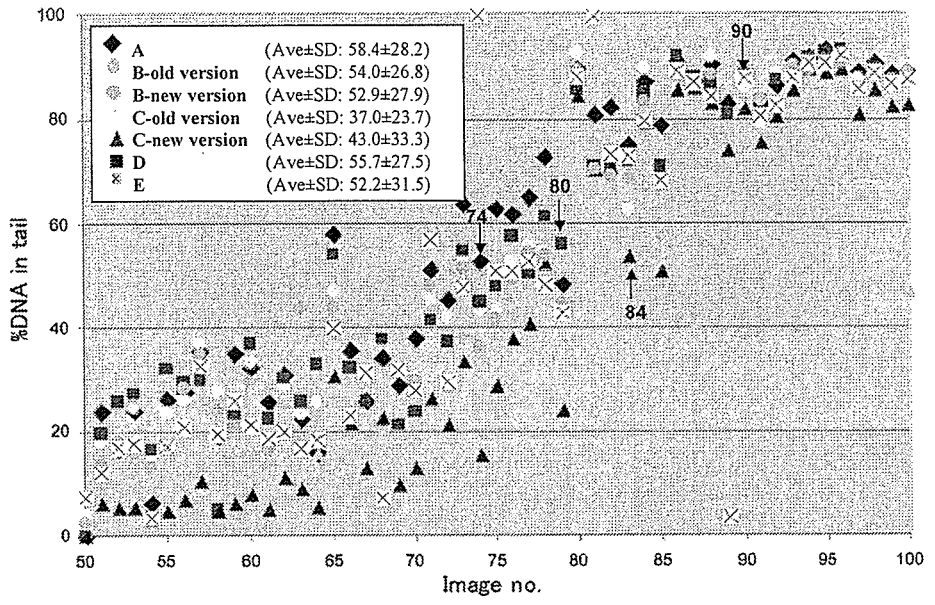
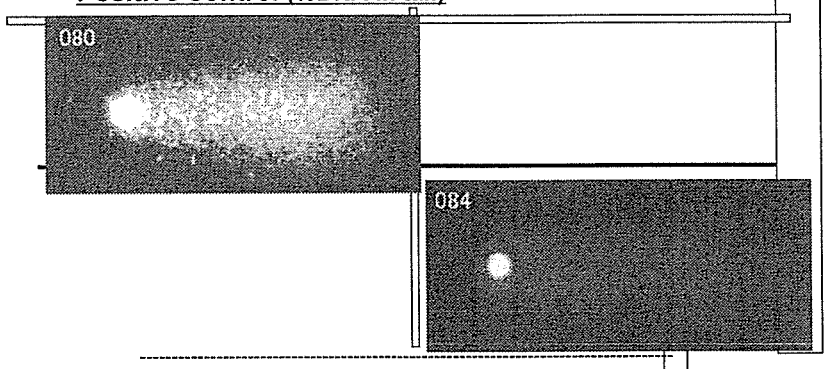
## Positive control (%DNA in tail)




MMS/JacVAM Joint Seminar, K. Yamakage, 2006

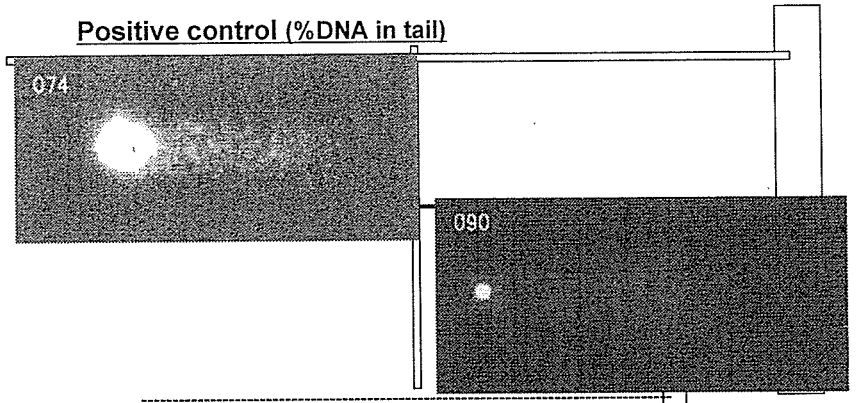
## Positive control (%DNA in tail)




| | %DNA in tail | |
|---|---|---|
| | Image no. 080 | Image no. 084 |
| A | 89.71 | 87.14 |
| B-old version | 92.66 | 89.76 |
| B-new version | 89.27 | 83.23 |
| C-old version | 84.00 | 78.00 |
| C-new version | 84.45 | 89.45 |
| D | 85.77 | 85.06 |
| E | 88.03 | 79.53 |

MMS/JacVAM Joint Seminar, K. Yamakage, 2006

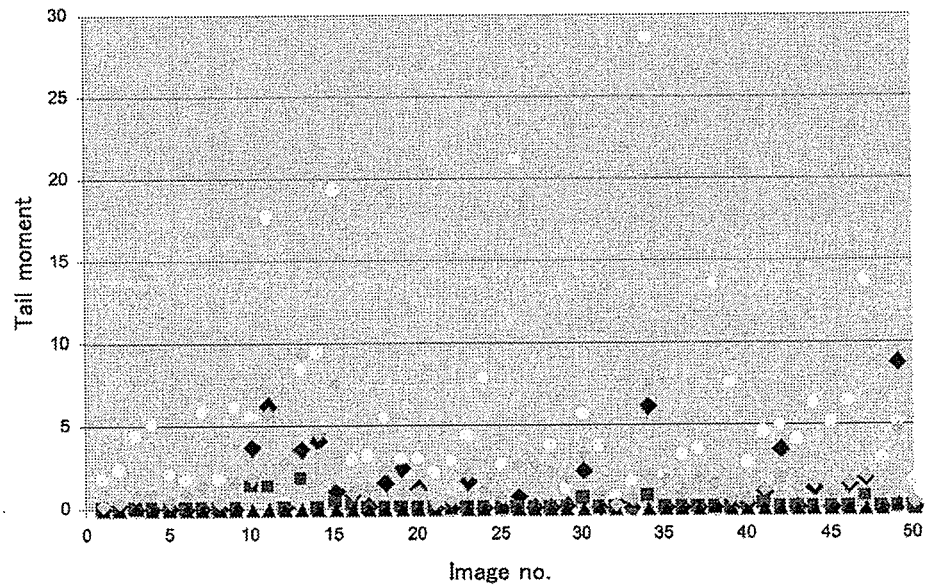
## Positive control (%DNA in tail)



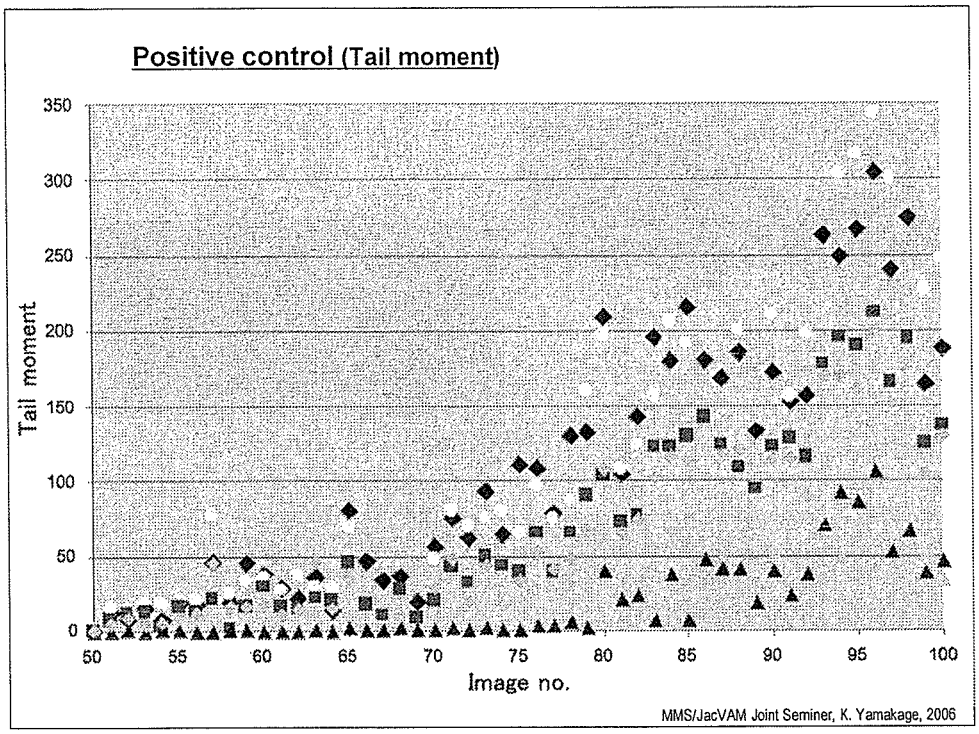

| | %DNA in tail | |
|---|---|---|
| | Image no. 074 | Image no. 090 |
| A | 52.85 | 88.51 |
| B-old version | 43.45 | 85.86 |
| B-new version | 36.28 | 87.59 |
| C-old version | 30.00 | 46.00 |
| C-new version | 15.69 | 82.12 |
| D | 44.86 | 88.36 |
| E | 99.75 | 87.78 |

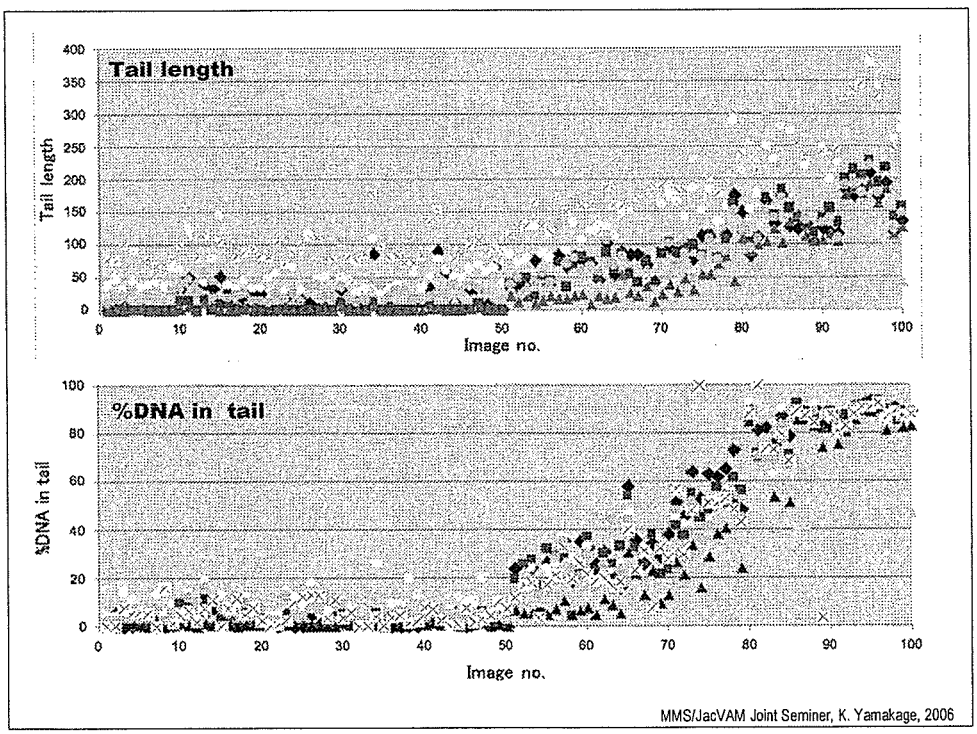
MMS/JacVAM Joint Seminar, K. Yamakage, 2006

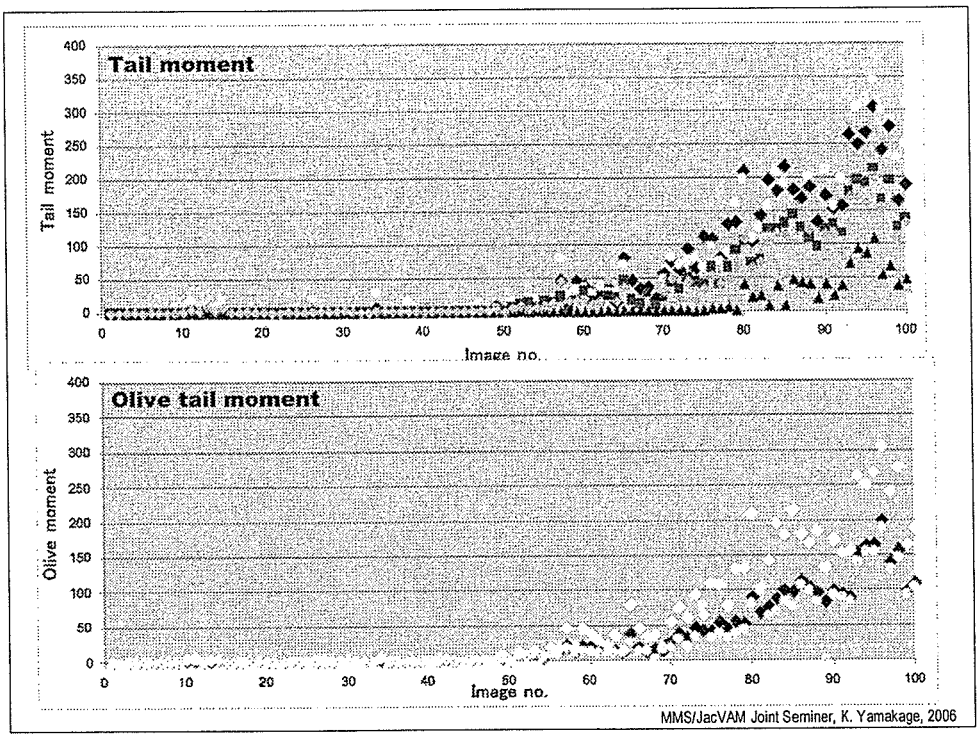
## Negative control (Tail moment)

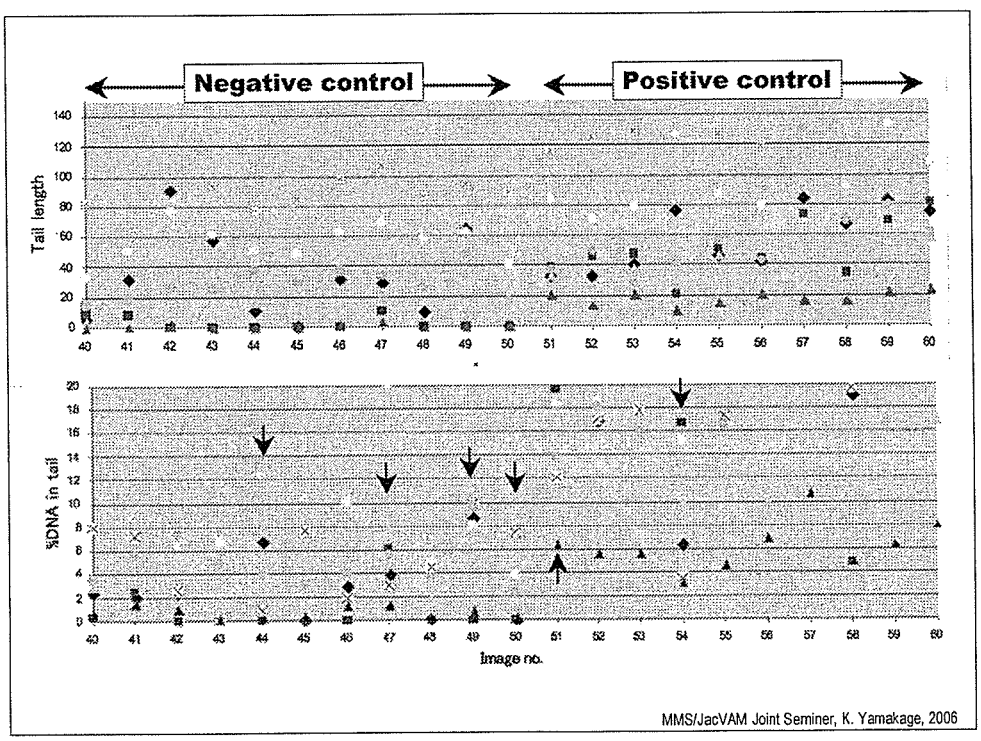


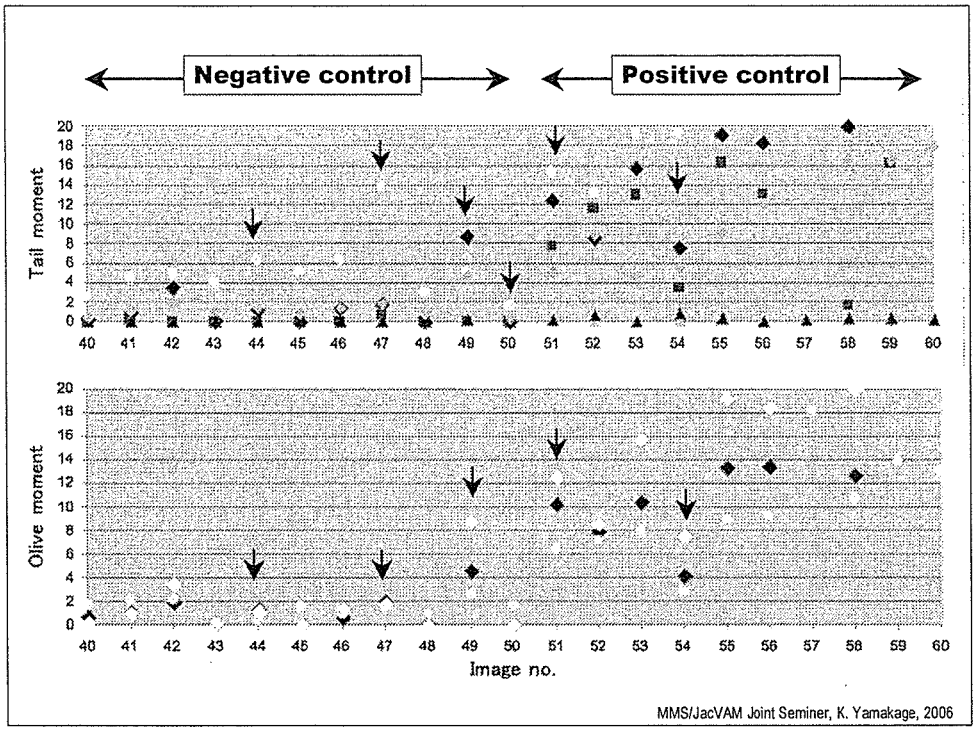

MMS/JacVAM Joint Seminar, K. Yamakage, 2006

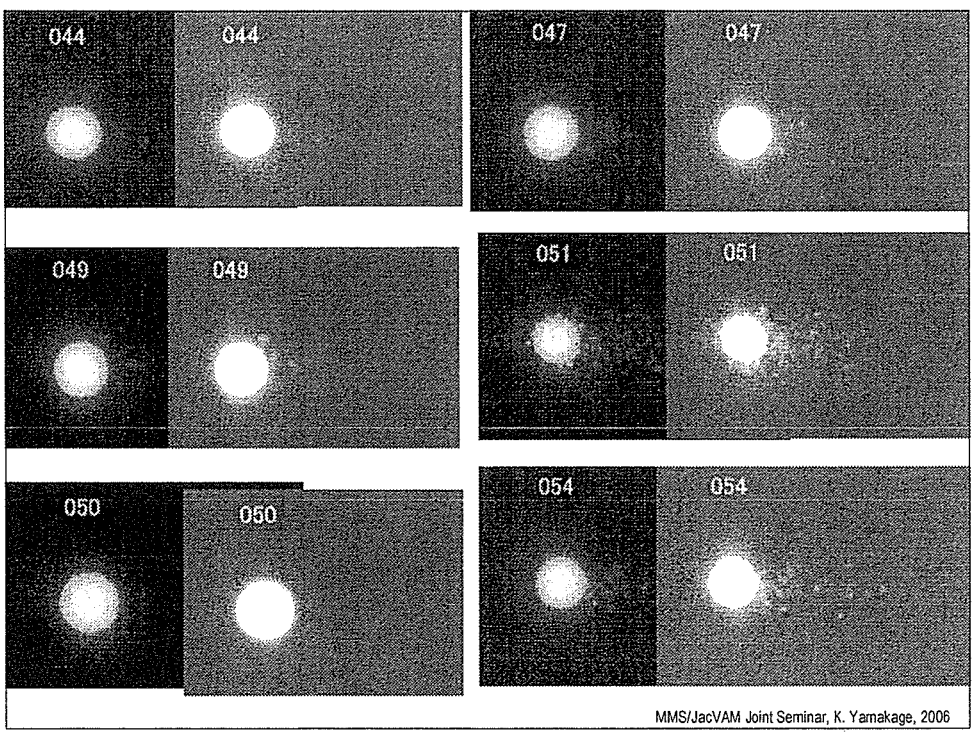

Positive control (Tail moment)
Tail moment
Image no.
MMS/JacVAM Joint Seminer, K. Yamakage, 2006


Tail length
Tail length
Image no.
%DNA in tail
%DNA in tail
Image no.
MMS/JacVAM Joint Seminer, K. Yamakage, 2006


Tail moment
Tail moment
Image no.
Olive tail moment
Olive moment
Image no.
MMS/JacVAM Joint Seminer, K. Yamakage, 2006


Negative control
Positive control
Tail length
%DNA in tail
Image no.
MMS/JacVAM Joint Seminer, K. Yamakage, 2006


Negative control
Positive control
Tail moment
Olive moment
Image no.
MMS/JacVAM Joint Seminer, K. Yamakage, 2006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MMS/JacVAM Joint Seminar, K. Yamakage, 2006

# Summary

- Three of seven image analyzers were impossible to measure some clear positive images.
- Differences (average values) of control images among image analyzers were 1.06-92.60 (tail length), 0.75-9.75 (%DNA in tail) and 0.02-5.68 (tail moment).
- Since overlapping of each parameter between control images and weak positive images were smaller in %DNA in tail than in tail length or tail moment, %DNA in tail might be more relaiable.

# JaCVAM initiative International validation on *in vivo* and *in vitro* comet assay

**Japanese Center for the Validation of Alternative Methods (JaCVAM)**

## 1 The purpose

1.1 To validate the *in vivo* comet assay as an alternative follow-up assay to the more commonly used *in vivo* liver UDS assay. Moreover, we would like to evaluate the use of the *in vivo* comet assay for the assessment of DNA damage by chemicals in multiple tissues and to investigate the correlation with carcinogenicity data in those tissues.

1) The intra- and inter-laboratory reproducibility of this assay will also be evaluated.
2) To clarify some technical aspects and to recommend the standard technical procedure of this assay, including whole cell *vs* isolated nuclei issue.
3) To discuss and recommend the method to assess cytotoxicity: histopathological method *vs* any other methods.

1.2 To validate the *in vitro* comet assay as a method of detecting potential DNA damaging effects of test chemicals and also as an alternative to the *in vivo* comet assay.

## 2 Organization

2.1 Management Team
M. Hayashi (JaCVAM/NIHS)
Y. Uno (MMS*/Mitsubishi Phama Co.)
T. Hurtung or any other representative (ECVAM)
L. Schechtman (ICCVAM/FDA)
R. Tice (NICEATM)

Secretariat
H. Kojima (JaCVAM/NIHS)

*Mammalian Mutagenicity Study Group, which is a sub-organization to the Japanese Environmental Mutagen Society

2.2 Consultation team
N. Asano (MMS/Nitto Denko Co.)
B. Burlinson (Huntingdon, UK)
M. Honma (NIHS)
D. Lovell (Statistician, University of Surrey)
T. Morita (NIHS)
N. Nakashima (OECD)
Y. Ohno (JaCVAM/NIHS)
T. Omori (Statistician, Kyoto University)
YF Sasaki (Hachinohe National College of Technology)
B. Young (Bio-Reliance, USA)

2.3 Local Committee
N. Asano (MMS/Nitto Denko Co.)
M. Hayashi (JaCVAM/NIHS)
M. Honma (NIHS)
H. Kojima (JaCVAM/NIHS)
T. Morita (NIHS)
M. Nakajima (MMS/Anpyo-Center)
T. Omori (Statistician, Kyoto University)
Y.F. Sasaki (Hachinohe National College of Technology)
Y. Uno (MMS/Mitsubishi Phama Co.)
K. Yamakage (MMS/FDSC)

2.4 SD Team for pre-validation
K. Yamakage (FDSC)
M. Nakajima (Anpyo-Center)
Patricia Escobar (Invitrogen)
B. Burlinson (Huntingdon)
P. Clay (Syngenta)

2.5 SD Team for main validation
FDSC (Dr. K. Yamakage)
Anpyo-Center (Mr. M. Nakajima)
Invitrogen (Dr. Patricia Escobar)
Huntingdon (Dr. B. Burlinson)
Syngenta (Dr. P. Clay)
Merck (Dr. R.D. Storer)

To be added up to approximately 10 qualified laboratories in total.

## 3 Time schedule

3.1 April 13, 2006 Yoga, Japan
Local Organizing Committee meeting,
3.2 August 14-15, 2006 Sapporo, Hokkaido
Management Team and Kick-off meeting
(Management Team members, Expert and Observer team, and representatives from laboratories for pre-validation)
3.3 September-November, 2006 *In vivo* pre-validation
3.4 December, 2006 Data cleaning and analysis
3.5 February-March, 2007 Management team meeting (telephone conference?) for the evaluation of the pre-validation study and planning for the main validation and also preparation of the pre-validation *in vitro* study
3.6 March, 2007 Preparation of the report for the MHLW budget
3.7 April-May, 2007 *In vivo* main validation/*in vitro* pre-validation
3.8 August, 2008 Management team meeting for the *in vitro* pre-validation

study and also for the main validation study

3.9 February-March, 2008 Management team meeting for the assessment of the *in vivo* main-validation study and the evaluation of *in vitro* pre-validation and planning the *in vitro* main validation study

3.10 Summer, 2008 Drafting of the *in vivo* comet assay test guideline and propose to OECD

3.11 February-March, 2009 Management team meeting for the assessment of the *in vitro* main-validation

3.12 Summer, 2009 Drafting of the *in vitro* comet assay test guideline and propose to OECD

**4 Success criteria**

To be discussed at the kickoff meeting in summer, 2006.

**5 Funding**

Grant form MHLW and MMS

**6 Pre-validation study**

The protocol used will be proposed for review at the Kick-off meeting
Negative (solvent) control; positive control (to be selected at the kick-off meeting); two dose levels of a positive control and coded (?) chemical.

1) Test animal species
Mouse
2) Study design

| Compound | Dose (mg/kg) | Number of animals |
|---|---|---|
| Corn oil (negative control) | 0 | 4 |
| EMS (positive control) | 200 | 4 |
| EMS (positive control) | 400 | 4 |
| Unknown | ? | 4 |
| Unknown | ? | 4 |

Twice repeat treatment at each laboratory.

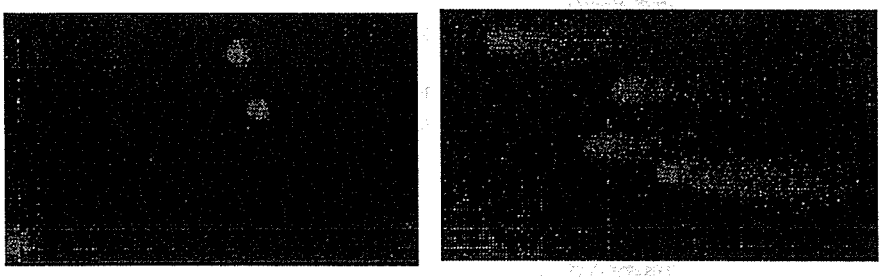
3) Route for administration
Oral gavage
4) Tissues to be investigated: Liver and stomach.
5) Preparation of whole cells or isolated nuclei
Each laboratory will use the mincing method to obtain whole cells and the homogenization method to obtain isolated nuclei.

**7** Main validation study will be discussed at the Management Team based on the outcomes of the pre-validation study.

**8** Others

Collaborate with the COMICS
Etc.

## *International validation study of in vivo & In vitro Comet assay*



Makoto HAYASHI / 2006

## Genotoxicity test

| | *In vitro* | *In vivo* |
|---|---|---|
| *DNA damage* | Rec-Assay<br>UDA<br>Comet assay<br>DNA strand break | UDS<br>Comet assay<br>DNA strand break |
| *Point mutation* | Ames assay<br>Mouse lumphoma<br>TKassay | Utilization of Transgenic animal |
| *Chrom. Aberration* | Chromosome aberration assay using mammalian cells | *In vivo* micronucleus test |

5

Committee on
MUTAGENICITY

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

# GUIDANCE ON A STRATEGY FOR TESTING OF CHEMICALS FOR MUTAGENICITY

*CHAIR*
*Professor Jim M Parry BSc PhD DSc*

December 2000

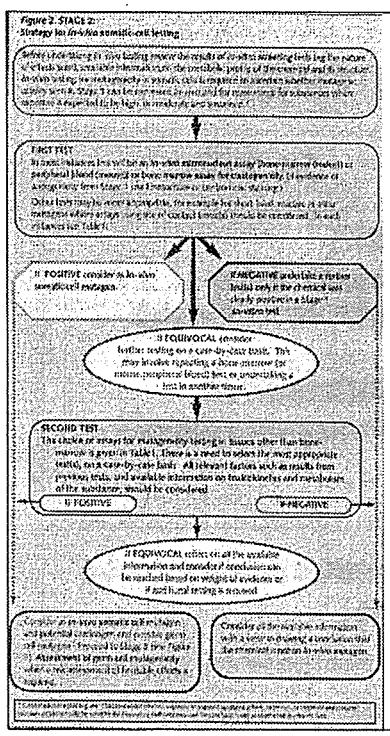
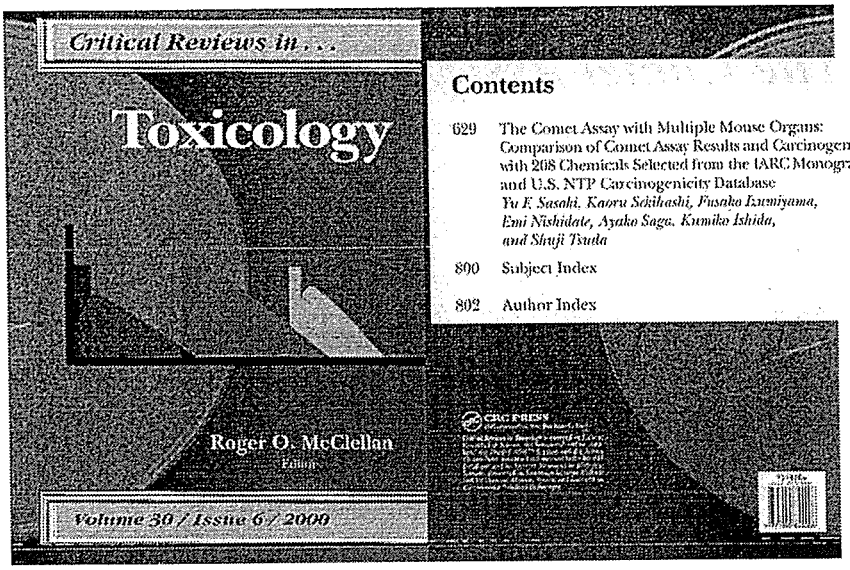



**Table 1: *In-vivo* assays for consideration in Stage 2 other than bone-marrow assays**

(These assays may also be applicable to the initial evaluation for effects in germ cell)

| Assay | Endpoint | OECD guideline | Main Attributes | Comments |
|---|---|---|---|---|
| Liver UDS | Thymidine incorporation outside S phase | Yes | Long history of use and acceptability by regulatory authorities | Limited use in tissues other than liver. Does not detect mutagenicity resulting from misrepair and non-repair. |
| Comet assay *(or other DNA strand-breakage assays)* | DNA strand breaks | No | Can be applied to a large range of tissues, including site-of-contact tissues. Relatively simple to undertake provided that a single cell suspension can be obtained. | May not detect some mutagens (such as those producing bulky adducts). Distinction between apoptosis, necrosis and genotoxicity requires expert judgement. |




Compound X

| Dose (mg/kg) | Sampling time (h) | Colon | | Stomach | |
|---|---|---|---|---|---|
| | | Exptl 1 | Exptl 2 | Exptl 1 | Exptl 2 |
| 0 | 0 | 5.6±0.9 | 8.1±3.5 | 5.9±0.7 | 9.7±1.9 |
| 1 | 3 | 13.0±2.0 ns | 13.8±4.7 ns | 8.6±1.5 ns | 9.2±1.6 ns |
| 10 | 3 | 25.6±1.7** | 13.6±6.6 ns | 8.3±1.3 ns | 19.6±4.2 ns |
| 100 | 3 | 29.4±3.2** | 7.9±4.1 ns | 13.1±1.2 ns | 13.7±4.7 ns |
| 1000 | 3 | 34.4±1.9** | 14.2±4.5 ns | 32.6±1.2** | 13.2±5.9 ns |
| 2000 | 3 | 40.4±3.5** | 16.3±5.1 ns | 9.3±2.0 ns | 17.7±9.1 ns |
| 2000 | 24 | 10.3±0.7ns | 9.7±3.3 ns | 16.2±1.1* | 17.8±4.3 ns |

# *The Comet Assay Working Group*

**4th International Workshop on Genotoxicity Testing**

**San Francisco, CA**
**September 10, 2005**

## Comet Assay Validation (1)

Validation discussed briefly; the need is to:

- Establish an international "Management Team"
- Obtain funding, at least for chemical purchase and distribution
- Review current status of the rodent alkaline Comet assay (need to obtain raw data)
- Identify most appropriate protocol(s)
- Identify chemicals to test coded in order to compare Comet assay performance against UDS, MN, & carcinogenicity test results
- Identify participating labs (preferably GLP-compliant)
- Develop optimal statistical methods for evaluating validation data

## Comet Assay Validation (2)

- Conduct phased/modular approach
  - Phase 1 - generate historical negative/positive control data
  - Phase 2 - test 3 coded substances to demonstrate cross lab performance (some labs may be excluded after this phase)
  - Phase 3 - test x coded substances to demonstrate reproducibility within and across labs
  - Phase 4 - test additional coded substances to demonstrate accuracy
- Data analyzed at each phase by the Management Team for lab performance and for assay relevance (accuracy) and reliability

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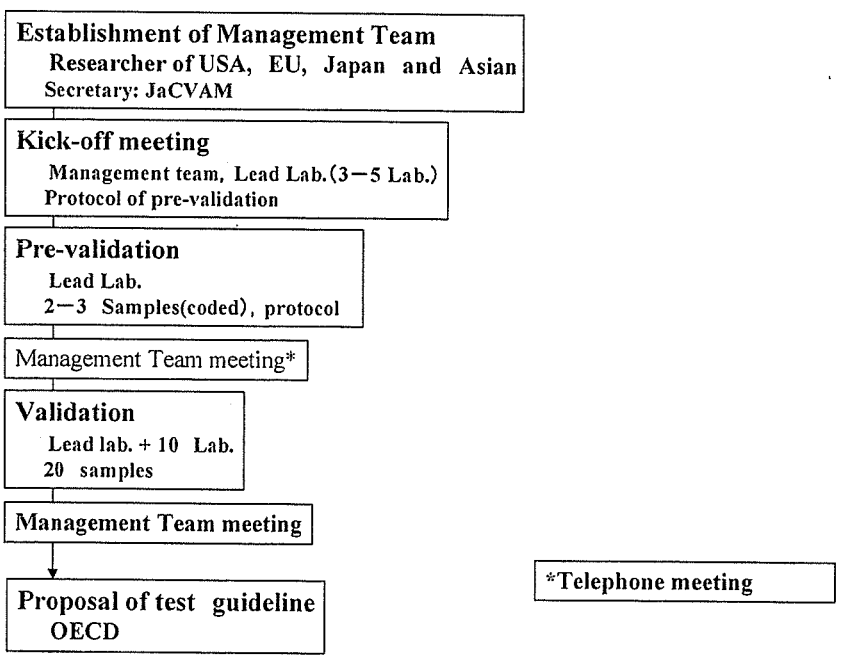


PROPOSED VALIDATION STUDY ROLES AND RESPONSIBILITIES*

**International Study Management Team**
Overall coordination/management
Approval of study design, protocols, time lines, participating laboratories, etc.
Test substance selection, acquisition, coding and distribution
Data evaluation/interpretation
Information exchange
Approval of all reports from the study

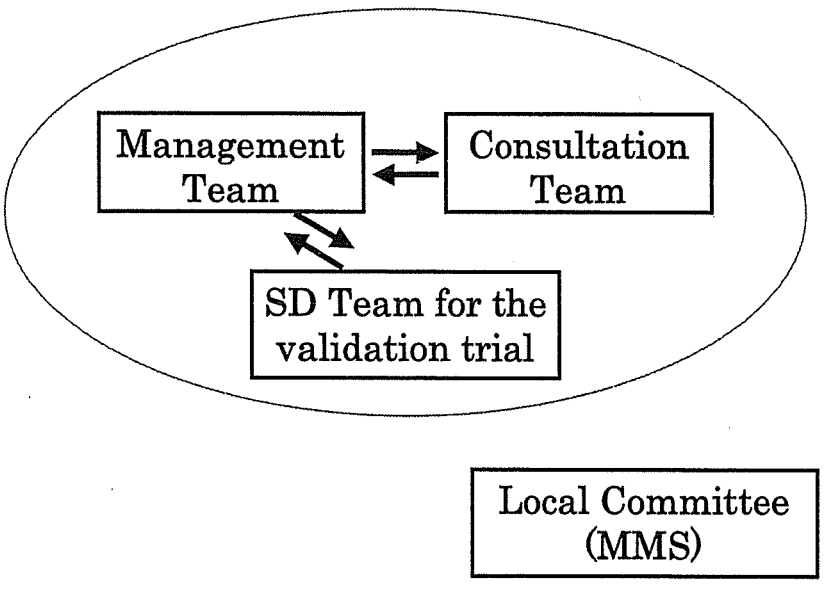
**Local Study Management Team**
Coordination/management of local participating laboratory
Manage contractual/financial considerations for local participating laboratory
Preliminary evaluation/interpretation of data from local participating laboratory
Information exchange with local participating laboratory
Preliminary review of reports from local participating laboratory

**Lead Laboratory**
Training/Instructions
Coordination of SOPs
Troubleshooting

**Participating Laboratories**
Data collection
Study conduct
Data evaluation

•OECD Series on Testing and Assessment Number 34:
•Guidance Document on the Validation and International Acceptance of
•New or Updated Test Methods for Hazard Assessment.

## JaCVAM Initiative International Validation Study on Comet Assay



## JaCVAM Initiative International Validation Study on Comet Assay

**Management Team**

Dr. M. Hayashi (JaCVAM/NIHS)
Dr. Y. Uno (MMS*/Mitsubishi Phama Co.)
Dr. T. Hurtung or any other representative (ECVAM)
Dr. L. Schechtman (ICCVAM/FDA)
Dr. R. Tice (NICEATM)

Secretariat
Dr. H. Kojima (JaCVAM/NIHS)

## JaCVAM Initiative International Validation Study on Comet Assay

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Y. Ohno (JaCVAM/NIHS)
T. Omori (Statistician, Kyoto University)
YF Sasaki (Hachinohe National College of Technology)
B. Young (Bio-Reliance, USA)

## JaCVAM Initiative International Validation Study on Comet Assay

**SD Team for the validation trial**

K. Yamakage (FDSC)
M. Nakajima (Anpyo-Center)
Patricia Escobar (Invitrogen)
B. Burlinson (Huntingdon)
P. Clay (Syngenta)

## JaCVAM Initiative International Validation Study on Comet Assay

**Local Committee (MMS)**

N. Asano (MMS/Nitto Denko Co.)
M. Hayashi (JaCVAM/NIHS)
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H. Kojima (JaCVAM/NIHS)
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Y.F. Sasaki (Hachinohe National College of Technology)
Y. Uno (MMS/Mitsubishi Phama Co.)
K. Yamakage (MMS/FDSC)

## Topics to be discussed and made consensus

***Protocol issues***

- Isolated nuclei *vs* whole cell
- Positive control and test chemical
- Animals, size of study, treatment, sampling
- Slide preparation, electrophoresis, staining
- Endpoint and analysis (including IA vs categorization)
- Other protocol issue

## Topics to be discussed and made consensus

- Cytotoxicity (histopathology vs others)
- Statistical analysis of data
- Success criteria
- Time schedule proposal