U.S. Food & Drug Administration, National Center for Toxicological Research Jefferson, Arkansas

Paul C. Howard, Ph.D.

Presentation to the:

Science Advisory Committee on Alternative Toxicological Methods (SACATM)

18-19 June 2008

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The opinions expressed by the speaker should not be considered official statement or policy of the FDA, or any other federal agency. The opinions expressed are his alone.

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FDA U.S. Food and Drug Administration U.S. Department of Health and Human Services

FDA's Mission Statement

The FDA is responsible for protecting the public health by assuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation.

The FDA is also responsible for advancing the public health by helping to speed innovations that make medicines and foods more effective, safer, and more affordable; and helping the public get the accurate, science-based information they need to use medicines and foods to improve their health.

Underlined for emphasis by P Howard

U.S. Food and Drug Administration

Mission – Protect the public health by assuring safe and effective medical products and safe foods for humans and animals.

- **Foods**
  - All interstate domestic and imported (including produce, fish, shellfish, shell eggs, milk) except meat and poultry.
  - Bottled water.
  - Wine (<7% alcohol).
  - Infant formula

Food Additives

  - Colors
  - Food containers

- **Cosmetics**

Dietary Supplements

Animal Feeds

Pharmaceuticals

  - Human (safety, efficacy)
  - Animal (safety, efficacy)

Medical Devices

Radiation Producing Devices

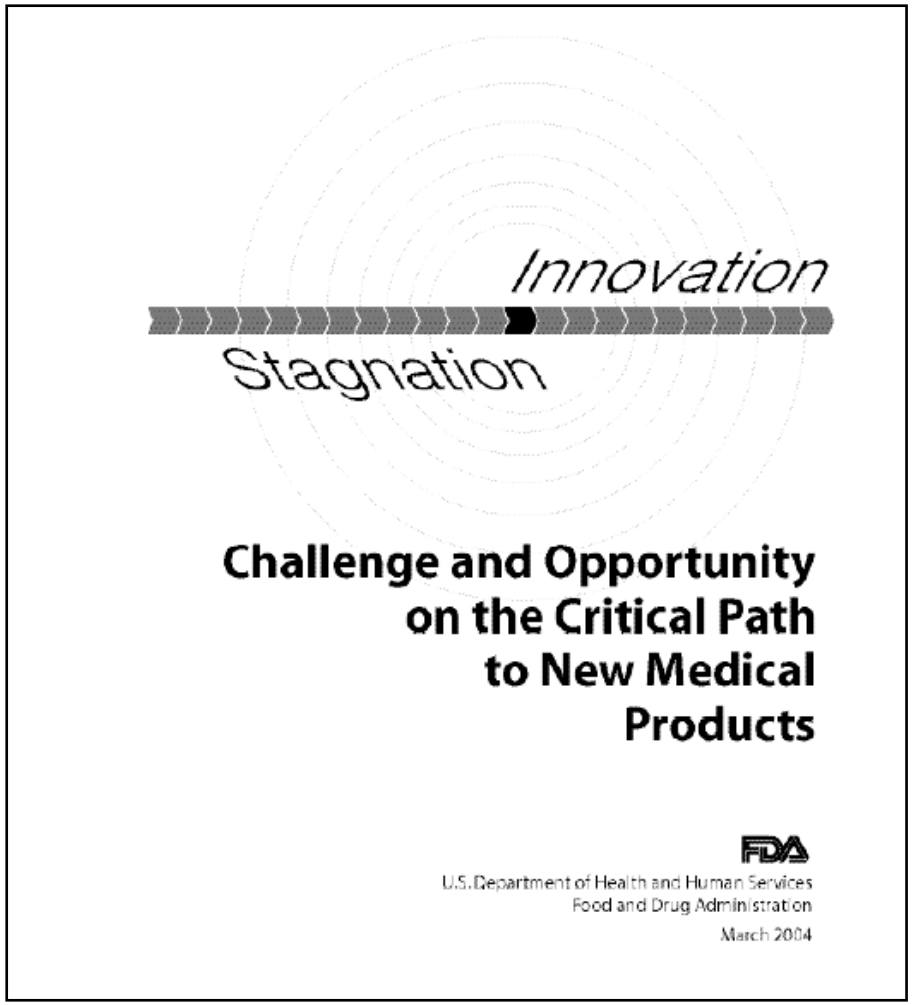
Vaccines

Blood Products

Tissues

Sterilants

Critical Path Initiative



Modernize the sciences through which the FDA-regulated products are developed, evaluated and manufactured.

Path to enhancing medical product development, with better tools for:

- Assessing safety *

- Demonstrating medical utility

- Characterization and manufacture

http://www.fda.gov/oc/initiatives/criticalpath/initiative.html

Provides description of:
(1) *the accomplishments of the Critical Path Initiative*, and
(2) *specific opportunities that could help speed development and approval of medical products.*

http://www.fda.gov/oc/initiatives/criticalpath/reports/opp_list.pdf

*Better Evaluation Tools

Biomarker Qualification and Standards

Qualifying Disease- and Disorder-Specific Biomarkers

Safety Biomarkers

Advancing the Use of New Imaging Techniques

Improving Predictions of Human Response from Disease Models

Streamlining Clinical Trials

Advancing Innovative Trial Designs

Improving Measurement of Patient Response

Streamlining the Clinical Trial Process

http://www.fda.gov/oc/initiatives/criticalpath/reports/opp_list.pdf

***Harnessing Bioinformatics**

Moving Manufacturing Into the 21st Century

Manufacturing Biologics
Manufacturing Devices
Manufacturing Drugs
*Nanotechnology

Developing Products to Address Urgent Public Health Needs

*Rapid Pathogen Identification
*Better Predictive Disease Models

***Specific At-Risk Populations- Pediatrics**

http://www.fda.gov/oc/initiatives/criticalpath/reports/opp_list.pdf

NCTR's Mission

NCTR conducts peer-reviewed scientific research and provides expert technical advice and training that enables FDA to make sound science-based regulatory decisions that improve the health of the American people. The research is focused towards understanding critical biological events in the expression of toxicity and towards developing methods and incorporating new technologies to improve the assessment of human exposure, susceptibility, and risk through the characterization of present models and the development of new models.

Underline by P. Howard for emphasis

NCTR Strategic Research Goals

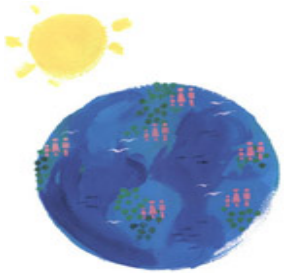
1) *Advance scientific approaches and tools to attain personalized nutrition and medicine;*

2) *Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process;*

3) *Develop and apply rapid detection technologies and testing platforms to assure food safety, biosecurity, food biodefense, and to combat terrorism.*

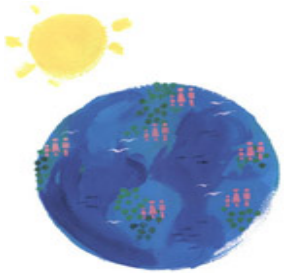
Toxicology Testing and Research at NCTR:

1) Investigator initiated (hypothesis based);
2) Provide data on specific material to support an FDA Center's risk identification or risk assessment;
3) FDA specified area of need (*e.g.* Critical Path Initiative);
4) Interagency requests for research expertise.



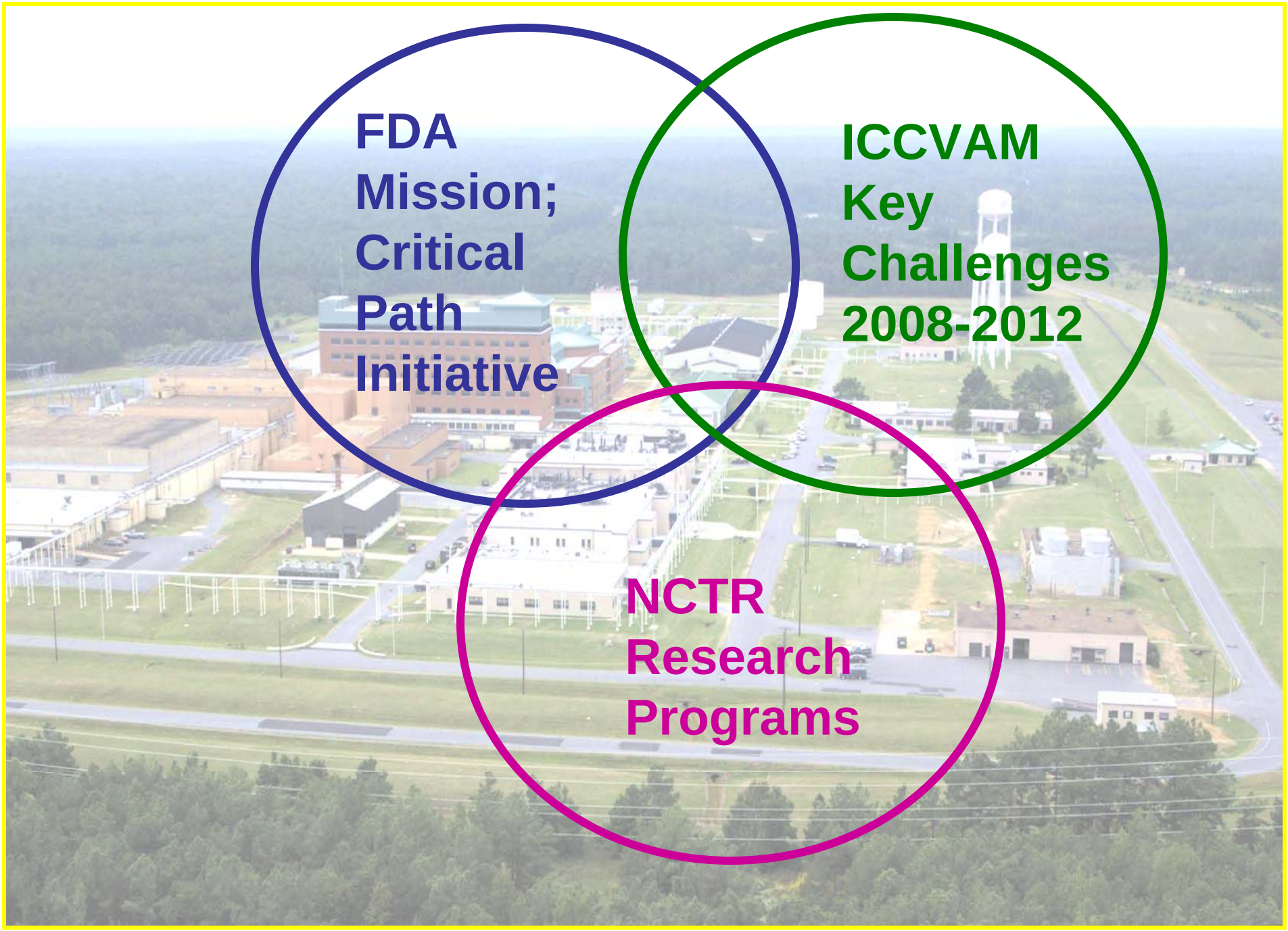
Four key challenges identified by ICCVAM for 2008-2012:

1) *Identifying priorities and conducting and facilitating alternative test method activities (Ocular Tox.; Biologics; Dermal Tox.; Acute Tox.; Immunotoxicity; Endocrine Disruptors; Pyrogens; Reproductive & Developmental Tox.; Chronic Tox. & Carcinogenicity; Neurotoxicity)
2) *Incorporating new science and technology (High Throughput Screening; other Animal Systems; Computational Approaches; Biomarkers of Toxicity; Toxicology Databases; Nanomaterials Testing).



3) Fostering regulatory acceptance and use of alternative methods;

4) Developing partnerships.


FDA Mission; Critical Path Initiative
ICCVAM Key Challenges 2008-2012
NCTR Research Programs

NCTR Research Divisions:

Biochemical Toxicology
Genetic and Reproductive Toxicology
Microbiology
Neurotoxicology
Personalized Nutrition and Medicine
Systems Toxicology
Veterinary Services

Research Projects -1

- Genotoxicity, Mutagenicity and Exposure Biomarkers of Acrylamide and its Metabolite, Glycidamide, in Rodents: HPRT and TK+/- Mutagenesis Assay
- DNA Adducts of Tamoxifen
- Toxicities of the Antiretroviral Drugs
- Food-borne Toxin Potencies Assessed Using Cultured Macrophage Cells
- Real-time PCR Assays for Ricin and Related Potential Bioterrorism Agents in Foods

Research Projects -2

- Thermodynamic Measurements for Inactivation of Bioterrorism Agents Ricin and Abrin.

- Chemical Inactivation of Protein Toxins on Food-Contact Surfaces.

- In Vitro Studies on Melamine and Cyanuric Acid Biochemical Toxicology.

- Determination of the Immunogenicity of Permanent Makeup Inks and Their Components Using a Modification of the LLNA (LPNA)

Research Projects -3

- Efficient Regulatory Method for Evaluating Chromosomal Damage: Analysis of Micronucleus in Different Rat Strains by Flow Cytometry

- DNA Adduct Formation, Mutations and Patterns of Gene Expression in Big Blue Rats Treated with the Botanical Carcinogens Riddelliine, Aristolochic Acid and Comfrey

- Measurement of Cancer-Associated Gene Mutation in Colon Tumor and Non-Tumor Tissue

Research Projects -4

- Evaluation of the Types of Genetic Events Detected by the Mouse Lymphoma Assay and the Role of the Assay in Mechanistically-Based Risk Assessment

- Analysis of p53 Codon 270 CGT to TGT Mutation in Simulated Solar Light-induced Skin Tumors and Exposed Mouse Skin

- Benefit/Risk Classification Models for Regulatory Decision Making in Personalized Medicine

Research Projects -5

- Characterization of Mutations in the PIG-A Gene in Human Lymphoblastoid Cells and C57Bl/6 Mice
- Neurotoxicity Assessment of Manganese Nanoparticles in PC12 Cells and in Mice
- The Role of Mitochondrial Energy Disruption in the Mechanism of Neurotoxicity: Neurophysiological, Neurochemical, and cDNA Microarray Approaches
- NMDA Antagonist/GABA Agonist-induced Cell Death in the Developing Rat Brain

Research Projects -6

- Development of "Mitochip" a Glass-based Oligonucleotide Microarray Containing Mitochondrial and Nuclear Genes Associated with Mitochondrial Function

- Development of a Novel Class Prediction Method, Decision Forest, for Analysis of Genomic and Proteomic Data

- Differential Gene Expression in Rodent and Human Primary Hepatocytes Exposed to the Peroxisome Proliferator Activated Receptor (PPAR) Alpha Agonists

Impact of Systems Toxicology on the 3 Rs

James C. Fuscoe, Ph.D.
Acting Director, Division of Systems Toxicology,
National Center for Toxicological Research
U.S. Food and Drug Administration

6th World Congress on
Alternatives and Animal Use in the
Life Sciences -Omics Technologies
August 24, 2007

Acknowledgement to Jim Fuscoe for next 4 slides.

What Do We Need for Genomics?

- Calibrated RNA samples
- Reliable benchmark datasets
- Metrics/Thresholds for assessing the performance achievable on microarray platforms
- Thorough and independent validation
- Guidelines for microarray QC and data analysis

>1,000 arrays

Pilot-I: RNA Samples

- 1st face-to-face meeting on Feb. 11, 2005 at FDA/NCTR
- Selection of two RNA samples from four candidates
- Five replicate microarrays for each RNA
- Four microarray platforms (AFX/AGL/GEH/ILM)
- **160** microarrays from seven test sites
- 2nd face-to-face meeting on May 2-3, 2005 at FDA/CDER

Pilot-II: Tissue Titration

- Selection of two of the 13 mixtures of **A** and **B**
- Three to five replicate microarrays for each mixture
- Four microarray platforms (AFX/AGL/GEH/ ILM)
- Two alternative platforms (TAQ/QGN)
- **200** microarrays from four platform providers
- TAQ/QGN validation data for ten tissue-specific genes

Main Study: Reference Datasets

- Four RNA samples (**A**, **B**, **C**, and **D**)
- Detailed QC data for total RNA and targets
- Five replicate microarrays for each RNA
- Seven microarray platforms (ABI/AFX/AGL/EPP/GEH/ILM/NCI)
- Three to six test sites for each microarray platform
- **534** microarrays from 24 test sites
- Three alternative technology platforms (TAQ/QGN/GEX)
- **1000** genes by TAQ; **245** genes by QGN; **207** genes by GEX

Microarray Platforms:

ABI: Applied Biosystems
AFX: Affymetrix
AGL: Agilent
EPP: Eppendorf
GEH: GE Healthcare
ILM: Illumina
NCI: NCI_Operon custom oligoarray

Alternative Technology Platforms:

GEX: StaRT-PCR from Gene Express
QGN: QuantiGene from Genospectra
TAQ: TaqMan® from Applied Biosystems

Data Analysis

- ~40 organizations are analyzing the datasets
- QC metrics and thresholds
- Precision and cross-lab/platform comparison
- Sequence-based cross-platform mapping
- Normalization and gene selection methods
- Validation of microarray results
- Titration datasets for assessing accuracy
- Performance of spike-in controls
- Modeling cross-hybridization
- One-color versus two-color designs
- Informatics tools
- Public deposition
- MAQC-3 in Palo Alto, CA, Dec. 1-2, 2005
- MAQC-4 in Boston, MA, Feb. 3-4, 2006

MAQC Publications (Sept. 2006)
MAQC Guidance (2006-2007)

Applications of "omics" in Systems Toxicology toward Refinement, Reduction and Replacement

Smaller Number of Animals (→ Humans)

Metabolomics

Proteomics

Biomarkers, Systems Tox.

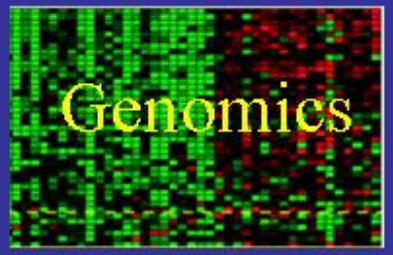
Applications of Technologies at NCTR toward Refinement, Reduction and Replacement

Smaller Number of Animals (→ Humans)

DNA methylation

Applied Tox

Metabolomics



Proteomics

SNPs, polymorphisms

Alternative Tests

Biomarkers, Systems Tox. R, R, R

Conclusion:

Through fulfilling it's role in the FDA & Critical Path, NCTR is addressing some components of two of the four Key Challenges for ICCVAM in 2008-2012.

(Identifying priorities and conducting and facilitating alternative test method activities;
Incorporating new science and technology)