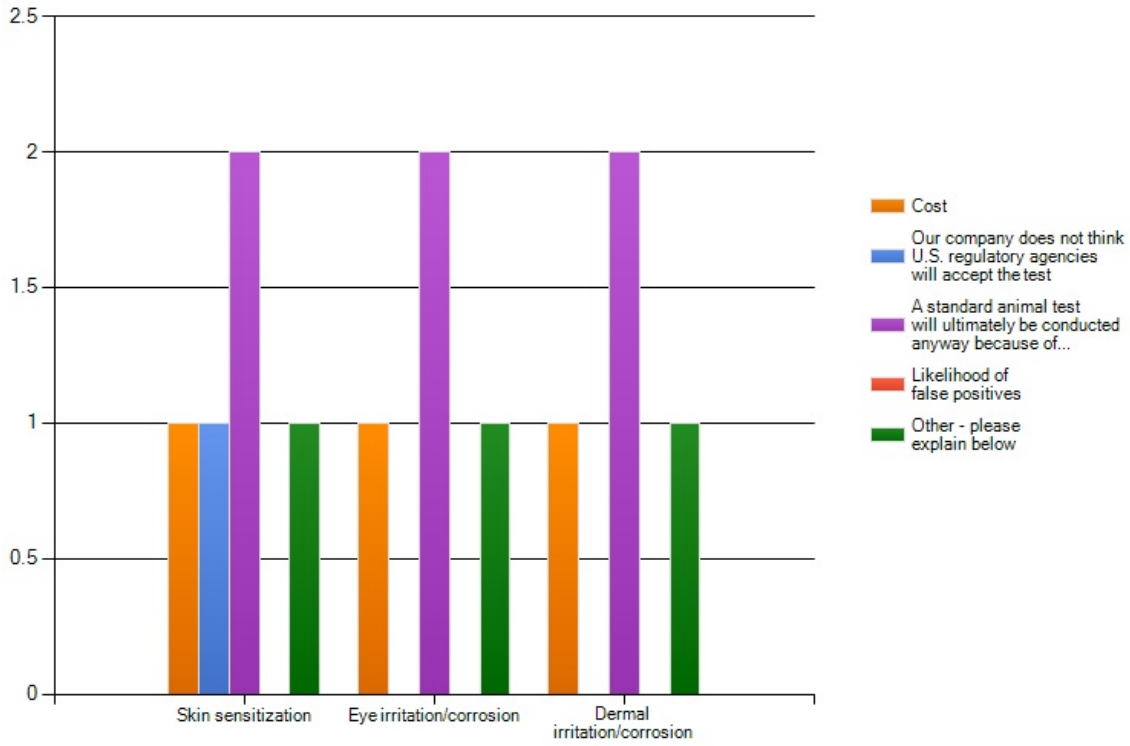
- "Accepted without comment, but often together with *in vivo* data"

Q4. If your company is not using ICCVAM-recommended alternative testing methods, why not?

Comment:

- "Not accepted by regulatory agency; not compatible with our company's chemistry; not applicable with insoluble compounds; lack of concordance with *in vivo* data."

Q5. For each alternative method below, if ICCVAM-recommended alternative methods are not being implemented at your company, what is the most likely reason(s). Check all that apply.



Comments:

- "Likelihood of false negatives; cost increases since *in vitro* often must be followed by *in vivo* testing in any case."
- "We are using all of these an[d] other alternative methods not yet reviewed or approved by ICCVAM."

Q6. For picking starting doses for acute oral toxicity testing, if in vitro cytotoxicity methods are not being implemented at your company, what is the most likely reason(s). Check all that apply.

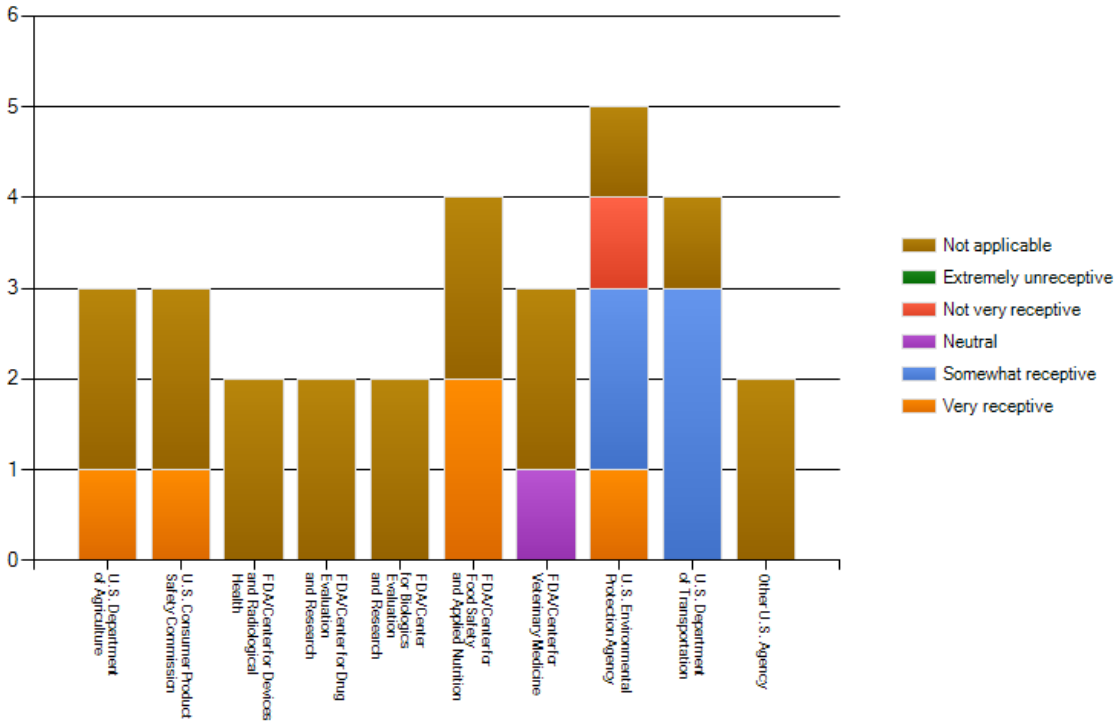
Cost 0%
Timing 0%
Just not practical - we can do a better job using experience 50%

Other – please explain 50%

Comments:

- "Starting doses are selected by our CROs based on weight-of-evidence evaluation of available data."
- Lack of concordance with *in vivo* data; in vitro LD_{50} values far lower than *in vivo."*

Q7. Based on your company's past experience in submitting data from ICCVAM-recommended alternative methods to U.S. regulatory agencies, please rank the receptivity of the agencies to these data.



If other U.S. Agency, please specify and include any additional comments for this question regarding reasons given for not accepting data from ICCVAM-recommended alternative methods.

Comment:

- "Our EHS division does not submit study reports/filings to US agencies."

Q8. We very much appreciate your response this inquiry. Your input carries a great deal of weight and your comments may be used verbatim, but without attribution. Please provide any additional comments on how alternative methods can be better implemented or more effectively approved.

Comments:

- "It would be fair to say that wherever possible the CRO's we use for EHS-related testing would follow protocols that reflect the ICCVAM recommendations. For example, the Rabbit Enucleated Eye Test (REET) is reportedly based on the protocol used by one UK-based CRO. The ocular toxicity recommendations are covered by protocols for the Bovine Corneal Opacity and Permeability Test Method (BCOP). The allergic contact dermatitis recommendations are

covered by the murine Local Lymph Node Assay (LLNA) protocol. For other protocols this may not always be as easy because ICCVAM requirements may not always be acceptable to those outlined in the OECD test guidelines and enforced by EU competent authorities. For acute systemic toxicity, we and our CRO's typically prefer the Acute Toxic Class Method to the Up-and-Down Procedure. CRO's take account of worldwide standards such as the Global Harmonization Scheme for classification and labeling of products when performing study designs. Further, UK-based CRO's have to take account of testing strategies that have been approved by the UK animal welfare Inspectorate as they are bound by UK law to ensure that they meet UK approved methodology and standards within study design and so have to adopt the expectations of the UK Government."

- "Increase collaboration with ECVAM, JACVAM etc; simplify methods evaluation and make it faster; get binding agreement from US Regulatory agencies not only to accept alternative in vitro data but also to REQUIRE it instead of in vivo data; drop request to confirm negative in vitro data with in vivo assay."
- "We continue to use many alternative methods for internal decision making. ICCVAM review and approval of alternative methods is helpful with some of our regulatory submissions but the list of ICCVAM approved methods is still limited. It may be more helpful for ICCVAM to consider domain-specfic approvals for methods rather than try to validate methods across all potential applications."

Results – CRO Survey

Q1. Is your CRO U.S. based or non-U.S. based?
Yes, it is headquartered in the U.S. 83.3%
No, it is headquartered outside the U.S. 16.7%

Q2. What are your CRO's testing capabilities?
In vitro primarily 16.7%
In vivo primarily 16.7%
Both *in vitro* and *in vivo* 66.7%
In vivo, but only after tests have been conducted *in vitro* 0%

Q3. SKIN SENSITIZATION testing at your CRO – Please approximate as a percentage (e.g., 35%) or a ratio (e.g., 98/280) the skin sensitization studies conducted or managed over the past two years (2010 - 2011) for the following:

Assay	Respondent 1	Respondent 2	Respondent 3	Respondent 4	Respondent 5
Local Lymph Node Assay	60 studies	55/89	2%	20%	0
Guinea Pig Sensitization	18 studies	34/89	0%	80%	0
Other screening method for this endpoint (please name methods)	0	0	0%		KerationoSens (10/10)
Not applicable	0	0	0	0	0

Q4. OCULAR IRRITATION/CORROSION testing at your CRO – Please approximate as a percentage (e.g., 35%) or a ratio (e.g., 98/280) the ocular irritation/corrosion studies conducted or managed over the past two years (2010 - 2011) for the following:

Assay	Respondent 1	Respondent 2	Respondent 3	Respondent 4	Respondent 5
In vivo Draize assay	8	141/1630	0%	100%	
ICCVAM-recommended alternative method for ocular irritation/corrosion	0	195/1630	0%		50%
Another screening method for ocular irritation/corrosion (please indicate which screening method)	10	1294/1630	0%		EpiOcular 50%
Not applicable	0	0	0	0	0

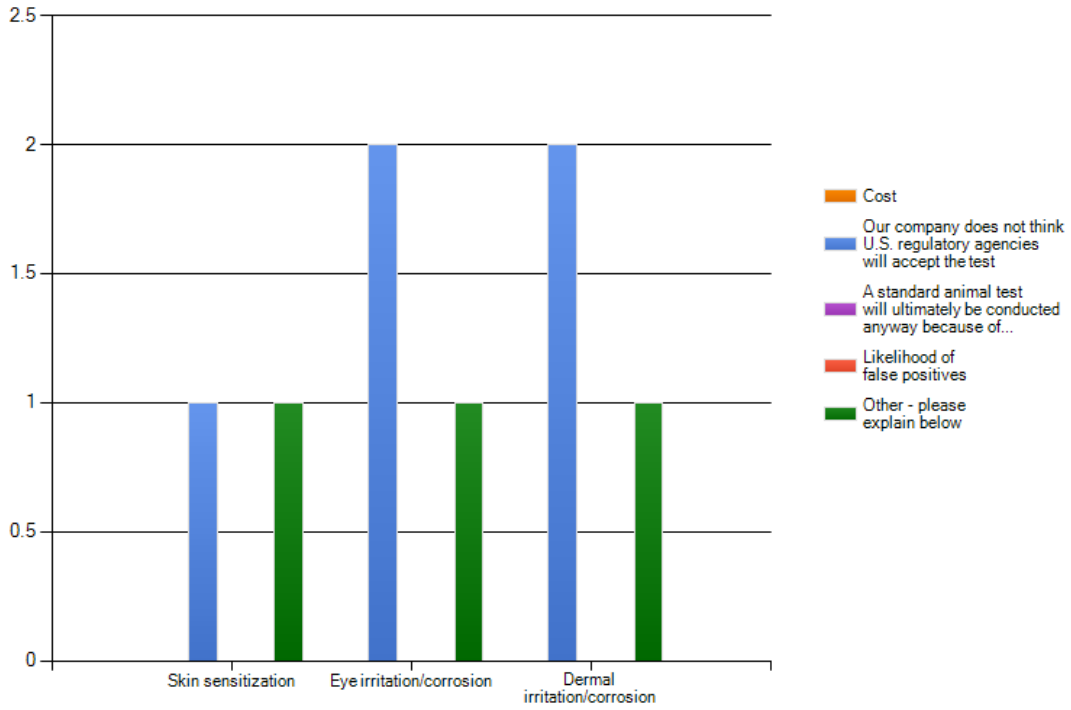
Q5. DERMAL IRRITATION/CORROSION testing at your CRO – Please approximate as a percentage (e.g., 35%) or a ratio (e.g., 98/280) the dermal irritation/corrosion studies conducted or managed over the past two years (2010 - 2011) for the following:

Assay	Respondent 1	Respondent 2	Respondent 3	Respondent 4	Respondent 5
In vivo dermal irritation/corrosion testing	*16*	*157/173*	*2%*	*100%*	
ICCVAM-recommended alternative method for dermal irritation/corrosion (please indicate which alternative method)	*0*	*16/173*	*0%*		*40%*
Another alternative screening method for dermal irritation/corrosion (please indicate which alternative method)	*EpiSkin 6*		*0%*		*Epiderm time course 60%*
Not applicable					

Q6 - 8. Use of in vitro cytotoxicity method to PICKING STARTING DOSES for acute oral systemic toxicity testing – Please approximate as a percentage (e.g., 35%) or a ratio (e.g., 98/280) the studies conducted or managed over the past two years (2010 - 2011) for the following:

Method	Respondent 1	Respondent 2	Respondent 3	Respondent 4	Respondent 5
Up-and-Down	3	0	0	0	100%
Toxic Class	3	0	0	0	
Fixed dose	3	0	0	0	

Q9. For each alternative method below, if ICCVAM-recommended alternative methods are not being done at your CRO, what is the most likely reason(s). Check all that apply.



Comments:

- "We do not believe that there is a fully accepted regulatory replacement for this test. Currently, these are screens."
- "We have been providing alternatives to *in vivo* irritation studies since 1990. Our clients have developed strategies to evaluate potential irritation, not only as a screening tool but to make decisions for potential clinical trials."
- "We do not do environmental chemical testing, only pharmaceutical products. None of our clients ever ask for these methods for evaluation of their products."
- "We believe this to be true under most circumstances."

Q10. For picking starting doses for acute oral toxicity testing, if in vitro cytotoxicity methods are not being done at your CRO, what is the most likely reason(s). Check all that apply.

Cost 25%
Timing 25%
Just not practical – we can do a better job using experience 25%
Other – please explain 75%
Comments

- "We have not investigated this test."
- "Most sponsors rely on knowledge of the chemistry of their products to estimate the starting levels for any of the acute oral toxicity testing. In the 2010 - 2011 time frame we performed 67 up and down and 38 acute toxic class oral studies. None were performed using cytotoxicity to estimate the starting points and in virtually all studies the estimated starting dose was correct."
- "*In vitro* tests are not remotely predictive of animal responses, especially for the types of pharmaceutical products we evaluate. The idea that an *in vitro* test is going to accurately predict the complex drug metabolism that goes on in an animal that impacts toxicity is amusing."
- "The majority of the studies we conduct are limit tests and most of these pass."

Q11. At your CRO, are most alternative methods that are being implemented?:

ICCVAM-recommended methods for regulatory use 25%
Screening methods for non-regulatory use 75%
Comments:

- "We have a very wide selection of *in vitro* tests, primarily offered at our [redacted] facility. Most of the tests are OECD test guideline driven or have been through ECVAM (some ICCVAM) validation."
- "Although some alternatives are being used as screening methods, most are being used to provide estimates of irritancy/nonirritancy for cosmetic and personal care products and ingredients not subject to regulatory review."
- "We use a significant number of *in vitro* ADMET tests to support early drug discovery, not to replace FDA mandated animal tests."
- "We have and will continue to adopt alternative methods once they become uniformly acceptable to the global regulatory agencies"

Q12. We very much appreciate your response this inquiry. Your input carries a great deal of weight and your comments may be used verbatim, but without attribution. Please provide any additional comments on how alternative methods can be better implemented or more effectively approved.

Comments:

- "We have a very wide selection of in vitro tests, primarily offered at our [redacted] facility. The tests include skin absorption (OECD 428), skin irritation (OECD 429), skin corrosion (OECD 431), eye irritation (ECVAM, SkinEthic HCE), *in vitro* cytotox for medical devices, *in vitro* metabolism (induction, inhibition, comparative metabolism), genetic toxicology and hERG (safety pharmacology ICH S7b). This group also evaluates new tests (eg MucilAir inhalation toxicity test) and will become involved in more formal validations (eg currently working on Vitosens and PPRA following ECVAM style validations)."
- "We have been involved in the development, promotion and use of alternative acute ocular testing methods since 1990. We have noted the reluctance of toxicologists to use alternatives without regulatory acceptance. The "we will accept positive results, but a negative result requires animal testing" is a cop-out to appease certain political entities. Acute ocular alternatives have been available for over 25 years. Get regulatory approval for a tiered

strategy to cover the spectrum from non-irritating to corrosive and the only rabbits you'll see will be at Easter."

- "We use *in vitro* tests extensively for early screening, but in the pharma space they are not even close to predictive enough to replace animal testing."

Recommendations

Based on discussions held during the IWG meetings and responses to the surveys, the IWG offers the following recommendations to ICCVAM and NICEATM:

- ICCVAM should regularly collect data regarding implementation of their recommended alternative testing methods from both regulated industry and U.S. regulatory agencies. A survey instrument and the intention to collect information should become part of ICCVAM efforts in the future.
- ICCVAM should generate a concise plan and timeline of implementation of methods and the resulting reduction in volume of animals used. There should be clear articulation of goals and anticipated milestones.
- The preliminary data from this survey should be shared with U.S. regulatory agencies and ICCVAM agencies should formally respond to this report.
- The current survey can be used as a starting point for assessment of implementation of ICCVAM-recommended methods. It can be enhanced and refined; additional granularity in the surveys could be incorporated, e.g., the LLNA could be broken down into non-radioactive/radioactive.
- When requesting data on implementation, specify numeric data regarding the kinds and numbers of assays submitted and accepted should be solicited. Further, ask how many assays were submitted resulting in requests to go back and do follow-up *in vivo* testing.
- Provide advance notice for the intent to request for data along with guidance on variables or parameters to be collected; data have been requested only informally in the past. Encourage industry and regulatory agencies to collect data on implementation on a continual basis, rather than only retrospectively when a request is made.
- Use initial industry-wide and agency-wide surveys to establish a benchmark for the current levels of implementation. This will be important for obtaining the trajectory of change in implementation.
- Each regulatory agency will require a unique survey tailored to its mission. EPA, DOT, and CPSC typically have a standard battery of tests on a substance which is the basis for the submission, so there will be an easier set of questions to ask. FDA typically holds more negotiations with drug companies and has continuing downstream dialogue. However, each agency can and should create some sort of guidance showing how alternative tests can be integrated into safety assessments and when outcomes of alternative tests are sufficient to halt further testing which may require use of laboratory animals.
- Make a goal of surveying agencies to determine how they accept data; what is the signal to move on to an *in vivo* test; are the *in vitro* tests just considered:
    - screening tests
    - supplementary/refinement tests, or
    - definitive/replacement tests

- Determine a regular interval period for implementation surveys to be repeated.
- Targeting the right people to receive implementation surveys in industry and U.S. agencies will be critical.
- Work closely with EPA to assure that ICCVAM-recommended methods are adopted and accepted in a timely way.
- Open a dialogue with FDA regarding the relevance of ICCVAM-recommended methods to FDA's mission.
- Encourage U.S. regulatory agencies to be more proactive in supporting alternatives and becoming involved in ICCVAM activities.
- At future SACATM meetings, provide input on alternatives used for device testing,
- The next generation of alternative test needs to be treated more thoughtfully in the sense of evaluating methods as part of an alternative scheme for assessing chemical or product safety.
- ICCVAM should work with ICATM to advocate for worldwide acceptance of alternative methods.