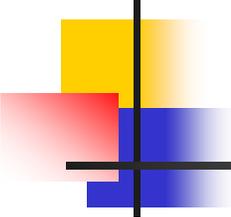


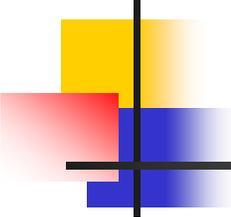
Who's Who in the Validation of Assays for the EDSP

Briefing for New EDMVAC Members
March 2, 2005



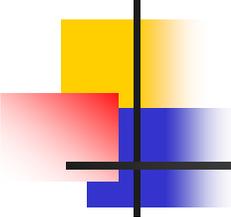
Objectives of Briefing

- To provide background on test method validation
- To delineate the roles of the organizations involved in the validation of EDSP assays
- To begin to communicate the strategy and assumptions EPA is using to accomplish validation



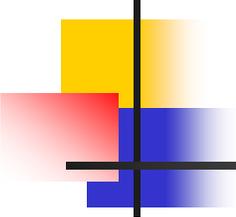
Cast of Characters

- EDSP team--staff in OSCP/OPPTS responsible for the validation of assays and development of the EDSP
- EDMVAC—a committee of technically qualified stakeholders under FACA to advise EPA on the validation of assays for the EDSP
- ICCVAM—an interagency committee set up by statute to validate test methods
- ORD—staff in EPA's Duluth and RTP labs who provide technical assistance to OSCP
- OECD—an international organization to avoid duplication of efforts and non-tariff barriers to trade



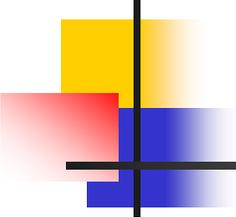
Outline of Briefing

- Part I-- Background on ICCVAM and the validation process
- Part II-- Organizational roles
- Part III-- Validation of assays for the EDSP



Evolution of ICCVAM

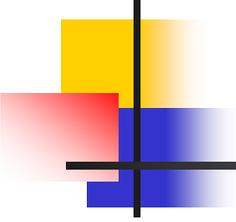
- National Toxicology Program
- 1978 ■ **Develop and validate improved test methods**
- NIH Revitalization Act: P.L. 103-43
- 1993 ■ **Develop and validate test methods for acute and chronic safety testing, including alternative methods that can reduce or eliminate the use of animals**
- **Establish criteria for validation and regulatory acceptance**
- **Develop process for regulatory acceptance of scientifically valid methods**



Evolution of ICCVAM (cont.)

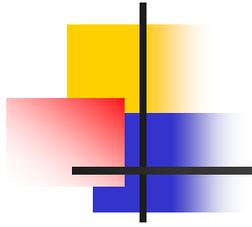
- Report of the ad hoc ICCVAM
- 1994** ■ ICCVAM established
- 1997**
 - Replaced ad hoc ICCVAM
 - Implemented NIEHS directives: P.L. 103-43
- 1998**
 - NTP Advisory Committee on Alternative Toxicology Methods
 - NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICETAM)
 - ICCVAM Reauthorization Act
 - Structure and role of ICCVAM
- 2000**
 - Requires the use of valid methods

ICCVAM and NICEATM Goals



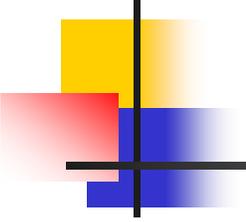
- To promote the scientific validation and regulatory acceptance of new alternative test methods that:
 - are more predictive of human health and ecological effects than current methods
 - refine, reduce, and replace animal use where scientifically feasible
- To contribute to improved public health
 - ⇒ Improved risk assessments
 - ⇒ Improved risk management
 - ⇒ Prevention of injury and disease!

Prerequisites for Using New Methods

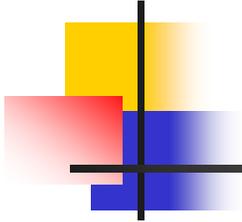


- Scientific Validation
 - Determination of the usefulness and limitations of a test method for a specific purpose
 - Determination of relevance and reliability
- Acceptance for Regulatory Use
 - Determination that the proposed use of data from the new test method will provide for comparable or better level of protection of human health or the environment than the current method or approach

What does Scientific Validation Involve?

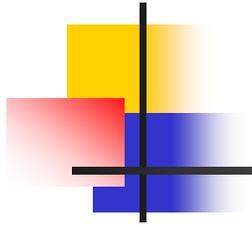
- 
- Determination of Relevance
 - The extent to which a test method will correctly predict or measure the biological effect of interest
 - e.g., accuracy, sensitivity, specificity, false negative rate/ false positive rate
 - Determination of Reliability
 - The extent to which a test can be performed reproducibly within and among laboratories over time.
 - **NOTE: Validation status is not immutable**
 - Subsequent data and experience can lead to loss or affirmation of validation status

Criteria for Test Method Validation



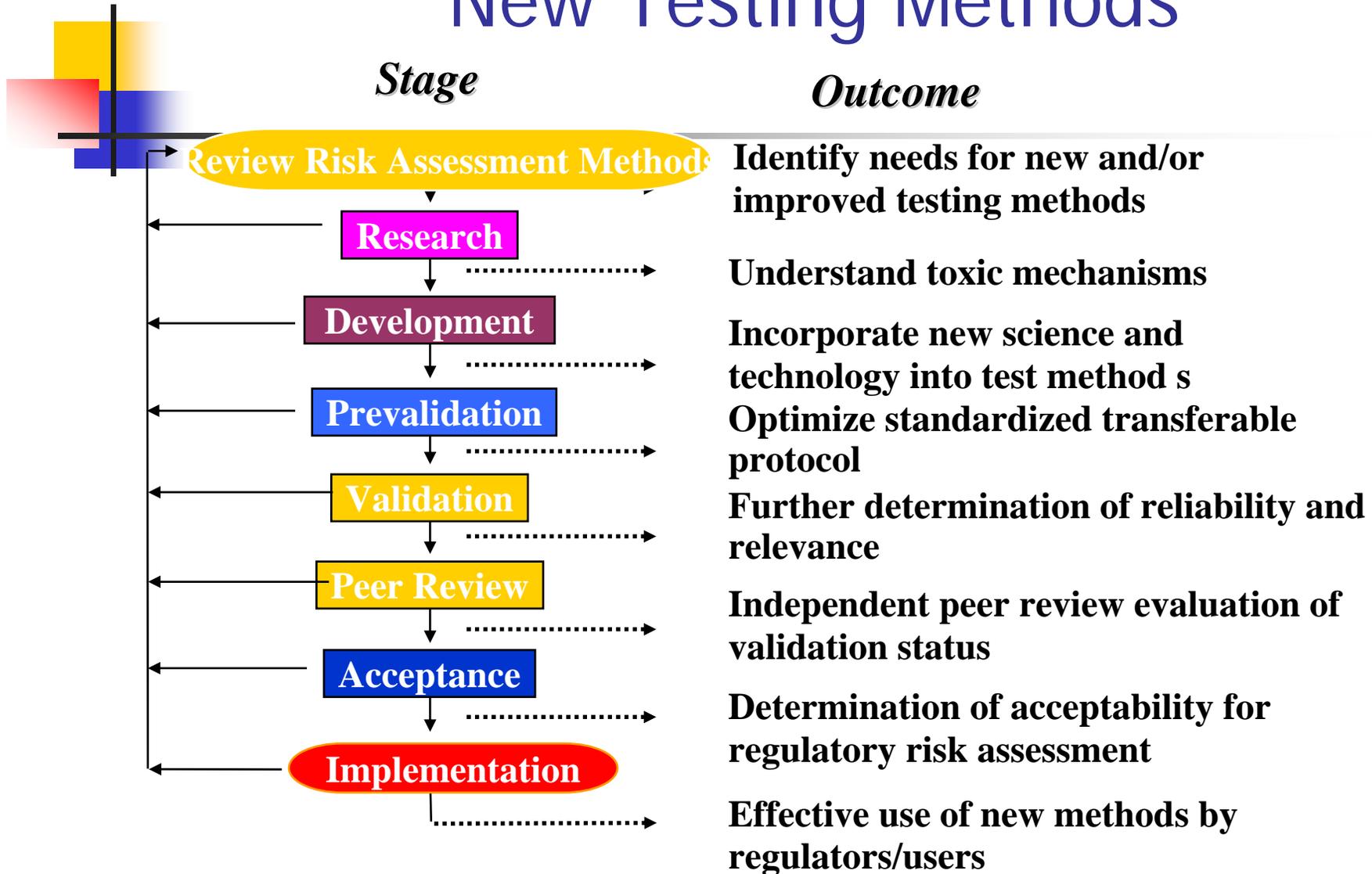
1. Clear statement of proposed use
 2. Biological basis/relationship to effect of interest
 3. Formal detailed protocol
 4. Reliability assessed
 5. Relevance assessed
 6. Limitations described
 7. All data available for review
 8. Data quality: *Ideally GLPs*
 9. Independent scientific peer review
-

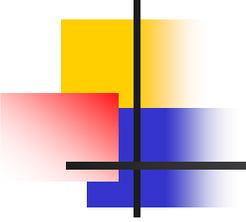
Criteria For Test Method Acceptance



1. Fits into the regulatory testing structure
 2. Adequately predicts the toxic endpoint of interest
 3. Generates data useful for risk assessment
 4. Adequate data available for specified uses
 5. Robust and transferable
 6. Time and cost-effective
 7. Adequate animal welfare consideration (3Rs)
-

Evolution Process for New Testing Methods

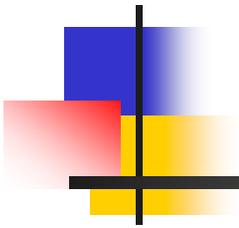


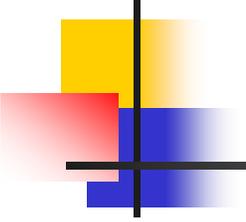


Validation Process Statutory Requirements

- EPA must use valid screens and tests in the EDSP (FQPA)
- The EDSP must be reviewed by the FIFRA Scientific Advisory Panel or Scientific Advisory Board (FQPA)

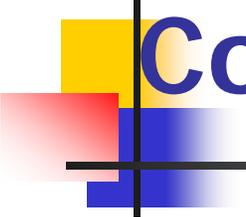
Part II: Organizational Roles





Process Realities for EDSP

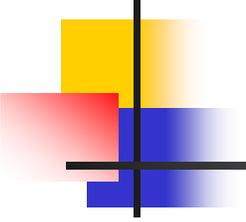
- Need validated test methods
- Follow ICCVAM guidance for validation of test protocols
- Stakeholder involvement throughout the process
- Process must be open under the FACA procedures
- International harmonization of guidelines through OECD
- Deadlines established in NRDC settlement agreement
- Deadlines in appropriations process



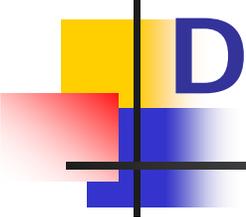
Challenge: How to Meet Conflicting Requirements

- Guidelines of international interest to be developed and validated through coordination in OECD
- Most domestic guidelines to be developed and validated by EPA with advice from EDMVAC
- ER/AR binding assay to be validated by ICCVAM.

EPA Process for Domestic Test Methods



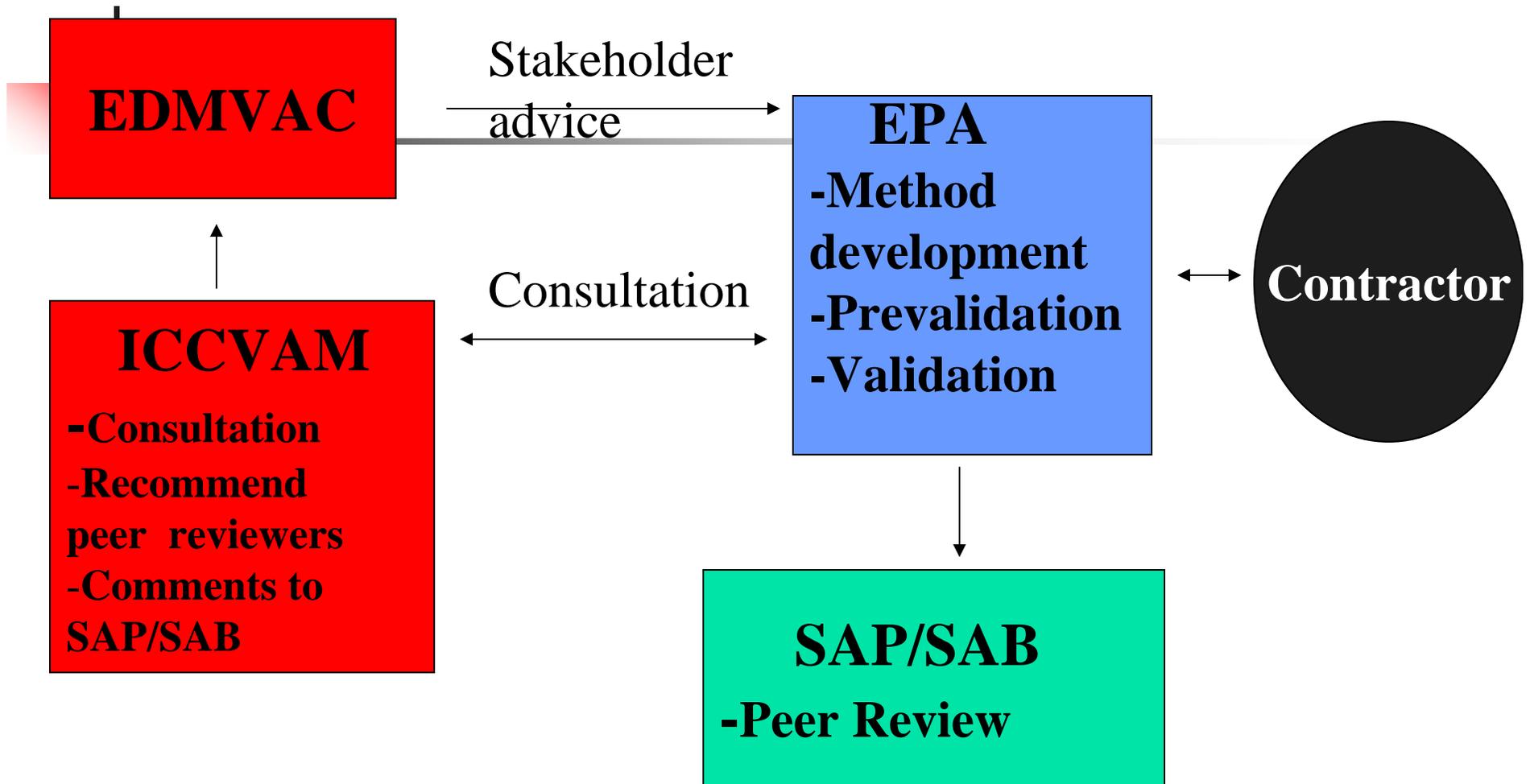
- For most domestic test methods, follow ICCVAM guidance with EPA process
- EDMVAC will advise EPA on design and execution of validation work and validation products
- ORD will develop new methods and provide technical advice to EDSP
- EDSP/OSCP will conduct literature reviews and laboratory work for methods optimization, standardization and validation through Contractor

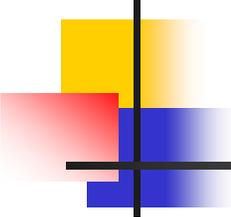


EPA Process for Domestic Guidelines

- Involve ICCVAM through representation on EDMVAC
- Consult with ICCVAM between pre-validation and validation and give opportunity to comment at peer review
- Conduct independent peer review

Process for Domestic Guidelines

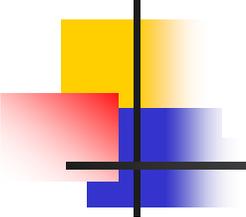




Process for Test Methods of International Interest

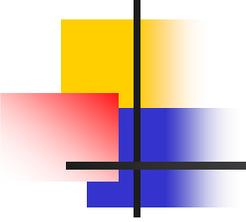
- Endocrine disruptor test methods of international interest to be developed and validated through coordination in Organization for Economic Cooperation and Development

OECD



(Organisation for Economic Co-Operation and Development)
Intergovernmental Organisation grouping 30 industrialised countries

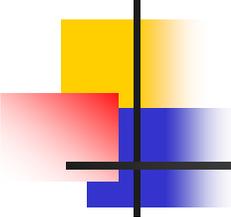
- Austria
- Belgium
- Czech Republic
- **Denmark**
- Finland
- France
- **Germany**
- Greece
- Hungary
- Ireland
- Italy
- Luxembourg
- **The Netherlands**
- Poland
- Portugal
- Slovak Republic
- Spain
- **Sweden**
- **United Kingdom**
- Iceland
- **Norway**
- **Switzerland**
- Turkey
- Canada
- Mexico
- **United states**
- Australia
- **Japan**
- New Zealand
- South Korea



1981 "MAD" DECISION

OECD Council Decision on Mutual Acceptance
of Data in an Assessment of Chemicals including Pesticides
C(81)30(Final)

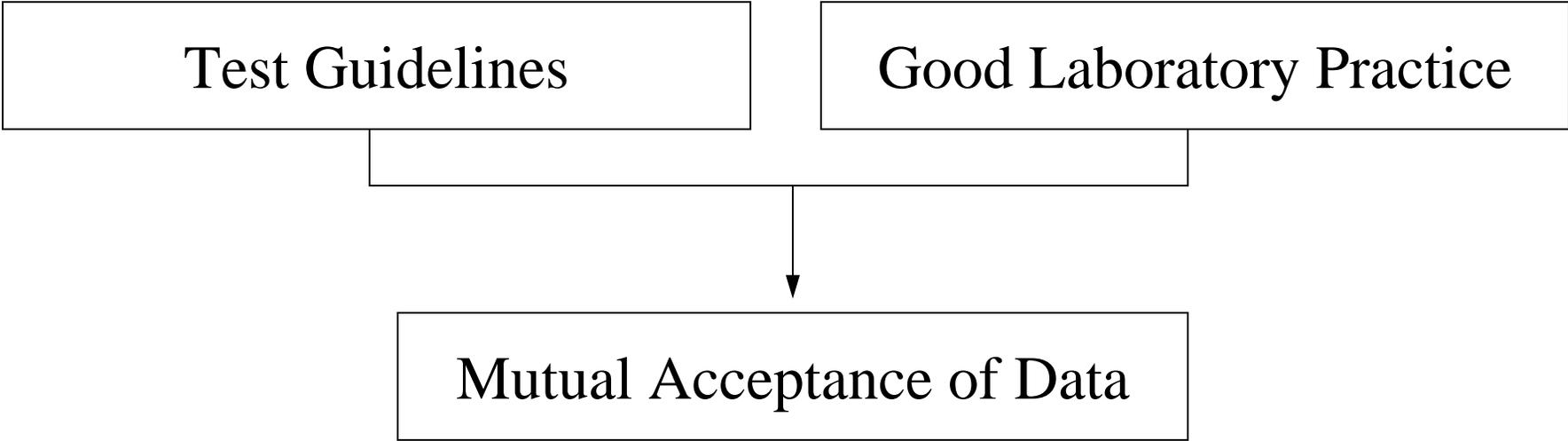
" Decides that the data generated in the testing of chemicals in an OECD member country in accordance with OECD Test Guidelines and OECD Principles of Good Laboratory Practice shall be accepted in other member countries for purposes of assessment and other uses relating to the protection of man and the environment."



DATA QUALITY ENSURED BY

Test Guidelines

Good Laboratory Practice



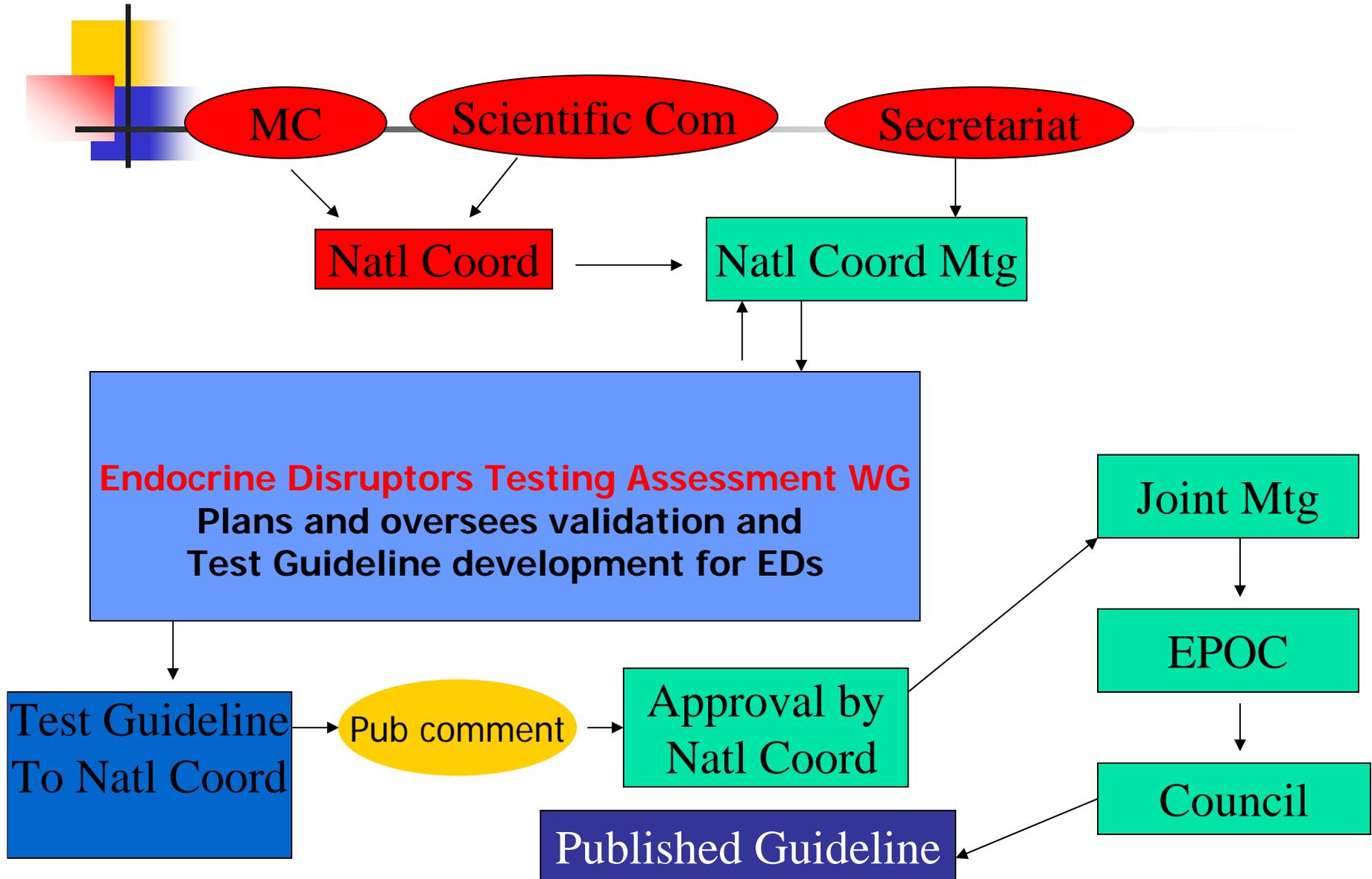
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graph TD; A[Test Guidelines] --- B[Good Laboratory Practice]; B --> C[Mutual Acceptance of Data];
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Mutual Acceptance of Data

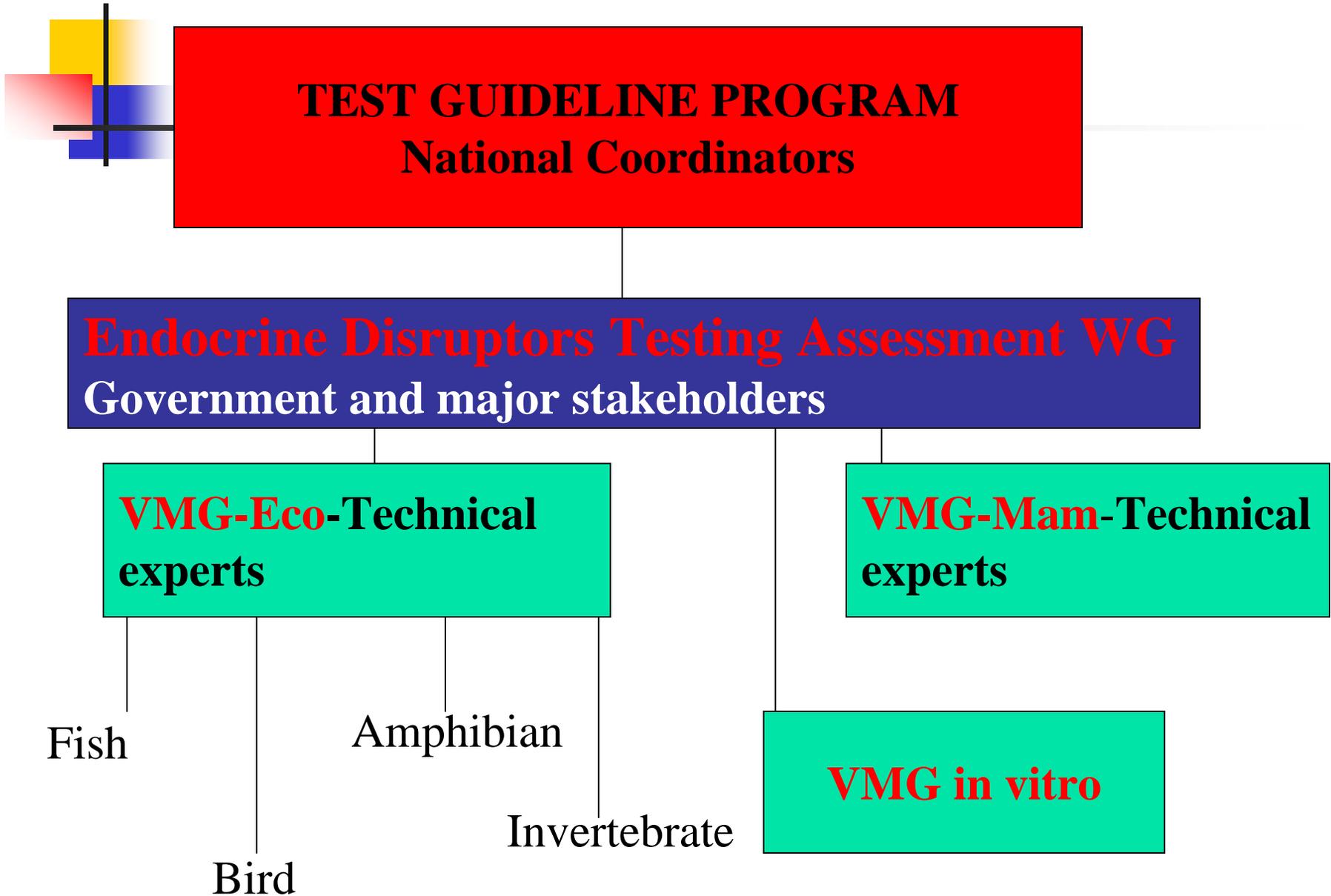
Avoid: duplication of testing by industry
non-tariff trade barriers

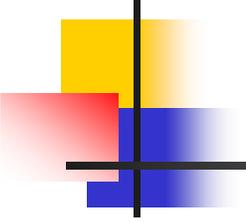
MAD Council Decisions open to non-members

OECD Test Guideline Process



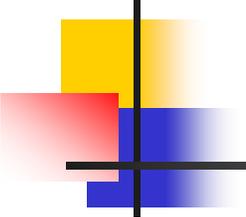
OECD ED Test Guideline Roles





Possible Modes of OECD Involvement in Test Method Validation

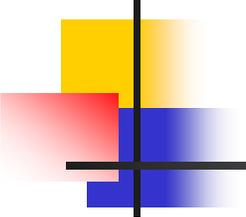
1. Coordinates prevalidation and validation
2. Lead country develops prevalidation data; OECD manages validation
3. Lead country develops all prevalidation and validation data for OECD guideline development



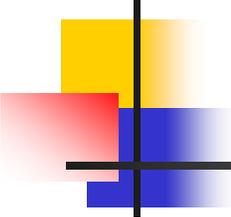
Role of EDTA

- Plan and execute pre-validation and validation of some endocrine test methods
- Oversee the development of test guidelines based on the validated procedures
- Provide review and quality control of documents prior to submission to National Coordinators

Process for International Guidelines



- OECD's Endocrine Disruptor Testing and Assessment workgroup will be primary vehicle for deliberation and stakeholder input
- US will be lead country or co-lead on most guidelines
 - Lead country coordinates technical work
 - US volunteering for lead country because we have resources and are mandated to meet schedule



Process for International Guidelines

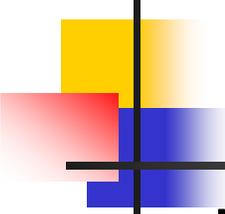
- Peer review by letter
- Validated test methods will be the basis for test guidelines
- National Coordinator comment process
- EDMVAC kept informed and will be asked for input for US position

Lead Organization for Validation

	EPA	OECD	ICCVAM
ER AR Binding			X
Steroidogenesis	X		
Aromatase	X		
Uterotrophic		X	
Hershberger		X	

Lead Organization for Validation

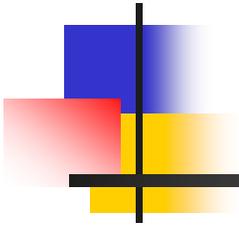
	EPA	OECD	ICCVAM
Pubertal female	X		
Pubertal male	X		
Frog assay		X	
Fish Repro Screen		X	
Adult male [U.S. industry]			

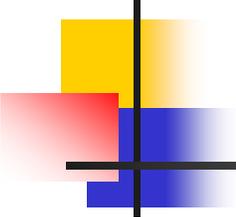


Lead Organization for Validation

Test	EPA	OECD	ICCVAM
Mammalian		?	
Avian		X	
Fish		X	
Amphibian		X	
Invertebrate	?	?	

Part III: Validation of Assays for the EDSP

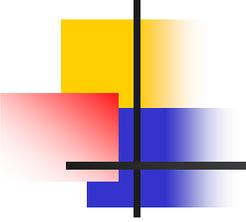




KEY CONCEPTS IN EPA VALIDATION APPROACH

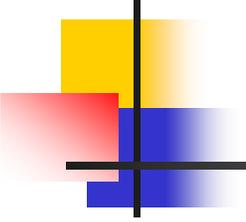
- Select core chemicals to permit comparison of assays
- Augment core chemicals with chemicals specific to each assay
- Run more chemicals in prevalidation than in validation phase to establish relevance of assay in prevalidation
- Select small number of chemicals in validation phase
- Empirically validate assay rather than battery
- Battery validation will be an analysis of the test results of individual assays

Validation Process for EDSP

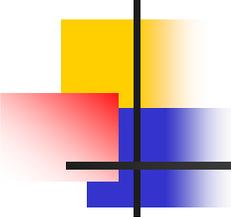


- Method development and preparation of **Detailed Review Paper (DRP)**
- Pre-validation
 - Demonstration of relevance
 - Development of standard optimized protocol
 - Determination of readiness for validation in consultation with EDMVS and ICCVAM
- Validation in multiple laboratories
 - Demonstrate reliability across labs and over time
- Independent peer review of validation effort

Detailed Review Paper

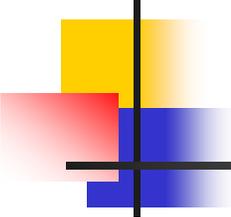


- Explains basic purpose of the assay and the context in which it will be used
- Explains scientific principles upon which the assay rests
- Reviews candidate protocols and compares them with respect to meeting purpose, cost and other practical considerations
- Identifies their developmental status and information needs
- Recommends a protocol for initiation of prevalidation



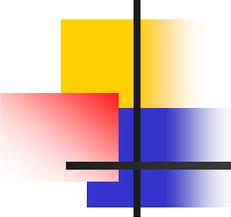
Prevalidation

- Types of prevalidation studies depend on state of development of protocol
 - **Protocol demonstration**
 - Confirms results in literature
 - Gives **lead laboratory** experience in conducting assay
 - Uses small number of chemicals



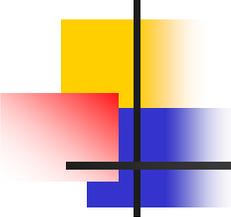
Prevalidation

- **Special studies** (as needed)
 - Address specific questions that arise from the DRP or during the course of the protocol demonstration
- **Protocol optimization study**
 - Conducted to choose optimum conditions, refine the protocol, and eliminate non-sensitive or duplicative endpoints
- **Multi-chemical study**
 - Primary test of relevance
 - Should state expected outcomes before conducting
 - Typically 8-12 chemicals whose mode of action is known for Tier 1, more for some in vitro and less for Tier 2
 - Conducted blind to eliminate bias



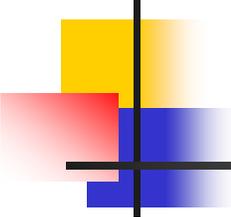
Prevalidation

- For new assays, at least one prevalidation study will be conducted in more than one laboratory to get an estimate of interlaboratory transferability
- **Prevalidation study report**
 - Summarizes and analyzes the results of prevalidation studies
 - Addresses question of readiness for validation
 - EDMVS review is essential before beginning interlaboratory validation



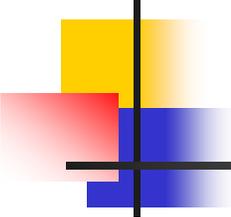
Validation

- Purpose:
 - Tests transferability of protocol to other laboratories
 - Determines reliability of protocol
 - Adds to understanding of relevance
- Approach:
 - Conduct identical studies in 3 laboratories
 - Use small number of chemicals in studies
 - Goal: one per major mode of action
 - Validation studies conducted according to GLPs
 - Flexibility needed for addition of new endpoints to existing guideline.



Validation

- **Validation report**
 - Will summarize data from each participating laboratory
 - Analyzes key parameters that allow a determination to be made regarding reliability
- **Integrated summary report**
 - Summarizes background data, prevalidation report and validation report
 - Addresses adequacy of validation
 - Follows ICCVAM BRD format



Peer Review

- An independent peer review panel will be convened to review groups of related assays
- Peer review panel could be convened by EPA (SAP/SAB joint panel), NICEATM, or a contractor
- All reports would go to the peer review panel. Raw data would be available upon request.