VALIDATION, THE MISSING LINK TO MOVE FROM RESEARCH TO TOOLS AND METHODS: EXPERIENCE AND EXPECTATIONS FROM THE FIELD

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Mutual Acceptance of Data (MAD)

- Council Decision 1981: "*Test data generated in any member country in accordance with OECD Test Guidelines and Principles of Good Laboratory Practice (GLP) shall be accepted in other member countries for assessment purposes and other uses relating to the protection of human health and the environment".*

Under MAD:

- Council Decision is legally binding on OECD member countries
- Countries can set their own data requirements and make their own assessment of the information provided by the tests.
- Countries cannot ask industry in an OECD country to do a test using a different method for which an agreed OECD TG exists
- "**Tested once, accepted everywhere**"

Major Points

- What is validation in an OECD context?
- What are the stages of validation in the development of an OECD Test Guideline.
- How do we move from research tools in the literature to developing and validating a method.

The Process:

Developer: *Comes forward with an assay developed for a regulatory purpose. How can they build confidence in this assay to be considered as a guideline?*

- Who needs the test? Is the test fit for purpose? Does it inform of human or eco-toxicity?
- Are regulatory agencies in need for the test and is the test going to be accepted and used by regulators?

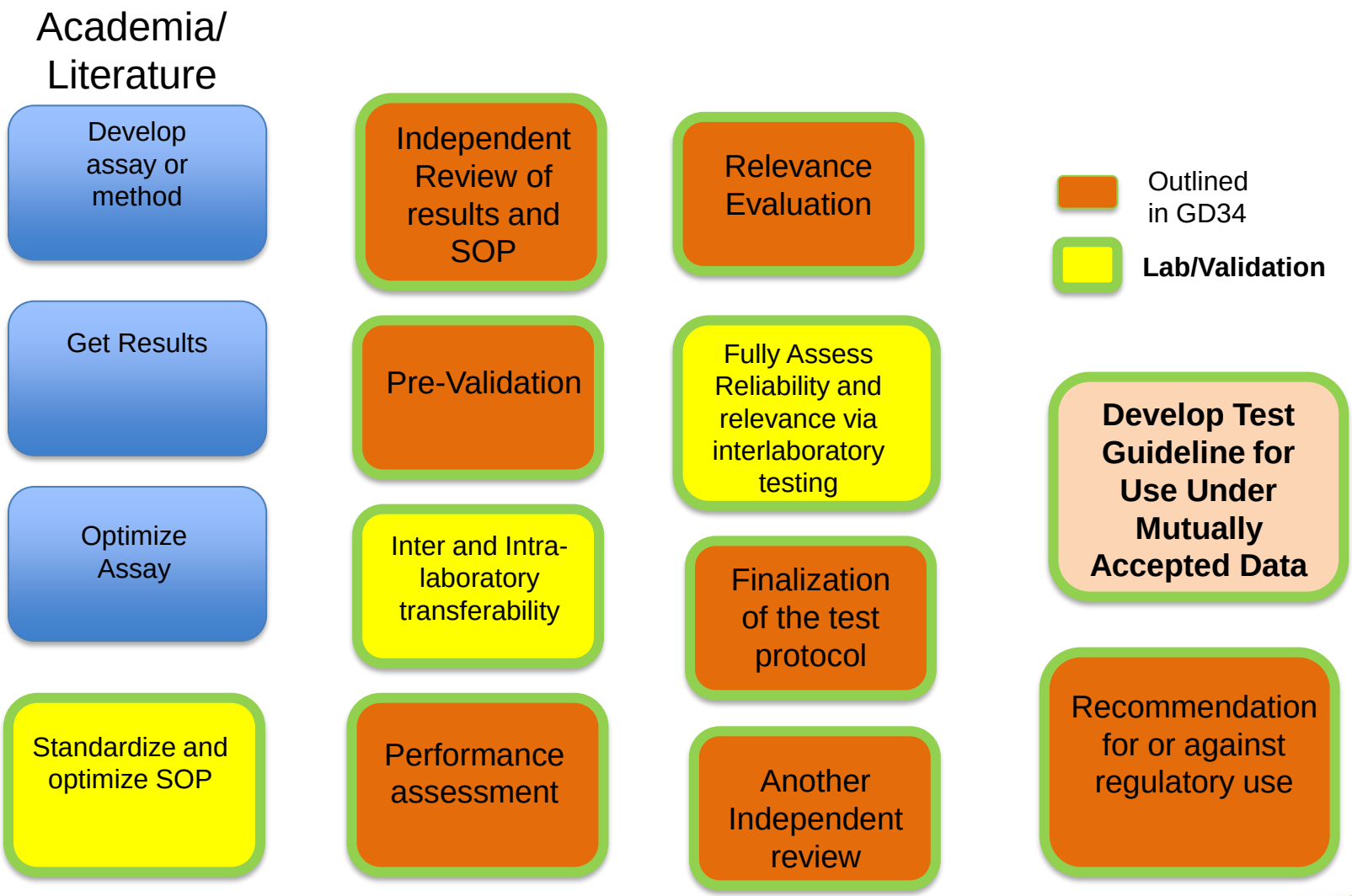
- What data does one need to provide in order to convince regulatory agencies to accept the data provided?

- Is it reproducible, is it sensitive and is it specific.

OECD GD 34 Drafted to Provide Guidance.

- What is regulatory need?
- Ring Tests How many labs and how many chemicals etc.

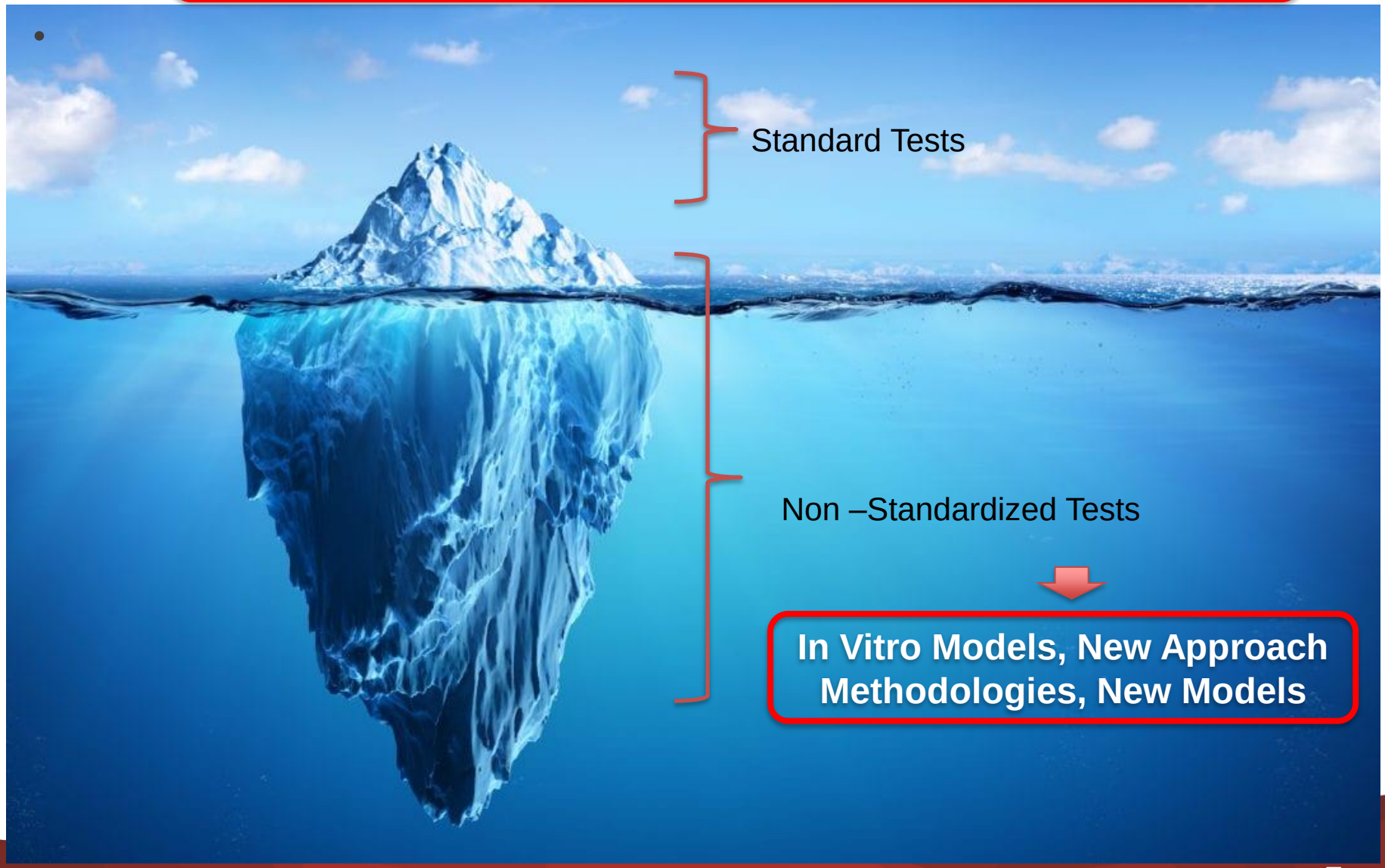
Guidance Document-34 Process for Review and Acceptance of New or Revised Approaches for Regulatory Decisions



- **THIS PROCESS IS VERY SLOW!**

The Challenge

Tens of Thousands of Chemicals and Potential Endocrine Disruptors



Endocrine Disruption

- Endocrine disruption is a broad term.

- Historically it was limited to EATS Estrogen Androgen Thyroid and Steroidogenesis.

- Now we know that it can include many other tissues, and that chemicals with endocrine disrupting effects can affect many health outcomes.
- GAPS:
  - Non-EATS testing (Metabolic Disease)

How can we speed things up?

- Evaluation of the New Methods based on:

- Reproducibility
- Biological Relevance i.e. predict human or eco-toxicological endpoint.
- Regulatory usefulness

- Incorporating methods not as a stand-alone but as an integrated approach.

Metabolic Disruption - Data Gaps

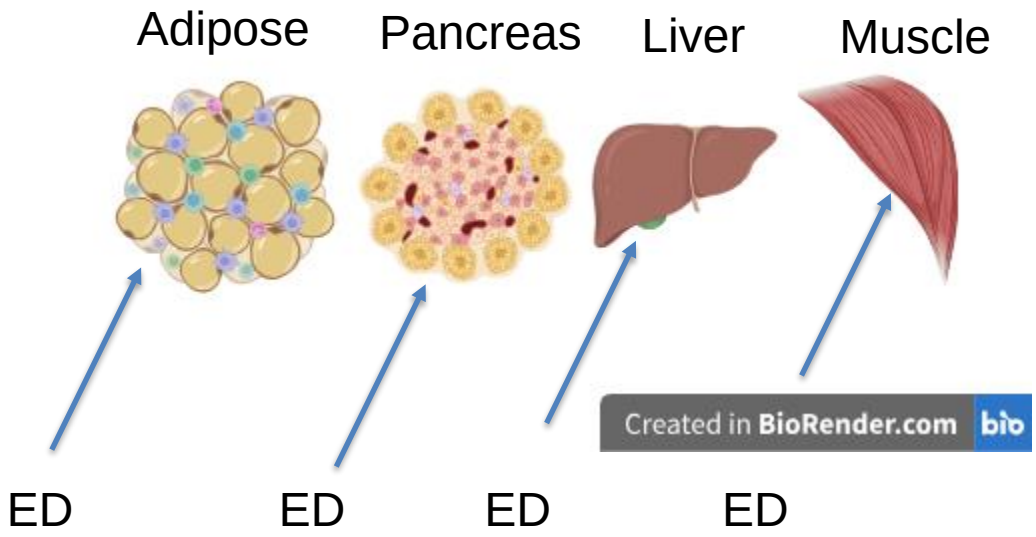
- Diabetes
- Liver disease, with some exceptions, rodent assays can detect steatosis.
- Obesity

- Endocrine-related cardiovascular effects (NOS, endothelins [air pollution])

- Health outcomes that are
  - vulnerable to environmental influences
  - Huge burden of morbidity and mortality to developed society
  - Very poorly evaluated in existing testing and assessment approaches

Endocrine Disruption includes Metabolic Disruption

- Animal studies have blind spots for this kind of disruption
- There are no test guidelines that are designed to test for, insulin resistance, insulin release, liver fibrosis etc.



SOPs in the Literature

- For a method to be reproducible and reliable we need a proper standardized method.

- Methods in the literature: How detailed are methods in the literature?
  - Not Very
  - Not consistent

- **Very important**: Can one rely on the results to make a regulatory decision?

Why there aren’t more labs participating in validation exercises?

- In order for a test and its results to be accepted by regulators they need to be reproducible.
  - The exact same experiment repeated multiple times in separate laboratories
- One cannot publish already published results.

- There is no interest from academia to **precisely** reproduce someone else's data

  - No benefit for career progression, acquiring funding, attracting students, etc

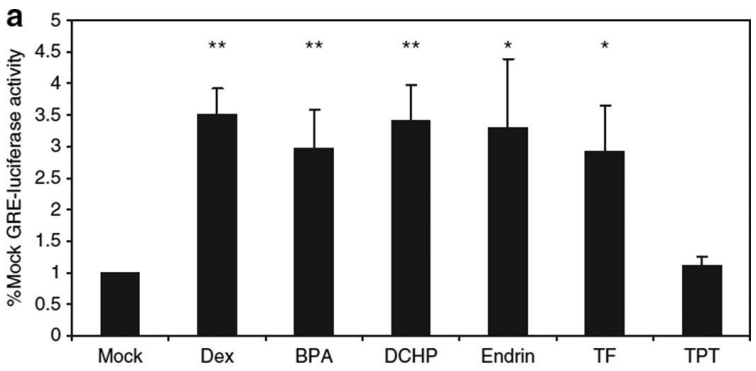
Case Study #1 The Glucocorticoid transactivation study

- In order to demonstrate specificity and sensitivity for a test guideline literature needs to be consulted to gather at least 20 positives, and 20 negatives.

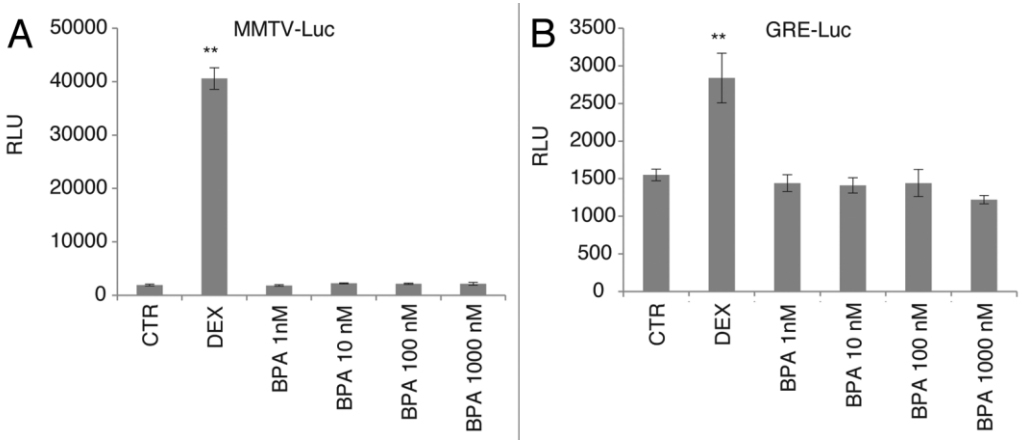
- This was an issue for the GR-TA.

- Reproducibility?

The Case of BPA Reproducibility for Glucocorticoid Receptor Transactivation



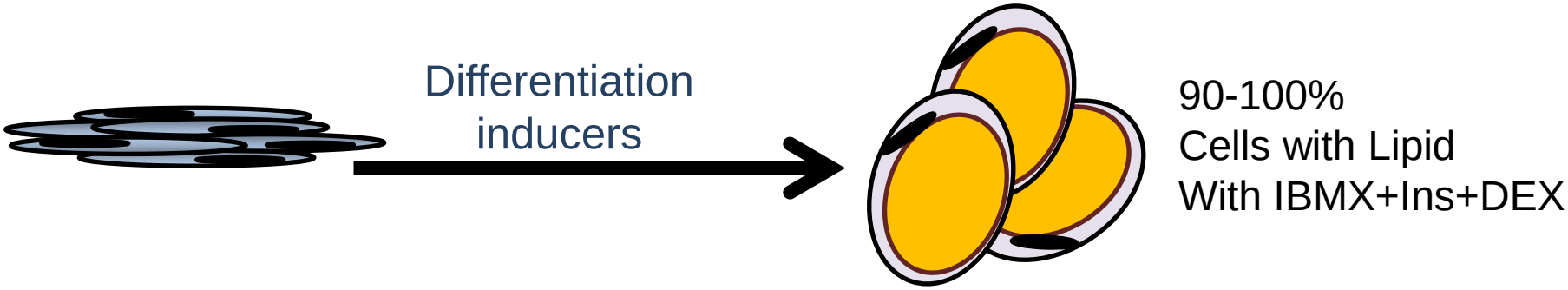
Sargis RM et al., Obesity (Silver Spring). 2010 Jul;18(7):1283-8.



Atlas E, et al. Adipocyte. 2014 Jul 1;3(3):170-9.

Case #2 Adipogenesis- In Defence of Clear Detailed SOPs

- Adipogenesis – Fat Cell Differentiation – Chemical Effects



- Some labs use IBMX Insulin and Dexamethasone + Chemical (Sensitivity?)
- Some other labs use only IBMX + Ins + Chemical
- Some labs use only chemical (Is it fit for purpose?)

SOPs

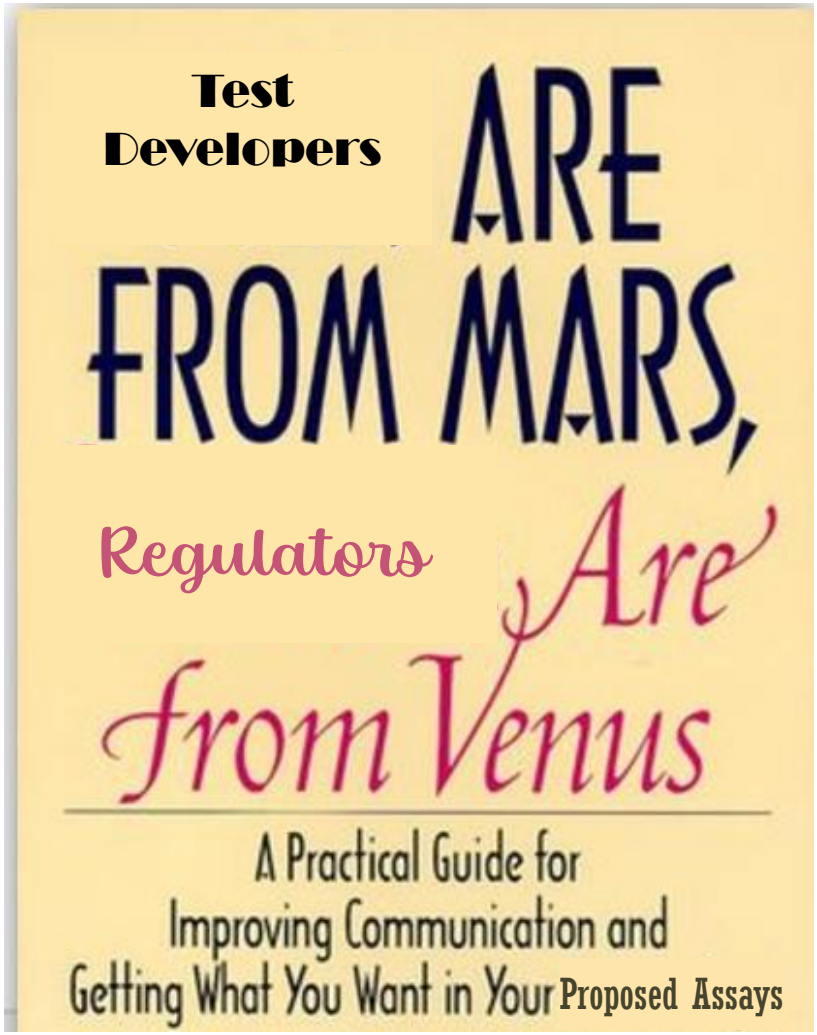
- Another example is in vitro assay for ER activation, estrogenicity
- Many labs are using the MCF-7 cell model
- However, in some publications we identified that chemicals that were supposed to be estrogenic were not detected in the high throughput transcriptomics in MCF-7 cells.
- Why?
- The media was not depleted from E2.
- THIS HIGHLIGHTS THE IMPORTANCE OF USING STANDARDIZED METHODS

NAMs

- New Approach Methodologies: NAMs
- As regulatory agencies are moving away from animal tests new tests and approaches are being developed and published.
- NEW CHALLENGES
- HOW DO WE MOVE FROM PUBLISHED TESTS AND RESULTS TO VALIDATED TESTS ACCEPTED BY ALL

SOPs

- NAMs are being developed at a very high rate
- There are methodologies that require expertise and thus detailed SOPs are crucial for the success of these methods.
- The US EPA is developing many high throughput screening for identifying Molecular Initiating Events (MIEs) (ToxCast).
- Gene Expression Analysis
- Cell Painting
- All these are done *in vitro,* ***but they are not standardized or accepted for regulatory purposes.***



Academic Test Developers need be aware of how test information is used to ensure test protocols are designed to be fit for this purpose

Moving forward

- More labs need to participate in the validation exercises.
- Clear MIEs with expected health outcomes need to be developed, maybe incorporated in AOPs.
- Methodologies to investigate the endocrine disruptor effects are advancing all the time in academic labs.
- As the field advances at such high velocity coordination and collaboration between academia, industry and regulatory is imperative to arrive at mutual acceptance of data.

- Thank You!