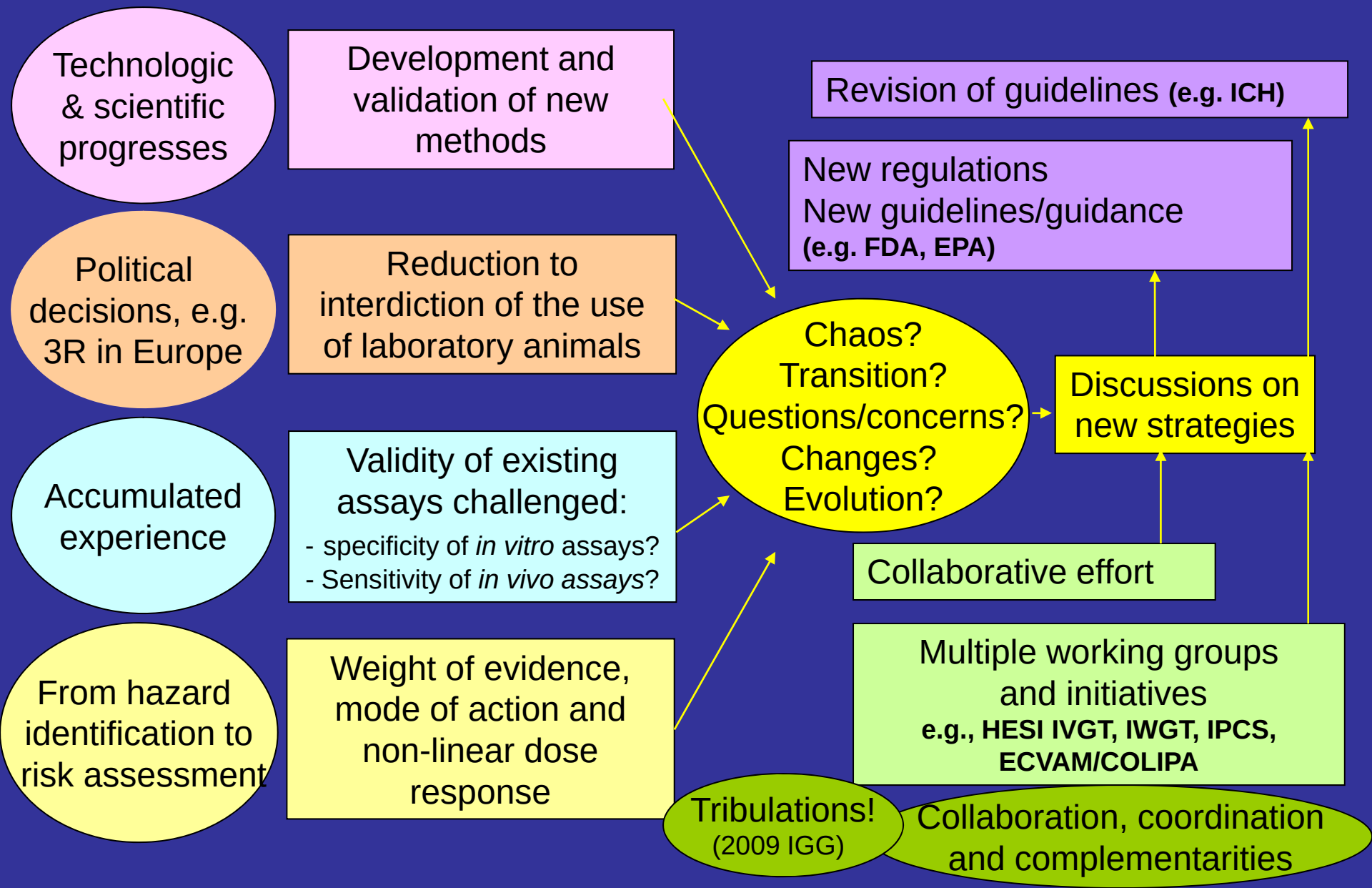
Approaches to follow-up on positive results in genetic toxicology tests in the context of human risk assessment

Véronique Thybaud (sanofi aventis)
Chair, ILSI/HESI IVGT

HESI Annual Meeting, May 12, 2010

Genetic toxicology: What's new? 変



What's new? 変

Relevance and follow-up of positive results in vitro? Many publications …

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Mutation Research 628 (2007) 31–55
Genetic Toxicology and Environmental Mutagenesis
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How to reduce false positive results when undertaking *in vitro* genotoxicity testing and thus avoid unnecessary follow-up animal tests: Report of an ECVAM Workshop☆

David Kirkland[a,*], Stefan Pfuhler[b], David Tweats[c], Marilyn Aardema[d], Raffaella Corvi[e], Firouz Darroudi[f], Azeddine Elhajouji[g], Hansruedi Glatt[h], Paul Hastwell[i], Makoto Hayashi[j], Peter Kasper[k], Stephan Kirchner[l], Anthony Lynch[i], Daniel Marzin[m], Daniela Maurici[e], Jean-Roc Meunier[n], Lutz Müller[l], Gerhard Nohynek[o], James Parry[c], Elizabeth Parry[c], Veronique Thybaud[p], Ray Tice[q], Jan van Benthem[r], Philippe Vanparys[s], Paul White[t]

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Mutation Research 488 (2001) 151–169
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Review

A review of the genotoxicity of marketed pharmaceuticals

Ronald D. Snyder[a,*], John W. Green[b]

[a] *DuPont Pharmaceuticals Company, Stine-Haskell Research Center, PO Box 50, H1/1710, Newark, DE 19714, USA*
[b] *Haskell Laboratory for Toxicology and Industrial Medicine, Stine-Haskell Research Center PO Box 50, H1/1710, Newark, DE 19714, USA*

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Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens I. Sensitivity, specificity and relative predictivity☆

David Kirkland[a,*], Marilyn Aardema[b], Leigh Henderson[c,1], Lutz Müller[d]

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Mutation Research 633 (2007) 67–79
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Current issues

Relevance and follow-up of positive results in *in vitro* genetic toxicity assays: An ILSI-HESI initiative☆

Véronique Thybaud[a], Marilyn Aardema[b], Daniel Casciano[c], Vicki Dellarco[d], Michelle R. Embry[e,*], B. Bhaskar Gollapudi[f], Makoto Hayashi[g], Michael P. Holsapple[e], David Jacobson-Kram[h], Peter Kasper[i], James T. MacGregor[j], Robert Rees[k]

Mutation Research 627 (2007) 41–58
Genetic Toxicology and Environmental Mutagenesis
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Strategy for genotoxicity testing: Hazard identification and risk assessment in relation to *in vitro* testing

V. Thybaud[a,*], M. Aardema[b], J. Clements[c], K. Dearfield[d], S. Galloway[e], M. Hayashi[f], D. Jacobson-Kram[g], D. Kirkland[c], J.T. MacGregor[h], D. Marzin[i], W. Ohyama[j], M. Schuler[k], H. Suzuki[l], E. Zeiger[m]

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Mutation Research 608 (2006) 29–42
Environmental Mutagenesis
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Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens II. Further analysis of mammalian cell results, relative predictivity and tumour profiles

David Kirkland[a,*], Marilyn Aardema[b], Lutz Müller[c], Makoto Hayashi[d]

[a] *Covance Laboratories Limited, Otley Road, Harrogate HG3 1PY, United Kingdom*
[b] *The Procter and Gamble Company, Miami Valley Laboratories, P.O. Box 538707, Cincinnati, OH 45253-8707, USA*
[c] *Non-Clinical Drug Safety, F Hoffmann-La Roche Ltd., PRBN-S, Bldg 7331b, CH-4070 Basel, Switzerland*
[d] *Division of Genetics and Mutagenesis, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*

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2005 International Workshop on Genotoxicity Testing (IWGT)

Follow-up of *in vitro* positive results

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Strategy for genotoxicity testing: Hazard identification and risk assessment in relation to *in vitro* testing

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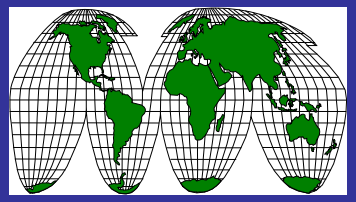

Follow-up of *in vitro* positive results

No or low concern, and no further testing required beyond the standard battery, when:

- non-reproducible or marginal *in vitro* positive results, i.e.,
  - results that are not consistently repeatable
  - weak effects without a strong dose relationship and values within or close to a range that could occur by chance variability (negative control historical data)
- results from other assays with a similar endpoint are negative

Follow-up of *in vitro* positive results

No or low concern, and no further testing required beyond the standard battery, when (Cont'd):

- Effects occur only at very high levels of cytotoxicity, but not at moderate levels, in the chromosomal aberration or mouse lymphoma tk+/- assays
- Absence of structural alerts or any other cause of concern.
- Similar considerations also described in regulatory documents
  - e.g. FDA guidance, Draft ICH S2(R1)

The *in Vitro* Genetic Toxicity Testing (IVGT) ILSI-HESI initiative on Relevance and Follow-up of Positive Results in *in vitro* Genetic Toxicity Testing

Context to HESI-IVGT Effort

- Relatively high rate of positive results in the in vitro mammalian cell assays and more importantly … low specificity
- Many *in vitro* results, especially in the *in vitro* chromosome damage tests, not confirmed in the in vivo genetic toxicology tests and/or in carcinogenicity studies
- Need to move from hazard identification to human risk assessment

The *in Vitro* Genetic Toxicity Testing (IVGT) ILSI-HESI initiative on Relevance and Follow-up of Positive Results in *in vitro* Genetic Toxicity Testing

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Current issues

Relevance and follow-up of positive results in *in vitro* genetic toxicity assays: An ILSI-HESI initiative☆

Véronique Thybaud[a], Marilyn Aardema[b], Daniel Casciano[c], Vicki Dellarco[d], Michelle R. Embry[e,*], B. Bhaskar Gollapudi[f], Makoto Hayashi[g], Michael P. Holsapple[e], David Jacobson-Kram[h], Peter Kasper[i], James T. MacGregor[j], Robert Rees[k]

HESI IVGT
"Review" Group
Follow-up strategies in case of (clear) positive results *in vitro*

Véronique Thybaud (Sanofi aventis), Chair
Kerry Dearfield (USDA), Co-chair

Working group:
Michael C. Cimino, Elisabeth Lorge, Laura Custer, Andreas Czich, Jim Harvey, Susan Hester, Jim Kim, David Kirkland, Dan Levy, Martha Moore, Gladys Ouédraogo-Arras, Maik Schuler, Willi Suter, Kevin Sweder, Kirk Tarlo, Jan van Benthem, Freddy van Goethem, Christine Witt

Decision process flow chart for follow-up actions

***In vitro* "clear" positive result from initial standard battery of genotoxicity tests**
e.g., bacterial gene mutation assay, mouse lymphoma assay, mammalian cell chromosome aberration or micronucleus tests

Step 1: Interpretation
Analyze all data/information, including:

Step 2: Weight of evidence (WOE) determination
Hypothesize a mode of action (MOA) for the adverse effect of concern (e.g., confoundin
ype of damage, DNA reactive ver
ctive mechanism) and **determine**
ere is "enough" information for a decis

Enough evidence to be considered genotoxic. No further testing.

Considered by WOE/MOA as low (negligible) concern for humans associated with the usage. No (more) follow-up testing.

ting.

Step 4: Follow-up because data or knowledge gaps
Follow-up testing does not necessarily mean a genotoxicity test. Decide if an additional *in vitro* test (or tests) is appropriate and sufficient, and if so, which one(s). If not, decide which *in vivo* test (or tests) is appropriate. Whatever test(s) is chosen, it must address the data/knowledge gap identified in step 2 and improve the WOE and assessment of risk for humans.

Decision

Decision process flow chart for follow-up actions

***In vitro* "clear" positive result from initial standard battery of genotoxicity tests**
e.g., bacterial gene mutation assay, mouse lymphoma assay, mammalian cell chromosome aberration or micronucleus tests

Step 1: Interpretation
Analyze all data/information, including: genotoxicity and other toxicity data, possible confounding factors, SAR, physico-chemical properties, *in silico* results, literature, metabolism and kinetics.

Step 2: Weight of evidence (WOE) determination
Hypothesize a mode of action (MOA) for the adverse effect of concern (e.g., confounding factors, type of damage, DNA reactive versus non-DNA reactive mechanism) and **determine via WOE if there is "enough" information for a decision**. **If there is a data or knowledge gap that needs to be addressed, then provide justification for follow-up testing.**

Step 3: Decision

Enough evidence to be considered genotoxic. No further testing.

Considered by WOE/MOA as low (negligible) concern for humans associated with the usage. No (more) follow-up testing.

Step 4: Follow-up because data or knowledge gaps
Follow-up testing does not necessarily mean a genotoxicity test. Decide if an additional *in vitro* test (or tests) is appropriate and sufficient, and if so, which one(s). If not, decide which *in vivo* test (or tests) is appropriate. Whatever test(s) is chosen, it must address the data/knowledge gap identified in step 2 and improve the WOE and assessment of risk for humans.

Step 5: Run additional test(s)

Step 6: Re-interpret & Decision

HESI IVGT Review group

Other points to consider:

- Are the effects biologically relevant?
- Are the effects cell specific or not?
- When *in vivo* data available are the effects also seen *in vivo*?
  - Possible concern to human risk lessened when all the available data, including appropriate negative *in vivo* follow-up test results, are considered in pointing toward a low level of concern.
- Is it reasonable to expect these effects to occur in humans under normal conditions of use?
  - e.g. therapeutic dose, environment contamination, food intake
  - for either an indirect or a direct interaction with DNA mechanism.

HESI IVGT Review group
Ranking of existing genotoxicity assays

- done for *in vitro* and *in vivo* existing assays
- four categories
- based on their strengths and weaknesses, and their ability to contribute to data interpretation
- from assays that are robust enough to be used in the standard battery to assays seldom used because better alternatives exist.

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Ranking of existing genotoxicity assays

Type of assay	Category	OECD Guideline(s)	Endpoint(s)	Strengths	Limitations	Opportunities	References
Gene mutations							
Gene mutation assays in transgenic models	2	No guideline	Gene mutations (point mutations including base pair substitutions and frameshift mutations) in mammals *in vivo.* Reporter genes (e.g., *lacZ*, *lacI*, *gpt*) in shuttle vectors (e.g., lambda phage). Some models (e.g., *spi*, plasmid) also have the ability to detect deletions.	Can be applied to any tissue. Relevant end-point: gene specific. No selective pressure on mutations, therefore accumulation of damage over time. Uses a small number of animals.	Labor intensive and expensive. Requires multiple dosing. Requires transgenic animals. Need to optimize protocols for different tissues, or to apply the recommended design (28 treatment days, sampling after 3 and/or 28-day recovery period). Mutamouse, Big Blue and *gpt* delta models do not detect large deletions. Relatively high mutant frequency background shown to impact the sensitivity.	Mutant sequencing for mechanistic information (mutational spectrum) and confirmation of mutation (increase in mutant frequency versus clonal effect). Quantitation of dose response possible.	Heddle et al. (2000), Thybaud et al. (2003), Lambert et al. (2005), OECD (2009)

Example

HESI IVGT Review group
Ranking of existing genotoxicity assays

Category #1 :

- Well characterized.
- Endpoints and underlying mechanisms understood (for the most part).
- Well-validated; used in many laboratories; background controlled; reproducible results within and between laboratories.
- OECD guidelines available (for most of them).
- May or may not be part of the standard battery of tests recommended for regulatory purpose.
- May be useful as follow-up testing.

HESI IVGT Review group
Ranking of existing genotoxicity assays

Category #1 :

- **i.e.**
- Bacterial Reverse Mutation Assay in *Salmonella typhimurium* and *Escherichia coli*
- *In Vitro* Gene Mutation Assay in Mammalian Cells
- Mouse Lymphoma Assay
- *In Vitro* Micronucleus Assay in Mammalian Cells
- *In Vitro* Chromosome Aberration Assay
- Mammalian Bone Marrow Chromosomal Aberration Test
- Mammalian Erythrocyte Micronucleus Test

HESI IVGT Review group
Ranking of existing genotoxicity assays

Category #2 :

- May be well-characterized.
- May be used by fewer laboratories / Less frequently used than category #1 tests.
- May have less historical data available than category #1 tests.
- May be more resource intensive than category #1 tests.
- May be useful as specific follow-up testing, e.g., mechanistic studies, to follow-up category #1 tests.

HESI IVGT Review group
Ranking of existing genotoxicity assays

Category #2 :

- **i.e.**
- *In Vitro* Comet Assay in Mammalian Cells
- *In Vitro* DNA Adducts in Mammalian Cells (different methods)
- Gene mutation assays in transgenic models
- Mammalian Spermatogonial Chromosomal Aberration Test
- Rodent Dominant Lethal Assay
- *In Vivo/In Vitro* Mammalian Unscheduled DNA Synthesis (UDS) Assay in liver cells
- *In Vivo* Mammalian Comet Assay
- *In vivo* DNA Adducts in Mammalian Cells (different methods)

HESI IVGT Review group
Ranking of existing genotoxicity assays

Category #3 :

- Promising new and upcoming assays
- Show promise but perhaps not well (or sufficiently) validated
- Strengths and limitations understood, but not fully defined
- May become category #2, or even category #1, test when the assay becomes more broadly validated and used (e.g., as a result of collaborative effort) and/or when a more thorough evaluation/analysis of accumulated data becomes available.

HESI IVGT Review group
Ranking of existing genotoxicity assays

Category #3 :

- **e.g.** Expanded Simple Tandem Repeat (ESTR) assay
- **More should be identified in the next future, e.g., by IVGT "Novel and emerging technologies" group**

- Identification of Mutagens w / QSAR Computational Toxicology (e.g. Vitic / Derek)

- Enzyme-DNA films for reactive metabolite screening using electro-optical arrays and mass spectrometry

- Yeast DEL assay

- Toxicogenomics analysis of genotoxic mechanisms

- Greenscreen GADD45a-GFP

- Humanized *in vitro* genotoxicity assays

- *In vitro* organ models / 3D human skin

- Flow cytometric methods for *in vitro* and *in vivo* micronuclei

- *In vivo* mutation assay based on the endogenous Pig-a locus

HESI IVGT Review group
Ranking of existing genotoxicity assays

Category #4 :

- Some may be well characterized
- Mechanism leading to the positive response unknown or less understood than in the other categories.
- Endpoints less useful for study of mechanism than the other categories
- May provide pertinent information in specific circumstance.
- May demonstrate technical and/or feasibility limitations (e.g. cost, resources, number of animal used).
- May not be well-validated (only a few laboratories perform the assay)
- Better alternatives exist.

HESI IVGT Review group
Ranking of existing genotoxicity assays

Category #4 :

- **i.e.**
- Gene Mutation Assay Bacterial DNA Damage or Repair Assay
- *In Vitro* and *In Vivo* Alkaline Elution in Mammalian Cells
- *In Vitro* Mammalian Unscheduled DNA Synthesis (UDS) Assay in Liver cells
- *In Vitro* and *In Vivo* Sister Chromatid Exchange Assay in Mammalian Cells
- Mouse Spot Test; Mouse Biochemical and visible Specific Locus Test
- Rodent Heritable Translocation Assay

HESI IVGT Review group - Potential follow-up assays

Assays that can be chosen		DNA adduct assay	UDS assay	Comet assay	Gene mutation assays: *In vitro* assays (e.g. hprt)	Gene mutation assays: Transgenic models (a)		Micronucleus assay: Micronuclei without centromere	Micronucleus assay: Micronuclei with centromere	Chromosome Aberration assay	Assays for non DNA reactive mechanisms
End-points detected by the above assays		DNA Primary damage: Adducts		DNA Primary damage: Breaks	Point mutations	Point mutations	Deletions	Structural chromosome damage	Numerical chromosome damage	Structural chromosome damage	
Follow-up in case of positive findings in the *in vitro* gene mutation assays: for mechanistic purpose and/or confirmation of the *in vitro* findings	*In vitro* assays	To evaluate DNA reactivity			To confirm the gene mutation end-point *in vitro*						To evaluate the evidence supporting non DNA mechanisms
	In vivo assays	To further evaluate DNA reactivity *in vivo*				To further evaluate the gene mutation end-point *in vivo*					
Follow-up in case of positive findings in the *in vitro* chromosome damage tests: for mechanistic purpose and/or confirmation of the *in vitro* findings	*In vitro* assays	To evaluate DNA reactivity						To confirm the induction the chromosome damage end-point *in vitro* and to differentiate clastogen from aneugen mechanism			
	In vivo assays	To further evaluate DNA reactivity *in vivo*					To further evaluate the chromosome damage end-point *in vivo*	To further evaluate chromosome damage end-point *in vivo*, and in case of *in vivo* findings to differentiate clastogen from aneugen mechanism			

HESI IVGT Review group - Potential follow-up assays

Endpoints and relationships between standard *in vitro* genotoxicity assays

Standard *in vitro* assays						
In vitro Assays	Ames Assay	Mammalian Gene Mutation Assays (e.g. HPRT)	Mouse Lymphoma Assay		Micro-nucleus Assays	Chromosome Aberration Assays
			Large colonies	Small colonies		
Endpoints detected *in vitro*	Gene mutation assay +++	+++	+++	-	-	-
	-	-	Structural chromosome damage +	+++	+++	+++
	-	-	Numerical chromosome damage +	+	+++	++
	-	-	-	-	Polyploidy ++	+++

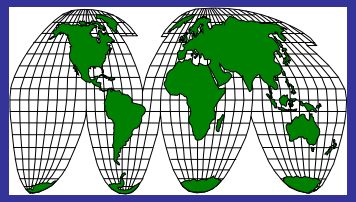
+++ efficiently detected ++ detected depending on test design + not always detected - not detected

HESI IVGT Review group - Potential follow-up assays

End-points detected by potential *in vivo* follow-up assays

Appropriate *in vivo* follow up assays						
In vivo Assay	Unscheduled DNA Synthesis	Transgenics	DNA Adducts	Comet Assay	Micro-nucleus Assays	Chromosome Aberration Assays
Follow up to *in vitro* positives	Follow up in vitro Gene mutation assay +++	 +++	 +++	 ++*	-	-
	-	Follow up Structural chromosome damage +	 +	 ++*	 +++	 +++
	-	-	-	-	Follow up numerical chromosome damage +++	 ++
	-	-	-	-	Follow up polyploidy ++	 +++

+++ efficiently detected ++ detected depending on test design or *not enough data available + not always detected - not detected

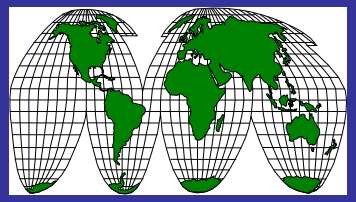


2009 International Workshop on Genotoxicity Testing

Follow-up of *in vivo* positive results

Véronique Thybaud (Sanofi aventis), Chair
Lutz Müller (Roche), Co-chair
Jim MacGregor (Consultant), Rapporteur

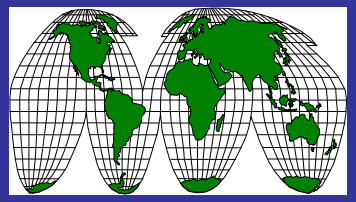
Working group:
Riccardo Crebelli, Kerry Dearfield, George Douglas, Peter B. Farmer, Elmar Gocke, Makoto Hayashi, David P. Lovell, Werner K. Lutz, Daniel Marzin, Martha Moore, Takehiko Nohmi, David H. Phillips and Jan Van Benthem



Follow-up of *in vivo* positive results

Key messages:

- *In vitro* test(s) positive results and the absence of genotoxic effects in appropriate *in vivo* endpoints in adequately exposed tissues in relevant animal species: negligible genotoxicity risk *in vivo* and absence of concern.
- Nonlinear response curves and operational threshold occur *in vivo* even with DNA-reactive agents.
- Consensus is needed on appropriate mathematical models and statistical analyses for defining thresholds and risk levels.



Key messages (cont'd):

- The better the information about mode of action and dose-response relationship, the more certain is the interpretation of dose-response relationship and determination of an acceptable exposure level in humans (exposure metrics are key).
- Need to continue the development of *in vivo* assays especially multi-endpoints, multi-species assays, with emphasis on those applicable to humans.
- To be further evaluated by HESI IVGT "Quantitative group".

Genetic toxicology: What's new? 変

Conclusion:

- Until recently regulatory documents and scientific workshops mainly focused on the definition and refinement of protocols and elaboration of standard batteries of test

  e.g. OECD guidelines, ICH S2 A and B guidelines, IWGT workshops on test protocols.

- Now they also consider the interpretation of the results (including positive findings), e.g., weight of evidence approach, recommendations for follow-up testing considering all available data including mode of action, and human risk assessment

  e.g. FDA and EPA guidance, IWGT workshops on strategy aspects, HESI IVGT, ICH S2(R1) draft.

Thank you!