Genetic toxicology: Why Thinking Outside of the Box?

Overview, Rationale and Objectives of the Workshop

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Sanofi

Workshop: "Genetic Toxicology: Opportunities to Integrate New Approaches"

ILSI Health and Environmental Sciences Institute

Silver Spring
April 24 and 25, 2012

Objectives

1. To improve the **scientific basis of the interpretation** of results from *in vitro* genetic toxicology tests for purposes of **accurate human risk assessment**.

2. To develop **follow-up strategies** for determining the **relevance of *in vitro* test results to human health**.

3. To provide a framework for the **integration of the *in vitro* testing results into a risk-based assessment** of the effects of chemical exposures to **human health**.

ILSI: International Life Sciences Institute

HESI: Health and Environmental Sciences Institue

2012 IVGT Membership

Industry Participation

AstraZeneca
Bayer Healthcare Pharma
BioReliance
Bristol-Myers Squibb
Covance
Dow Chemical
GlaxoSmithKline
ILS Inc.
Janssen Pharmaceuticals
L'Oreal
Mitsubishi Tanabe Pharma
Novartis
Pfizer Inc.
Procter & Gamble
Sanofi
Servier
Takeda

Government / Research Institution Participation

Federal Institute for Drugs and Medical Devices (BfArM, Germany)
Health Canada
National Center for Toxicological Research, U.S. FDA
National Institute for Public Health and the Environment (RIVM, NL)
National Institute of Health Sciences (Japan)
U.S. Department of Agriculture
U.S. Environmental Protection Agency
U.S. Food and Drug Administration
U.S. National Institute of Environmental Health Sciences/Division of the National Toxicology Program

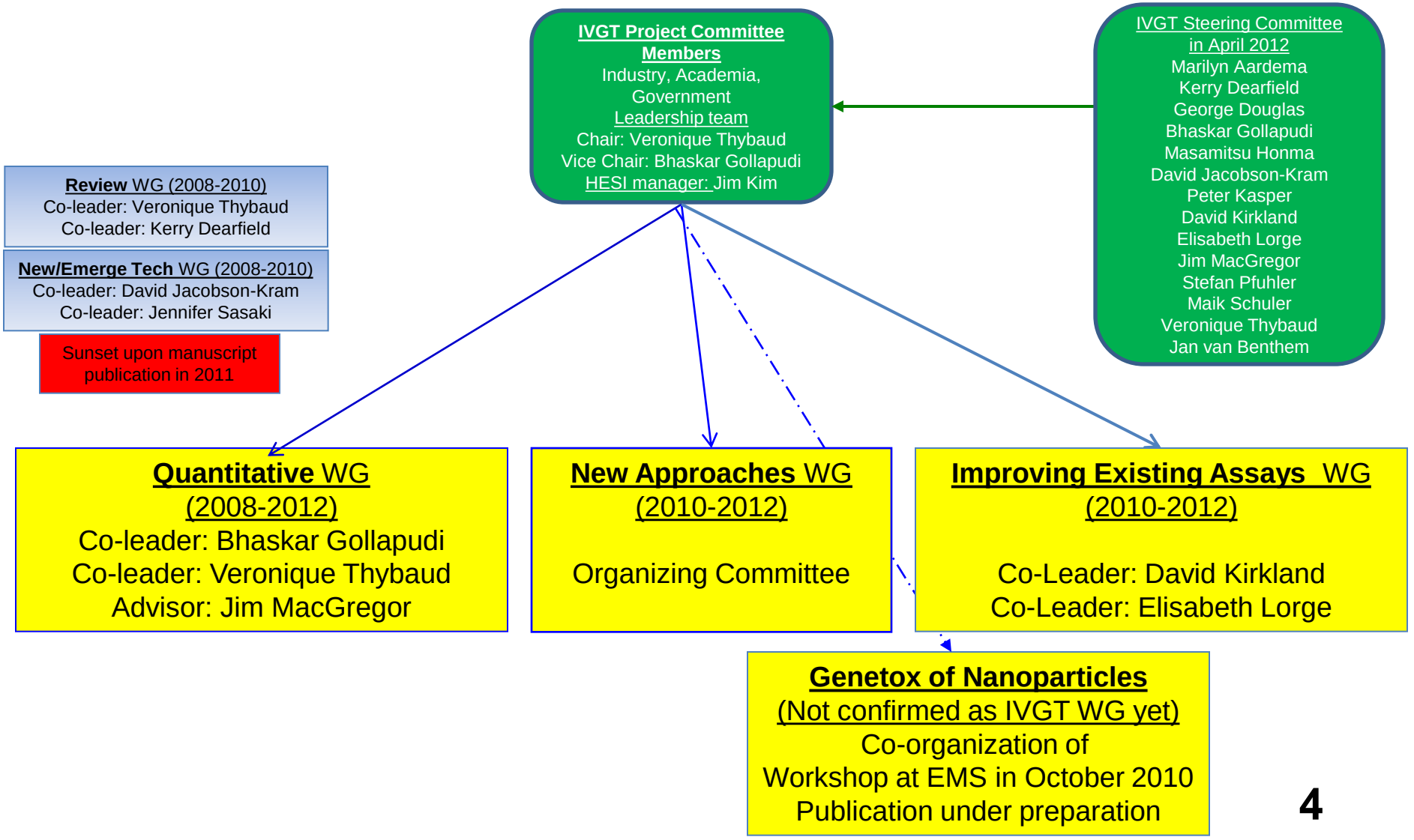
Academic Participation

New York Medical College
Swansea University
University of Ottawa
University of Surrey
St. George's University of London

Consultant Participation

Errol Zeiger Consulting
David Kirkland Genetox Consulting
Jim MacGregor Toxicology Consulting Services

2012 IVGT organization



2012 IVGT organization

Workshop Organizing Committee

• Marilyn Aardema	BioReliance (USA)
• Jan van Benthem	RIVM (Netherlands)
• Laura Custer	Bristol-Myers Squibb (USA)
• Bhaskar Gollapudi	The Dow Chemical Co. (USA)
• Masa Honma	National Institute of Health Sciences (Japan)
• James Kim	ILSI Health and Environmental Sciences Institute (USA)
• Manju Manjanatha	U.S. FDA / NCTR (USA)
• Stefan Pfuhler	Procter & Gamble (USA)
• Leon Stankowski	BioReliance (USA)
• Véronique Thybaud	Sanofi (France)
• Paul White	Health Canada (Canada)
• Kristine Witt	NIEHS/DNTP (USA)
• Errol Zeiger	Consultant (USA)

Purpose of this workshop

- To bring together expertise from both within and outside the discipline of genetic toxicology

- To consider the impact that our improved understanding of biology and new technologies might have on our ability to perform genetic toxicology studies to yield information that is more relevant to human hazard and risk assessment for genetic damage

- To define ways of bridging genetic toxicology to other disciplines, and to identify potential synergies that would result in new approaches to inform more accurate genotoxicity risk assessment.

Purpose of Applied Genetic Toxicology

- To detect genotoxic potential (DNA primary damage, gene mutations, structural and numerical chromosome damage) in particular formation of stable and transmissible mutations
  - Hazard identification

- To avoid exposure to genotoxic agents that would induce mutations in somatic and germ cells and contribute to adverse effects in the exposed population and future generation (i.e., diseases such as cancer, aging, malformations)
  - Risk assessment
  - Risk management.

From Exposure to Genotoxic Agent to Adverse Effects on Human Health

Exposure & Metabolic activation

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Electrophilic reactive compound & Metabolite

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Interactions with DNA [and/or other cellular components]

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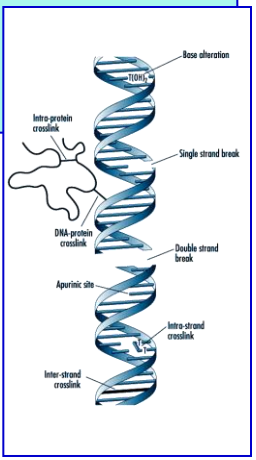
DNA primary damage

DNA primary damage
- adducts, modified bases, abasic sites
- simple- and double- DNA strand breaks
- inter- and intra-strand crosslink



Apoptosis | **Replication** | **DNA repair**

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MUTATION

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If other keys events occur

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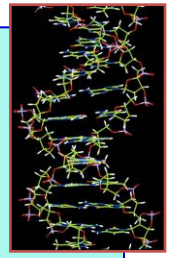
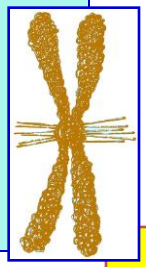
Adverse effects on human health
Somatic cells: cancer, other diseases, aging
Germ cells: malformations, genetic diseases

Mutations

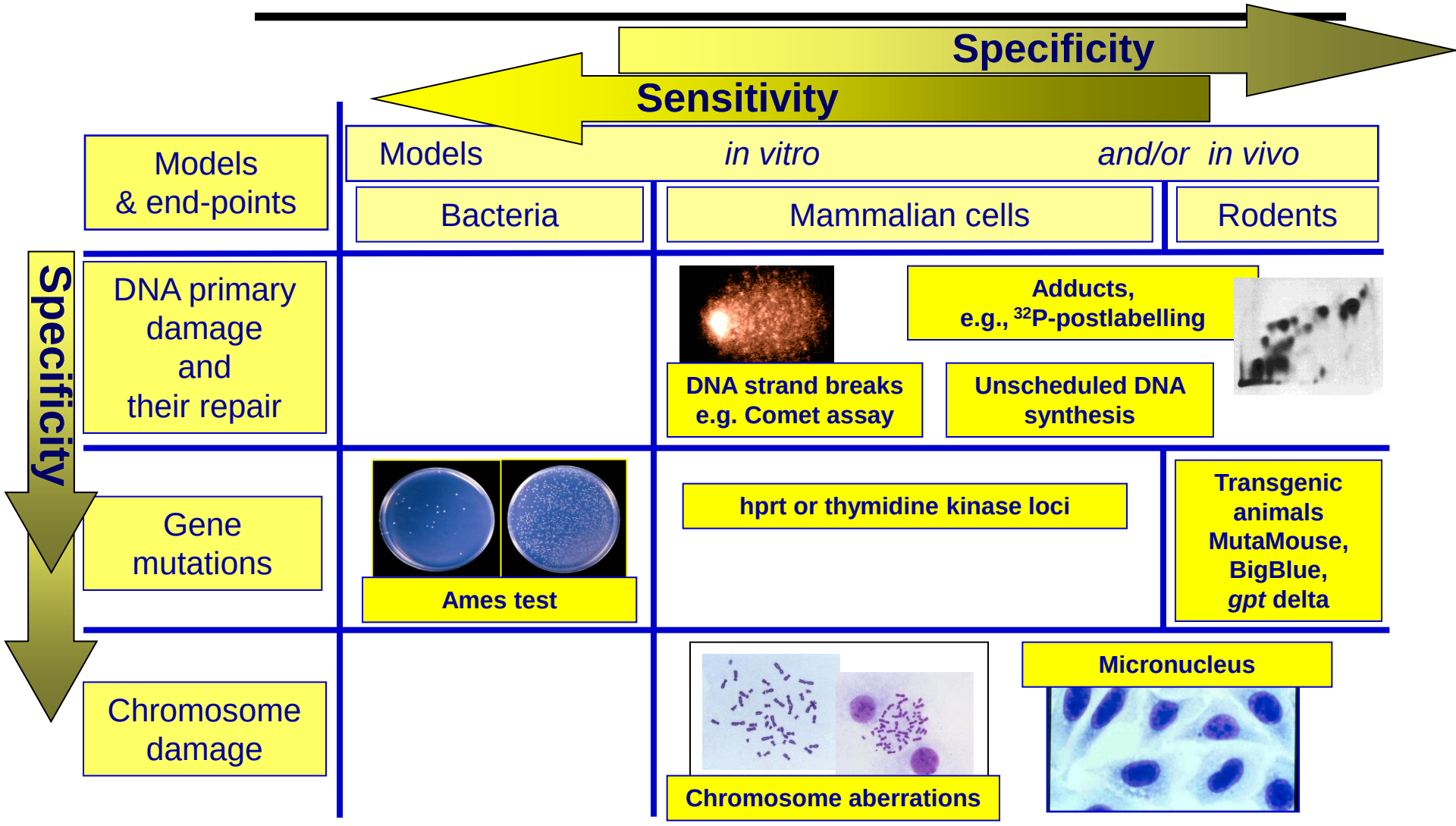
Genes
- base pair substitutions
- deletions
- insertions



Chromosomes
- Structural and numerical damage



Surrogate Models and End-Points



Most Commonly Used Assays

- Gene mutation test in bacteria (Ames test)
- *In vitro* chromosomal damage assays in mammalian cells
  - Metaphase chromosome aberration and micronucleus tests
- *In vitro* gene mutation assays in mammalian cells
  - At thymidine kinase locus, e.g. mouse lymphoma Tk gene mutation assay (also detecting chromosomal damage)
  - At HPRT locus
- *In vivo* chromosomal damage tests, generally for using rodent hematopoietic cells (when *in vivo* assays acceptable)
  - Metaphase chromosome aberration and micronucleus tests
- Additional *in vivo* assays in somatic and/or germ cells (when *in vivo* assays acceptable)

➢ Some of the above tests have been in use for more than 30 years.

Follow-Up in Case of Positive Results

- In case of *in vitro* positive results: A second *in vivo* assay in appropriate somatic tissue(s)
  - DNA strand breaks (e.g., Comet assay) or DNA adducts
  - Gene mutation in transgenic animals (e.g., MutaMouse, BigBlue, *gpt* delta)
- In case of *in vivo* positive results and for some regulations (e.g. REACH) : an assay in Germ cells
  - Dominant lethal test
  - Spermatogonial chromosome aberrations
  - Gene mutation in transgenic animals (e.g., MutaMouse, BigBlue, *gpt* delta)
- More weight generally given to *in vivo* results

Follow-Up in Case of Positive Results

- Mechanistic studies
  - DNA-reactive versus non DNA-reactive compounds
    - Primary target different from DNA (e.g. mitotic spindle, topoisomerases) and irrelevant mechanisms because obtained under non physiological experimental conditions

  ➢ Need for multi-parametric approach
    - Genotoxicity, cell cycle, toxicity, apoptosis, any other mechanistic information and key markers
- Quantitative approach
  - Linear versus non-linear dose response
    - No genotoxic effect level and safety/uncertainty factors
    - Mechanisms underlying the non-linear dose-response

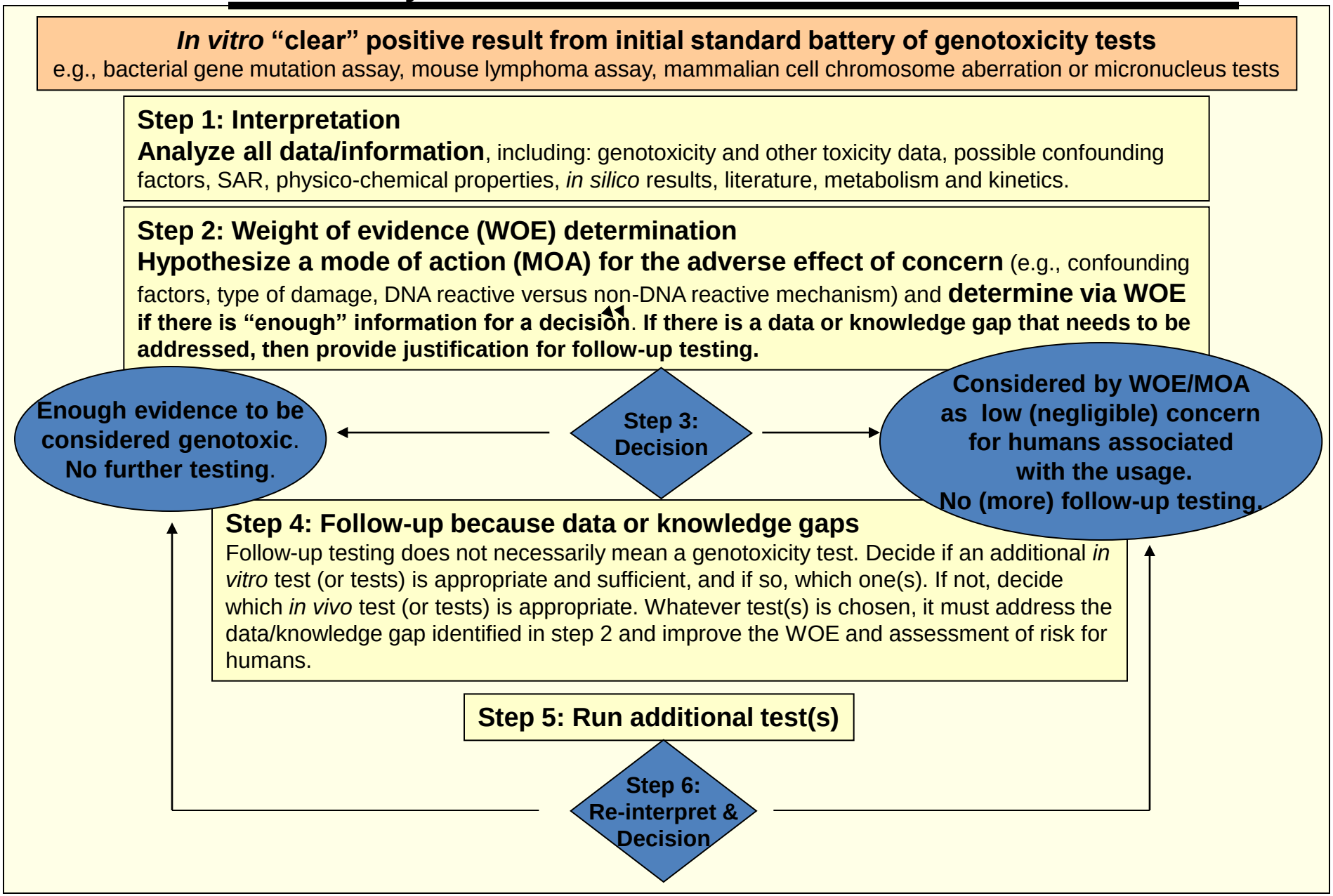
  ➢ Need for models relevant to human and criteria for extrapolation from *in vitro* to *in vivo* and from *in vivo* to human.

IVGT Workgroup
Review of Existing Assays and Flow chart

- Review of existing assays
- Development of a flow chart
  - for follow up actions in case of positive results in the *in vitro* assays to assist in risk-based decision making

Dearfield et al. (2011) Follow-Up Actions from Positive Results of *In Vitro* Genetic Toxicity Testing. Environmental Molecular Mutagenesis. 52; 177-204.

Follow-up actions in case of positive results in the *in vitro* assays

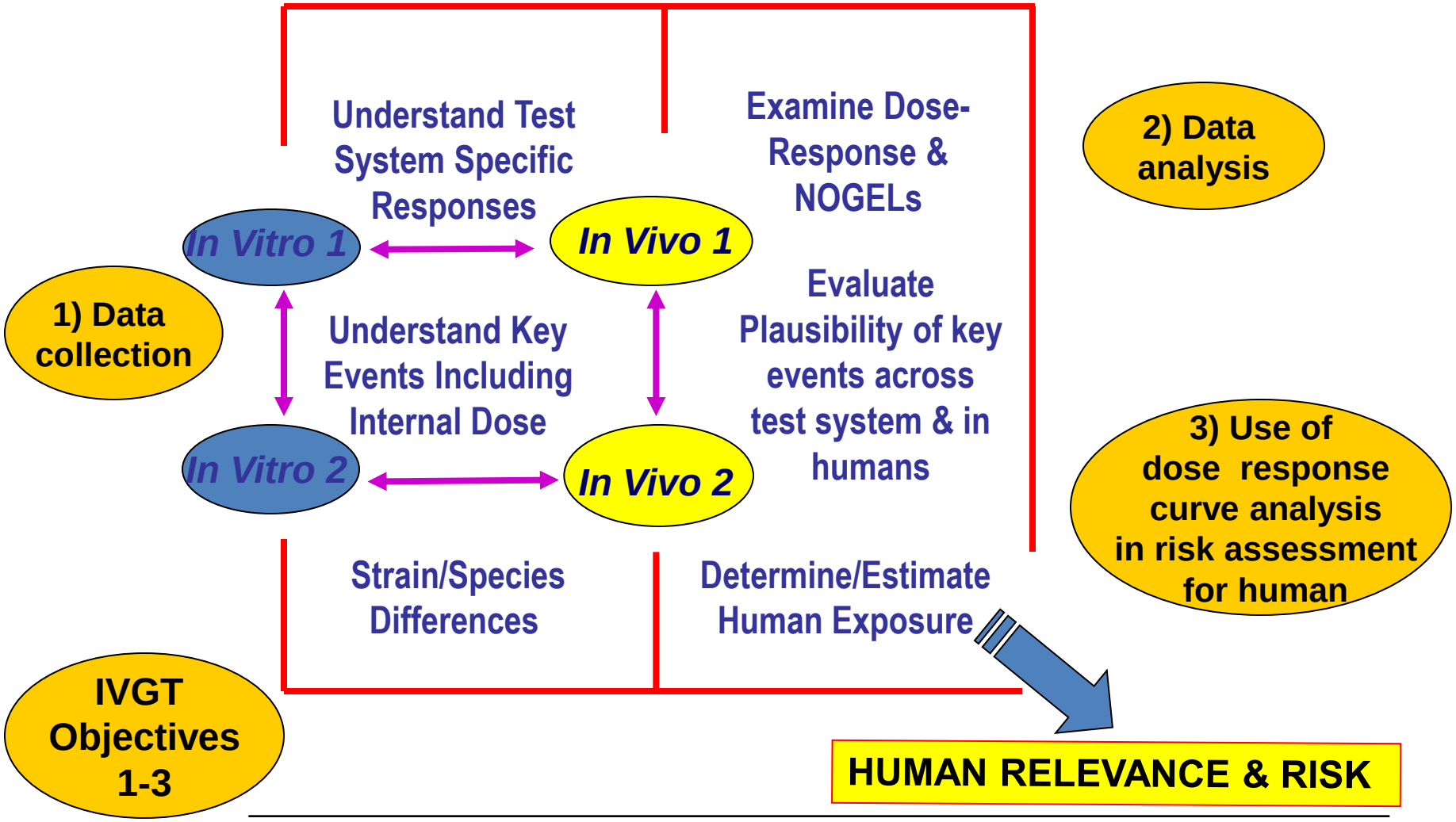


IVGT Workgroup
Review of New and Emerging Technologies

	Mature	Maturing	Emerging
In silico	Leadscope, MultiCASE, Derek for Windows, Vitic		
Moving beyond the traditional battery		Greenscreen	*Pig-a* mutation
Replacement or improvement of existing assays	Comet assay, *In vivo* micronucleus (flow cytometry)	3D skin models	Transgenic *in vitro* systems, "Humanized" test systems
Mechanistic understanding and Follow-up	Comet assay	Yeast DEL assay, *In vitro* micronucleus (flow cytometry)	Enzyme-DNA films, Toxicogenomics, DNA adductome, *Pig-a* mutation

Lynch et al. (2011) New and Emerging Technologies for Genetic Toxicity Testing. Environmental and Molecular Mutagenesis. 52; 205-223.

IVGT Workgroup
From qualitative to quantitative evaluation



Remaining issues and gaps

- Relevance of surrogate models
  - Ability to mimic human cells (DNA repair, metabolic activation, genetic stability) and whole body complexity
- Relevance of measured end-points
  - Need to consider all key events (including epigenetic factors) that might contribute to DNA damage, and impact response to DNA damage and DNA integrity
- Extrapolation to human
  - Need for appropriate safety and uncertainty factors
  - Based on protective mechanisms and genetic polymorphism.

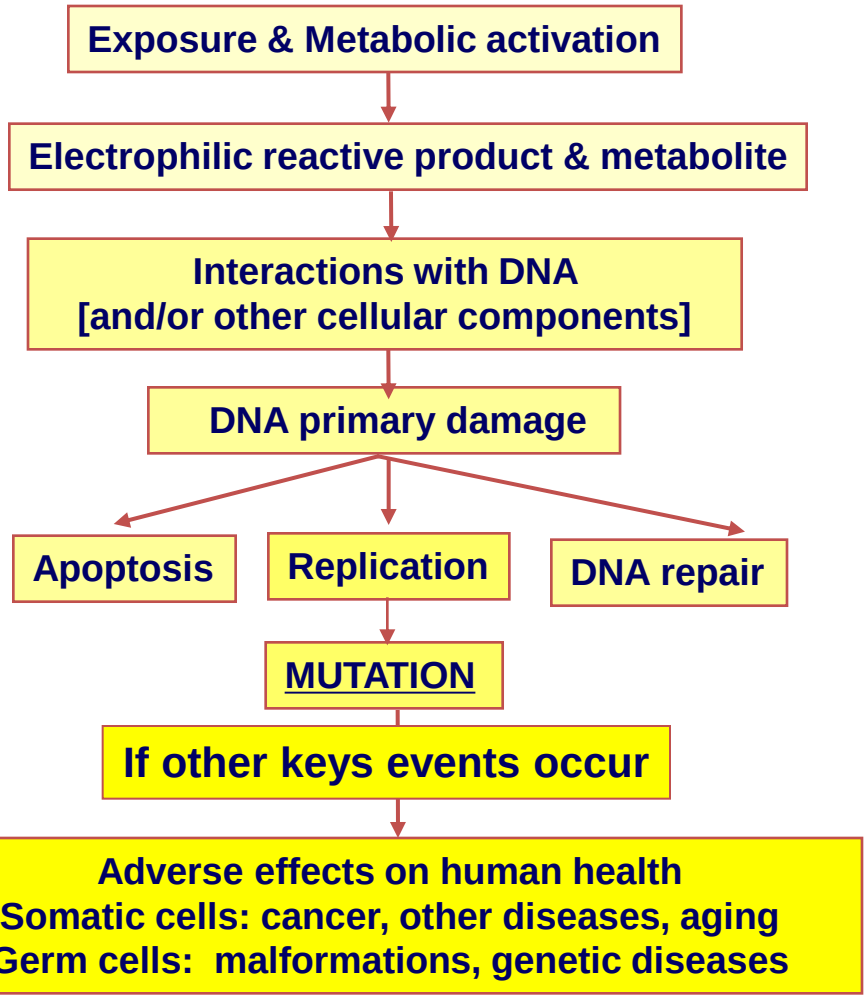
Remaining issues and gaps

- Two options
  - To improve the existing assays
    - On going IVGT activities
  - To benefit from the new approaches used in other biology domains and “think outside of the box”
    - Experimental models
    - Technologies
    - Understanding of biological mechanism.

Benefit Expected from New Approaches

Experimental models more relevant to human:
- Primary and stem cells
- 3-D models
- Human and/or humanized models

Methodologies:
- Measurement of multiple parameters in parallel to put genotoxicity measurement into context
- High throughput methods
- Intra-cellular or intra-organ visualization of damage



Knowledge on key events contributing to cell response to genotoxic stress and other events able to impact DNA integrity:
- Mechanistic understanding of non-DNA reactive mechanisms and epigenetic mechanisms
- Determination of mechanisms underlying dose-response curves (defense mechanisms, genetic susceptibility)

Objectives of the Workshop

- Technologies and tools are being developed in fields outside of genetic toxicology
  - Experimental models that would help increasing relevance to human
  - Technologies that would allow the measurement of multiple parameters for better understanding on mode of action are now available
- Our understanding of molecular biology has increased exponentially in recent years
  - particularly in areas such as epigenetics, miRNA, and genetic structure.

Organization of the workshop

- Three sessions
  - Session 1: Alternative experimental models to improve genetic toxicity testing
  - Session 2: Biomarkers of epigenetic changes and their applicability to genetic toxicology
  - Session 3*:* New technologies and approaches

- For each session
  - Introduction
  - 3-4 Lectures (including SWOT analysis)
  - Discussion based on a list of question
  - SWOT: Strengths/Weaknesses/Opportunities/Threats

Could technologies and experimental models recently developed in fields outside genetic toxicology, and progress made in this understanding of molecular biology help…

…moving genetic toxicology forward from purely a hazard identification science to better informing the human risk ...

Thank you!