In vitro Cell Transformation Assays

The use of the SHE cell transformation assay in hazard and risk assessment for industrial chemicals under REACH

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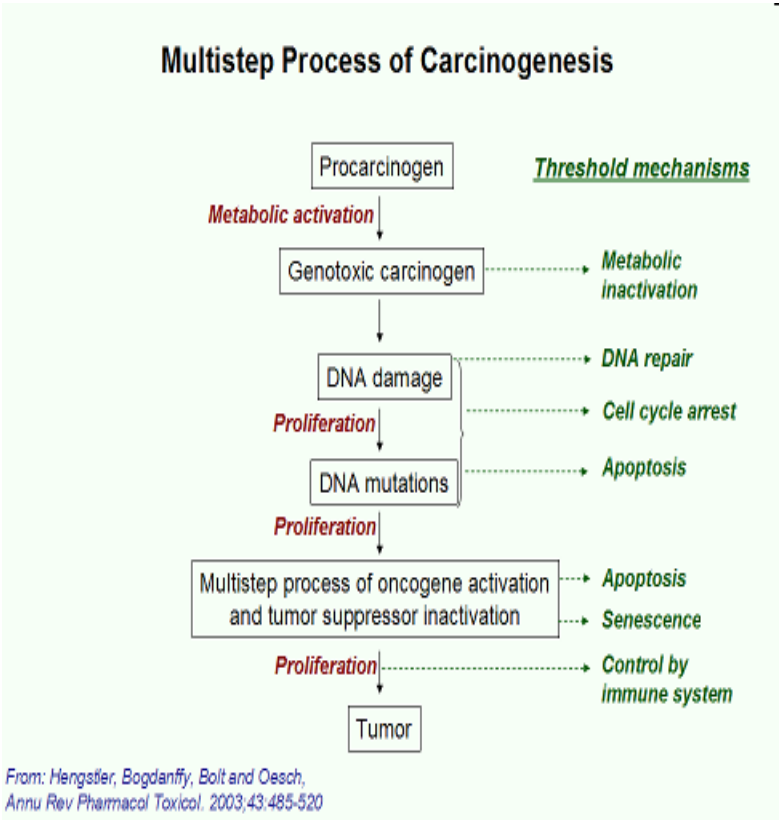
Carcinogenicity

- Carcinogenesis is a complex long-term multifactorial process and consists of sequence of stages
- Two categories based on the mode of action: genotoxic carcinogens versus non-genotoxic carcinogens
- Chronic toxicity studies for identification of hyperplastic and preneoplastic responses related to tumour growth – 2 years bioassays

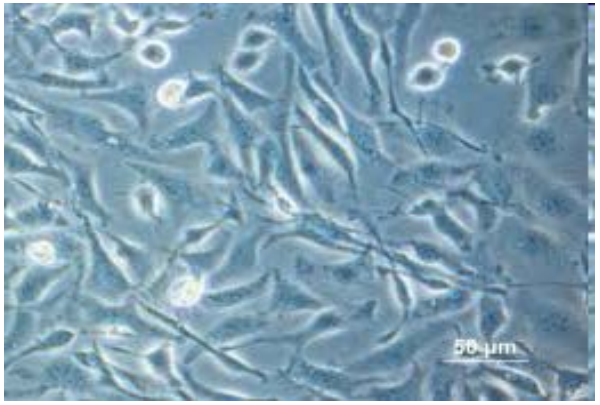


Non-genotoxic carcinogens

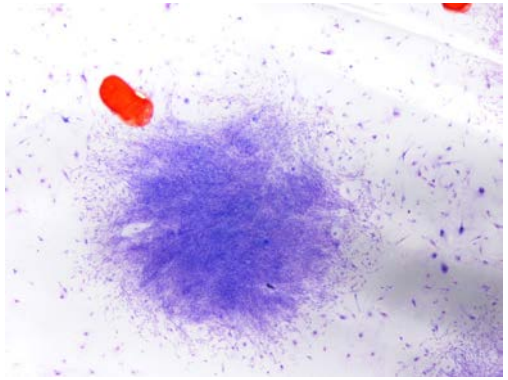
- Increase cell growth/proliferation (increase DNA synthesis, decrease apoptosis)
- Modulation of intercellular communication (hormonal disruption, inhibition of gap junctional intercellular communication)
- Modulation of gene expression (hypomethylation, activation of transcription factors)
- High complexity of the carcinogenicity process
- Data from repeated-dose toxicity studies (NOAEL) are used for quantitative risk assessment of non-genotoxic carcinogens

SHE Assay

- Cells are diploid, karyotypically stable
- Cell population is comprised of multiple cell types and cells at various stages of differentation
- Metabolically competent
- Study Duration 9 days
- Cells demonstrate a multistage of neoplastic transformation similar to that observed *in vivo*

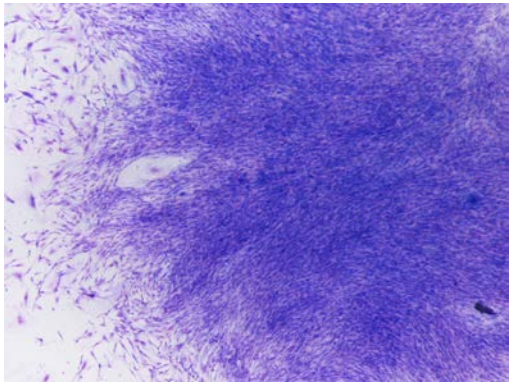


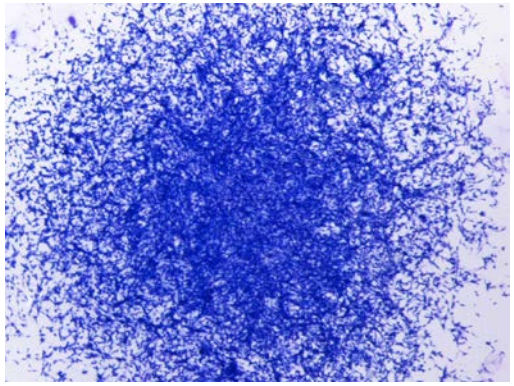
Transformed and non-transformed colonies – SHE assay 6.7



Normal colonies

Transformed colonies





Standard and Low pH SHE Assay

Standard-Assay

Large percentage (80%) of serum lots do not support the MT phenotype

50% or less of cell isolates support the MT phenotype

Low MT frequencies difficult to apply statistics

Dose-response for induction of MT often not observed

Low pH-Assay

75% of serum lots support the MT phenotype

Greater than 90% of cell isolates support the MT phenotype

Higher frequencies of MT allow for use of statistics

Dose-response frequently observed, and toxicity not required to obtain the MT phenotype

Predictivity – OECD 2008

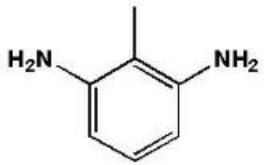
	SHE pH 6.7	SHE pH > 7.0
Chemicals	109	203
Concordance	74%	80%
Sensitivity	74%	86%
Specificity	75%	64%
False +	25%	36%
False -	26%	13%

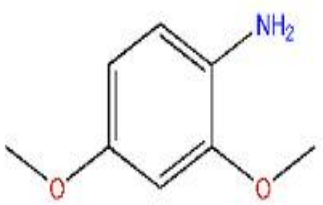
Use of the SHE Cell Transformation Assay

- Clarification of the meaning of positive results from genotoxicity assays, to be used in the weight of evidence assessment
- Data of these assays can be useful where genotoxicity data for a certain substance class have limited predictive capacity
- Investigation of compounds with structural alerts for carcinogenicity
- Use of in vitro cell transformation data to demonstrate differences or similarities across a chemical category
- Investigation of tumour promoting activity

SHE Assay Results for Aromatic Amines

- 45 aromatice amines with carcinogenicity data
- 36 carcinogens: SHE was positive for 32/36 = 89% sensitivity
- 9 non-carcinogens: SHE was negative for 9/9 = 100% specificity
- Overall concordance was 41/45 = 91**%**





Case Metal Powder and Metal Alloy

- REACH substance > 1000 annual tonnes
- Classified by IARC as Group 1 carcinogen
- Positive effects in the rodent bioassay in mice and rats
- Positive effects in genotoxicity tests using metal salts
- Goal re-classification based on experimental in vitro data
- Both metal powder and a metal alloy were investigated
- Due to inert properties extracts of the compounds were investigated

Case Metal Powder and Metal Alloy

- Standard in vitro genotoxicity test battery was performed
- SHE cell transformation assay
- Examination of the extracts on the content of metal ion release (653 ng/L to 165 µg/L,respectively)
- No genotoxic effects in the genotoxicity studies performed

SHE assay results – Metal Powder

Extract concentration in %	Relative plating efficiency in %*	Morphological transformation frequency in %
Exposure period 7 days		
Negative control[1]	100.0	0.59
Solvent control[2]	100.0	0.26
Positive control[3]	93.3	**3.04**[S]
Negative control (extracted)[1]	100.0	0.61
25.0	101.0	**1.45**[S]
37.5	103.8	**1.41**[S]
50.0	96.3	**1.59**[S]
75.0	96.6	**2.50**[S]
100.0	106.9	**1.98**[S]

SHE assay results – Metal alloy

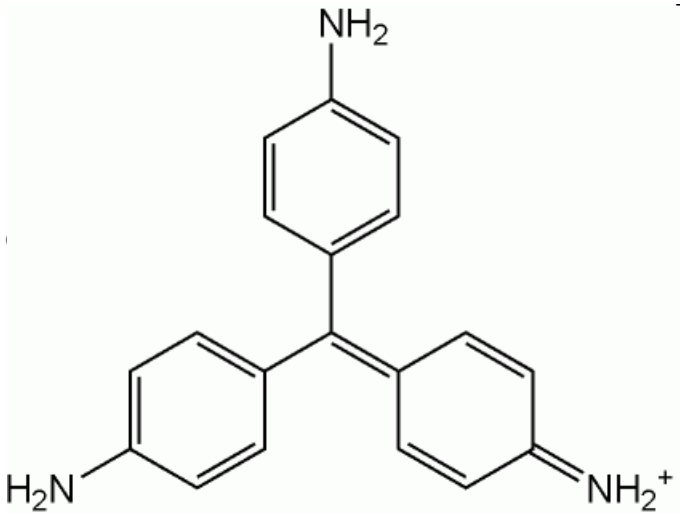
Extract concentration in %	Relative plating efficiency in %	Morphological transformation frequency in %
Exposure period 7 days		
Negative control[1]	77.0	0.58
Solvent control[2]	100.0	0.25
Positive control[3]	96.5	**2.34[S]**
Negative control (extracted)[1]	100.0	0.40
2.0	91.5	**1.99[S]**
4.0	88.2	**2.13[S]**
6.0	82.0	**2.34[S]**
7.0	71.8	**2.79[S]**
8.0	68.7	**3.06[S]**
10.0	44.1	**3.28[S]**
12.0	23.9	**3.41[S]**
14.0	14.8	**4.50[S]**

Conclusion on metal powder and alloy

- Metal ions lead to significant increase in cell transformation
- Carcinogenicity of the metal ions is based on non-genotoxic mechanism
- A threshold maybe established which may lead to a re-classification of the metal

Case Compound with structural alert

- REACH substance > 1000 annual tonnes
- Certain similarity in chemical structure to pararosaniline
- No data on genotoxicty and carcinogenicity available
- Pararosaniline cause cancer in animals
- Pararosaniline is mutagenic
- Performance of the SHE assay for investigation of potential carcinogenic effect



SHE assay results of alerted compound

Test item concentration in µg/mL	Relative plating efficiency in %	Morphological transfomation frequency in %
	Exposure period 7 days	
Negative control[1]	97.9	0.50
Solvent control[2]	100.0	0.61
Positive control[3]	112.4	3.30[S]
10.0	116.2	0.68
12.5	110.0	**1.27[S]**
15.0	109.8	**1.50[S]**
17.5	106.5	**1.26[S]**
20.0	91.9	**2.36[S]**
22.5[P]	62.2	**1.78[S]**
25.0[P]	66.8	**2.52[S]**
27.5[P]	57.5	**2.47[S]**
30.0[P]	48.0	**3.89[S]**

Conclusion on alerted compound

- Significant increase in morphologically transformed colonies
- Compound needs authorisation according to REACH regulations
- Categorisation to a certain chemical category by SHE cell transformation data is possible
- Waiving and read-across can be confirmed and supported by SHE cell transformation data

CTA for carcinogenicty safety assessment of cosmetic ingredient

- Ban of animal experiments for cosmetics since March 2009 and March 2013
- No in vivo genotoxicity tests as follow-up of in vitro positives
- Risk from non-genotoxic carcinogens cannot be sufficiently evaluated since the repeated dose toxicity test is not allowed anymore
- CTA able to detect both genotoxic and non-genotoxic compounds
- CTA have the potential to contribute to a weight of evidence approach

Projects included in the Work Plan of the Test Guidelines Programme

Assay	Inclusion in the work plan	Lead country	Status of the work
SHE	2003	France	EC Validation and Peer Review Reports available since Feb. 2011
Balb/c 3T3	2007	Japan	EC Validation and Peer Review Reports available since Feb. 2011
Bhas 42		Japan	Validation Report not yet available

WNT meeting (1)

In April 2011, most National Coordinators agreed that the CTAs could be very useful as part of a testing battery and weight of evidence approach for identifying non-genotoxic substances which may be carcinogenic and for which carcinogenicity testing is not specifically required.

WNT meeting (2)

Terms of Reference for the expert group:

Review all available information

Identify the applicability domain

Discuss possible pathways and mechanisms of action

Discuss the need for specific targeted prospective testing before finalizing one or several Test Guideline(s)

Recommendation of the expert group to the WNT

Expert Meeting at December 14. and 15, 2011:

- Applicability domain and Regulatory Use of CTA – already provided
- MOA or mechanism of CTA – Use of biomarkers, immage analysis, Differences in methylation profiles of DNA, Co-operation with the OECD working group « Adverse Outcome Pathways »
- Processing of an OECD Guideline for the SHE assay based on the provided data - Lead is France
- Generation of additional data for the Balb/3T3 Test is necessary before an OECD guideline can be developed – Lead is Japan
- Bhas42 validation report will be provided to ECVAM and will be evaluated
- OECD WNT meeting at April 24-27, 2012 in Paris

What is necessary in future

- Problem of subjectivity in scoring MT should be solved: training, automation, molecular/biochemical markers – photocatalogue published in special issue in Mutation Research
- Need for a consistent protocol across labs – SOP published in special issue in Mutation Research
- Further increase in data base will contribute to the acceptance of the assay
- Establishment of assays using human cells

Thank you very much for your attention