

## Area: carcinogenicity

**Test purpose: screening and ITS**

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### Impact on animal use

The reports on "Alternative (Non-animal) Methods for Chemicals Testing: Current Status and Future Prospects" (Worth and Balls, 2002) as well as the report on "Alternative methods for Cosmetic testing: Current status and future prospects" (Eskes and Zuang, 2005) have identified advanced and most promising in vitro methods that have the potential to reduce the number of animals in the area of carcinogenicity.

The process of carcinogenesis is recognized as resulting from a sequence of stages and complex biological interactions. The modeling of such complex adverse effects cannot be accomplished at present by the use of non animal tests.

The experts suggested that the carcinogenic potential of a substance could be detected by a combination of the existing in vitro genotoxicity, the cell transformation and the gap junction intercellular communication assays. The cell transformation assay is currently under validation. However, the exact impact of these tests can only be assessed if testing strategies are available.

Test method		Status of validation	Information needed	Timelines
Carcinogenicity	Cell transformation assay Balb/C 3t3 Cell transformation assay SHE cells Chairman MT : Philippe Vanparys	<b>Under pre-validation, phase 2: testing 5 coded chemicals</b>	In vivo and in vitro (CTA) animal data	Ending expected spring 2007

### Information sought

1. Need for data obtained in CTA by industry (methods being very costly, this should avoid additional time and costs) together with in vivo data (GLP rodent carcinogenicity assay)
2. Need for substances (a few grams) disclosed or undisclosed from industry if both in vitro and in vivo data available

### METHODS PROFILES (see list of criteria attached)

CRITERION	CTA (SHE)	CTA (BALB 3T3)
Regulatory requirement	YES	YES
Predictivity	↑↑↑	↑↑
Applicability across sectors	Cosm, Pharm, Chem, Crops	Cosm, Pharm, Chem, Crops
Range of substances concerned	↑↑↑	↑↑
Transferability	↑↑↑	↑↑
Impact of numbers of animal used	Replacement of >400 rats per assay	Replacement of >400 rats per assay
Relevance for screening	YES	YES
Stand alone method or part of a future strategy	Future strategy	Future strategy
Availability of in vivo/in vitro data and substances	High/medium to many/high	High/low to medium/high
Overall cost vs. current assays	↓↓↓	↓↓↓

# ADDITIONAL INFORMATION

## 1) Historical background

- CTA developed in the '70s
- Used by chemical, cosmetics and drug companies
- ECVAM Workshop on Cell Transformation Assay (CTA) Combes et al., ATLA 27, 745-767, 1999)
- OECD Draft Detailed Review Paper DRP31 (2000-2006)
- Expert meeting at ECVAM, April 2004 (15 experts)

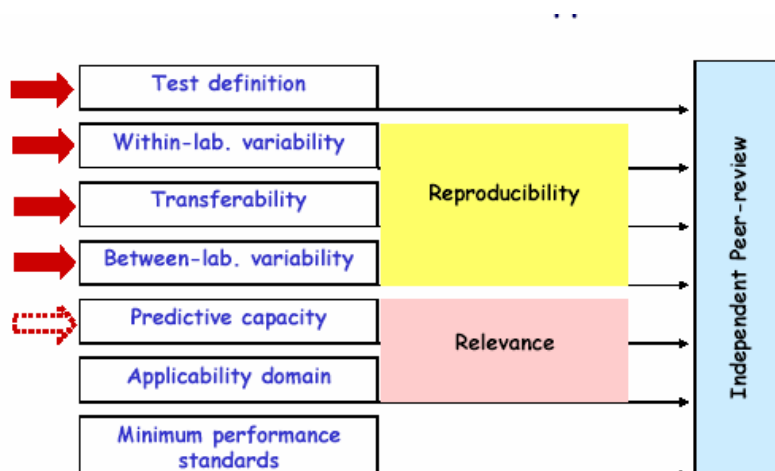
## 2) CTA expert meeting, ECVAM, 2004

- Review status of the art
- Decision to prevalidate CTA
- Two assays: SHE and Balb/C 3T3
- Set up of Validation Management Team
  - Chair: P. Vanparys (J&J);
  - members from industry, academia, FDA
- International effort (Europe, US, Japan)

## 3) Prevalidation CTA

<p><b>Balb/c 3T3</b> <b>Mouse fibroblast cell line</b></p> <ul style="list-style-type: none"> <li>▪ ECVAM</li> <li>▪ Hatano Res. Institute (Japan)</li> <li>▪ RCC (Germany)</li> </ul>	<p><b>SHE (low pH)</b> <b>Syrian Hamster Embryo primary cells</b></p> <ul style="list-style-type: none"> <li>▪ BASF (Germany)</li> <li>▪ RCC (Germany)</li> <li>▪ Bioreliance (USA)</li> <li>▪ Uni of Metz (France- standard pH)</li> </ul>
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## 4) Modular validation approach



## 5) SHE CTA Phase I

- Training
- 2 protocols: low pH, standard pH
- SOPs
- 1 un-coded and 1 coded compound
- Production of photos catalogue (for scoring)
- Data
  - cytotoxicity as relative plating efficiency
  - cell transformation as morphological transformation

## 6) Balb/c 3T3 Phase I

- Training
- Optimization/standardization of protocol and determination of most suitable cell clone
- SOPs
- Production of photos catalogue (for scoring)
- 1 un-coded and 1 coded compound
- Data – cytotoxicity
  - Crystal Violet (CV)
  - Colony Forming Efficiency
  - morphological transformation

## 7) Next steps

### Prevalidation

- Test 5 coded chemicals (carcinogens and non carcinogens)
- End Phase II: September 2007
- Assessment of
  - standardization,
  - within-lab variability,
  - transferability,
  - between-lab variability,
  - preliminary predictive capacity

### Validation

- Confirmation and assessment of predictive capacity.

## 8) OECD Environment, Health and Safety Publications: Series on Testing and Assessment

### DETAILED REVIEW PAPER ON CELL TRANSFORMATION ASSAYS FOR DETECTION OF CHEMICAL CARCINOGENS

Purpose: to evaluate the performance of the three CTAs in predicting mammalian carcinogenicity (SHE, Balb/c 3T3, C3H)

- 1st draft circulated for comments 2001

- 2nd draft circulated in 2002
- 3rd draft circulated in 2006

	<b>SHE</b>	<b>BALB</b>	<b>C3H</b>
<b>Concordance (%)</b>	86	69	78
<b>Sensitivity (%)</b>	90	77	80
<b>Specificity (%)</b>	74	49	68
<b>Total number of chemicals</b>	258	178	135
<b>Non carcinogens</b>	28	33	18

## 9) Information needed

### Data from industry:

Which companies perform/have performed CTA?

- CTA (SHE and Balb/C 3T3)
  - Detailed protocol and data evaluation
  - How to present the data to be discussed directly with the experts and VMT
- GLP rodent cancer bioassay
- Other data for determination of carcinogenicity assessment if available
- Really **RELEVANT** if data from both CTA and rodent bioassay are available.

### Substances from industry

- Compounds disclosed or undisclosed
- Only if data on both CTA and bioassay are available
- Quantity: a few grams

## Criteria for prioritization

The following criteria were agreed at the WG5 Workshop as important for the prioritization of validation studies:

### A – Ethics

- the number of animals used by the animal and the alternative test (impact data) in the overall context of the actual use of the respective methods,
- the level of diminution of the test severity or 3Rs benefit offered
- the increased level of safety introduced

### B – Regulatory demand

- Would the test address regulatory needs ?
  - Does the method cover a specific regulatory testing purpose ?
  - Is it strictly applicable ? ie hazard/risk
  - Are there clear agreed rules/ criteria for how test results would be used for decision making : ie Regulatory and/or Safety
  - Would the test offer full replacement for safety assessment or be used in a tiered strategy ?
- Are there legislative timelines ?

### C - Industrial incentive

- Does it have a high probability to be accepted by regulators (liability aspects) ?
- the applicability of the test in different sectors,
- the applicability for all chemicals / or for materials of limited range,
- the frequency of use of the method
- method transferability,
- need for special equipment, trained staff, resources (overall cost)
- reduced time
- Could the test be used for screening purposes?(HTS)
- are there patents involved, would the test be commercially available?

### D – Development status

- relevant and consistent responses with positive and negative reference materials
- level of advancement of the test, (optimised protocol , standard operating procedures )and of the method (prediction model)
- part of an overall strategy (basic research still missing),
- availability of data (optimization studies , prevalidation , publications ),
- Is this test making use of the best scientific knowledge available?
- probability for success