Stemina
BIOMARKER DISCOVERY

ICCVAM Public Statement
May 27, 2021

Elizabeth Donley, JD, MBA, MS
Chief Executive Officer

Stemina's *In Vitro* Toxicology Assays

Exposure-based prediction of toxicity potential using targeted metabolomics and human pluripotent stem cells and differentiated cells

devTOX^{qP} Accurately Predicts Developmental Toxicity

- First paper published with EPA in 2011 on devTOX
- Extensive validation studies including 2020 Toxicological Sciences paper with EPA on 1,065 ToxCast Phase I and II compounds
- Predictive in a diverse chemical set
  - Pharmaceuticals, agrichemicals, cosmetics, industrial, and environmental chemicals
  - Data compared to published human and *in vivo* results
- Internal study of representative compounds from each category

Compound Set	N	Accuracy*	Sensitivity	Specificity	PPV	NPV
All	124	87%	88%	86%	88%	86%
Pharma	65	90%	87%	92%	94%	83%

*Accuracy reported is balanced accuracy ($\frac{Sensitivity+Specificity}{2}$)

Assay Developers Need a Clear Process for Validation of New Alternative Methods (NAMs)

- Provide specific and clear criteria about information needed for validation or qualification
- Provide a clear process, **with timeline,** for deliverables and validation decision
- Clear assessment of the accuracy and ability of the NAM to provide information for a specific context of use
- ICCVAM as the inter-agency committee is best positioned to provide a single process for qualification of alternatives
  - ICCVAM should provide a new and coordinated process for validation
  - NAM developers cannot afford to run a separate process for every agency and cannot afford to support a multi-year process with no defined timeline

The Regulated Public Needs a Clear Context of Use for New Alternative Methods (NAMs)

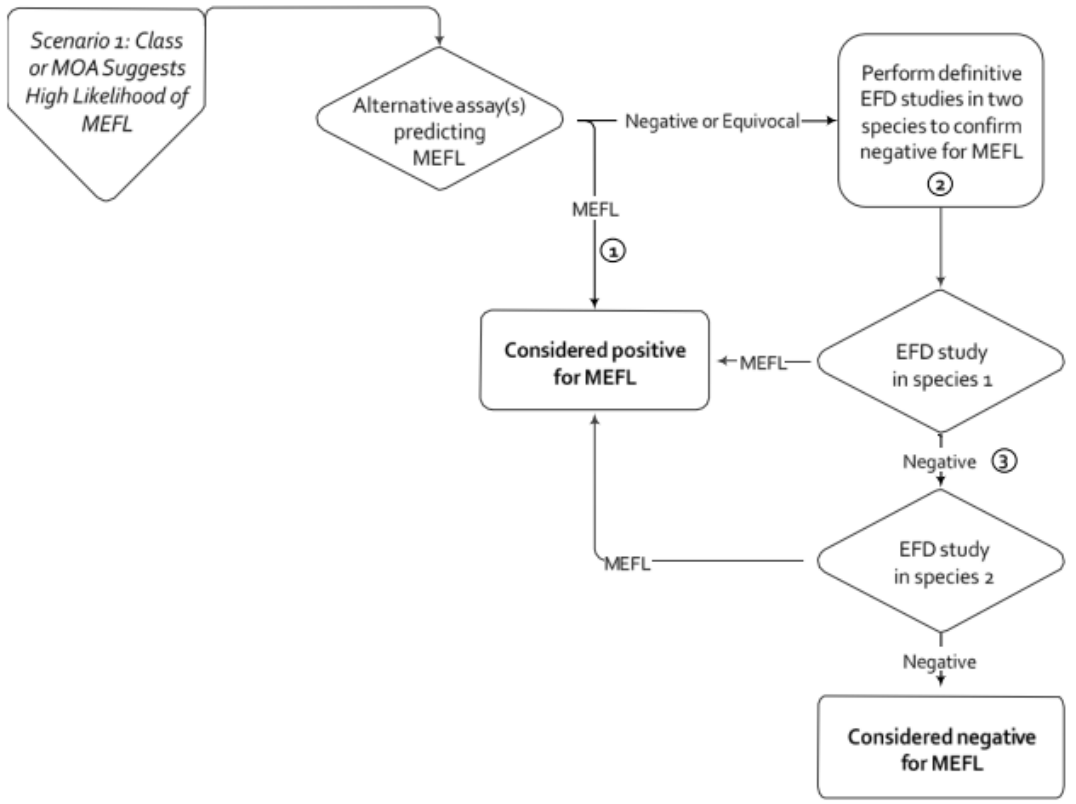
- Provide specific and clear criteria about where and how validated NAMs should and could be used by the regulated public
- Provide case studies for context of use to provide guidance for use of NAMs in candidate compound regulatory approval
- Provide methods for incorporating NAMs in reducing, refining and replacing animals in toxicity testing
  - Examples include read-across and weight of evidence comparison and correlation of compound analog data in vivo with NAM risk assessment data for the candidate compound

New ICH S5(R3) Guidelines Support Use of Qualified Alternative Assays

Let's Use Them to Harmonize Globally

- Guidance provides example scenarios where **qualified** alternative assays could be applied in place of/in conjunction with *in vivo* EFD studies.
  - Pharmaceuticals intended to treat severely debilitating/life-threatening diseases or late-life onset diseases
  - Pharmaceuticals expected to be embryo-fetal toxicants
    - **Stemina has submitted an LOI for the Biomarker Qualification Program for this scenario**

Figure 1: Use of Alternative Assays for Pharmaceuticals Expected to be EFD Toxicants



1) No additional assessment is warranted if unequivocal MEFL signal is observed at clinically relevant extrapolated exposures.
2) Alternatively, pEFD studies can be used; however, negative results should be confirmed by a definitive study in the relevant species
3) Conducting *in vivo* EFD studies in series, as shown, can permit reduction in animal use, as 2nd *in vivo* assay is not warranted if the first study is positive.

Tox21 Success and the 3R's Require a Clear Validation Process

- devTOX is an example of a NAM that:
  - has been peer-reviewed and published 5 times in high profile journals in a decade from 2011 to 2021
  - Including two joint publications with EPA
- devTOX consistently outperforms the required in vivo tests
- devTOX is the only validated human system for assessing species specific response for developmental toxicity (e.g. potential for developmental toxicity in a human embryo)

Yet devTOX has not had the opportunity to assist with the Tox21 objectives and the 3R's because there has not been a process to do so at any agency since the last ICCVAM submission in 2013!

Select Stemina Publications

Simms, L, et al. Curr Res Toxicol. (2020). The use of human induced pluripotent stem cells to screen for developmental toxicity potential indicates reduced potential for non-combusted products, when compared to cigarettes.

Palmer, JA, et al. Toxicol Sci. (2020). A Targeted Metabolomics-Based Assay Using Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Identifies Structural and Functional Cardiotoxicity.

Zurlinden, TJ, et al. Toxicol Sci. (2020). Profiling the ToxCast Library With a Pluripotent Human (H9) Stem Cell Line-Based Biomarker Assay for Developmental Toxicity.

Palmer, JA, et al. Reprod Toxicol. (2017). A human induced pluripotent stem cell-based in vitro assay predicts developmental toxicity through a retinoic acid receptor-mediated pathway for a series of related retinoid analogues.

Zhu, H, et. al. ALTEX. (2016). Supporting read-across using biological data.

Palmer, JA, et. al. Birth Defects Res B Dev Reprod Toxicol. (2013). Establishment and Assessment of a New Human Embryonic Stem Cell-Based Biomarker Assay for Developmental Toxicity Screening.

For a complete list visit our website: https://stemina.com/resources/journal-publications/