Other recent activities

Other recent activities [1]

HPV chemicals programme

Manual for Investigation of HPV Chemicals

http://www.oecd.org/document/7/0,2340,en_2649_34379_1947463_1_1_1_1,00.html

4.3 Guidance for the Initial Assessment of Health Effects

Table: Status of the HPV Chemicals (As of March 2006)

Status of the HPV Chemicals		Number
Information gathering and data review		434
SIDS Testing Plan submitted & reviewed		69
Draft SIAR (EU-RAR) submitted to the CDG		15
SIAR prepared for SIAM-22		83
SIAR discussed, not finalised		16
SIAR assessed	documents not received	167
	documents received	26
	Available on the web	52
SIAR published *	by UNEP	272
	by EU	63
	Total **	1191

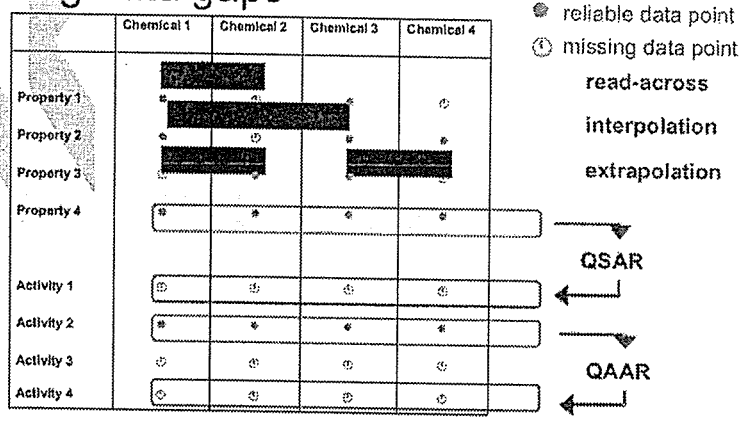
*: 6 chemical has been published by both UNEP and EU.

**: Including 51 non-HPV chemicals

Other recent activities [2]

Category Approach

Filling data gaps



Other recent activities [3]

1. (Q)SARs
2. Exposure Assessment
3. Classification and Labelling (GHS)
4. Integrated Approach to Testing and Assessment
5. Global Portal
6. Safety of Manufactured Nanomaterials

WEBSITE

- Information on Programmes
- Documents (not Test Guidelines)
- Databases
- News

http://www.oecd.org/ehs

OECD OCDE

5

資料 10

The in vitro Comet assay
-Study Plan-

Masamitsu Honma
Division of Genetics and Mutagenesis
National Institute of Health Sciences

Protocol Issues

1. Cells, Cell lines
2. Treatment
3. Cytotoxic parameter, dose selection
4. Cell preparation, slide preparation
5. Electrophoresis
6. Microscopic observation
7. Comet analysis
8. Statistical analysis
9. Others

1. Cells, Cell lines
   - TK6 (Human lymphoblatoid cell line)
   - WTK-1 (TK6 progenitor cell, p53-mutant)

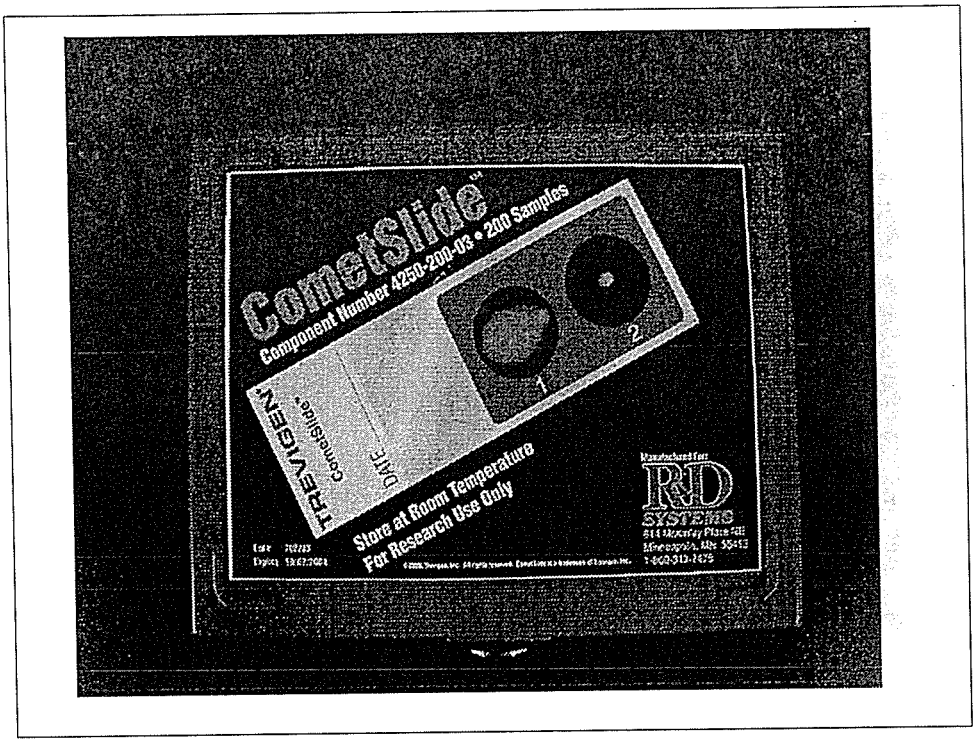
2. Treatment
   - 4h treatment
   - with/without S9 (Rat liver, human liver)

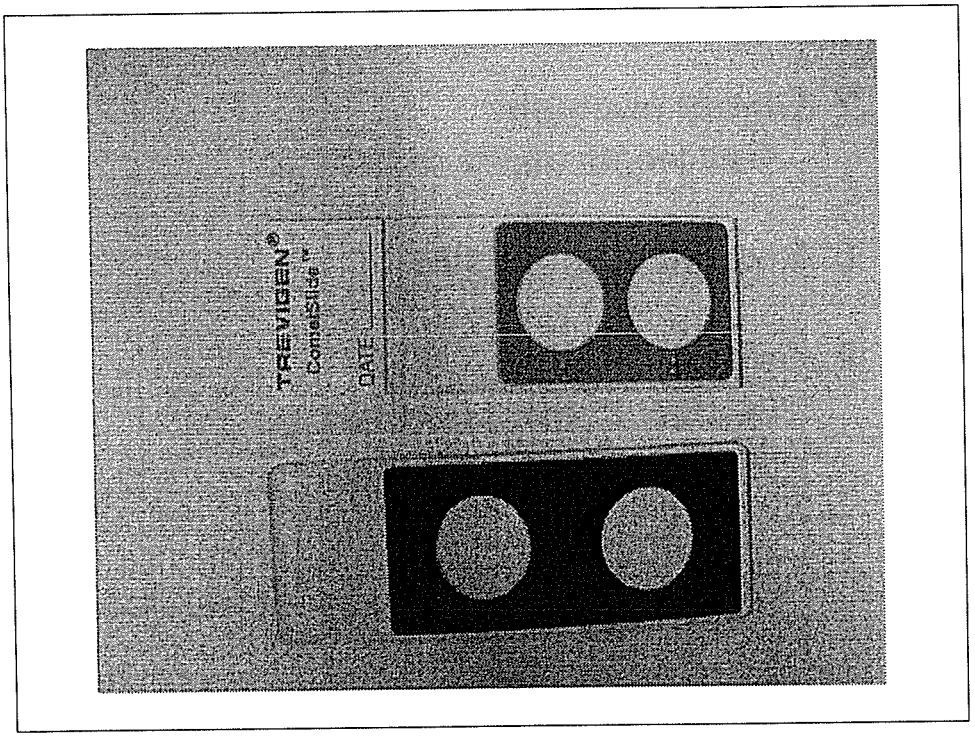
3. Cytotoxic parameter, dose selection
   - Relative survival and/or relative growth
   - Top dose: 80-90% cytotoxicity

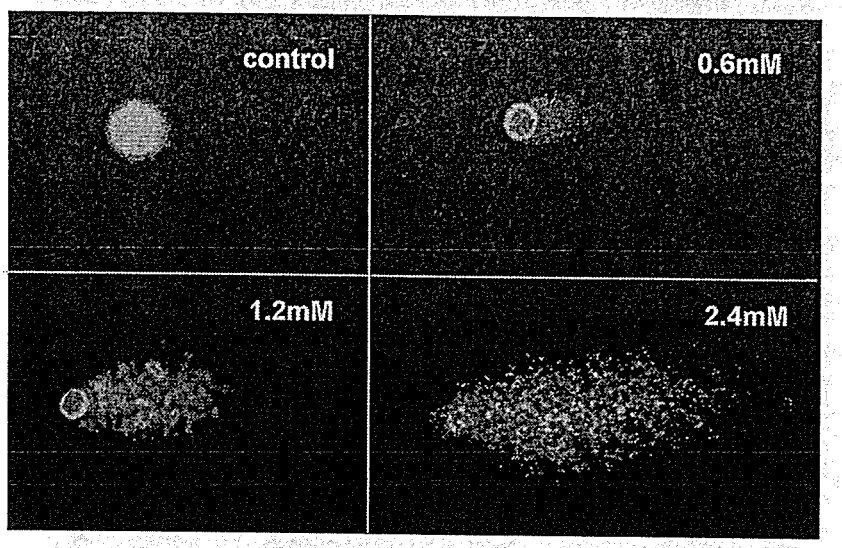
4. Cell preparation, slide preparation
   -
   - Trevigen "CometAssay™" kit

5. Electrophoresis
   - pH>13 Alkanine electrophoresis
   - 300mA (CC), 30-40V, 15-30min

6. Microscopic observation
   - SYBR green staining
   - X 200
   - Capturing 50 cells by CCD camera


CometSlide™
Component Number 4250-200-03 • 200 Samples
TREVIGEN®
CometSlide™
DATE
1
2
Store at Room Temperature
For Research Use Only
R&D
SYSTEMS


TREVIGEN®
CometSlide™
DATE



7. Comet analysis
   - Tail length and % of DNA

8. Statistical analysis

9. Others
   - Neutral Comet assay
   - Multi-endpoint analysis
     - Micronuclei test
     - TK-mutation assay
     - Molecular analysis of TK-mutants
     - Gene expression analysis (p53, GADD45, γH2AX etc.)

Memo about statistical test

2006/08/11
Takashi Omori

1. Some considerations

- The percentage of DNA in tail is one of parameters to measure the DNA damage. This parameter may become a primary indicator in our study.
- In the study, for one group of an experiment, four animals are used, and for one animal of an experiment, 150 of the parameter are observed. Therefore we obtain 600 values of parameters for each animal. However, since the random allocation is done for animal, the statistical analysis method should be constructed for the unit of random allocation. In addition to that, it would be better to use information of 150 data observed repeatedly for each animal.
- Because of the percentage, the range of this parameter is $[0,1]$. The variance of the parameter depends upon the mean of the parameter. That is, the variance relates to the mean. For these data, very often the logit transformation is applied. Unfortunately 0 for the percentage of DNA in tail is often observed especially in negative control group, and the logit transformation can't apply when data take 0.
- To resolve these considerations, we conducted a small examination for the possibility of a two stage analysis. In the first stage, the average and the standard deviation for each individual animal of the parameter is calculated. Since the numbers of the parameter is 150 for each individual, the value of average is expected to be over 0 for all groups. In the second stage, we apply a statistical analysis for the averages with their standard deviations obtained in the first stage. Based on the logit transformation of the average, the statistical test for the difference between the examined chemical group and the negative control group is able to construct.

2. Notation

i: group id ($i = 0, 1, 2, \ldots i, \ldots, I, I+1$)

where i=0: negative control group; i=I+1: positive control group

j: animal id ($j = 1, \ldots, n_i$)

k: observation for repetition within an animal ($k = 1, \ldots, m_{ij}$)

z_{ijk} : The k th observation of the percentage of DNA in tail on the j th animal of the i th group.

x_{ij} : The sample mean of the percentage of DNA in tails on the j th animal of the i th group;

$$x_{ij} = \frac{1}{m_{ij}} \sum_k z_{ijk} .$$

s_{ij}^2 : The sample variance of the percentage of DNA in tails on the j th animal of the i th group;

$$s_{ij}^2 = \frac{1}{m_{ij} - 1} \sum_k \left(z_{ijk} - x_{ij}\right)^2 .$$

$V(x_{ij})$: The variance of x_{ij};

$$V(x_{ij}) = s_{ij}^2 / m_{ij} .$$

y_{ij} : The logit transformation of x_{ij};

$$y_{ij} = \ln \frac{x_{ij}}{1 - x_{ij}} .$$

$V(y_{ij})$: The variance of y_{ij};

$$V(y_{ij}) \approx \left(\frac{1}{x_{ij}} + \frac{1}{1 - x_{ij}}\right)^2 V(x_{ij})$$ (This is obtained by the delta method).

$\bar{y}_i$: The mean of y_{ij};

$$\bar{y}_i = \frac{1}{n_i} \sum_j y_{ij} .$$

$V(\bar{y}_i)$: The variance of $\bar{y}_i$;

$$V(\bar{y}_i) = \frac{1}{n_i^2} V\left(\sum_j y_{ij}\right) = \frac{1}{n_i^2} \sum_j V(y_{ij}) = \frac{1}{n_i^2} \sum_j \left(\frac{1}{x_{ij}} + \frac{1}{1 - x_{ij}}\right)^2 \frac{s_{ij}^2}{m_{ij}} .$$

$V(\bar{y}_i - \bar{y}_{i'})$: The variance of the difference between $\bar{y}_i$ and $\bar{y}_{i'}$;

$$V(\bar{y}_i - \bar{y}_{i'}) = V(\bar{y}_i) + V(\bar{y}_{i'})$$

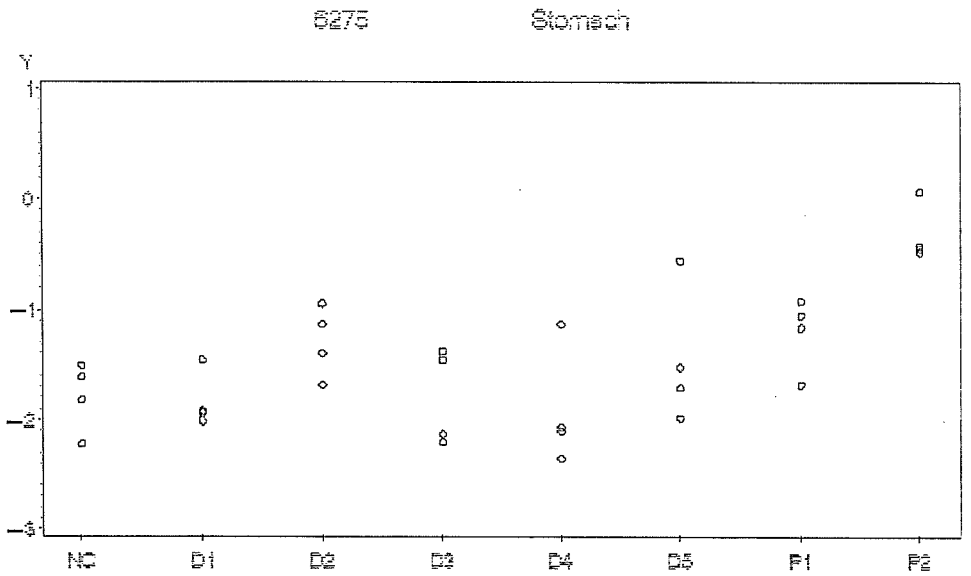
3. Test statistics

- Suppose η_i is the population mean of y_{ij} for i th group. We can construct a test for the null hypothesis $H_0 : \eta_i = \eta_{i'}$ using by;

$$\frac{\bar{y}_i - \bar{y}_{i'}}{\sqrt{V(\bar{y}_i - \bar{y}_{i'})}} \sim N(0,1)$$

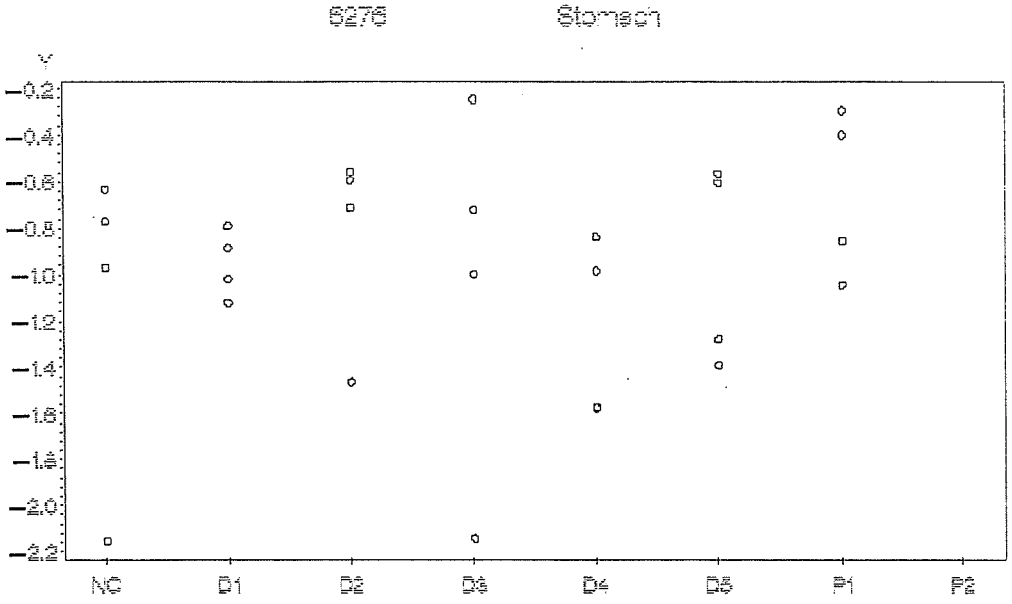
4. Examples

- Example 1



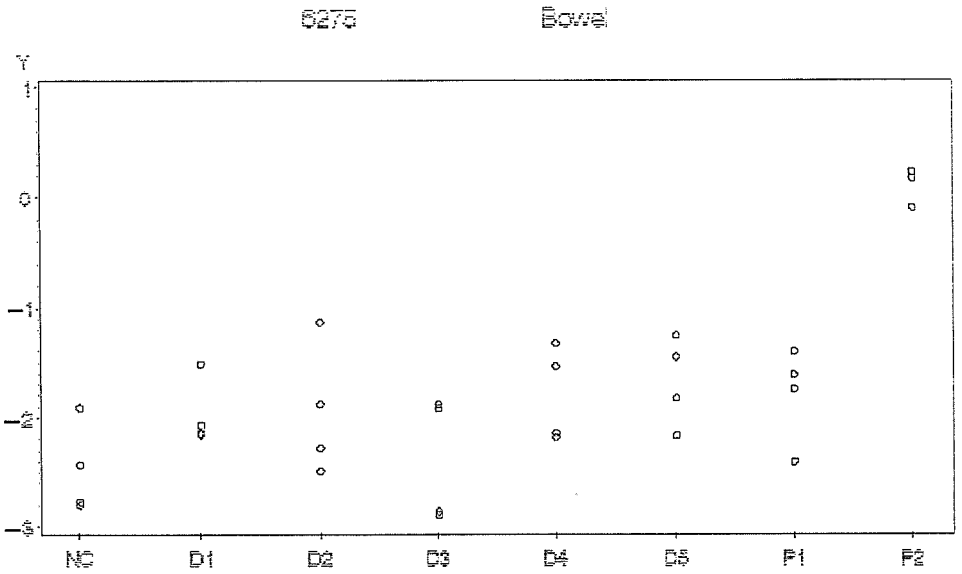
	p-value (one side)
NC-D1	0.709
NC-D2	<0.001
NC-D3	0.512
NC-D4	0.928
NC-D5	<0.001
NC-P1	<0.001
NC-P2	<0.001

- Example 2



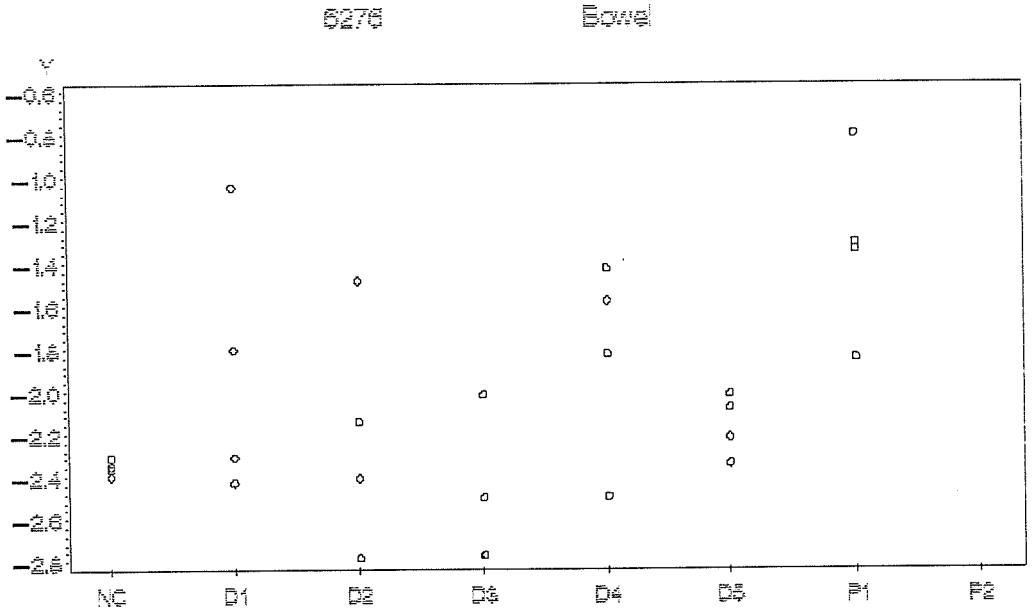
	p-value (one side)
NC-D1	0.003
NC-D2	<0.001
NC-D3	0.057
NC-D4	0.951
NC-D5	0.011
NC-P1	<0.001

- Example 3



	p-value (one side)
NC-D1	<0.001
NC-D2	<0.001
NC-D3	0.182
NC-D4	<0.001
NC-D5	<0.001
NC-P1	<0.001
NC-P2	<0.001

- Example 4



	p-value (one side)
NC-D1	<0.001
NC-D2	0.043
NC-D3	0.965
NC-D4	<0.001
NC-D5	0.011
NC-P1	<0.001

5. Further consideration

- We would like to know whether p-values in the examples are reasonable for experimenters.
- There may be better transformation than the logit transformation, for example the square root transformation.
- Though this method does not take the multiplicity into consideration, it will be possible to incorporate it in the method.

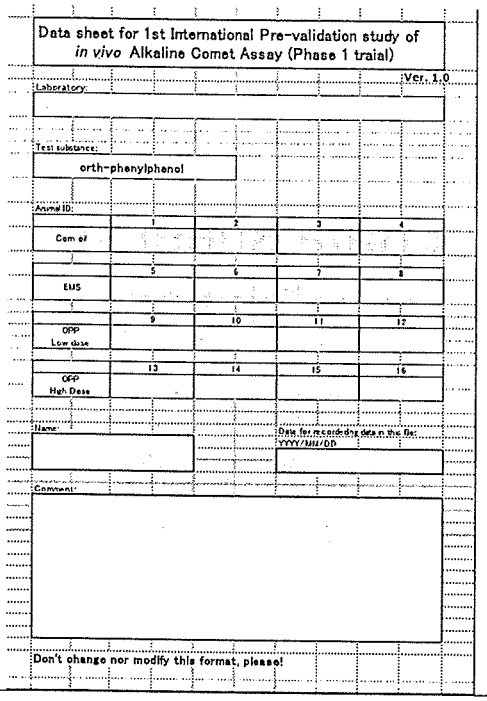
About data sheet ver.1.0 for our validation study

Takashi Omori

06 Aug 03

Introduction

- In the validation study, we have to keep reliable data from each facility
- To do so, we have developed data sheets by Microsoft Excel
- This slide explain the data sheet for our pre-validation study *in vivo* alkaline comet assay



Data sheet for 1st International Pre-validation study of *in vivo* Alkaline Comet Assay (Phase 1 traial)

Ver. 1.0

Laboratory:

Test substance:

orth-phenylphenol

Animal ID:

Cont	1	2	3	4
EMS	5	6	7	8
OPP Low dose	9	10	11	12
OPP High Dose	13	14	15	16

Name:

Date for recording data in the file: YYYY/MM/DD

Comment:

Don't change nor modify this format, please!

First of all

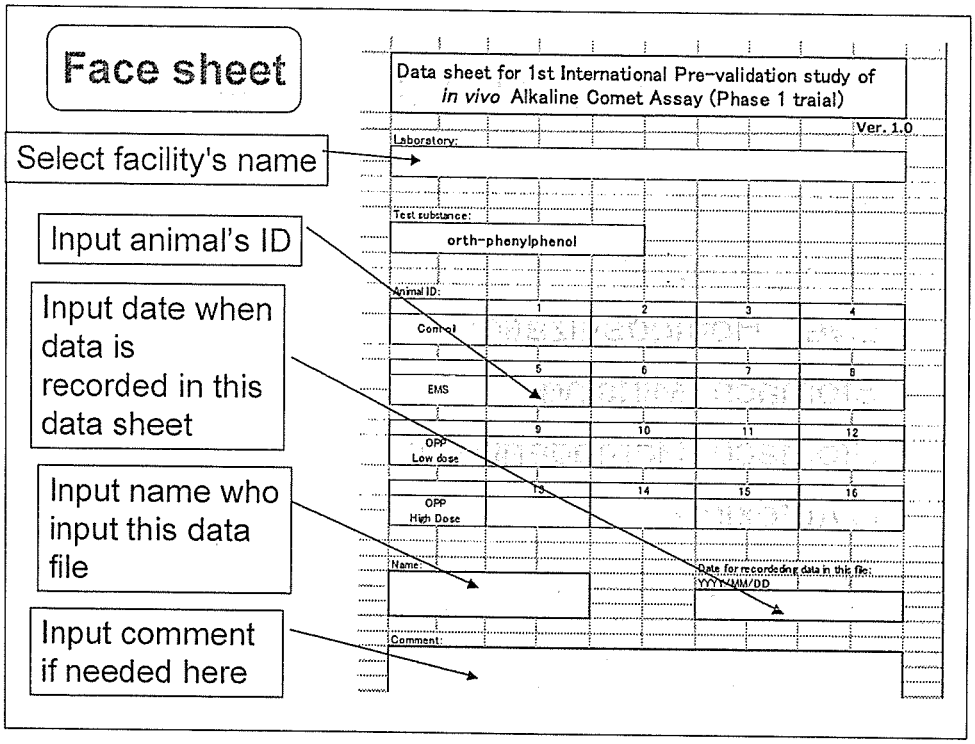
- Don't change and modify the format of this file, please!
  - Before data analysis, we will construct a data-base for this study
  - Data files from each facility will be automatically imported to data-base

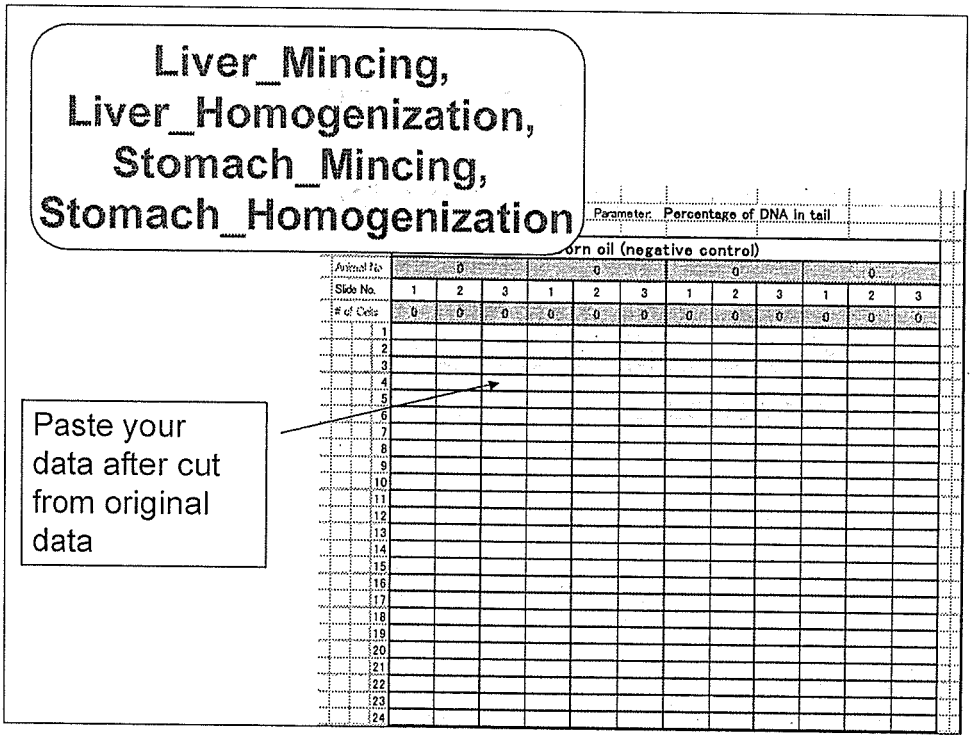
Contents of data sheet

- Face sheet
- Liver_Mincing
- Liver_Homogenization
- Stomach_Mincing
- Stomach_Homogenization
- Cytotoxicty
- Summary

Colors of cell

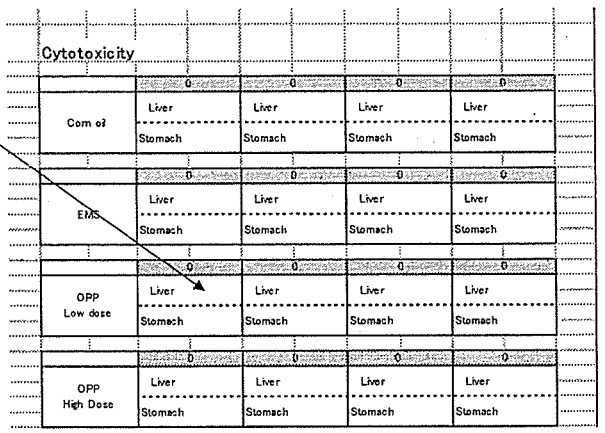
- There are 3 kind of colors for cell
  - Sky blue: Input or paste data or information
  - Yellow: Select from list
  - Pink: Inputted or selected data or information is referred


Face sheet
Select facility's name
Input animal's ID
Input date when data is recorded in this data sheet
Input name who input this data file
Input comment if needed here
Data sheet for 1st International Pre-validation study of
in vivo Alkaline Comet Assay (Phase 1 traial)
Ver. 1.0
Laboratory:
Test substance:
orth-phenylphenol
Animal ID:
Control
EMS
OPP Low dose
OPP High Dose
Name:
Date for recording data in this file:
YYYY/MM/DD
Comment:

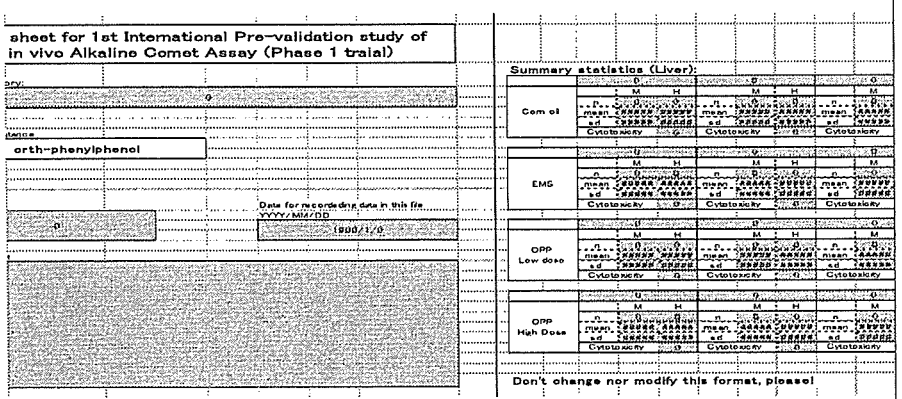

Liver_Mincing,
Liver_Homogenization,
Stomach_Mincing,
Stomach_Homogenization
Parameter: Percentage of DNA in tail
Paste your data after cut from original data

Cytotoxicity

Select the result of cytotoxicity



Summary



Finally, your data is summarized in this sheet.
You can check your data using this sheet.