Appendix 8.1

Version 4, October 30, 2014

Study plan for the validation trial on Vitrigel-EIT as alternative to eye irritation testing

Conducted by

Vitrigel-EIT Validation Management Team

INDEX

1. Background

Using scaffold made of collagen "Vitrigel", Vitrigel-EIT (Eye Irritation Test) is an important test method for evaluating the eye irritation toxicity of chemicals because of its technical simplicity, short-term test period.

The aim of this study is to (pre)validate the Vitrigel-EIT to assess transferability and between-laboratory variability, in order to incorporate this test for screening the eye irritation of chemicals in accordance with the Globally Harmonized System of Classification and Labelling of Chemicals：GHS） categories. The Vitrigel-EIT for multi-phase validation studies (validation trial) will be undertaken i) in accordance with the principles and criteria documented in the OECD No. 34 Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment [OECD, 2005], ii) according to the Modular Approach to validation [Hartung et al., 2004] ,iii) according to the concept discussed on validation trail with participation of GLP Test Facilities [Cooper-Hannan et al., 1999] where the whole concept of validation trial is described in the context of GLP.

The studies part of a validation trial should ideally be performed in accordance with GLP [OECD, 1998-2007; FDA, 1999; EPA, 1998a&b; JSQA, 2010; SCC, 2010]. As a minimum, but not necessary limited, use of standard operating procedures (SOP), adequate data recording, reporting and record keeping are essential.

A general conceptional framework [Hartung et al., 2004; OECD, 2005] will be used for documenting all the study to assess the validation status of a test method, called “modular approach” to validation. In this approach, the information needed to support the validity of the method is organized into modules that provide the following information:

Module 1: Test Definition

Module 2: Within-laboratory repeatability and reproducibility

Module 3: Between-laboratory transferability

Module 4: Between-laboratory reproducibility

Module 5: Predictive capacity

Module 6: Applicability domain

Module 7: Performance standards

The Modular approach as introduced by Hartung et al., allows using datasets from various data sources and studies. This advantage is used in the following proposal to assess the scientific validity of the Vitrigel-EIT.

2. Objective of the study

The validation trial will assess the reliability (reproducibility within and between laboratories) and relevance (predictive capacity) of the Vitrigel-EIT with a challenging set of test substances (test items) for which high quality *in vitro and in vivo* data are available. The *in vivo* data of chemicals should be used some types of GHS or EPA category classified by individual animal data.

3. Validation Management Team（VMT）

The VMT encompasses collective expertise with the test, in the underlying science and the scientific design, management and evaluation of a validation trial.

The VMT, which plays a central role overseeing the conduct of the validation trial, includes: Approval with date and signature of all protocols, study plans and study reports and amendments

Table 1. Members for Vitrigel-EIT Validation Management Team

Table 1. Members for Vitrigel-EIT Validation Management Team

Name	**Role and expertise**	**Affiliation**
Trial coordinator Hajime Kojima	Trial coordinator, Chemical management and Quality assurance	Japanese Center for the Validation of Alternative Methods (JaCVAM), National Institute of Health Sciences (NIHS)
Lead lab Toshiaki Takezawa Hiroyuki Yamaguchi	Developer of this assay and expertise underlying science	National Institute of Agrobiological Sciences
Tadashi Uchino	Quality assurance	NIHS
Takashi Sozu	Data analysis and biostatistics dossier	Kyoto Univ.
Liaison members		
ICCVAM liaison Nicole Kleinstreuer	Validation study expertise	NICEATM/ICCVAM, USA
ECVAM liaison Michael-Wilhelm SCHAEFFER	Validation study expertise	ECVAM, Italy
KoCVAM liaison Lim, Chae-Hyung	Validation study expertise	KoCVAM, Korea
Taiwan liaison Wannhsin Chen	Validation study expertise	Industrial Technology Research Institute, Taiwan

3.1 Participating Test Facilities

The laboratories participating in the study are defined as follow:

Test Facility 1: Mika Watanabe, Hatano Res. Inst., Food and Drug Safety Center

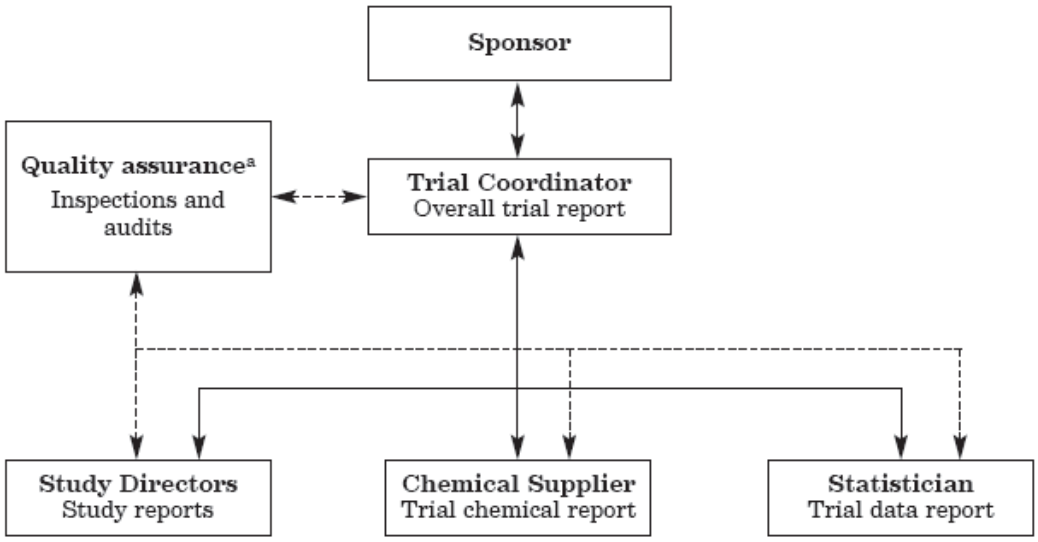
Test Facility 2: Takayuki Fukuda, BOZO RESEARCH CENTER.

Test Facility 3: Kunihiko Yamashita, Daicel Corporation

Information relevant for Modules 2, 3, 4 performed by all laboratories. Data obtained by these laboratories have demonstrated that the Vitrigel-EIT is transferable and reproducible between experienced laboratories. Two laboratories (facility 1 and 2) are GLP lab and third lab is non-GLP laboratory. Three facilities will be the laboratory participating in this validation trail acting as unexperienced laboratory to assess between laboratory transferability, reliability and relevance of the Vitrigel-EIT under non-GLP conditions (GLP spirit).

3.2 Management structure

The management structure of the validation trail is shown in **Figure 1**



[a]Several Quality Assurance units might be involved in a multi-study trial.
Dashed lines indicate assurance staff involvement.

Figure 1: Management Structure of the Vitrigel-EIT validation trial

1) Chemical management group

The members of chemical management group are elected by recommendation of the Vitrigel-EIT VMT. They prepare a tentative list of test chemicals and works with the VMT to make a final decision on the test chemicals to be used in the validation trial. The coded test chemicals listed are distributed by chemical supplier, JaCVAM.

2) Data analysis group

The members of data analysis group are elected by recommendation of the Vitrigel-EIT VMT, and check and analyze the data obtained in this validation trail from a third-party standpoint. They also take charge of statistical processing in this validation trail.

3) Quality assurance group

The members of quality assurance group are elected by recommendation of the Vitrigel-EIT VMT. They audit protocol, test chemical preparation record forms, blank data sheets, etc. and distributes them to the research laboratories participating in this validation trail. They also collects filled out forms and data sheets after completion of experiments, pointing out omissions or flaws in recording, if any, and requesting correction of such errors in the submitted documents from study directors.

4) Lead laboratory

The lead laboratory representing the test method is responsible for providing the test method protocol and the eventually necessary test chemical preparation record forms, blank data sheets, etc. The trail coordinator has to ensure that such data recording or calculation blank have been validated before distribution to the test facilities involved in the validation trail. The lead laboratory is also responsible for providing, if necessary, new versions of the protocols during the entire validation trail. The lead lab and the other participating test facilities might be contacted by the VMT for technical issues.

3.3 Sponsor

The Vitrigel-EIT study completing the validation trail for assessing the validity of Vitrigel-EIT will be financed by the Ministry of Agriculture, Forestry and Fisheries, Japan.

Management Office of Validation Trial (MOVT)

Toshiaki Takezawa, National Institute of Agrobiological Sciences

TEL:+81- 29-838-6294 e-mail: t.takezawa@affrc.go.jp

MOVT will support the Vitrigel-EIT validation trail by assuring that reliability is assessed. At the same time, preliminary results of the test method can be evaluated. For this purpose MOVT will support:

- the financial aspects related to the trial coordination of a validation trial (e.g. organization of VMT meetings where also the involved test facilities can be invited for technical clarifications to the VMT, the publication of the multi-side results)
- the test, reference and control item purchase, coding and distribution to the test facility
- the availability of the test systems to the participating laboratories by supporting the lead laboratory with the logistics for delivering the test system to the facility
- the independent data analysis and statistical support (biostatistician) based on the study reports generated
- the other costs for participating laboratories

3.4 Trial Coordination

Hajime Kojima was appointed as the trail coordinator with well-defined roles and responsibilities to coordinate the study and to establish a VMT by supporting of JaCVAM.

The name and location of the trail coordinator should be identified in each individual study plan. For the Vitrigel-EIT validation trial, the trail coordinator has direct access to the test item coding.

The trail coordinator's responsibilities include:

a) Establishment of/support to lead laboratory, including meeting organization

b) Communication and coordination with test Facilities

c) Recording of document and data flow between facilities

d) Assessing and documenting the impact of any amendments and/or deviations from study plans and/or protocol on the quality and integrity of the validation trail

e) Ensuring that the individual study reports are forwarded, in a timely manner, for data and statistical analysis

f) Preparing the study plan and report, which can be based on the study reports from the lead laboratories and other test facilities involved in the validation trial, and should reflect the overall

g) The communication of the results of the trail into the public domain

The role of trail coordinator (as the formal representative of the VMT and the single contact point with the study directors) is of fundamental importance. The trial coordinator is the single critical point of control and must ensure clear lines of communication between the involved test facilities in the trial. The communication line of the trail coordinator is with the study directors of the different test facilities. The study directors are the single point of contact with the trial coordinator (unless otherwise communicated by the participating Test Facilities) to assure a transparent and recorded documentation flow during the trail. The trail coordinator should also ensure that appropriate arrangements have been made for the supply of the test systems, and test, control and reference items, which meet the requirements of the trail, and that there are appropriate test method protocols (dated signature by the trial coordinator and the Lead Laboratories) and, if appropriate, validated data recording, data analysis, data reporting sheets

for the test method.

It is the responsibility of the VMT to approve the study plans send for approval by the test facilities, and any amendments to the study plan, by dated signature.

3.5 [Module 1]Test definition

The lead laboratory will be responsible for issuing a training agenda to the trail coordinator for further distribution to the all test facility giving details what training aspects will be covered during the training of the other Study Directors and Study Personnel at the lead laboratory. Furthermore, after the training, the lead laboratory will issue to the trail coordinator a training report and indicating if critical observations are made by the other test facilities regarding the Vitrigel-EIT method protocols. In case any critical observations are made a new version of the Vitrigel-EIT method protocols might necessary be issued to the other test facilities before initiating the between-laboratory transferability. For the transfer of Vitrigel-EIT method to the all test facility, the Phase 0 study using 5 non-coded chemicals was performed to know technical transfer among participant laboratories.

3.6 [Module 2] Within-laboratory reproducibility

The within-laboratory reproducibility of the all test facility has been done by an independent biostatistical analysis, under the JaCVAM. The proportion of concordance at each laboratory should be equal or more than 80 % as acceptance criteria.

3.7 [Module 3] Between-laboratory transferability

This between-laboratory transferability (Module 3, identical to ICCVAM proficiency testing phase) is performed in order to assess the successful transfer of the assay to a test facility

unexperienced with that particular test method but having knowledge of similar test systems and endpoint detection methods.

Each test item will be tested in triplicate in 3 independent runs to know within-lab reproducibility according to the Vitrigel-EIT method protocol describing the details of the experimental design (Phase I). The 10 test items selected for the phase I study are tested. The all facility will prepare a study according to internal GLP spirit. This plan will be submitted to the trail coordinator and lead laboratory for approval.

The results of the within-laboratory reproducibility and transferability will be reviewed before progressing with module 4 on the between laboratory reproducibility. If the transferability data do not meet test acceptance criteria, the trial coordinator representing the VMT will try to identify the problems and make corrections where needed after discussion with VMT. At the end of the testing, the test facilities will submit a QC certified copy of whole study dossier to the trail coordinator (study plan in GLP spirit, raw data, records and data analysis, study report in GLP spirit).

3.8 [Module 4] Between-laboratory reproducibility

Thirty coded test items have been selected to confirm the between-laboratory reproducibility in the phase II study.

At the end of the testing, the test facilities will submit a QC certified copy of whole study dossier to the trial coordinator (study plan in GLP spirit, raw data, records and data analysis, study report in GLP spirit). The proportion of concordance between-laboratory reproducibility should be equal or more than 80% as acceptance criteria.

3.9 [Module 5] Predictive capacity

The predictive capacity has been assessed using forty coded test chemicals in the phase I & II study. Depending on the statistical analysis the preliminary design for validation as well as the automatisation of the test leading to an increased dataset will be considered.

3.10 [Module 6] Applicability domain

The necessity for Applicability domain will be subject to a VMT decision once the data of the between laboratory reproducibility has been assessed.

3.11 [Module 7] Performance standards

The necessity for performance standard will be subject to a VMT decision once the data of the between laboratory reproducibility has been assessed.

4 Protocol

In this validation trail, the protocol (ver. 1.51e) will be used (attached Document #A). This protocol will make up a draft by the lead laboratory and be finalized by VMT.

5 Chemicals

5.1 Chemicals Selection

Test chemicals have been selected by chemical repository based on published papers on in vivo eye irritation test and validation studies for in vitro alternative assays on eye irritation test in accordance with GHS or EPA categories.

The applied selection criteria were:

- Some types of GHS or EPA categories
- information on mode/site of action

- coverage of range of relevant chemical classes and product classes quality and quantity of reference data (*in vivo* and *in vitro*)
- high quality data derived from animals and (if available) also humans
- knowledge on interspecies variations (for example: variability with regard to the uptake of chemicals, metabolism, etc.)
- coverage of range of toxic effects/potencies
- chemicals that do not need metabolic activation
- appropriate negative and positive controls
- physical and chemical properties (feasibility of use in the experimental set-up as defined by the CAS No.)
- single chemical entities or formulations of known high purity
- availability
- costs

In the first phase of the selection procedure, the Chemical Management Group identified and collected several existing lists of potential chemical sensitizing in order to establish a primary database. These chemicals had originally been compiled by international experts for various purposes e.g. as reference compounds for validation studies. An extensive literature research was performed by the Chemical Management Group in order to insure that the preselected chemical fulfilled the selection criteria described above.

Emphasis was laid on the fact that different potencies (strong, weak and no activity) have been chosen. In addition, it was decided that at least 20% of the total substances to be tested should be negative in order to increase the statistical power of the data analysis.

In the first phase Vitrigel-EIT validation study with data generation at the test facilities, 10

chemicals will be tested three times in each test chemical for within-laboratory reproducibility and confirmation of between-lab transferability. After discussion of Phase I & II results, detailed test planning of the Phase III will be determined. At this moment, 36 chemicals will be planned in the phase III for between-lab reproducibility and predictive capacity (Table 2).

Table 2. Outline of test planning in each validation stages.

Stage	Labs	Chemicals		Information obtained
Phase 0	3	5 non-coded	→	- Confirmation of technical transferability by 3 trials in each laboratory
Phase I	3	10 coded	→	- Between-lab transferability & reproducibility, within-lab reproducibility by 3 trials in each laboratory
Phase II	3	10 coded	→	- Between-lab reproducibility by 1 trials in each laboratory
Phase III	3	36 coded	→	- Between-lab reproducibility and predictivity by 1 trial in each laboratory

(Planning of Phase II will be determined after discussion of the results of Phase I)

5.2 Chemicals Acquisition, Coding and Distribution

The assessment of within-laboratory reproducibility (Module 2), between laboratory transferability (Module 3) and between laboratory reproducibility (Module 4) in the all test facilities have been performed with coded chemicals. This Vitrigel-EIT study plans describes the generation of the missing data sets under coded test item. If the results obtained are not very similar to the previous obtained sets, the VMT has to assess if coded chemicals need to be tested in the all test facilities.

The coding will be supervised by the trail coordinator, in collaboration with the chemical repository responsible of coding and distribution of test, reference and control items for the validation trial.

5.3 Handling

Each test facility shall receive through the trail coordinator essential information about the test chemicals (physical state, weight or volume of sample, specific density for liquid test chemicals, and storage instructions). Moreover, the Test Facility Manager should receive the safety information concerning the hazards identification and exposure controls/personal protection.

6 Records and archiving

At the end of the trail, the Vitrigel-EIT validation reports are prepared by the trail coordinator or the VMT personnel who appointed by the trail coordinator. The report summarizes the goals, procedures, results and conclusions of a validation trial. This represents the whole multi-phase validation studies, including archiving and, as such, will cover several study reports, as well as reports for test item supply, data management and statistics. The trail coordinator oversees the preparation of the study report. The trail coordinator will be representing the VMT discussions responsible for preparation of the scientific conclusions. Signatories to the report include the trail coordinator, the statistician, and the Test Facility Managers and Study Directors of the involved test facilities. Although the Study Directors may not be involved with the preparation of the report, their signatures confirm that the report is an accurate reflection of the management and study events. The report should contain a statement, signed by

the trail coordinator, commenting on the accuracy and completeness of the report and identifying any significant issues which could have affected the integrity of the , including matters of GLP compliance. A QA statement will be included in the report, in order to identify what QA monitoring was done and to confirm whether or not the report is an accurate reflection of the multi-study data.

7 Study timeline

An approximate schedule for Vitrigel-EIT validation trail is shown in Table 3.

Duration of this validation trail is around fifteen -month from September 2013 to February 2015.

Table 3. Schedule of Vitrigel-EIT validation trail

Month	Activity
Feb/2013-May/ 2013	Establish the VMT, selection of participating laboratories, deliberation, decision and read-through of draft study plan, deliberation and decision of study protocol
May/ 2013	Lecture & training for technical transfer to participants at NIHS (Tokyo)
June-August/2013	Pre-validation study (Phase 0) to confirm technical transfer from lead laboratory to participating laboratories using 5 non-coded chemicals
September-2013	The test data will be managed by Sozu
October/2-3, 2013	**1st VMT meeting** (kick off): Confirmation of test schedule, protocol, No. of test chemicals *etc.and* evaluation of the outcome of Phase-0 and planning of Phase-I study
October-December/2013	**Re-test of phase -0 study using the revised protocol**
March-April/ 2014	**PhaseI study** to know between-lab transferability & reproducibility, and within-lab. reproducibility using 10 chemicals, 3 tests are conducted for each chemical independently
May/2014	The test data will be analyzed by Sozu
June/2014	**2nd VMT meeting:** evaluation of the outcome of PhaseI and planning of Phase-II study
July-September/ 2014	**PhaseII study** using 10 chemicals, 1 test, to know between-laboratory reproducibility
September/2014	The test data will be analyzed by Sozu and mail meeting: evaluation of the outcome of PhaseII study
November-January/ 2014	**Phase-III study** using 10 chemicals, 1 test, to know between-laboratory reproducibility
January/2014	**3rd VMT meeting:** evaluation of the outcome of PhaseIII study
August/2015	The validation report finalized.

Abbreviations

CAS: Chemical Abstracts Service
GLP: Good Laboratory Practice
JaCVAM: Japanese Centre for the Validation of Alternative Methods
NIHS: National Institute of Health Sciences
OECD: Organization for Economic Co-operation and Development
QC: Quality Control
TG: Test Guideline
VMT: Validation Management Team
MOVT: Management Office of Validation Trial

Annex1

The Schedule of Vitrigel-EIT validation trail was revised in the **2nd VMT meeting, Tokyo, on June 4, 2014.**

The Phase-IIa study will be conducted with the protocol ver.1.61e.

Purpose: Re-evaluation of between-laboratory reproducibility using the new 10 coded chemicals with the revised protocol on Vitrigel-EIT. In case where the proportion of between-laboratory reproducibility is equal or more than 80%, the phase III study will be conducted.

June/2014	**2nd VMT meeting:** evaluation of the outcome of Phase-I and planning of Phase-II and III study
July-August/ 2014	**Phase-IIa study** using 10 chemicals, 1 test, to know between-laboratory reproducibility.
September/2014	The test data will be analyzed by Sozu
October/2014	The International teleconference will be held.
November-December/2014	**Phase-III study** using 30 chemicals, 1 test, to know between-laboratory reproducibility and predictive capacity
January/2015	The test data will be analyzed by Sozu
February/2015	**3rd VMT meeting:** evaluation of the outcome of Phase-III study
2015	The validation report will be finalized.

Annex2

The Phase-III study will be conducted with the protocol ver.1.71 e.

Purpose: Re-evaluation of between-laboratory reproducibility and predictivity using the 36 coded chemicals with the revised protocol on Vitrigel-EIT. In case where the proportion of between-laboratory reproducibility is equal or more than 80%, the phase III study will be conducted.

November-December/2014	**Phase-III study** using 36 chemicals, 1 test, to know between-laboratory reproducibility and predictive capacity
January/2015	The test data will be analyzed by Sozu
February/2015	**3rd VMT meeting:** evaluation of the outcome of Phase-III study
2015	The validation report will be finalized.

Appendix8.2

Standard Protocol for the Vitrigel-EIT method

Hiroyuki Yamaguchi[1, 2] and Toshiaki Takezawa[1] Version 1.80e April 2, 2015

[1]Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, [2]Kanto Chemical Co., Inc.

1. Principle of the method

The Vitrigel-EIT method is a highly sensitive means of testing for eye irritation potency by analyzing a series of transepithelial electrical resistance (TEER) measurements taken over time after exposure to a test chemical. A typical protocol for this test method is as follows.

2. Applicability domain

The following substances are not suitable for testing with this method and are excluded from the applicability domain.

1. Chemicals that have a pH level of 5 or less in solution (see section 5.5.3, step 3).
2. Solids that have both a logP value of at least 2.5 and a density of either less than 0.95 g/cm^3 or more than 1.10 g/cm^3.

3. Materials to be procured by the user

- HCE-T cells (Cell Bank, Riken BioResource Center, RCB2280)
- Clean bench or biological safety cabinet
- CO_2 incubator (37°C, 5% CO_2 in air)
- Phase-contrast microscope
- Water bath
- Centrifuge (700 × g or greater)
- Safety pipette filler
- Micropipettes (100 µL, 1000 µL)
- Tweezers
- 70% Ethanol
- Balance
- Computer (Windows OS with USB port)
- Liquid nitrogen freezer (for cell storage)
- Universal pH test paper (ADVANTEC, 07011030)

4. Vitrigel-EIT kit contents (for 72 tests)

*Other equivalent reagents or materials are acceptable.

Name	Maker	Item code	Quantity	Store at
ad-MED Vitrigel™ comprise 12 collagen vitrigel membrane (CVM) chambers set in a 12-well plate 	Kanto	08360-96	6 plates	r.t.[1]
Culture medium (prepared by Kanto) Composition D-MEM/F-12 5% fetal bovine serum, 5 μg/ml human insulin, 10 ng/ml human epidermal growth factor, 2.5 % dimethyl sulfoxide, 100 U/ml penicillin, and 100 μg/ml streptomycin	 LT[2] Sigma LT LT Sigma LT	 11330-032 F2442 12585-014 PHG0311 D2650 15140-148	1 bottle	2–8°C
0.05% trypsin-0.02% EDTA-2 Na solution	LT	25300-054	10 bottles	−20°C or lower
PBS	Sigma	D8537	1 bottle	2–8°C
0.4% trypan blue solution	LT	15250-061	1 vial	r.t.
Negative control reagent (saline) (prepared by Kanto)			1 vial	2–8°C
Positive control reagent (benzalkonium chloride)	Sigma	B6294	1 vial	r.t.
Reference control reagent (99.5% ethanol)	Kanto	14033-00	1 vial	r.t.
Dimethyl sulfoxide	Sigma	D2650	1 vial	r.t.
TEER recorder (prototype)	Kanto		1 set	r.t.
Tissue culture flask (T-75)	BD Falcon	353024	3 packages	r.t.
5ml Pipette	BD Falcon	357529	1 package	r.t.
15ml Plastic tube	BD Falcon	352096	1 package	r.t.
1.5ml Plastic tube	As one	1-7521-01		
Hemocytometer	TGK	OC-C-S02	1 package	r.t.
Chambers without membrane and 12-well plate for TEER recorder check	Kanto		1 set	r.t.

NB1: room temperature, NB2: Life Technologies

5. Procedure

5.1 Thawing and initial culturing of HCE-T cells

1. Warm culture medium in a water bath at 37°C.
2. Pour 14 ml of the culture medium into a T-75 tissue culture flask and spread it over the bottom surface.
3. Transfer a cryotube of human corneal epithelium (HCE) T cells from the liquid nitrogen freezer to the water bath and agitate gently until completely thawed.
4. Open the cryotube, make sure any sunken HCE-T cells are suspended in the medium, then add the total cell suspension to the culture medium in the T-75 flask, and mix until uniform. Then, transfer the flask to the CO_2 incubator (37°C, 5% CO_2 in air) to start culturing the HCE-T cells.
5. After culturing the cells for at least 2 but no more than 6 hours, observe the morphology of the cells under a phase-contrast microscope. If most of the cells are well attached, as shown in Fig. 1 (a few non-attached cells can be ignored), replace the culture medium with a fresh one and continue the culturing. If not well attached, dispose of the failed cell culture and repeat steps 1 to 4 using a different cryotube of HCE-T cells.

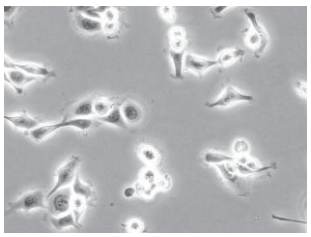


Fig. 1 Phase-contrast microphotograph of HCE-T cells cultured for two hours.

6. After culturing the cells for a few days, observe the morphology of the cells under a phase-contrast microscope. If the cells have proliferated into a monolayer of between 80 and 100% confluency, as shown in Fig. 2, proceed to section 5.2 to passage the cells as described. If not, dispose of the failed culture and repeat steps 1 to 5 using a different cryotube of HCE-T cells.

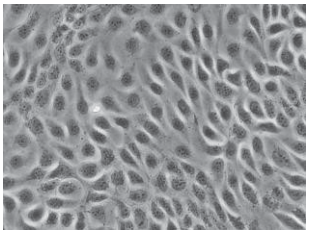


Fig. 2 Phase-contrast microphotograph of HCE-T cells in a min. 80% confluent monolayer appropriate for passaging.

5.2 Passaging HCE-T cells

1. Prepare four new T-75 flasks with 14 ml of fresh culture medium in each flask.
2. Warm the culture medium bottles, PBS, and trypsin solution in a water bath at 37°C.
3. Remove the culture medium from the flask of HCE-T cells that have proliferated into a min. 80% confluent monolayer. Pour 10 ml of PBS into the flask and spread it over the bottom surface to rinse the cells.
4. Remove the PBS from the flask. Pour 5 ml of trypsin solution into the flask, spread it over the bottom surface, and the remove 4.3 ml of the solution. Incubate the flask in a CO_2 incubator for five minutes. Surplus trypsin solution can be stored in a freezer and reused.
5. Tap the bottom and side walls of the flask several times to detach the cells from the

bottom surface, as shown in Fig. 3.

6. Pour 4 ml of fresh culture medium into the flask and suspend the cells by pipetting as quickly as possible.
7. Add 1.0 to 1.2 ml of the cell suspension to the culture medium in each of the four new flasks described in step 1 and mix until uniform. Next, place the flasks in a CO_2 incubator to start passaging HCE-T cells in the four new flasks. Thereafter, change the culture medium every other day.

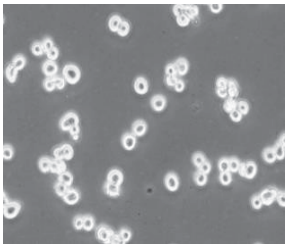


Fig. 3 Phase-contrast microphotograph of HCE-T cells after trypsinization

8. Allow the subculture to proliferate in as many or as few flasks as needed (ordinarily, two to eight).

5.3 Cryopreservation of HCE-T cells

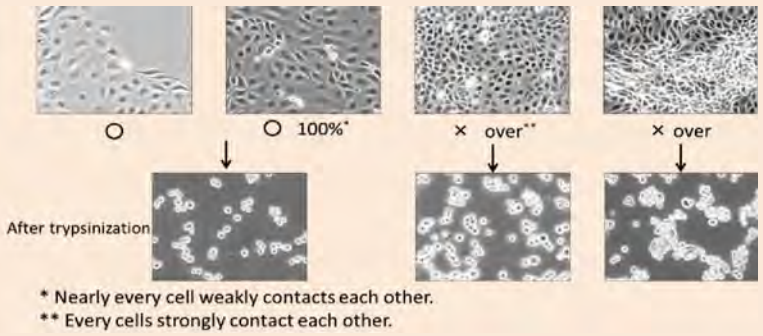
Prepare eight cryotubes of HCE-T cells from the four T-75 flasks prepared in section 5.2 by transferring half of the cells in each flask to a cryotube, as described below.

1. Prepare a culture medium with 20% DMSO by adding 0.8 ml of DMSO to 3.2 ml of culture medium and mixing until uniform.
2. Follow steps 2 to 6 of the procedure described in section 5.2 to create four flasks, in which HCE-T cells have proliferated into a monolayer of between 80 and 100% confluency.
3. Collect the cell suspension in a 50 ml centrifuge tube and spin at 700 × g for three minutes at room temperature.
4. Remove the supernatant and tap the tube several times to loosen the multicellular pellet.
5. Suspend the cells in 4 ml of a fresh culture medium.
6. Add 4 ml of the culture medium with 20% DMSO to the cell suspension and gently mix by pipetting. Quickly dispense 1 ml of the cell suspension to each cryotube.
7. Place the cryotubes in a programmable freezer (or equivalent) and cool at a rate of -1°C/minute to a temperature of -80°C. Preserve the cryotubes in a liquid nitrogen freezer.

5.4 Preparation of Vitrigel-HCE models

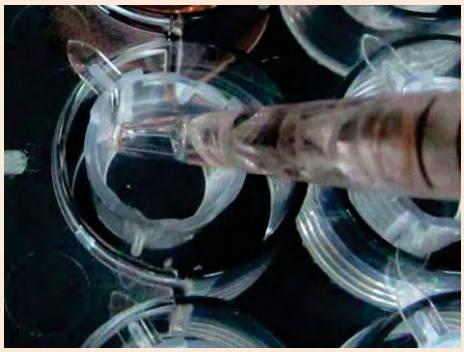
1. Follow the procedure described in section 5.1. to thaw HCE-T cells preserved in the cryotubes.
2. Initiate culturing and allow the HCE-T cells to proliferate into a monolayer of between 50 and 100% confluency. About 80 models can be prepared from a single T-75 flask in which the cells have proliferated into a confluent monolayer.

Caution:
Do not use HCE-T cells in an over-confluent state, because cell-to-cell aggregates are difficult to separate.



3. Follow steps 2 to 6 of the procedure described in section 5.2.
4. Observe the morphology of the cells under a phase-contrast microscope. Proceed to step 5 only if the culture comprises well-separated, individual cells. If cellular aggregates comprising six cells or more are observed over more than 20% of the surface area, dispose of the failed cell culture and return to step 1 of this section.
5. Transfer the cell suspension to a 50-ml tube. Resuspend the cells uniformly and then immediately dispense 100 µL of the cell suspension into a 1.5-ml tube.
6. Add 100 µL of trypan blue solution to the cell suspension and mix well by pipetting. Count the numbers of living and dead cells by using a hemocytometer and calculate the survival rate and density of the cell suspension. If the cell survival rate is lower than 95%, dispose of the failed cell culture and return to step 1 of this section.

Caution:
Do not push a collagen vitrigel membrane with the pipette tip. Use the guide on the side wall of the chamber to fix the pipette tip temporarily.



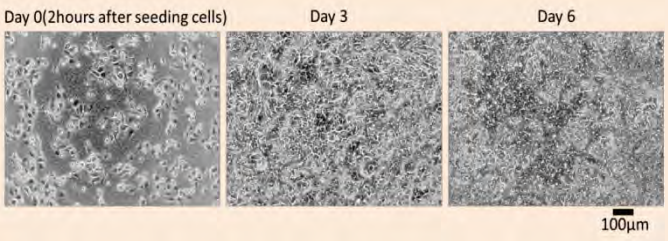
7. For each collagen vitrigel membrane chamber pre-set in a well of a 12-well plate, first pour 1.5 ml of culture medium into the well on the outside the chamber and then pour 0.5 ml of culture medium directly into the chamber itself. Let stand at room temperature for at least 10 minutes but no more than 2 hours.
8. Remove the culture medium from the inside of each collagen vitrigel membrane chamber and pour 0.5 ml of the cell suspension at a density of 1.2×10^5 cells/ml. Transfer the plate

to a CO_2 incubator and culture for two days.

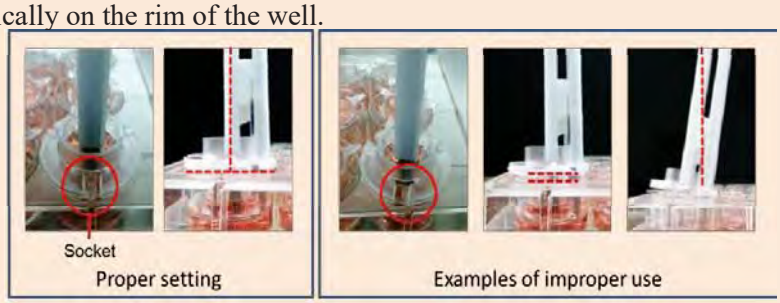
9. Change the culture medium in the cell outside the chamber and remove the culture medium from inside the chamber to start the air–liquid interface culture, then culture for four days in a CO_2 incubator.
10. Change the culture medium in the cell outside the chamber on the fifth day after seeding.
11. On the sixth day after seeding, pour 0.5 ml of culture medium directly into the inside of the chamber. Adjust the temperature of the model to 28±2°C. Set the electrode to the TEER recorder on the chamber. Measure an initial TEER value for each HCE model before exposure to a test chemical and record the measured values on the data sheet.
12. HCE models with an initial TEER value of 140–220 $\Omega \cdot cm^2$ are acceptable for use in testing. HCE models that pass the above acceptance criterion should be used in a chemical exposure test within the same day.

Caution:
Although no technique for measuring temperature is specified, we recommend pouring culture medium into an empty well of the 12-well plate before adjusting the temperature for all models, then measuring the temperature of the medium inside the wells. If there is no empty well available, use an empty well on a new 12-well plate.

Phase-contrast microphotographs of HCE-T cells cultured in a CVM chamber. It is important to seed cells uniformly. The cells gradually form multilayers.



Set the electrode to the TEER recorder in the chamber by fixing the three chamber arms in the three sockets of the adaptor. The adapter of electrode should stand vertically on the rim of the well.



Caution:
Do not leave a model outside a CO_2 incubator without culture medium in a chamber. Do not leave a model outside a CO_2 incubator over 2 hours.

5.5 Performing tests using the Vitrigel-EIT method

5.5.1 Checking the TEER recorder

1. Pour 3 ml of a 0.45% saline solution at 25±5°C in one well of a 12-well plate and insert a chamber without membrane in the well.
2. Pour 3 ml of a 0.9% saline solution at 25±5°C in one well of 12-well plate and insert a chamber without membrane in the well.
3. Measure the TEER value in both wells. If the TEER recorder is functioning normally, the measured values will satisfy the following conditions:

   (TEER value of 0.45% saline) − (TEER value of 0.9% saline) ≥ 60 Ω·cm^2

5.5.2 Testing positive, negative, and reference control reagents

1. Equilibrate the culture medium to an ambient temperature of between 22 and 30°C.
2. The Vitrigel-EIT method uses a saline solution as negative control, a benzalkonium-chloride solution as positive control, and an ethanol solution as reference control. Prepare each in a 2.5% (w/v) reagent solution by adding 0.1–0.2 g of sodium chloride, benzalkonium chloride, and ethanol to an appropriate volume of culture medium in a 15-ml tube and mixing until uniform. As long as the proper concentration is maintained, the total quantity of the prepared reagent is unimportant.

   Caution:
   To minimize volatilization, 15-ml tubes should be sealed tightly when not in use.

3. Adjust the temperature of the reagent solution to 28±2°C.
4. Adjust the temperature of a model to 28±2°C.

   Caution:
   Measure temperature by immersing a thermometer in the reagent solution or culture medium.

5. Remove the culture medium from inside the chamber and set the electrode to the TEER recorder on the chamber.

   Caution:
   Add the control reagent within 5 minutes of removing the culture medium from inside the chamber.

6. Bearing in mind the importance of performing the test within the required timeframe, quickly pour 500 µL of the control reagent into the inside of a chamber (within 2-seconds time) and click the Start icon on the measurement software within 2 to 5 seconds of exposure to the control reagent. TEER values are automatically recorded every 10 seconds for 3 minutes thereafter. If the differential between the initial TEER value and the TEER

value at 0 seconds is more than ±40 Ω·cm^2, reject the results and retest using a different HCE model.

7. Input the measured TEER values to the data sheet (software), as described in section 5.5.4.
8. Once the measured TEER values are input, the data is automatically analyzed to determine whether or not the success criteria have been satisfied as show below. If the data is marked Pass on the data sheet, proceed to the next test. If the data is marked NG on the data sheet, redo the test starting with preparation of a Vitrigel-HCE model, as described in section 5.4.

Success criteria

Negative control: The plateau level is 5% or less of the TEER value at 0 seconds.

Positive control: The plateau level is 40% or more of the TEER value at 0 seconds.

Reference control: The plateau level is 10% or more of the TEER value at 0 seconds.

9. Rinse the electrode with pure water, and wipe it with an absorbent paper towel after every test.

5.5.3 Testing test chemicals

1. Equilibrate the culture medium to an ambient temperature of between 22 and 30°C.
2. Prepare a 2.5% (w/v) test chemical solution by adding 0.1–0.2 g of each test chemical to an appropriate volume of culture medium in a 15-ml tube. As long as the proper concentration is maintained, the total quantity of the prepared reagent is unimportant. Mix manually until the test chemical dissolves or for a maximum of one minute. If the test chemical does not dissolve readily, try using the following techniques in the following order to dissolve it: a) mix mechanically for a maximum of one minute using a vortex mixer, b) sonication for a maximum of 20 minutes, or c) heating to a maximum temperature of 70°C. After trying each technique, adjust the temperature of each test chemical solution to 28±2°C and check solubility. Move to the next step of the procedure once the test chemical solution is well dissolved or homogeneously dispersed.

> **Caution:**
> To minimize volatilization, the 15-ml tubes should be tightly sealed when not in use. The parameters (time, temperature, intensity, etc.) for dissolving test chemicals may be adjusted to accommodate the physiochemical properties of each test chemical.

3. Measure the pH level of the test chemical solution using universal pH test paper.
4. Adjust the temperature of the test chemical solution to 28±2°C.
5. Adjust the temperature of a model to 28±2°C.

> **Caution:**
> Measure temperature by immersing a thermometer in the test chemical or culture medium.

6. Remove the culture medium from inside the chamber and set the electrode to the TEER

recorder on the chamber.

7. Bearing in mind the importance of performing the test within the required timeframe, quickly pour 500 µL of the test chemical solution into the inside of a chamber (within 2-seconds time) and click the Start icon on the measurement software within 2 to 5 seconds of exposure to the test chemical solution. TEER values are automatically recorded every 10 seconds for 3 minutes thereafter. If the differential between the initial TEER value and the TEER value at 0 second is more than 40 $\Omega \cdot cm^2$, reject the results and retest using a different HCE
model.

Caution:
For test chemicals that are insoluble, a homogeneous suspension of the test chemical may be prepared immediately before testing.

8. Input the measured TEER values to the data sheet (software), as described in step 5.5.4.
9. Rinse the electrode with pure water or 70% ethanol, and wipe it with an absorbent paper towel after every test.

5.5.4 Automatic analysis system utilizing a data sheet

1. Three individual tests are performed for each test chemical, and the measured TEER values are input to software data sheet running on PC automatically analyze the data. The mean TEER values for the three tests are plotted on a timeline to create a profile of TEER values (dP/dT), which are analyzed for three parameters: time lag (t_1), intensity ($-[P_2 - P_1]/[t_2 - t_1]$), and plateau level ($100 - P_2$). Time lag (t_1) is defined as the maximum time a profile was maintained at $0 \geq dP/dT > -0.03\%$/second. The starting time of plateau level (t_2) after the profile was maintained at ($dP/dT \leq -0.03\%$/second) for a particular period of time was defined as the initial time at which the profile was maintained at $0 \geq dP\ (P_3 - P_2)/dT\ (t_3 - t_2) > -0.03\%$/seconds. The time ($t_3$) is represented in the equation ($t_3 = t_2 + 30$ seconds) because the plateau level was evaluated by the profile for 30 seconds. P_1, P_2, and P_3 are the percentages against the initial TEER value at t_1, t_2, and t_3 after exposure to the test chemical, as shown in Fig. 4.

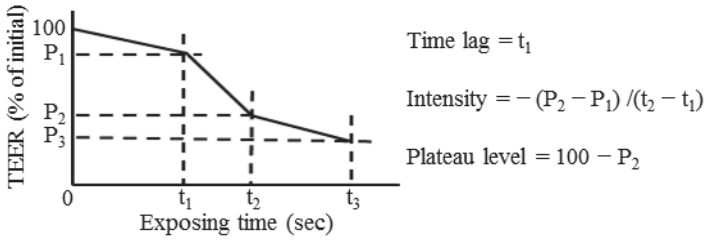


Fig. 4 Schematic analysis of a TEER profile after exposure to a test chemical
Here, t_1 and t_2 represent time lag and starting time of plateau level, respectively. t_3 is defined as $t_3 = t_2 + 30$ seconds. P_1, P_2, and P_3 are the percentages against the initial TEER value at times t_1, t_2, and t_3, respectively.

2. Eye irritation potency was predicted using the following criteria.

Criteria	Result
Time lag ≤ 180 or Intensity ≥ 0.05 or Plateau level > 5.0	Irritant (I)
Time lag > 180 and Intensity < 0.05 and Plateau level ≤ 5.0	Non-irritant (NI)

Revision history

Version 1.60e

Date: June 30, 2014 Description of change

4.411, Procedure 4.5.2., step 1, 3 and Procedure 4.5.3., step1, 3 Replaced the word “room temperature” to “ambient temperature for the experiment” Added an attention about the ambient temperature at procedure 4.4., step 11

Reason

Control of the ambient temperature is important for success the experiment.

Procedure 4.5.2., step 1, 4.5.3., step 1

Change the ambient temperature for a TEER test as follows.

between 18 and 30

↓

between 22 and 30

Reason

Temperature of a HCE model affects values of time dependent TEER change.

Procedure 4.5.2., step 7

Included the reference control as acceptance criteria, and changed the acceptance criterion as follows. Plateau level is between 10% and 30%, inclusive.

↓

Plateau level is between 10% and 40%, inclusive. Reason

At the 2nd VMT meeting, all members agreed to should be included the reference control as the acceptance criteria for quality preservative of the model.

Expand the acceptance criteria on ground of the test results by the read laboratory.

Procedure 4.5.3., step 2, 5th sentence

Changed the description of the procedure for propagating a test chemical solution as follows.

Old:

If the test chemical has not been dissolved, try to dissolve it by the mechanical mixture for a maximum 1-minute period using a voltex, by the sonication for a maximum

20-minute period, or by the heating to 70

New:

If the test chemical has not been dissolved, try to dissolve it by selecting an appropriate technique(s) from the following; mechanical mixture for a maximum 1-minute period

using a vortex mixer, sonication for a maximum 20-minute period, or heating to maximum 70
Reason
At the phase 1 study, some members misunderstood the procedure and they thought that all of three techniques should be done.Replaced the word “voltex” to “vortex mixer” (corrected a typo).

Procedure 4.5.2., step 2 and 4.5.3., step 2
Added an attention “To avoid volatilizing chemicals, the 15ml tube should be tightly lidded after weighing a test chemical to the tube except for adding a culture medium and sampling the 2.5% test chemical solution.”.
Reason
Clarify the attention that the tube should be tightly lidded during the experiment to avoid volatilizing chemicals.

Procedure 4.5.2., step 5 and 4.5.3., step 5
Added a sentence “In case the increasing and decreasing of 40 Ω・cm2 and more is occurred between the initial TEER value and the TEER value at 0 second, reject the experiment and retest using another HCE model.”
Reason
In case the TEER value at 0 second evidently differs from the initial TEER value, it indicates some technical failure in the experiment (e.g., contamination of electrical nose, improper use of electrode).

Version 1.70e
Date: October 15, 2014 Description of change

Procedure 4.4., step 11, Procedure 4.5.2., step 4 and Procedure 4.5.3., step 4
Replaced the word “Let stand for 10 minutes (within 2 hours) at the ambient temperature for the experiment.” to “Adjust the temperature of the model to 282 Added an attention about the way of measuring temperature at procedure 4.4., step 11
Reason
Temperature control of a model is important for success the experiment.

Procedure 4.5.2., step 1 and Procedure 4.5.3., step 1
Replaced the word “ambient temperature for the experiment” to “between 22 to 30
Remove attentions at 4.5.2., step 1 and Procedure 4.5.3., step 1

Reason
Remove the word “ambient temperature”.

Procedure 4.5.2., step 3 and Procedure 4.5.3., step 3
Replaced the word “with the ambient temperature for the experiment” to “to 282 Added an attention about the way of measuring temperature at procedure 4.5.2., step 3 and Procedure 4.5.3., step 3
Reason
Temperature control of a 2.5 w/v% reagent solution is important for success the experiment.

Procedure 4.5.2., step 7
Changed the acceptance criterion of reference control as follows. Plateau level is between 10% and 30%, inclusive.
↓
Plateau level is level is 10% or more
Reason
The upper limit of acceptance criterion will be decided at the end of phase III validation study.

Version 1.80e
March 31, 2015

Description of changes

Section 2. Applicability domain

A new section was added to define an applicability domain based on the test results of 132 chemicals at the lead laboratory.

Section 3. Materials to be procured by the user

The term universal pH test paper (ADVANTEC, 07011030) was added to section 3, because an additional step was added to measure the pH level of the test chemical solution using universal pH test paper.

Section 4. Vitrigel-EIT kit contents

Formerly Section 2, this section was renumbered to accommodate the addition of the new Section 2.

Section 5. Procedure

Formerly Section 5, this section was renumbered to accommodate the addition of the new Section 2.

Section 5.5.3, Step 2

Changed the description as follows.

Old:

If the test chemical has not been dissolved, try to dissolve it by selecting an appropriate technique(s) from the following; mechanical mixture for a maximum 1-minute period using a vortex mixer, sonication for a maximum 20-minute period, or heating to maximum 70°C.

New:

If the test chemical does not dissolve readily, try using the following techniques in the following order to dissolve it: a) mix mechanically for a maximum of one minute using a vortex mixer, b) sonication for a maximum of 20 minutes, or c) heating to a maximum temperature of 70°C. After trying each technique, adjust the temperature of each test chemical solution to 28±2°C and check solubility. Move to the next step of the procedure once the test chemical solution is well dissolved or homogeneously dispersed.

Also, a condition was added to the Caution box at the end of Step 2, which reads:

> The parameters (time, temperature, intensity, etc.) for dissolving test chemicals may be adjusted to accommodate the physiochemical properties of each test chemical.

This was done due to problems with between-laboratory reproducibility during Phase III, when there were non-concordant results for some test chemicals. These discrepancies were attributed to differences in techniques used to dissolve the test chemicals, so we specified procedures for dissolving test chemicals.

Procedure 5.3.3., step 3

Add an extra procedure “Measure pH level of each 2.5% test chemical solution was using Universal pH test paper.”

Reason for revision

Add a procedure in association with setting applicability domains.

Vitrigel-EIT method　バリデーション試験　モデル作製記録用紙 Version 1.3, October 31, 2013

モデル作製記録

項目	記載欄
実験者名	
実施日	年　　月　　日

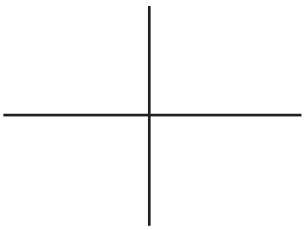
１．HCE-T 細胞（理研 BRC, Code#RCB2280）

項目	記載欄
Lot #	RCB2280
フラスコ数（2 個以下）	
細胞播種日	年　　月　　日
50%～100%コンフルエントの範囲にある	□Yes　□No

２．試薬・器具

項目	記載欄
培養液 lot #	
トリプシン溶液 lot #	
コラーゲンビトリゲルチャンバーlot #	

３．トリプシン処理・細胞数計測

項目	記載欄
トリプシン添加時刻、培養液添加時刻	＿＿：＿＿～＿＿：＿＿
概ね 5 個以下の細胞塊に分散しているか	□Yes　□No
細胞数計測 （　　）生細胞数 | （　　）生細胞数 （　　）生細胞数 | （　　）生細胞数	
死細胞率 5%以下か？	□Yes　□No　（　　%）
細胞懸濁液濃度	＿＿＿＿cells/ ml

４．コラーゲンビトリゲル膜チャンバーへの細胞播種

項目	記載欄
1.2×10^5cells/ml 細胞懸濁液調製	細胞懸濁液＿＿ml + 培養液＿ml
チャンバーへの播種開始時刻、終了時刻	＿＿：＿＿～＿＿：＿＿
2 日培地交換、気相培養開始・終了時刻	＿＿：＿＿～＿＿：＿＿
5 日目培地交換開始時刻・終了時刻	＿＿：＿＿～＿＿：＿＿

施設名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

実験責任者名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　月　日

事務局確認 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　月　日

機器校正・点検記録

マイクロピペット（100μl）

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

施設名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

実験責任者名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

事務局確認 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

機器校正・点検記録

マイクロピペット（1000μl）

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

施設名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

実験責任者名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

事務局確認 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

機器校正・点検記録

天秤

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

施設名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

実験責任者名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

事務局確認 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

機器校正・点検記録

CO_2インキュベーター

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

施設名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

実験責任者名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

事務局確認 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

機器校正・点検記録

ウォーターバス

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

施設名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

実験責任者名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

事務局確認 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

機器校正・点検記録

遠心分離機

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

項目	記載欄
型番	
管理番号	
確認日	年　　月　　日
確認者	
備考	

施設名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

実験責任者名 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

事務局確認 ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

HCE-T 細胞培養記録

解凍

項目	記載欄
実験者名	
実施日	年　　月　　日
凍結細胞 lot#	
培養液 lot#	
細胞解凍時刻	:
培養液交換時刻	:

継代培養

項目	記載欄
実験者名	
実施日、開始時刻	年　　月　　日　　:
前回継代日	年　　月　　日
トリプシン処理時間	min
細胞希釈倍率	
継代後の継代数	

項目	記載欄
実験者名	
実施日、開始時刻	年　　月　　日　　:
前回継代日	年　　月　　日
トリプシン処理時間	min
細胞希釈倍率	
継代後の継代数	

項目	記載欄
実験者名	
実施日、開始時刻	年　　月　　日　　:
前回継代日	年　　月　　日
トリプシン処理時間	min
細胞希釈倍率	
継代後の継代数	

項目	記載欄
実験者名	
実施日、開始時刻	年　　月　　日　　:
前回継代日	年　　月　　日
トリプシン処理時間	min
細胞希釈倍率	
継代後の継代数	

培養液交換

実施者名	実施日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日
	年　月　日

施設名 _____

実験責任者名 _____　年　月　日

事務局確認 _____　年　月　日

曝露試験記録

項目	記載欄
実験者名	
実施日	年　　月　　日

１．TEER 装置の性能チェック

（0.45%NaCl の TEER 値）－（0.9%NaCl の TEER 値）≧ 60Ω・cm² か？	（＿＿＿）－（＿＿＿）＝＿＿＿

２．被験物質の調製

被験物質名	
秤量値(0.1-0.2g)	＿＿g
培養液添加量	＿＿＿＿ml
培養液添加時刻	＿＿：＿＿
混合方法	□転倒混和（1min 以内） □ボルテックス（1min 以内） □加熱（70℃以下, ＿時間） □超音波処理（20min 以内）
溶解性	□溶解 □不溶（□懸濁　□沈降　□浮遊）

３．曝露試験

モデル＃	＃＿＿＿＿　＃＿＿＿＿　＃＿＿＿＿
モデルをインキュベーターから取り出した時刻	＿＿：＿＿　＿＿：＿＿　＿＿：＿＿
チャンバー内に培養液を添加した時刻	＿＿：＿＿　＿＿：＿＿　＿＿：＿＿
モデル温度（測定方法）	℃（　　）
被験物質温度（測定補法）	℃（　　）
被験物質曝露開始時刻 TEER 測定スタートは曝露から 被験物質曝露終了時刻	＿＿：＿＿　＿＿：＿＿　＿＿：＿＿ ＿＿秒後　＿＿秒後　＿＿秒後 ＿＿：＿＿　＿＿：＿＿　＿＿：＿＿

４．添付書類

曝露試験データシート

施設名　＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

実験責任者名　＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

事務局確認　＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿　　年　　月　　日

薬品使用記録

項目	記載欄
薬品名	
CAS 番号	
製造元	
規格（グレード）	
メーカーコード	
ロット番号	
外観・性状	
保管条件	
受領日	
受領量	
保管場所	
開封日	
備考	

使用履歴

使用年月日	使用者	使用目的	風袋込み重量 (g)		備考
			使用前	使用後	

施設名 _____

実験責任者名 _____ 年　月　日

事務局確認 _____ 年　月　日

試験日	
実施者名	
被験物質名	

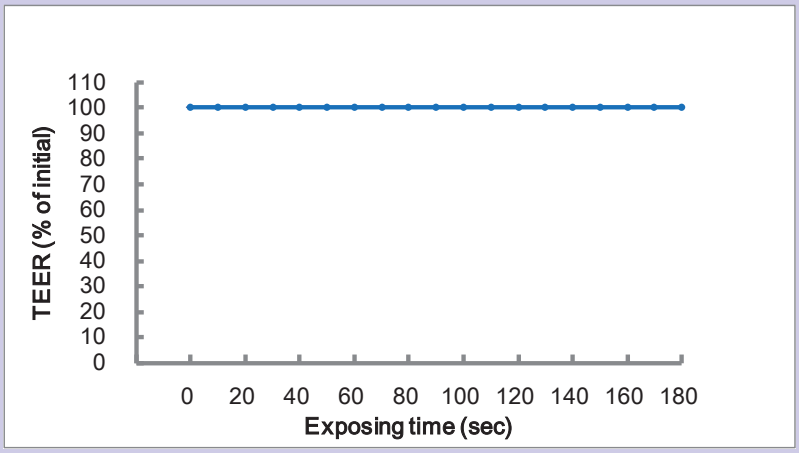
Test #	1	2	3
Model #			
Initial			
0			
10			
20			
30			
40			
50			
60			
70			
80			
90			
100			
110			
120			
130			
140			
150			
160			
170			
180			

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.00	NI
Plateau level	0	NI



Appendix8.4

Report on the selection of test substances for Vitrigel-EIT validation study

2015/6/30

Vitrigel-EIT Validation Management Team (VMT)

In this report, the selection process of test substances was described for the Vitrigel-EIT test validation study.

The objective of this study was to evaluate the within- and between-laboratory reproducibility and predictability of the Vitrigel-EIT on eye irritation (consistency with the two categories, Irritant and Non-irritant) in accordance with an initial step of bottom-up approach.

As a complementary study, the validation management team (VMT) evaluated the predictability of the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (UN GHS) with three classifications (Category 1, Category 2, Non-irritant).

For this purpose, phase I, phase II and phase III studies were conducted by three laboratories using the test substances as shown in Table 1. These test substances were selected by the VMT members not included the delegate of three laboratories. All test substances distributed by The Japanses Ceter for the Validation of Alternative methods (JaCVAM).

Table 1. Breakdown of the Vitrigel-EIT validation study

Phase	The number of the test substances	The number of the repetitions	Examination
0	5	3	Within- laboratory transferability
I	10	3	Between- laboratory transferability & Within- and between- laboratory reproducibility
II	10	1	Between- laboratory reproducibility
III	36	1	Between- laboratory reproducibility and predictability

1. Phase 0 study

In the phase 0 study, five test substances were selected by the VMT for Within- laboratory transferability as shown in Table 2. The VMT distributed 3 runs per each non-coded test substance to each laboratory.

Table 2. List of the 5 substances selected for phase 0 study in Vitrigel-EIT validation study

No.	Chemical	CAS	Solid/ Liquid	Supplier
Reference control	Ethanol	64-17-5	Liquid	Wako Pure Chemical
1	Benzalkonium chloride	8001-54-5	Solid	MP Biomedicals.Inc.
2	Isopropanol	67-63-0	Liquid	Wako Pure Chemical
3	Glycerol	56-81-5	Liquid	Wako Pure Chemical
4	n-Hexanol	111-27-3	Liquid	Sigma
5	Silicon Dioxide n-Hydrate	7699-41-4	Solid	Wako Pure Chemical

2. Phase I study

In the phase I study, ten test substances were selected by the VMT for within- and between- laboratory reproducibility as shown in Table 3. All substances were selected in consideration of valance of UN GHS labeling, solid: liquid from the following lists.

- The existing in individual animal data on test substances were available for classifying the eye irritating hazard under UN GHS.
- Test substances had already been evaluated in other i*n vitro* eye irritation tests.

Ten test substances comprising 5 irritants and 5 non-irritants, and 5 solids and 5 liquids were listed in Table 3. For within- and between-laboratory reproducibility, the VMT distributed 3 sets per each coded test substance to each laboratory. Three sets were tested separately, but the order in which they were tested dose not matter. That is, the VMT distributed 30 coded test substances (10 test substances) in the phase I study to each laboratory.

Table 3. List of the 10 substances selected for phase I in Vitrigel-EIT validation study

No.	Test substance	CAS No.	Solid/ Liquid	Supplier	Storage	Lab. Code			GHS
						VA	**VB**	**VC**	
1-1	Imidazole	288-32-4	solid	Wako	rt	VA001 VA018 VA025	VB008 VB015 VB021	VC005 VC012 VC029	1
1-2	Cyclohexanol	108-93-0	liquid	Sigma Aldrich	rt	VA002 VA019 VA026	VB009 VB016 VB022	VC006 VC013 VC030	1
1-3	3,3-Dithiodipropionic acid	1119-62-6	solid	Wako	rt	VA003 VA020 VA027	VB010 VB017 VB023	VC007 VC014 VC021	2A
1-4	Acetone	67-64-1	liquid	Wako	rt	VA004 VA011 VA028	VB001 VB018 VB024	VC008 VC015 VC022	2A
1-5	3-Chloropropionitrile	542-76-7	liquid	Aldrich	rt	VA005 VA012 VA029	VB002 VB019 VB025	VC009 VC016 VC023	2B
1-6	n,n-Dimethylguanidine sulfate	598-65-2	solid	Aldrich	rt	VA006 VA013 VA030	VB003 VB020 VB026	VC010 VC017 VC024	NC
1-7	Toluene	108-88-3	liquid	Sigma Aldrich	rt	VA007 VA014 VA021	VB004 VB011 VB027	VC001 VC018 VC025	NC
1-8	3-Methoxy-1,2-propanediol	623-39-2	liquid	TCI	rt	VA008 VA015 VA022	VB005 VB012 VB028	VC002 VC019 VC026	NC
1-9	Gluconolactone	90-80-2	solid	TCI	rt	VA009 VA016 VA023	VB006 VB013 VB029	VC003 VC020 VC027	NC
1-10	Ammonium nitrate	6484-52-2	solid	Sigma Aldrich	rt	VA010 VA017 VA024	VB007 VB014 VB030	VC004 VC011 VC028	2B

rt: room temp, NC: Not classified

Set 1
Set 2
Set 3

VA	FDSC : FOOD AND DRUG SAFETY CENTER , HATANO RESEARCH INSTITUTE
VB	BoZo Research Center Inc.
VC	Daicel Corporation

3. Phase II study

In the phase II study, ten test substances were selected by the VMT for between- laboratory reproducibility as shown in Table 4. All substances were selected in consideration of valance of UN GHS labeling, solid: liquid from the following lists.

Ten test substances comprising four UN GHS Category 1, three GHS Category 2A and 2B, and three Not classified, and 5 solids and 5 liquids were listed in Table 4.

Table 4. List of 10 test substances selected for phase II in Vitrigel-EIT validation study

No.	Test substance	CAS No.	Solid/ Liquid	Supplier	Storage	Lab. Code			GHS
						VA	**VB**	**VC**	
2-1	Imidazole	288-32-4	Solid	Wako	rt	VA101	VB108	VC105	1
2-2	Cyclohexanol	108-93-0	Liquid	Sigma-Aldrich	rt	VA102	VB109	VC106	1
2-3	Cyclopentanol	96-41-3	Liquid	Aldrich	rt	VA103	VB110	VC107	2A/2B
2-4	SDS	151-21-3	Solid	Wako	rt	VA104	VB101	VC108	1
2-5	2-Methyl-1-pentanol	105-30-6	Liquid	Aldrich	rt	VA105	VB102	VC109	2B
2-6	Sodium salicylate	54-21-7	Solid	Wako	rt	VA106	VB103	VC110	1
2-7	α-Hexylcinnamaldehyde	101-86-0	Liquid	Wako	rt	VA107	VB104	VC101	2A/2B
2-8	n,n-Dimethylguanidine sulfate	598-65-2	Solid	Aldrich	rt	VA108	VB105	VC102	NC
2-9	Toluene	108-88-3	Liquid	Sigma-Aldrich	4℃	VA109	VB106	VC103	NC
2-10	Gluconolactone	90-80-2	Solid	TCI	rt	VA110	VB107	VC104	NC

rt: room temp, NC: Not classified

VA	FDSC：FOOD AND DRUG SAFETY CENTER , HATANO RESEARCH INSTITUTE
VB	BoZo Research Center Inc.
VC	Daicel Corporation

4. Phase III study

In the phase III study, thirty-seven test substances were selected by the VMT for between- laboratory reproducibility and predictability as shown in Table 5. All substances were selected in consideration of valance of UN GHS labeling, solid: liquid from the following lists.

According to the objective in the study plan, the coded 37 test substances were prepared to evaluate the predictability and to confirm between-laboratory reproducibility of Vitrigel-EIT validation studies. The coded 37 test substances were distributed to each laboratory for phase III validation study.

In the list, the test substances selected in 3-dimensional corneal model (such as EpiOcular) validation study by the

European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM)[1)], and the substances selected in the Short Time Exposure (STE) test validation study by the Japanese Center for the Validation of Alternative Methods (JaCVAM) and Independent peer review by Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)[2,3)], and OptiSafe™ evaluation by NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

In the phase III study, all test substances were selected in consideration of valance of UN GHS labeling, solid: liquid from the following lists.

- The existing in individual animal data on test substances were available for classifying the eye irritating hazard under UN GHS. Test substances had already been evaluated in other *in vitro* eye irritation tests (Table 6)
- The valance of solid : liquid is uniformity (Table 7)

From total 37 test substances, the one test substance, No.VC213: Sodium chloroacetate (No. 3-16), which a chemical master at Lab.C missed to open the name. Therefore, this chemical was eliminated in the list and Cyclopentanol as an alternative chemical coded and distributed by JaCVAM.

Table 5. List of 37 test substances selected for phase III in Vitrigel-EIT validation study

No.	Test substance	CAS No.	Solid/ Liquid	Supplier	Storage	Lab. Code			GHS
						VA	**VB**	**VC**	
3-1	2,5-Dimethyl-2,5-hexanediol	110-03-2	Solid	Aldrich	rt	VA225	VB201	VC228	1
3-2	2-Benzyl-4-chlorophenol	120-32-1	Solid	Wako	rt	VA226	VB202	VC229	1
3-3	2,2-Dimethyl butanoic acid	595-37-9	Liquid	Wako	rt	VA227	VB203	VC230	1
3-4	Captan	133-06-2	Solid	TCI	rt	VA228	VB204	VC201	1
3-5	tetra-N-Octylammonium bromide	14866-33-2	Solid	Wako	rt	VA229	VB205	VC202	1
3-6	Butanol	71-36-3	Liquid	Wako	rt	VA230	VB206	VC203	1
3-7	3-(2-Aminoethylamino)propyl] trimethoxysilane	1760-24-3	Liquid	Aldrich	rt	VA201	VB207	VC204	1
3-8	Dodecyl sodium sulfate	151-21-3	Solid	Wako	rt	VA202	VB208	VC205	1
3-9	m-Phenylenediamine	108-45-2	Solid	Wako	4℃	VA203	VB209	VC206	1
3-10	Tetraethylene glycol	17831-71-9	Liquid	TCI	rt	VA204	VB210	VC207	1
3-30	Imidazole	288-32-4	Solid	Wako	rt	VA224	VB230	VC227	1
3-32	Sodium salicylate	54-21-7	Solid	Sigma-Aldrich	rt	VA232	VB233	VC236	1
3-11	gamma-Butyrolactone	96-48-0	Liquid	Aldrich	rt	VA205	VB211	VC208	2A
3-12	Methyl acetate	79-20-9	Liquid	Sigma-Aldrich	rt	VA206	VB212	VC209	2A
3-13	Myristyl alcohol	112-72-1	Solid	Aldrich	rt	VA207	VB213	VC210	2A

3-14	2,6-Dichlorobenzoyl chloride	4659-45-4	Liquid	Aldrich	rt	VA208	VB214	VC211	2A
3-15	Dibenzyl phosphate	1623-08-1	Solid	TCI	-20°C	VA209	VB215	VC212	2A
~~3-16~~	~~Sodium chloroacetate~~	~~3926-62-3~~	~~Solid~~	~~Sigma-Aldrich~~	~~rt~~	~~VA210~~	~~VB216~~	~~VC213~~	~~2B~~
3-17	1-(2-Propoxy-1-methylethoxy)-2-propanol	29911-27-1	Liquid	Aldrich	rt	VA211	VB217	VC214	2B
3-18	Camphene	79-92-5	Solid	Aldrich	rt	VA212	VB218	VC215	2B
3-19	Ethyl-2-methylacetoacetate	609-14-3	Liquid	Aldrich	rt	VA213	VB219	VC216	2B
3-20	Propylene glycol propyl ether	1569-01-3	Liquid	Aldrich	rt	VA214	VB220	VC217	2A or 2B
3-31	2-Methyl-1-pentanol	105-30-6	Liquid	Aldrich	rt	VA231	VB232	VC235	2B
3-33	alpha-Hexylcinnamaldehyde	101-86-0	Liquid	Wako	rt	VA233	VB234	VC231	2A/2B
3-37	Cyclopentanol	96-41-3	Liquid	Aldrich	rt	VA237	VB237	VC237	2A/2B
3-21	Methyl amyl ketone	110-43-0	Liquid	Sigma-Aldrich	rt	VA215	VB221	VC218	NC
3-22	2-(n-Dodecylthio)ethanol (2-Hydroxyethyl n-dodecyl sulfide)	1462-55-1	Liquid	Frontier Scientific, Inc.	rt	VA216	VB222	VC219	NC
3-23	iso-Octylthioglycolate	25103-09-7	Liquid	Wako	rt	VA217	VB223	VC220	NC
3-24	2,4-Difluoronitrobenzene	446-35-5	Liquid	Wako	rt	VA218	VB224	VC221	NC
3-25	tetra-Aminopyrimidine sulfate	5392-28-9	Solid	Aldrich	rt	VA219	VB225	VC222	NC
3-26	2,4-Pentanediol	625-69-4	Liquid	Aldrich	rt	VA220	VB226	VC223	NC
3-27	iso-Octyl acrylate	29590-42-9	Liquid	Aldrich	rt	VA221	VB227	VC224	NC
3-28	Metasilicic acid (Silicon Dioxide n-Hydrate)	7699-41-4	Solid	Wako	rt	VA222	VB228	VC225	NC
3-29	Potassium tetrafluorobroate	14075-53-7	Solid	Aldrich	rt	VA223	VB229	VC226	NC
3-34	n,n-Dimethylguanidine sulfate	598-65-2	Solid	TCI	rt	VA234	VB235	VC232	NC
3-35	Toluene	108-88-3	Liquid	Sigma-Aldrich	rt	VA235	VB236	VC233	NC
3-36	Gluconolactone	90-80-2	Solid	Wako	rt	VA236	VB231	VC234	NC

rt: room temp, NC: Not classified

VA	FDSC : FOOD AND DRUG SAFETY CENTER , HATANO RESEARCH INSTITUTE
VB	BoZo Research Center Inc.
VC	Daicel Corporation

Table 6. Distribution of test substances (lank of *in vivo*) selected for Vitrigel-EIT validation study

GHS				Total
Category 1	Category 2A/2B	Category 2B	No	
12	8	4	12	36

Table 7. Distribution of test substances (chemical properties) selected for Vitrigel-EIT validation study

Solid	Liquid	Total
16	20	36

References

1) Eye Irritation Validation Study on Human Tissue Models: Statistical Analysis and Reporting on the EpiOcular™ EIT
2) ICCVAM (2015) http://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/ocular/ste/index.html
3) Ohno Y, Kaneko T, Inoue T, Morikawa Y, Yoshida T, Fujii A, Masuda M, Ohno T, Hayashi M, Momma J, Uchiyama T, Chiba K, Ikeda N, Imanishi Y, Itakagaki H, Kakishima H, Kasai Y, Kurishita A, Kojima H, Matsukawa K, Nakamura T, Ohkoshi K, Okumura H, Saijo K, Sakamoto K, Suzuki T, Takano K, Tatsumi H, Tani N, Usami M, Watanabe R.: Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (1) Overview of the validation study and Draize scores for the evaluation of the tests.Toxicol In Vitro. 13(1):73-98(1999)

Appendix8.5.1

Statistical analysis report for Vitrigel-EIT validation study (Phase 1)

Appendix: Submitted data sheets

Takuto
Nakayama
Takashi Sozu

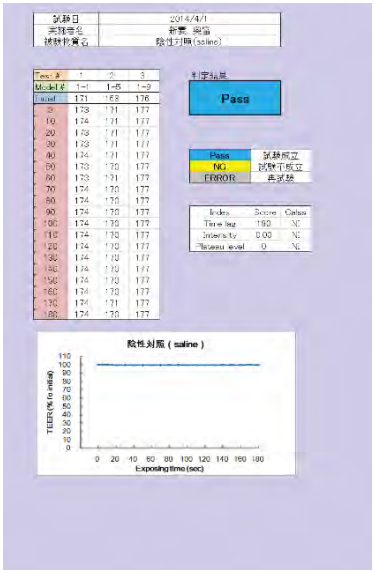
Department of Management Science, Faculty of Engineering,
Tokyo University of Science

8 February 2016

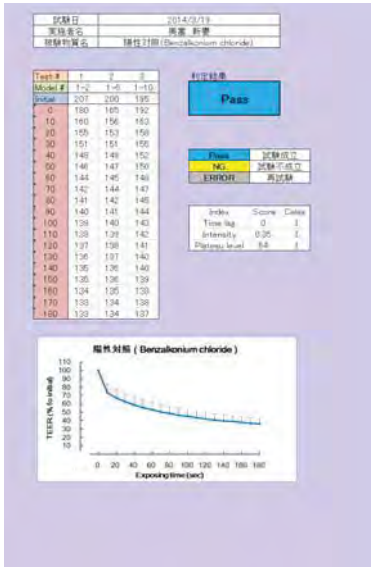
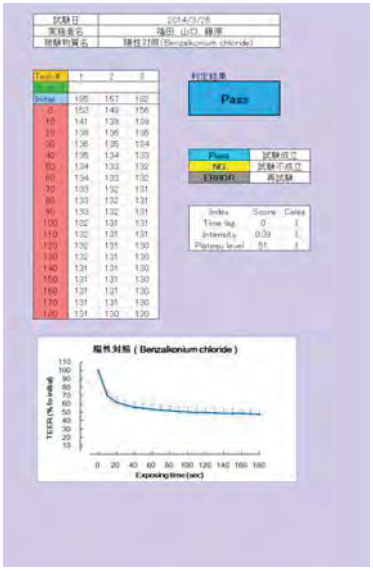
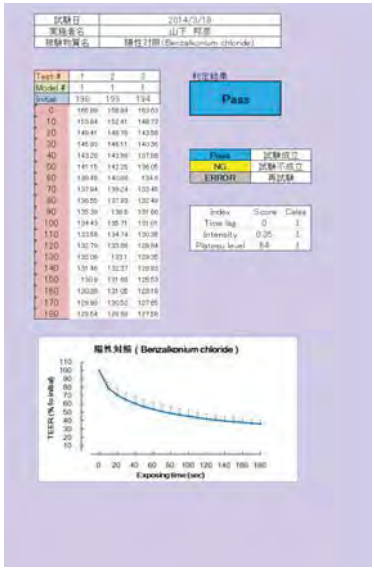
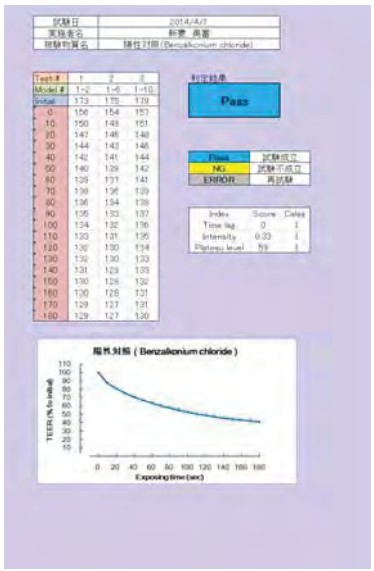
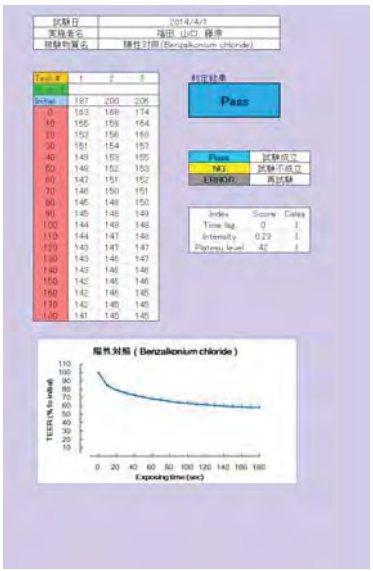
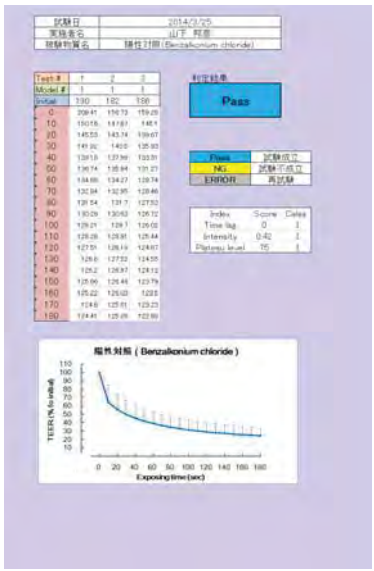
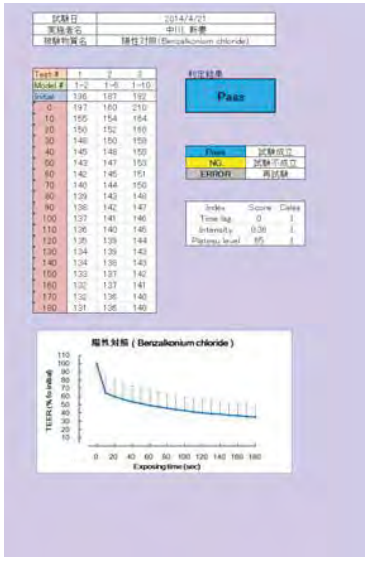
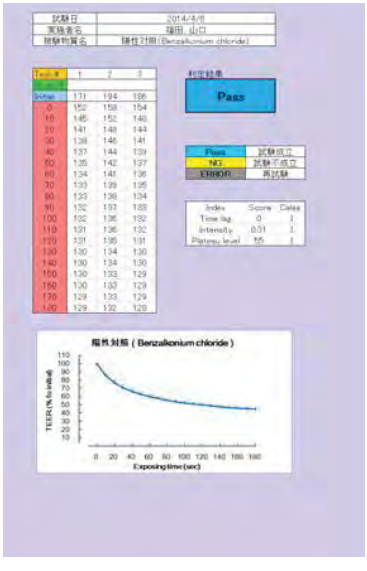
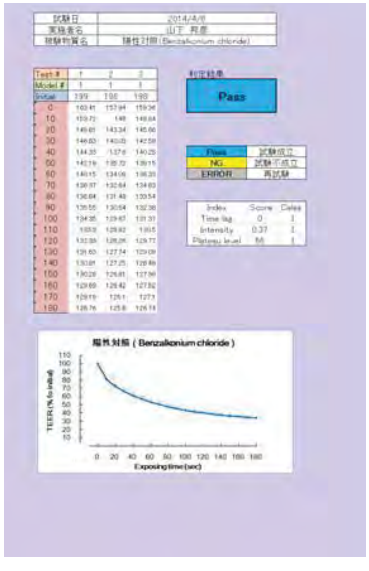
Chemicals lists

No.	Chemicals	GHS
Negative control	Saline	
Positive control	Benzalkonium chloride	
Reference control	Ethanol	
21	n,n-Dimethylguanidine sulfate	Not classified
23	Toluene	
28	3-Methoxy-1,2-propanediol	
30	Gluconolactone	
4	Imidazole	Category 1
6	Cyclohexanol	
9	3,3-Dithiodipropionic acid	Category 2A
10	Acetone	
16	3-Chloropropionitrile	Category 2B
17	Ammonium nitrate	

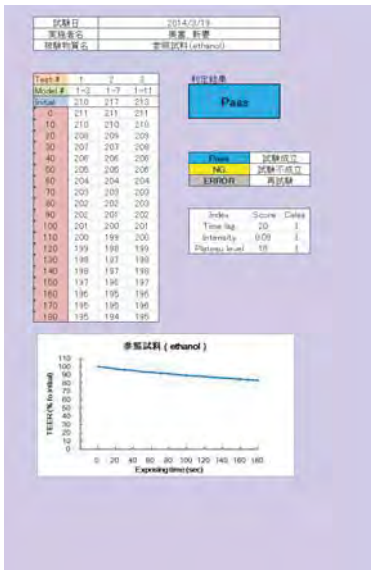
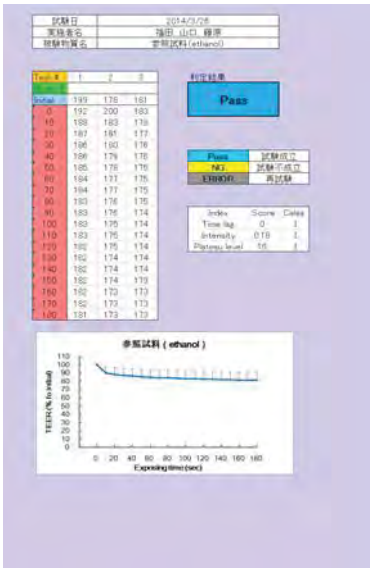
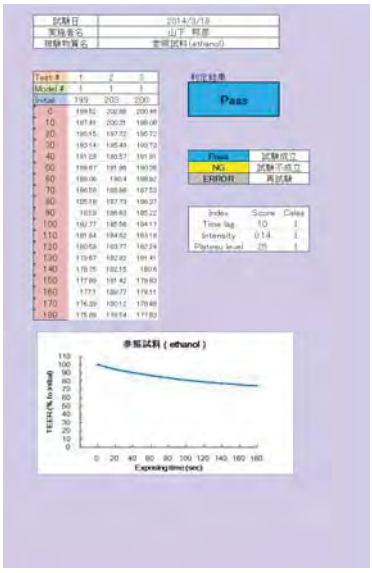
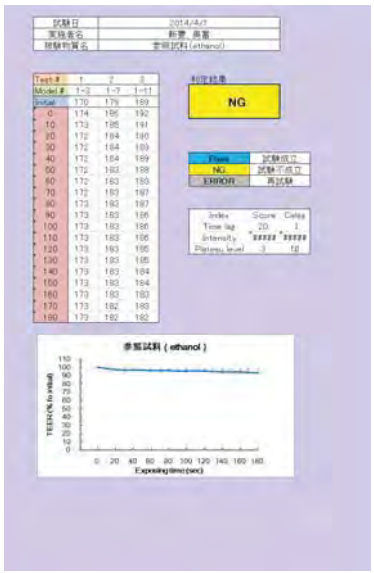
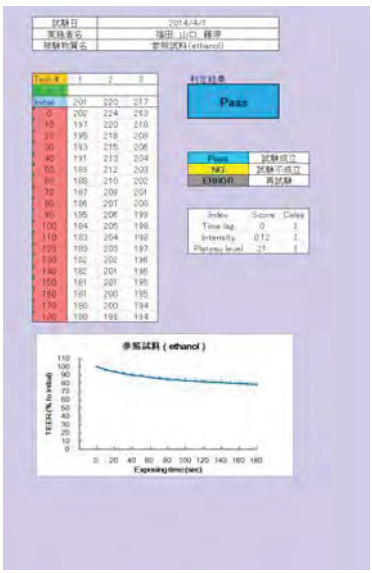
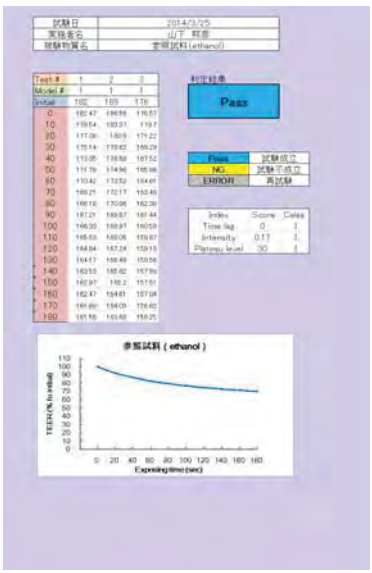
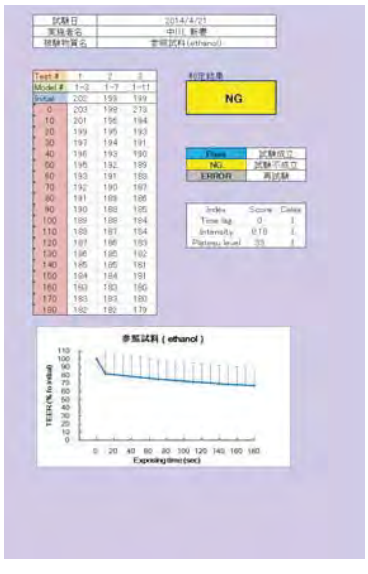
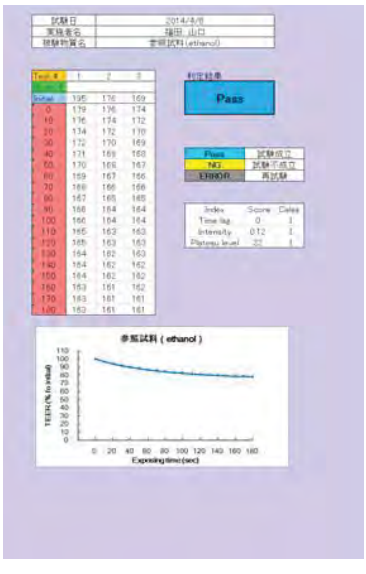
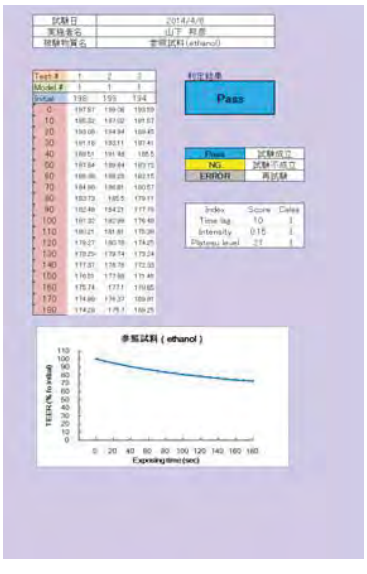
Negative control

FDSC	BoZo	Daicel
1st		
		
2nd		
		
3rd		
		

Positive control

FDSC	BoZo	Daicel
1st		
		
2nd		
		
3rd		
		

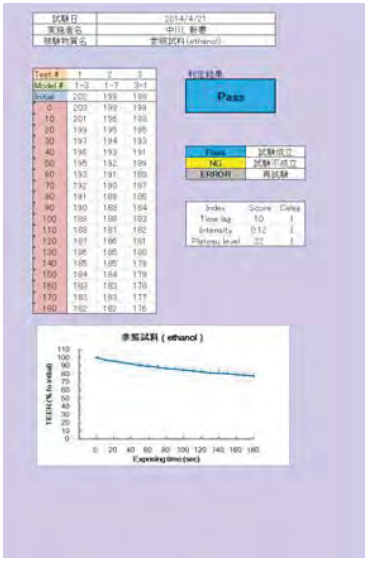
Reference

FDSC	BoZo	Daicel
1st		
		
2nd		
		
3rd		
		

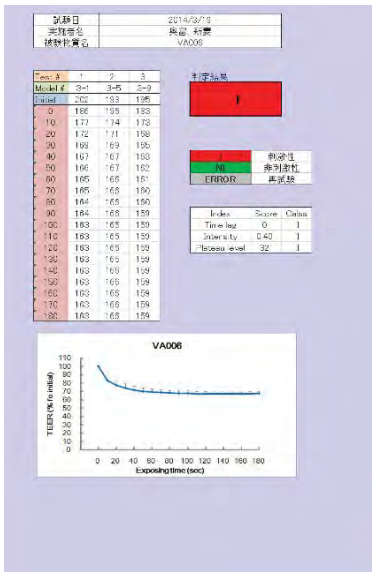
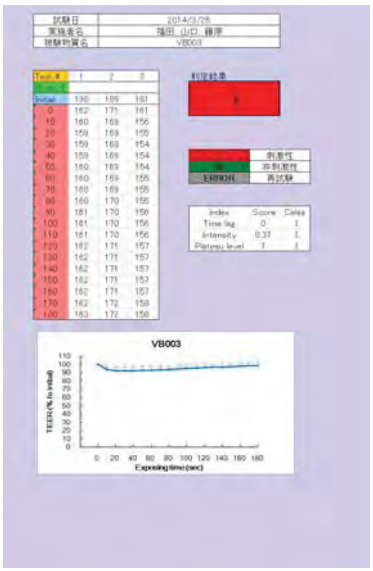
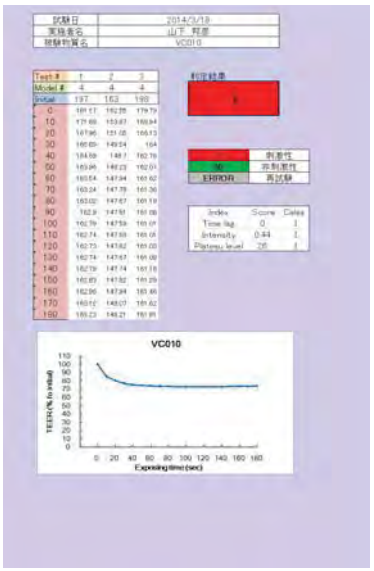
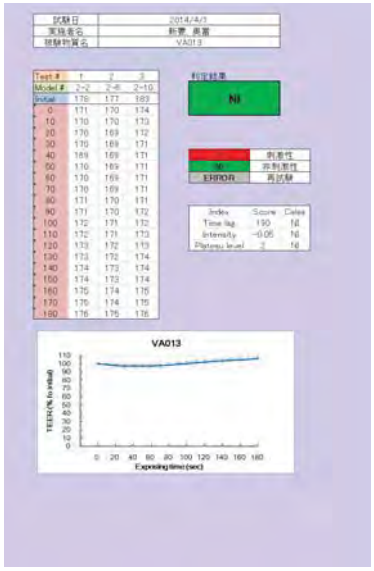
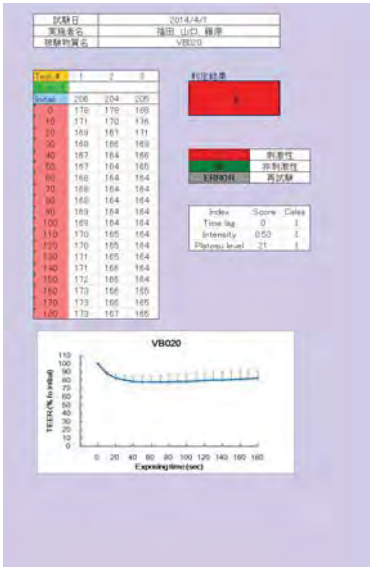
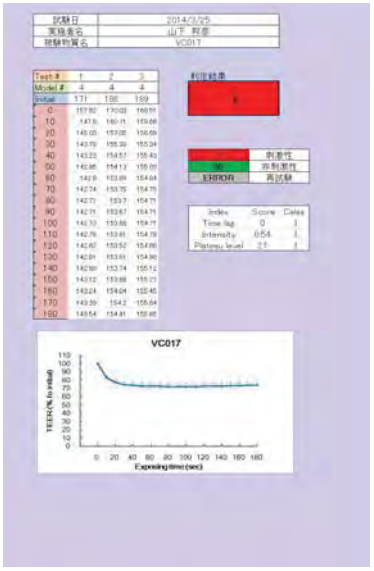
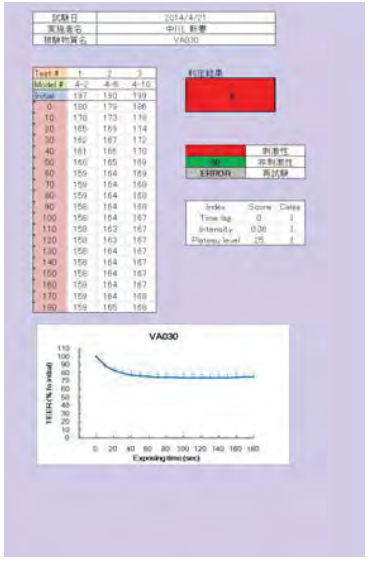
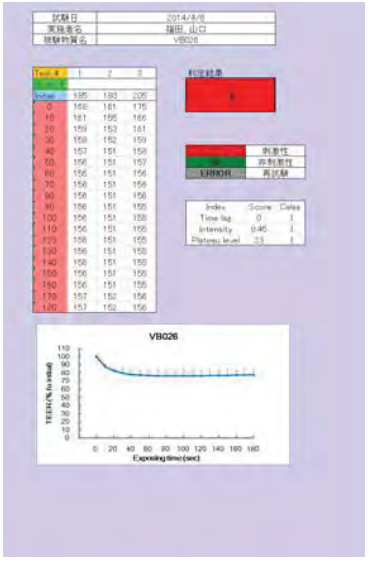
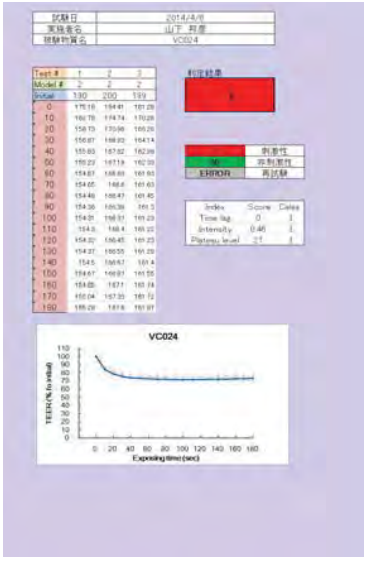
Reference

FDSC BoZo Daicel

3rd(再)



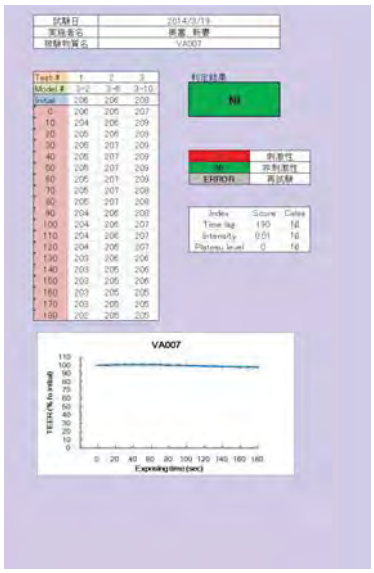
Not classified
No.21

	FDSC	BoZo	Daicel
1st			
2nd			
3rd			

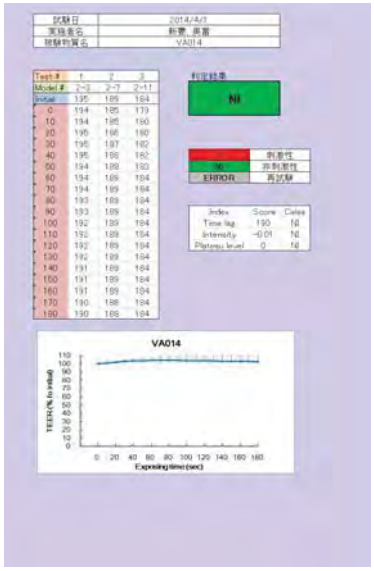
Not classified
No.23

FDSC

1st

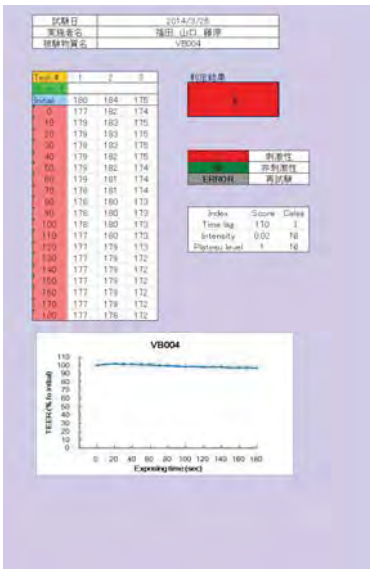


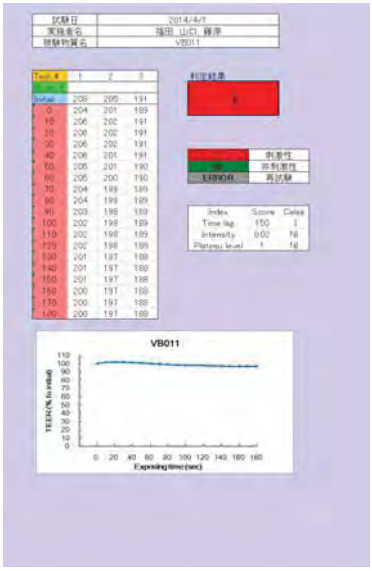
2nd

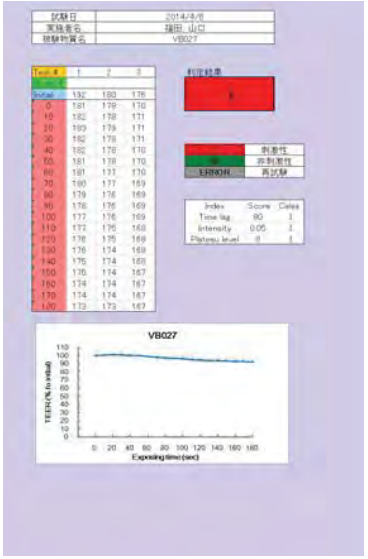


3rd

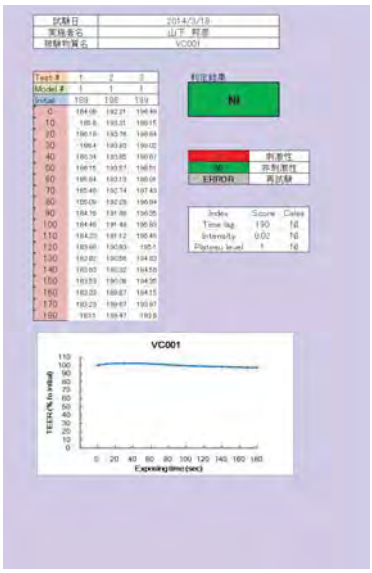
BoZo

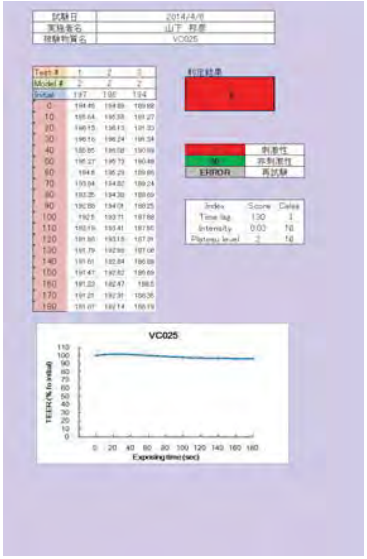






Daicel

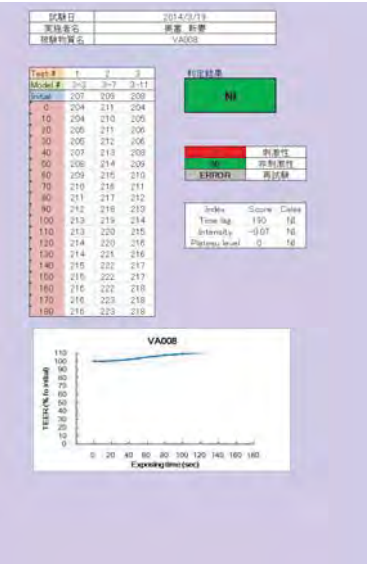




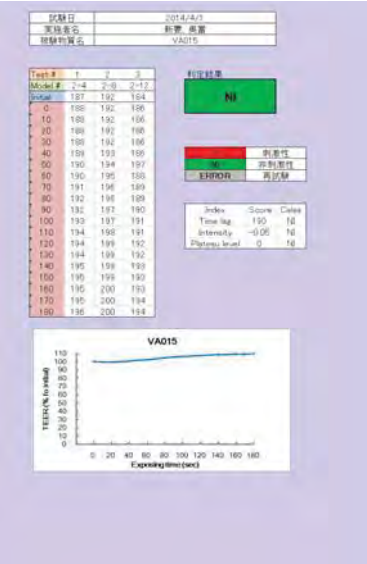
Not classified
No.28

FDSC

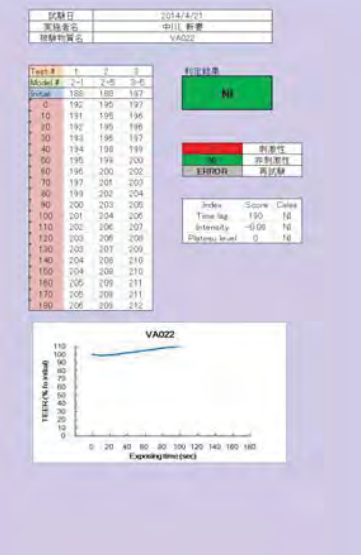
1st



2nd

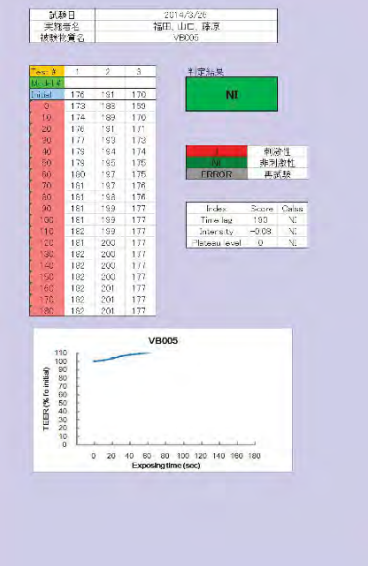


3rd

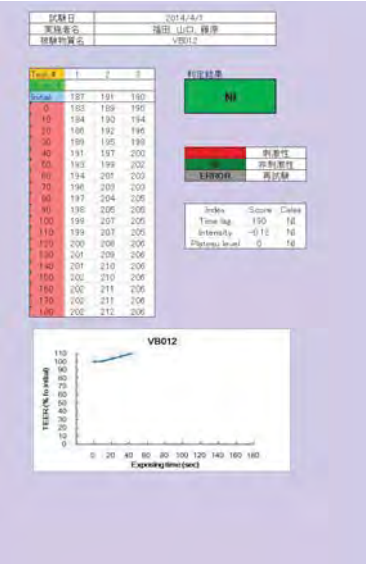


BoZo

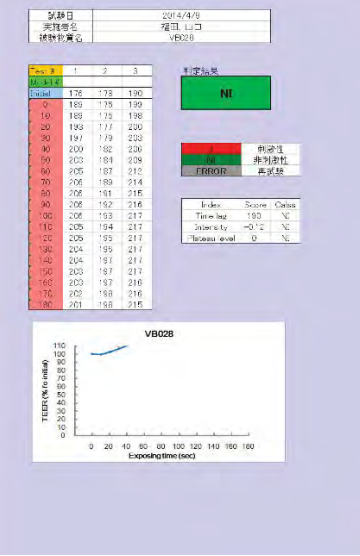
1st



2nd



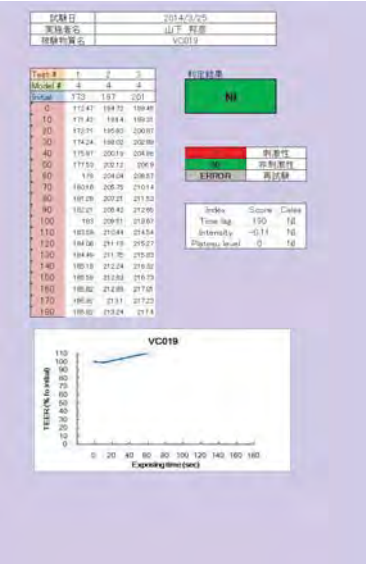
3rd



Daicel

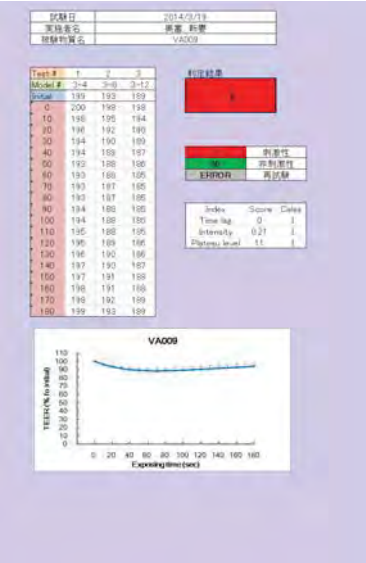
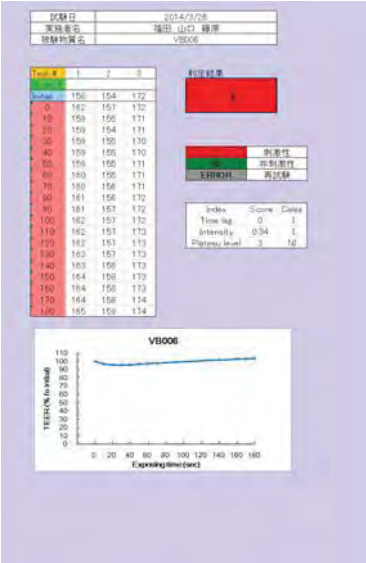
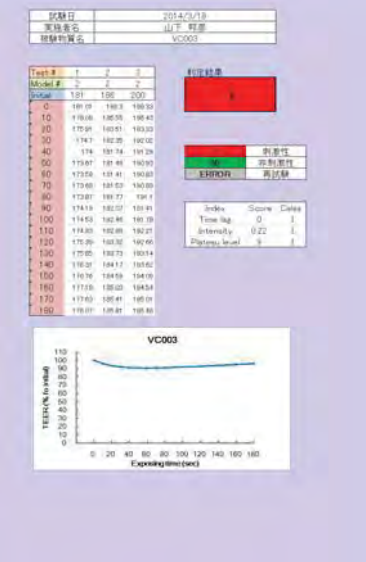
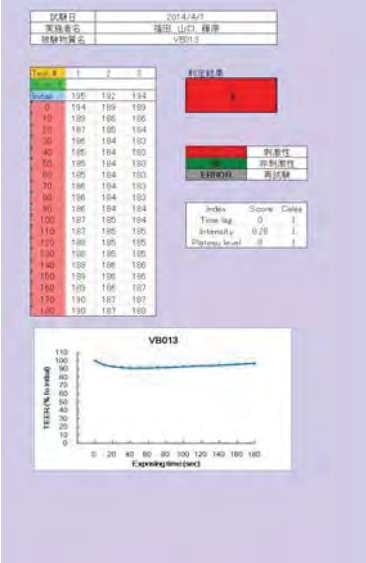
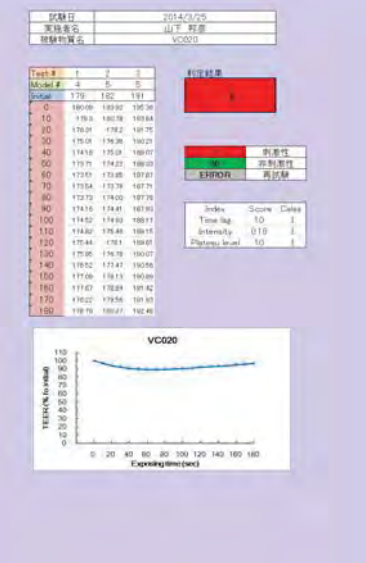
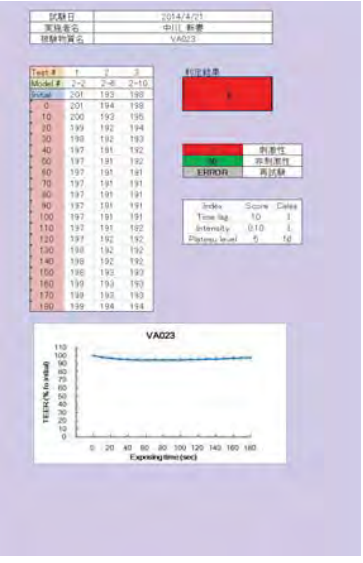
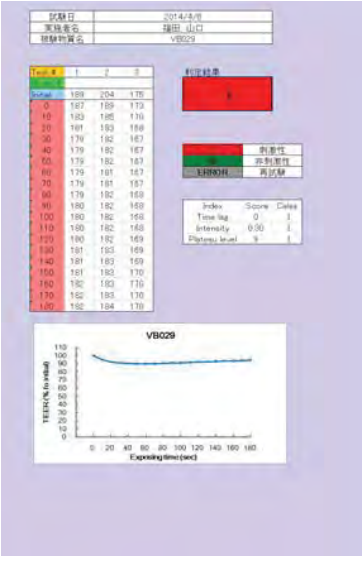
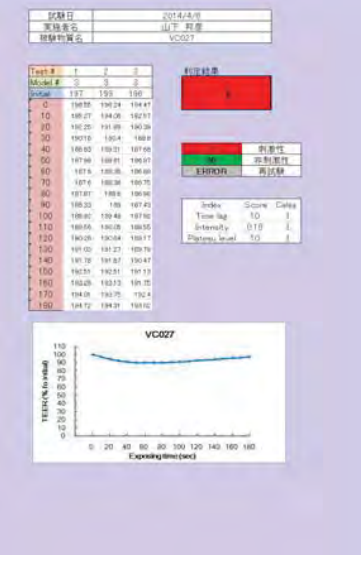
1st

2nd



3rd

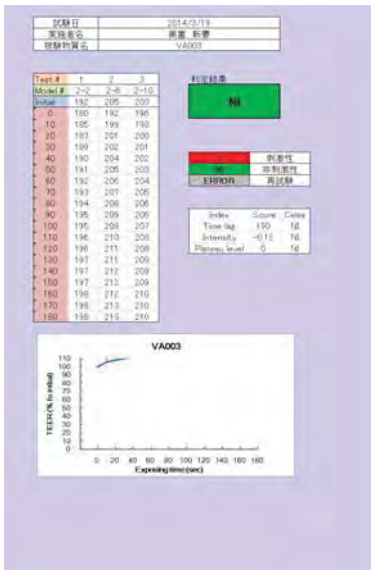
Not classified
No.30

FDSC	BoZo	Daicel
1st		
		
2nd		
		
3rd		
		

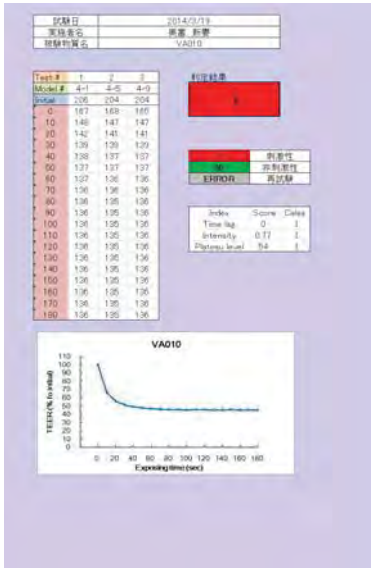
Category 2A
No.9

FDSC

1st



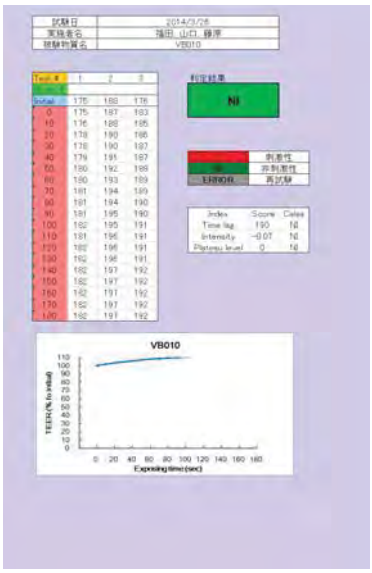
2nd



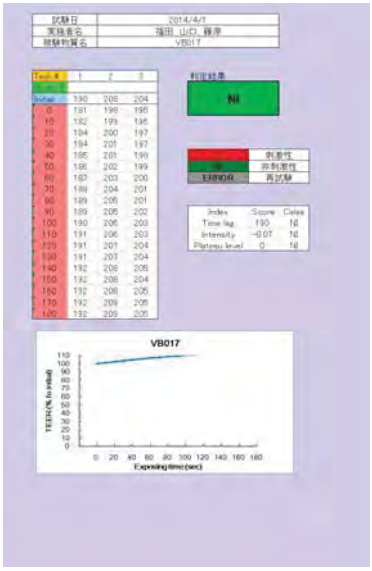
3rd

BoZo

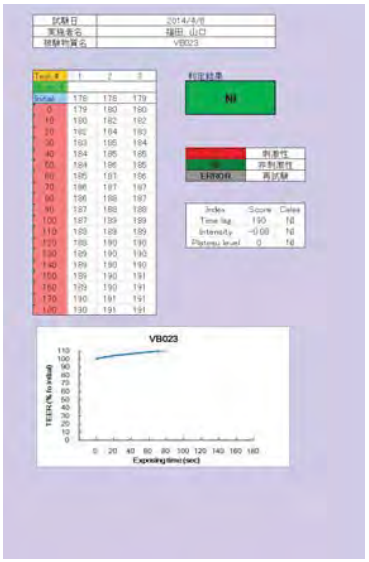
1st



2nd

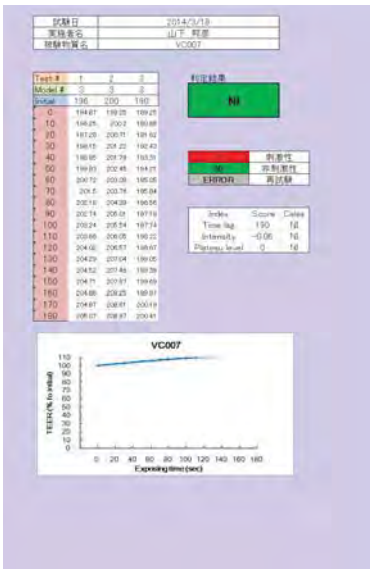


3rd

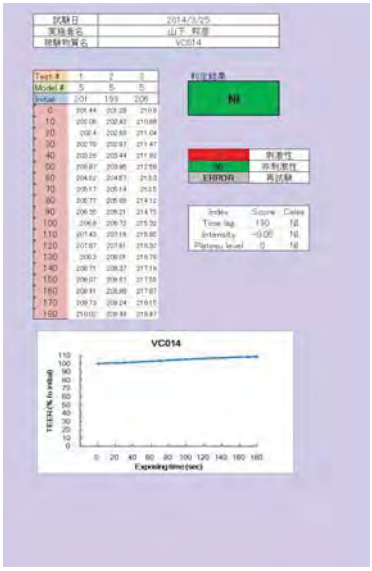


Daicel

1st



2nd

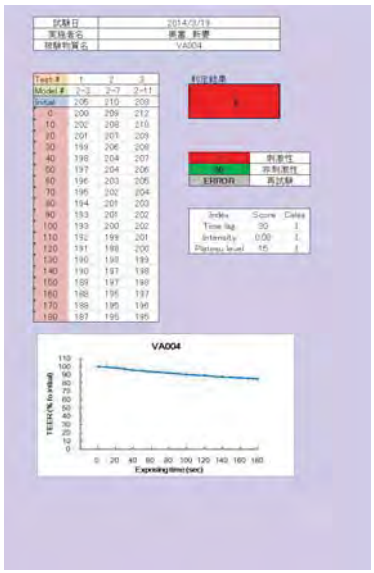


3rd

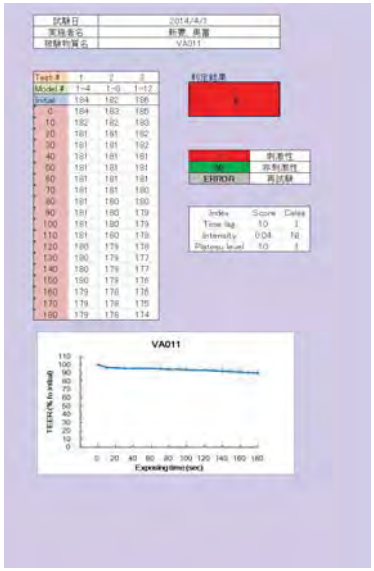
Category 2A
No.10

FDSC

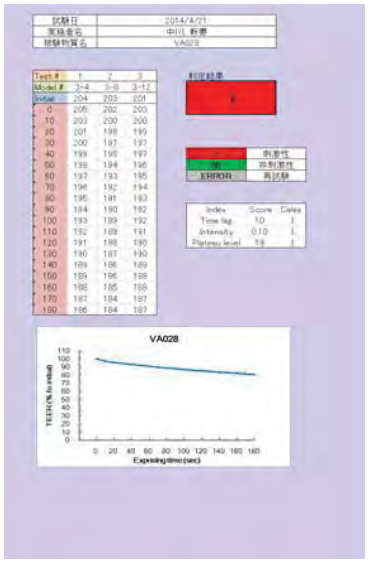
1st



2nd

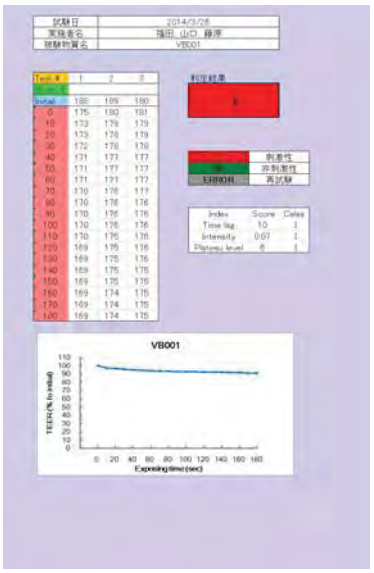


3rd

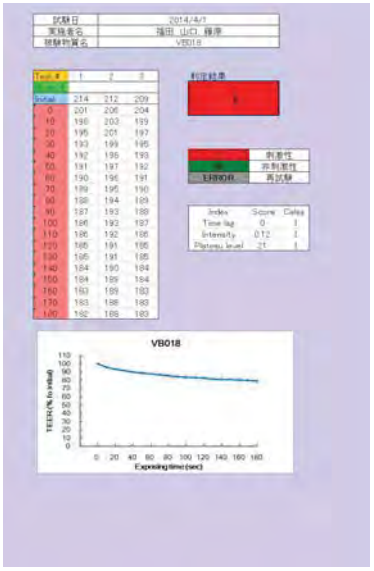


BoZo

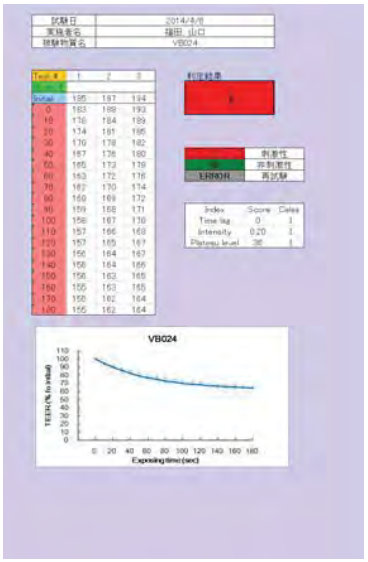
1st



2nd

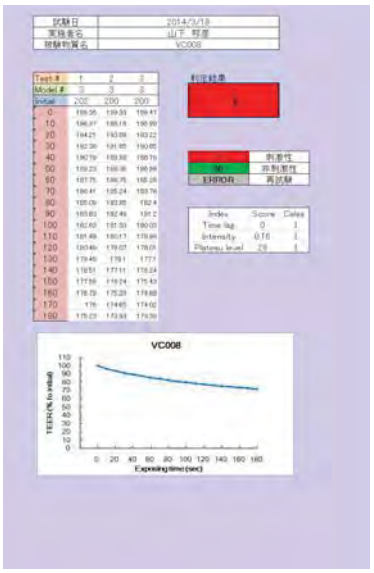


3rd

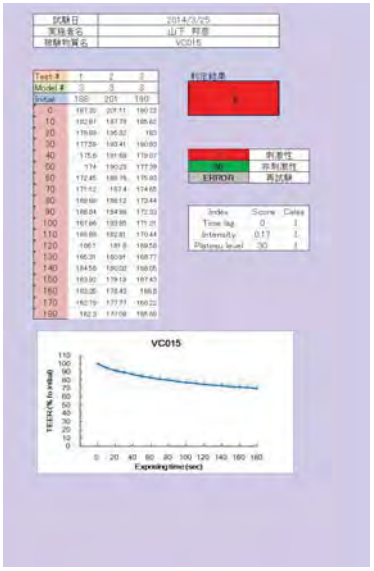


Daicel

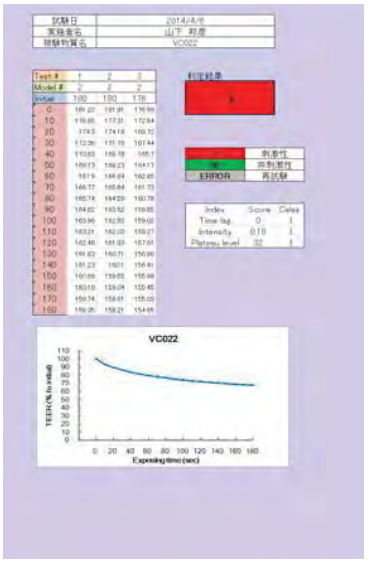
1st



2nd



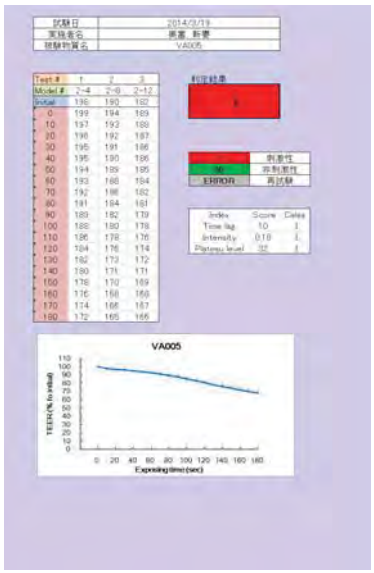
3rd



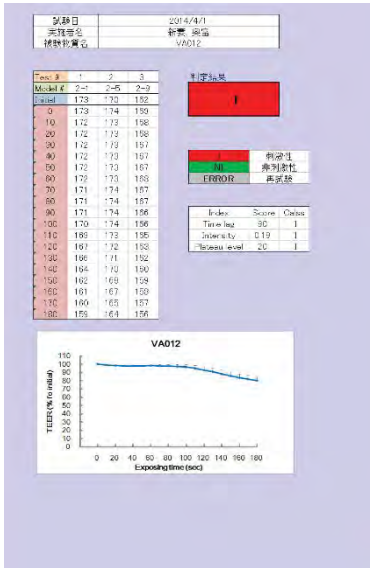
Category 2B
No.16

FDSC

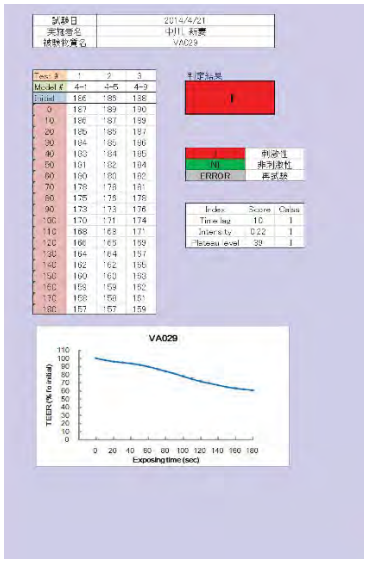
1st



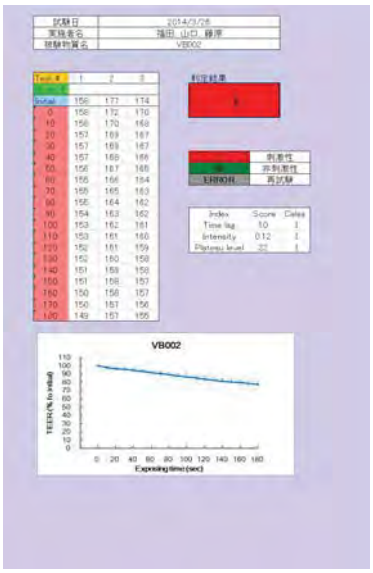
2nd

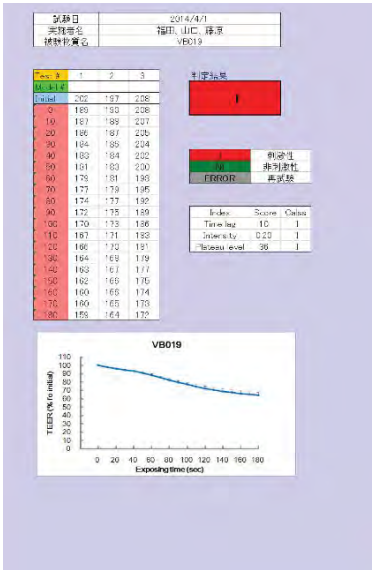


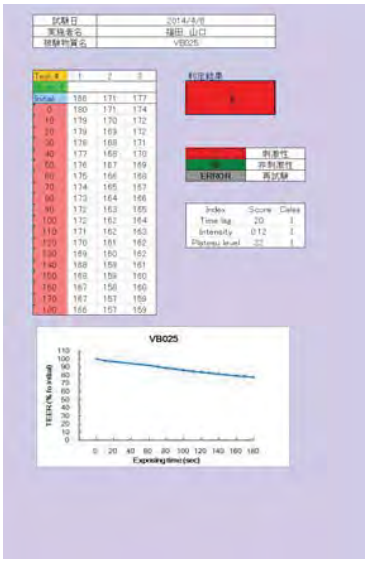
3rd



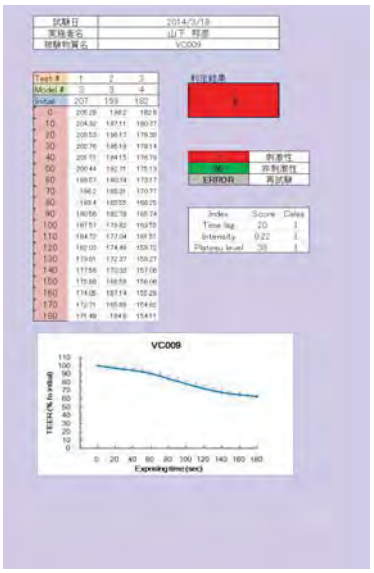
BoZo

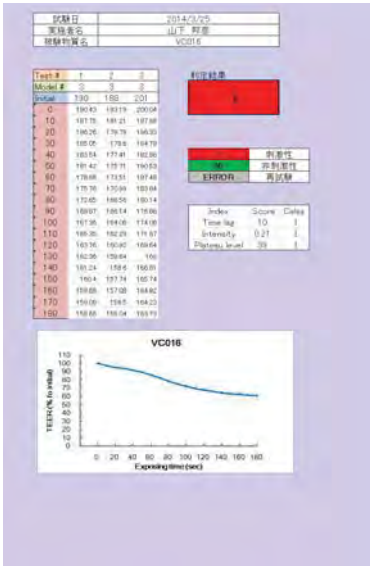


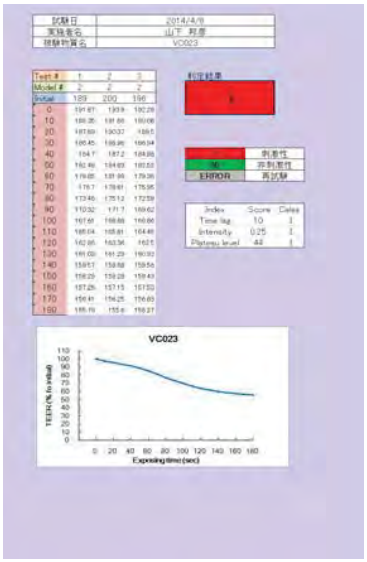




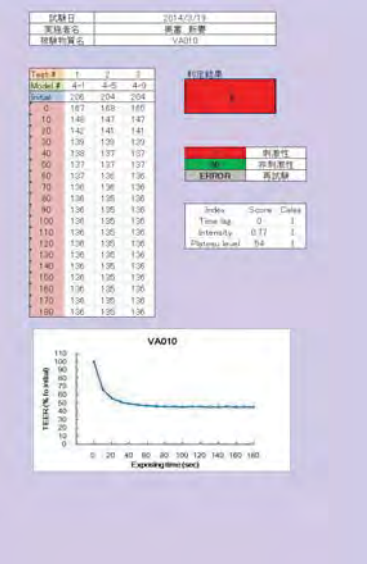
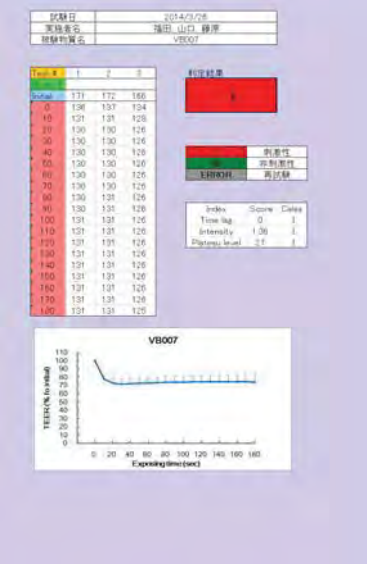
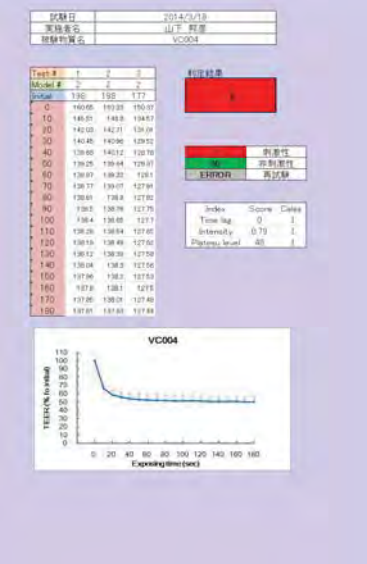
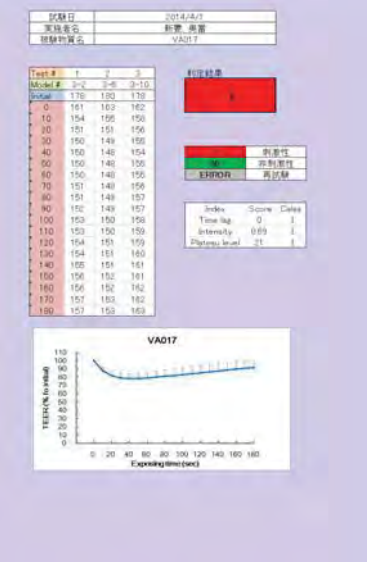
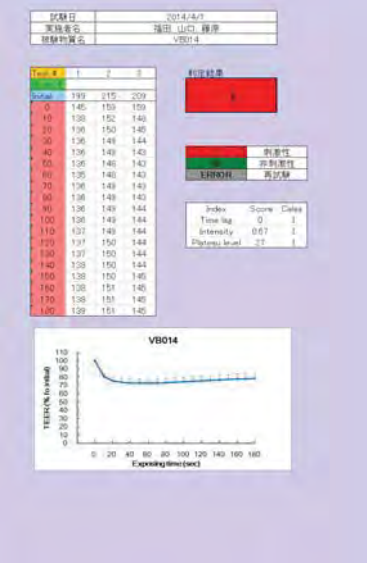
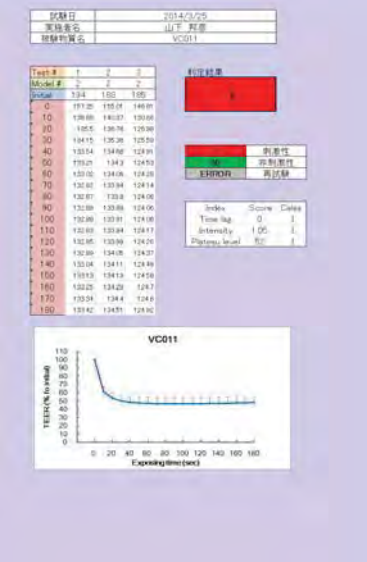
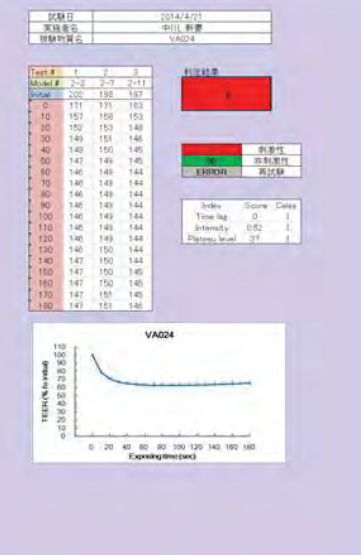
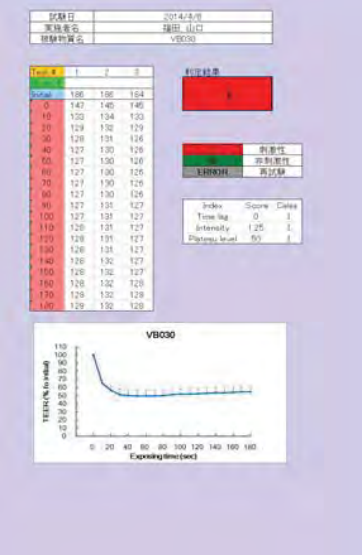
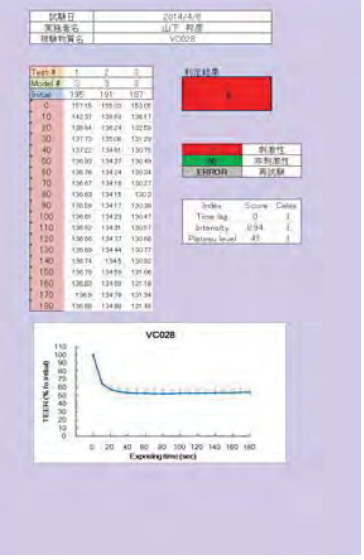
Daicel







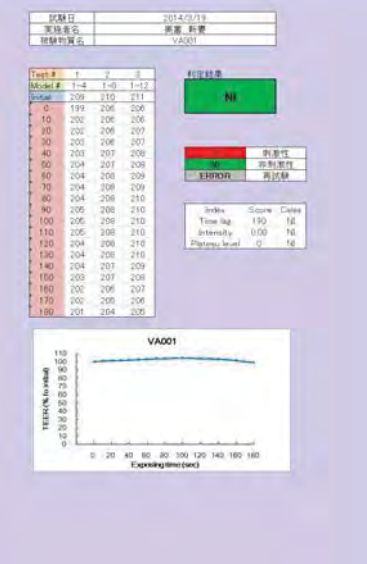
Category 2B
No.17

FDSC	BoZo	Daicel
1st		
		
2nd		
		
3rd		
		

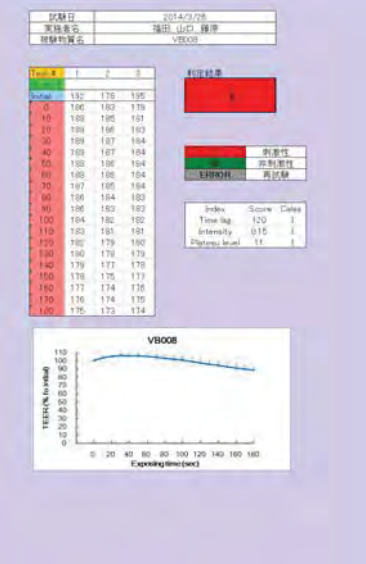
Category 1
No.4

FDSC

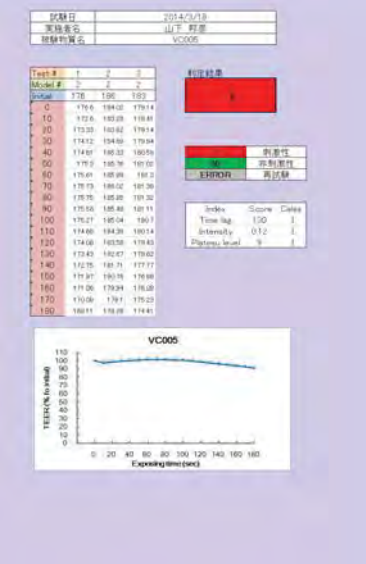
1st



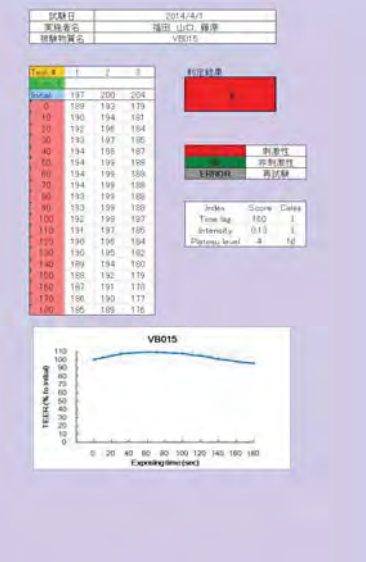
BoZo

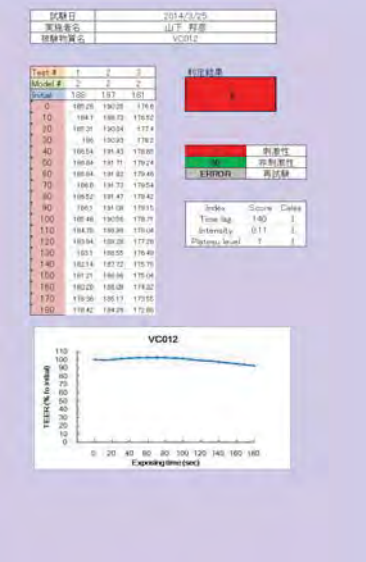


Daicel

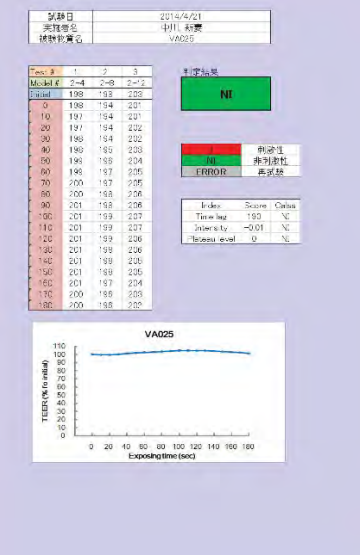


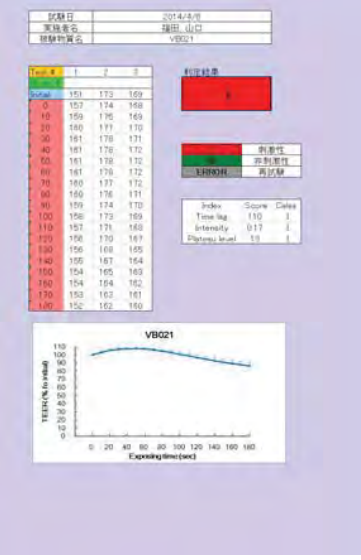
2nd

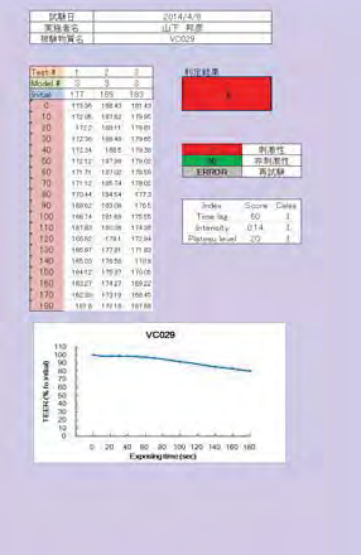




3rd



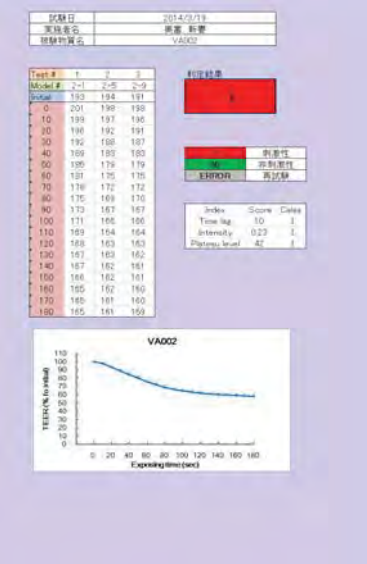




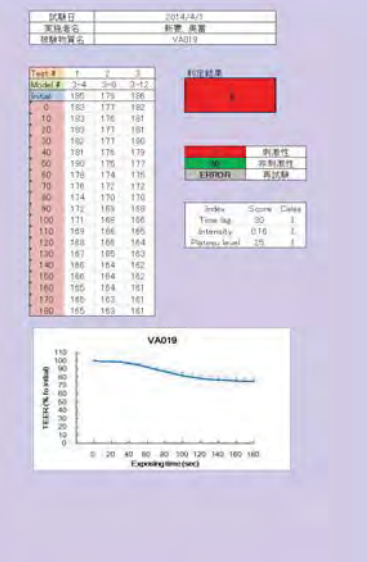
Category 1
No.6

FDSC

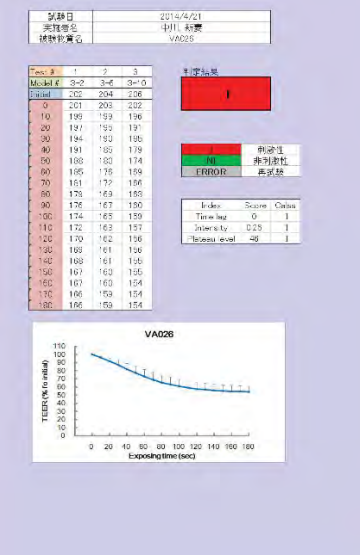
1st



2nd

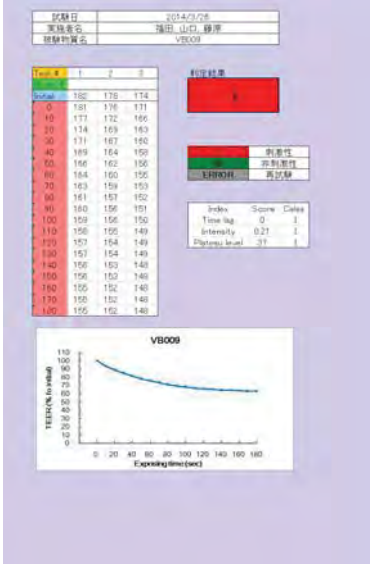


3rd

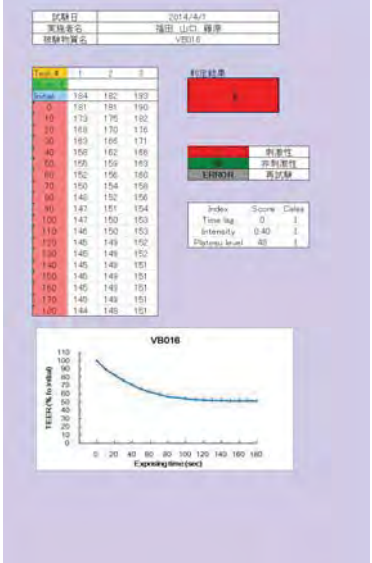


BoZo

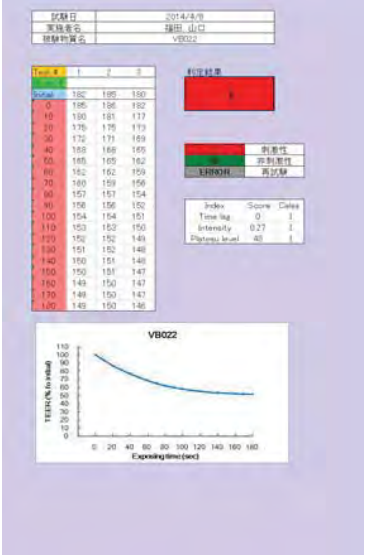
1st



2nd

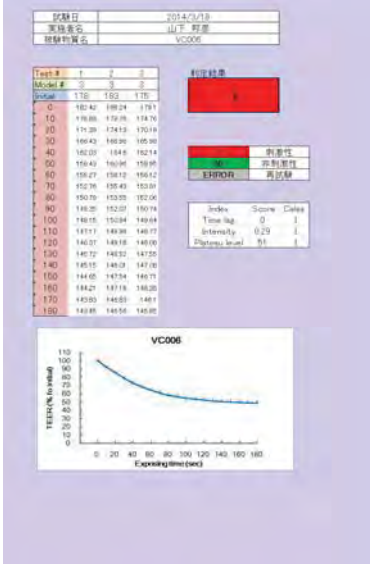


3rd

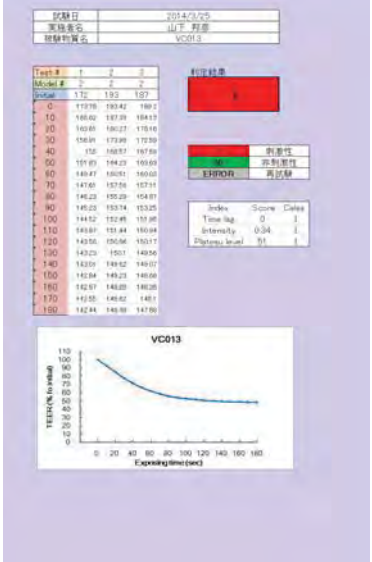


Daicel

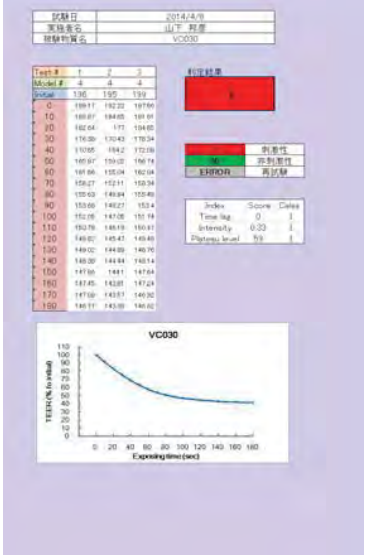
1st



2nd



3rd



Appendix8.5.2

Statistical analysis report for Vitrigel-EIT validation study (Phase 2)

Appendix: Submitted data sheets

Takuto Nakayama
Takashi Sozu

Department of Management Science, Faculty of Engineering,
Tokyo University of Science

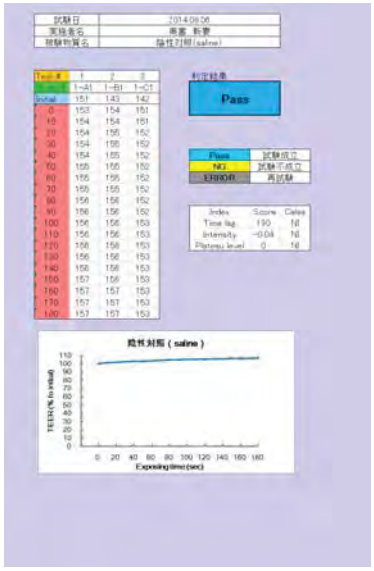
8 February 2016

Chemicals

No.	Chemicals	GHS
Negative control	Saline	
Positive control	Benzalkonium chloride	
Reference control	Ethanol	
8	n,n-Dimethylguanidine sulfate	Not classified
9	Toluene	
10	Gluconolactone	
3	Cyclopentanol	Category 2A/2B
7	α-Hexylcinnamaldehyde	
5	2-Methyl-1-pentanol	Category 2B
1	Imidazole	Category 1
2	Cyclohexanol	
4	Sodium Dodecyl Sulphate	
6	Sodium salicylate	

Negative control

FDSC

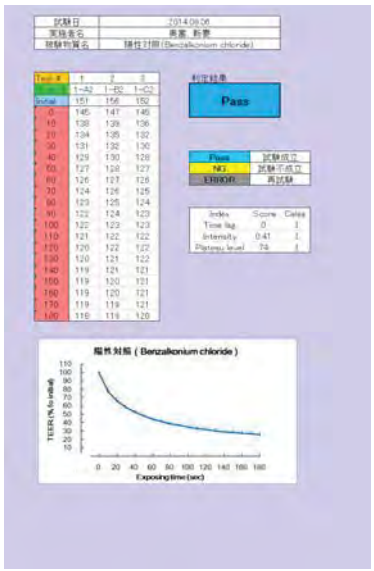


BoZo

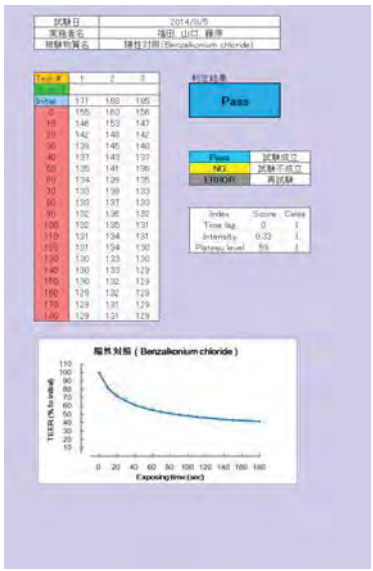
Daicel

Positive control

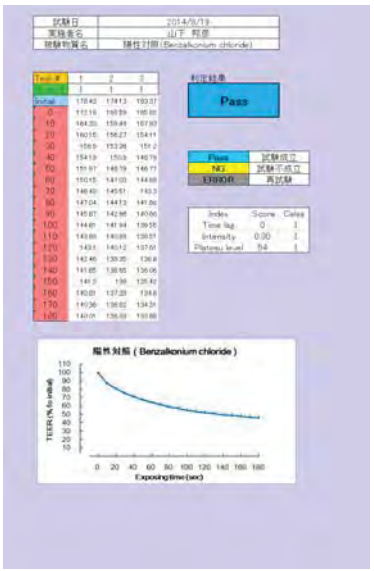
FDSC



BoZo

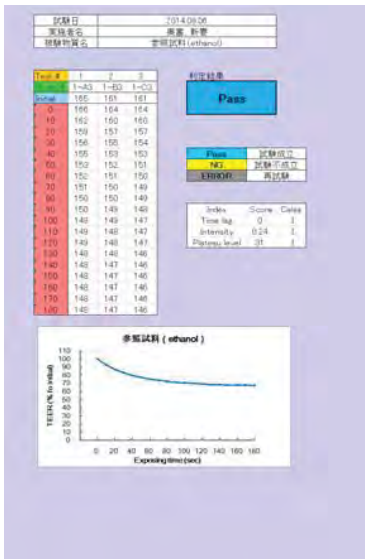


Daicel

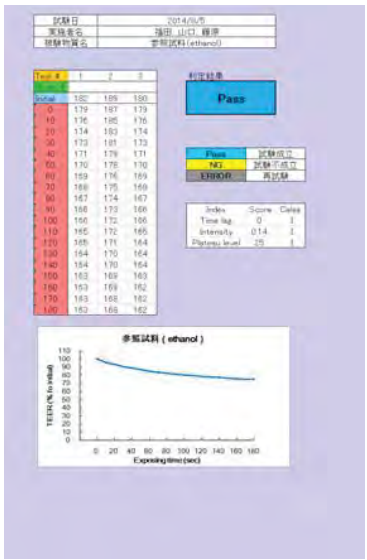


Reference

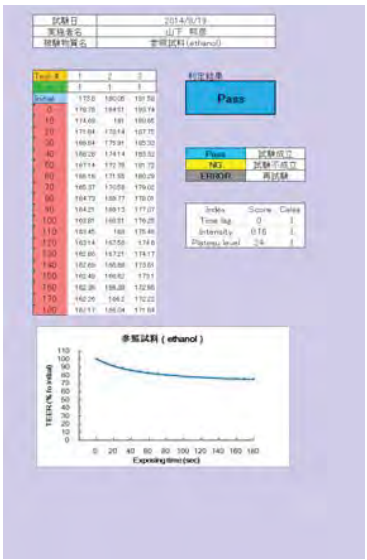
FDSC



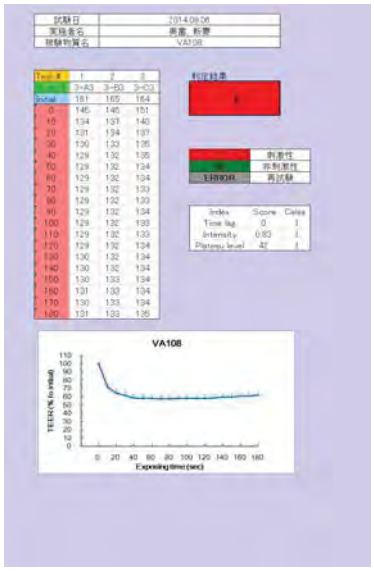
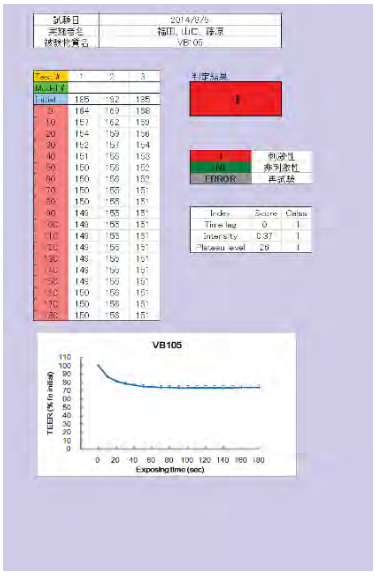
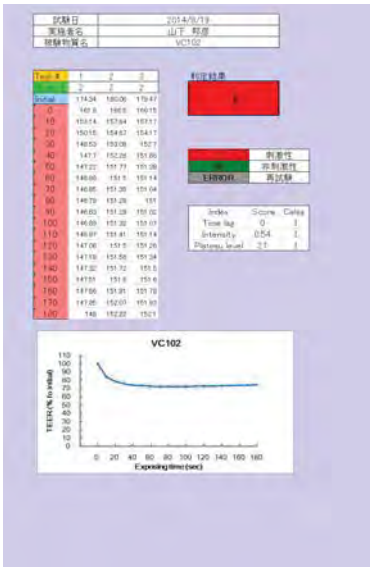
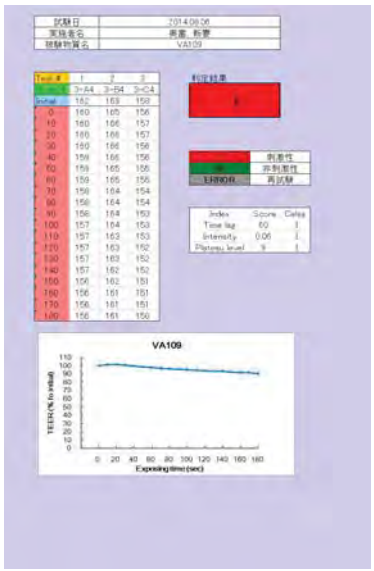
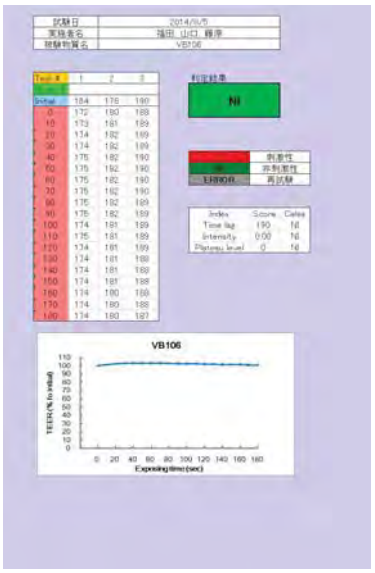
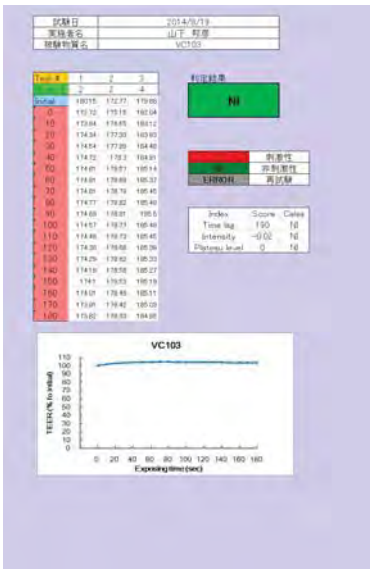
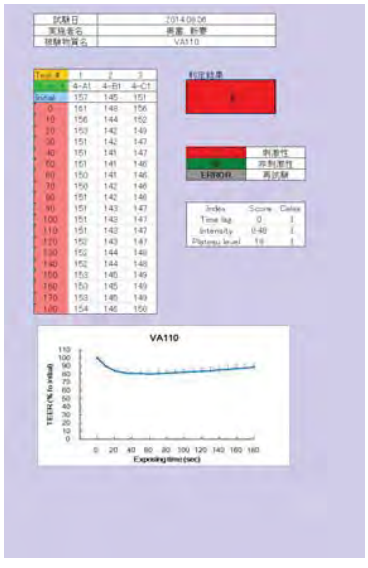
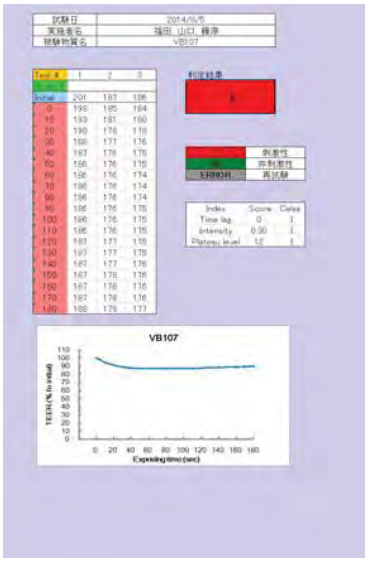
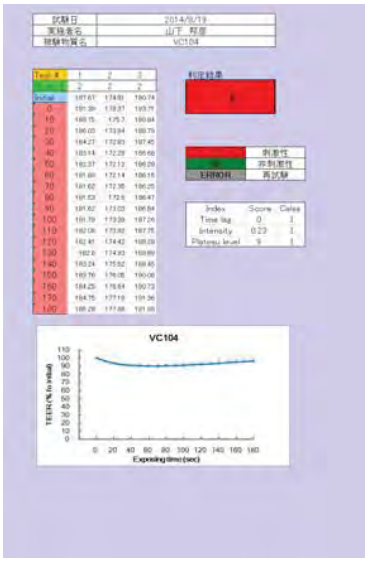
BoZo



Daicel



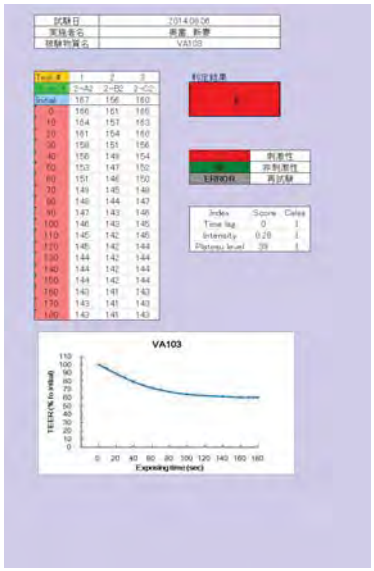
Not classified

FDSC	BoZo	Daicel
No.8		
		
No.9		
		
No.10		
		

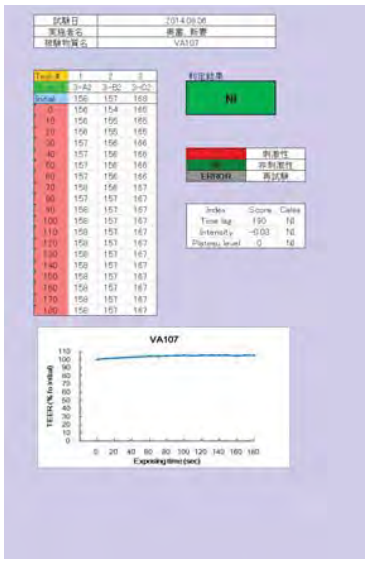
Category 2A/2B

FDSC

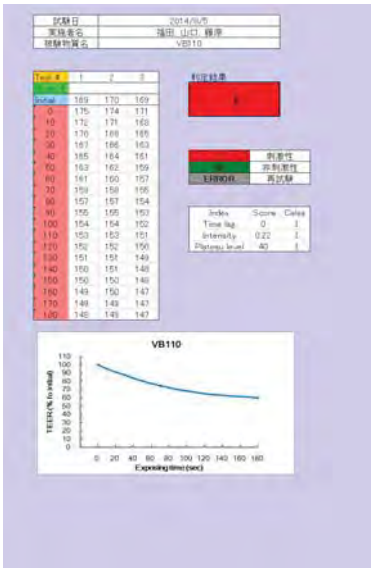
No.3

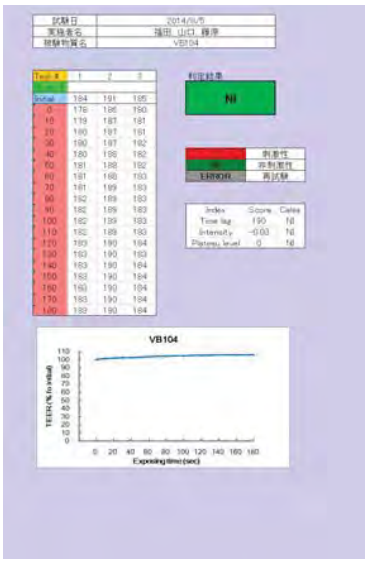


No.7

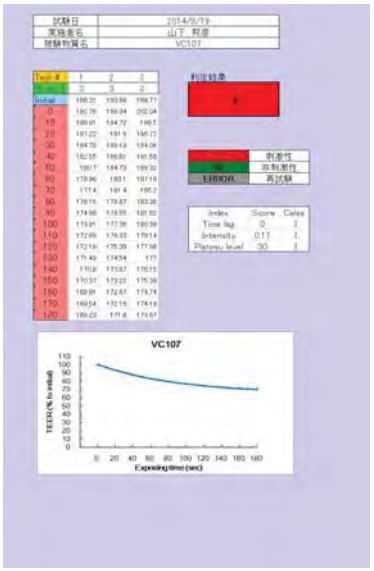


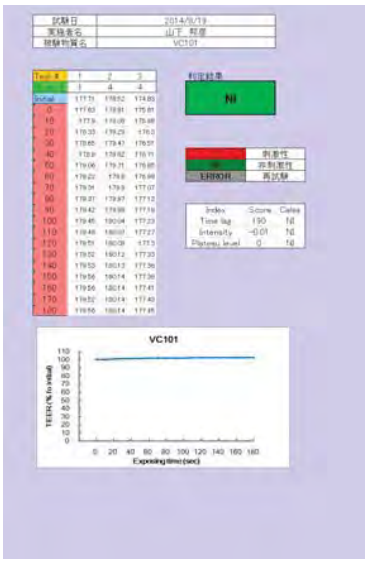
BoZo





Daicel

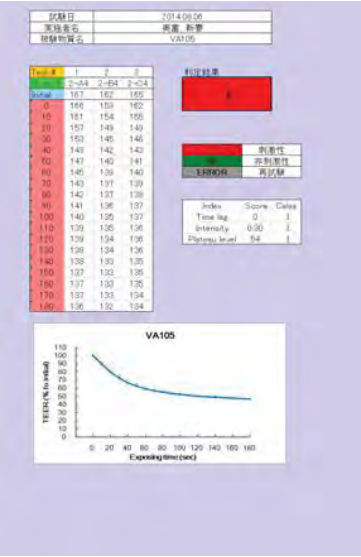




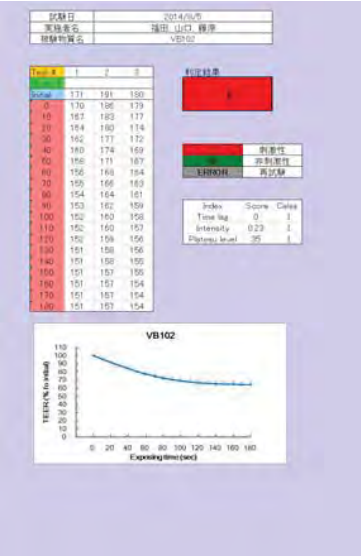
Category 2B

FDSC

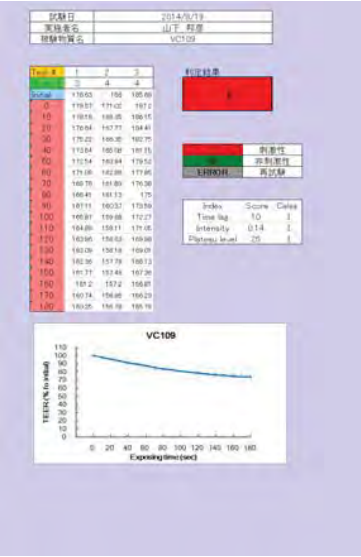
No.5



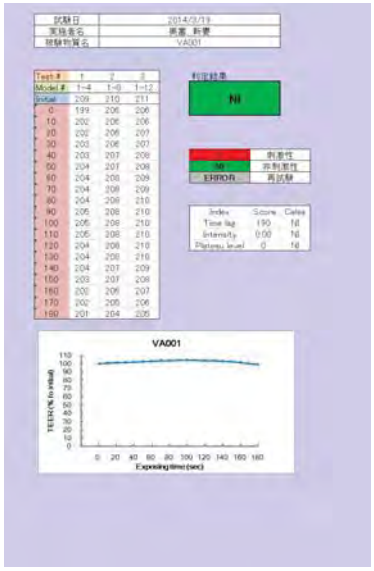
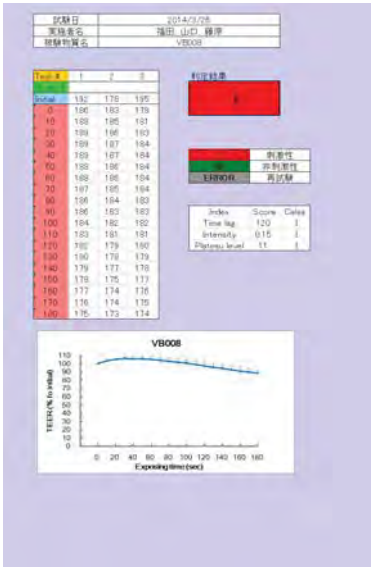
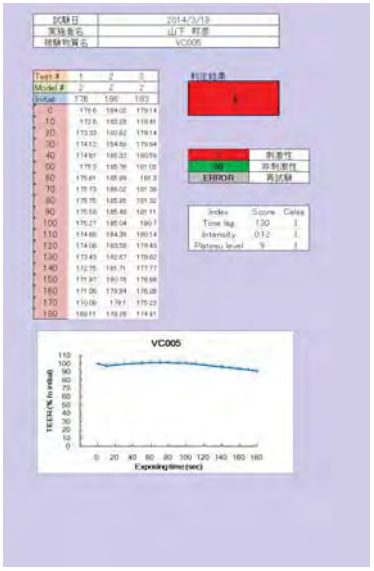
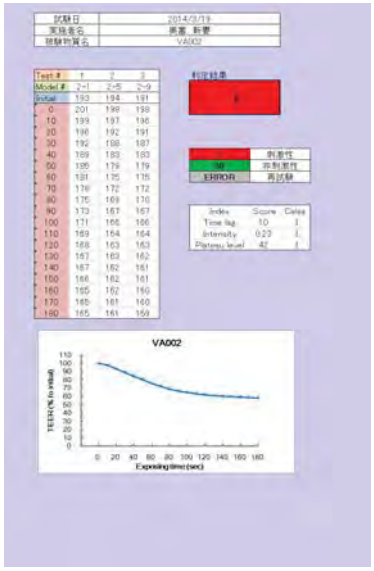
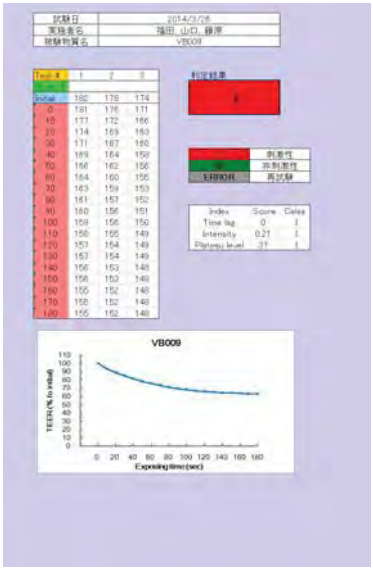
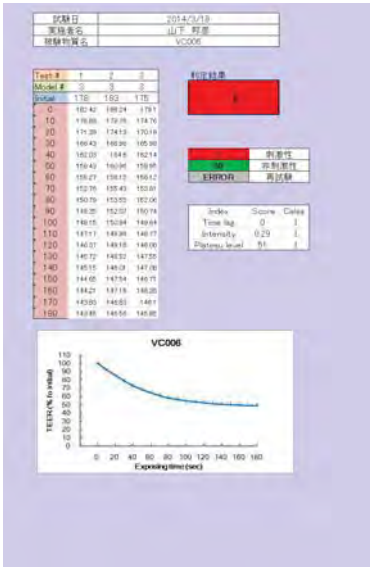
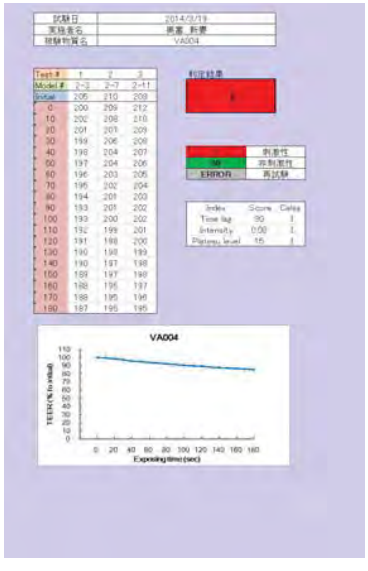
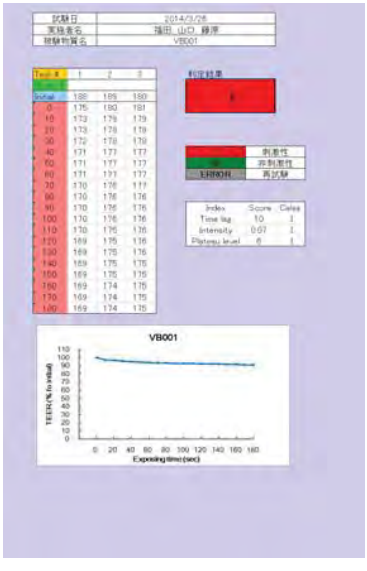
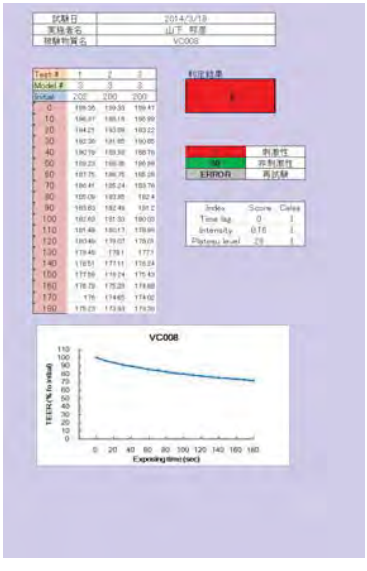
BoZo



Daicel



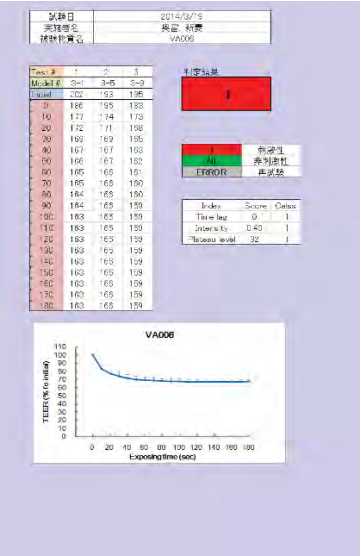
Category1

FDSC	BoZo	Daicel
No.1		
		
No.2		
		
No.4		
		

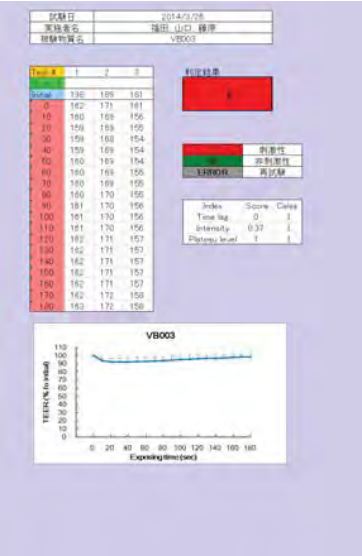
Category1

FDSC

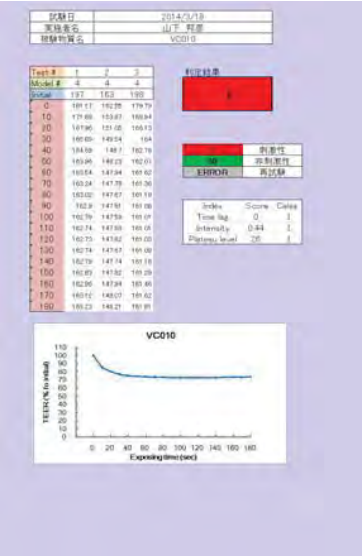
No.6



BoZo



Daicel



Appendix8.5.3

Statistical analysis report for Vitrigel-EIT validation study (Phase 2 additional) Appendix: Submitted data sheets

Takuto Nakayama
Takashi Sozu

Department of Management Science, Faculty of Engineering,
Tokyo University of Science

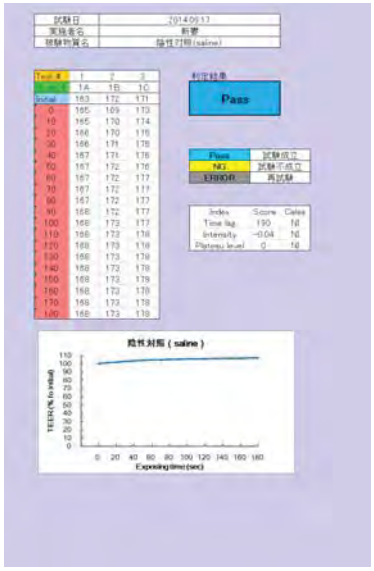
8 February 2016

Chemicals

No.	Chemicals	GHS
Negative control	Saline	
Positive control	Benzalkonium chloride	
Reference control	Ethanol	
1	Imidazole	Category 1

Negative control

FDSC

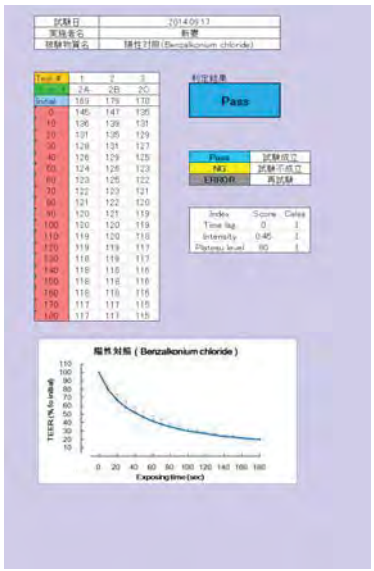


BoZo

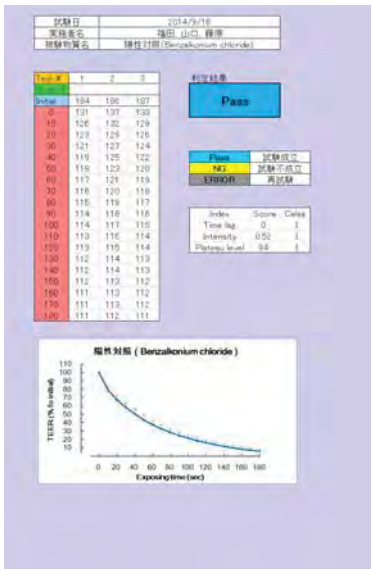
Daicel

Positive control

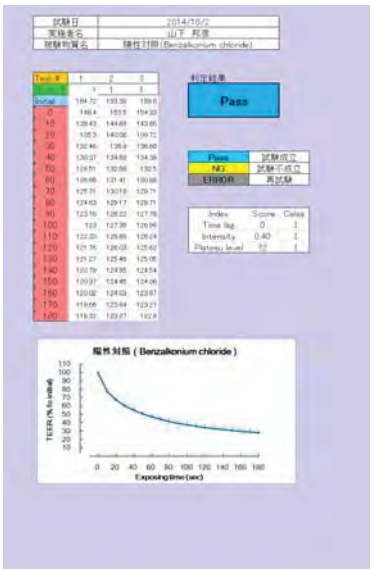
FDSC



BoZo

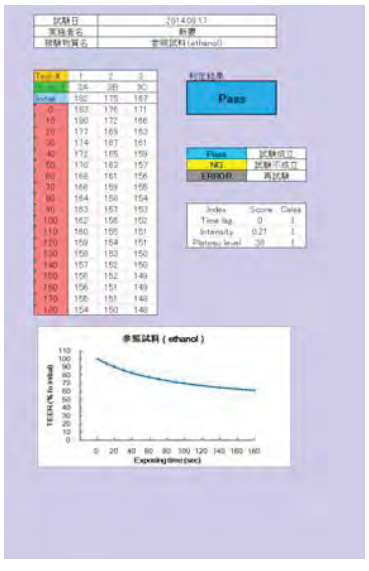


Daicel

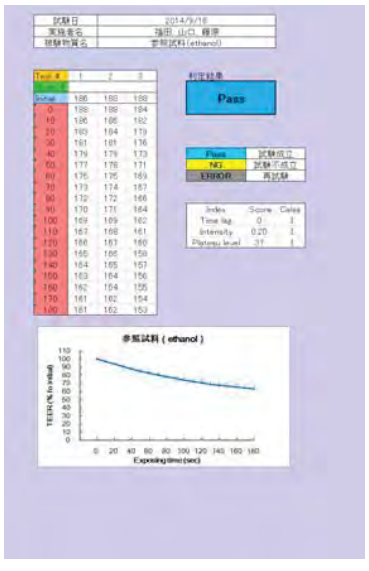


Reference

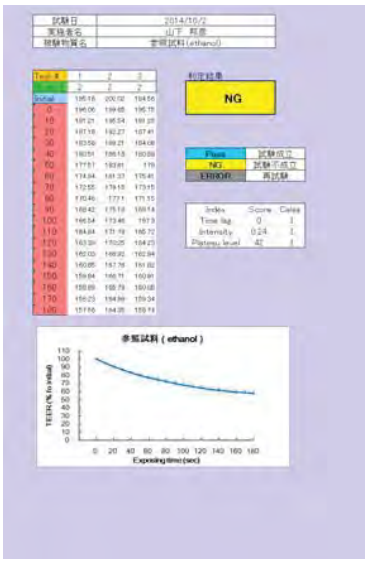
FDSC



BoZo



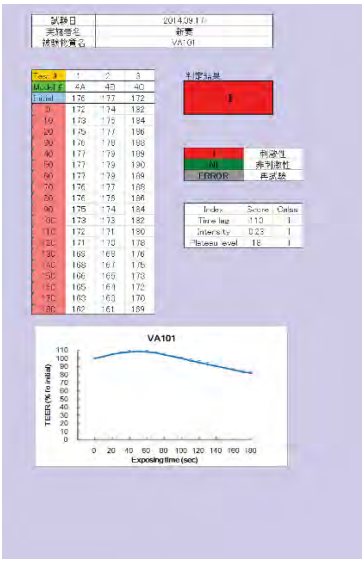
Daicel



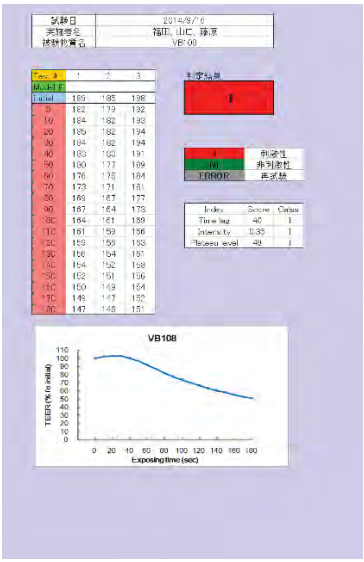
Category1

FDSC

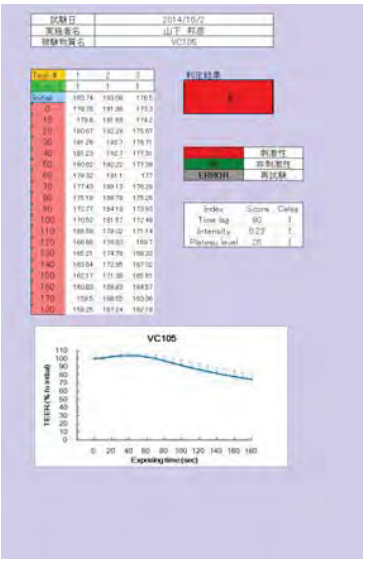
No.1



BoZo



Daicel



Appendix8.5.4

Statistical analysis report for Vitrigel-EIT validation study (Phase 3)

Appendix: Submitted data sheets

Takuto Nakayama
Takashi Sozu

Department of Management Science, Faculty of Engineering,
Tokyo University of Science

1 June 2015

Chemicals

No.	Chemicals	GHS
Negative control	Saline	
Positive control	Benzalkonium chloride	
Reference control	Ethanol	
21	Methyl amyl ketone	Not classified
22	2-(n-Dodecylthio)ethanol	
23	iso-Octylthioglycolate	
24	2,4-Difluoronitrobenzene	
25	tetra-Aminopyrimidine sulfate	
26	2,4-Pentanedio	
27	iso-Octyl acrylate	
28	Silicon Dioxide n-Hydrate	
29	Potassium tetrafluoroborate	
34	n,n-Dimethylguanidine sulfate	
35	Toluene	
36	Gluconolactone	
11	gamma-Butyrolactone	Category2A,2A/2B, 2B
12	Methyl acetate	
13	Myristyl alcohol	
14	2,6-Dichlorobenzoyl chloride	
15	Dibenzyl phosphate	
16	Sodium chloroacetate	
17	1-(2-Propoxy-1-methylethoxy)-2-propanol	
18	Camphene	
19	Ethyl-2-methylacetoacetate	
20	Propylene glycol propyl ether	
31	2-Methyl-1-pentanol	
33	α-Hexylcinnamaldehyde	
37	Cyclopentanol	

No.	Chemicals	GHS
1	2,5-Dimethyl-2,5-hexanediol	Category 1
2	2-Benzyl-4-chlorophenol	
3	2,2-Dimethyl butanoic acid	
4	Captan	
5	tetra-N-Octylammonium bromide	
6	Butanol	
7	3-(2-Aminoethylamino)propyl]trimethoxysilane	
8	Dodecyl sodium sulfate	
9	m-Phenylenediamine	
10	Tetraethylene glycol	
30	Imidazole	
32	Sodium salicylate	

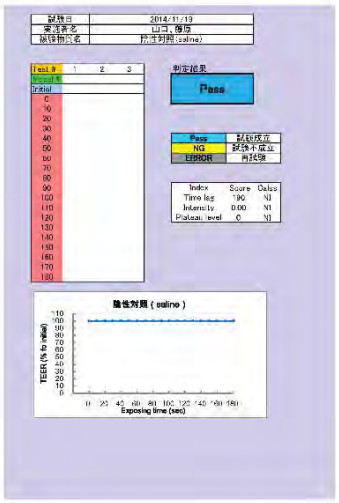
Negative control

FDSC

BoZo

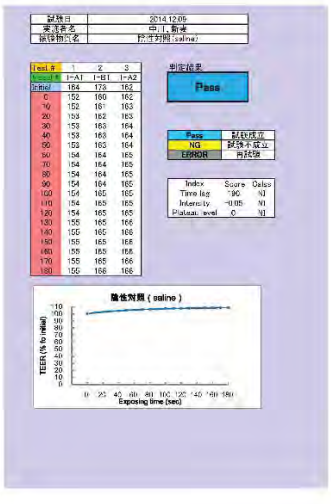
Daicel

1st



2nd

3rd



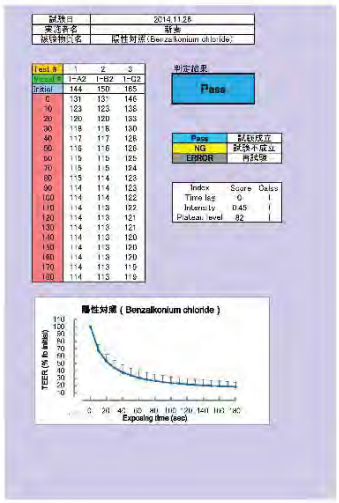
Positive control

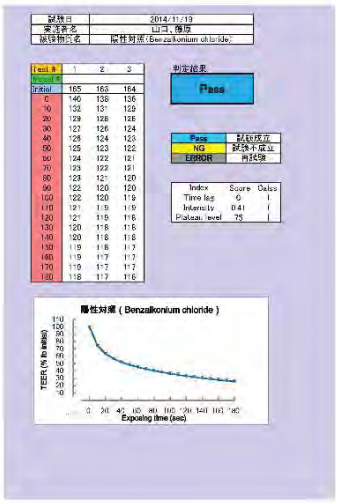
FDSC

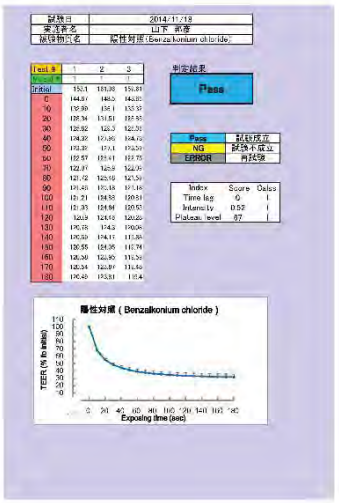
BoZo

Daicel

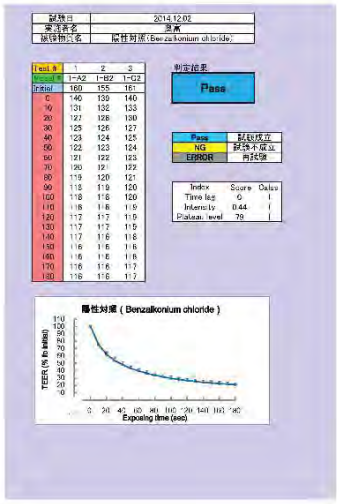
1st

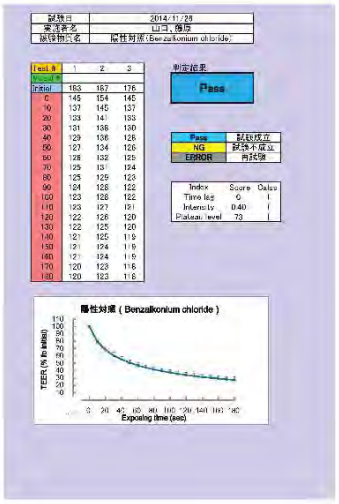


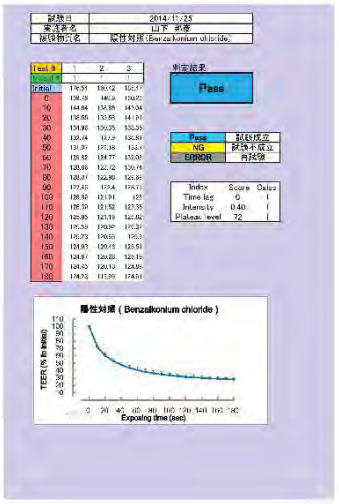




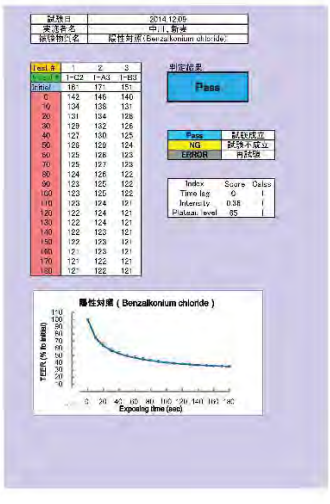
2nd

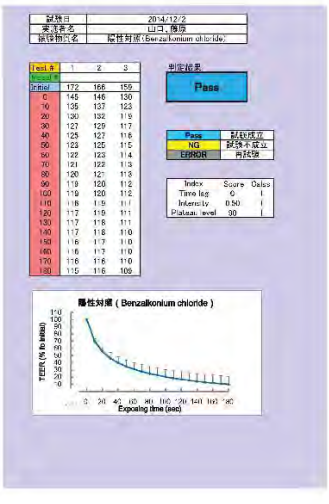






3rd





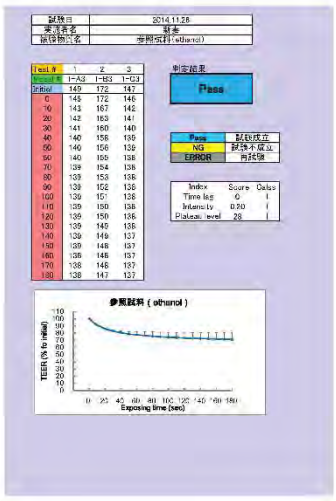
Reference

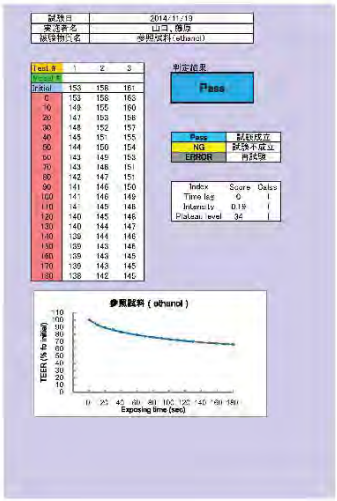
FDSC

BoZo

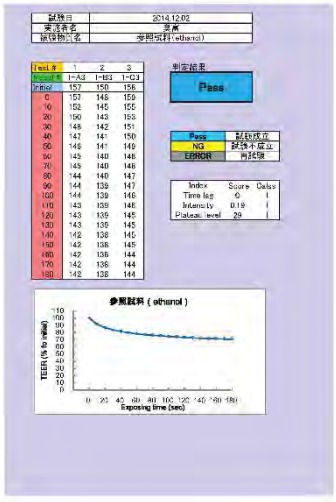
Daicel

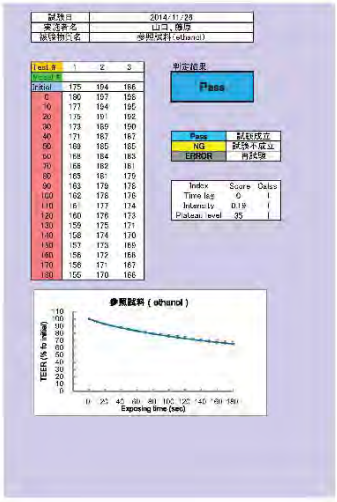
1st

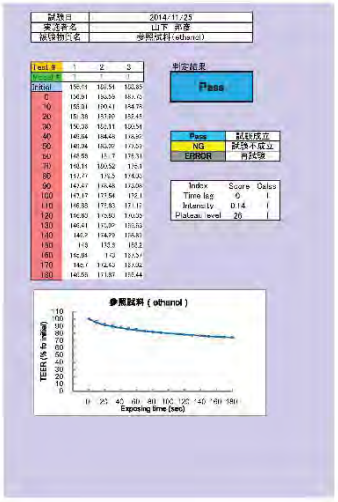




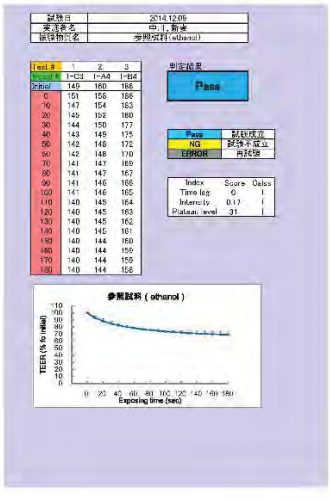
2nd

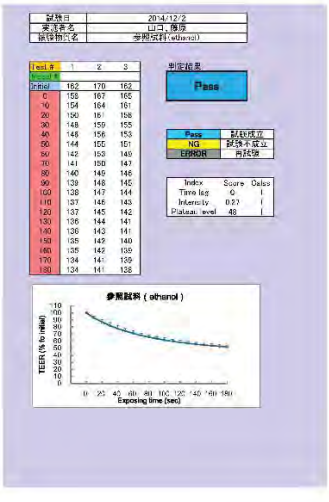






3rd





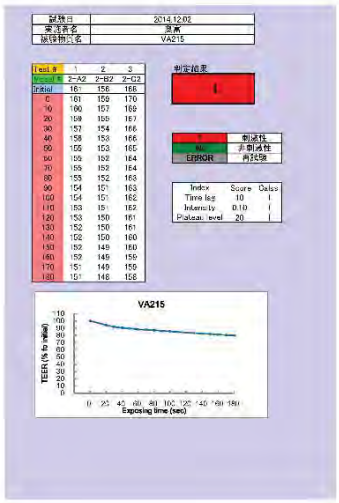
Not classified

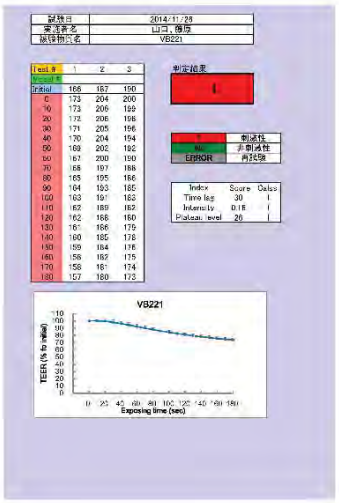
FDSC

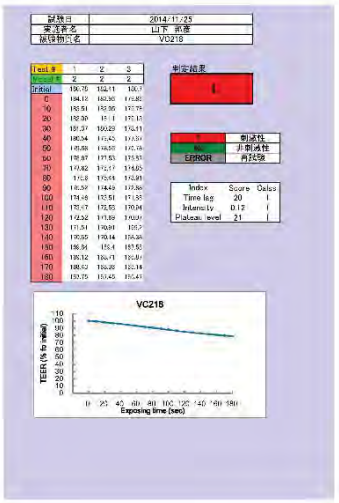
BoZo

Daicel

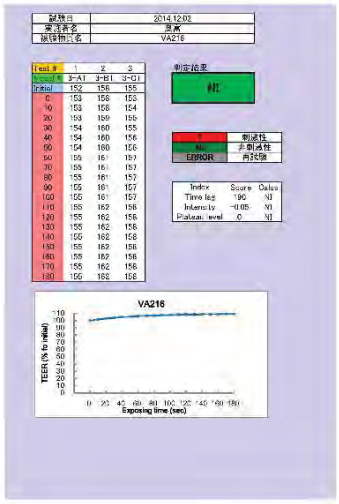
No.21

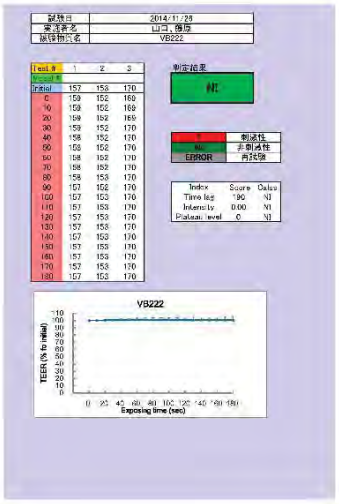


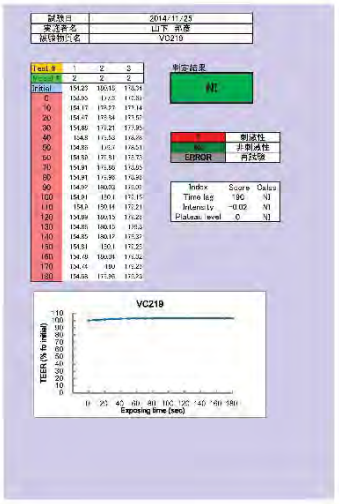




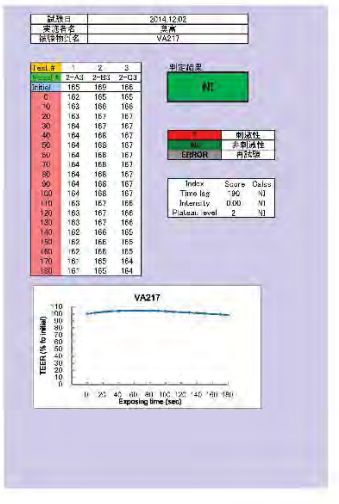
No.22

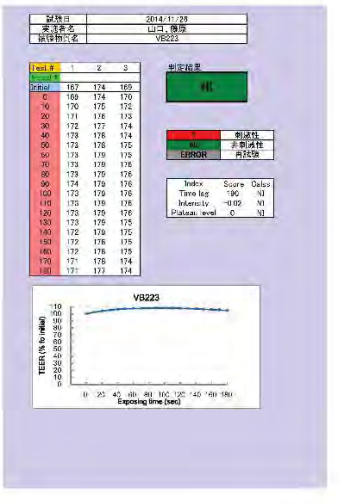


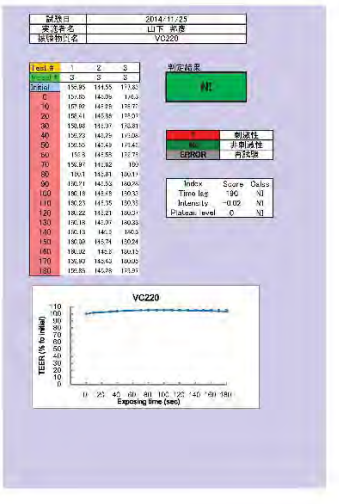




No.23







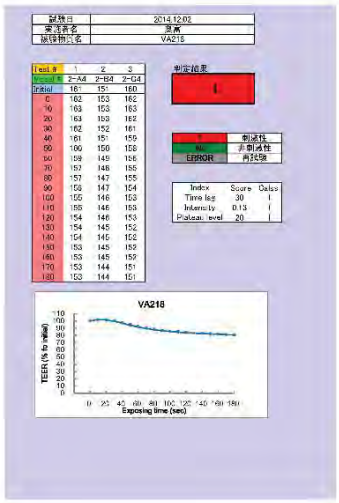
Not classified

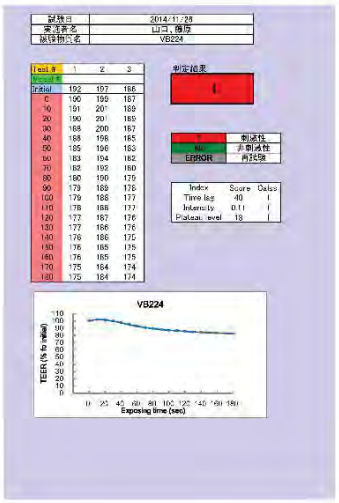
FDSC

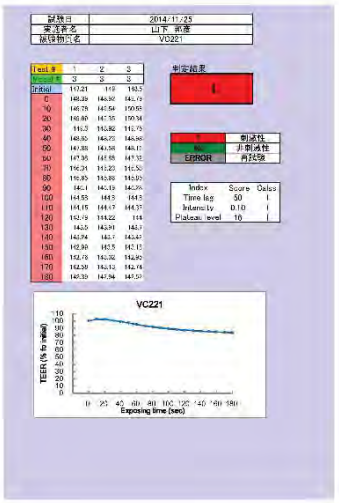
BoZo

Daicel

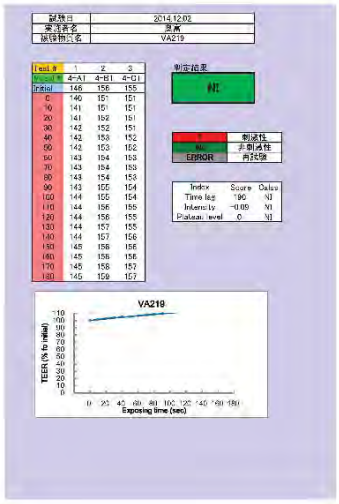
No.24

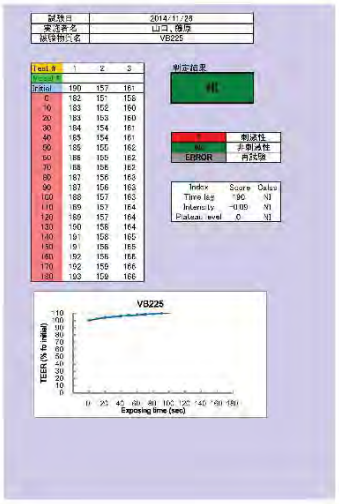


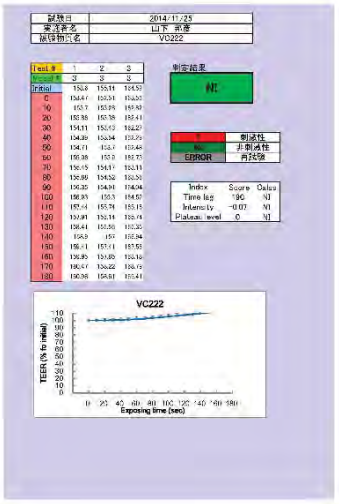




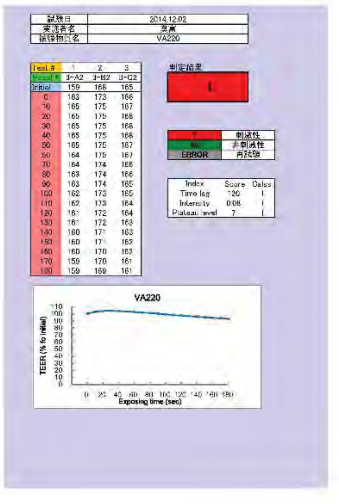
No.25

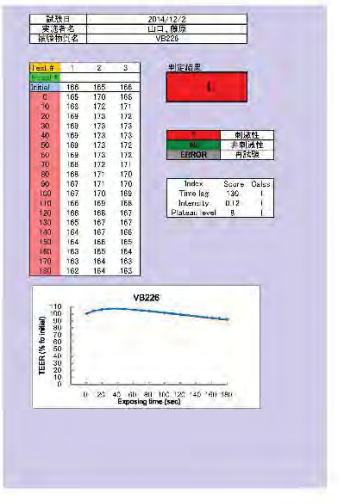


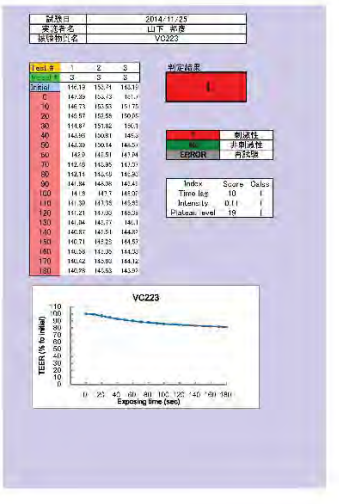




No.26







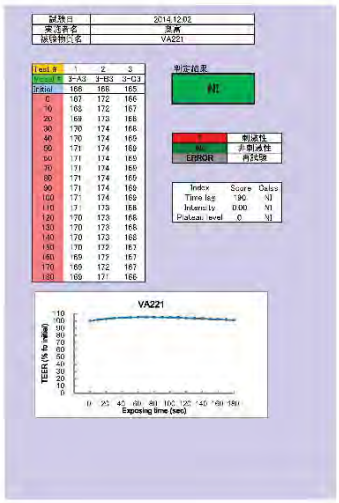
Not classified

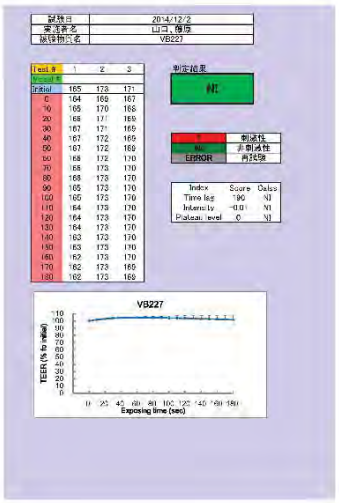
FDSC

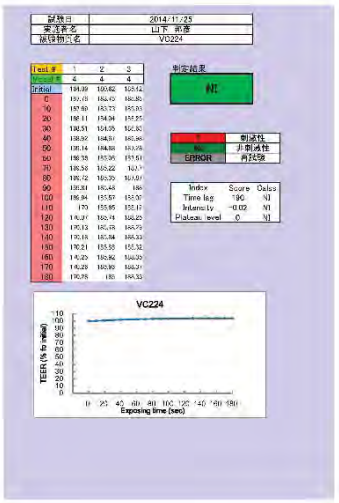
BoZo

Daicel

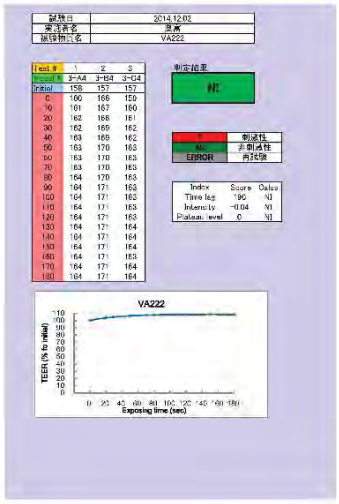
No.27

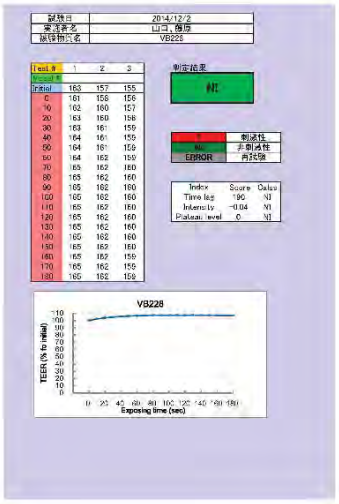


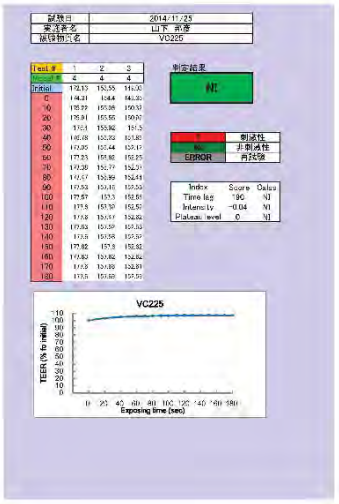




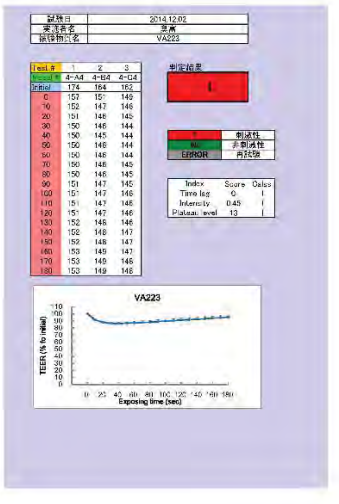
No.28

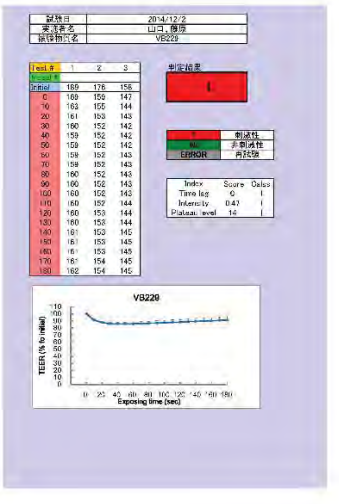


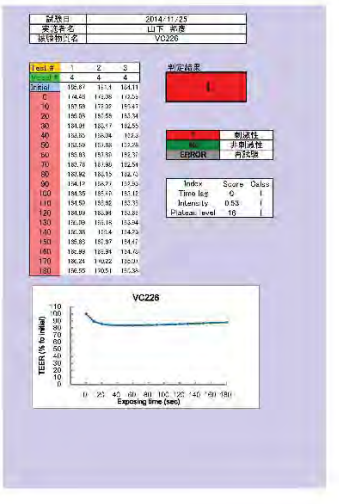




No.29







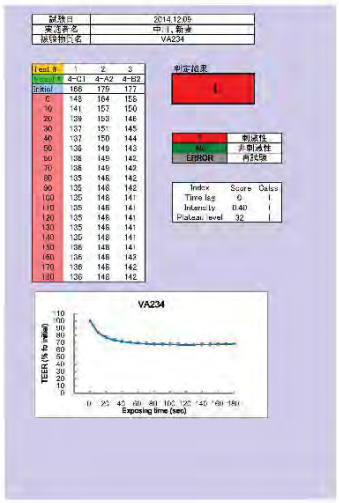
Not classified

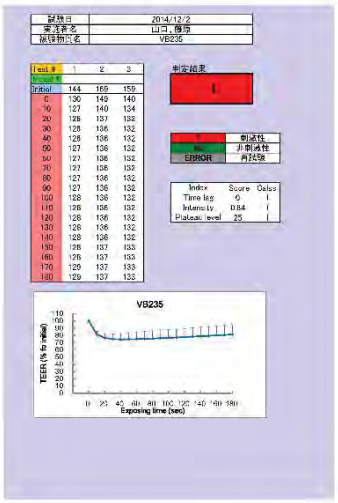
FDSC

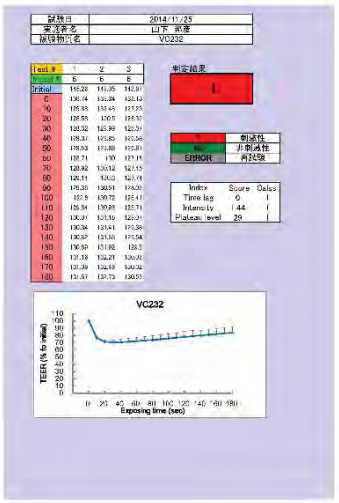
BoZo

Daicel

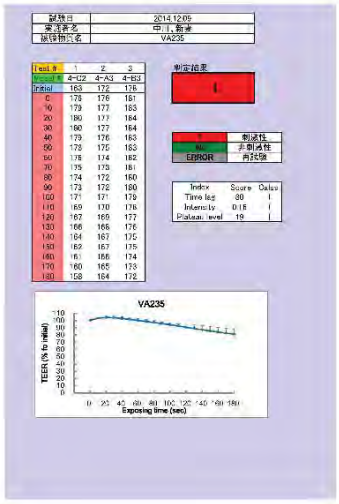
No.34

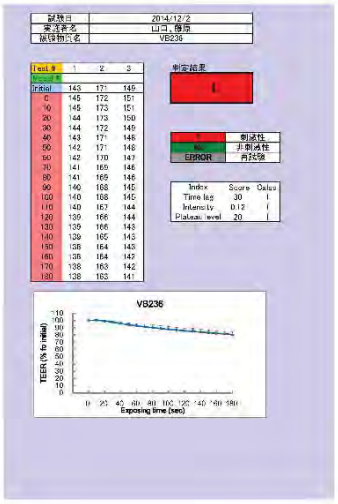


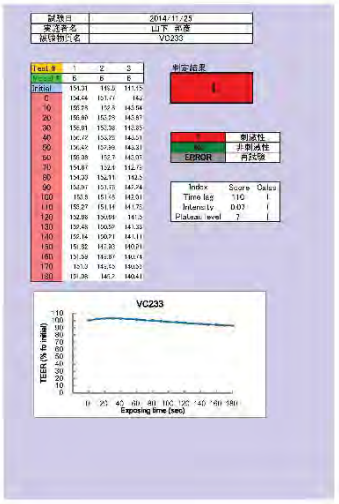




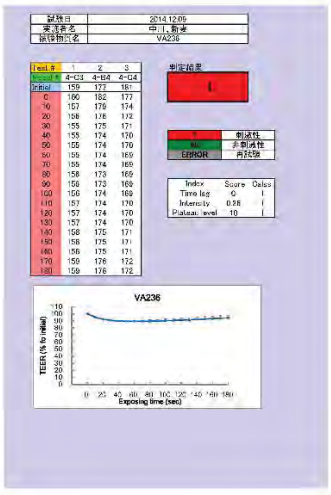
No.35

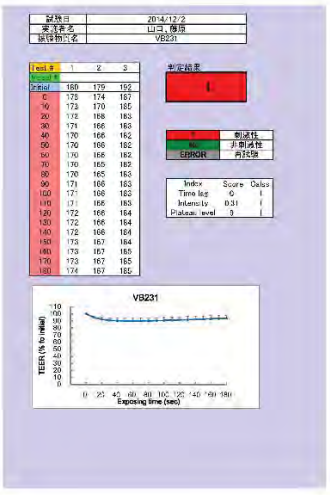


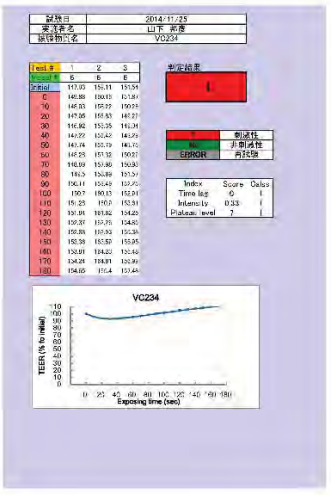




No.36







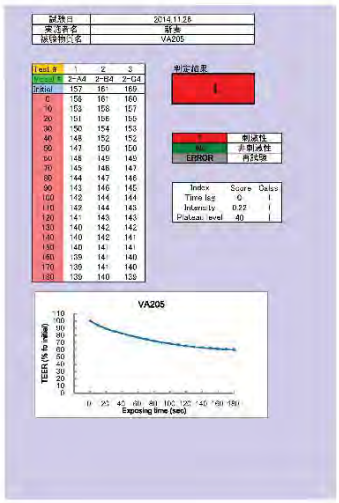
Category 2A, 2A/2B, 2B

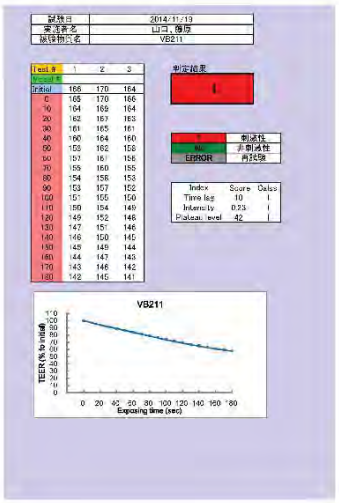
FDSC

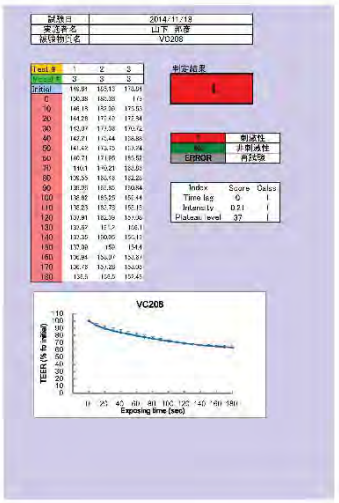
BoZo

Daicel

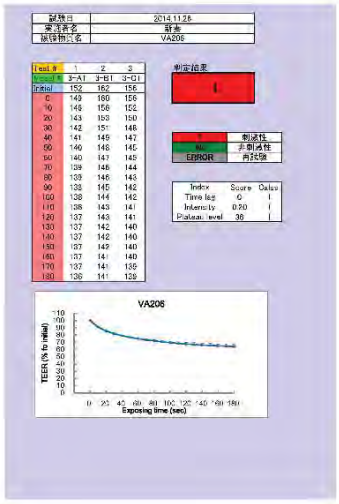
No.11

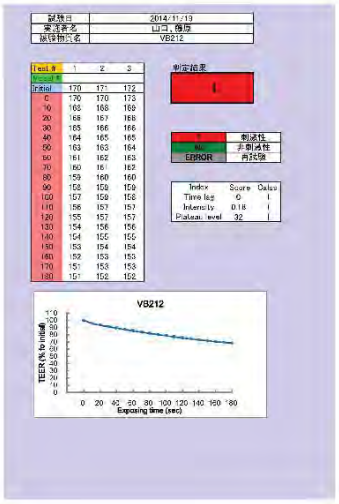


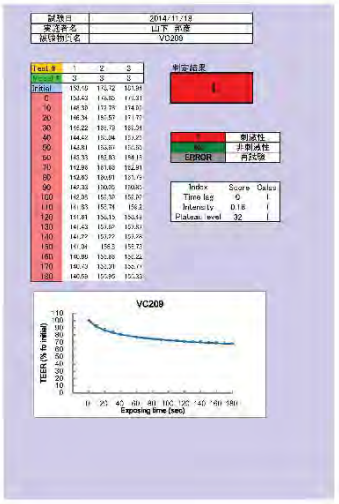




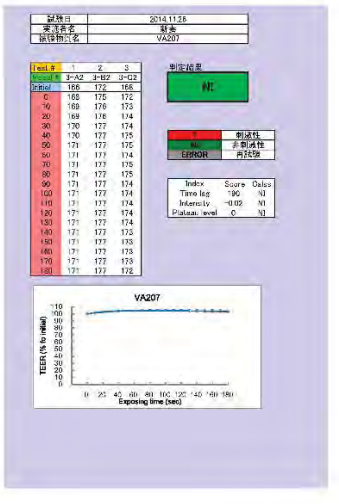
No.12

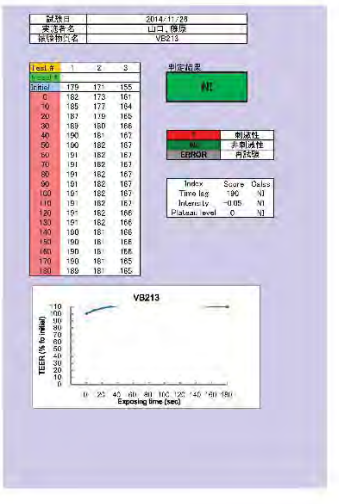


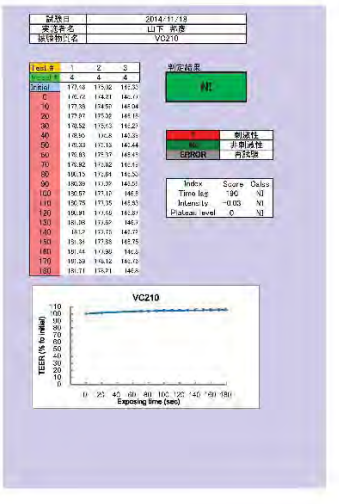




No.13







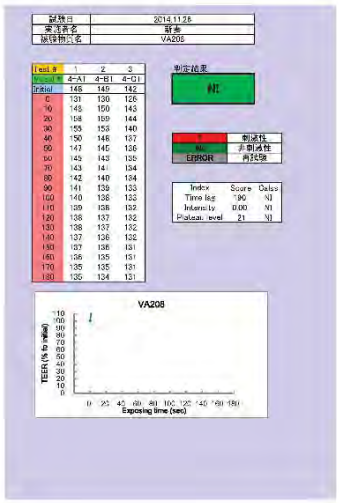
Category 2A, 2A/2B, 2B

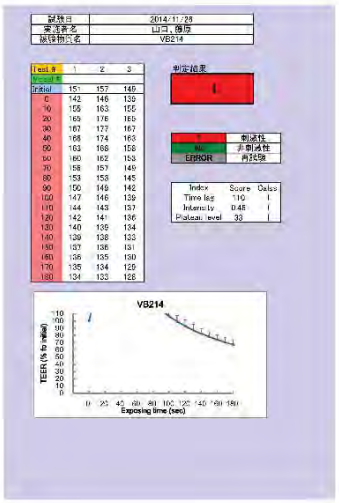
FDSC

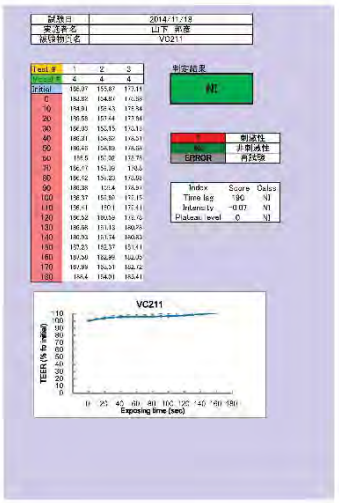
BoZo

Daicel

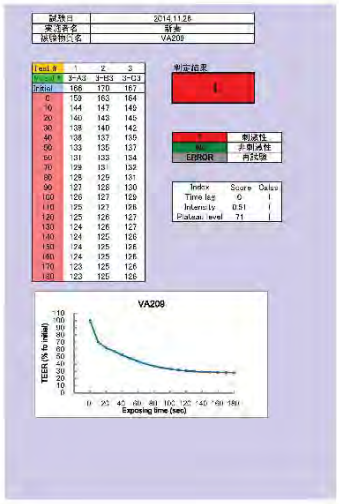
No.14

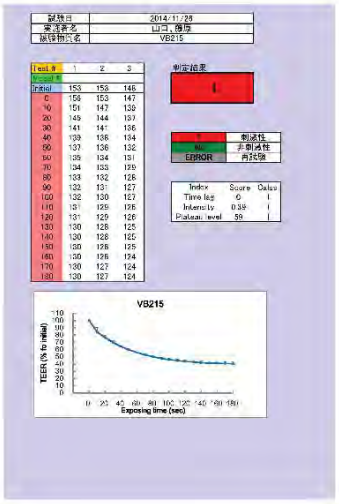


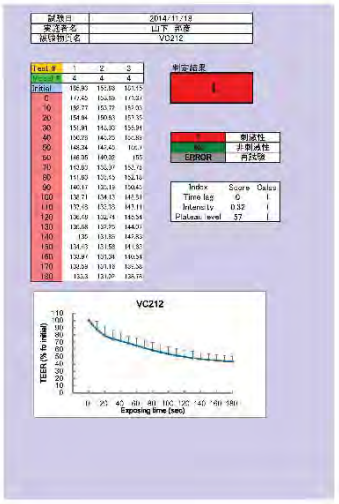




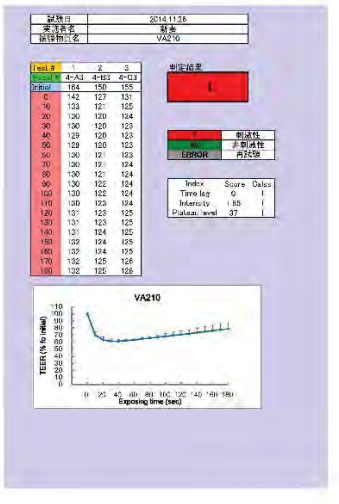
No.15

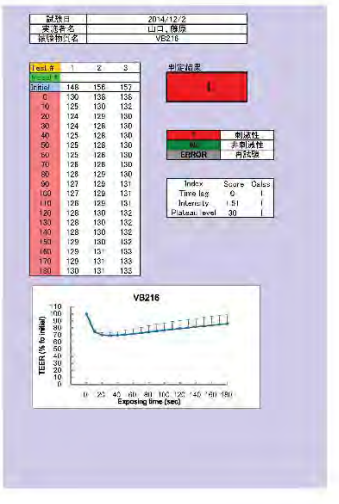


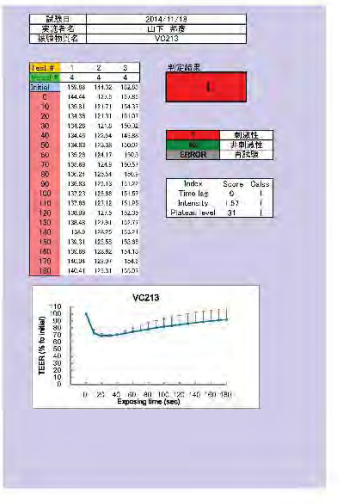




No.16







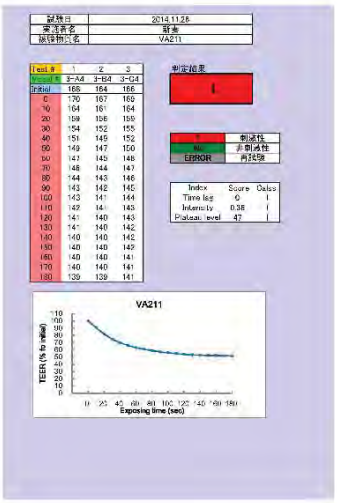
Category 2A, 2A/2B, 2B

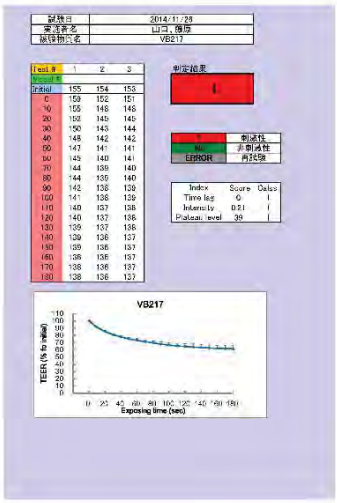
FDSC

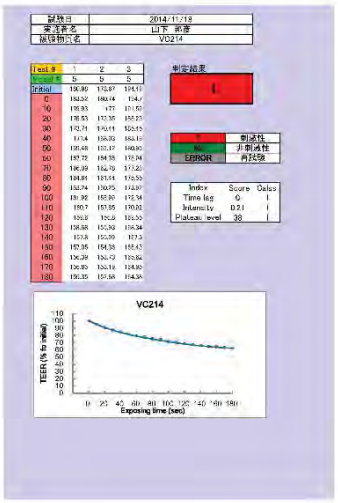
BoZo

Daicel

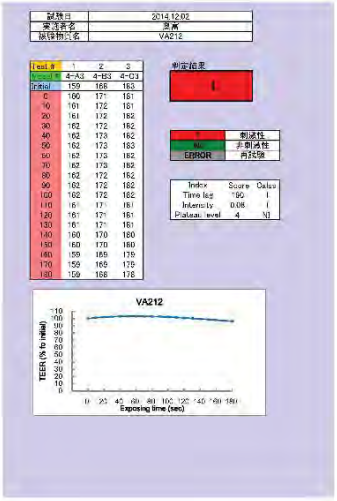
No.17

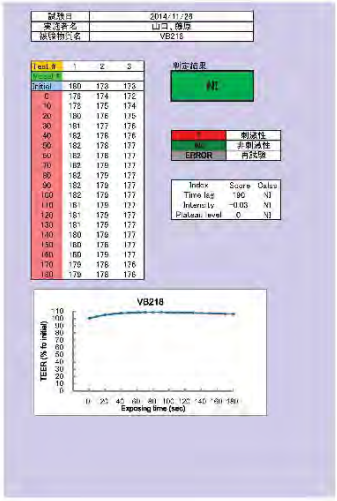


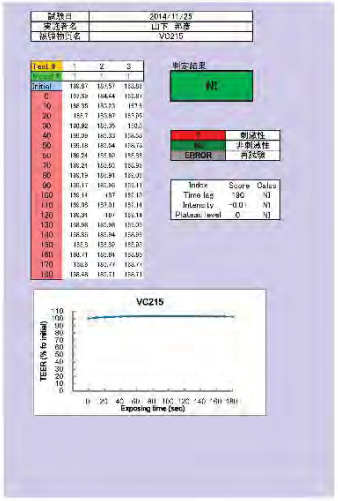




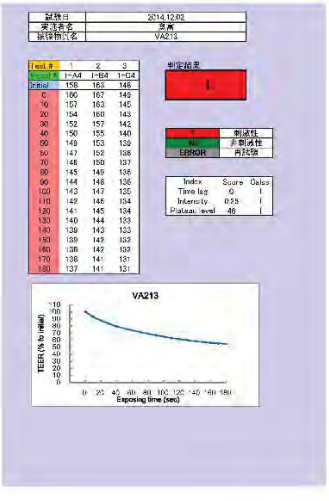
No.18

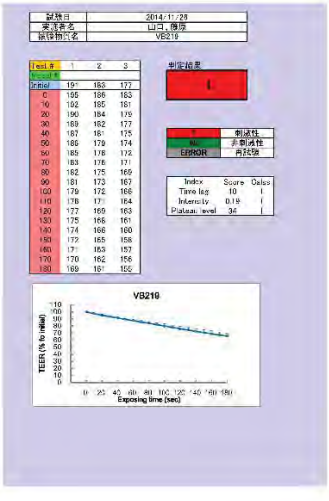


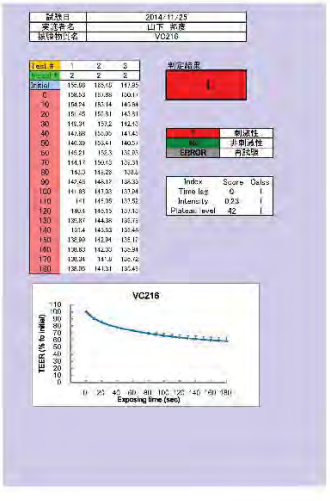




No.19







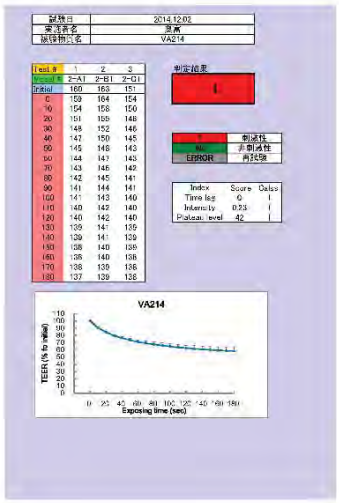
Category 2A, 2A/2B, 2B

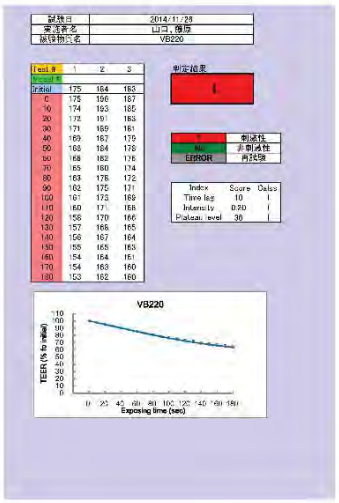
FDSC

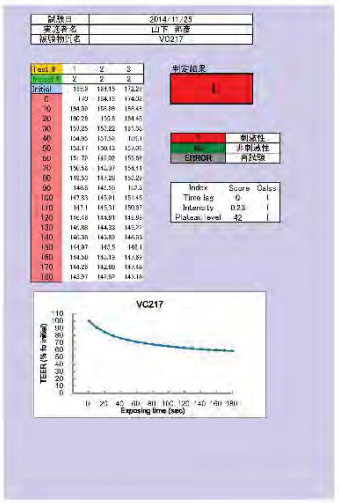
BoZo

Daicel

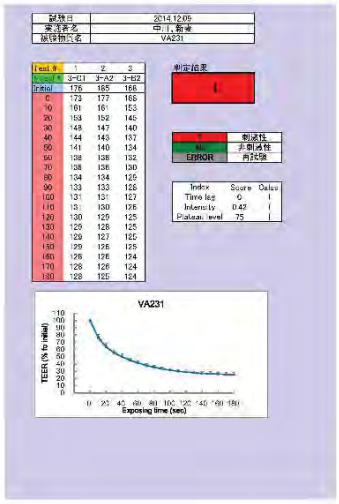
No.20

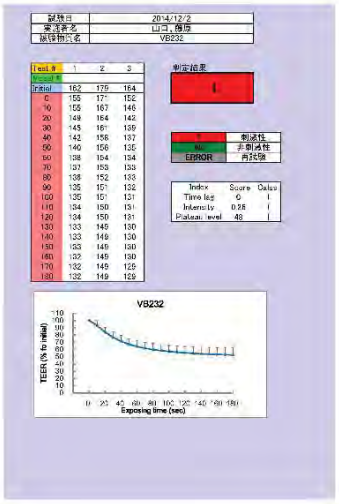


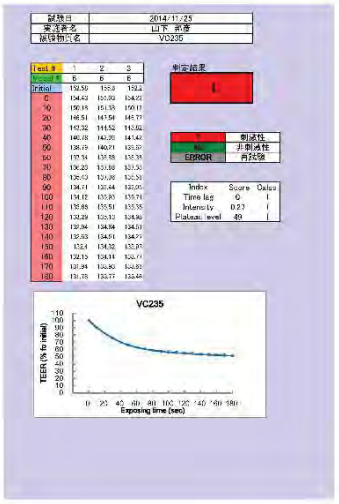




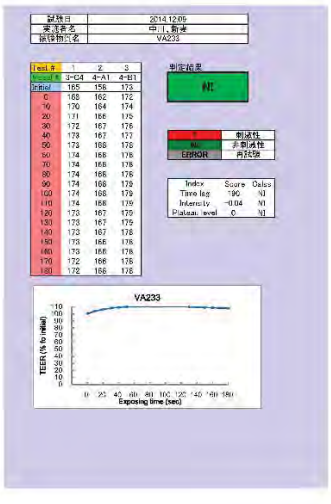
No.31

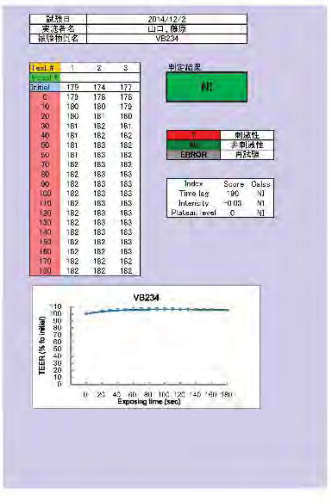


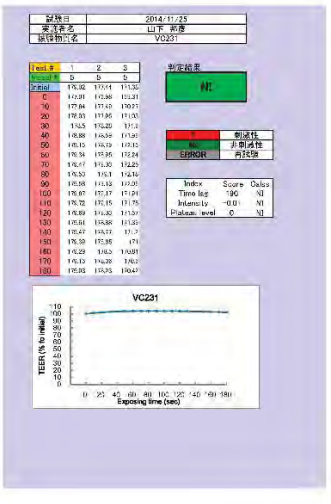




No.33



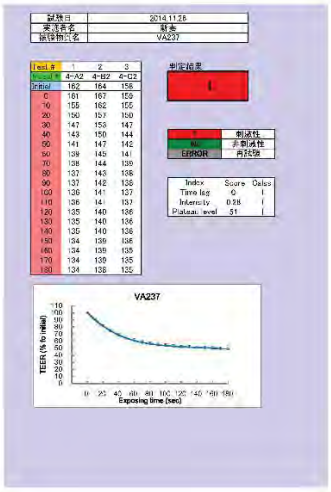




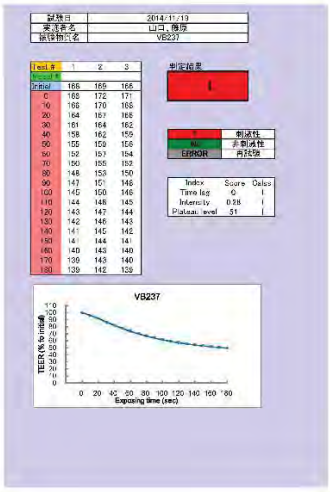
Category 2A, 2A/2B, 2B

FDSC

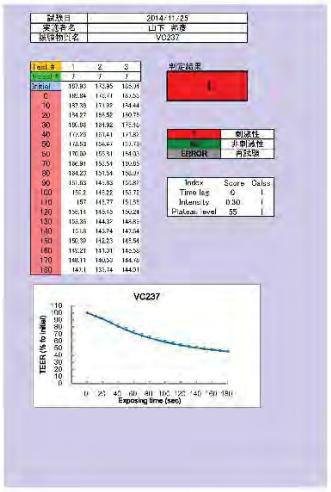
No.37



BoZo



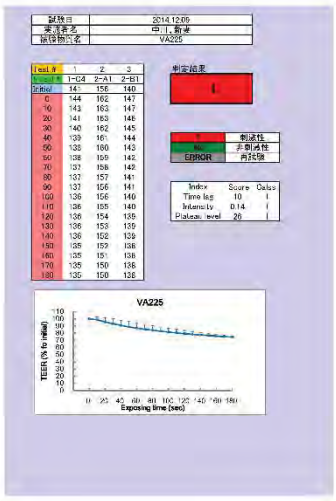
Daicel



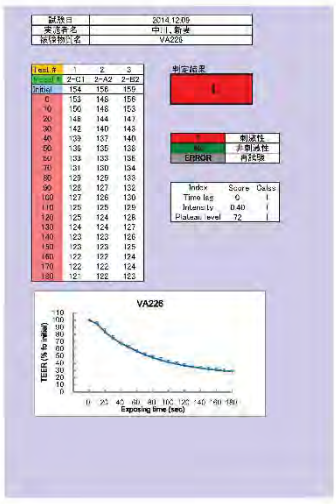
Category 1

FDSC

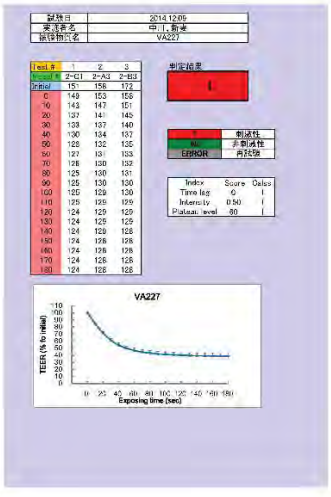
No.1



No.2

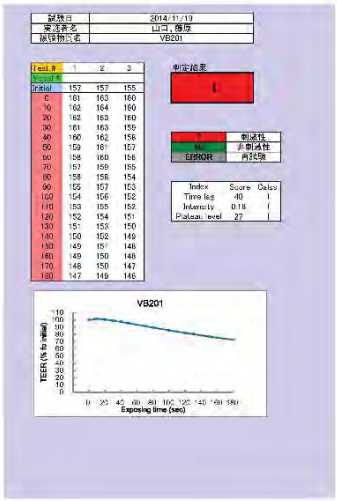


No.3

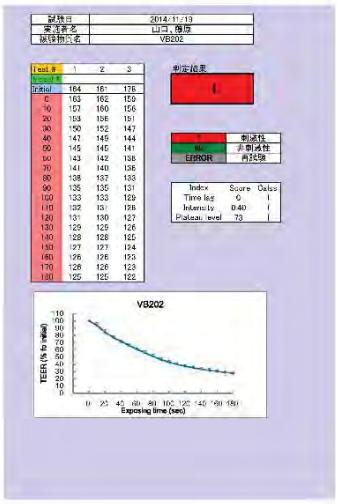


BoZo

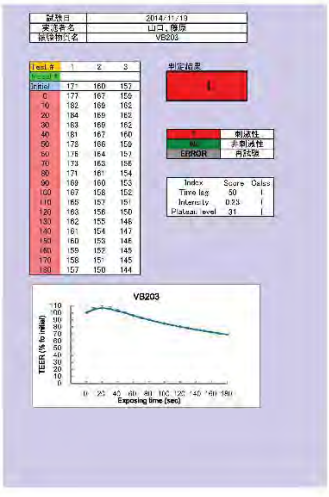
No.1



No.2

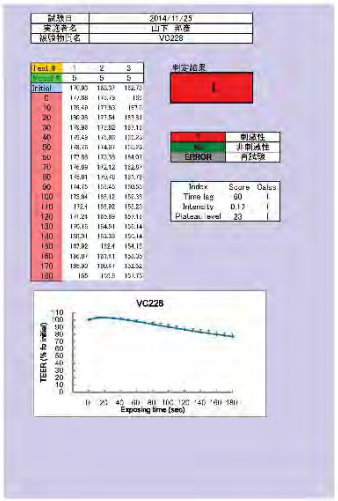


No.3

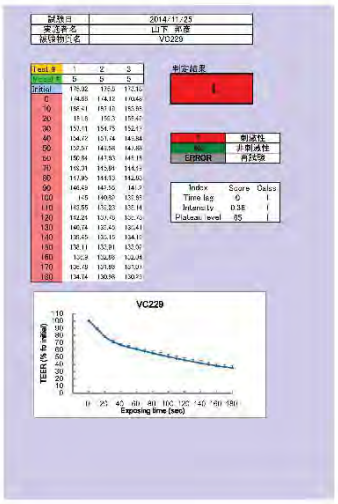


Daicel

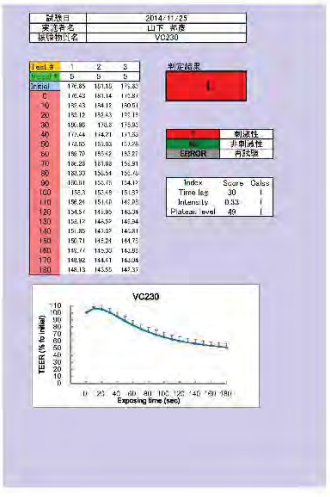
No.1



No.2



No.3



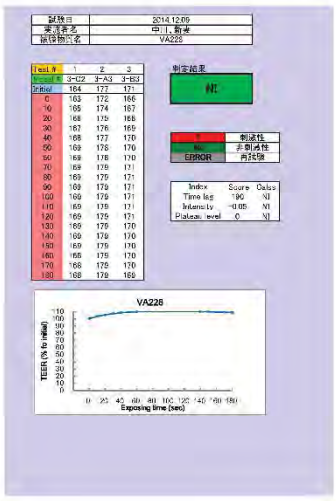
Category 1

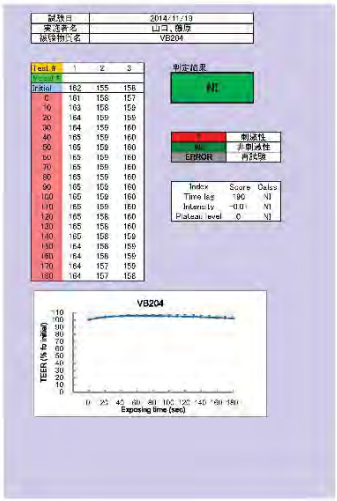
FDSC

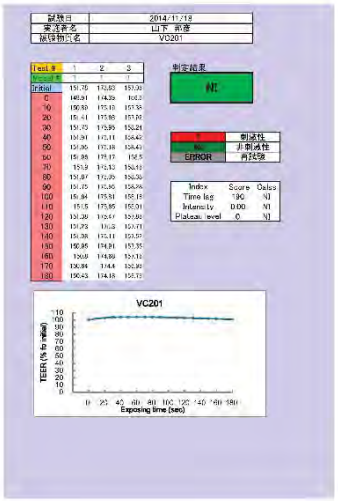
BoZo

Daicel

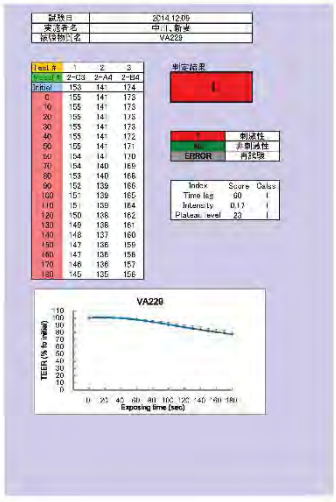
No.4

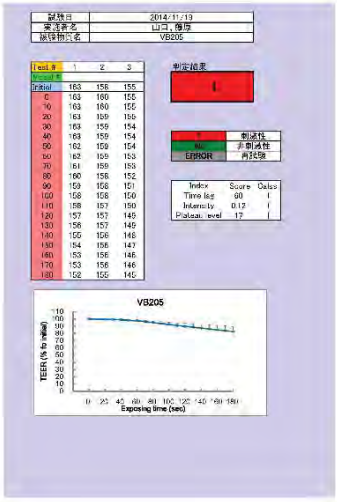


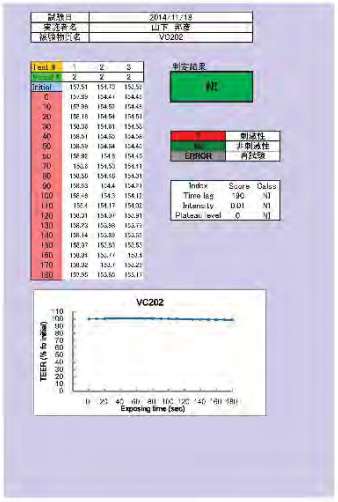




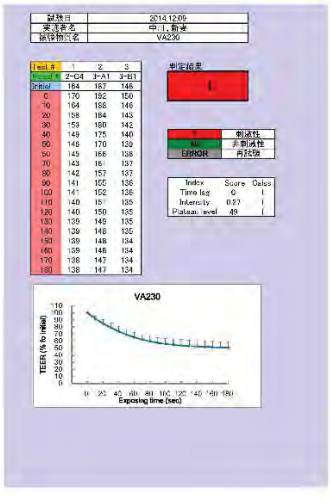
No.5

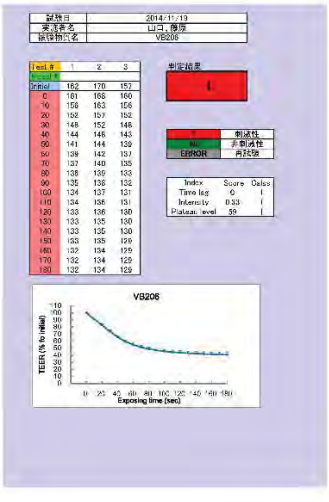


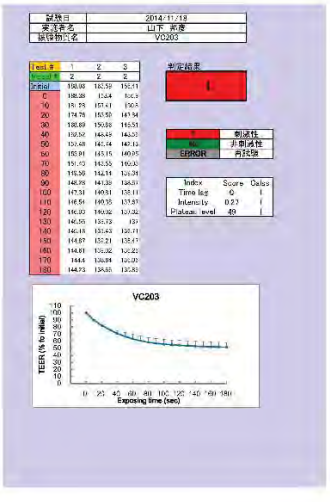




No.6







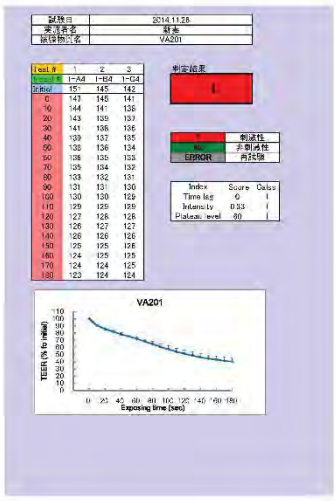
Category 1

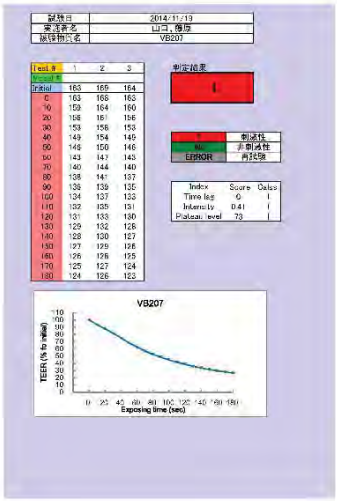
FDSC

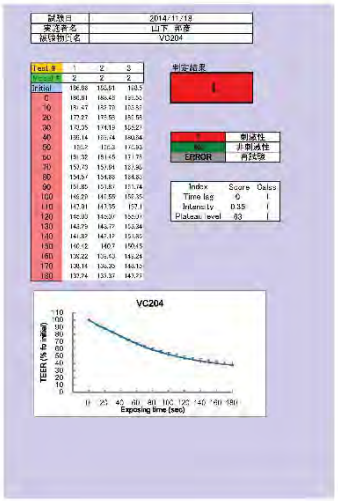
BoZo

Daicel

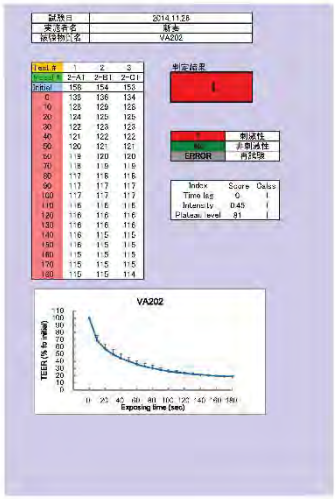
No.7

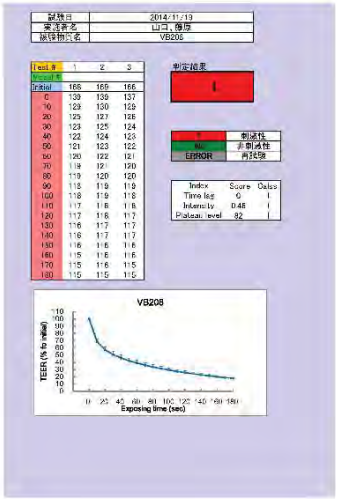


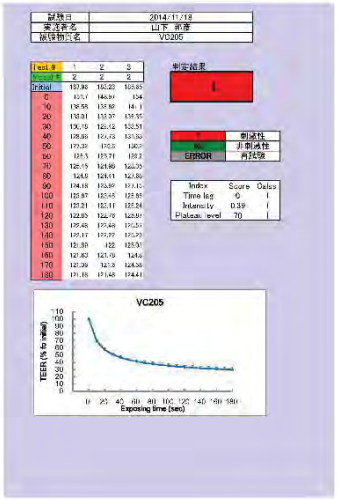




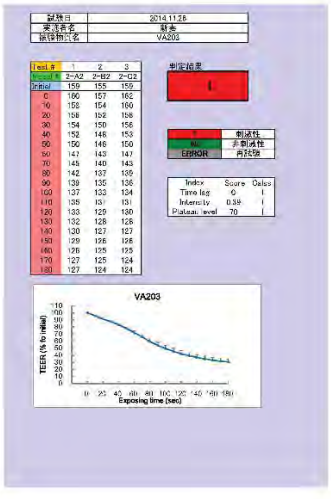
No.8

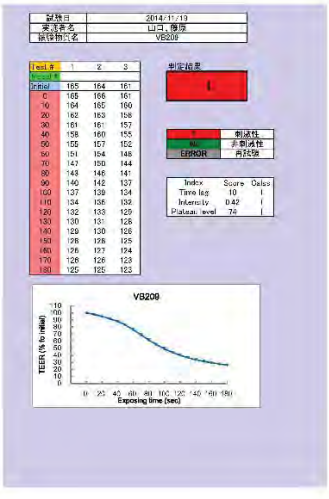


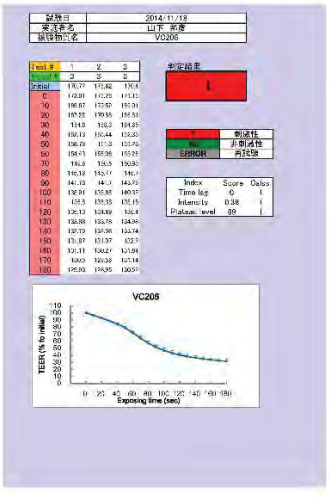




No.9







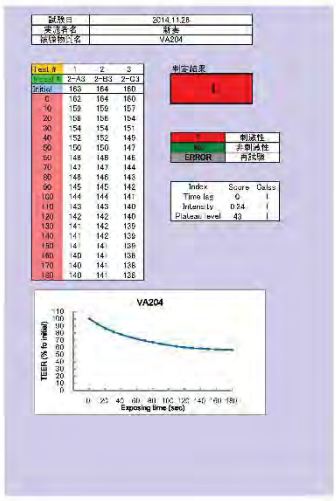
Category 1

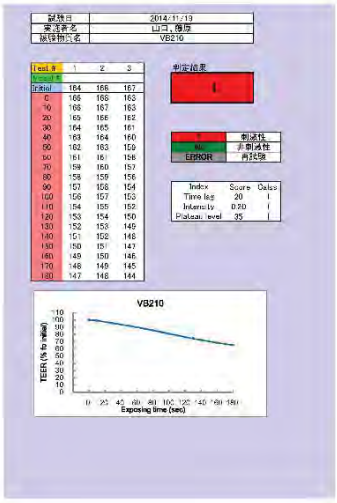
FDSC

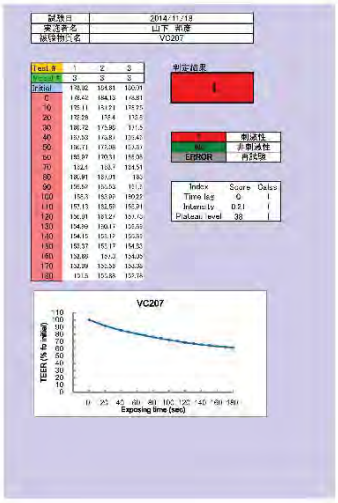
BoZo

Daicel

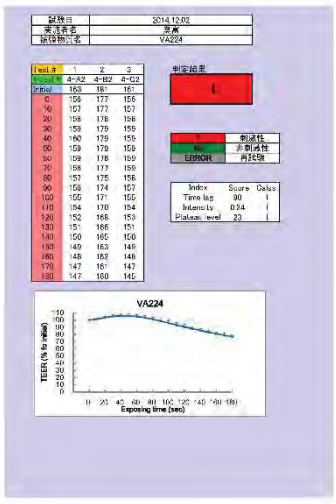
No.10

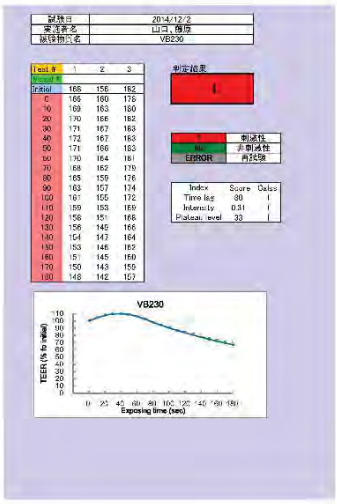


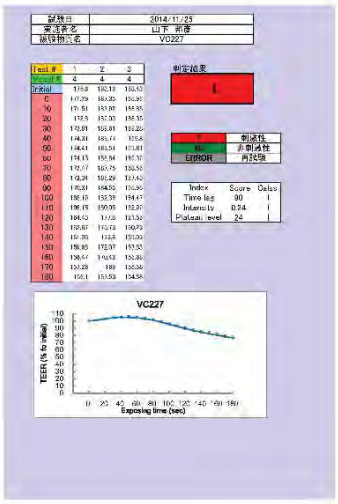




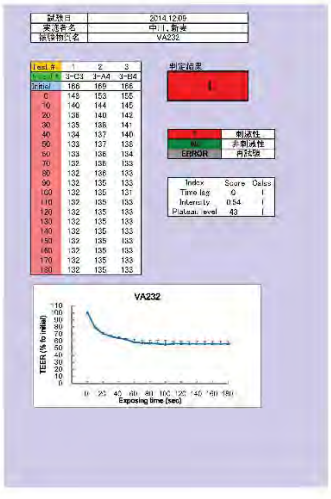
No.30

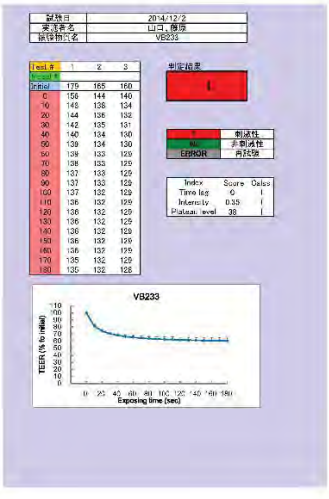


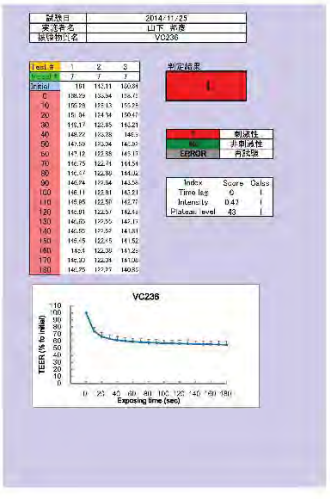




No.32







Appendix8.6

Report on the Quality assurance for Vitrigel-EIT validation study

2015/6/30

Vitrigel-EIT Validation Management Team (VMT)

In this report, the quality assurance (QA) was described for the Vitrigel-EIT test validation study.

The objective of this study was to evaluate the within- and between-laboratory reproducibility and predictability of the Vitrigel-EIT on eye irritation (consistency with the two categories, Irritant and Non-irritant) in accordance with an initial step of bottom-up approach.

As a complementary study, the validation management team (VMT) evaluated the predictability of the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (UN GHS) with three classifications (Category 1, Category 2, Non-irritant) with four classifications.

For this purpose, phase I, phase II and phase III studies were conducted by three laboratories as shown in Table 1.

Table 1: Breakdown of the Vitrigel-EIT validation study

Phase	The number of the test substances	The number of the repetitions	Examination
0	5	3	Within- laboratory transferability
I	10	3	Between- laboratory transferability & Within- and between- laboratory reproducibility
II	10	1	Between- laboratory reproducibility
III	36	1	Between- laboratory reproducibility and predictability

The members of quality assurance group are elected by recommendation of the Vitrigel-EIT VMT.

Hajime Kojima doubled a responsibility of quality assurance as trail coordinator. He audited test chemical preparation record forms, blank data sheets, etc. and distributes them to the research laboratories participating in this validation trail. They also collects filled out forms and data sheets after completion of experiments, pointing out omissions or flaws in recording, if any, and requesting correction of such errors in the submitted documents from study directors.

QA should be done based on OECD QUALITY ASSURANCE AND GLP (OECD Series on Principles of GLP and Compliance Monitoring Number 4 : Revised, 1999).

1. Phase I study

Two results of reference (ethanol) at FDSC did not achieve the success criteria at the protocol. These results at FDSC may be caused by quality preservative of the model. Analyzing the phase I validation data, the range of reference control should be revised and used at the next phase study. Reference control should be included in the acceptance criteria in the protocol for quality preservative of the model.

2. Phase II study

The results of phase 2 study was sufficient with acceptance criteria for between-laboratory reproducibility. However, the concern remains negative data by test chemical No.1, which is GHS category 1. All participants requested to make a clear reason. Lead lab. suggested this test may subject to tested temperature. Lead lab. collected data at high temp.(28℃). Therefore, all participants agreed to perform an additional study using test chemical No.1 in high temp. As the results, All data was positive in the modified conditions. It is necessary to revise the protocol to set up the rigorous temperature conditions.

For predictivity, the VMT judged the data at phase 2 study cannot use with that of phase 3 study. The chemical numbers (30) at the original study plan will be modified in accordance with requesting the test facilities and reduced on 4 chemicals (GHS category1 ： 2chemicals,category2:1 chemical, no:1chemical).

3. Phase III study

During the experiment period, JaCVAM received a question on Chemical No. 213 from the study director at Lab.C during the validation study. He knew this chemical is a deleterious substance and its name. Considering the possibility of code open on this chemical at this lab., JaCVAM determined to delete this chemical from the list soon, and distributed new one (No. 237) to all laboratories. This chemical is similar level of GHS category with No. 213.

From the record, the following issues should be checked.

1) Periods from exposure a test chemical solution to start a measurement

Lab.A: 4 sec.

Lab.B: 3 sec.

Lab.C: 2 sec.

2) Temperature at models

Lab.A: 27.0-28.7℃ (Culture medium)

Lab.B: 26.4-28.0℃(Water bath)

Lab.C: 26.9-28.4℃ (Culture medium)

3)Solubility of test chemicals

No. of insoluble chemicals

Lab.A: 21 sediment: 4, supernatant:2(No. 212, 216)

Lab.B: 19 sediment: 10, supernatant:7(No.213,221,222,223,232,234,236)

Lab.C: 17 sediment: 7, supernatant:10 (No. 202,210,218, 219,220, 224, 230, 231, 233, 235)

4) Other issues

- At Lab.C, the different batch of frozen cell lines used by mixture.

- At Lab.A, chemical No. 216 was tested twice. The former data were not approved.

The all aforementioned issues reported at the VMT meeting and all members judged minor issues and these issues agreed not correlated with data analysis.

4. References

1) OECD Series on Principles of GLP and Compliance Monitoring Number 4 : Revised (1999)

Draft summary record of the Vitrigel-EIT International VMT 1st Meeting

Date: Wednesday, October 2, 2013, 11:00 – 17:00, 20:00-21:10 (Teleconference)
Venue: Daicel Corporation, Room 14M and Shinagawa Annex (Tokyo)
Participants: Toshiaki Takezawa (National Institute of Agrobiological Sciences: NIAS), Hiroyuki Yamaguchi （NIAS, Kanto Chemical Co.,）, Takashi Sozu (Kyoto Univ.), Takeru Nitsuma, Mika Watanabe (Hatano Res. Inst., Food and Drug Safety Center), Takayuki Fukuda, Sho Fujiwara, Noriko Yamaguchi(BoZo RESEARCH CENTER), Kunihiko Yamashita (Daicel Corporation), Michael-Wilhelm SCHAEFFER (EURL ECVAM: Teleconference only), Nicole Kleinstreuer (NICEATM/ICCVAM), Chae-Hyung Lim (KoCVAM), Hajime Kojima (JaCVAM)

1. Welcome address

Toshiaki Takezawa (TT) spoke welcome address to the participated members. All members introduced themselves. Hajime Kojima (HK) explained agenda and a house keeping for this meeting. .

2. Introduction of Vitrigel-EIT

TT presented the Vitrigel project, the collagen vitrigel membrane (CVM) developed by TT, is composed of high-density collagen fibrils equivalent to connective tissues *in vivo* and the project used this CVM is on-going in the field of basic research, medical devices and alternative to animal testing supported by MAFF(Ministry of Agriculture, Forestry and Fisheries). Hiroyuki Yamaguchi (HY) and TT developed a new eye irritancy test method using CVM and human corneal epithelium, such as " Vitrigel-EIT". HY introduced regarding the development of this test method.

3. Phase 0 study

HK presented the study plan of this validation study. As various points were suggested by Nicole and Lim, the study plan was revised. However, the main purpose and success criteria of this validation study were agreed by all members.

HY talked about the protocol of this assay including a measurement of TEER (Trans Epithelial Electric Resistance) as an endpoint in this assay. After that, Takashi Sozu (TS) presented the result of phase 0 study. He mentioned the Intra-laboratory reproducibility of glycerol obtained at Bozo was poor and Intra-laboratory reproducibility of ethanol (positive control) obtained at Daicel did not achieve the success criteria. All data were stable excluding No.2 data of glycerol obtained at Bozo and data of ethanol were in narrow range. Nicole requested the change of positive control from ethanol to benzalconium chloride, because the results of positive control should be strong and stable. She proposed ethanol was set up as a reference control and should be determined its range using lab data after phase I validation study. The other VMT members were agreed her suggestions and the protocol will be revised by lead lab.

4. Phase I study

HY mentioned the revised points of protocol by the results of phase 0 study. Major revised points addressed

1) Positive control and reference control

2) Optimal temperature for the assay (after experiments by lead laboratory)

Using the revised protocol, the VMT requested the additional assays to Bozo and Diacel. They agreed this proposal. Confirming the modification of additional results, all members agreed to process on to phase I study. The new timeline was approved by all members.

Date	schedule
October/2013	The revised protocol will be proposed by lead lab.
November-December/2013	**Re-test of phase -0 study using the revised protocol**
March-April/ 2014	**Phase-I study** to know between-lab transferability & reproducibility, and within-lab. reproducibility using 10 coded chemicals, 3 tests are conducted for each chemical independently
May/2014	The test data will be analyzed by Sozu
June/2014	**2nd VMT meeting:** evaluation of the outcome of Phase-I and planning of Phase-II study

In the teleconference, all members explained introduction of Vitrigel-EIT and the outline of this meeting to Michael-Wilhelm SCHAEFFER.

Appendix 8.8 Predictive capacity of the Vitrigel-EIT for 57 chemicals

No.	Chemical	Class	CAS No.	Supplier	Physical state	Density (g/cm^3)	logP	pH[a]	GHS[b]	EPA[c]	Draize score		Test date	Protocol ver.	Temperature ℃	Scores[d]			Judgment[e]
											Score	Ref.				Lag time	Intensity	Plateau level	
1	2-ethyl-1-hexanol	Alcohols	104-76-7	Wako	Liquid	0.83	3.04	7	2A	II	51.3	h	2011/9/15	*	30	0	0.23	41.0	I
2	3-methoxy-1,2-propanediol	Alcohols	623-39-2	TCI	Liquid	1.11	-1.13	7	NC	IV	0	h	2011/9/15	*	30	>180	-0.10	0.0	NI
3	cyclohexanol	Alcohols	108-93-0	Sigma-Aldrich	Liquid	0.96	1.23	7	1	I	79.8	h	2011/9/15	*	30	0	0.31	56.0	I
4	isopropyl alcohol	Alcohols	67-63-0	Wako	Liquid	0.78	0.05	7	2A	III	30.5	h	2011/9/15	*	30	0	0.30	27.0	I
5	*n*-hexanol	Alcohols	111-27-3	Aldrich	Liquid	0.82	2.03	7	2A	II	64.8	h	2011/9/15	*	30	0	0.33	59.0	I
6	3,3-dimethylpentane	Alkanes	562-49-2	Aldrich	Liquid	0.69	3.74	7	NC	IV	0	h	2011/10/20	*	30	>180	-0.01	0.0	NI
7	acetone	Ketones	67-64-1	Wako	Liquid	0.79	-0.24	7	2A	II	65.8	h	2011/10/20	*	30	0	0.21	10.0	I
8	methyl isobutyl ketone	Ketones	108-10-1	TCI	Liquid	0.80	1.31	7	NC	III	4.8	h	2011/10/20	*	30	0	0.25	32.0	I
9	polyethylene glycol 400	Polyols	25322-68-3	TCI	Liquid	1.13	-1.41	7	NC	IV	0	h	2011/9/15	*	30	>180	-0.01	2.0	NI
10	propylene glycol	Polyols	57-55-6	Wako	Liquid	1.04	-0.92	7	NC	IV	1.3	h	2011/10/6	*	30	>180	0.00	0.0	NI
11	benzalkonium chloride	Surfactants (cationic)	8001-54-5	Sigma-Aldrich	Solid	0.99	1.68	7	1	I	108[f]	h	2011/10/6	*	30	0	1.00	90.0	I
12	cetylpyridinium bromide	Surfactants (cationic)	140-72-7	TCI	Solid	-	1.77	7	1	I	89.7[f]	h	2011/10/6	*	30	0	1.16	81.0	I
13	Triton X-100	Surfactants (nonionic)	9002-93-1	Sigma-Aldrich	Liquid	1.06	4.89	7	1	I	68.7[f]	h	2011/10/6	*	30	0	0.92	83.0	I
14	Tween20	Surfactants (nonionic)	9005-64-5	Sigma-Aldrich	Liquid	1.10	6.12	7	NC	III	4	h	2011/10/6	*	30	>180	-0.03	0.0	NI
15	2-ethylhexyl p-dimethyl-amino benzoate	UV absorbing agents	21245-02-3	Aldrich	Liquid	1.00	4.70	7	NC	IV	0	h	2011/9/15	*	30	>180	-0.04	0.0	NI
16	sodium hydroxide	Inorganic chemicals	1310-73-2	Wako	Solid	2.10	-	>11	1	I	108[f]	h	2011/10/6	*	30	0	13.27	133.0	I
17	3-methyl-pentynol	Alcohols	77-75-8	Sigma	Liquid	0.87	0.71	8	1	-	-	-	2012/4/27	*	28	0	0.60	36.0	I
18	butyl cellosolve	Alcohols	111-76-2	Sigma	Liquid	0.90	0.83	8	1	II	68.7	h	2012/4/27	*	28	0	0.48	58.0	I
19	2-methylbutanoic acid	Carboxylic acids	116-53-0	Sigma	Liquid	0.93	1.18	4	1	I	-	-	2014/1/21	1.4e	26	>180	0.02	-0.7	NI
20	nonylphenyl-polyethylene glycol	Polyols	9016-45-9	Wako	Solid	1.06	6.51	7	1	-	-	-	2014/7/15	1.61e	28	40	0.20	30.1	I
21	distearyldimethylammonium chloride	Surfactants (cationic)	107-64-2	Wako	Solid	0.86	1.01	7	1	I	96.3	i	2013/7/9	1.3e	28	90	0.03	1.0	I
22	acid red 92	Color additives	18472-87-2	Wako	Solid	2.16	7.13	unmeasurable	2	I	71	i	2012/4/27	*	28	0	0.74	82.0	I
23	ethyl acetate	Esters(acetate)	141-78-6	Sigma	Liquid	0.90	0.73	8	2B	III	18	h	2012/4/27	*	28	0	0.29	52.0	I
24	3,3'-dithiodipropionic acid	Acids	1119-62-6	Wako	Solid	1.45	-0.15	4	2B	II	31.7	h	2013/10/29	1.4e	28	>180	-0.02	0.0	NI
25	ammonium nitrate	Organic salts	6484-52-2	Sigma	Solid	1.72	-	8	2B	III	18.3	h	2012/4/27	*	28	0	2.07	62.0	I
26	ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate	Esters	96568-04-6	TCI	Solid	1.43	2.30	5	2B	III	-	-	2013/10/29	1.4e	28	>180	0.00	1.0	NI
27	3-chloropropionitrile	Nitriles	542-76-7	Wako	Liquid	1.16	0.18	5	2B	III	13.7	h	2013/7/9	1.3e	28	10	0.31	56.0	I
28	triethanolamine	Alkanolamines	102-71-6	Wako	Liquid	1.12	-1.00	9	NC	III	8	i	2012/4/27	*	28	0	0.17	31.0	I
29	1,5-hexadiene	Alkanes	592-42-7	Sigma	Liquid	0.69	2.87	7	NC	III	4.7	h	2013/7/9	1.3e	28	>180	-0.01	0.0	NI
30	isopropyl bromide	Hydrocarbons	75-26-3	Sigma	Liquid	1.31	2.14	8	NC	IV	2.7	h	2012/4/27	*	28	>180	0.00	0.0	NI
31	*n*-octyl bromide	Hydrocarbons	111-83-1	Sigma	Liquid	1.11	4.89	8	NC	IV	0	h	2012/4/27	*	28	>180	-0.05	0.0	NI
32	cyclopentasiloxane	Silicon compounds	541-02-6	Sigma	Liquid	0.96	8.03	8	NC	-	-	-	2012/4/27	*	28	>180	-0.02	0.0	NI
33	sucrose fatty acid ester	Polyols, Esters	–	TCI	Solid	-	0.35	7	2	II	28.3	i	2013/10/29	1.4e	28	0	0.24	42.0	I
34	styrene	Aromatics	100-42-5	Sigma-Aldrich	Liquid	0.91	2.95	7	NC	III	6.8	h	2013/10/29	1.4e	28	>180	-0.01	0.0	NI
35	potassium laurate	Surfactants (anionic)	10124-65-9	Woko Pure	Solid	1.12	4.57	7	1	I	33.7[f]	j	2013/10/29	1.4e	28	0	0.40	71.0	I
36	methyl cyanoacetate	Esters, Nitrile compounds	105-34-0	Sigma-Aldrich	Solid	1.12	-0.47	7	2A	II	27.7	h	2013/10/29	1.4e	28	20	0.07	14.0	I
37	cyclohexanone	Ketones, Hydrocarbons (cyclic)	108-94-1	Sigma-Aldrich	Liquid	0.95	0.81	7	NC	III	-	-	2013/10/29	1.4e	28	10	0.26	48.0	I
38	tetrahydrofuran	Furans	109-99-9	Sigma-Aldrich	Liquid	0.89	0.46	7	1	-	-	-	2013/10/29	1.4e	28	0	0.22	40.0	I
39	6-methylpurine	Bases	2004-03-7	Sigma-Aldrich	Solid	1.40	-1.50	7	2B	-	-	-	2014/1/21	1.4e	26	>180	-0.05	0.0	NI
40	lactic acid	Carboxylic acids	50-21-5	Alfa Aesar	Liquid	1.20	-0.72	3	1	I	102.7	j	2013/11/19	1.4e	27	>180	-0.30	0.0	NI
41	sodium 2-naphthalenesulfonate	Organic salts	532-02-5	Sigma-Aldrich	Solid	-	-0.12	7	2	III	-	-	2013/11/19	1.4e	27	0	0.54	59.0	I
42	domiphen bromide	Surfactants (cationic)	538-71-6	Sigma-Aldrich	Solid	-	2.91	7	1	I	96.3[f]	j	2013/11/19	1.4e	27	0	0.32	57.1	I
43	di(2-ethylhexyl) sodiumsulfosuccinate	Surfactants (anionic)	577-11-7	Sigma-Aldrich	Solid	1.10	1.94	7	1	I	57[f]	j	2013/10/29	1.4e	28	0	0.24	35.0	I
44	cetylpyridinium chloride	Surfactants (cationic)	6004-24-6	Sigma-Aldrich	Solid	1.00	1.77	7	1	I	94.7[f]	j	2013/11/19	1.4e	27	0	0.33	59.0	I
45	polyoxyethylene hydrogenated castoroil (60E.O.)	Alkoxylated alcohols, Polymeric ethers	61788-85-0	Woko Pure	Liquid	1.02	-	7	NC	IV	0[f]	j	2014/1/21	1.4e	26	>180	-0.01	0.0	NI
46	2-benzyloxyethanol	Alcohols, Ethers	622-08-2	Wako	Liquid	1.06	2.23	7	2A	II	-	-	2013/11/19	1.4e	27	10	0.32	57.7	I
47	benzethonium chloride	Surfactants (cationic)	121-54-0	Sigma-Aldrich	Solid	1.00	2.90	7	1	-	67	j	2013/11/19	1.4e	27	0	0.38	68.3	I
48	dimethyl sulfoxide	Thioethers	67-68-5	Sigma-Aldrich	Liquid	1.10	-1.35	7	NC	III	7.3	j	2014/1/21	1.4e	26	>180	-0.11	0.0	NI
49	citric acid	Carboxylic acids	77-92-9	Sigma-Aldrich	Solid	1.67	-1.64	3	2A	-	-	-	2013/11/19	1.4e	27	30	0.20	15.7	I
50	isobutyl alcohol	Alcohols	78-83-1	Wako	Liquid	0.81	0.76	7	2A	I	60.3	j	2013/11/19	1.4e	27	10	0.24	44.1	I
51	isobutanal	Aldehyde	78-84-2	Wako	Liquid	0.79	0.89	6	2B	III	-	-	2013/11/19	1.4e	27	30	0.17	27.5	I
52	glycolic acid	Carboxylic acids	79-14-1	Wako	Solid	1.49	-1.11	4	2	III	17.3[f]	j	2013/11/19	1.4e	27	0	0.37	3.7	I
53	polyoxyethylene 23 lauryl ether	Surfactants (nonionic)	9002-92-0	Sigma-Aldrich	Liquid	1.02	3.54	7	NC	III	0	j	2014/1/21	1.4e	26	>180	-0.01	0.0	NI
54	polyethylene glycol monostearate (10E.O.)	Surfactants (nonionic)	9004-99-3	Wako	Solid	1.00	6.63	7	NC	-	0	i	2013/10/29	1.4e	28	>180	-0.02	0.0	NI
55	Tween80	Surfactants (nonionic)	9005-65-6	Sigma-Aldrich	Liquid	1.06	6.12	7	NC	IV	0[f]	j	2014/1/21	1.4e	26	>180	-0.02	0.0	NI
56	1,2,4-trimethylbenzene	Hydrocarbons	95-63-6	Sigma-Aldrich	Liquid	0.88	3.63	7	NC	-	4.7	j	2014/1/21	1.4e	26	>180	-0.01	0.0	NI
57	1,3-di-isopropylbenzene	Aromatics	99-62-7	Sigma-Aldrich	Liquid	0.86	4.35	7	NC	IV	2	h	2014/1/21	1.4e	26	>180	0.00	0.0	NI

Note -, unknown; 1, GHS Category 1; 2, GHS Category 2; 2A, GHS Category 2A; 2B, GHS Category2B; NC, not classified (United Nations, 2013); I, EPA Category I; II, EPA Category II III, EPA Category III; IV, EPA Category IV (EPA, 1998);
*, Test was conducted in accordance with the description as previously reported in "Toxicol. Sci. 135(2): 347-355, 2013".

[a] pH of 2.5(w/v)% test chemical solution

[b] United Nations, 2013. Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Fifth revised edition. New York and Geneva (ST/SG/AC.10/30/Rev.5).

[c] EPA, 1998. Health Effects Test Guidelines OPPTS 870.2400 Acute Eye Irritation. United States Environmental Protection Agency, Washington, DC. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0156-0006 [19 June 2015]

[d] These scores were calculated from the average time-dependent profile of TEER values in three-independent experiments

[e] I, irritant; NI, non-irritant

[f] Data from 10% exposure condition.

[g] Data from 15% exposure condition.

[h] European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC).1998. Eye irritation: reference chemicals data bank (Second Edition),ECETOC technical report No. 48. ECETOC, Brussels, Belgium.

[i] Ohno Y, et al. 1999. Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (1) Overview of the validation study and Draize scores for the evaluation of the tests. Toxicol. in Vitro 13, 73-98.

[j] Takahashi Y, et al. 2011. The Short Time Exposure (STE) test for predicting eye irritation potential: Intra-laboratory reproducibility and correspondence to globally harmonized system (GHS) and EU eye irritation classification for 109 chemicals. Toxicol. in Vitro 25, 1425-1434.

Appendix8.9

Table. List of proficiency chemicals

No.	Test chemical	CAS No.	State	Density (g/cm^3)	logP	pH	GHS	Vitrigel-EIT
1	Imidazole	288-32-4	Solid	1.03	-0.08	9	Category1	Positive
2	Sodium Dodecyl Sulphate	151-21-3	Solid	0.40	1.60	7		Positive
3	Sodium salicylate	54-21-7	Solid	0.32	0.42	7		Positive
4	Camphene	79-92-5	Solid	0.84	1.94	7	Category 2A or 2B	Positive or False negative
5	2-Methyl-1-pentanol	105-30-6	Liquid	0.83	1.76	7		Positive
6	α-Hexylcinnamaldehyde	101-86-0	Liquid	0.95	5.12	7		False negative
7	iso-Octylthioglycolate	25103-09-7	Liquid	0.97	4.36	7	No Category	Negative
8	3-Methoxy-1,2-propanediol	623-39-2	Liquid	1.11	-1.13	7		Negative
9	Toluene	108-88-3	Liquid	0.87	2.73	7		False positive
10	Silicon Dioxide n-Hydrate	7699-41-4	Solid	1.58	-	7		Negative

Research article

Journal of AppliedToxicology

Received: 19 June 2015, Revised: 14 September 2015, Accepted: 21 September 2015 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jat.3254

Predictive performance of the Vitrigel-eye irritancy test method using 118 chemicals

Hiroyuki Yamaguchi[a,c], Hajime Kojima[b] and Toshiaki Takezawa[a]*

ABSTRACT: We recently developed a novel Vitrigel-eye irritancy test (EIT) method. The Vitrigel-EIT method is composed of two parts, i.e., the construction of a human corneal epithelium (HCE) model in a collagen vitrigel membrane chamber and the prediction of eye irritancy by analyzing the time-dependent profile of transepithelial electrical resistance values for 3 min after exposing a chemical to the HCE model. In this study, we estimated the predictive performance of Vitrigel-EIT method by testing a total of 118 chemicals. The category determined by the Vitrigel-EIT method in comparison to the globally harmonized system classification revealed that the sensitivity, specificity and accuracy were 90.1%, 65.9% and 80.5%, respectively. Here, five of seven false-negative chemicals were acidic chemicals inducing the irregular rising of transepithelial electrical resistance values. In case of eliminating the test chemical solutions showing pH 5 or lower, the sensitivity, specificity and accuracy were improved to 96.8%, 67.4% and 84.4%, respectively. Meanwhile, nine of 16 false-positive chemicals were classified irritant by the US Environmental Protection Agency. In addition, the disappearance of ZO-1, a tight junction-associated protein and MUC1, a cell membrane-spanning mucin was immunohistologically confirmed in the HCE models after exposing not only eye irritant chemicals but also false-positive chemicals, suggesting that such false-positive chemicals have an eye irritant potential. These data demonstrated that the Vitrigel-EIT method could provide excellent predictive performance to judge the widespread eye irritancy, including very mild irritant chemicals. We hope that the Vitrigel-EIT method contributes to the development of safe commodity chemicals. Copyright © 2015 The Authors. *Journal of Applied Toxicology* published by John Wiley & Sons Ltd.

Keywords: collagen vitrigel membrane; corneal epithelium; eye irritation test; HCE T cells; predictive performance; transepithelial electrical resistance

Introduction

The prediction of eye irritation following chemical exposure is required for the development of not only cosmetics and consumer products but also drugs. Test methods *in vivo*, *ex vivo* and *in vitro* have been developed to predict eye irritation (Yamaguchi *et al.*, 2013). Concerning eye irritation tests (EITs) *in vivo*, the Draize rabbit EIT has been mainly utilized for evaluating cosmetic ingredients (Draize *et al.*, 1944; OECD, 2012a) and pharmaceutical agents (Uematsu *et al.*, 2007). Regarding those *ex vivo*, excised bovine corneas and chicken eyes successfully contributed to the establishment of Organisation for Economic Co-operation and Development (OECD) Test Guidelines as the bovine corneal opacity permeability test (OECD, 2013) and isolated chicken eye test (OECD, 2009), respectively. In addition, concerning those *in vitro*, various kinds of two- and three-dimensional cell culture systems have been proposed. The fluorescein leakage test method was adopted as an OECD test guideline (OECD, 2012b). The draft test guideline of short time exposure test using a monolayer culture system of Statens Seruminstitut rabbit cornea cells was published by OECD (ICCVAM, 2013; OECD, 2014; Sakaguchi *et al.*, 2011; Takahashi *et al.*, 2011). *In vitro* test methods using three-dimensional culture models have an advantage that these models can directly expose water-insoluble chemicals (Cotovio *et al.*, 2010; Jung *et al.*, 2011; Katoh *et al.*, 2013). The EpiOcular-EIT using a tissue culture model reconstructed by culturing normal human epidermal keratinocytes is currently under peer review process aiming for an OECD test guideline (Kaluzhny *et al.*, 2011; Pfannenbecker *et al.*, 2013). However, no *ex vivo* and *in vitro* test methods have the performance to replace fully the Draize-EIT that has been adopted for regulatory purposes. Therefore, a tiered approach of combining several test methods that used different complementary indicators was proposed (Scott *et al.*, 2010). In *ex vivo* test methods such as the bovine corneal opacity permeability and isolated chicken eye tests, the degree of tissue damage based on change in corneal opacity, corneal permeability of fluorescein and thickness of cornea was utilized as an indicator. In *in vitro* test methods using three-dimensional culture models fabricated in various culture inserts, cellular viability measured by the MTT assay has been used as a major indicator. However, the MTT assay has some limitations. The MTT solution could penetrate only two or three layers from the basal side because this solution was placed under the basal side of the models. This procedure may

*Correspondence to: Toshiaki Takezawa, Division of Animal Sciences, National Institute of Agrobiological Sciences, 1-2 Ohwashi, Tsukuba, Ibaraki, 305-8634, Japan. E-mail: t.takezawa@affrc.go.jp

[a]Division of Animal Sciences, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan

[b]Japanese Center for the Validation of Alternative Methods (JaCVAM), Biological Safety Research Center, National Institute of Health Sciences, Setagaya, Tokyo, Japan

[c]Isehara Research Laboratory, Technology and Development Division, Kanto Chemical Co., Inc., Isehara, Kanagawa, Japan

underestimate the mild irritancy that has toxic effects only in the superficial layer of the corneal epithelium (Pauly *et al.*, 2009). To overcome this issue, different indicators such as occludin, interleukin-8 and MUC1 have been proposed (Meloni *et al.*, 2010; Song and Joo, 2004). The most apical part of the lateral membrane in the superficial epithelial cells contains the junctional complex, including tight junctions, which thus directly contribute to the first line of defense in the cornea. Therefore, the structural and functional change of tight junction-associated protein such as occludin and ZO-1 was reported as an early marker of eye irritancy (Meloni *et al.*, 2010).

A collagen vitrigel membrane (CVM) we previously developed is composed of high-density collagen fibrils equivalent to connective tissues *in vivo* and is easily handled with tweezers. In addition, it possesses excellent transparency and permeability of protein with high molecular weight and consequently the various studies utilizing it as a cell culture scaffold advances so well (Takezawa *et al.*, 2004, 2007a_c). We established a preparation method of a corneal epithelium model utilizing an air_liquid interface culture system that facilitates induction of layering rabbit corneal epithelial cells cultured on the CVM scaffold (Takezawa *et al.*, 2008). To overcome species differences between human and rabbit in sensitivity to exogenous chemicals, we developed a human corneal epithelium (HCE) model by three-dimensionally culturing HCE-T cells on the CVM scaffold (Takezawa *et al.*, 2011a). Here, the scaffold was fabricated on a polyethylene terephthalate (PET) membrane of a Millicell chamber appropriate for the transepithelial electrical resistance (TEER) assay of epithelial cells. TEER is known as a suitable method for evaluating the integrity of the tight junction of corneal epithelium *in vivo* (Uematsu *et al.*, 2007). We examined four chemicals using the HCE model, and consequently demonstrated that the time-dependent relative changes of TEER are useful indicators to assess ocular irritancy effects of chemicals even in the middle category (Takezawa *et al.*, 2011a). However, this model is inappropriate for immunohistological analyses due to difficulty in preparing its frozen sections, including the PET membrane. To overcome this inconvenience we recently developed a novel chamber merely accompanying a CVM without the PET membrane and established its mass production process (Takezawa *et al.*, 2011b, 2012). Recently, we established a new test method to extrapolate the widespread eye irritancy by briefly analyzing the time-dependent profile of TEER after exposing chemicals to a HCE model reconstructed in a CVM chamber. Here, we named the new test method as a "Vitrigel-EIT method." Thirty chemicals were successfully classified into the irritant or non-irritant category without false negatives by the Vitrigel-EIT method (Yamaguchi *et al.*, 2013).

In this study, we aimed to estimate the predictive performance of the Vitrigel-EIT method by testing a total of 118 chemicals, including the previous 30 ones. In addition, we intended to clarify the mechanism-based reason for raising false-negative and false-positive reactions by measuring the pH level of the test chemical solution and observing the immunohistology of HCE models after exposing test chemicals, respectively. The immunohistological observation was performed for ZO-1, a tight junction-associated protein and MUC1, a cell membrane-spanning mucin.

Materials and methods

Antibodies and reagents

The rabbit polyclonal antibody for ZO-1 and mouse monoclonal antibody for MUC1 were purchased from Life Technologies Corp. (Grand Island, NY, USA) and Sanbio BV (Uden, the Netherlands), respectively. A goat Alexa Fluor 555-conjugated secondary antibody for rabbit IgG and a goat Alexa Fluor 488-conjugated secondary antibody for mouse IgG were purchased from Life Technologies Corp. Hoechst33342 was purchased from Dojindo Laboratories (Kumamoto, Japan). Normal goat serum was purchased from Sigma-Aldrich (St. Louis, MO, USA). Tissue-Tek optimal cutting temperature (OCT) compound was purchased from Sakura Finetek Japan (Tokyo, Japan). All other reagents not specified above were of the highest grade.

Human corneal epithelium T-cell culture

A SV40-immortalized HCE cell strain (HCE-T cells, RCB no. 2280) was obtained from the RIKEN BioResource Center (Tsukuba, Japan). The cells were maintained in the following culture medium: 1 : 1 mixture of Dulbecco's modified eagle medium and nutrient mixture F-12 supplemented with 5% heat-inactivated fetal bovine serum, 5 $\mu g\ ml^{-1}$ recombinant human insulin, 10 $ng\ ml^{-1}$ recombinant human epidermal growth factor, 0.5% dimethyl sulfoxide 100 units ml^{-1} penicillin and 100 $\mu g\ ml^{-1}$ streptomycin (Araki-Sasaki *et al.*, 1995; Yamasaki *et al.*, 2009). Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Preparation of collagen vitrigel membrane chambers

A collagen xerogel membrane chamber (ad-MED Vitrigel™) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). The collagen xerogel membrane chamber was set in the well of a 12-well plate. Then, the collagen xerogel membrane was immersed in the above culture medium by pouring 1.5 ml outside and 0.5 ml inside the chamber in the well for 10 min to convert the xerogel into a vitrigel immediately before use.

Reconstruction of a human corneal epithelium model

A culture medium outside the chamber in the well of a 12-well plate was changed to 1.5 ml of the fresh medium. The medium inside the chamber was removed and 0.5 ml of a cell suspension in a culture medium at a density of 1.2×10^5 cells ml^{-1} was poured on to the CVM of the chamber and cultured for 2 days at 37 °C. Subsequently, the medium inside the chamber was removed and the cells were cultured for 4 days under the air_liquid interface to fabricate a HCE model. The medium outside the chamber was changed every day or the third day during the culture period on the air_liquid interface to simplify the previous culture procedure (Yamaguchi *et al.*, 2013).

Immunohistology of human corneal epithelium models after exposing test chemicals

The HCE models after exposing test chemicals were isolated from the plastic cylinder of the chamber and fixed for 5 min in methanol kept on ice immediately after sufficiently chilling it at -45 °C. Then, they were embedded in an OCT compound after removing the excessive methanol around them with an absorbent paper towel, frozen in liquid nitrogen and stored at _80 °C. The samples were vertically cut into cross-sections with a thickness of 5 μm against the CVM using a cryostat (CM3050S; Leica Microsystems, Wetzlar, Germany). The frozen sections spread on a glass slide were dried out for 60 min at room temperature. The frozen sections were immersed in phosphate-buffered saline (PBS) for 5 min to remove the OCT compound, and incubated with PBS containing 1% normal

Journal of AppliedToxicology

goat serum for 30 min to block non-specific adsorption of antibodies. Then, the first antibodies against ZO-1 or MUC1 in PBS containing 1% normal goat serum were applied and incubated for 16 h, followed by washing them with PBS three times. Alexa fluor 555- or Alexa fluor 488-conjugated secondary antibodies were applied and incubated for 3 h, followed by washing them with PBS three times. Subsequently, cell nuclei were counterstained with Hoechst33342. Sections were observed by a laser scanning confocal microscope (FV1000; Olympus, Tokyo, Japan).

Calculation of transepithelial electrical resistance values of a human corneal epithelium model

Each HCE model in a CVM chamber as a sample, a CVM chamber as a blank were subjected to the measurement of electrical resistance (R_{sample} and R_{blank}, respectively) by the method as previously described (Yamaguchi *et al.*, 2013). The TEER value was calculated using the following formula:

$$\text{TEER} = (R_{sample} - R_{blank}) \times \text{effective surface area } (1.0\ \text{cm}^2).$$

Exposure experiment of test chemicals in the Vitrigel-eye irritancy test method

Eighty-eight test chemicals were selected according to the globally harmonized system of classification and labeling (GHS) classification for eye irritation (United Nations, 2013). The information on the total 118 test chemicals, including the previously tested 30 chemicals is shown in Table 1. Every test chemical solution was prepared in a culture medium at a concentration of 2.5 (weight/volume) % appropriate for measuring TEER values without being influenced by the test chemical-dependent electrical resistance. Here, the chemicals were dissolved in the medium by using an appropriate technique(s) as follows: vortex mixing within 1 min, sonication within 20 min and/or heating in a water bath <70 °C. In case test chemicals are insoluble or immiscible by the above technique(s), the test chemical solution was prepared as a homogeneous suspension that the chemical was mixed well in the medium by vortex within 1 min immediately before use. The pH level of each 2.5 w/v % test chemical solution was measured using Universal pH test paper from ADVANTEC (Tokyo, Japan).

The HCE models on day 6 were subjected to the exposure experiment of test chemicals. At first, 500 µl of culture medium was poured in the chamber and the value of the R_{sample}, before chemical exposures, was measured to obtain the initial TEER value of each model. Next, the medium inside the chamber was changed to 500 µl of test chemical solution and the periodical values of R_{sample} were measured by the TEER recorder at intervals of 10 s for 3 min after exposure of each test solution. Three independent models were subjected to the exposure experiment for each test solution to plot the average time-dependent profile of TEER values on a chart. The chemical exposure experiment was conducted in the ambient temperature of 28 ± 2 °C.

Eye irritant potential of test chemicals using the Vitrigel-eye irritancy test method

The average time-dependent profile of TEER values after exposing each test chemical solution in three-independent experiments was analyzed by three indexes for time lag, intensity and plateau level. The score of each index was calculated by the formula as previously described (Yamaguchi *et al.*, 2013). The eye irritant potential of test chemicals was classified into two categories, irritant and non-irritant, according to the criteria for the scores of three indexes shown in Table 2.

Subsequently, the correlation with the GHS classification of 118 test chemicals was estimated by calculating sensitivity, specificity and accuracy in accordance with the following formula. (The correlation with the US Environmental Protection Agency [EPA] classification of 98 test chemicals was also estimated in a similar manner except for 20 chemicals unknown as EPA classification.)

$$\text{Sensitivity}(\%) = A/(A+B)\times 100$$
$$\text{Specificity}(\%) = D/(C+D)\times 100$$
$$\text{Accuracy}(\%) = (A+D)/(A+B+C+D)\times 100$$

Here, A, B, C and D represent the number of chemicals categorized as irritant by both the traditional GHS or EPA classification and judgment of the Vitrigel-EIT method, irritant by the traditional GHS or EPA classification and non-irritant by judgment of the Vitrigel-EIT method, non-irritant by the traditional GHS or EPA classification and irritant by judgment of the Vitrigel-EIT method, and non-irritant by both the traditional GHS or EPA classification and judgment of the Vitrigel-EIT method, respectively.

Results

Predictive performance of the Vitrigel-eye irritancy test method

The final judgment of the 118 test chemicals using the Vitrigel-EIT method and the pH level of each test chemical solution are shown in Table 3. Here, 80 test chemicals were classified as irritant and the other 38 chemicals as non-irritant.

The classification by this test method was in accordance with the GHS categories on 95 test chemicals in a total of 118 chemicals. Meanwhile, seven chemicals were predicted as non-irritant among the 71 irritant chemicals classified in categories 1, 2, 2A and 2B by the GHS, indicating a 9.9% false-negative rate. In addition, 16 chemicals were predicted as irritant among the 47 non-irritant chemicals using the GHS classification, indicating a 34.1% false-positive rate. Therefore, the sensitivity, specificity and accuracy were 90.1%, 65.9% and 80.5%, respectively.

In addition, the classification using this test method was in accordance with the EPA categories on 82 test chemicals in a total of 98 chemicals. Meanwhile, 14 chemicals were predicted as non-irritant among the 79 irritant chemicals classified in to categories I, II and III by the EPA, indicating a 17.7% false-negative rate. In addition, two chemicals were predicted as irritant among the 19 non-irritant chemicals using the EPA classification, indicating a 10.5% false-positive rate. Therefore, the sensitivity, specificity and accuracy were 82.3%, 89.5% and 83.7%, respectively.

Distribution of pH levels in test chemical solutions

The pH levels of 118 test chemical solutions could be measured except for one solution that was inappropriate for the pH test paper and found distributed in the wide range from 3 to more than 11 as shown in Table 3. Here, in the nine chemical

Table 1. List of the 118 test chemicals

Chemical	Class	CAS no.	Supplier	GHS class[a]	EPA class[b]	Draize score
Methoxyethyl acrylate	Acrylates	3121-61-7	Sigma-Aldrich	1	> III	45[c]
Cyclohexanol	Alcohols	108-93-0	Sigma-Aldrich	1	I	79.8[c]
2,5-dimethyl-2,5-hexanediol	Alcohols	110-03-2	Sigma	1	I	28.3[c]
Diethylethanolamine	Amines	100-37-8	Sigma	1	I	94.7[e]
m-phenylenediamine	Amines	108-45-2	Wako Pure	1	I	–
Acetic acid	Carboxylic acids	64-19-7	Wako Pure	1	I	68[d,f]
2-methylbutanoic acid	Carboxylic acids	116-53-0	Sigma	1	I	–
Imidazole	Heterocyclics	288-32-4	Sigma	1	I	59.3[c]
Promethazine hydrochloride	Miscellaneous	58-33-3	Sigma	1	I	71.3[c]
Sodium salicylate	Organic salts	54-21-7	Wako Pure	1	I	83.7[d]
Lactic acid	Carboxylic acids	50-21-5	Alfa Aesar	1	I	102.7[e]
Pyridine	Heterocyclics	110-86-1	Sigma-Aldrich	1	I	48[c]
Sodium hydroxide	Inorganic chemicals	1310-73-2	Wako Pure	1	I	108[c,f]
Potassium laurate	Surfactants (anionic)	10124-65-9	Wako Pure	1	I	33.7[e,f]
di(2-ethylhexyl) sodium sulfosuccinate	Surfactants (anionic)	577-11-7	Sigma-Aldrich	1	I	57[d,f]
Cetyltrimethylammonium bromide	Surfactants (cationic)	57-09-0	Sigma	1	I	96[e,f]
Stearyltrimethylammonium chloride	Surfactants (cationic)	112-03-8	Wako Pure	1	I	91.3[d,f]
Benzalkonium chloride	Surfactants (cationic)	8001-54-5	Sigma-Aldrich	1	I	108[c,f]
Distearyldimethylammonium chloride	Surfactants (cationic)	107-64-2	Wako Pure	1	I	96.3[d]
Cetylpyridinium bromide	Surfactants (cationic)	140-72-7	TCI	1	I	89.7[c,f]
Domiphen bromide	Surfactants (cationic)	538-71-6	Sigma-Aldrich	1	I	96.3[e,f]
Cetylpyridinium chloride	Surfactants (cationic)	6004-24-6	Sigma-Aldrich	1	I	94.7[d,f]
Triton X-100	Surfactants (non-ionic)	9002-93-1	Sigma-Aldrich	1	I	68.7[c,f]
Butyl cellosolve	Alcohols	111-76-2	Sigma	1	II	68.7[c]
Monoethanolamine	Alkanolamines	141-43-5	Sigma-Aldrich	1	III	23.3[d,f]
Sodium lauryl sulfate	Surfactants (anionic)	151-21-3	Wako Pure	1	III	59.2[c,g]
3-methyl-pentynol	Alcohols	77-75-8	Sigma	1	–	–
Nonylphenyl-polyethylene glycol	Polyols	9016-45-9	Wako Pure	1	–	–
Tetrahydrofuran	Furans	109-99-9	Sigma-Aldrich	1	–	–
Benzethonium chloride	Surfactants (cationic)	121-54-0	Sigma-Aldrich	1	–	67[e]
Benzyl alcohol	Alcohols	100-51-6	Sigma	2	I	31[d]
Acid red 92	Color additives	18472-87-2	Wako Pure	2	I	71[d]
Sucrose fatty acid ester	Polyols, esters	-	TCI	2	II	28.3[d]
2-ethoxyethyl acetate	Esters (acetate)	111-15-9	Sigma	2	III	15[c]
Glycolic acid	Carboxylic acids	79-14-1	Wako Pure	2	III	17.3[e,f]
Sodium 2-naphthalenesulfonate	Organic salts	532-02-5	Sigma-Aldrich	2	III	–
Diisopropanolamine	Alcohols	110-97-4	Sigma-Aldrich	2	–	9.7[e,f]
Butanol	Alcohols	71-36-3	Wako Pure	2A	I	60.8[c]
Ethanol	Alcohols	64-17-5	Wako Pure	2A	I	24[c]
Isobutyl alcohol	Alcohols	78-83-1	Wako Pure	2A	I	60.3[e]
n-hexanol	Alcohols	111-27-3	Aldrich	2A	II	64.8[c]
2-ethyl-1-hexanol	Alcohols	104-76-7	Wako Pure	2A	II	51.3[c]
1-octanol	Alcohols	111-87-5	Wako Pure	2A	II	41[c]

(Continues)

Journal of AppliedToxicology

Table 1. (Continued)

Chemical	Class	CAS no.	Supplier	GHS class[a]	EPA class[b]	Draize score
Cyclopentanol	Alcohols	96-41-3	Aldrich	2A	II	21.7[d]
2-benzyloxyethanol	Alcohols, ethers	622-08-2	Wako Pure	2A	II	–
Methyl acetate	Esters	79-20-9	Sigma-Aldrich	2A	II	39.5[c]
Methyl cyanoacetate	Esters, nitrile compounds	105-34-0	Sigma-Aldrich	2A	II	27.7[c]
Butyrolactone	Lactone	96-48-0	Sigma-Aldrich	2A	II	43[c]
Acetone	Ketones	67-64-1	Wako Pure	2A	II	65.8[c]
Isopropyl alcohol	Alcohols	67-63-0	Wako Pure	2A	III	30.5[c]
Myristyl alcohol	Fatty alcohols	112-72-1	Sigma-Aldrich	2A	III	4[c]
Methyl ethyl ketone (2-butanone)	Ketones	78-93-3	TCI	2A	III	50[c]
Hexyl cinnamic aldehyde	Aldehyde	101-86-0	Wako Pure	2A	–	–
Citric acid	Carboxylic acids	77-92-9	Sigma-Aldrich	2A	–	–
Potassium sorbate	Organic salts	24634-61-5	Sigma-Aldrich	2A	–	–
Calcium thioglycolate	Organic salts	814-71-1	Wako Pure	2A	–	52.3[e]
Propasol solvent P	Alcohols	1569-01-3	Sigma	2B	II	–
3,3′-dithiodipropionic acid	Acids	1119-62-6	Wako Pure	2B	II	31.7[c]
2-methyl-1-pentanol	Alcohols	105-30-6	TCI	2B	III	13[c]
n-butanal	Aldehydes	123-72-8	Sigma	2B	III	–
Ethyl acetate	Esters (acetate)	141-78-6	Sigma	2B	III	18[c]
Camphene	Hydrocarbons	79-92-5	Sigma	2B	III	–
Isobutanal	Aldehyde	78-84-2	Wako Pure	2B	III	–
Di(propylene glycol) propyl ether	Alkoxylated alcohols	29911-27-1	Aldrich	2B	III	–
Ethyl-2-methylacetoacetate	Esters	609-14-3	Sigma-Aldrich	2B	III	18[c]
Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate	Esters	96568-04-6	TCI	2B	III	–
3-chloropropionitrile	Nitriles	542-76-7	Wako Pure	2B	III	13.7[c]
Ammonium nitrate	Organic salts	6484-52-2	Sigma	2B	III	18.3[c]
Sodium monochloroacetate	Organic salts, halogen compounds	3926-62-3	Aldrich	2B	III	–
n-lauroylsarcosine sodium salt	Sarcosine derivatives	137-16-6	Sigma-Aldrich	2B	III	–
6-methylpurine	Bases	2004-03-7	Sigma-Aldrich	2B	–	–
Xylene	Aromatics	1330-20-7	Wako Pure	NC	II	9[c]
Toluene	Hydrocarbons	108-88-3	Wako Pure	NC	III	9[c]
1,5-hexadiene	Alkanes	592-42-7	Sigma	NC	III	4.7[c]
Triethanolamine	Alkanolamines	102-71-6	Wako Pure	NC	III	8[d]
N,N-Dimethylguanidine sulfate	Organic salts	598-65-2	TCI	NC	III	6.7[c]
Styrene	Aromatics	100-42-5	Sigma-Aldrich	NC	III	6.8[c]
Methyl cyclopentane	Cycloalkanes	96-37-7	TCI	NC	III	3.7[c]
Butyl acetate	Esters	123-86-4	Sigma-Aldrich	NC	III	7.5[c]
Ethyl trimethyl acetate	Esters	3938-95-2	Sigma-Aldrich	NC	III	3.8[c]
2,2-dimethyl-3-pentanol	Fatty alcohols	3970-62-5	Sigma-Aldrich	NC	III	8.3[c]
1,2,3-trichloropropane	Hydrocarbons	96-18-4	Aldrich	NC	III	8.7[c]
Dodecane	Hydrocarbons	112-40-3	Sigma-Aldrich	NC	III	2[c]
Methyl isobutyl ketone	Ketones	108-10-1	TCI	NC	III	4.8[c]
Methyl pentyl ketone	Ketones	110-43-0	Wako Pure	NC	III	–
Cyclohexanone	Ketones, hydrocarbons (cyclic)	108-94-1	Sigma-Aldrich	NC	III	–

(*Continues*)

Table 1. (Continued)

Chemical	Class	CAS no.	Supplier	GHS class[a]	EPA class[b]	Draize score
Tween20	Surfactants (non-ionic)	9005-64-5	Sigma-Aldrich	NC	III	4[c]
Polyoxyethylene 23 lauryl ether	Surfactants (non-ionic)	9002-92-0	Sigma-Aldrich	NC	III	0[e]
Dimethyl sulfoxide	Thioethers	67-68-5	Sigma-Aldrich	NC	III	7.3[e]
2,4-pentandiol	Alcohols	625-69-4	Sigma	NC	IV	1.3[c]
3-methoxy-1.2-propanediol	Alcohols	623-39-2	TCI	NC	IV	0[c]
Isopropyl bromide	Hydrocarbons	75-26-3	Sigma	NC	IV	2.7[c]
n-octyl bromide	Hydrocarbons	111-83-1	Sigma	NC	IV	0[c]
Gluconolactone	Lactone	90-80-2	TCI	NC	IV	2[e]
Glycerol	Polyols	56-81-5	Wako Pure	NC	IV	1.7[c]
Propylene glycol	Polyols	57-55-6	Wako Pure	NC	IV	1.3[c]
Polyethylene glycol 400	Polyols	25322-68-3	TCI	NC	IV	0[c]
Iso-octyl acrylate	Acrylates	29590-42-9	Sigma-Aldrich	NC	IV	0.7[c]
3,3-Dimethylpentane	Alkanes	562-49-2	Aldrich	NC	IV	0[c]
1,9-decadiene	Alkenes	1647-16-1	Sigma-Aldrich	NC	IV	2[c]
Polyoxyethylene hydrogenated castoroil (60E.O.)	Alkoxylated alcohols, polymeric ethers	61788-85-0	Wako Pure	NC	IV	0[d,f]
1,3-di-isopropylbenzene	Aromatics	99-62-7	Sigma-Aldrich	NC	IV	2[c]
Isopropyl myristate	Esters	110-27-0	Sigma-Aldrich	NC	IV	0[d]
Ethylhexyl salicylate	Esters, ultraviolet absorbing agents	118-60-5	Sigma-Aldrich	NC	IV	–
2-methylpentane	Hydrocarbons	107-83-5	Sigma-Aldrich	NC	IV	2[c]
Diisobutyl ketone	Ketones	108-83-8	Sigma-Aldrich	NC	IV	0.7[c]
Tween80	Surfactants (non-ionic)	9005-65-6	Sigma-Aldrich	NC	IV	0[e,f]
2-ethylhexyl p-dimethyl-amino benzoate	Ultraviolet absorbing agents	21245-02-3	Aldrich	NC	IV	0[c]
Cyclopentasiloxane	Silicon compounds	541-02-6	Sigma	NC	–	–
EDTA, di-potassium	Amines	25102-12-9	Sigma-Aldrich	NC	–	10.3[e]
Betaine monohydrate	Amino acids	590-47-6	Sigma-Aldrich	NC	–	5.3[e]
1,2,4-trimethylbenzene	Hydrocarbons	95-63-6	Sigma-Aldrich	NC	–	4.7[e]
Petroleum ether	Hydrocarbons	8032-32-4	Sigma-Aldrich	NC	–	2[e]
Hexane	Hydrocarbons	110-54-3	Sigma-Aldrich	NC	–	0[e]
Silic anhydride	Inorganic chemicals	7631-86-9	Wako Pure	NC	–	2.7[d]
2,4-pentanedione	Ketones	123-54-6	Sigma-Aldrich	NC	–	14[e]
3-glycidoxypropyltrimethoxysilane	Organosilicon compounds	2530-83-8	Sigma-Aldrich	NC	–	2[e]
Polyethylene glycol monostearate (10E.O.)	Surfactants (non-ionic)	9004-99-3	Wako Pure	NC	> III	0[d]

–, unknown; 1, category 1 (irreversible effects on the eye); 2, category 2 (irritating to eyes); 2A, category 2A (irritating to eyes); 2B, category 2B (mildly irritating to eyes); NC, not classified; I, category I (corrosive (irreversible destruction of cornea tissue) or corneal involvement or irritation persisting for more than 21 days); II, category II (corneal involvement or other eye irritation clearing in 8–21 days); III, category III (corneal involvement or other eye irritation clearing in 7 days or less); IV, category IV (minimal effects clearing in less than 24 h).

[a]GHS category (United Nations, 2013).

[b]EPA category (EPA, 1998).

[c]ECETOC (1998).

[d]Ohno *et al.* (1999).

[e]Takahashi *et al.* (2011).

[f]Data from 10% exposure condition.

[g]Data from 15% exposure condition.

Journal of AppliedToxicology

Table 2. Criteria for the judgment using the Vitrigel-eye irritancy test method

Judgment	Criteria
Irritant	Score of time lag ≤ 180 or score of intensity ≥ 0.05 or score of plateau level > 5
Non-irritant	Score of time lag > 180 and score of intensity < 0.05 and score of plateau level ≤ 5

Table 3. Summary data of the results by Vitrigel-eye irritancy test method for 118 test chemicals

Chemical	pH [a]	Score[b]			
		Lag time	Intensity	Plateau level	Final judgment
Methoxyethyl acrylate	7	0	0.24	42.7	I
Cyclohexanol	7	0	0.31	56.0	I
2,5-dimethyl-2,5-hexanediol	7	80	0.15	18.0	I
Diethylethanolamine	10	0	0.66	66.0	I
m-phenylenediamine	8	10	0.35	62.0	I
Acetic acid	4	>180	0.00	−51.0	NI
2-methylbutanoic acid	4	>180	0.02	−0.7	NI
Imidazole	9	100	0.26	22.0	I
Promethazine hydrochloride	6	0	0.69	69.0	I
Sodium salicylate	7	0	1.01	60.0	I
Lactic acid	3	>180	−0.30	0.0	NI
Pyridine	7	10	0.18	32.7	I
Sodium hydroxide	≥11	0	13.27	133.0	I
Potassium laurate	7	0	0.40	71.0	I
Di(2-ethylhexyl) sodium sulfosuccinate	7	0	0.24	35.0	I
Cetyltrimethylammonium bromide	7	0	0.35	63.0	I
Stearyltrimethylammonium chloride	7	0	0.32	57.0	I
Benzalkonium chloride	7	0	1.00	90.0	I
Distearyldimethylammonium chloride	7	90	0.03	1.0	I
Cetylpyridinium bromide	7	0	1.16	81.0	I
Domiphen bromide	7	0	0.32	57.1	I
Cetylpyridinium chloride	7	0	0.33	59.0	I
Triton X-100	7	0	0.92	83.0	I
Butyl cellosolve	8	0	0.48	58.0	I
Monoethanolamine	≥11	0	0.36	65.4	I
Sodium lauryl sulfate	7	0	0.70	84.0	I
3-methyl-pentynol	8	0	0.60	36.0	I
Nonylphenyl-polyethylene glycol	7	40	0.20	30.1	I
Tetrahydrofuran	7	0	0.22	40.0	I
Benzethonium chloride	7	0	0.38	68.3	I
Benzyl alcohol	7	0	0.27	49.0	I
Acid red 92	-	0	0.74	82.0	I
Sucrose fatty acid ester	7	0	0.24	42.0	I
2-ethoxyethyl acetate	7	0	0.20	37.0	I
Glycolic acid	4	0	0.37	3.7	I
Sodium 2-naphthalenesulfonate	7	0	0.54	59.0	I
Diisopropanolamine	9	0	0.24	43.1	I
Butanol	8	0	0.89	53.0	I
Ethanol	7	10	0.14	26.0	I
Isobutyl alcohol	7	10	0.24	44.1	I
n-hexanol	7	0	0.33	59.0	I
2-ethyl-1-hexanol	7	0	0.23	41.0	I
1-octanol	7	10	0.16	29.0	I
Cyclopentanol	7	10	0.20	35.7	I
2-benzyloxyethanol	7	10	0.32	57.7	I
Methyl acetate	7	10	0.16	29.2	I
Methyl cyanoacetate	7	20	0.07	14.0	I
Butyrolactone	7	60	0.11	16.1	I
Acetone	7	0	0.21	10.0	I

(Continues)

Table 3. (Continued)

Chemical	pH[a]	Score[b]			
		Lag time	Intensity	Plateau level	Final judgment
Isopropyl alcohol	7	0	0.30	27.0	I
Myristyl alcohol	7	>180	−0.03	0.0	NI
Methyl ethyl ketone (2-butanone)	7	0	0.21	37.0	I
Hexyl cinnamic aldehyde	7	0	0.57	6.0	I
Citric acid	3	30	0.20	15.7	I
Potassium sorbate	7	0	0.72	21.7	I
Calcium thioglycolate	10	0	0.53	53.4	I
Propasol solvent P	8	0	0.38	57.0	I
3,3′-dithiodipropionic acid	4	>180	−0.02	0.0	NI
2-methyl-1-pentanol	7	0	0.77	46.0	I
n-butanal	7	80	0.15	18.0	I
Ethyl acetate	8	0	0.29	52.0	I
Camphene	7	100	0.04	4.0	I
Isobutanal	6	30	0.17	27.5	I
Di(propylene glycol) propyl ether	7	0	0.22	40.4	I
Ethyl-2-methylacetoacetate	7	10	0.16	29.7	I
Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate	5	>180	0.00	0.0	NI
3-chloropropionitrile	5	10	0.31	56.0	I
Ammonium nitrate	8	0	2.07	62.0	I
Sodium monochloroacetate	7	0	0.78	31.4	I
n-lauroylsarcosine sodium salt	6	0	0.35	63.6	I
6-methylpurine	7	>180	−0.05	0.0	NI
Xylene	7	>180	−0.01	0.0	NI
Toluene	7	140	0.02	0.0	I
1,5-hexadiene	7	>180	−0.01	0.0	NI
Triethanolamine	9	0	0.17	31.0	I
N,N-Dimethylguanidine sulfate	7	0	1.35	41.0	I
Styrene	7	>180	−0.01	0.0	NI
Methyl cyclopentane	7	>180	−0.02	0.0	NI
Butyl acetate	7	10	0.15	26.3	I
Ethyl trimethyl acetate	7	110	0.07	7.2	I
2,2-dimethyl-3-pentanol	7	10	0.20	35.7	I
1,2,3-trichloropropane	7	80	0.11	14.2	I
Dodecane	7	>180	−0.02	0.0	NI
Methyl isobutyl ketone	7	0	0.25	32.0	I
Methyl pentyl ketone	7	50	0.20	9.0	I
Cyclohexanone	7	10	0.26	48.0	I
Tween 20	7	>180	−0.03	0.0	NI
Polyoxyethylene 23 lauryl ether	7	>180	−0.01	0.0	NI
Dimethyl sulfoxide	7	>180	−0.11	0.0	NI
2,4-pentandiol	8	70	0.07	8.0	I
3-methoxy-1,2-propanediol	7	>180	−0.10	0.0	NI
Isopropyl bromide	8	>180	0.00	0.0	NI
n-octyl bromide	8	>180	−0.05	0.0	NI
Gluconolactone	6	>180	0.00	0.0	NI
Glycerol	7	0	0.34	20.4	I
Propylene glycol	7	>180	0.00	0.0	NI
Polyethylene glycol 400	7	>180	−0.01	2.0	NI
Iso-octyl acrylate	7	>180	−0.02	0.0	NI
3,3-dimethylpentane	7	>180	−0.02	2.0	NI
1,9-decadiene	7	>180	−0.01	0.0	NI
Polyoxyethylene hydrogenated castoroil (60E.O.)	7	>180	−0.01	0.0	NI
1,3-di-isopropylbenzene	7	>180	0.00	0.0	NI
Isopropyl myristate	7	>180	0.00	0.0	NI
Ethylhexyl salicylate	7	>180	−0.02	0.0	NI
2-methylpentane	7	>180	−0.01	0.0	NI
Diisobutyl ketone	7	>180	0.00	0.0	NI
Tween 80	7	>180	−0.02	0.0	NI

(Continues)

Table 3. (Continued)

Chemical	pH [a]	Score[b]			
		Lag time	Intensity	Plateau level	Final judgment
2-ethylhexyl *p*-dimethyl-amino benzoate	7	>180	−0.04	0.0	NI
Cyclopentasiloxane	8	>180	−0.02	0.0	NI
EDTA, di-potassium	5	0	0.38	37.8	I
Betaine monohydrate	7	0	0.26	21.1	I
1,2,4-trimethylbenzene	7	>180	−0.01	0.0	NI
Petroleum ether	7	>180	−0.03	0.0	NI
Hexane	7	>180	−0.01	0.0	NI
Silic anhydride	7	>180	−0.05	0.0	NI
2,4-pentanedione	6	10	0.15	28.2	I
3-glycidoxypropyltrimethoxysilane	7	0	0.11	20.2	I
Polyethylene glycol monostearate (10E.O.)	7	>180	−0.02	0.0	NI

–, not tested; I, irritant; NI, non-irritant.
[a]pH 2.5 (w/v)% test chemical solution.
[b]These scores were calculated from the average time-dependent profile of transepithelial electrical resistance values in three independent experiments.

solutions falling under the pH ranges of less than 5, four chemicals were irritant whereas five chemicals were non-irritant and false-negative by the Vitrigel-EIT. The other two false-negative chemicals judged as non-irritant by the Vitrigel-EIT were pH 7 in their solution.

Immunohistological characteristics of human corneal epithelium models after exposing test chemicals

In the HCE model after exposing polyoxyethylene 23 lauryl ether, Tween 80 or polyoxyethylene hydrogenated castor oil (60E.O.),

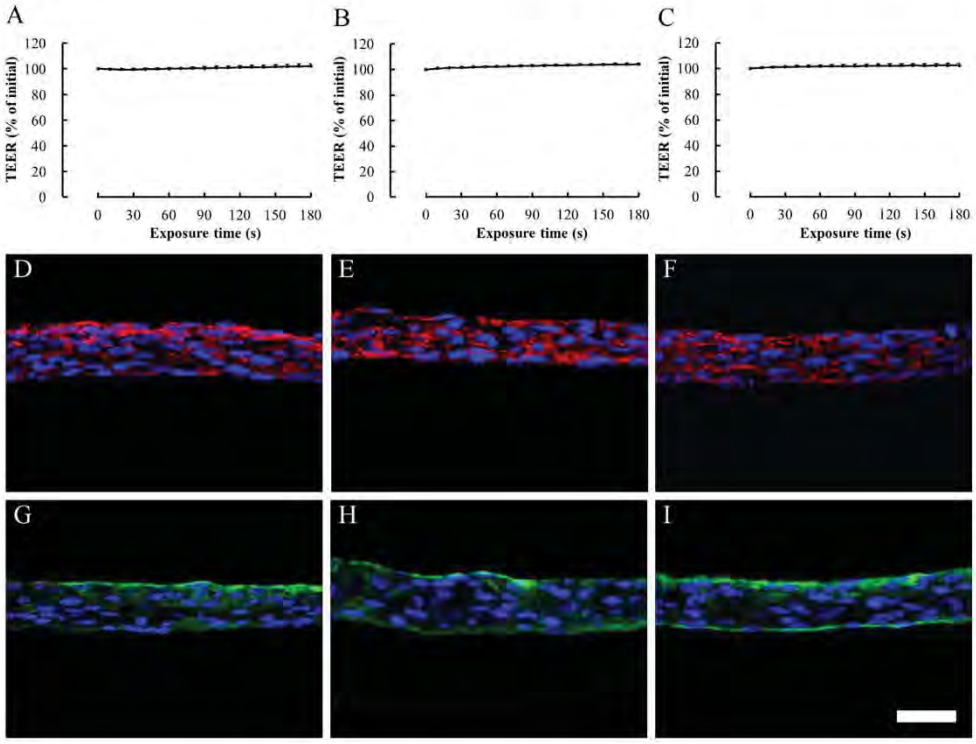


Figure 1. The average time-dependent profile of TEER values in three independent experiments and immunohistological characteristics of human corneal epithelium models using test chemicals that were classified in category NC by the globally harmonized system of classification and labeling and classified as a non-irritant using the Vitrigel-eye irritancy test method. The average time-dependent profile of TEER values after exposing polyoxyethylene 23 lauryl ether (A), Tween 80 (B) and polyoxyethylene hydrogenated castor oil (60E.O.) (C). Cross-sections of the human corneal epithelium models after exposing polyoxyethylene 23 lauryl ether (D,G), Tween 80 (E,H) and polyoxyethylene hydrogenated castor oil (60E.O.) (F,I) were stained with antibodies for ZO-1 (D–F) and MUC1 (G–I). Nuclei of cells were stained with Hoechst 33342. Scale bars represent 50 μm. TEER, transepithelial electrical resistance.

the time-dependent relative changes of TEER values were almost nothing (Fig. 1A–C). Therefore, these chemicals were all classified as non-irritants using the Vitrigel-EIT method. Here, ZO-1 was abundantly expressed in the lateral and basal surfaces of cells in the superficial layer in comparison to the other layers (Fig. 1D–F). MUC1 was merely expressed in the apical surface of cells in the superficial layer (Fig. 1G–I). These immunohistological observations demonstrated that the HCE models maintained healthy morphology even after exposing the test chemicals classified in to category NC by the GHS and classified as non-irritant by the Vitrigel-EIT method.

In the HCE model after exposing potassium laurate, butanol or propanol solvent P, the time-dependent relative changes of TEER values were rapidly decreased (Fig. 2A–C). Therefore, these chemicals were all classified into irritant by the Vitrigel-EIT method. Here, ZO-1 and MUC1 expressions were remarkably disappeared in the HCE models (Fig. 2D–I). These immunohistological characteristics demonstrated that the HCE models lost their barrier function after exposing the test chemicals that were classified in to category 1, 2A and 2B by the GHS and classified as irritant by the Vitrigel-EIT method.

Meanwhile, in the HCE model after exposing triethanolamine, methyl isobutyl ketone or glycerol, the time-dependent relative changes of the TEER values were slowly decreased (Fig. 3A–C). Therefore, these chemicals were all classified into irritant by the Vitrigel-EIT method. Here, the ZO-1 and MUC1 expressions were partially disappeared in the HCE models (Fig. 3D–I). These immunohistological characteristics demonstrated that the HCE models lost their barrier function after exposing the false-positive test chemicals classified in to category NC by GHS whereas they were classified as irritant by the Vitrigel-EIT method.

Discussion

We developed the Vitrigel-EIT method by measuring the time-dependent profiles of the TEER values for 3 min after exposing 30 test chemicals as previously reported (Yamaguchi *et al.*, 2013). However, generally more than 100 chemicals should be tested for estimating the predictive performance of an EIT method. In this study, we tested a total of 118 test chemicals with a variety of physical and chemical properties. Consequently, this test method indicated the good predictive performance comparable to other test methods currently in development aiming for the OECD test guidelines. For example, the sensitivity, specificity and accuracy of the EpiOcular-EIT were 98.1%, 72.9% and 84.8%, respectively (Kaluzhny *et al.*, 2011). In addition, the sensitivity, specificity and accuracy of the short time exposure test in a bottom–up approach were 88%, 80% and 85%, respectively (ICCVAM, 2013). However, some chemicals were classified as false-negatives or false-positives. It is important to clarify the mechanism-based reason for raising false-negative and false-positive reactions, particularly for establishing an *in vitro* test method that can truly extrapolate an *in vivo* reaction after exposing a chemical. To overcome this issue, we tried to clarify the mechanism for raising false-negative and false-positive reactions.

Regarding the false-negative reactions, five of the seven false-negative chemicals were acidic and their 2.5 (w/v)% solutions for

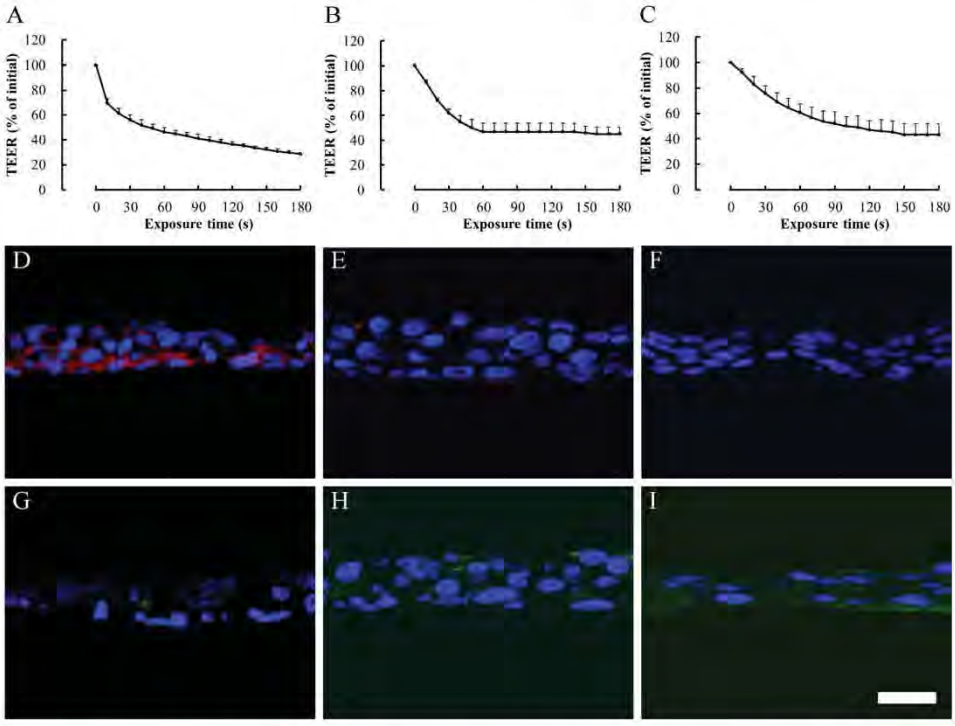


Figure 2. The average time-dependent profile of TEER values in three independent experiments and immunohistological characteristics of human corneal epithelium models using test chemicals that were classified as category 1, 2A and 2B using the globally harmonized system of classification and labeling and classified as an irritant by the Vitrigel-eye irritancy test method. The average time-dependent profile of TEER values after exposing potassium laurate (A), butanol (B) and propasol solvent P (C). Cross-sections of the human corneal epithelium models after exposing potassium laurate (D,G), butanol (E,H) and propanol solvent P (F,I) were stained with antibodies for ZO-1 (D–F) and MUC1 (G–I). Nuclei of cells were stained with Hoechst 33342. Scale bars represent 50 μm. TEER, transepithelial electrical resistance.

Journal of AppliedToxicology

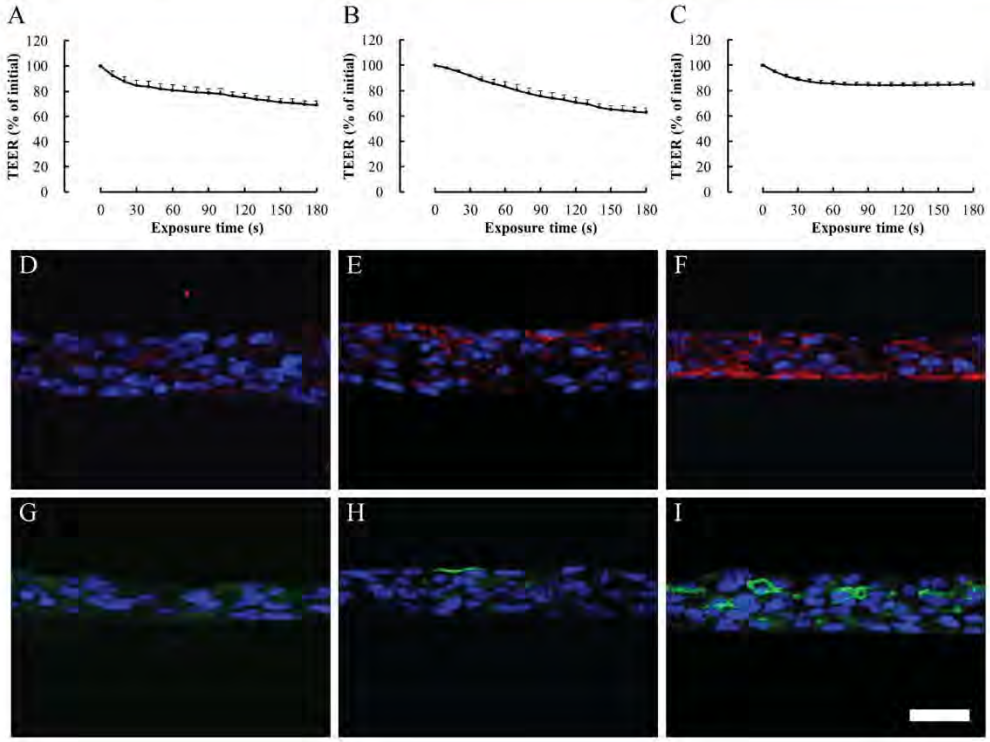


Figure 3. The average time-dependent profile of TEER values in three independent experiments and immunohistological characteristics of the human corneal epithelium models using test chemicals that were classified as category NC using the globally harmonized system of classification and labeling, whereas they were classified as an irritant using the Vitrigel-eye irritancy test method. The average time-dependent profile of TEER values after exposing triethanolamine (A), methyl isobutyl ketone (B) and glycerol (C). Cross-sections of the human corneal epithelium models after exposing triethanolamine (D,G), methyl isobutyl ketone (E,H) and glycerol (F,I) were stained with antibodies for ZO-1 (D–F) and MUC1 (G–I). Nuclei of cells were stained with Hoechst 33342. Scale bars represent 50 μm. TEER, transepithelial electrical resistance.

exposure experiments indicated the pH level lower than 5. In addition, the TEER values of the HCE models after exposing the five acidic false-negative chemical solutions were increased from the initial TEER values. Interestingly, it was reported that isolated rabbit esophageal mucosal epithelium and normal human bronchial epithelial cell layers in culture increased their TEER values when they were exposed to weak acidic solutions (Farré *et al.*, 2008; Oshima *et al.*, 2012). In the case of the nine test chemicals showing pH 5 or lower in their exposure solutions that were excluded from total 118 chemicals, the sensitivity, specificity and accuracy on the 109 chemicals were improved from 90.1%, 65.9% and 80.5% to 96.8%, 67.4% and 84.4%, respectively. One of two non-acidic false-negative chemicals was myristyl alcohol. Myristyl alcohol is a rugged waxy solid and water-insoluble at room temperature. In the Draize-EIT, solid chemicals have the potential to injure eyes due to their mechanical stress inducing scratching on living tissues (Kaluzhny *et al.*, 2011; Takahashi *et al.*, 2011; York and Steiling, 1998). In the Vitrigel-EIT method, solid chemicals were applied to the HCE models as its homogeneous suspension and kept stationary for 3 min without stirring or shaking. Therefore, one speculation for the false-negative result on myristyl alcohol is the deference of test conditions between the Draize-EIT and the Vitrigel-EIT method. Another non-acidic false-negative chemical was 6-methylpurine. It is a powdery solid and water-soluble. We are currently investigating the reason for the false-negative judgment with 6-methylpurine; however, it is still unknown at present.

Regarding the false-positive reactions, Draize scores provided an interesting viewpoint. The average of Draize scores for 14 false-positive chemicals and that for 27 non-irritant chemicals revealed 6.5 and 2.2 except for six chemicals unknown as Draize scores, respectively. These values suggest that the eye irritant potential of false-positive chemicals is stronger than that of non-irritant chemicals. Moreover, one of the 16 false-positive chemicals and eight of those were classified into categories II and III by the EPA, respectively. Interestingly, it was reported that about 30% chemicals classified into category III by the EPA were labeled as not-classified by GHS (ICCVAM, 2010). The high rate of reducing eye irritant chemicals by using the criteria of GHS compared to that of EPA is attributable to the difference in the classification system between GHS and EPA as briefly mentioned below. In the classification system, each chemical was tested using at least three animals in both the GHS and EPA. The eye irritancy of test chemicals in animals was estimated using the criteria for four indexes, i.e., corneal opacity, conjunctival redness, swelling and iritis. Here, each index has three endpoints for the time, i.e., 24, 48 and 72 h after the test chemical administration. In the case of the GHS classification, test chemicals were evaluated in two steps, i.e., each test chemical was first categorized according to the value of the three endpoints for the four indexes in each animal, and next the category involving the over half result in all tested animals was finally judged as the classification of the test chemical (United Nations, 2013). On the other hand, in the case of the EPA classification, test chemicals were evaluated in one step, i.e., the

classification of each test chemical was determined based on the maximum score for each endpoint in any animal (EPA, 1998).

The HCE model appropriate for the Vitrigel-EIT method possessed about six cell layers, expressed the HCE-related proteins and developed the barrier function, suggesting the model well reflects HCE *in vivo* (Yamaguchi *et al.*, 2013). In particular, ZO-1 is one of the tight junction-related proteins and associated with the principal barrier that separates the eye from the outside environment (Yi *et al.*, 2000). In addition, MUC1 is one of the cell membrane spanning mucin families expressed in the superficial layer of corneal epithelium and plays a protective role against the adherence of pathogens (Song and Joo, 2004). Those reports suggest that ZO-1 and MUC1 expressions are essential for maintaining healthy corneal epithelium.

In the current study on immunohistological analyses, ZO-1 and MUC1 expressions in HCE models were maintained after exposing chemicals judged as non-irritant (Fig. 1D–I) and disappeared after exposing chemicals judged as irritants (Fig. 2D–I) by the GHS classification and the Vitrigel-EIT method. By contrast, those expressions disappeared in the model after exposing chemicals judged as non-irritant by the GHS classification and as irritant by the Vitrigel-EIT method, i.e., false-positive chemicals (Fig. 3D–I). These data demonstrated that such false-positive chemicals induced the unhealthy conditions for the HCE models, suggesting that the chemicals have an eye irritant potential.

In addition, gluconolactone is classified as a non-irritant chemical using the GHS. However, gluconolactone is hydrolyzed by water to form gluconic acid, which is a severe eye irritant chemical. In case the test chemical solution of gluconolactone is left for more than 6 min before exposing it to HCE models, it was judged as irritant by the Vitrigel-EIT method due to the coexistence of gluconic acid (data not shown). This suggests that such a test chemical solution revealing hydrolyzability should be prepared immediately before the chemical exposure experiment.

In this study, we demonstrated that the Vitrigel-EIT method could estimate the widespread eye irritancy of various chemicals with very few false-negatives. The validation study of the Vitrigel-EIT method has been conducted by the international validation management team organized in association with the International Collaboration on Alternative Test Methods. Hereafter, we intend to decide the applicability domain of the Vitrigel-EIT method and to describe it in the final protocol for the validation report. We hope that such an effort for registering the Vitrigel-EIT method as a new test guideline for the OECD contributes to the development of safe commodity chemicals.

Conflict of interest

Mr. Yamaguchi and Dr. Takezawa have a patent Cell culture chamber, method for producing same, tissue model using cell culture chamber, and method for producing same issued.

Acknowledgments

This work was supported by Agri-Health Translational Research Project (no. 6320) from the Ministry of Agriculture, Forestry and Fisheries of Japan.

References

Araki-Sasaki K, Ohashi Y, Sasabe T, Hayashi K, Watanabe H, Tano Y, Handa H. 1995. An SV40-immortalized human corneal epithelial cell line and its characterization. *Invest. Ophthalmol. Vis. Sci.* **36**: 614–621.

Cotovio J, Grandidier MH, Lelièvre D, Bremond C, Amsellem C, Maloug S, Ovigne JM, Loisel-Joubert S, Van Der Lee A, Minondo AM, Capallere C, Bertino B, Alépée N, Tinos-Tessonneaud E, De Brugerolle de Fraissinette A, Meunier JR, Leclaire J. 2010. *In vitro* assessment of eye irritancy using the Reconstructed Human Corneal Epithelial SkinEthic HCE model: Application to 435 substances from consumer products industry. *Toxicol. In Vitro* **24**: 523–537.

Draize JH, Woodard G, Calvery HO. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* **82**: 377–390.

European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). 1998. Eye irritation: reference chemicals data bank (Second Edition), ECETOC technical report No. 48. ECETOC, Brussels, Belgium.

EPA. 1998. Health Effects Test Guidelines OPPTS 870.2400 Acute Eye Irritation. United States Environmental Protection Agency: Washington, DC. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0156-0006 (accessed 19 June 2015).

Farré R, van Malenstein H, De Vos R, Geboes K, Depoortere I, Vanden Berghe P, Fornari F, Blondeau K, Mertens V, Tack J, Sifrim D. 2008. Short exposure of oesophageal mucosa to bile acids, both in acidic and weakly acidic conditions, can impair mucosal integrity and provoke dilated intercellular spaces. *Gut* **57**: 1366–1374.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Current Validation Status of In Vitro Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products. Appendix J NICEATM Analysis: Reduced Eye Hazard Labeling Resulting from Using Globally Harmonized System (GHS) Instead of Current U.S. Regulatory Classification Criteria. NIH Publication No. 10-7553. Research Triangle Park, NC: National Institute of Environmental Health Sciences. http://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/invitro-2010/appj-analysis.pdf (accessed 19 June 2015).

ICCVAM. 2013. Short Time Exposure (STE) Test Method Summary Review Document, NIH. Research Triangle Park, NC: National Institute of Environmental Health Sciences. http://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/STE-SRD-NICEATM-508.pdf (accessed 19 June 2015).

Jung KM, Lee SH, Ryu YH, Jang WH, Jung HS, Han JH, Seok SH, Park JH, Son Y, Park YH, Lim KM. 2011. A new 3D reconstituted human corneal epithelium model as an alternative method for the eye irritation test. *Toxicol. In Vitro* **25**: 403–410.

Kaluzhny Y, Kandàrovà H, Hayden P, Kubilus J, d'Argembeau-Thornton L, Klausner M. 2011. Development of the EpiOcularTM Eye Irritation Test for Hazard Identification and Labelling of Eye Irritating Chemicals in Response to the Requirements of the EU Cosmetics Directive and REACH Legislation. *ATLA* **39**: 339–364.

Katoh M, Hamajima F, Ogasawara KH. 2013. Establishment of a new in vitro test method for evaluation of eye irritancy using a reconstructed human corneal epithelial model, LabCyte CORNEA-MODEL. *Toxicol. In Vitro* **27**: 2184–2192.

Meloni M, Pauly A, De Servi B, Le Varlet B, Baudouin C. 2010. Occludin gene expression as an early in vitro sign for mild eye irritation assessment. *Toxicol. in Vitro* **24**: 276–285.

OECD. 2009. *OECD Guidelines for Testing of Chemicals; Test guideline 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants*. OECD: Paris.

OECD. 2012a. *OECD Guidelines for Testing of Chemicals; Test guideline 405: Acute Eye Irritation/Corrosion*. OECD: Paris.

OECD. 2012b. *OECD Guidelines for Testing of Chemicals; Test guideline 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants*. OECD: Paris.

OECD. 2013. *OECD Guidelines for Testing of Chemicals; Test guideline 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*. OECD: Paris.

OECD. 2014. *Draft Guidelines for Testing of Chemicals: The Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*. OECD: Paris.

Ohno Y, Kaneko T, Inoue T, Morikawa Y, Yoshida Y, Fujii A, Masuda M, Ohno T, Hayashi M, Momma J, Uchiyama T, Chiba K, Ikeda N, Imanishi Y, Itakagaki H, Kakishima H, Kasai Y, Kurishita A, Kojima H, Matsukawa K, Nakamura T, Ohkoshi K, Okumura H, Saijo K, Sakamoto K, Suzuki T, Takano K, Tatsumi H, Tani N, Usami M, Watanabe R. 1999. Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (1) Overview of the validation study and Draize scores for the evaluation of the tests. *Toxicol. In Vitro* **13**: 73–98.

Oshima T, Koseki J, Chen X, Matsumoto T, Miwa H. 2012. Acid modulates the squamous epithelial barrier function by modulating the localization of claudins in the superficial layers. *Lab. Invest.* **92**: 22–31.

Pauly A, Meloni M, Baudouin FB, Warnet JM, Baudouin C. 2009. Multiple endpoint analysis of the 3D-reconstituted corneal epithelium after treatment with benzalkonium chloride: Early detection of toxic damage. *Invest. Ophthalmol. Vis. Sci.* **50**: 1644–1652.

Pfannenbecker U, Bessou-Touya S, Faller C, Harbell J, Jacob T, Raabe H, Tailhardat M, Alépée N, De Smedt A, De Wever B, Jones P, Kaluzhny Y, Le Varlet B, McNamee P, Marrec-Fairley M, Van Goethem F. 2013. Cosmetics Europe multi-laboratory pre-validation of the EpiOcular™ reconstituted human tissue test method for the prediction of eye irritation. *Toxicol. In Vitro* **27**: 619–626.

Sakaguchi H, Ota N, Omori T, Kuwahara H, Sozu T, Takagi Y, Takahashi Y, Tanigawa K, Nakanishi M, Nakamura T, Morimoto T, Wakuri S, Okamoto Y, Sakaguchi M, Hayashi T, Hanji T, Watanabe S. 2011. Validation study of the Short Time Exposure (STE) test to assess the eye irritation potential of chemicals. *Toxicol. In Vitro* **25**: 796–809.

Scott L, Eskes C, Hoffmann S, Adriaens E, Alepée N, Bufo M, Clothier R, Facchini D, Faller C, Guest R, Harbell J, Hartung T, Kamp H, Varlet BL, Meloni M, McNamee P, Osborne R, Pape W, Pfannenbecker U, Prinsen M, Seaman C, Spielmann H, Stokes W, Trouba K, Berghe CV, Goethem FV, Vassallo M, Vinardell P, Zuang V. 2010. A proposed eye irritation testing strategy to reduce and replace in vivo studies using Bottom–Up and Top–Down approaches. *Toxicol. In Vitro* **24**: 1–9.

Song IK, Joo CK. 2004. Morphological and functional changes in the rat cornea with an ethanol-mediated epithelial flap. *Invest. Ophthalmol. Vis. Sci.* **45**: 423–428.

Takahashi Y, Hayashi K, Abo T, Koike M, Sakaguchi H, Nishiyama N. 2011. The Short Time Exposure (STE) test for predicting eye irritation potential: Intra-laboratory reproducibility and correspondence to globally harmonized system (GHS) and EU eye irritation classification for 109 chemicals. *Toxicol. In Vitro* **25**: 1425–1434.

Takezawa T, Ozaki K, Nitani A, Takabayashi C, Shimo-Oka T. 2004. Collagen vitrigel: a novel scaffold that can facilitate a three-dimensional culture for reconstructing organoids. *Cell Transplant.* **13**: 463–473.

Takezawa T, Ozaki K, Takabayashi C. 2007a. Reconstruction of hard connective tissue utilizing a pressed silk sheet and type-I collagen as the scaffold for fibroblasts. *Tissue Eng.* **13**: 1357–1366.

Takezawa T, Nitani A, Shimo-Oka T, Takayama Y. 2007b. A protein-permeable scaffold of a collagen vitrigel membrane useful for reconstructing crosstalk models between two different cell types. *Cells Tissues Organs* **185**: 237–241.

Takezawa T, Takeuchi T, Nitani A, Takayama Y, Kino-Oka M, Taya M, Enosawa S. 2007c. Collagen vitrigel membrane useful for paracrine assays *in vitro* and drug delivery systems *in vivo*. *J. Biotechnol.* **131**: 76–83.

Takezawa T, McIntosh-Ambrose W, Elisseeff JH. 2008. A novel culture model of rabbit corneal epithelium utilizing a handy scaffold of collagen vitrigel membrane and its cryopreservation. *AATEX* **13** (Suppl.): 176.

Takezawa T, Nishikawa K, Wang PC. 2011a. Development of a human corneal epithelium model utilizing a collagen vitrigel membrane and the changes of its barrier function induced by exposing eye irritant chemicals. *Toxicol. In Vitro* **25**: 1237–1241.

Takezawa T, Aoki S, Oshikata A, Okamoto C, Yamaguchi H, Narisawa Y, Toda S. 2011b. A novel material of high density collagen fibrils: A collagen xerogel membrane and its application to transplantation in vivo and a culture chamber in vitro. *24th European Conference on Biomaterials*, 831 (Abstract).

Takezawa T, Aoki S, Oshikata A, Okamoto C, Yamaguchi H, Narisawa Y, Toda S. 2012. A novel material of high density collagen fibrils: A collagen xerogel membrane and its application to transplantation in vivo and a culture chamber in vitro. In *24th European Conference on Biomaterials*, International Proceedings Division (ed.). Medimond: Bologna, 181–185.

Uematsu M, Kumagami T, Kusano M, Yamada K, Mishima K, Fujimura K, Sasaki H, Kitaoka T. 2007. Acute corneal epithelial change after instillation of benzalkonium chloride evaluated using a newly developed in vivo corneal transepithelial electric resistance measurement method. *Ophthalmic Res.* **39**: 308–314.

United Nations. 2013. *Globally Harmonized System of Classification and Labeling of Chemicals (GHS)*, 5th revised (ST/SG/AC.10/30/Rev.5) edn. : New York.

Yamaguchi H, Kojima H, Takezawa T. 2013. Vitrigel-eye irritancy test method using HCE-T cells. *Toxicol. Sci.* **135**: 347–355.

Yamasaki K, Kawasaki S, Young RD, Fukuoka H, Tanioka H, Nakatsukasa M, Quantock AJ, Kinoshita S. 2009. Genomic aberrations and cellular heterogeneity in SV40-immortalized human corneal epithelial cells. *Invest. Ophthalmol. Vis. Sci.* **50**: 604–613.

Yi XJ, Wang Y, Yu FSX. 2000. Corneal epithelial tight junctions and their response to lipopolysaccharide challenge. *Invest. Ophthalmol. Vis. Sci.* **41**: 4093–4100.

York M, Steiling W. 1998. A critical review of the assessment of eye irritation potential using the Draize rabbit eye test. *J. Appl. Toxicol.* **18**: 233–240.

Appendex 8.11 The raw data for the 118 in-house chemicals

試験日	2014/1/21
実施者名	yamaguchi
被験物質名	#118; 1,3-di-isopropylbenzene

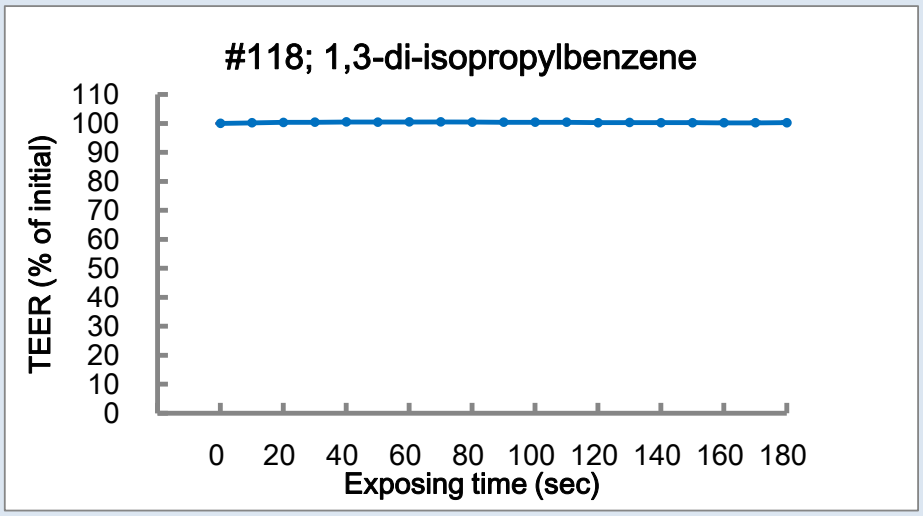
Test #	1	2	3
Model #	14011525	14011526	14011527
Initial	212	200	196
0	214	205	203
10	214	205	203
20	214	206	203
30	214	206	203
40	214	206	203
50	214	206	203
60	214	206	203
70	214	206	203
80	214	206	203
90	214	206	203
100	214	206	203
110	214	206	203
120	214	205	203
130	214	205	203
140	214	205	203
150	214	205	202
160	214	205	202
170	214	205	202
180	215	205	202

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.00	NI
Plateau level	0	NI



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#117; butyrolactone

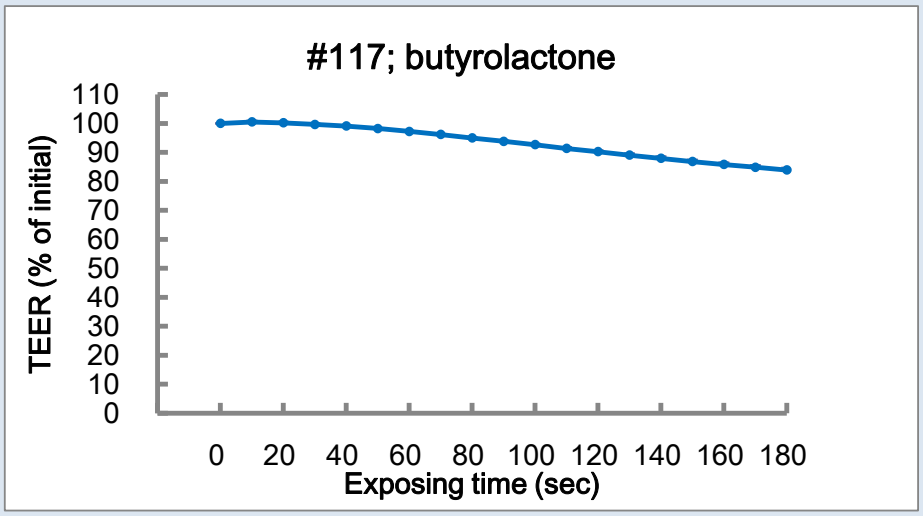
Test #	1	2	3
Model #	13112722	13112723	13112724
Initial	195	198	195
0	197	198	198
10	197	199	199
20	197	198	199
30	196	198	198
40	196	197	197
50	195	197	197
60	194	196	196
70	193	195	195
80	192	193	194
90	191	192	193
100	190	191	192
110	189	190	191
120	189	189	190
130	188	188	189
140	187	187	188
150	186	186	187
160	185	185	186
170	184	184	185
180	183	183	184

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	60	I
Intensity	0.11	I
Plateau level	16	I



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#116; cyclopentanol

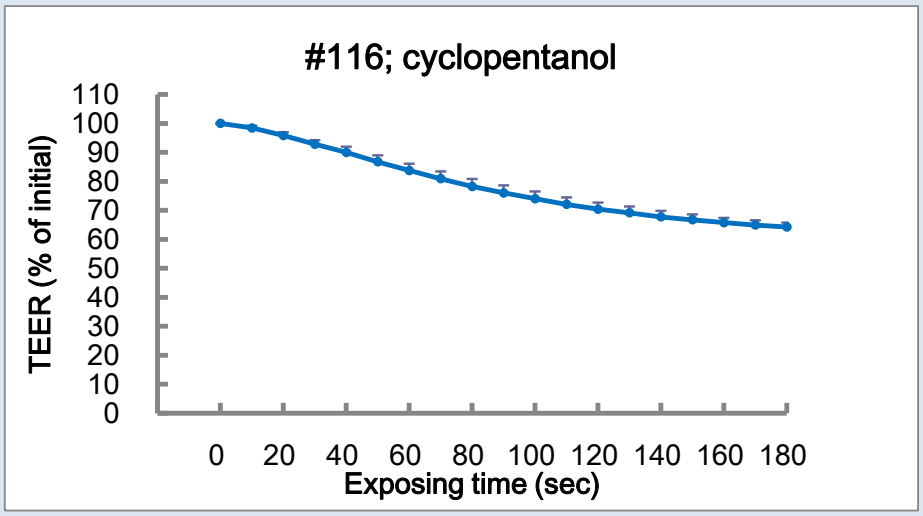
Test #	1	2	3
Model #	13112719	13112720	13112721
Initial	208	210	211
0	211	213	214
10	209	212	213
20	205	209	211
30	202	206	207
40	199	203	205
50	195	200	202
60	192	197	199
70	189	194	196
80	186	191	193
90	184	189	191
100	182	187	189
110	180	185	187
120	178	184	185
130	177	182	184
140	176	181	182
150	175	180	181
160	175	179	179
170	174	178	178
180	173	177	178

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.20	I
Plateau level	36	I



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#115; 1,2,3-trichloropropane

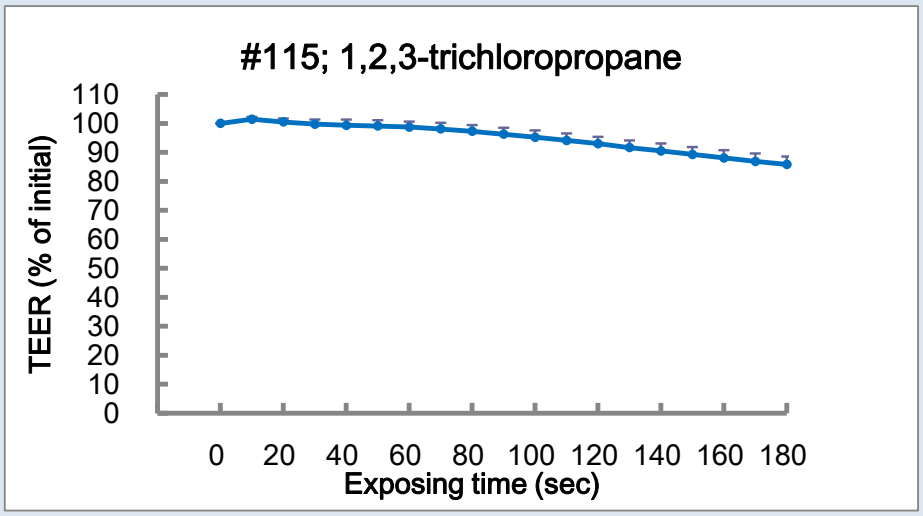
Test #	1	2	3
Model #	13112716	13112717	13112718
Initial	190	197	189
0	189	198	191
10	190	199	193
20	190	198	192
30	189	197	192
40	189	196	192
50	188	196	192
60	188	196	191
70	187	195	191
80	187	194	190
90	186	193	190
100	185	193	189
110	184	191	188
120	183	190	187
130	182	189	186
140	181	188	185
150	180	187	184
160	179	186	183
170	178	185	183
180	177	184	182

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	80	I
Intensity	0.11	I
Plateau level	14	I



試験日	2014/1/21
実施者名	yamaguchi
被験物質名	#114; 1,2,4-trimethylbenzene

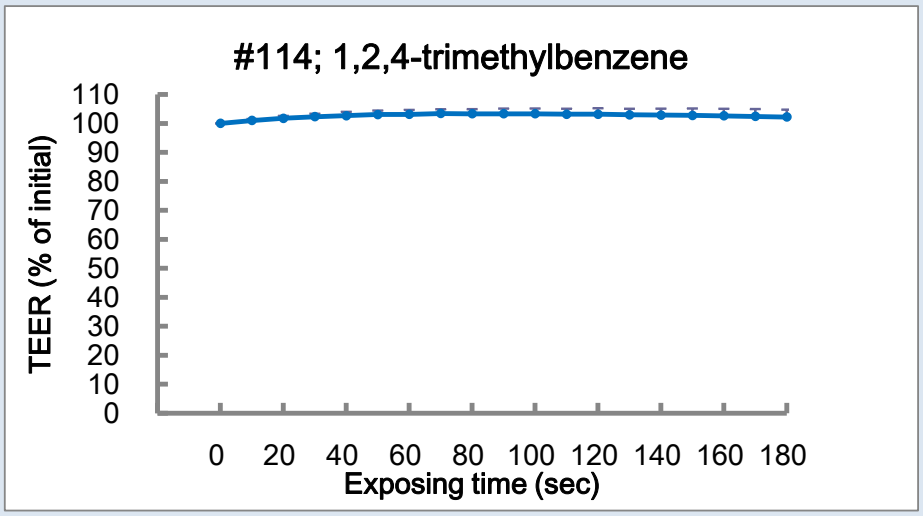
Test #	1	2	3
Model #	14011522	14011523	14011524
Initial	203	189	189
0	202	190	192
10	204	191	192
20	205	191	192
30	205	192	193
40	206	192	193
50	206	192	193
60	207	192	193
70	207	193	193
80	207	193	193
90	207	193	193
100	207	193	193
110	207	193	193
120	207	193	192
130	207	193	192
140	207	192	192
150	207	192	192
160	207	192	192
170	207	192	191
180	206	192	191

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2014/1/21
実施者名	yamaguchi
被験物質名	#113; Tween80

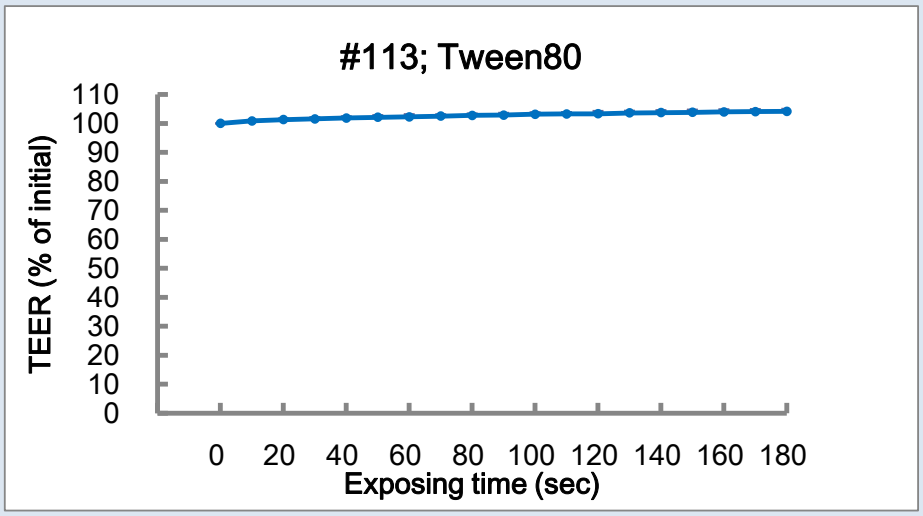
Test #	1	2	3
Model #	14011519	14011520	14011521
Initial	199	223	217
0	197	221	214
10	198	221	215
20	199	222	216
30	199	222	216
40	199	222	216
50	200	222	217
60	200	222	217
70	200	223	217
80	200	223	218
90	200	223	218
100	200	224	218
110	201	224	218
120	201	223	218
130	201	224	219
140	201	224	219
150	201	224	219
160	201	224	219
170	201	224	219
180	201	224	219

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#112; polyethylene glycol monostearate (10E.O.)

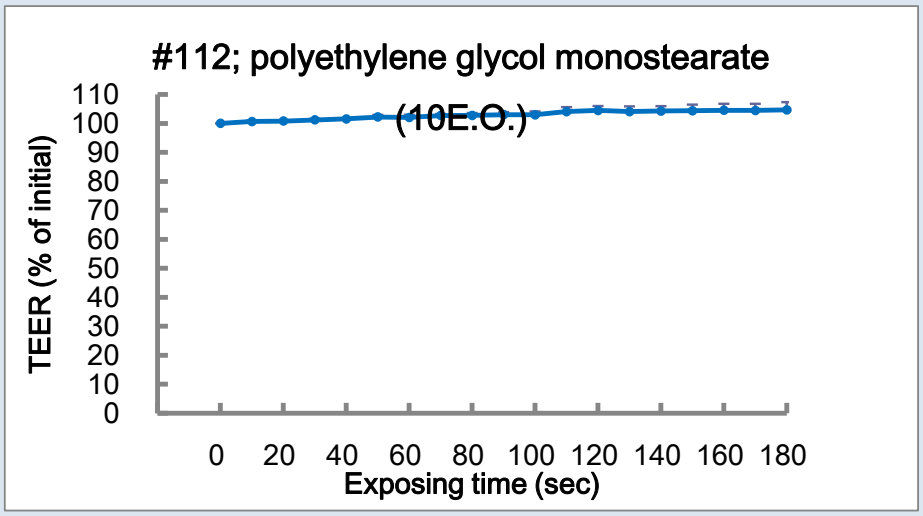
Test #	1	2	3
Model #	13102304	13102305	13102306
Initial	161	169	171
0	185	192	197
10	185	193	198
20	186	193	197
30	186	194	198
40	186	194	198
50	186	195	198
60	187	195	198
70	187	195	199
80	187	195	199
90	187	196	199
100	188	196	198
110	189	196	199
120	189	196	199
130	189	196	199
140	190	196	199
150	190	196	199
160	190	196	199
170	190	196	199
180	191	196	199

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2014/1/21
実施者名	yamaguchi
被験物質名	#111; polyoxyethylene 23 lauryl ether

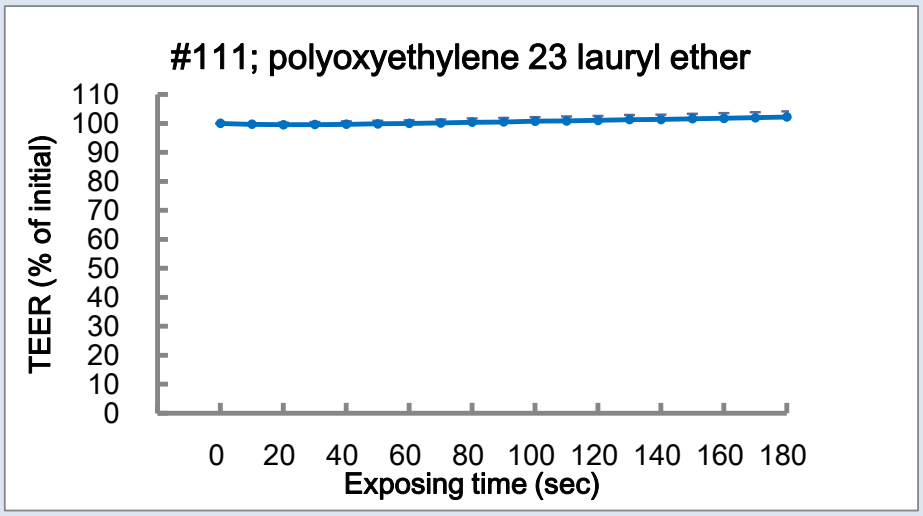
Test #	1	2	3
Model #	14011516	14011517	14011518
Initial	205	219	192
0	205	219	193
10	205	218	193
20	205	218	193
30	205	217	193
40	206	217	193
50	206	217	193
60	206	218	193
70	206	218	193
80	206	218	193
90	207	218	194
100	207	218	194
110	207	218	194
120	207	218	194
130	207	218	194
140	208	218	194
150	208	219	195
160	208	219	195
170	208	219	195
180	208	219	195

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#110; calcium thioglycollate

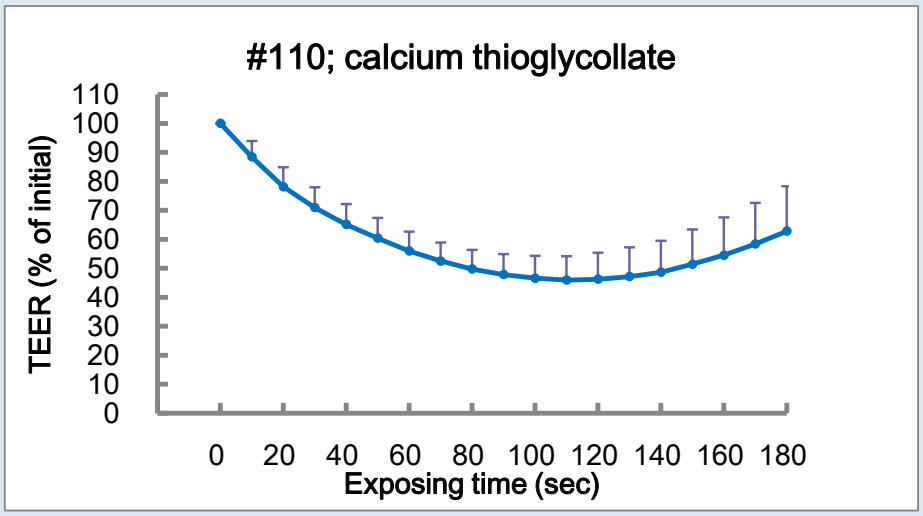
Test #	1	2	3
Model #	13112713	13112714	13112715
Initial	206	212	167
0	200	210	167
10	187	196	164
20	177	184	159
30	171	177	155
40	165	171	152
50	161	166	149
60	158	161	146
70	155	158	144
80	152	155	143
90	150	153	142
100	149	151	142
110	148	150	142
120	148	150	142
130	148	150	143
140	149	151	145
150	151	153	147
160	154	156	150
170	157	159	153
180	160	162	156

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.53	I
Plateau level	53	I



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#109; petroleum ether

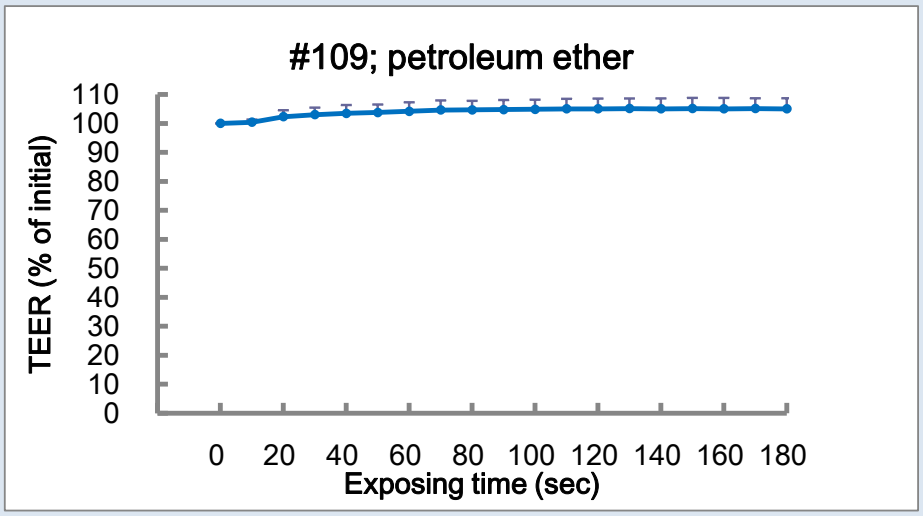
Test #	1	2	3
Model #	13112710	13112711	13112712
Initial	205	214	210
0	206	207	209
10	205	208	211
20	206	212	211
30	207	213	212
40	207	214	212
50	207	214	212
60	208	215	213
70	208	216	213
80	208	215	213
90	208	216	213
100	208	216	213
110	208	216	213
120	208	216	213
130	208	216	213
140	208	216	213
150	208	216	213
160	208	216	213
170	208	216	214
180	208	216	213

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.03	NI
Plateau level	0	NI



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#108; methyl acetate

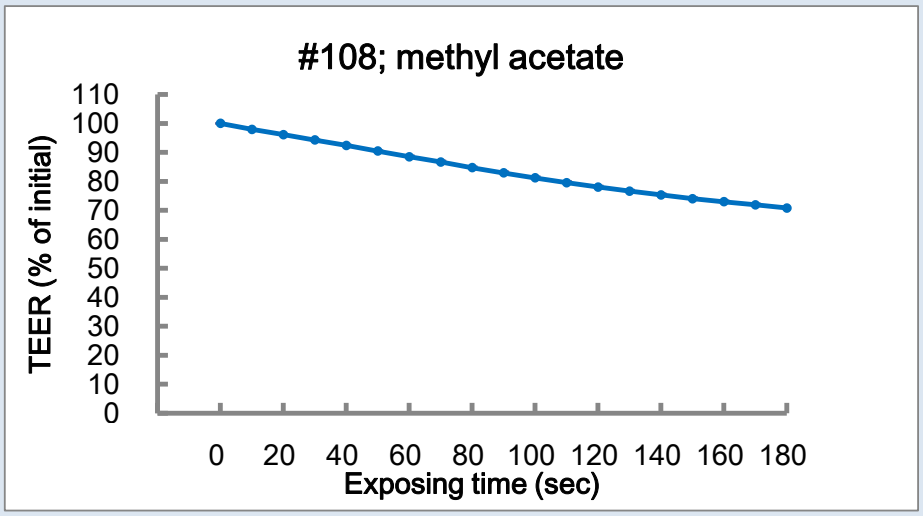
Test #	1	2	3
Model #	13111334	13111335	13111336
Initial	215	227	228
0	228	228	230
10	225	225	227
20	223	223	225
30	221	221	223
40	219	219	220
50	216	216	218
60	214	214	215
70	212	212	213
80	210	210	211
90	208	208	209
100	206	206	207
110	204	204	205
120	202	202	203
130	200	200	201
140	199	199	200
150	197	197	198
160	196	196	197
170	195	195	195
180	193	193	194

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.16	I
Plateau level	29	I



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#107; glycolic acid

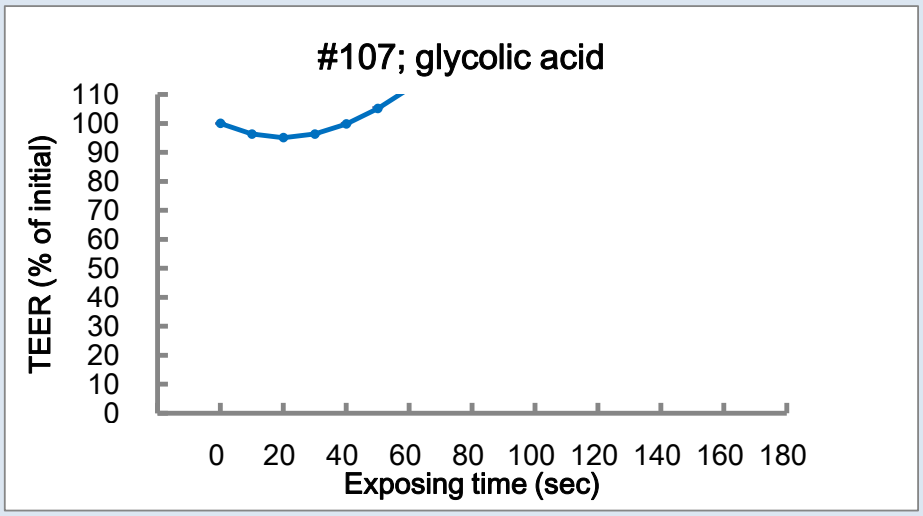
Test #	1	2	3
Model #	13111331	13111332	13111333
Initial	227	236	231
0	220	222	223
10	216	218	219
20	214	217	217
30	216	218	219
40	220	222	223
50	226	227	229
60	234	235	237
70	242	243	247
80	252	252	257
90	262	261	268
100	271	269	279
110	279	276	287
120	286	282	294
130	292	287	300
140	297	291	305
150	301	295	309
160	304	298	312
170	307	300	315
180	309	302	317

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.37	I
Plateau level	4	NI



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#106; isobutanal

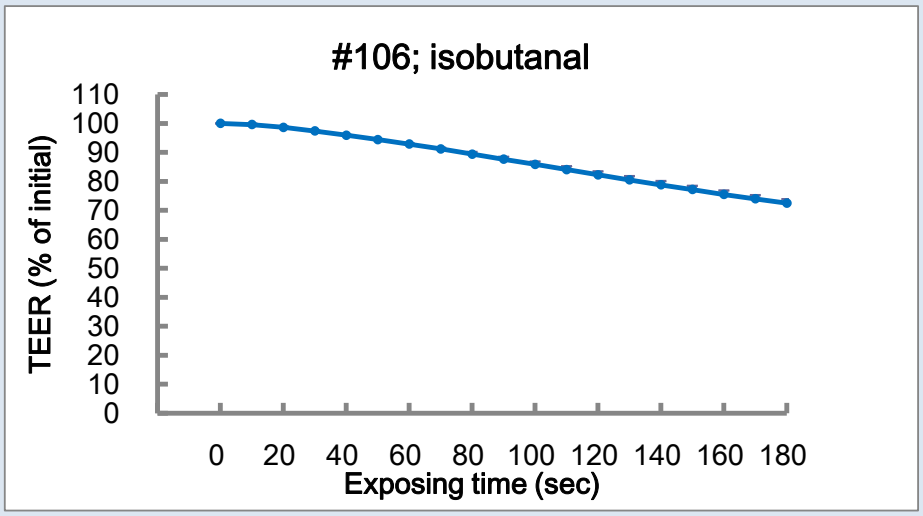
Test #	1	2	3
Model #	13111328	13111329	13111330
Initial	228	225	235
0	226	229	236
10	226	228	235
20	225	227	234
30	223	226	232
40	221	224	231
50	220	222	229
60	218	220	227
70	216	218	225
80	213	216	224
90	211	213	222
100	209	211	220
110	207	209	218
120	205	207	215
130	203	205	213
140	201	203	211
150	199	201	209
160	197	199	207
170	195	197	205
180	193	195	203

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	30	I
Intensity	0.17	I
Plateau level	28	I



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#105; isobutyl alcohol

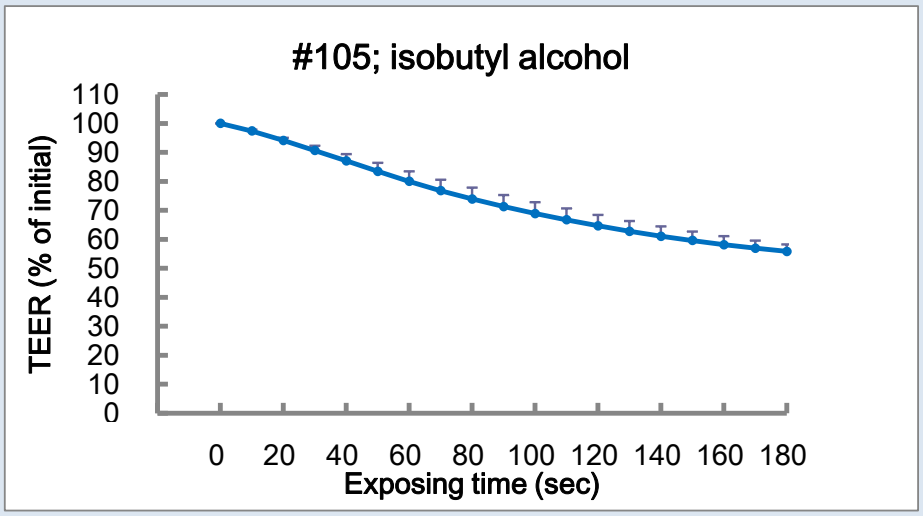
Test #	1	2	3
Model #	13111325	13111326	13111327
Initial	211	211	228
0	220	227	237
10	217	223	235
20	213	219	231
30	209	214	228
40	205	210	224
50	200	205	221
60	196	200	217
70	193	196	213
80	190	193	210
90	187	189	207
100	184	187	203
110	182	184	201
120	180	182	198
130	178	180	195
140	176	178	193
150	175	176	190
160	173	175	188
170	172	174	186
180	171	173	184

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.24	I
Plateau level	44	I



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#104; citric acid

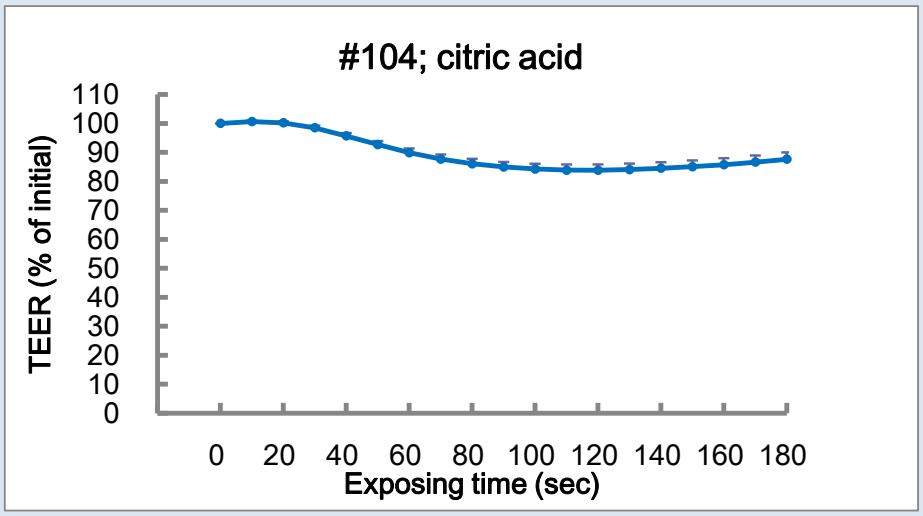
Test #	1	2	3
Model #	13111322	13111323	13111324
Initial	229	232	222
0	222	226	217
10	224	227	217
20	223	226	217
30	221	225	214
40	218	222	211
50	215	219	208
60	211	216	205
70	208	214	202
80	206	212	200
90	205	211	199
100	204	210	199
110	204	210	198
120	203	210	198
130	204	210	198
140	204	211	199
150	205	212	199
160	205	212	200
170	206	213	201
180	207	215	202

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	30	I
Intensity	0.20	I
Plateau level	16	I



試験日	2013/5/14
実施者名	yamaguchi
被験物質名	#103; silicic anhydride

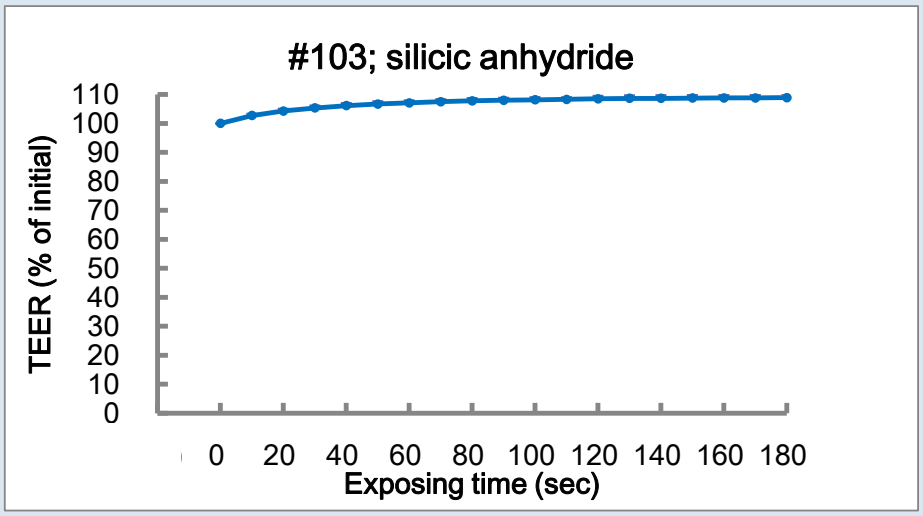
Test #	1	2	3
Model #	13050901	13050903	13050904
Initial	175	170	173
0	171	170	168
10	173	172	170
20	174	173	171
30	174	174	172
40	175	174	172
50	175	174	172
60	175	175	173
70	176	175	173
80	176	175	173
90	176	175	173
100	176	176	173
110	176	175	173
120	176	176	173
130	176	176	173
140	176	176	173
150	177	176	173
160	177	176	173
170	177	176	173
180	177	176	173

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.05	NI
Plateau level	0	NI



試験日	2014/1/21
実施者名	yamaguchi
被験物質名	#102; dimethyl sulfoxide

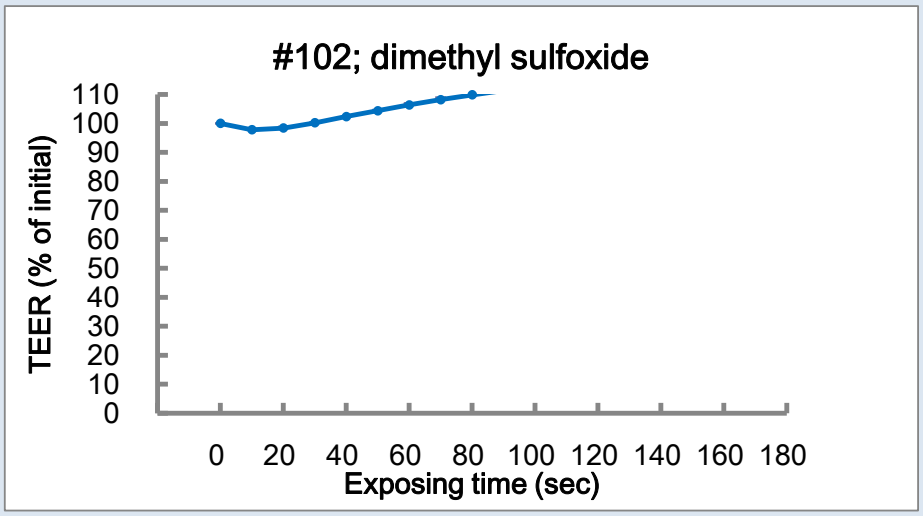
Test #	1	2	3
Model #	14011513	14011514	14011515
Initial	186	189	191
0	190	198	201
10	188	196	199
20	188	197	200
30	190	199	202
40	192	200	203
50	193	202	205
60	195	204	207
70	197	205	208
80	198	207	210
90	200	208	211
100	201	209	212
110	202	210	213
120	203	211	214
130	204	212	215
140	204	213	216
150	205	214	217
160	206	215	218
170	206	215	218
180	207	216	219

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.11	NI
Plateau level	0	NI



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#101; benzethonium chloride

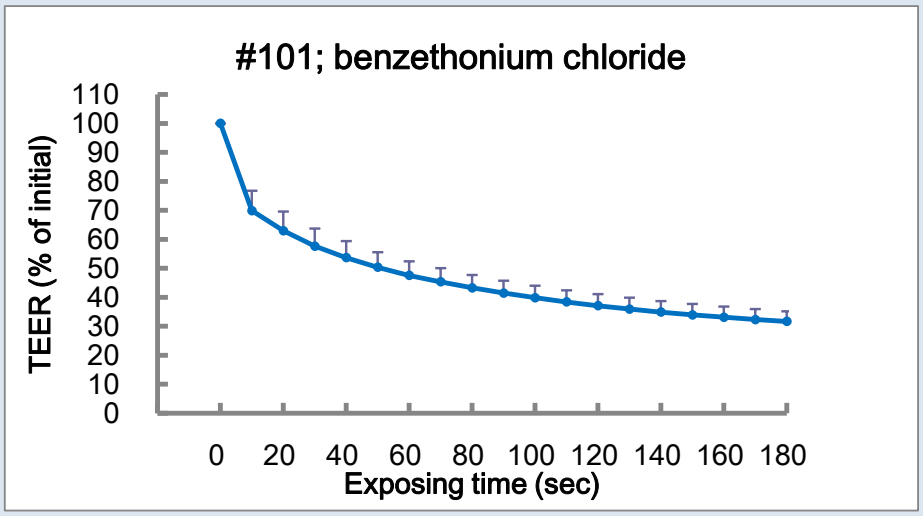
Test #	1	2	3
Model #	13111319	13111320	13111321
Initial	223	230	227
0	171	181	170
10	154	154	155
20	150	149	151
30	147	146	148
40	144	144	145
50	142	142	142
60	140	140	141
70	139	138	139
80	138	137	138
90	137	136	137
100	135	135	136
110	134	134	135
120	134	133	134
130	133	132	133
140	132	132	133
150	132	131	132
160	131	131	132
170	131	130	131
180	130	130	131

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.38	I
Plateau level	68	I



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#100; 2-benzyloxyethanol

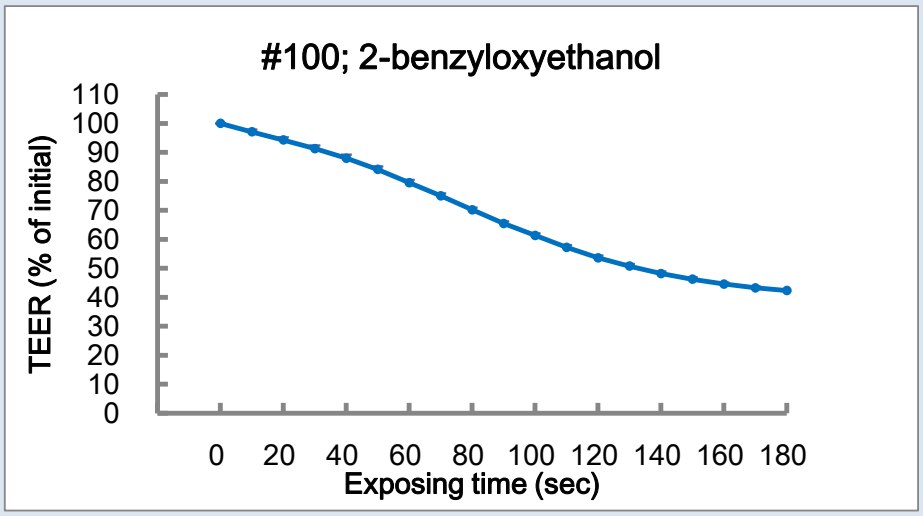
Test #	1	2	3
Model #	13111316	13111317	13111318
Initial	223	231	222
0	227	235	228
10	223	231	225
20	220	228	222
30	216	224	218
40	212	220	215
50	207	216	210
60	202	210	204
70	197	205	199
80	192	199	193
90	186	193	187
100	182	188	182
110	177	183	177
120	173	178	172
130	170	175	169
140	167	171	166
150	164	169	164
160	162	167	162
170	161	165	160
180	159	164	160

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.32	I
Plateau level	58	I



試験日	2014/1/21
実施者名	yamaguchi
被験物質名	#99; polyoxyethylene hydrogenated castoroil (60E.O)

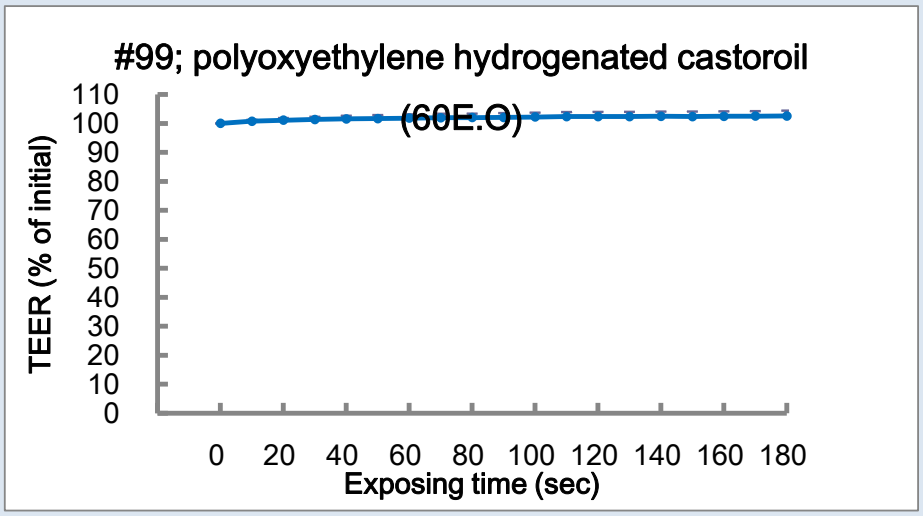
Test #	1	2	3
Model #	14011510	14011511	14011512
Initial	194	189	202
0	200	196	208
10	200	197	209
20	200	198	209
30	201	198	209
40	201	198	209
50	201	199	209
60	201	199	209
70	201	199	209
80	201	199	209
90	201	199	209
100	201	199	209
110	201	199	210
120	201	200	210
130	201	200	210
140	201	200	210
150	201	200	209
160	201	200	210
170	201	200	210
180	201	200	210

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#98; ethyl-2-methylacetoacetate

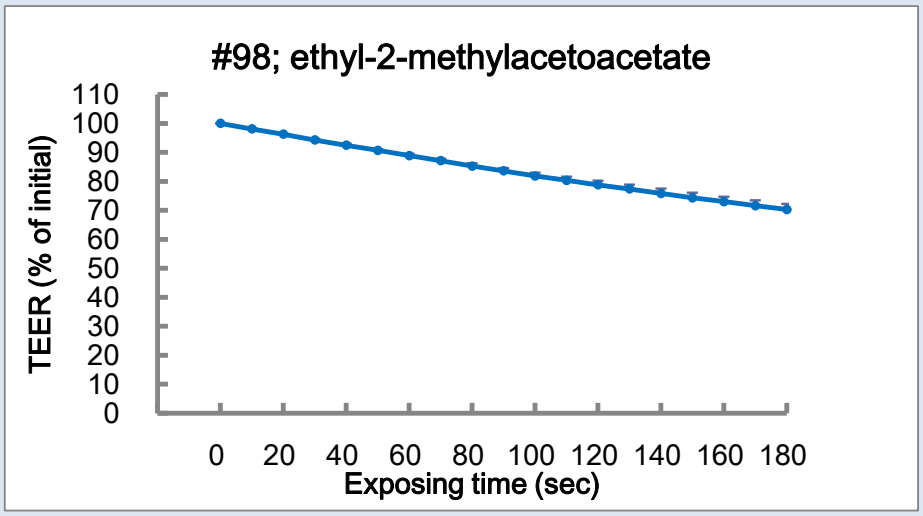
Test #	1	2	3
Model #	13112704	13112705	13112706
Initial	223	223	216
0	227	224	222
10	224	222	220
20	222	220	218
30	220	218	215
40	218	216	213
50	216	214	211
60	213	212	209
70	211	211	207
80	209	209	205
90	207	207	203
100	205	205	201
110	203	204	199
120	201	202	197
130	200	200	195
140	198	199	193
150	196	197	191
160	195	196	190
170	193	194	188
180	192	193	187

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.16	I
Plateau level	30	I



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#97; cetylpyridinium chloride

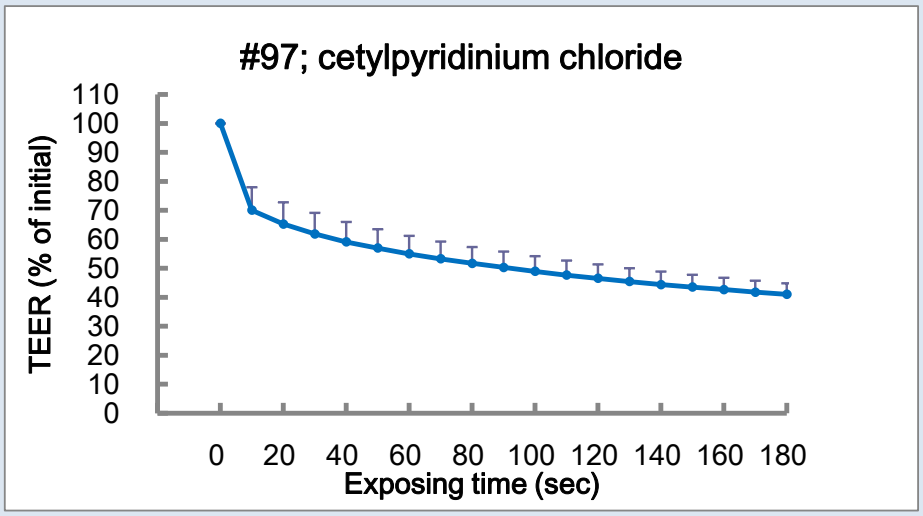
Test #	1	2	3
Model #	13111313	13111314	13111315
Initial	222	223	208
0	161	181	174
10	150	155	154
20	148	152	151
30	146	150	148
40	144	148	146
50	143	147	145
60	142	146	143
70	141	145	142
80	140	144	141
90	139	143	140
100	138	142	140
110	137	141	139
120	137	141	138
130	136	140	138
140	135	139	137
150	135	139	137
160	134	138	136
170	134	138	136
180	133	137	135

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.33	I
Plateau level	59	I



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#96; betaine monohydrate

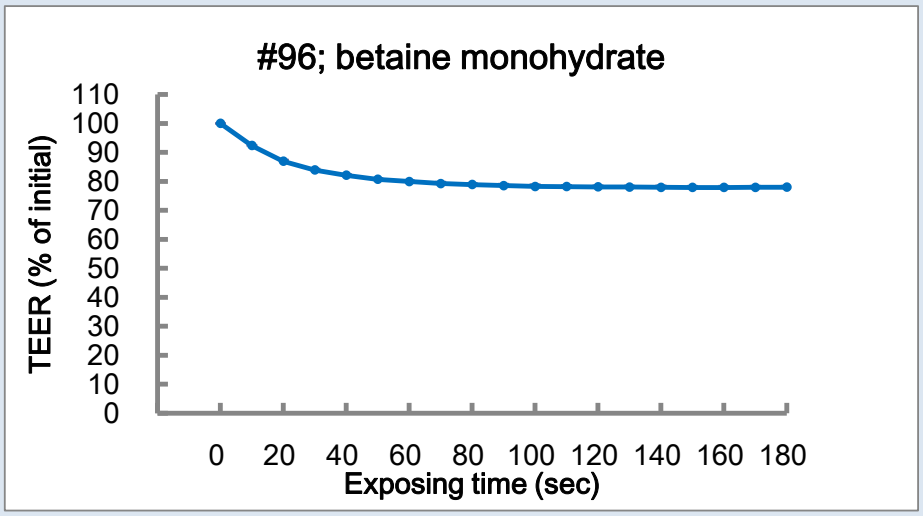
Test #	1	2	3
Model #	13120434	13120435	13120436
Initial	191	196	187
0	193	198	189
10	186	191	183
20	182	187	178
30	179	184	176
40	178	182	174
50	177	181	173
60	176	180	173
70	176	180	172
80	175	180	172
90	175	179	171
100	175	179	171
110	175	179	171
120	175	179	171
130	175	179	171
140	175	179	171
150	175	178	171
160	175	178	171
170	175	178	171
180	175	178	171

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.26	I
Plateau level	21	I



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#95; di(2-ethylhexyl) sodiumsulfosuccinate

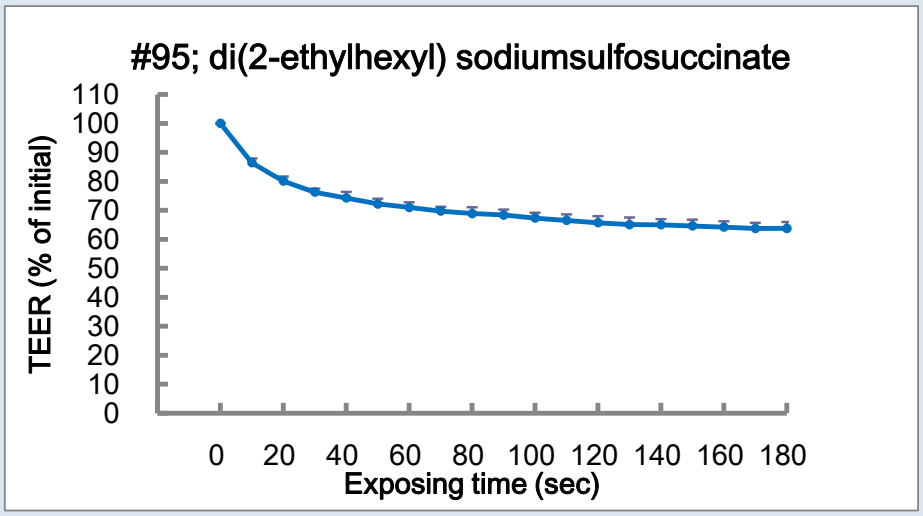
Test #	1	2	3
Model #	13102333	13102301	13102302
Initial	173	163	166
0	179	179	184
10	171	169	173
20	167	165	168
30	164	162	166
40	163	160	164
50	162	159	162
60	161	158	161
70	160	158	161
80	160	156	160
90	159	156	160
100	158	156	159
110	158	155	158
120	157	154	158
130	157	154	157
140	157	154	157
150	157	153	157
160	156	153	157
170	156	153	156
180	156	153	156

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.24	I
Plateau level	35	I



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#94; domiphen bromide

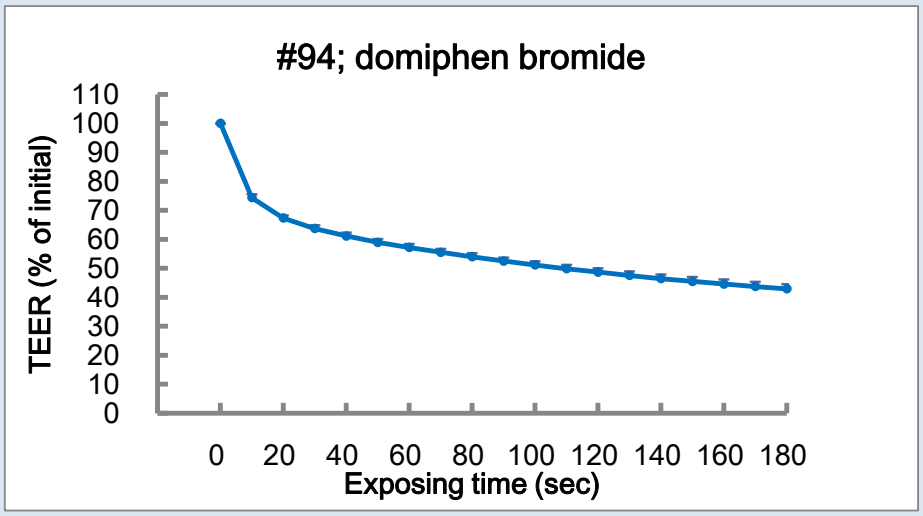
Test #	1	2	3
Model #	13111310	13111311	13111312
Initial	241	238	231
0	190	186	181
10	169	168	162
20	164	162	157
30	162	159	154
40	160	157	152
50	158	155	151
60	156	154	150
70	155	152	148
80	154	151	147
90	153	150	146
100	151	149	145
110	150	148	144
120	149	148	143
130	149	147	143
140	148	146	142
150	147	145	141
160	146	145	140
170	145	144	140
180	145	144	139

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.32	I
Plateau level	57	I



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#93; sodium 2-naphthalenesulfonate

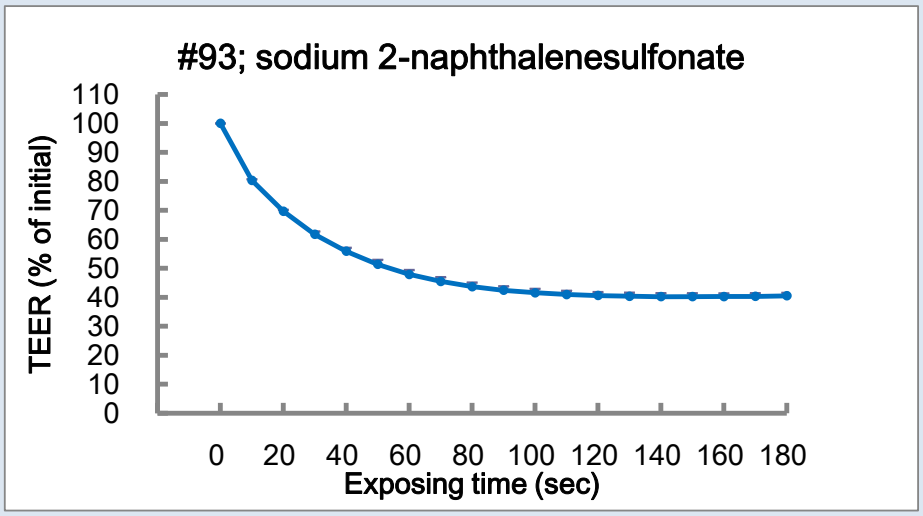
Test #	1	2	3
Model #	13111307	13111308	13111309
Initial	231	239	239
0	211	218	213
10	191	197	193
20	181	185	182
30	174	176	173
40	168	170	167
50	164	165	162
60	160	162	158
70	158	159	156
80	156	157	154
90	154	156	152
100	154	155	152
110	153	154	151
120	152	154	151
130	152	154	150
140	152	153	150
150	152	154	150
160	152	154	150
170	152	154	150
180	152	154	151

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.54	I
Plateau level	59	I



試験日	2013/11/19
実施者名	yamaguchi
被験物質名	#92; lactic acid

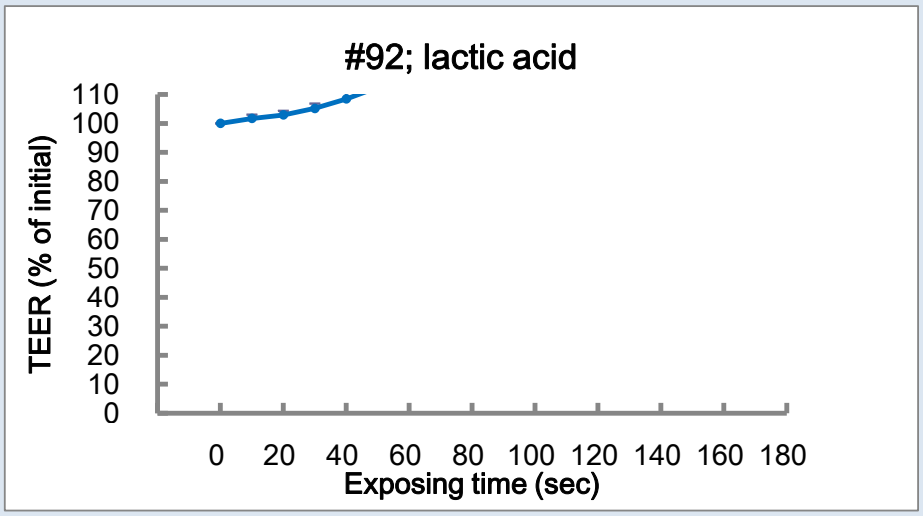
Test #	1	2	3
Model #	13111304	13111305	13111306
Initial	237	242	234
0	225	237	230
10	229	238	231
20	230	239	233
30	233	241	236
40	237	245	240
50	242	249	245
60	248	254	251
70	253	259	256
80	258	264	261
90	263	268	266
100	268	272	271
110	272	276	275
120	276	280	279
130	280	284	283
140	284	287	287
150	288	290	290
160	291	294	293
170	294	296	296
180	297	299	298

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.30	NI
Plateau level	0	NI



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#91; 2,2-dimethyl-3-pentanol

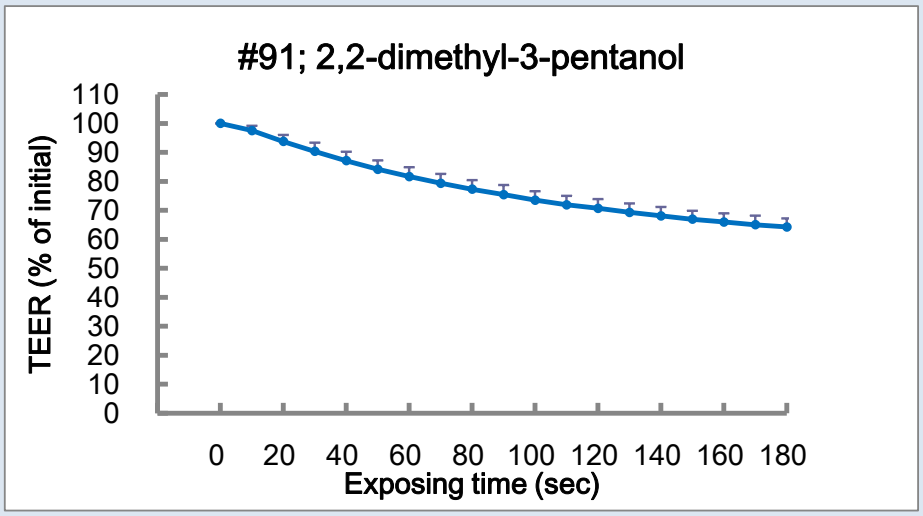
Test #	1	2	3
Model #	13120428	13120429	13120430
Initial	191	205	193
0	192	209	197
10	190	205	196
20	188	200	193
30	185	196	190
40	183	193	187
50	180	190	184
60	179	187	182
70	177	185	180
80	175	183	178
90	174	181	176
100	172	180	175
110	171	178	173
120	170	177	172
130	169	175	171
140	168	174	170
150	167	173	169
160	166	172	168
170	165	171	167
180	164	171	167

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.20	I
Plateau level	36	I



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#90; ethyl trimethyl acetate

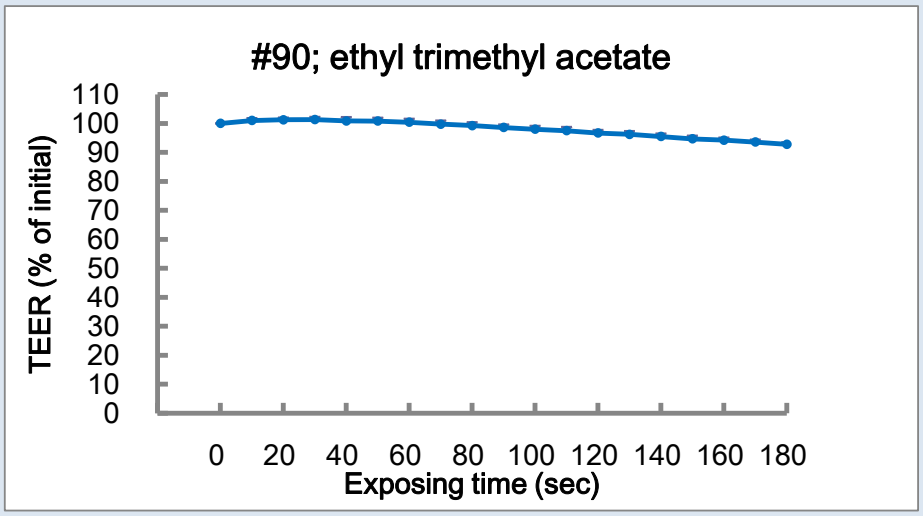
Test #	1	2	3
Model #	13120431	13120432	13120433
Initial	189	188	184
0	190	194	190
10	190	196	191
20	190	196	191
30	190	196	191
40	190	196	191
50	190	196	191
60	189	196	191
70	189	195	190
80	188	195	189
90	188	194	189
100	187	193	189
110	187	193	188
120	186	192	188
130	186	192	187
140	185	191	187
150	185	191	186
160	185	190	186
170	184	190	185
180	184	189	184

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	110	I
Intensity	0.07	I
Plateau level	7	I



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#89; sodium monochloroacetate

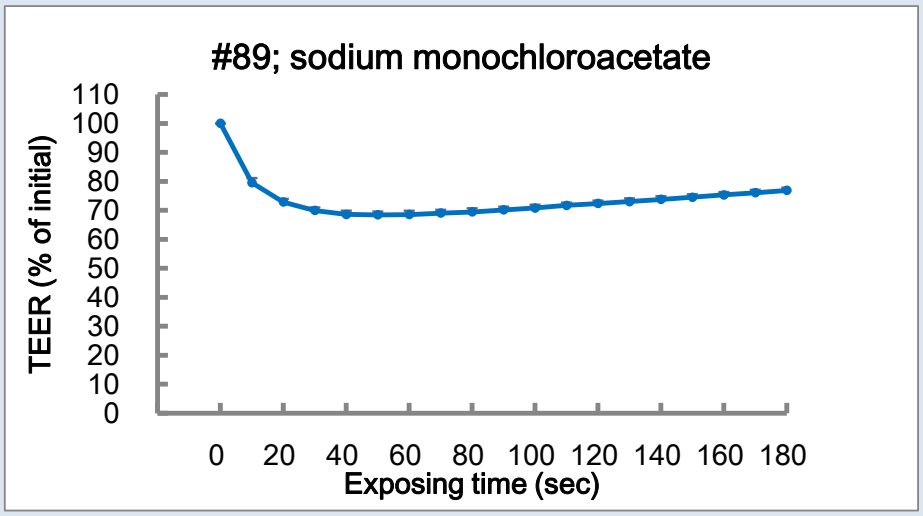
Test #	1	2	3
Model #	13110834	13110835	13110836
Initial	188	194	195
0	177	180	177
10	163	165	165
20	159	161	160
30	157	159	158
40	156	157	157
50	156	157	157
60	156	157	157
70	156	158	157
80	156	158	157
90	157	159	158
100	157	159	158
110	158	160	159
120	158	160	159
130	159	161	160
140	159	161	160
150	160	162	161
160	160	162	161
170	161	163	162
180	162	163	162

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.78	I
Plateau level	31	I



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#88; methoxyethyl acrylate

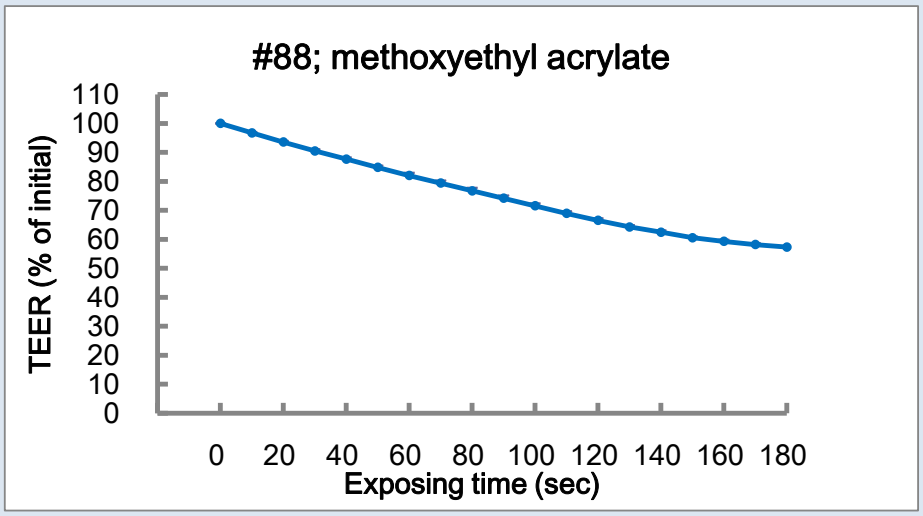
Test #	1	2	3
Model #	13110831	13110832	13110833
Initial	189	194	189
0	190	194	191
10	187	191	189
20	184	188	186
30	182	185	184
40	180	183	182
50	177	181	179
60	175	178	177
70	173	176	175
80	171	174	173
90	169	171	171
100	167	169	169
110	165	167	167
120	163	165	165
130	161	163	163
140	160	162	161
150	158	160	160
160	157	159	159
170	157	158	157
180	156	158	157

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.24	I
Plateau level	43	I



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#87; di(propylene glycol) propyl ether

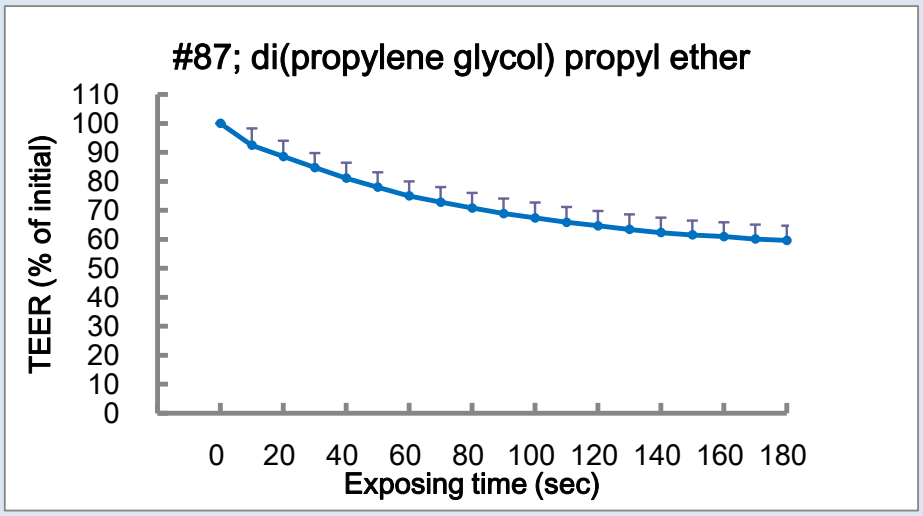
Test #	1	2	3
Model #	13110828	13110829	13110830
Initial	183	191	185
0	188	191	195
10	185	187	183
20	182	184	180
30	179	181	177
40	176	178	173
50	173	175	171
60	171	173	169
70	169	172	167
80	168	170	165
90	166	168	163
100	165	167	162
110	164	166	161
120	162	165	160
130	161	164	159
140	161	163	158
150	160	163	157
160	159	162	157
170	159	161	156
180	158	161	156

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.22	I
Plateau level	40	I



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#86; iso-octyl acrylate

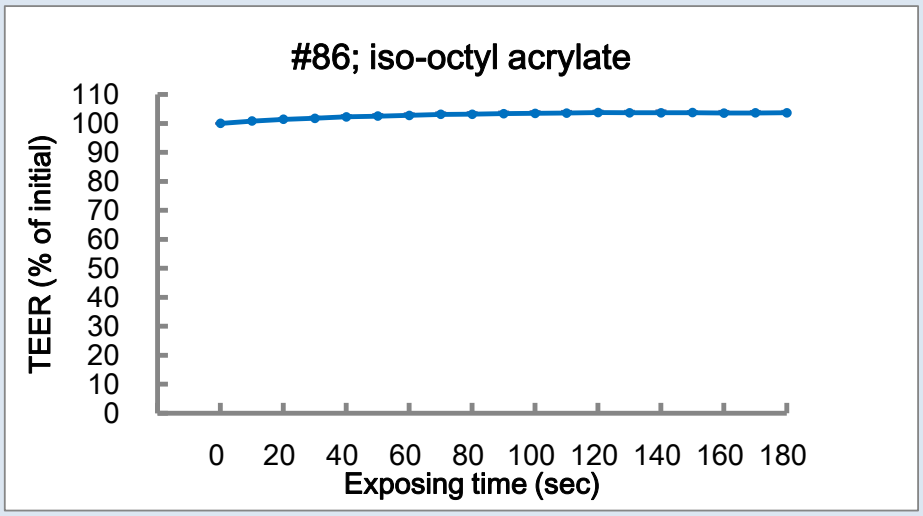
Test #	1	2	3
Model #	13112731	13112732	13112733
Initial	203	205	219
0	203	204	220
10	204	205	221
20	204	206	221
30	204	206	222
40	205	207	222
50	205	207	222
60	206	207	223
70	206	207	223
80	206	208	223
90	206	208	223
100	206	208	223
110	206	208	223
120	207	208	224
130	206	208	224
140	206	208	223
150	206	208	224
160	206	208	224
170	206	208	224
180	207	208	224

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#85; 3-glycidoxypropyltrimethoxysilane

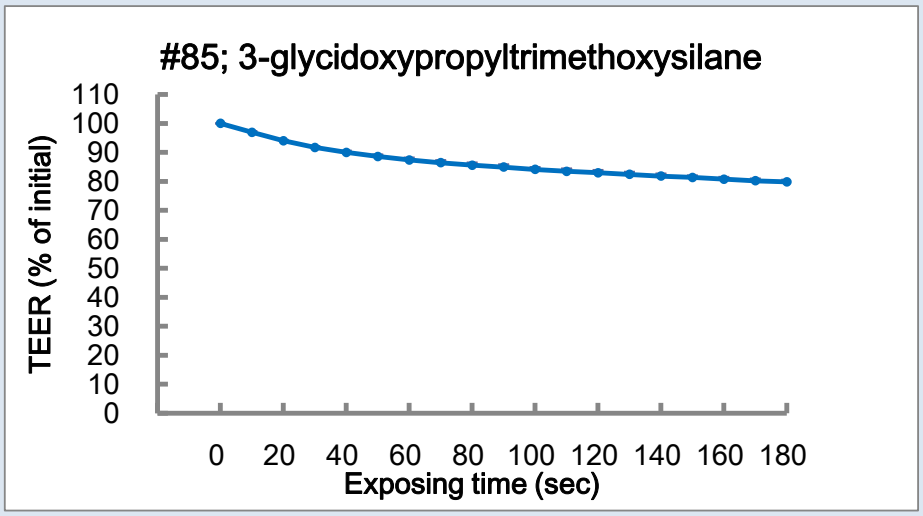
Test #	1	2	3
Model #	13112728	13112729	13112730
Initial	199	196	203
0	202	199	205
10	199	197	202
20	196	194	200
30	194	192	198
40	192	190	196
50	191	189	195
60	190	188	194
70	189	187	193
80	188	186	192
90	188	185	192
100	187	185	191
110	186	184	190
120	186	184	190
130	185	183	189
140	185	183	189
150	185	182	188
160	184	182	188
170	183	181	187
180	183	181	187

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.11	I
Plateau level	20	I



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#84; EDTA,di-potassium

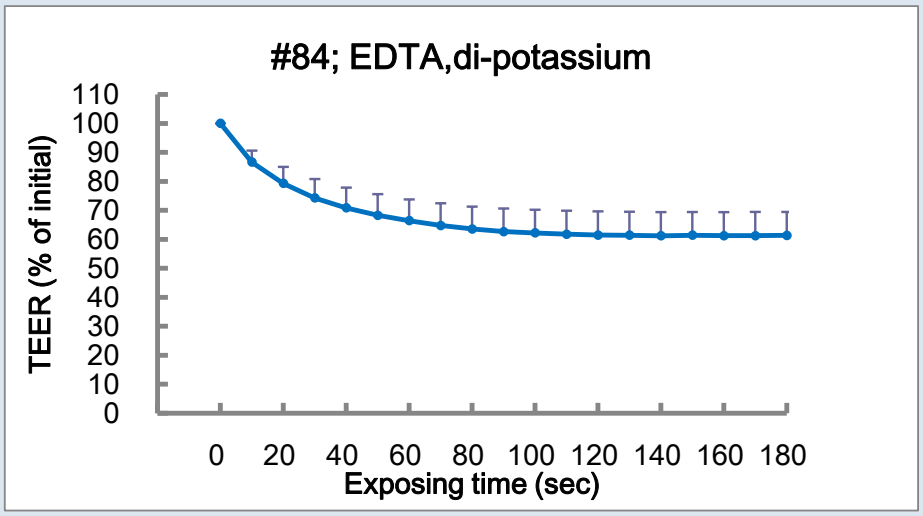
Test #	1	2	3
Model #	13112725	13112726	13112727
Initial	180	192	189
0	171	176	177
10	161	170	166
20	156	167	161
30	153	164	157
40	151	162	154
50	149	161	153
60	148	159	151
70	147	158	150
80	147	158	149
90	146	157	148
100	146	157	148
110	146	157	147
120	146	157	147
130	145	157	147
140	145	156	147
150	146	156	147
160	145	156	147
170	145	156	147
180	145	156	147

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.38	I
Plateau level	38	I



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#83; potassium sorbate

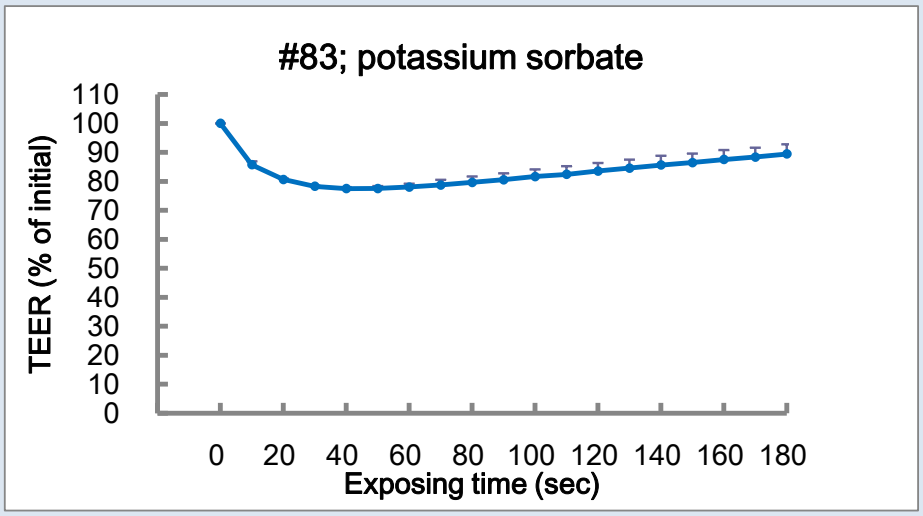
Test #	1	2	3
Model #	13110825	13110826	13110827
Initial	176	184	175
0	163	171	171
10	156	162	162
20	153	159	159
30	151	157	158
40	151	157	158
50	150	157	158
60	151	158	158
70	151	159	159
80	151	159	159
90	151	160	160
100	152	161	160
110	152	161	161
120	153	162	162
130	153	163	162
140	153	164	163
150	154	164	163
160	154	165	164
170	155	165	164
180	155	166	165

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.72	I
Plateau level	22	I



試験日	2014/1/21
実施者名	yamaguchi
被験物質名	#82; 6-methyl purine

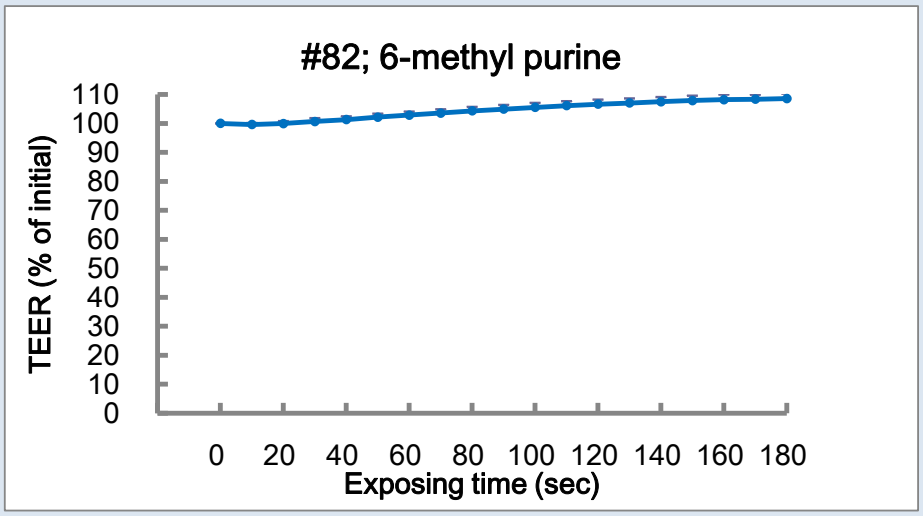
Test #	1	2	3
Model #	14011507	14011508	14011509
Initial	200	201	198
0	199	200	197
10	199	199	198
20	199	200	198
30	199	200	199
40	200	201	200
50	201	201	201
60	201	202	201
70	202	203	202
80	202	203	203
90	203	204	203
100	203	204	204
110	204	205	204
120	204	205	205
130	205	206	205
140	205	206	206
150	205	206	206
160	206	207	206
170	206	207	206
180	206	207	207

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.05	NI
Plateau level	0	NI



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#81; 1,9-decadiene

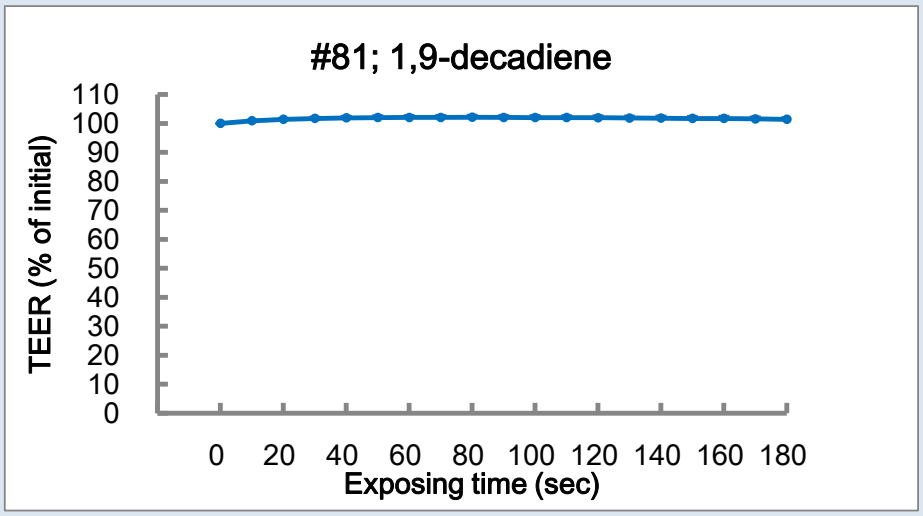
Test #	1	2	3
Model #	13110821	13110822	13110823
Initial	187	194	192
0	189	192	191
10	190	193	192
20	191	193	192
30	191	193	192
40	191	194	193
50	191	194	193
60	191	194	193
70	191	194	192
80	192	194	193
90	191	194	193
100	191	194	192
110	191	194	192
120	191	194	192
130	191	194	192
140	191	194	192
150	191	194	192
160	191	194	192
170	191	194	192
180	191	194	192

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#80; monoethanolamine

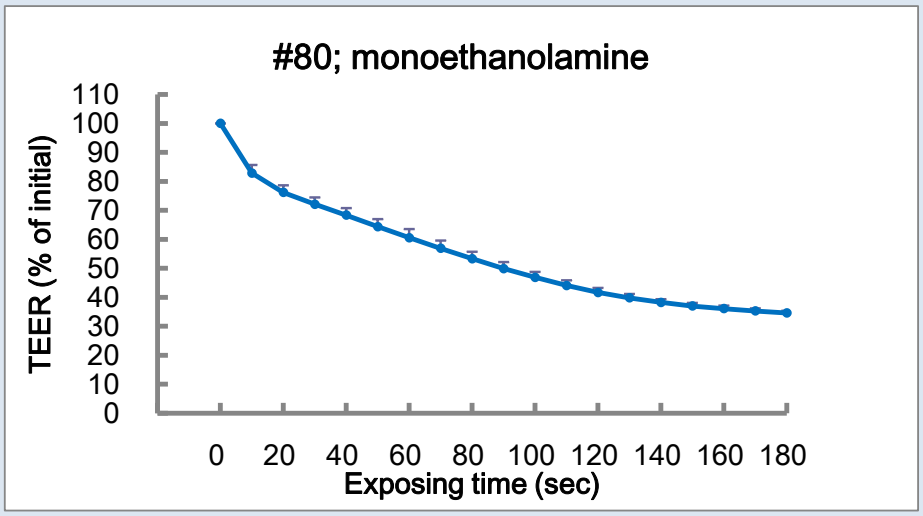
Test #	1	2	3
Model #	13110817	13110818	13110819
Initial	196	190	192
0	192	183	186
10	176	171	175
20	170	166	169
30	167	164	165
40	164	162	162
50	161	159	158
60	158	157	155
70	155	154	152
80	153	151	150
90	150	148	147
100	147	146	145
110	145	143	143
120	143	142	141
130	142	140	140
140	141	139	139
150	140	138	138
160	139	137	137
170	138	136	137
180	138	136	136

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.36	I
Plateau level	65	I



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#79; n-lauroylsarcosine sodium salt

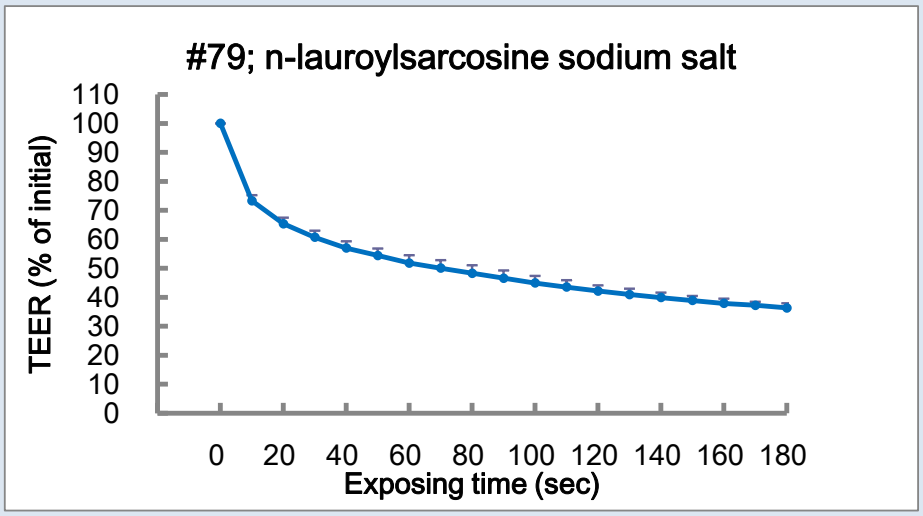
Test #	1	2	3
Model #	13120425	13120426	13120427
Initial	196	177	180
0	175	170	167
10	156	154	153
20	151	150	148
30	148	147	146
40	145	145	144
50	144	143	142
60	142	142	141
70	141	141	140
80	140	139	139
90	138	138	138
100	138	137	137
110	137	136	136
120	136	135	135
130	135	135	134
140	135	134	134
150	134	134	133
160	134	133	132
170	133	133	132
180	133	133	131

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.35	I
Plateau level	64	I



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#78; xylene

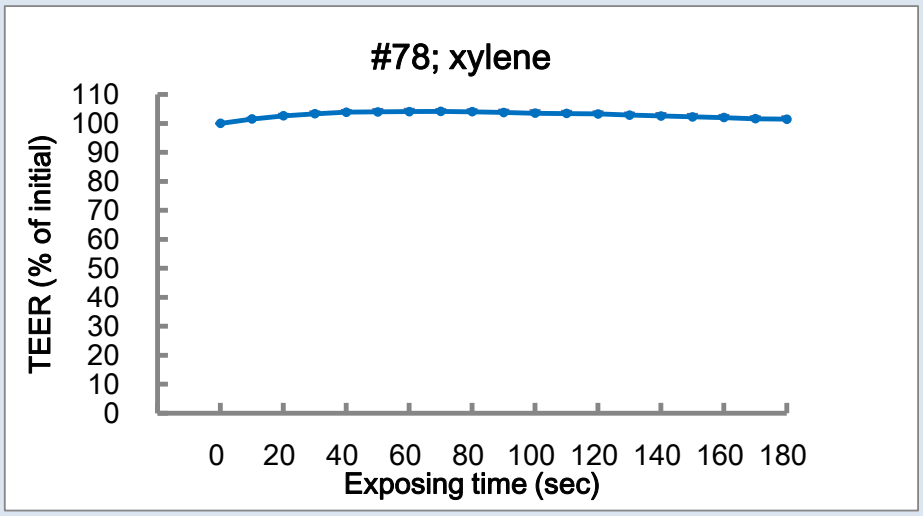
Test #	1	2	3
Model #	13120422	13120423	13120424
Initial	191	192	187
0	190	192	187
10	191	193	188
20	192	194	189
30	192	194	190
40	193	194	190
50	193	195	191
60	193	195	191
70	193	195	191
80	193	195	191
90	193	194	191
100	193	194	190
110	193	194	190
120	192	194	190
130	192	194	190
140	192	193	190
150	192	193	189
160	192	193	189
170	191	192	189
180	191	192	189

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#77; butyl acetate

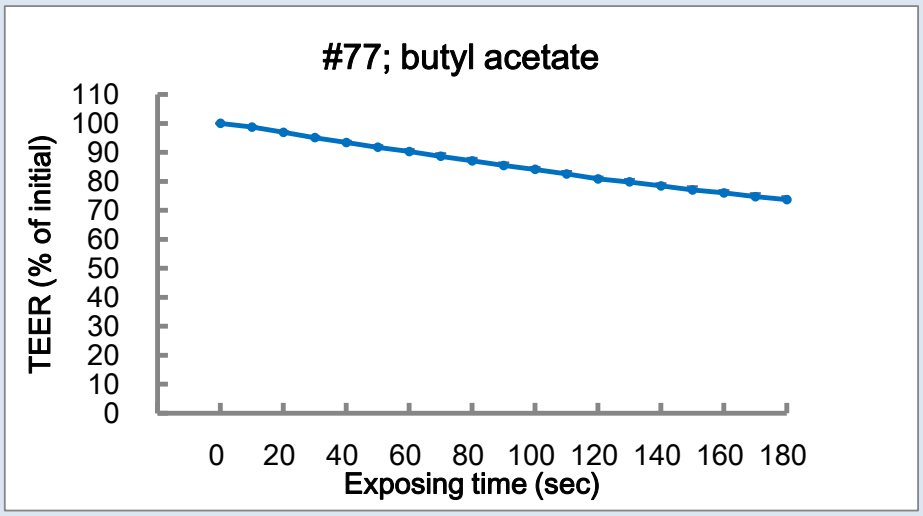
Test #	1	2	3
Model #	13120419	13120420	13120421
Initial	196	199	188
0	198	200	190
10	197	199	189
20	195	197	187
30	194	196	186
40	192	194	184
50	191	193	183
60	190	192	182
70	188	190	180
80	187	189	179
90	185	188	178
100	184	186	177
110	183	185	175
120	181	183	174
130	180	182	173
140	179	181	172
150	178	180	171
160	177	179	170
170	176	178	169
180	175	177	168

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.15	I
Plateau level	26	I



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#76; 2,4-pentanedione

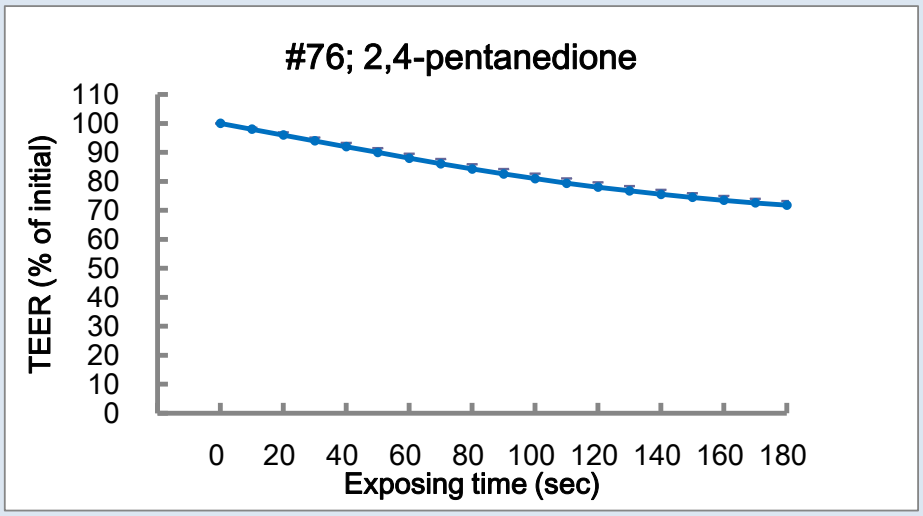
Test #	1	2	3
Model #	13120416	13120417	13120418
Initial	193	197	187
0	194	204	193
10	192	203	191
20	191	201	189
30	189	200	187
40	187	198	185
50	186	196	184
60	184	194	182
70	183	192	180
80	182	190	179
90	180	189	177
100	179	187	176
110	178	185	175
120	177	184	173
130	175	183	172
140	174	182	171
150	173	181	171
160	173	180	170
170	172	179	169
180	171	178	168

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.15	I
Plateau level	28	I



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#75; ethylhexyl salicylate

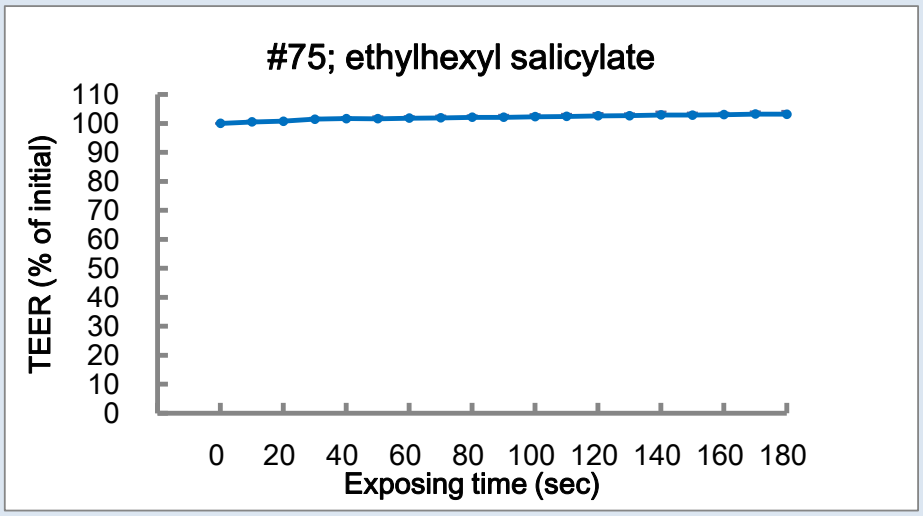
Test #	1	2	3
Model #	13120413	13120414	13120415
Initial	174	185	171
0	176	191	181
10	177	191	182
20	177	191	182
30	177	192	182
40	178	192	182
50	178	192	182
60	178	192	182
70	178	192	182
80	178	192	182
90	178	192	182
100	178	192	183
110	179	192	183
120	179	193	183
130	179	193	183
140	179	193	183
150	179	193	183
160	179	193	183
170	179	193	183
180	179	193	183

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#74; myristyl alcohol

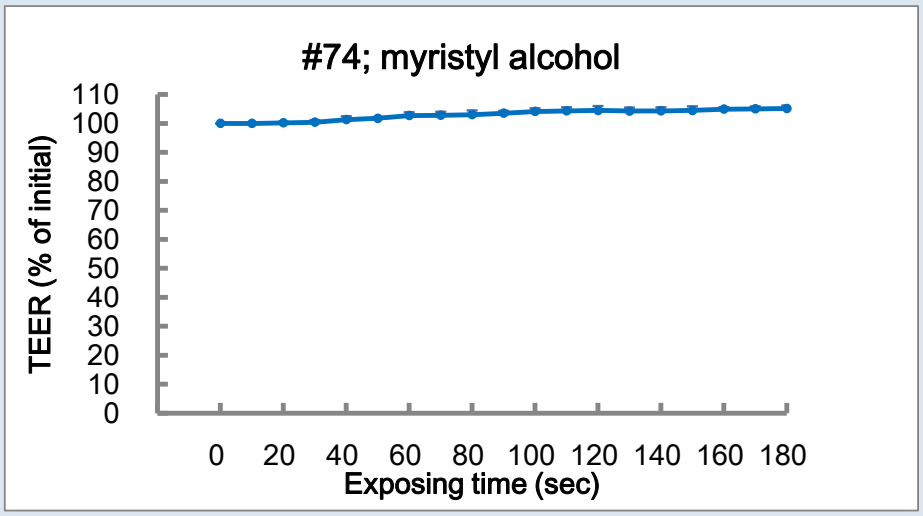
Test #	1	2	3
Model #	13102332	13102334	13102336
Initial	183	173	176
0	183	173	179
10	183	173	179
20	183	173	179
30	183	173	179
40	184	174	179
50	184	174	180
60	185	175	180
70	185	175	180
80	186	175	180
90	186	175	181
100	186	176	181
110	186	176	181
120	187	176	181
130	186	176	181
140	186	176	181
150	187	176	181
160	187	176	182
170	187	176	182
180	187	176	182

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.03	NI
Plateau level	0	NI



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#73; dodecane

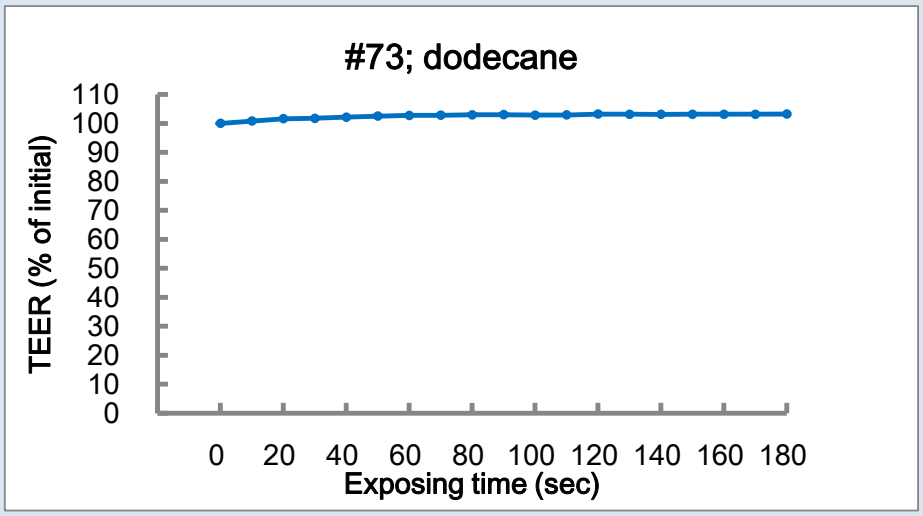
Test #	1	2	3
Model #	13120410	13120411	13120412
Initial	184	188	186
0	189	191	188
10	189	192	189
20	190	192	189
30	190	192	189
40	191	192	189
50	190	193	190
60	191	193	190
70	191	193	190
80	191	193	190
90	191	193	190
100	191	193	190
110	191	193	190
120	191	193	190
130	191	193	190
140	191	193	190
150	191	193	190
160	191	193	190
170	191	193	190
180	191	193	190

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#72; diisopropanolamine

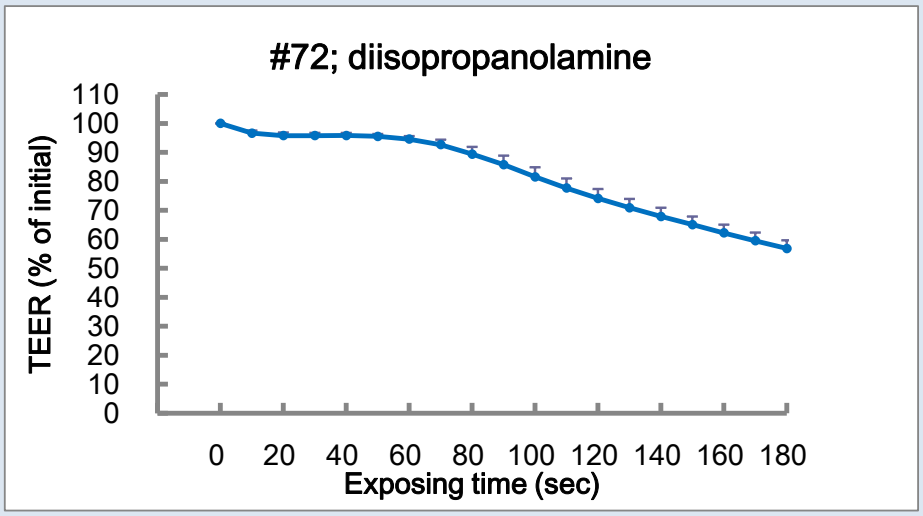
Test #	1	2	3
Model #	13110813	13110814	13110816
Initial	186	185	181
0	188	190	183
10	186	187	181
20	185	186	180
30	185	186	180
40	185	186	181
50	184	186	181
60	183	185	180
70	181	184	179
80	177	182	177
90	174	179	175
100	171	176	172
110	168	173	169
120	165	170	167
130	163	167	164
140	161	164	162
150	159	162	160
160	157	159	158
170	155	157	156
180	152	155	154

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.24	I
Plateau level	43	I



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#71; pyridine

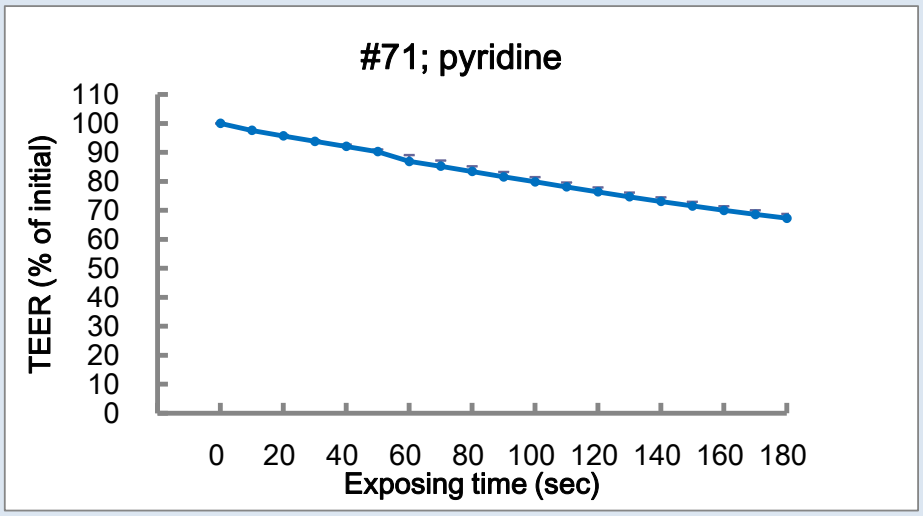
Test #	1	2	3
Model #	13110810	13110811	13110812
Initial	198	196	195
0	197	196	194
10	195	194	192
20	193	192	190
30	192	190	189
40	190	189	187
50	189	188	185
60	187	182	184
70	186	181	182
80	184	180	180
90	182	178	179
100	181	177	177
110	179	176	176
120	178	174	174
130	176	173	173
140	175	172	171
150	174	170	170
160	172	169	168
170	171	168	167
180	170	167	166

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.18	I
Plateau level	33	I



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#70; hexane

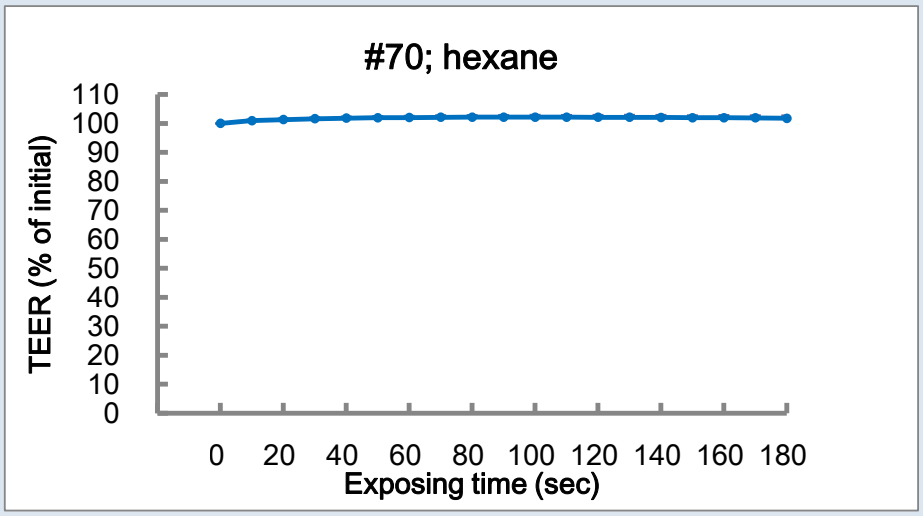
Test #	1	2	3
Model #	13110807	13110808	13110809
Initial	194	194	200
0	194	193	197
10	194	194	198
20	195	194	198
30	195	195	198
40	195	195	199
50	195	195	199
60	195	195	199
70	195	195	199
80	195	195	199
90	195	195	199
100	195	195	199
110	195	195	199
120	195	195	199
130	195	195	198
140	195	195	198
150	195	195	198
160	195	195	198
170	195	195	198
180	195	195	198

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2013/11/13
実施者名	yamaguchi
被験物質名	#69; isopropyl myristate

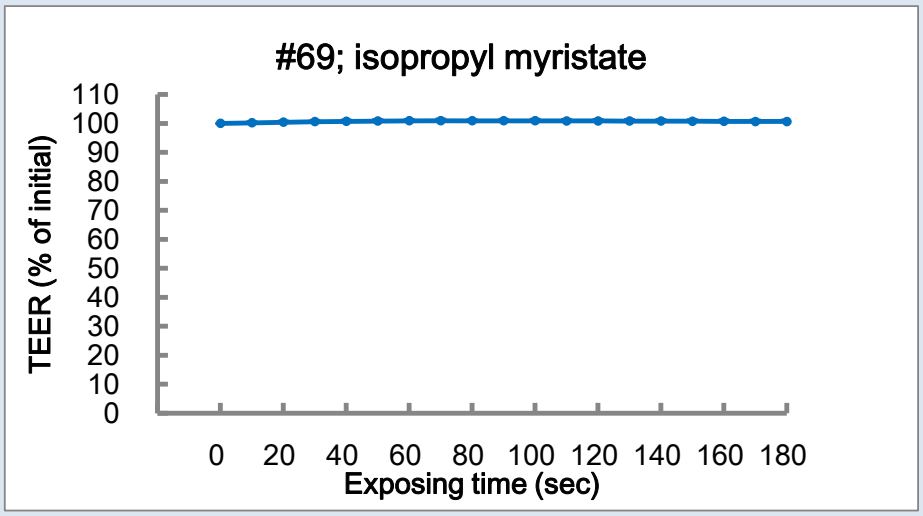
Test #	1	2	3
Model #	13110804	13110805	13110806
Initial	189	195	191
0	192	196	192
10	191	197	192
20	191	197	193
30	192	197	193
40	192	197	193
50	192	197	193
60	192	197	193
70	192	197	193
80	192	197	193
90	192	197	193
100	192	197	193
110	192	197	193
120	192	197	193
130	192	197	193
140	192	197	193
150	192	197	193
160	192	197	193
170	192	197	193
180	192	197	193

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.00	NI
Plateau level	0	NI



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#68; tetrahydrofuran

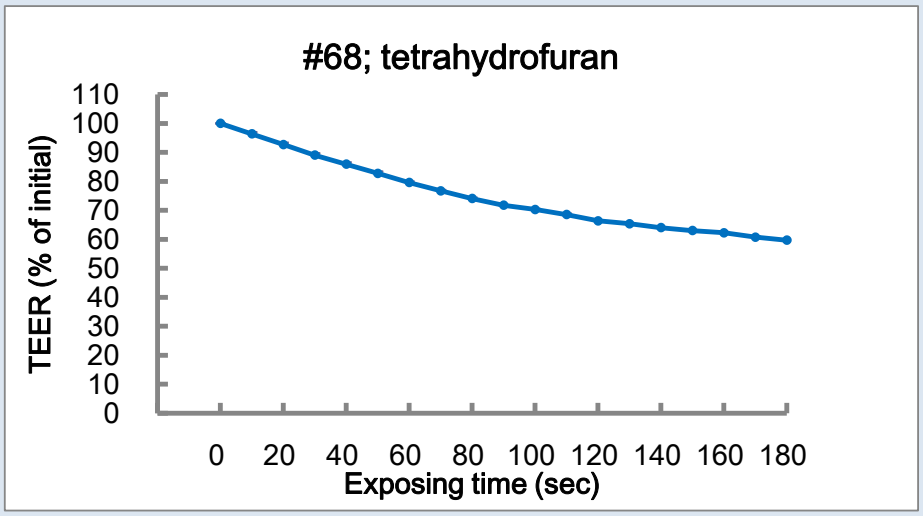
Test #	1	2	3
Model #	13102323	13102324	13102324
Initial	185	178	178
0	200	191	191
10	196	189	189
20	193	186	186
30	190	183	183
40	187	180	180
50	184	178	178
60	182	175	175
70	179	172	172
80	177	170	170
90	175	168	168
100	173	167	167
110	171	166	166
120	170	164	164
130	169	163	163
140	168	162	162
150	167	161	161
160	166	161	161
170	165	159	159
180	164	158	158

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.22	I
Plateau level	40	I



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#67; cyclohexanone

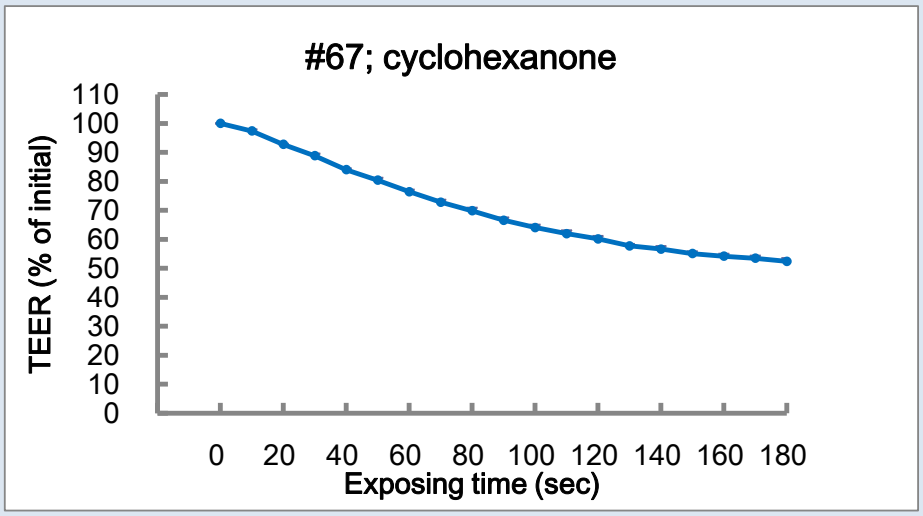
Test #	1	2	3
Model #	13102320	13102321	13102322
Initial	181	174	172
0	192	188	185
10	189	186	183
20	186	183	179
30	183	180	176
40	179	176	172
50	176	173	169
60	173	170	166
70	170	168	164
80	168	165	161
90	165	163	159
100	163	161	157
110	161	159	156
120	159	158	154
130	157	156	153
140	157	155	151
150	155	154	151
160	154	153	150
170	153	153	150
180	152	152	149

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.26	I
Plateau level	48	I



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#66; diisobutyl ketone

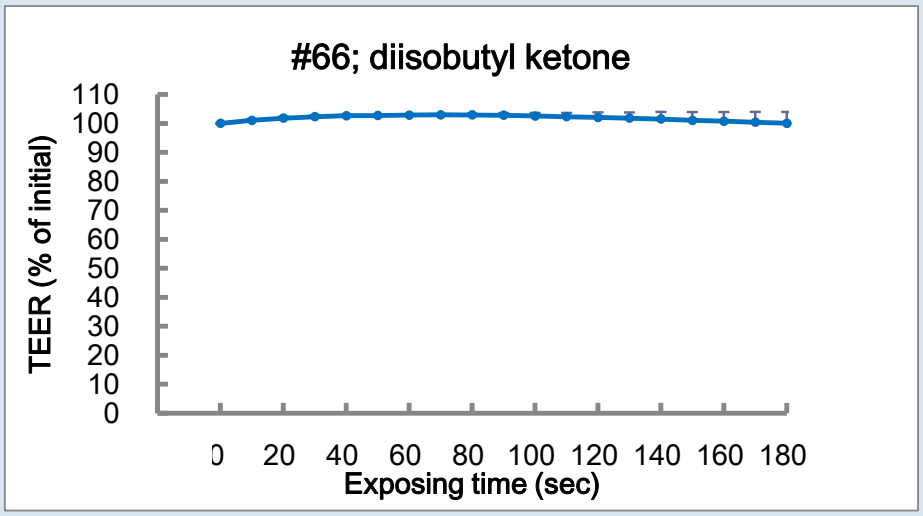
Test #	1	2	3
Model #	13120407	13120408	13120409
Initial	192	196	190
0	193	197	193
10	195	197	194
20	195	198	195
30	196	198	195
40	196	199	195
50	196	199	195
60	196	199	195
70	196	200	195
80	196	200	195
90	195	200	195
100	195	200	195
110	195	200	194
120	194	200	194
130	194	200	194
140	193	201	193
150	193	201	193
160	192	201	192
170	192	201	192
180	191	201	191

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.00	NI
Plateau level	0	NI



試験日	2013/12/10
実施者名	yamaguchi
被験物質名	#65; 2-methylpentane

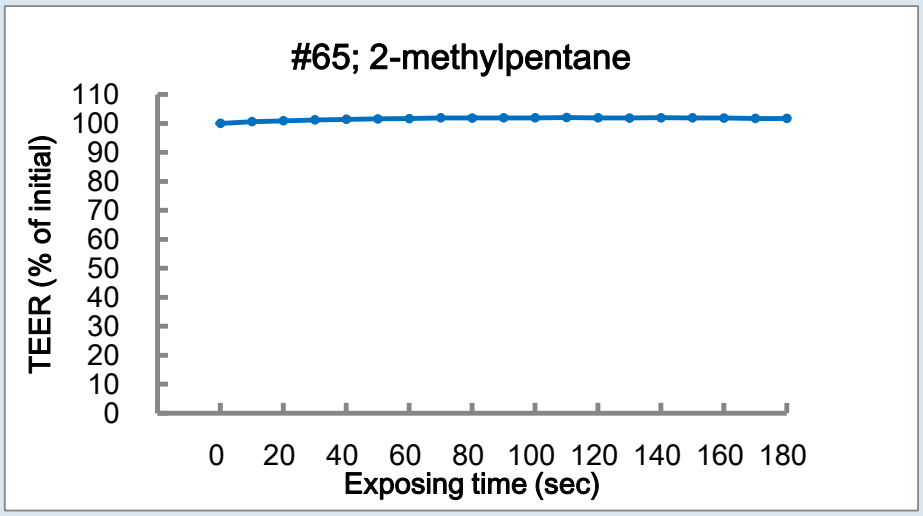
Test #	1	2	3
Model #	13120404	13120405	13120406
Initial	186	191	190
0	189	194	191
10	190	195	191
20	190	195	192
30	190	195	192
40	190	195	192
50	191	196	192
60	191	196	192
70	191	196	193
80	191	196	192
90	191	196	192
100	191	196	192
110	191	196	193
120	191	196	192
130	191	196	193
140	191	196	192
150	191	196	192
160	191	196	193
170	191	196	192
180	191	196	192

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#64; methyl cyanoacetate

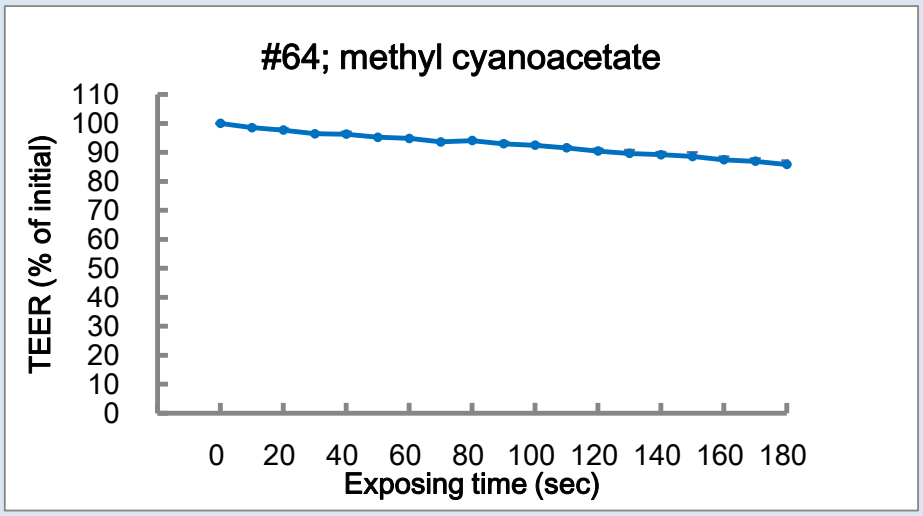
Test #	1	2	3
Model #	13102317	13102318	13102319
Initial	185	175	177
0	198	193	188
10	196	192	187
20	195	192	187
30	195	190	186
40	194	191	185
50	194	189	185
60	194	189	184
70	192	188	183
80	193	188	184
90	191	188	183
100	191	187	182
110	190	187	182
120	189	186	181
130	189	186	179
140	188	185	179
150	188	185	178
160	187	184	178
170	186	183	178
180	185	183	176

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	20	I
Intensity	0.07	I
Plateau level	14	I



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#63; potassium laurate

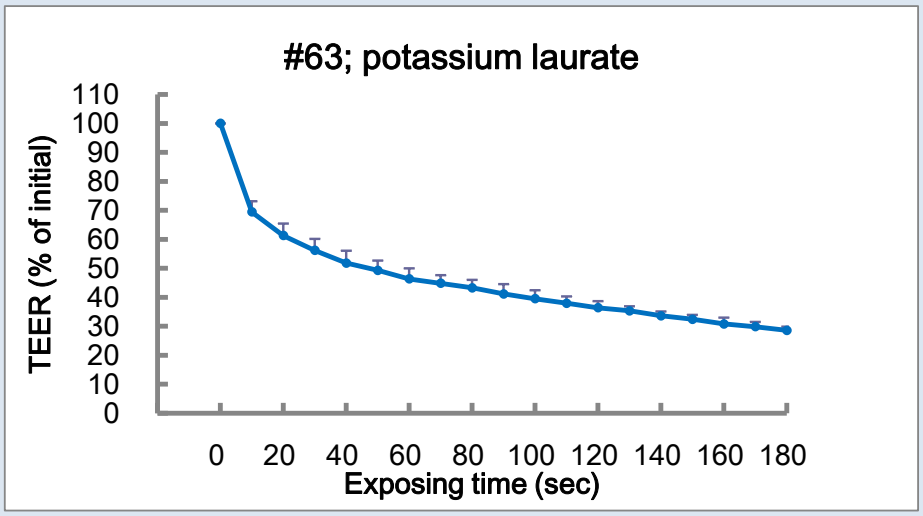
Test #	1	2	3
Model #	13102314	13102315	13102316
Initial	178	176	172
0	160	156	157
10	143	142	144
20	139	138	141
30	136	136	138
40	134	134	136
50	133	133	134
60	131	132	133
70	131	131	132
80	130	130	131
90	129	129	130
100	128	129	130
110	128	128	129
120	127	127	128
130	127	126	127
140	126	125	126
150	125	125	126
160	124	125	125
170	124	124	125
180	124	123	124

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.40	I
Plateau level	71	I



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#62; styrene

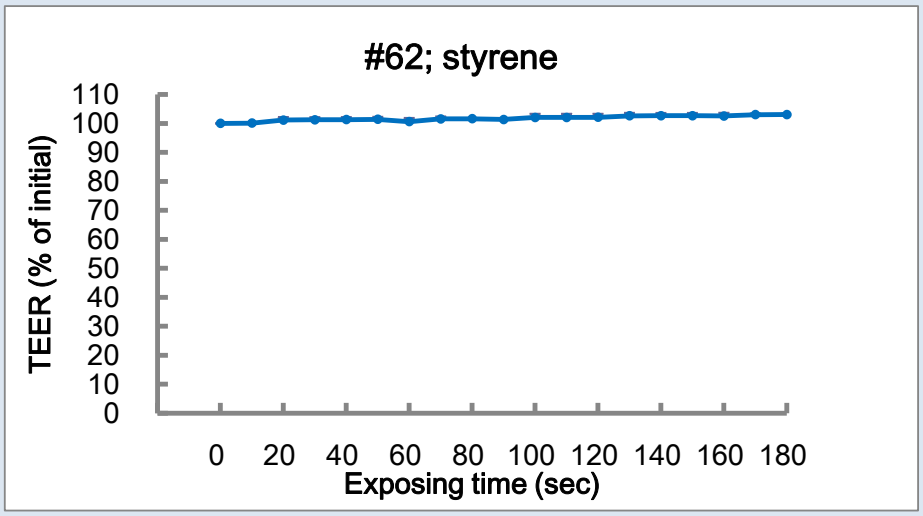
Test #	1	2	3
Model #	13102313	13102313	13102313
Initial	175	163	178
0	179	168	180
10	179	168	180
20	180	169	180
30	180	169	180
40	180	169	180
50	180	169	180
60	180	169	180
70	181	169	181
80	180	169	181
90	180	169	181
100	180	170	181
110	180	170	181
120	180	170	181
130	180	170	182
140	180	170	182
150	180	170	182
160	180	170	182
170	181	170	182
180	181	170	182

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#61; sucrose fatty acid ester

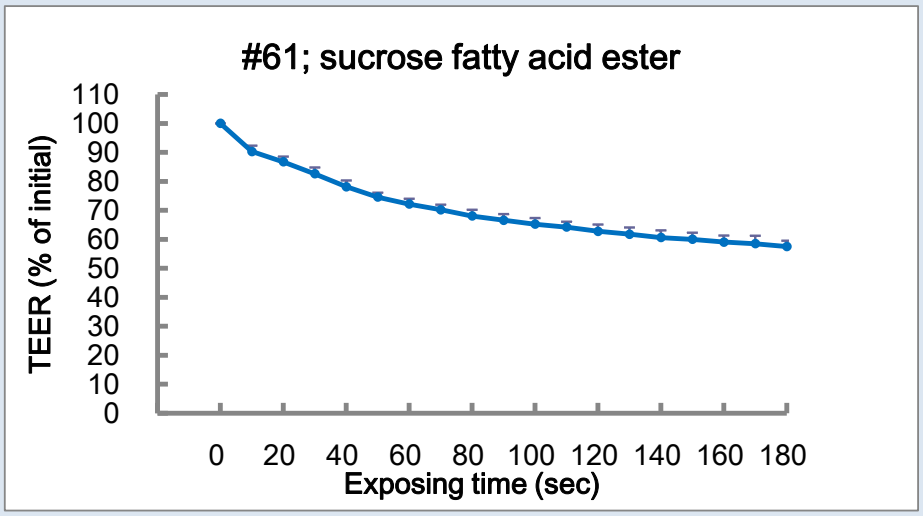
Test #	1	2	3
Model #	13102310	13102311	13102312
Initial	160	162	166
0	176	181	184
10	170	174	175
20	167	172	173
30	164	170	170
40	161	167	166
50	159	164	164
60	157	162	163
70	155	161	162
80	154	160	160
90	153	159	159
100	152	158	158
110	151	157	157
120	150	156	156
130	149	155	155
140	149	155	154
150	148	154	154
160	147	153	153
170	147	153	153
180	147	152	152

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.24	I
Plateau level	42	I



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#60; cycropentasiloxane

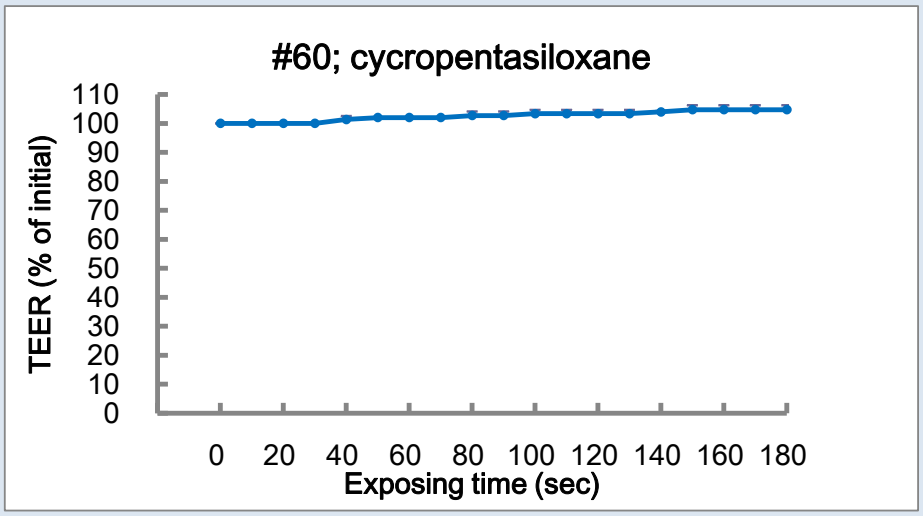
Test #	1	2	3
Model #	12042319	12042320	12042321
Initial	107	108	116
0	113	107	117
10	113	107	117
20	113	107	117
30	113	107	117
40	114	108	117
50	114	108	118
60	114	108	118
70	114	108	118
80	114	109	118
90	114	109	118
100	115	109	118
110	115	109	118
120	115	109	118
130	115	109	118
140	115	109	119
150	115	110	119
160	115	110	119
170	115	110	119
180	115	110	119

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#59; n-octyl bromide

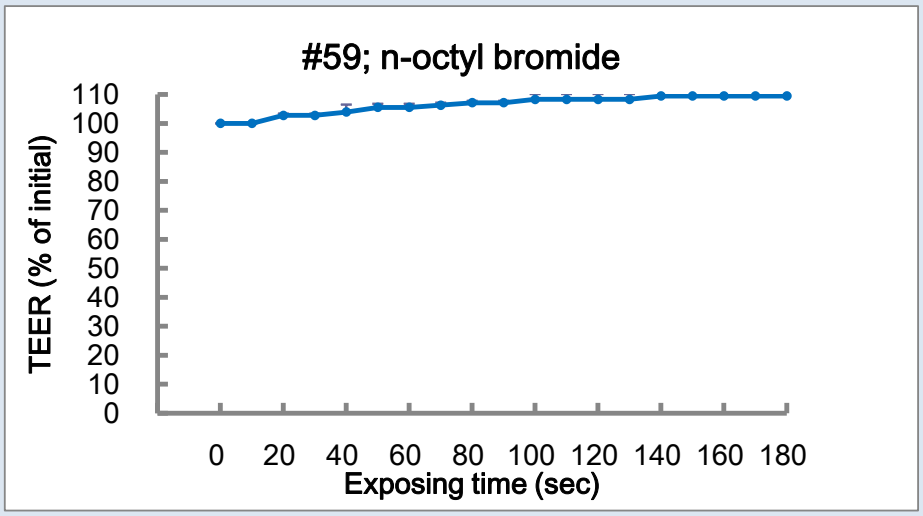
Test #	1	2	3
Model #	12042322	12042323	12042324
Initial	96	115	103
0	94	105	90
10	94	105	90
20	95	106	91
30	95	106	91
40	95	106	92
50	96	107	92
60	96	107	92
70	96	108	92
80	97	108	92
90	97	108	92
100	97	108	93
110	97	108	93
120	97	108	93
130	97	108	93
140	97	108	94
150	97	108	94
160	97	108	94
170	97	108	94
180	97	108	94

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.05	NI
Plateau level	0	NI



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#58; isopropyl bromide

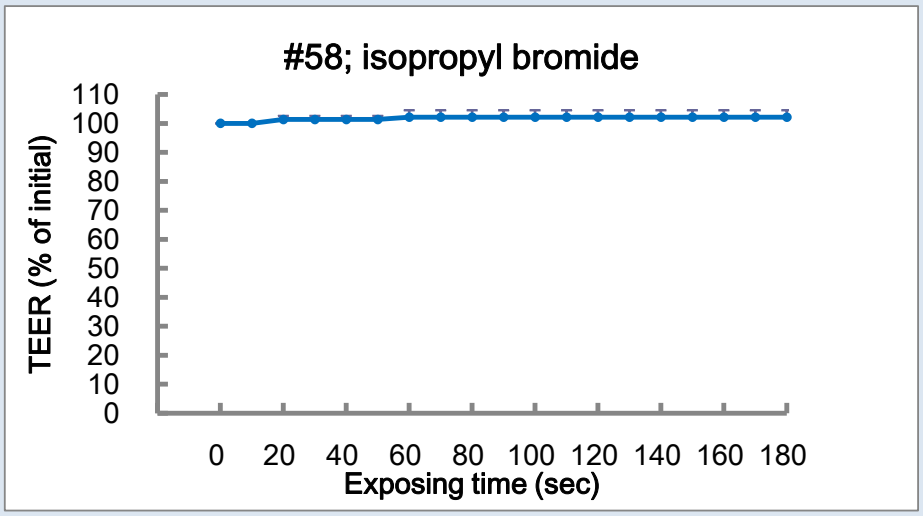
Test #	1	2	3
Model #	12042325	12042326	12042327
Initial	104	119	112
0	104	119	104
10	104	119	104
20	104	120	105
30	104	120	105
40	104	120	105
50	104	120	105
60	104	120	106
70	104	120	106
80	104	120	106
90	104	120	106
100	104	120	106
110	104	120	106
120	104	120	106
130	104	120	106
140	104	120	106
150	104	120	106
160	104	120	106
170	104	120	106
180	104	120	106

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2013/7/9
実施者名	yamaguchi
被験物質名	#57; 1,5-hexadine

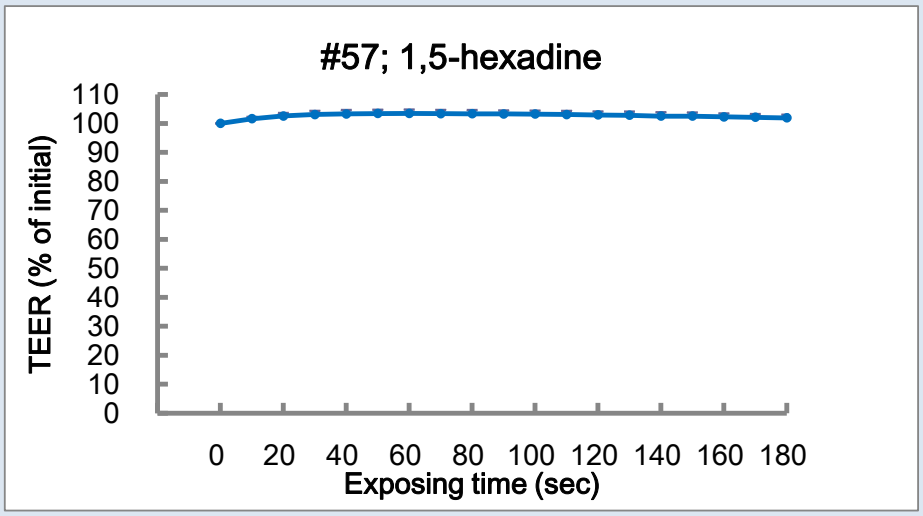
Test #	1	2	3
Model #	4	5	6
Initial	184	197	192
0	186	195	194
10	188	196	195
20	189	197	196
30	190	197	196
40	190	197	196
50	190	197	197
60	190	197	197
70	190	197	197
80	190	197	197
90	190	197	197
100	190	197	197
110	190	197	196
120	189	197	196
130	189	197	196
140	189	196	196
150	189	196	196
160	189	196	196
170	189	196	196
180	189	196	195

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#56; triethanolamine

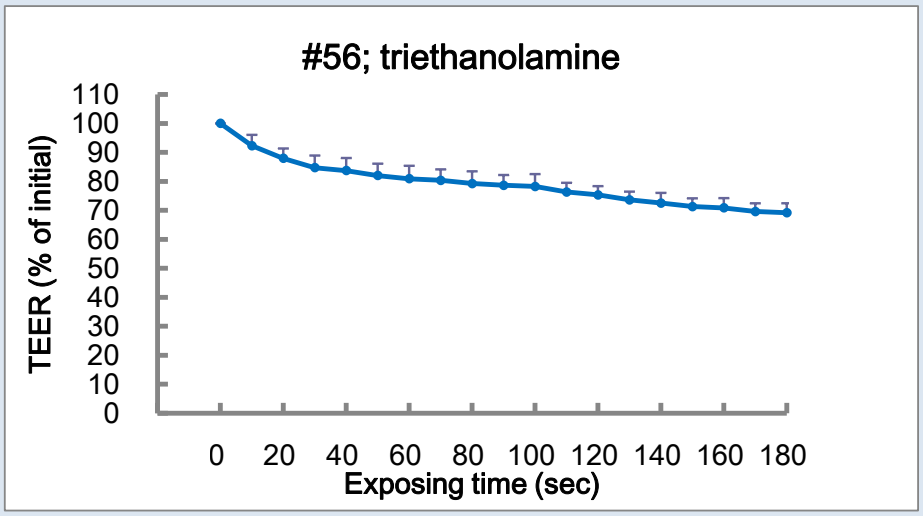
Test #	1	2	3
Model #	12042328	12042329	12042330
Initial	104	119	112
0	113	121	130
10	110	118	121
20	108	115	118
30	106	114	115
40	106	113	114
50	105	112	113
60	104	112	112
70	104	111	112
80	103	111	111
90	103	110	111
100	103	110	110
110	101	109	110
120	101	108	109
130	100	107	108
140	99	107	107
150	98	106	107
160	98	106	106
170	97	105	106
180	97	105	105

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.17	I
Plateau level	31	I



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#55; 2,4-pentanediol

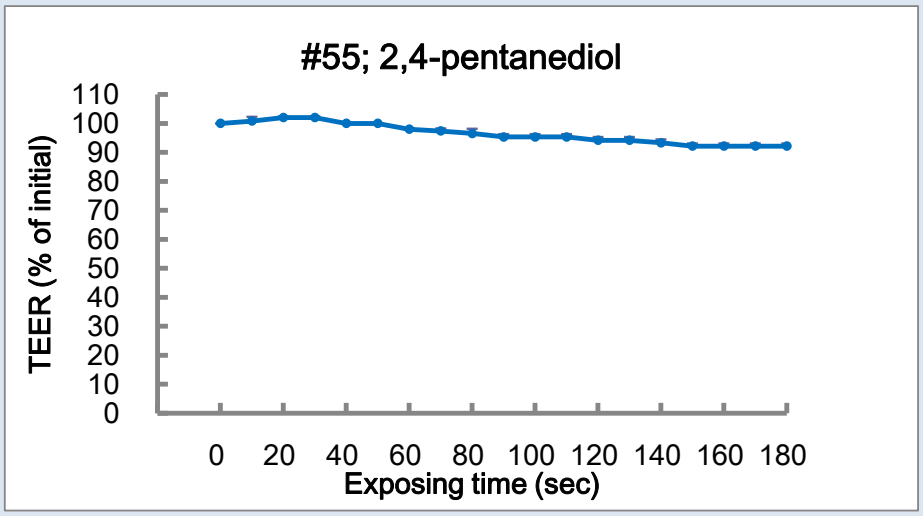
Test #	1	2	3
Model #	12042331	12042332	12042333
Initial	117	98	103
0	112	100	112
10	112	101	112
20	113	101	113
30	113	101	113
40	112	100	112
50	112	100	112
60	111	99	111
70	111	99	110
80	111	98	110
90	110	98	109
100	110	98	109
110	110	98	109
120	109	98	108
130	109	98	108
140	109	97	108
150	108	97	107
160	108	97	107
170	108	97	107
180	108	97	107

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	70	I
Intensity	0.07	I
Plateau level	8	I



試験日	2013/7/9
実施者名	yamaguchi
被験物質名	#54; 3-chloropropionitrile

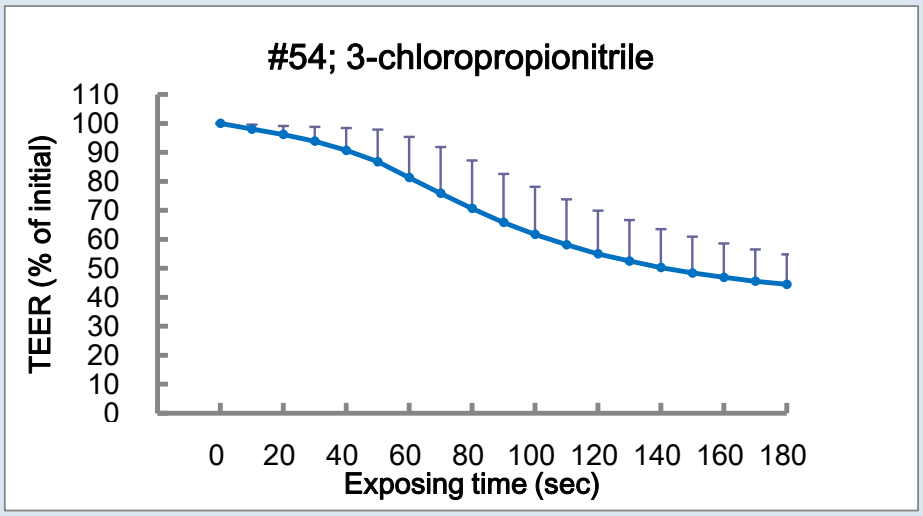
Test #	1	2	3
Model #	7	8	9
Initial	158	176	185
0	166	177	174
10	164	177	172
20	162	176	172
30	160	174	172
40	157	172	172
50	153	168	172
60	148	164	171
70	144	160	169
80	141	156	166
90	138	153	163
100	136	150	160
110	135	148	157
120	133	146	155
130	132	144	153
140	132	142	151
150	131	141	149
160	131	140	148
170	130	139	146
180	130	139	145

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.31	I
Plateau level	56	I



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#53; 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate

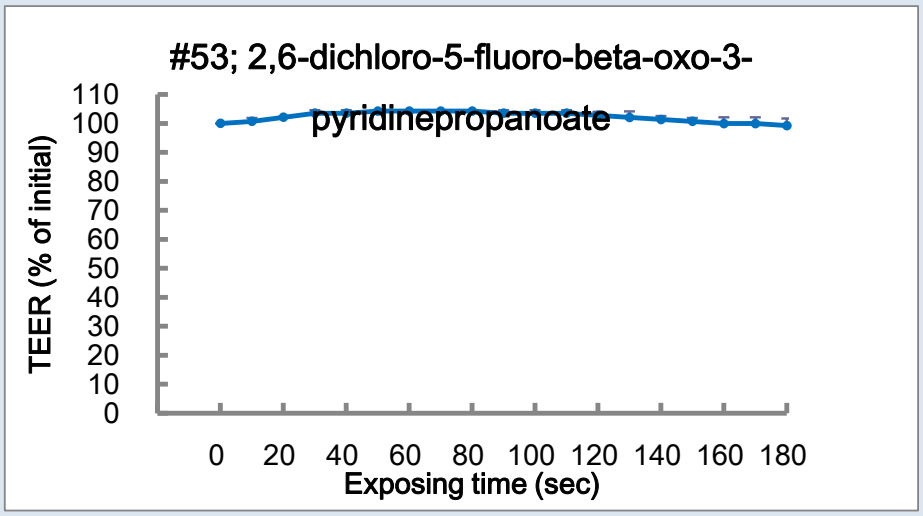
Test #	1	2	3
Model #	12042334	12042335	12042336
Initial	109	116	110
0	109	110	105
10	110	110	105
20	110	111	106
30	111	112	106
40	111	112	106
50	111	112	107
60	111	112	107
70	111	112	107
80	111	112	107
90	111	112	106
100	111	112	106
110	111	112	106
120	111	111	106
130	111	111	105
140	110	111	105
150	110	110	105
160	110	110	104
170	110	110	104
180	110	109	104

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.00	NI
Plateau level	0	NI



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#52; ammonium nitrate

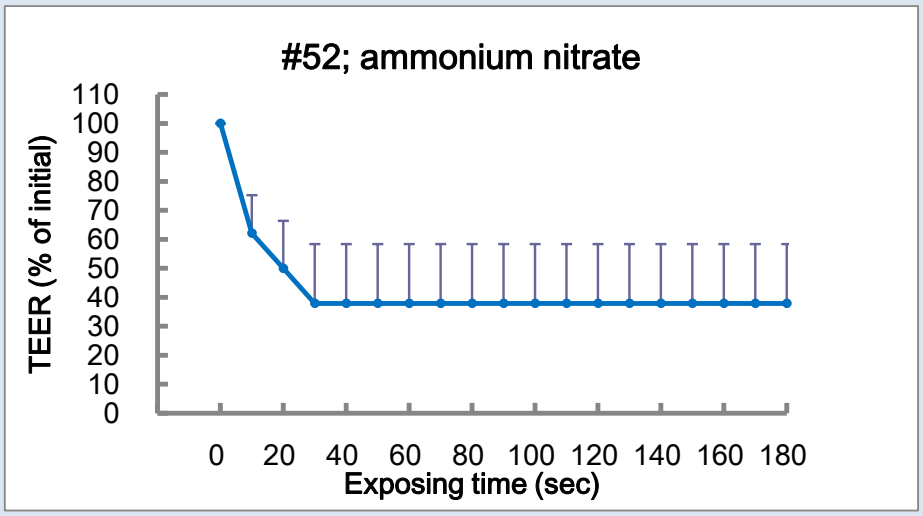
Test #	1	2	3
Model #	12042338	12042339	12042340
Initial	110	107	98
0	83	72	71
10	78	67	66
20	76	66	64
30	74	65	62
40	74	65	62
50	74	65	62
60	74	65	62
70	74	65	62
80	74	65	62
90	74	65	62
100	74	65	62
110	74	65	62
120	74	65	62
130	74	65	62
140	74	65	62
150	74	65	62
160	74	65	62
170	74	65	62
180	74	65	62

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	2.07	I
Plateau level	62	I



試験日	2013/10/29
実施者名	yamaguchi
被験物質名	#51; camphene

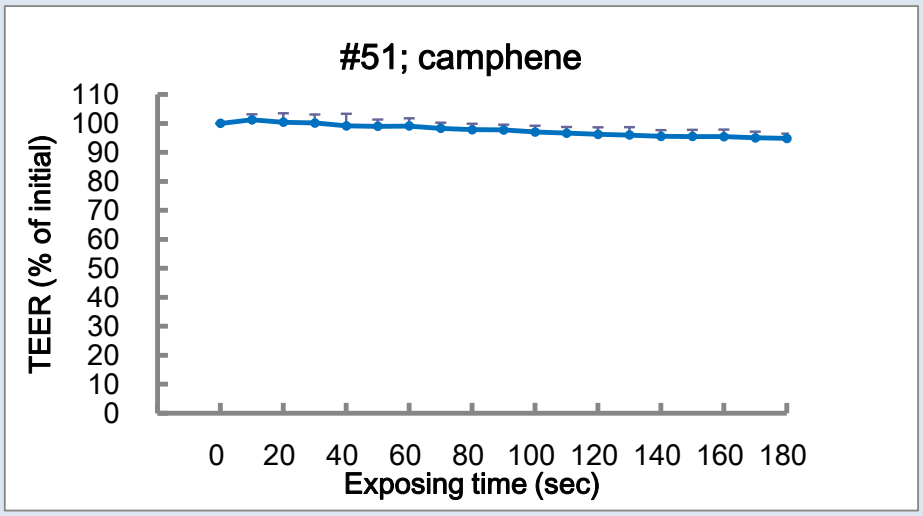
Test #	1	2	3
Model #	13102307	13102308	1310209
Initial	179	170	168
0	197	192	184
10	198	192	186
20	198	190	186
30	198	190	186
40	197	188	186
50	196	189	185
60	195	190	186
70	195	190	185
80	194	189	184
90	195	189	184
100	194	188	184
110	194	188	183
120	193	187	183
130	194	187	183
140	193	187	182
150	193	186	182
160	193	186	182
170	193	186	182
180	193	187	181

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	100	I
Intensity	0.04	NI
Plateau level	4	NI



試験日	2013/7/9
実施者名	yamaguchi
被験物質名	#50; 3,3-dithiodipropionic acid

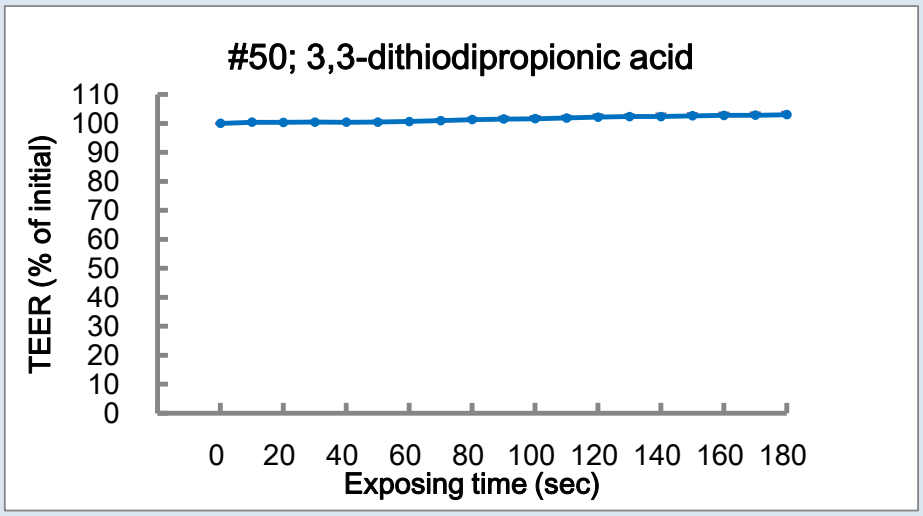
Test #	1	2	3
Model #	13	14	15
Initial	205	193	190
0	208	195	198
10	208	195	199
20	208	195	199
30	208	195	199
40	208	195	199
50	208	195	199
60	208	195	199
70	208	196	199
80	209	196	199
90	209	197	200
100	209	197	200
110	209	197	200
120	209	197	200
130	209	197	200
140	209	197	201
150	209	197	201
160	210	198	201
170	210	198	201
180	210	198	201

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#49; ethyl acetate

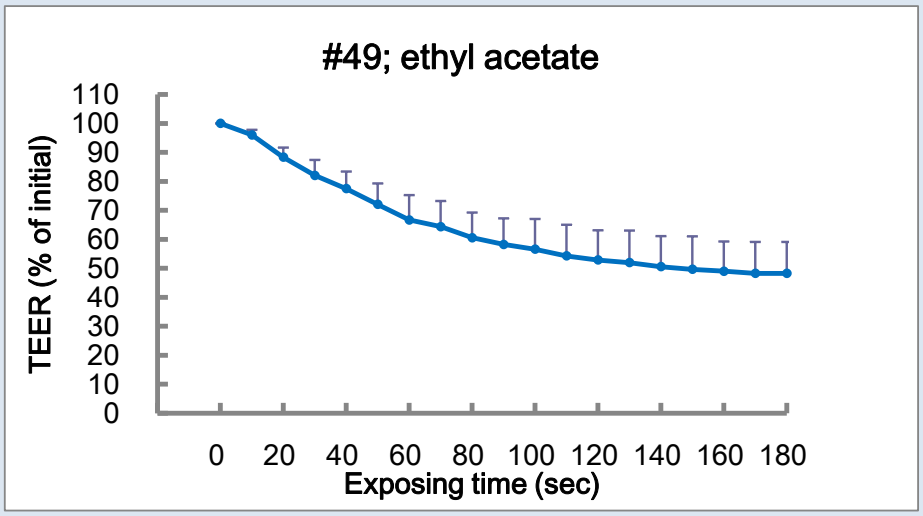
Test #	1	2	3
Model #	12042316	12042317	12042318
Initial	101	105	98
0	109	105	97
10	108	103	95
20	105	99	92
30	103	96	89
40	101	94	87
50	99	91	85
60	97	88	83
70	96	87	82
80	94	86	80
90	93	85	79
100	93	84	78
110	92	83	77
120	91	82	77
130	91	82	76
140	90	81	76
150	90	81	75
160	89	81	75
170	89	80	75
180	89	80	75

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.29	I
Plateau level	52	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#48; n-butanal

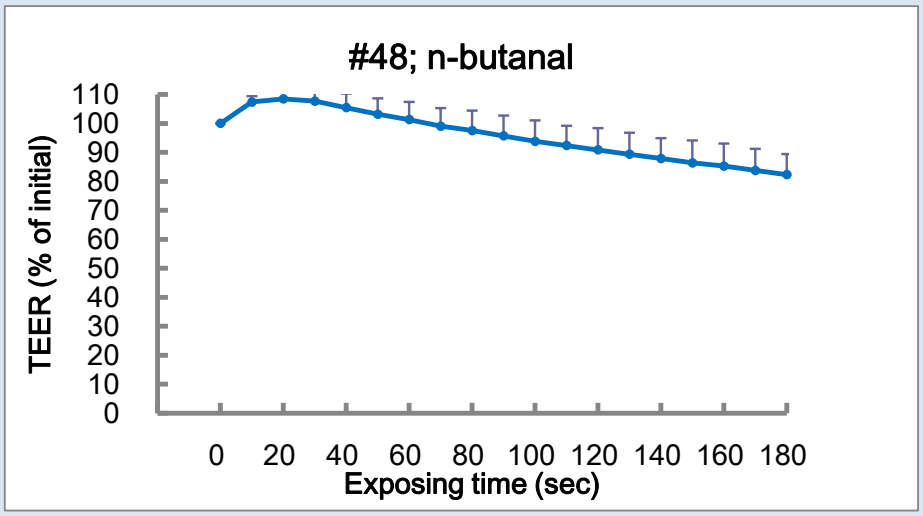
Test #	1	2	3
Model #	12110228	12110229	12110230
Initial	176	172	154
0	166	168	162
10	172	177	167
20	173	179	167
30	172	179	166
40	170	178	163
50	169	176	160
60	167	175	158
70	165	173	156
80	164	172	154
90	163	170	152
100	162	168	150
110	161	166	149
120	160	165	147
130	158	164	146
140	157	162	145
150	156	161	143
160	155	160	142
170	154	158	141
180	153	156	140

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	80	I
Intensity	0.15	I
Plateau level	18	I



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#47; propanol solvent P

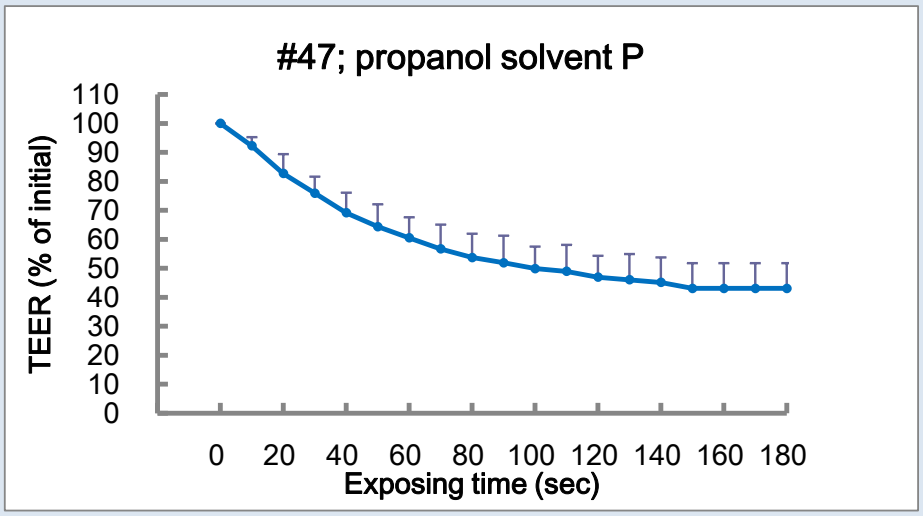
Test #	1	2	3
Model #	12042313	12042314	12042315
Initial	104	94	103
0	104	89	99
10	100	87	97
20	95	85	94
30	93	83	91
40	90	81	89
50	88	80	87
60	87	79	85
70	85	78	84
80	84	77	83
90	83	77	82
100	83	76	81
110	82	76	81
120	82	75	80
130	81	75	80
140	81	75	79
150	80	74	79
160	80	74	79
170	80	74	79
180	80	74	79

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.38	I
Plateau level	57	I



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#46; butanol

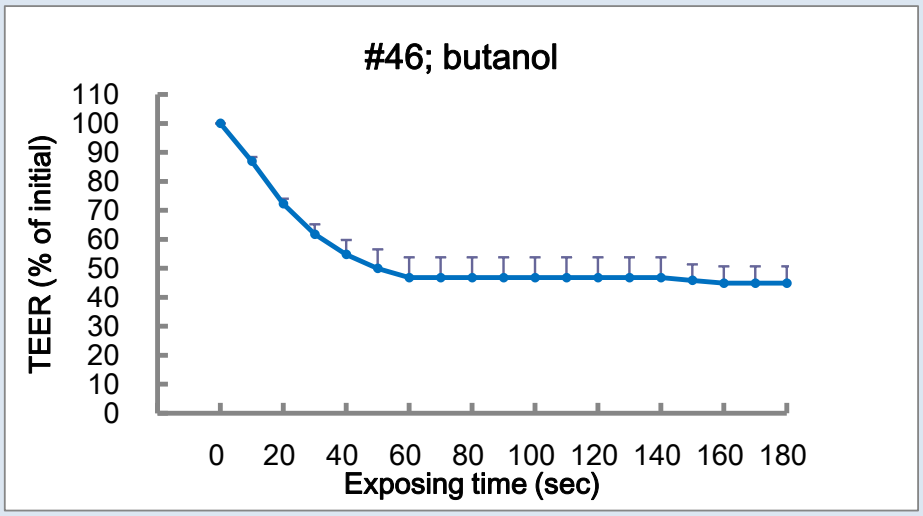
Test #	1	2	3
Model #	12042310	12042311	12042312
Initial	115	113	102
0	114	107	99
10	107	103	94
20	99	97	90
30	93	93	87
40	88	91	85
50	85	89	84
60	83	88	83
70	83	88	83
80	83	88	83
90	83	88	83
100	83	88	83
110	83	88	83
120	83	88	83
130	83	88	83
140	83	88	83
150	83	88	82
160	83	87	82
170	83	87	82
180	83	87	82

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.89	I
Plateau level	53	I



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#45; acid red 92

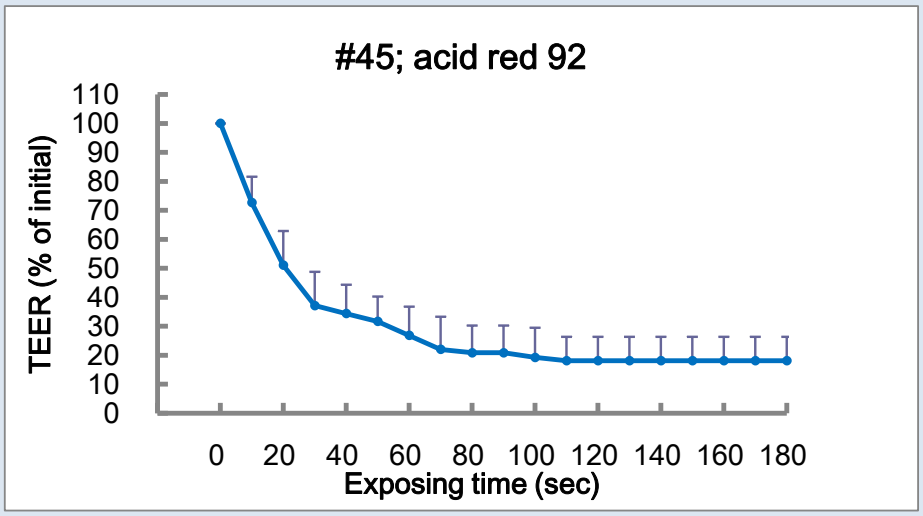
Test #	1	2	3
Model #	12042307	12042308	12042309
Initial	95	123	117
0	74	96	89
10	68	90	84
20	64	84	80
30	62	81	76
40	62	80	75
50	62	79	74
60	61	78	73
70	60	77	72
80	60	76	72
90	60	76	72
100	60	76	71
110	60	75	71
120	60	75	71
130	60	75	71
140	60	75	71
150	60	75	71
160	60	75	71
170	60	75	71
180	60	75	71

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.74	I
Plateau level	82	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#44; 2-ethoxyethyl acetate

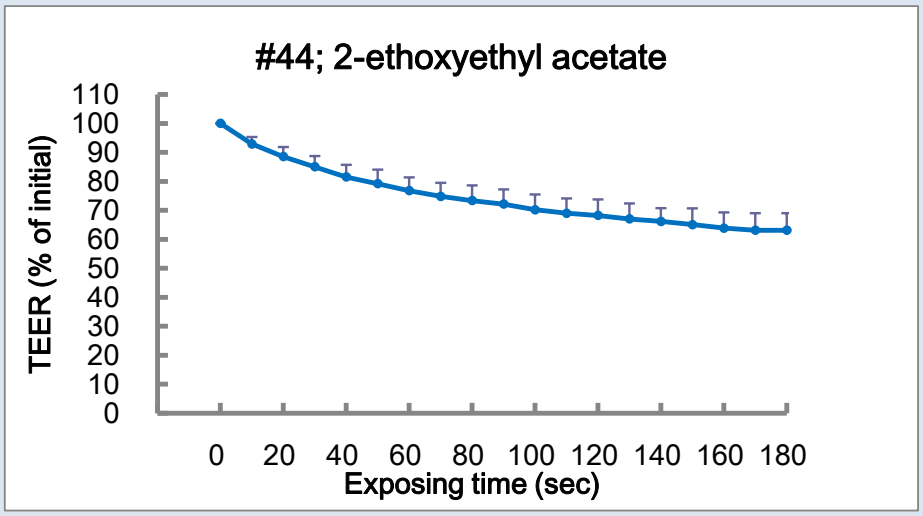
Test #	1	2	3
Model #	12110225	12110226	12110227
Initial	162	147	150
0	162	141	169
10	158	136	160
20	155	133	155
30	152	131	151
40	149	129	147
50	147	128	144
60	145	126	142
70	143	125	140
80	141	125	138
90	140	124	137
100	138	123	135
110	137	122	134
120	136	122	133
130	135	121	132
140	134	120	132
150	133	120	130
160	132	119	129
170	131	119	128
180	131	119	128

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.20	I
Plateau level	37	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#43; benzyl alcohol

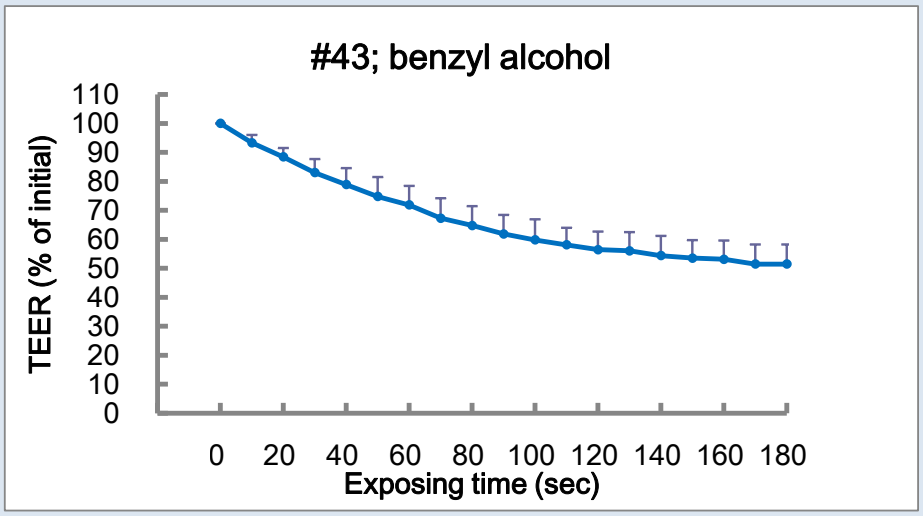
Test #	1	2	3
Model #	12110222	12110223	12110224
Initial	170	119	140
0	164	111	135
10	160	107	131
20	154	105	129
30	150	102	126
40	146	100	124
50	142	98	122
60	139	97	120
70	135	95	117
80	133	94	115
90	130	93	113
100	128	92	112
110	126	92	110
120	125	91	109
130	124	91	109
140	123	90	108
150	122	90	107
160	121	90	107
170	120	89	106
180	120	89	106

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.27	I
Plateau level	49	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#42; promethazine hydrochloride

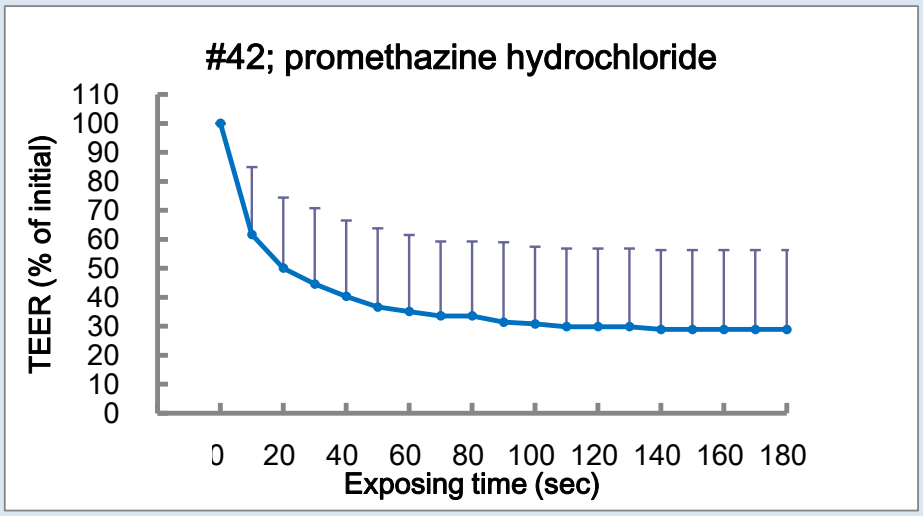
Test #	1	2	3
Model #	12110219	12110220	12110221
Initial	149	105	152
0	105	91	132
10	94	73	121
20	88	70	117
30	86	68	115
40	84	67	113
50	82	66	112
60	81	66	111
70	80	66	110
80	80	66	110
90	79	65	110
100	79	65	109
110	78	65	109
120	78	65	109
130	78	65	109
140	77	65	109
150	77	65	109
160	77	65	109
170	77	65	109
180	77	65	109

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.69	I
Plateau level	69	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#41; stearyltrimethylammonium chloride

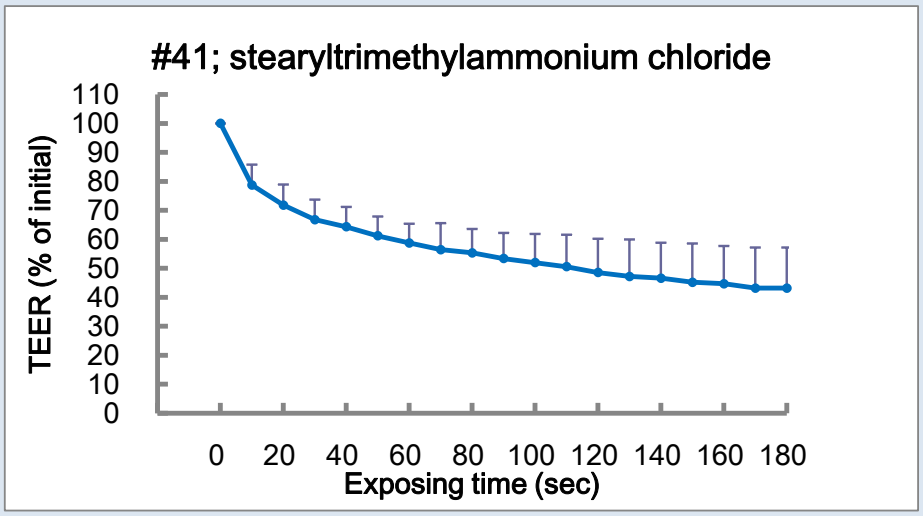
Test #	1	2	3
Model #	12110216	12110217	12110218
Initial	149	136	146
0	116	124	143
10	106	111	134
20	103	108	129
30	101	106	125
40	100	105	123
50	99	103	121
60	98	102	119
70	96	102	118
80	96	101	117
90	95	100	116
100	94	100	115
110	93	100	114
120	92	99	113
130	91	99	112
140	91	98	112
150	90	98	111
160	90	98	110
170	89	97	110
180	89	97	110

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.32	I
Plateau level	57	I



試験日	2013/7/9
実施者名	yamaguchi
被験物質名	#40; distearyldimethylammonium chloride

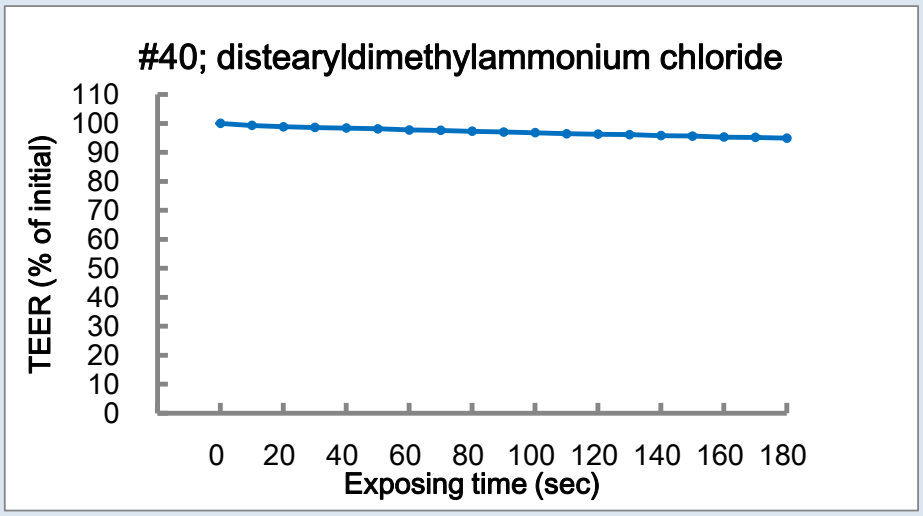
Test #	1	2	3
Model #	16	17	18
Initial	217	208	218
0	220	208	215
10	220	207	214
20	219	207	214
30	219	206	213
40	218	206	214
50	218	206	213
60	218	206	213
70	217	205	213
80	217	205	212
90	217	205	212
100	217	204	212
110	216	204	211
120	216	204	211
130	216	203	211
140	216	203	211
150	215	203	211
160	215	203	210
170	215	202	210
180	215	202	210

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	90	I
Intensity	0.03	NI
Plateau level	1	NI



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#39; cetyltrimethylammonium bromide

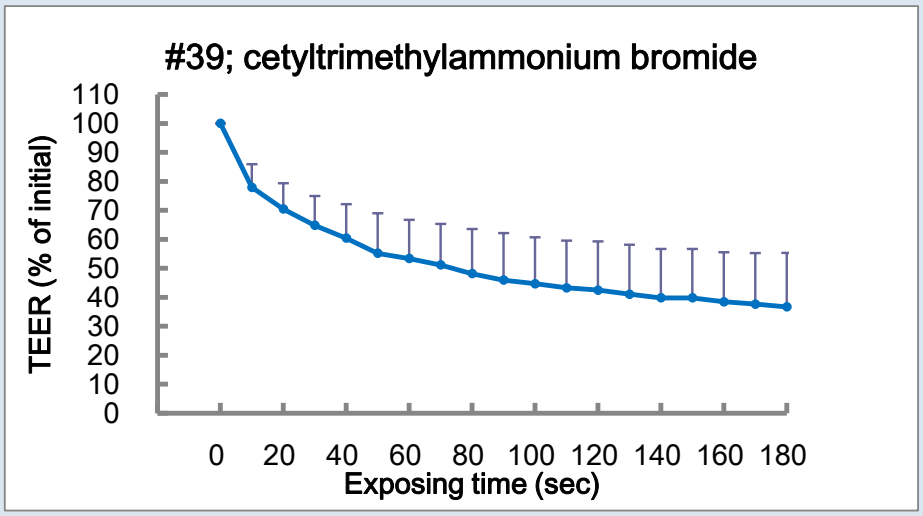
Test #	1	2	3
Model #	12110212	12110214	12110215
Initial	123	129	132
0	115	112	145
10	105	102	135
20	102	99	130
30	99	97	127
40	97	95	125
50	94	93	123
60	94	92	121
70	93	91	120
80	91	90	119
90	90	89	118
100	89	89	117
110	89	88	116
120	88	88	116
130	88	87	115
140	87	87	114
150	87	87	114
160	87	86	113
170	86	86	113
180	86	85	113

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.35	I
Plateau level	63	I



試験日	2014/7/15
実施者名	yamaguchi
被験物質名	#38, nonylphenyl-polyethylene glycol

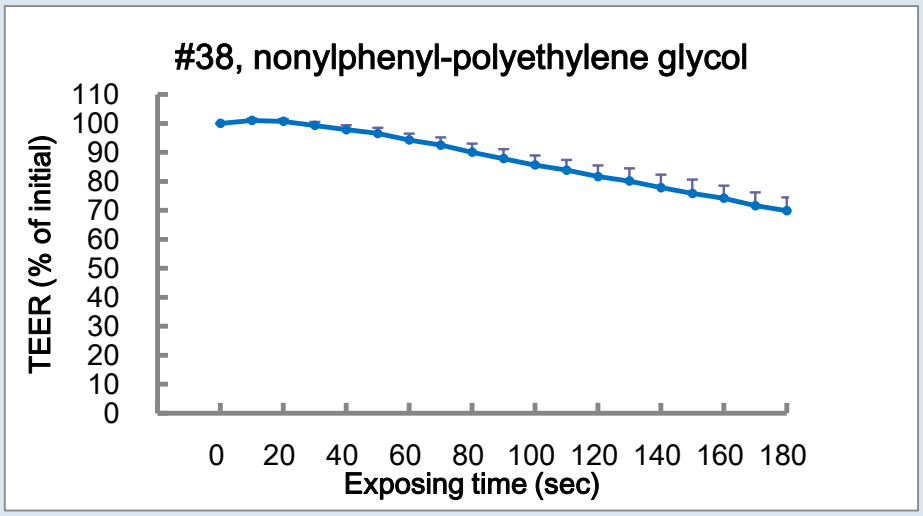
Test #	1	2	3
Model #	14070901	14070902	14070903
Initial	206	195	204
0	210	203	207
10	210	205	208
20	209	205	208
30	208	203	207
40	206	202	206
50	204	201	205
60	202	199	203
70	199	197	202
80	197	195	200
90	194	193	198
100	192	192	195
110	190	190	194
120	187	188	191
130	185	187	190
140	182	185	188
150	180	183	186
160	179	182	184
170	176	180	181
180	174	178	180

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	40	I
Intensity	0.20	I
Plateau level	30	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#37; acetic acid

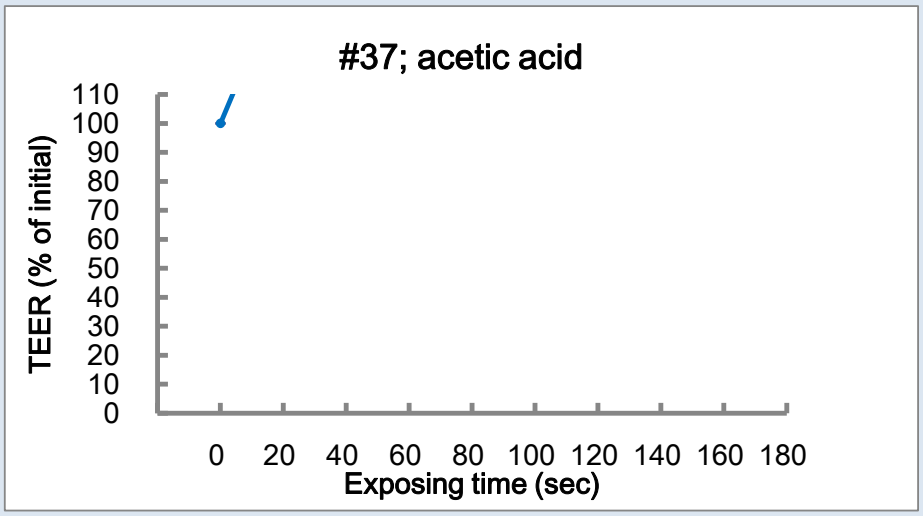
Test #	1	2	3
Model #	12110207	12110208	12110209
Initial	152	146	126
0	151	142	148
10	163	158	178
20	182	180	198
30	200	192	208
40	220	201	211
50	227	206	210
60	229	207	208
70	228	207	205
80	227	205	201
90	226	202	196
100	224	200	192
110	220	197	189
120	218	195	185
130	214	193	182
140	212	191	178
150	210	188	176
160	207	186	173
170	206	184	171
180	204	182	168

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.00	NI
Plateau level	−51	NI



試験日	2014/1/21
実施者名	yamaguchi
被験物質名	#36; 2-methylbutanoic acid

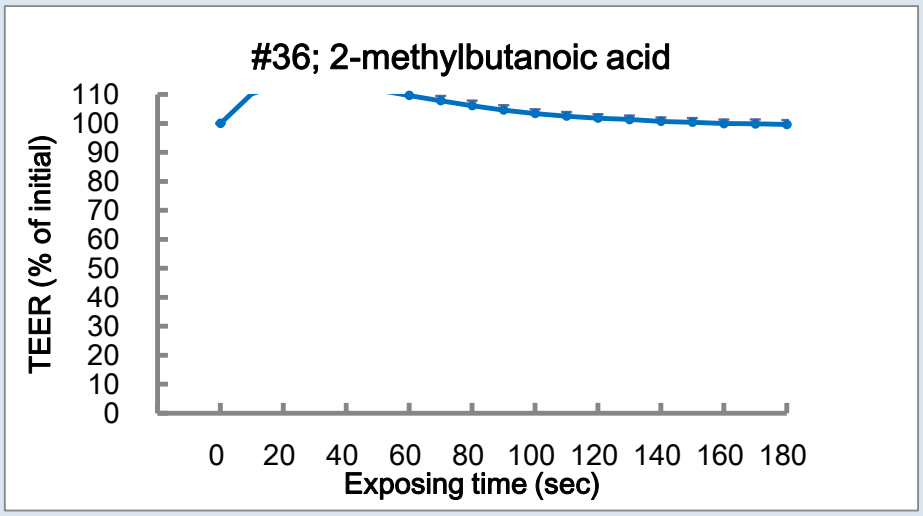
Test #	1	2	3
Model #	14011504	14011505	14011506
Initial	203	210	212
0	203	212	210
10	214	222	219
20	218	225	222
30	218	226	223
40	217	225	222
50	215	223	220
60	214	221	219
70	212	219	217
80	210	218	215
90	209	216	214
100	208	215	212
110	207	214	211
120	206	214	211
130	205	213	210
140	205	213	210
150	204	213	209
160	204	212	209
170	204	212	209
180	203	212	208

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.02	NI
Plateau level	−1	NI



試験日	2013/7/9
実施者名	yamaguchi
被験物質名	#35; imidazole

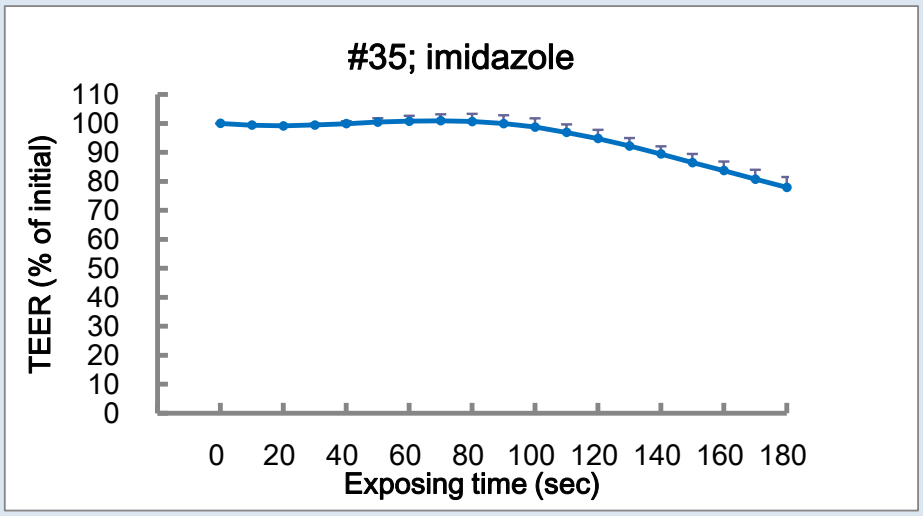
Test #	1	2	3
Model #	19	20	21
Initial	236	217	195
0	225	221	198
10	224	220	197
20	223	221	197
30	223	221	197
40	224	222	198
50	224	223	198
60	224	224	198
70	223	225	198
80	222	225	198
90	221	224	198
100	219	223	197
110	218	221	195
120	215	218	194
130	212	215	192
140	209	211	190
150	205	207	188
160	202	203	186
170	199	200	184
180	196	196	182

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	100	I
Intensity	0.26	I
Plateau level	22	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#34; m-phenylenediamine

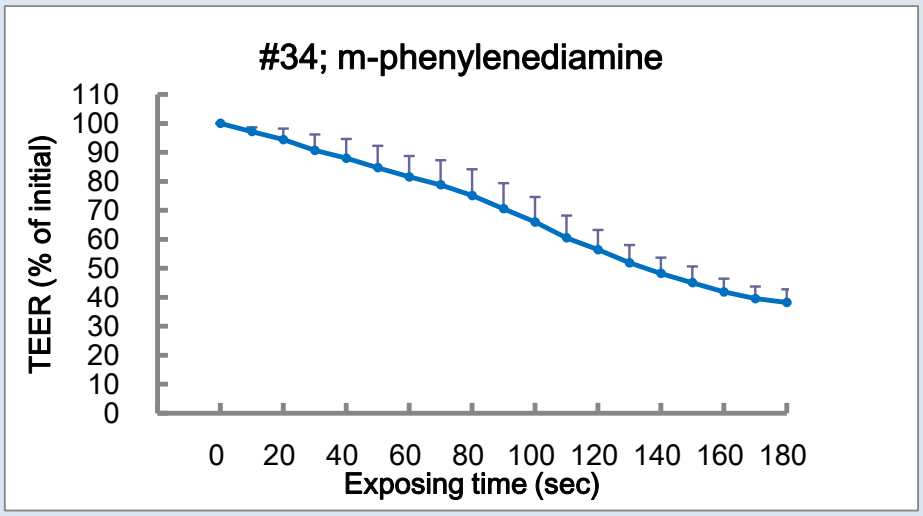
Test #	1	2	3
Model #	12110204	12110205	12110206
Initial	149	156	126
0	151	154	139
10	149	153	136
20	148	152	132
30	146	150	128
40	145	148	125
50	143	146	122
60	141	143	120
70	140	141	117
80	138	138	114
90	135	134	111
100	132	130	108
110	128	125	105
120	125	121	103
130	122	116	101
140	119	113	99
150	117	110	97
160	114	107	96
170	112	105	95
180	111	103	95

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.35	I
Plateau level	62	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#33; diethylethanolamine

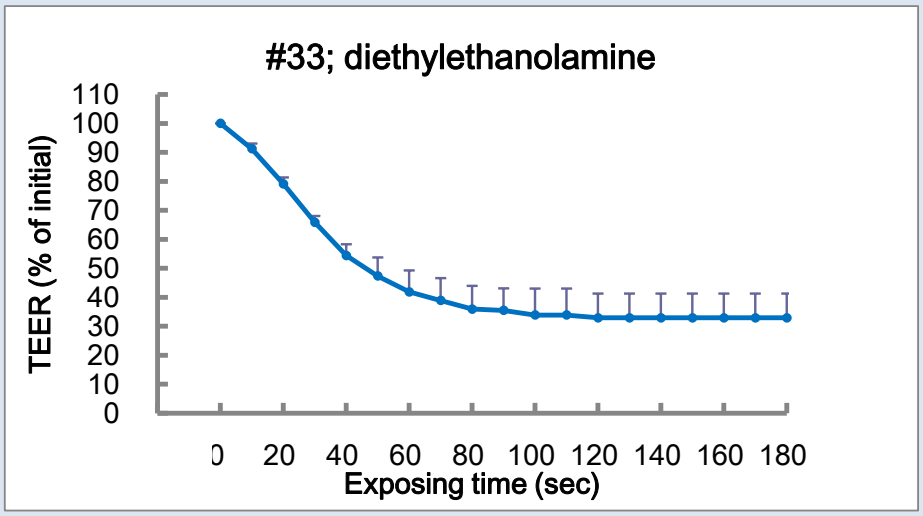
Test #	1	2	3
Model #	12110201	12110202	12110203
Initial	117	133	123
0	103	137	144
10	101	130	137
20	96	123	130
30	92	114	120
40	88	107	112
50	85	103	108
60	83	99	105
70	82	97	103
80	81	95	101
90	81	95	100
100	80	94	100
110	80	94	100
120	80	93	99
130	80	93	99
140	80	93	99
150	80	93	99
160	80	93	99
170	80	93	99
180	80	93	99

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.66	I
Plateau level	66	I



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#32; butyl cellosolve

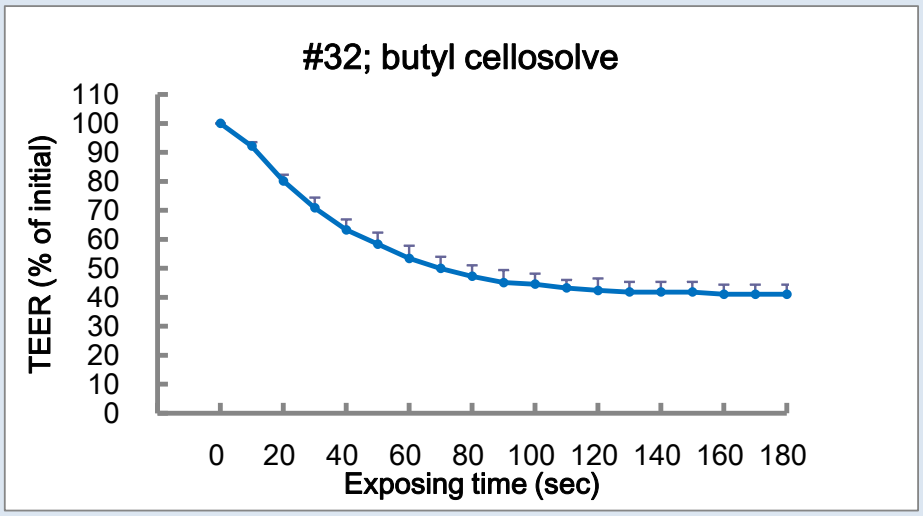
Test #	1	2	3
Model #	12042304	12042305	12042306
Initial	107	107	129
0	107	106	130
10	104	102	126
20	99	97	119
30	95	93	114
40	92	90	109
50	90	88	106
60	88	86	103
70	87	84	101
80	86	83	99
90	85	82	98
100	85	82	97
110	85	81	96
120	84	81	96
130	84	81	95
140	84	81	95
150	84	81	95
160	84	80	95
170	84	80	95
180	84	80	95

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.48	I
Plateau level	58	I



試験日	2012/4/27
実施者名	yamaguchi
被験物質名	#31; methyl pentynol

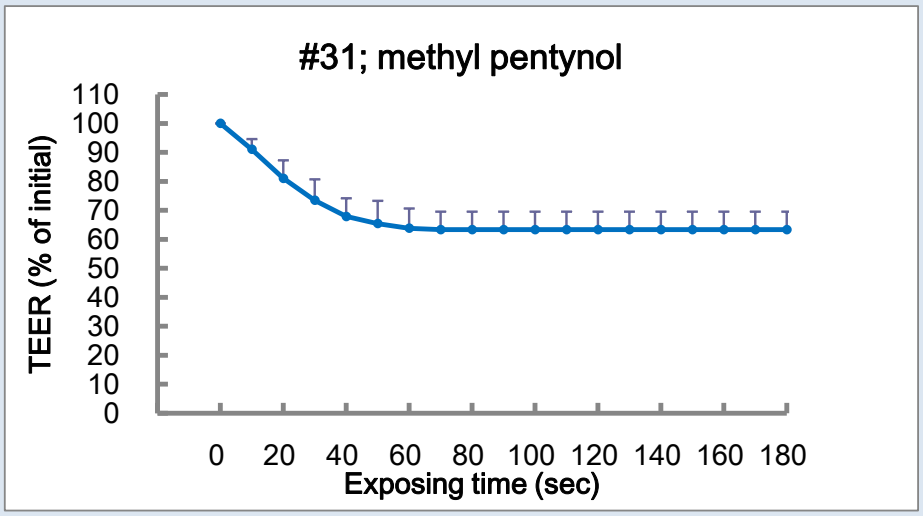
Test #	1	2	3
Model #	12042301	12042302	12042303
Initial	113	119	122
0	115	137	122
10	109	132	118
20	103	126	113
30	99	122	108
40	97	118	104
50	95	117	103
60	95	116	101
70	95	115	101
80	95	115	101
90	95	115	101
100	95	115	101
110	95	115	101
120	95	115	101
130	95	115	101
140	95	115	101
150	95	115	101
160	95	115	101
170	95	115	101
180	95	115	101

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.60	I
Plateau level	36	I



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#30; sodium hydroxide

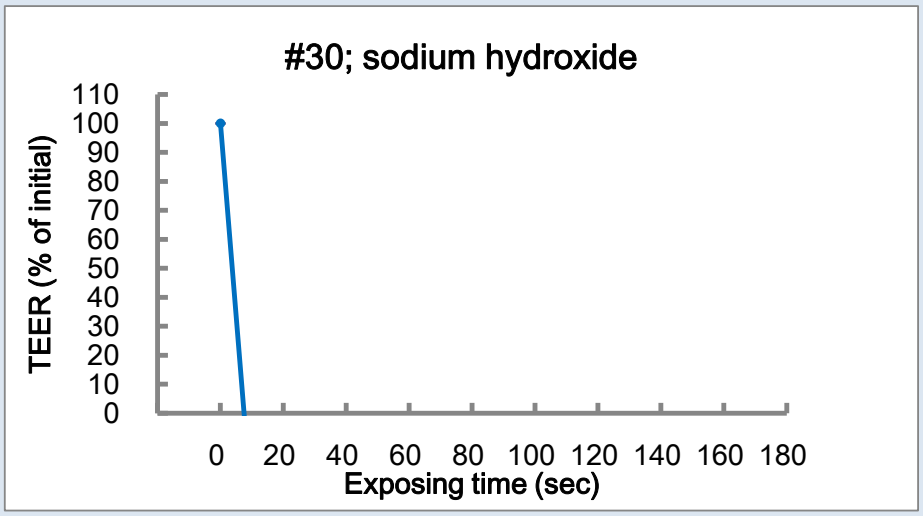
Test #	1	2	3
Model #	11093030	11093031	11093032
Initial	119	104	99
0	124	100	98
10	55	46	49
20	55	46	49
30	55	46	49
40	55	46	49
50	55	46	49
60	55	46	49
70	55	46	49
80	55	46	49
90	55	46	49
100	55	46	49
110	55	46	49
120	55	46	49
130	55	46	49
140	55	46	49
150	55	46	49
160	55	46	49
170	55	46	49
180	55	46	49

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	13.27	I
Plateau level	133	I



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#29; 2-ethylhexyl p-dimethyl-amino benzoate

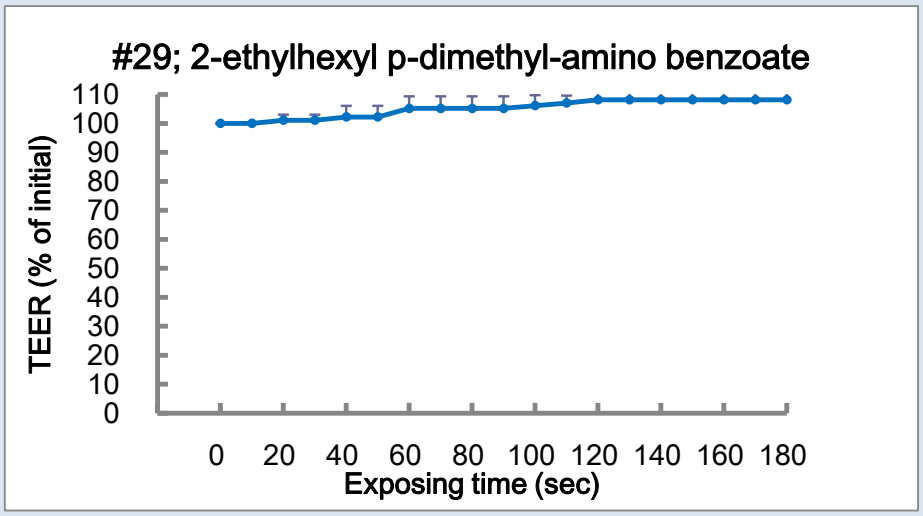
Test #	1	2	3
Model #	11093027	11093028	11093029
Initial	104	103	107
0	97	94	101
10	97	94	101
20	98	94	101
30	98	94	101
40	99	94	101
50	99	94	101
60	100	95	102
70	100	95	102
80	100	95	102
90	100	95	102
100	100	95	103
110	100	96	103
120	101	96	103
130	101	96	103
140	101	96	103
150	101	96	103
160	101	96	103
170	101	96	103
180	101	96	103

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.04	NI
Plateau level	0	NI



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#28; Tween20

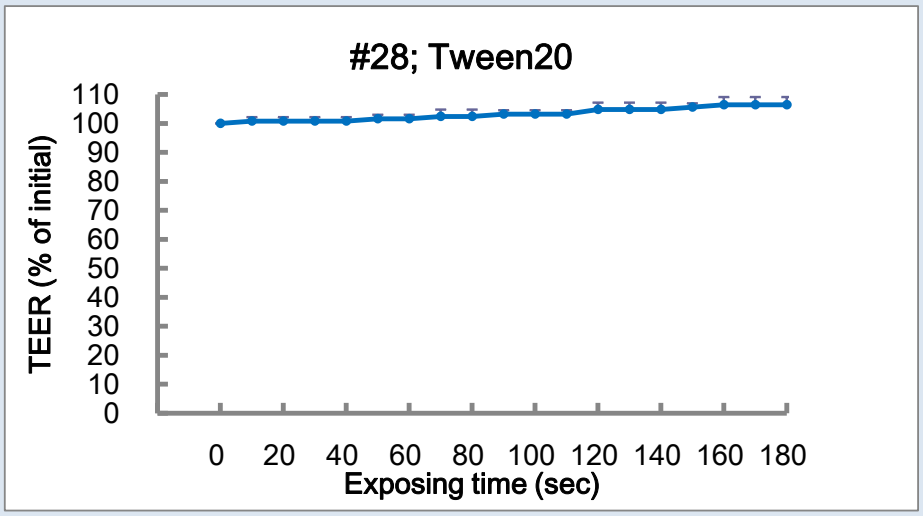
Test #	1	2	3
Model #	11093022	11093023	11093024
Initial	104	113	99
0	102	111	101
10	103	111	101
20	103	111	101
30	103	111	101
40	103	111	101
50	103	111	102
60	103	111	102
70	104	111	102
80	104	111	102
90	104	112	102
100	104	112	102
110	104	112	102
120	105	112	103
130	105	112	103
140	105	112	103
150	105	113	103
160	106	113	103
170	106	113	103
180	106	113	103

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.03	NI
Plateau level	0	NI



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#27; Triton X-100

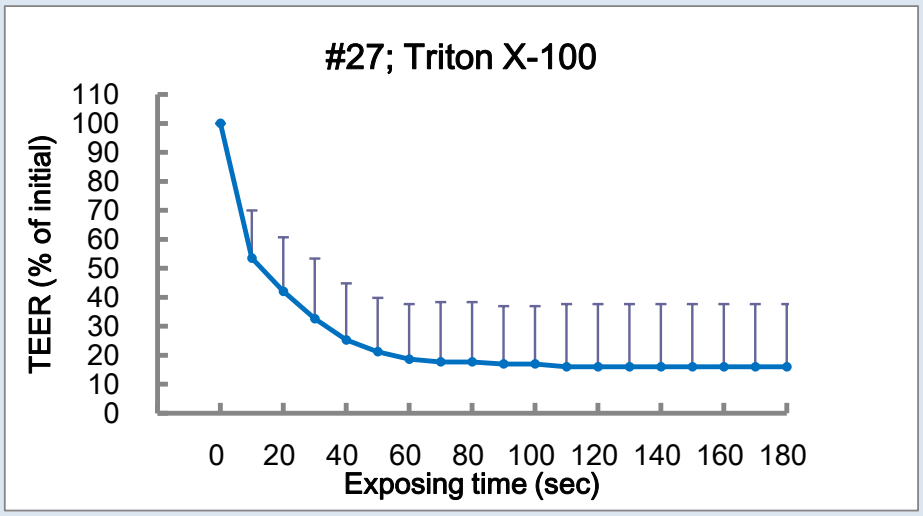
Test #	1	2	3
Model #	11093019	11093020	11093021
Initial	100	121	106
0	102	113	103
10	86	97	81
20	84	90	76
30	80	87	72
40	77	83	70
50	75	81	69
60	74	80	68
70	74	80	67
80	74	80	67
90	74	79	67
100	74	79	67
110	74	79	66
120	74	79	66
130	74	79	66
140	74	79	66
150	74	79	66
160	74	79	66
170	74	79	66
180	74	79	66

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.92	I
Plateau level	83	I



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#26; sodium laulyl sulfate

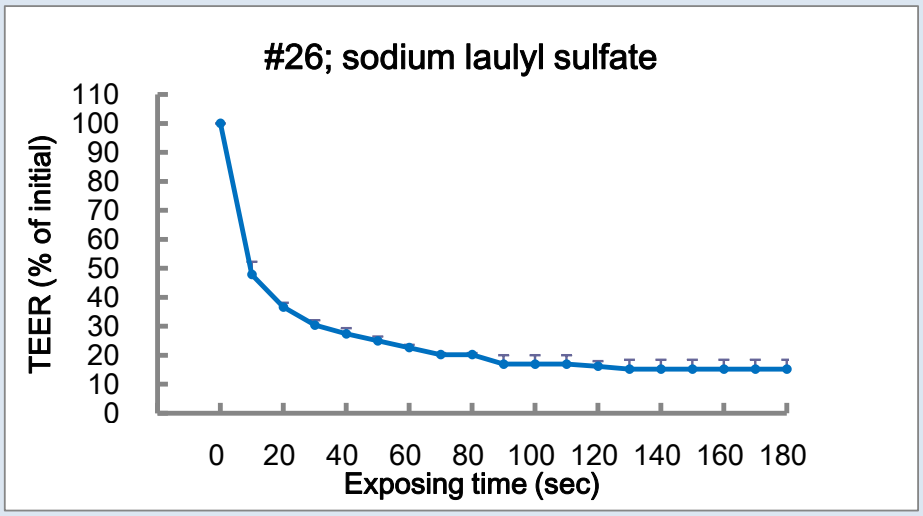
Test #	1	2	3
Model #	11093016	11093017	11093018
Initial	106	98	102
0	108	94	103
10	80	78	79
20	75	73	75
30	72	71	72
40	70	70	71
50	69	69	70
60	68	68	69
70	67	67	68
80	67	67	68
90	65	65	68
100	65	65	68
110	65	65	68
120	65	65	67
130	65	64	67
140	65	64	67
150	65	64	67
160	65	64	67
170	65	64	67
180	65	64	67

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.70	I
Plateau level	84	I



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#25; cetylpyridinium bromide

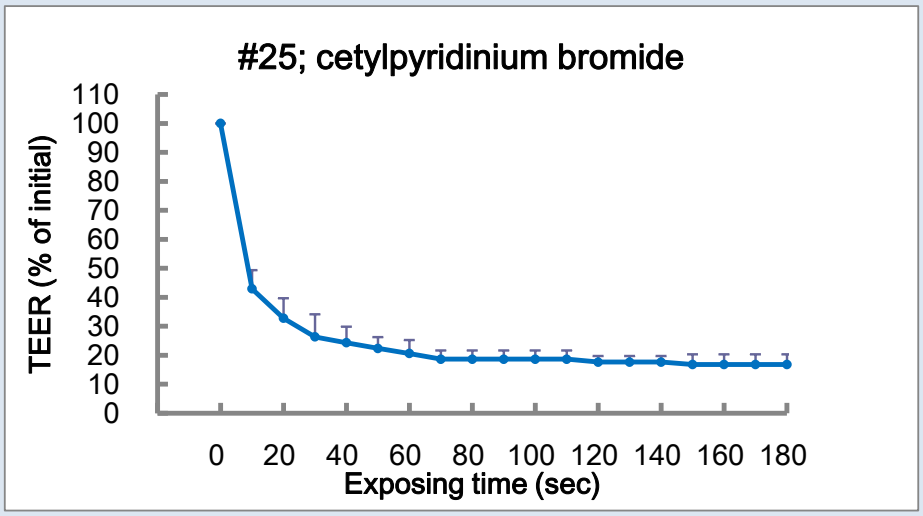
Test #	1	2	3
Model #	11093013	11093014	11093015
Initial	87	99	98
0	90	101	100
10	73	76	80
20	70	72	76
30	68	69	74
40	66	69	74
50	65	69	73
60	65	68	72
70	64	68	71
80	64	68	71
90	64	68	71
100	64	68	71
110	64	68	71
120	63	68	71
130	63	68	71
140	63	68	71
150	63	67	71
160	63	67	71
170	63	67	71
180	63	67	71

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	1.16	I
Plateau level	81	I



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#24; benzalkonium chloride

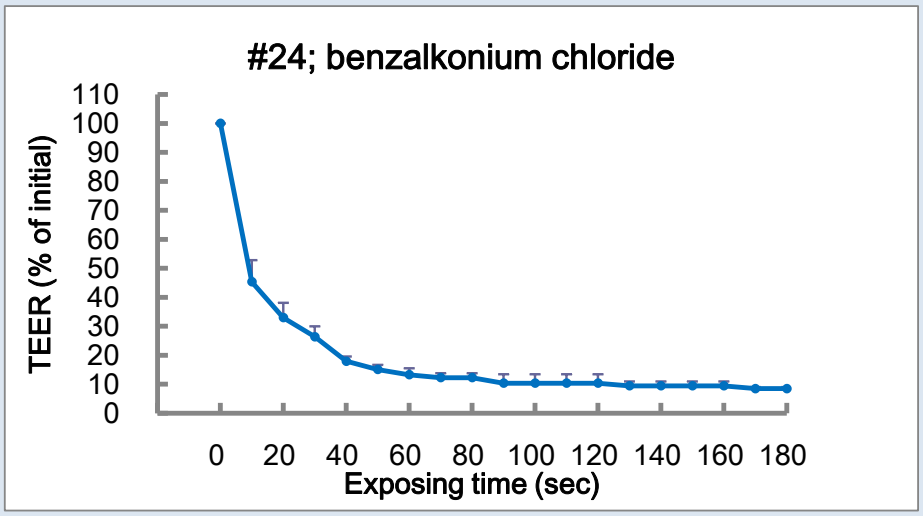
Test #	1	2	3
Model #	11093010	11093011	11093012
Initial	96	98	95
0	96	98	95
10	73	81	77
20	70	76	72
30	68	73	70
40	65	69	68
50	64	68	67
60	63	67	67
70	63	67	66
80	63	67	66
90	62	67	65
100	62	67	65
110	62	67	65
120	62	67	65
130	62	66	65
140	62	66	65
150	62	66	65
160	62	66	65
170	62	65	65
180	62	65	65

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	1.00	I
Plateau level	90	I



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#23; propylene glycol

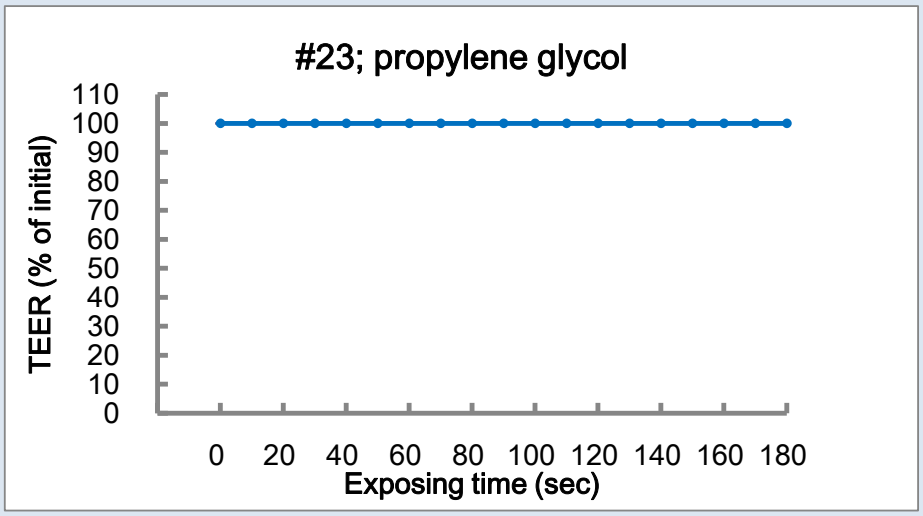
Test #	1	2	3
Model #	11093007	11093008	11093009
Initial	87	83	92
0	89	87	91
10	89	87	91
20	89	87	91
30	89	87	91
40	89	87	91
50	89	87	91
60	89	87	91
70	89	87	91
80	89	87	91
90	89	87	91
100	89	87	91
110	89	87	91
120	89	87	91
130	89	87	91
140	89	87	91
150	89	87	91
160	89	87	91
170	89	87	91
180	89	87	91

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.00	NI
Plateau level	0	NI



試験日	2011/10/6
実施者名	yamaguchi
被験物質名	#22; polyethylene glycol 400

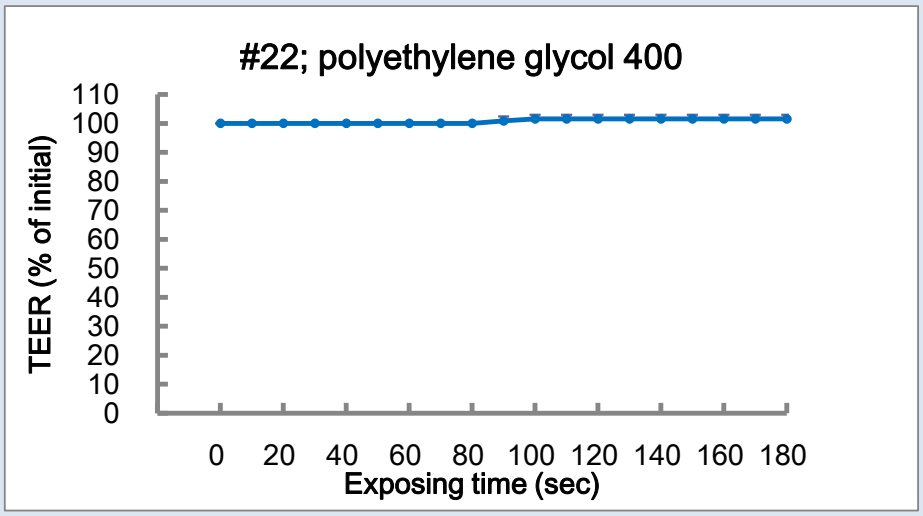
Test #	1	2	3
Model #	11093004	11093005	11093006
Initial	106	113	91
0	101	107	85
10	101	107	85
20	101	107	85
30	101	107	85
40	101	107	85
50	101	107	85
60	101	107	85
70	101	107	85
80	101	107	85
90	102	107	85
100	102	108	85
110	102	108	85
120	102	108	85
130	102	108	85
140	102	108	85
150	102	108	85
160	102	108	85
170	102	108	85
180	102	108	85

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2013/12/3
実施者名	yamaguchi
被験物質名	#21; glycerol

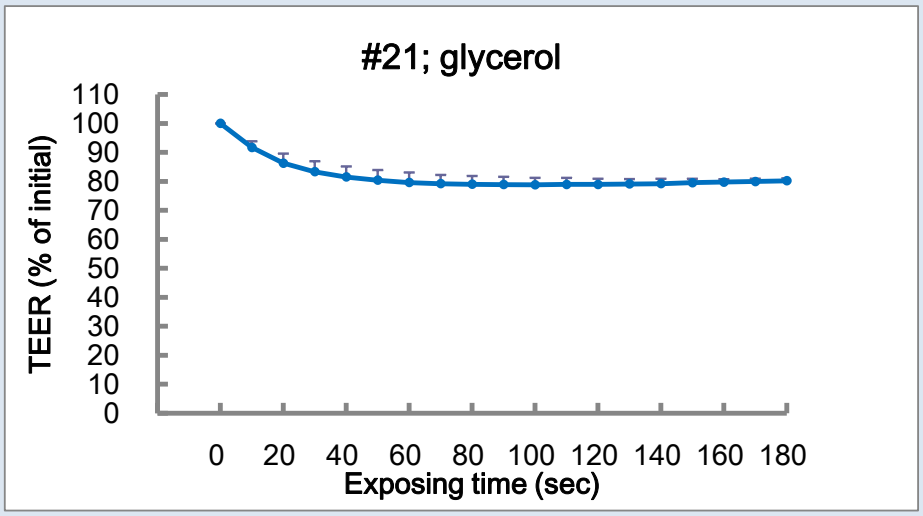
Test #	1	2	3
Model #	13112707	13112708	13112734
Initial	223	232	193
0	222	232	183
10	215	223	175
20	209	218	170
30	206	215	167
40	204	212	166
50	202	211	165
60	201	210	165
70	200	209	165
80	200	209	165
90	200	209	165
100	199	208	165
110	199	208	165
120	199	208	166
130	199	208	166
140	200	208	166
150	200	209	167
160	200	208	167
170	200	209	167
180	200	209	168

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.34	I
Plateau level	20	I



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#20; sodium salycylate

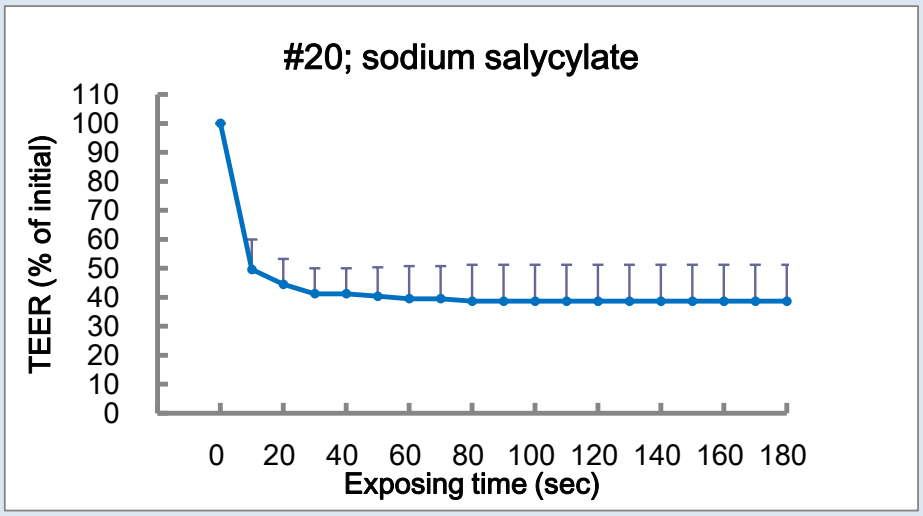
Test #	1	2	3
Model #	11101425	11101426	11101427
Initial	91	106	112
0	98	133	138
10	74	100	108
20	73	95	104
30	72	92	102
40	72	92	102
50	71	92	102
60	70	92	102
70	70	92	102
80	69	92	102
90	69	92	102
100	69	92	102
110	69	92	102
120	69	92	102
130	69	92	102
140	69	92	102
150	69	92	102
160	69	92	102
170	69	92	102
180	69	92	102

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	1.01	I
Plateau level	60	I



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#19; n,n-dimethylguanidine sulfate

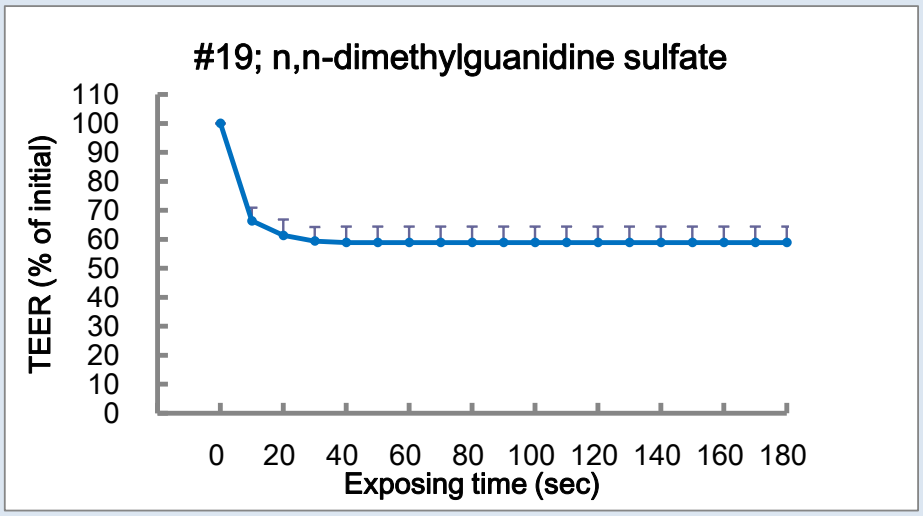
Test #	1	2	3
Model #	11101422	11101423	11101424
Initial	108	110	114
0	129	130	131
10	106	109	108
20	102	106	105
30	101	104	104
40	100	104	104
50	100	104	104
60	100	104	104
70	100	104	104
80	100	104	104
90	100	104	104
100	100	104	104
110	100	104	104
120	100	104	104
130	100	104	104
140	100	104	104
150	100	104	104
160	100	104	104
170	100	104	104
180	100	104	104

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	1.35	I
Plateau level	41	I



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#18; methyl isobutyl ketone

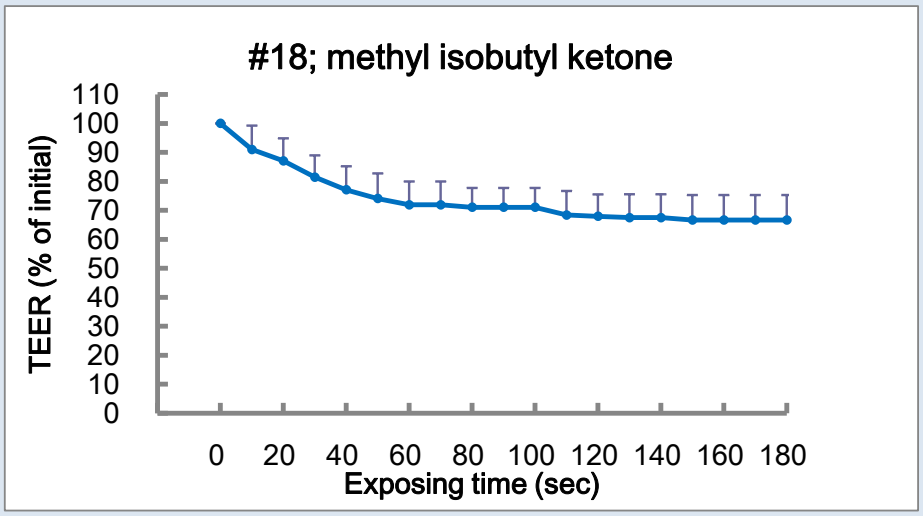
Test #	1	2	3
Model #	11101431	11101432	11101433
Initial	105	107	110
0	138	137	139
10	132	123	138
20	130	120	134
30	125	116	130
40	120	113	128
50	118	110	126
60	116	109	124
70	116	109	124
80	116	109	122
90	116	109	122
100	116	109	122
110	110	109	122
120	110	109	121
130	109	109	121
140	109	109	121
150	109	107	121
160	109	107	121
170	109	107	121
180	109	107	121

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.25	I
Plateau level	32	I



試験日	2011/11/3
実施者名	yamaguchi
被験物質名	#17; methyl ethyl ketone

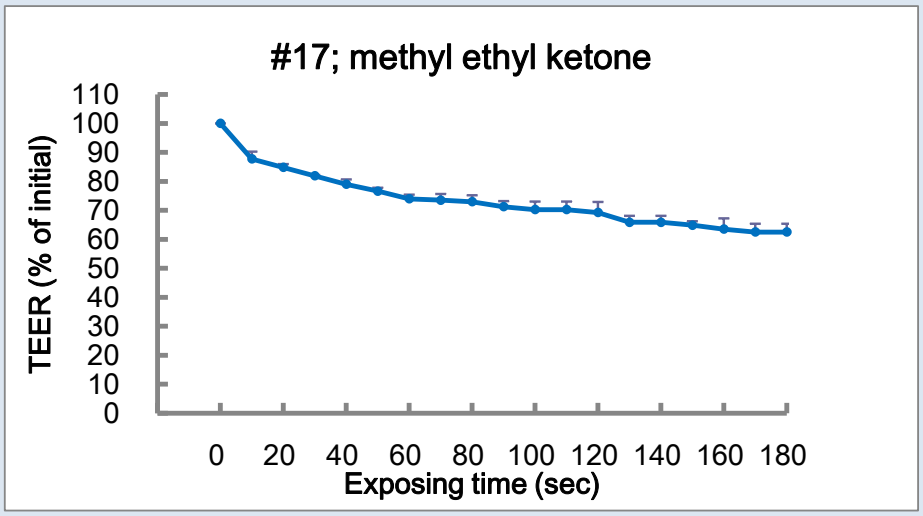
Test #	1	2	3
Model #	11102813	11102814	11102815
Initial	110	108	113
0	114	127	138
10	109	120	127
20	107	118	126
30	105	116	125
40	103	114	124
50	103	113	120
60	102	111	118
70	102	111	117
80	102	110	117
90	101	109	116
100	101	108	115
110	101	108	115
120	101	107	114
130	96	107	114
140	96	107	114
150	96	106	113
160	94	106	113
170	94	105	112
180	94	105	112

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.21	I
Plateau level	37	I



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#16; metyl pentyl ketone

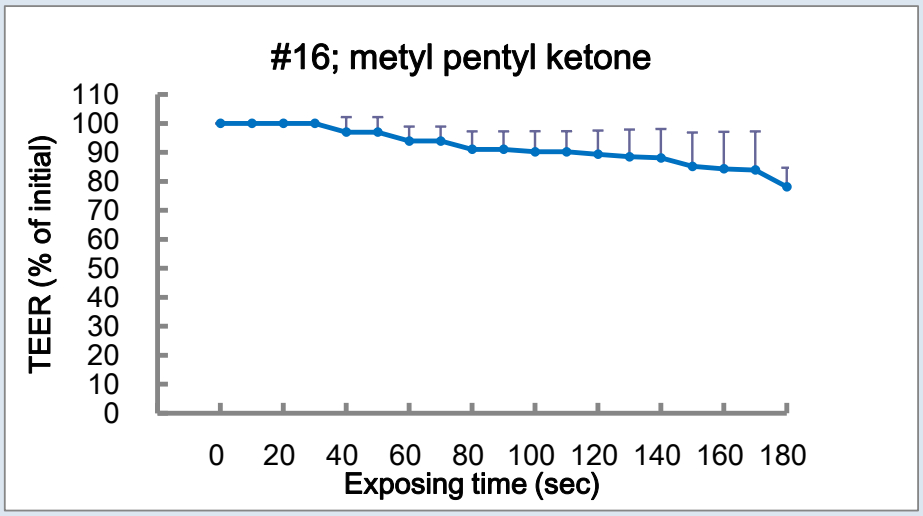
Test #	1	2	3
Model #	11101416	11101417	11101430
Initial	90	103	100
0	113	138	139
10	113	138	139
20	113	138	139
30	113	138	139
40	113	138	132
50	113	138	132
60	112	134	130
70	112	134	130
80	112	128	129
90	112	128	129
100	112	128	127
110	112	128	127
120	112	128	125
130	112	128	123
140	112	128	122
150	112	123	120
160	112	123	118
170	112	123	117
180	103	123	116

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	50	I
Intensity	0.20	I
Plateau level	9	I



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#15; gluconolactone

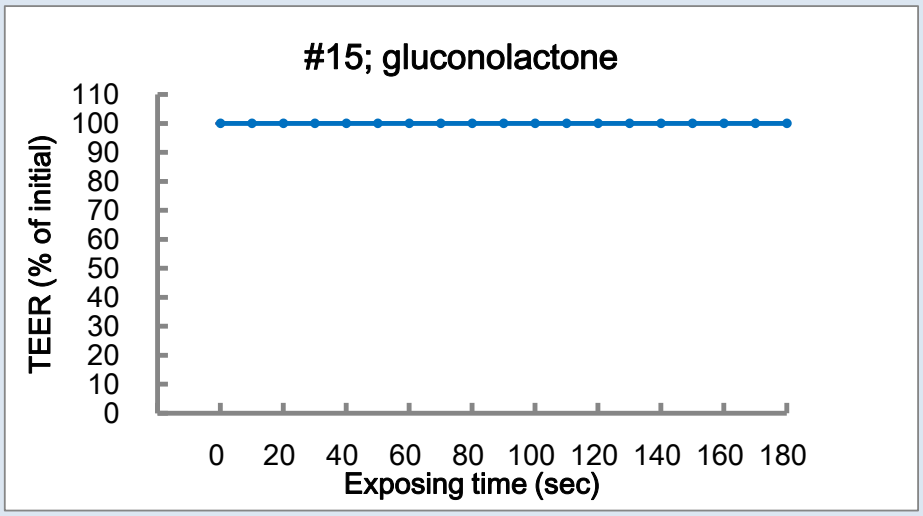
Test #	1	2	3
Model #	11101413	11101414	11101415
Initial	110	113	113
0	135	148	143
10	135	148	143
20	135	148	143
30	135	148	143
40	135	148	143
50	135	148	143
60	135	148	143
70	135	148	143
80	135	148	143
90	135	148	143
100	135	148	143
110	135	148	143
120	135	148	143
130	135	148	143
140	135	148	143
150	135	148	143
160	135	148	143
170	135	148	143
180	135	148	143

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	0.00	NI
Plateau level	0	NI



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#14; acetone

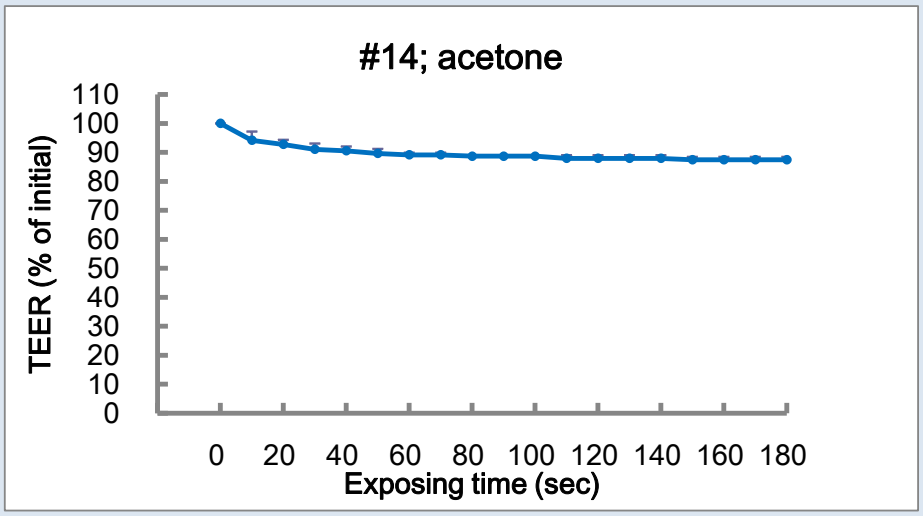
Test #	1	2	3
Model #	11101411	11101421	11101419
Initial	107	105	89
0	131	106	91
10	127	102	90
20	126	102	89
30	126	101	88
40	125	101	88
50	125	101	87
60	124	101	87
70	124	101	87
80	123	101	87
90	123	101	87
100	123	101	87
110	123	100	87
120	123	100	87
130	123	100	87
140	123	100	87
150	122	100	87
160	122	100	87
170	122	100	87
180	122	100	87

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.21	I
Plateau level	10	I



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#13; toluene

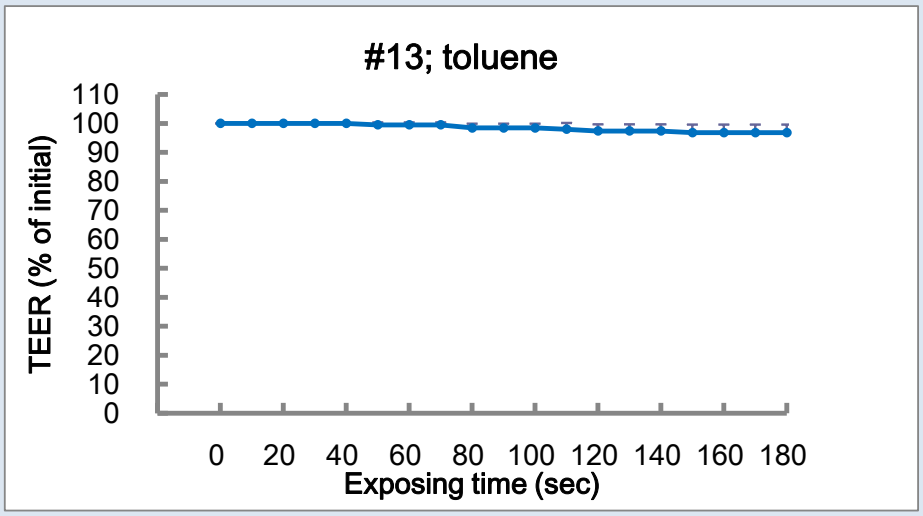
Test #	1	2	3
Model #	11101407	11101408	11101409
Initial	100	92	120
0	124	116	135
10	124	116	135
20	124	116	135
30	124	116	135
40	124	116	135
50	124	116	134
60	124	116	134
70	124	116	134
80	124	115	133
90	124	115	133
100	124	115	133
110	124	115	132
120	124	114	132
130	124	114	132
140	124	114	132
150	124	113	132
160	124	113	132
170	124	113	132
180	124	113	132

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	140	I
Intensity	0.02	NI
Plateau level	0	NI



試験日	2011/11/3
実施者名	yamaguchi
被験物質名	#12; methyl cyclopentane

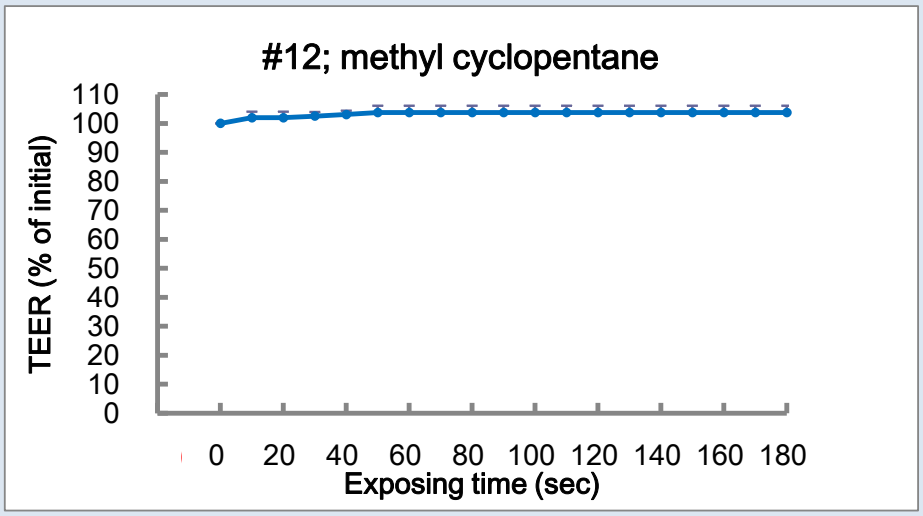
Test #	1	2	3
Model #	11102810	11102811	11102812
Initial	110	108	113
0	107	118	128
10	109	119	128
20	109	119	128
30	109	119	129
40	109	120	129
50	110	120	129
60	110	120	129
70	110	120	129
80	110	120	129
90	110	120	129
100	110	120	129
110	110	120	129
120	110	120	129
130	110	120	129
140	110	120	129
150	110	120	129
160	110	120	129
170	110	120	129
180	110	120	129

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.02	NI
Plateau level	0	NI



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#11; 3,3-dimethylpentane

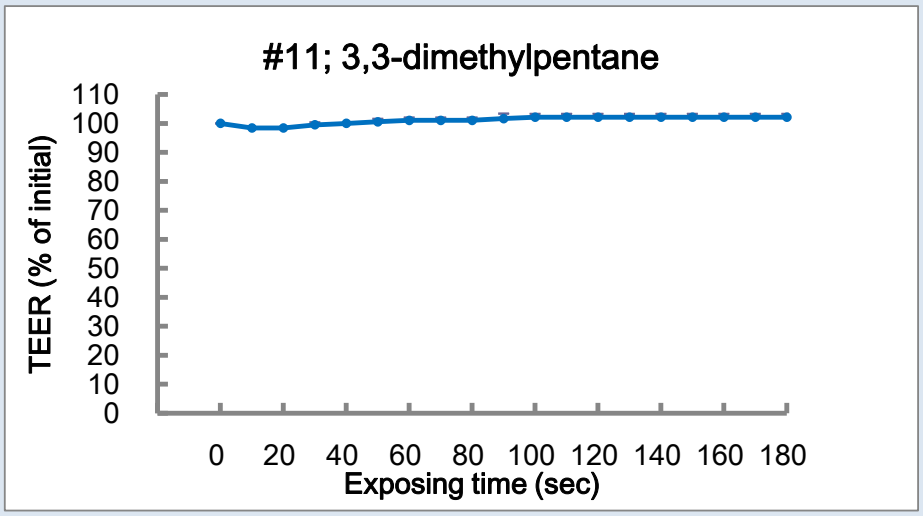
Test #	1	2	3
Model #	11101404	11101405	11101406
Initial	100	99	110
0	116	130	130
10	115	129	129
20	115	129	129
30	116	130	129
40	116	130	130
50	117	130	130
60	117	130	131
70	117	130	131
80	117	130	131
90	118	130	131
100	118	131	131
110	118	131	131
120	118	131	131
130	118	131	131
140	118	131	131
150	118	131	131
160	118	131	131
170	118	131	131
180	118	131	131

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.01	NI
Plateau level	0	NI



試験日	2011/11/3
実施者名	yamaguchi
被験物質名	#10; n-hexanol

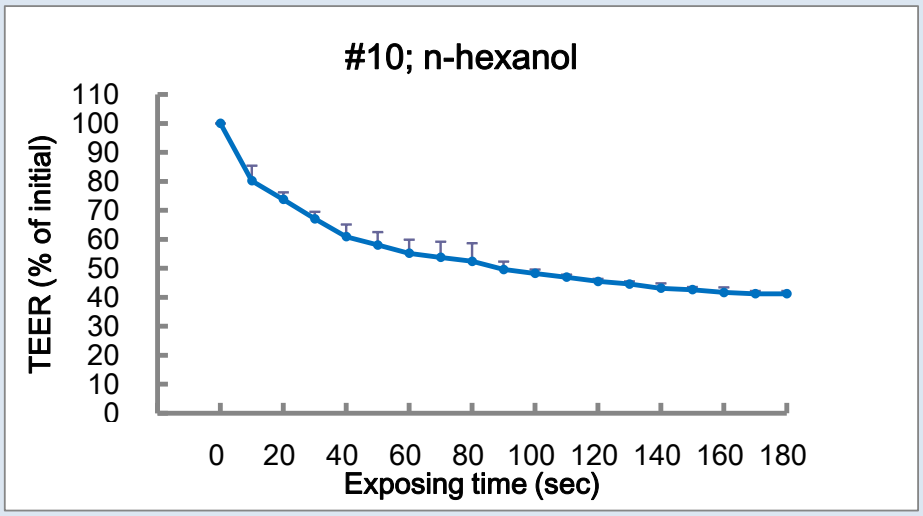
Test #	1	2	3
Model #	11102807	11102808	11102809
Initial	111	102	123
0	138	125	143
10	126	109	130
20	120	107	124
30	115	103	119
40	113	98	113
50	111	96	111
60	109	94	109
70	109	93	107
80	109	92	105
90	104	92	104
100	102	92	103
110	101	92	101
120	100	91	100
130	99	91	99
140	99	89	98
150	98	89	98
160	98	88	97
170	97	88	97
180	97	88	97

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.33	I
Plateau level	59	I



試験日	2011/9/15
実施者名	yamaguchi
被験物質名	#9; isopropyl alcohol

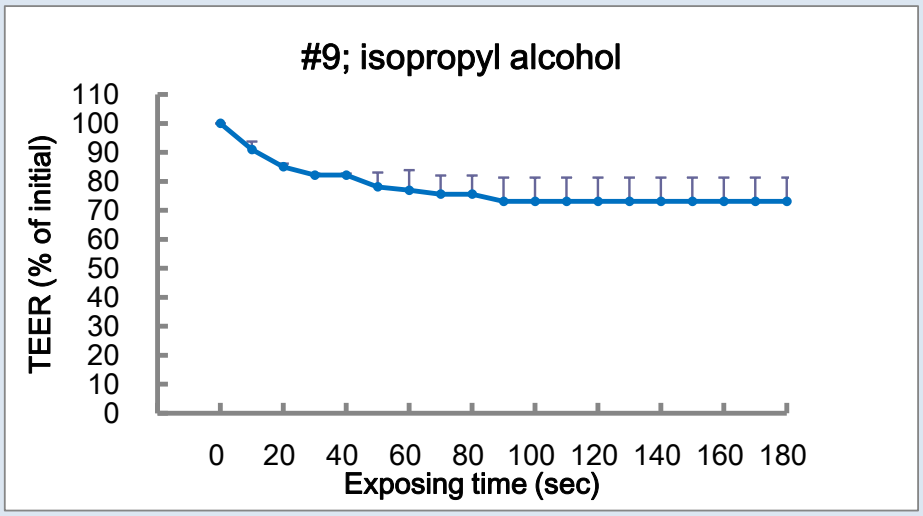
Test #	1	2	3
Model #	11090926	11090927	11090928
Initial	96	92	112
0	96	92	112
10	92	90	108
20	91	88	104
30	90	87	103
40	90	87	103
50	90	84	102
60	90	83	102
70	90	83	100
80	90	83	100
90	90	82	98
100	90	82	98
110	90	82	98
120	90	82	98
130	90	82	98
140	90	82	98
150	90	82	98
160	90	82	98
170	90	82	98
180	90	82	98

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.30	I
Plateau level	27	I



試験日	2011/11/3
実施者名	yamaguchi
被験物質名	#8; hexyl cinnamic aldehyde

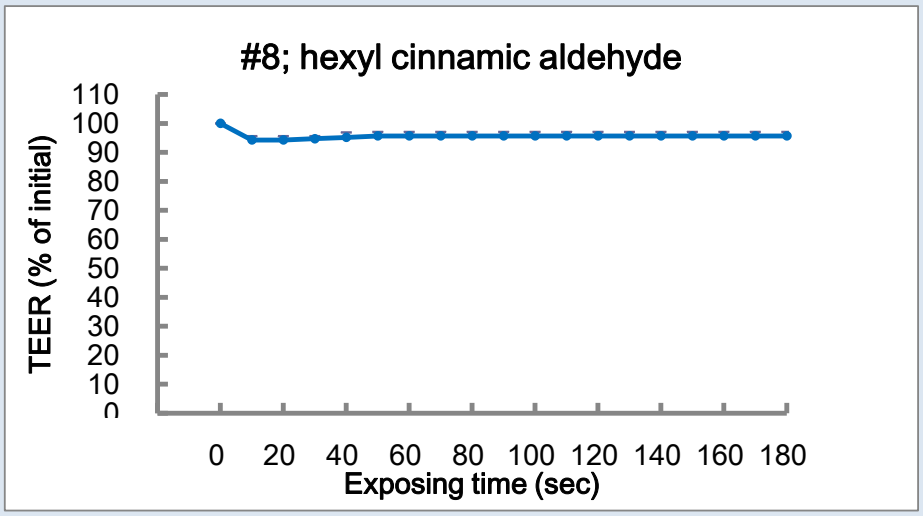
Test #	1	2	3
Model #	11102804	11102805	11102806
Initial	107	112	117
0	124	137	140
10	120	134	135
20	120	134	135
30	120	134	136
40	120	135	136
50	121	135	136
60	121	135	136
70	121	135	136
80	121	135	136
90	121	135	136
100	121	135	136
110	121	135	136
120	121	135	136
130	121	135	136
140	121	135	136
150	121	135	136
160	121	135	136
170	121	135	136
180	121	135	136

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.57	I
Plateau level	6	I



試験日	2012/11/8
実施者名	yamaguchi
被験物質名	#7; ethanol

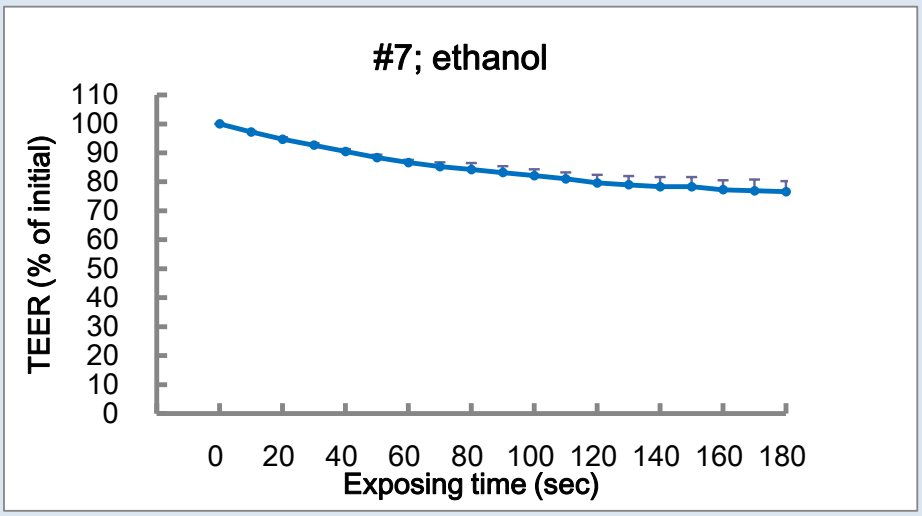
Test #	1	2	3
Model #	12110234	12110235	12110236
Initial	172	182	150
0	173	175	158
10	170	172	156
20	168	170	153
30	165	168	152
40	163	166	150
50	161	164	148
60	159	162	147
70	157	161	146
80	155	160	146
90	154	159	145
100	153	158	144
110	152	157	143
120	150	156	142
130	149	155	142
140	148	154	142
150	148	154	142
160	147	153	141
170	146	153	141
180	146	152	141

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.12	I
Plateau level	23	I



試験日	2011/9/15
実施者名	yamaguchi
被験物質名	#6; ciclohexanol

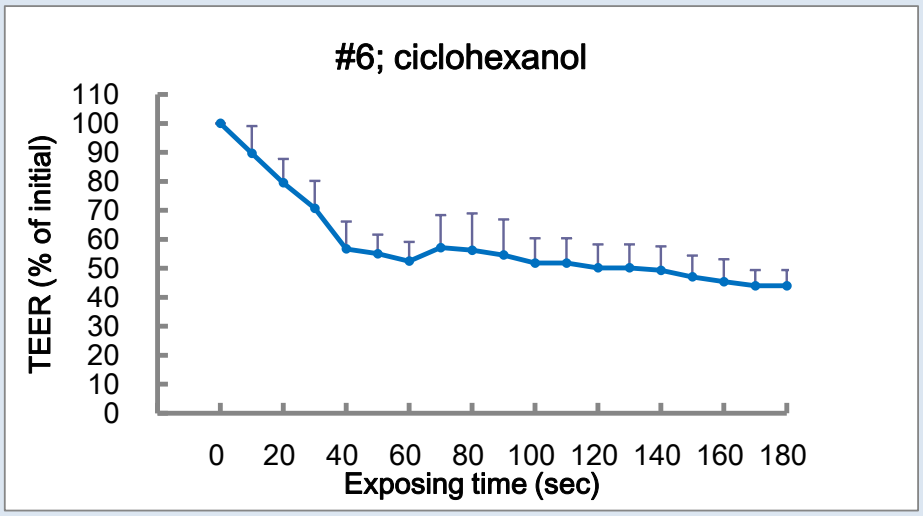
Test #	1	2	3
Model #	11090923	11090924	11090925
Initial	103	86	103
0	103	86	103
10	98	86	96
20	95	83	92
30	92	81	88
40	90	74	85
50	88	74	85
60	87	74	83
70	87	78	82
80	87	78	81
90	85	78	81
100	85	76	81
110	85	76	81
120	83	76	81
130	83	76	81
140	82	76	81
150	82	75	80
160	80	75	80
170	80	74	80
180	80	74	80

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.31	I
Plateau level	56	I



試験日	2011/9/15
実施者名	yamaguchi
被験物質名	#5; 3-methoxy-1,2-propanediol

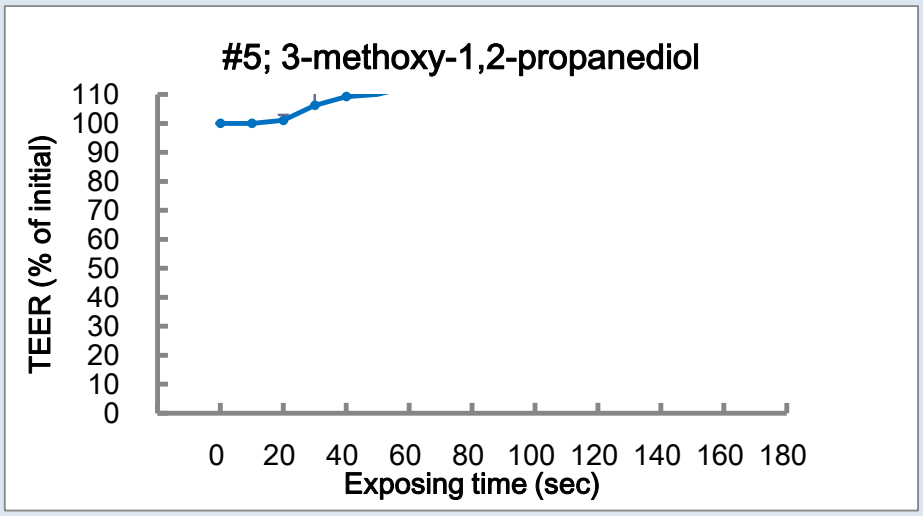
Test #	1	2	3
Model #	11090929	11090930	11090931
Initial	113	93	92
0	108	93	92
10	108	93	92
20	108	93	93
30	109	94	96
40	110	95	97
50	111	95	97
60	113	96	97
70	113	96	98
80	117	96	98
90	117	97	98
100	116	97	98
110	116	97	98
120	116	97	98
130	116	98	98
140	116	98	98
150	116	98	98
160	116	98	98
170	116	98	98
180	116	98	98

判定結果

NI

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	190	NI
Intensity	−0.10	NI
Plateau level	0	NI



試験日	2011/9/15
実施者名	yamaguchi
被験物質名	#4; 2-methyl-1-pentanol

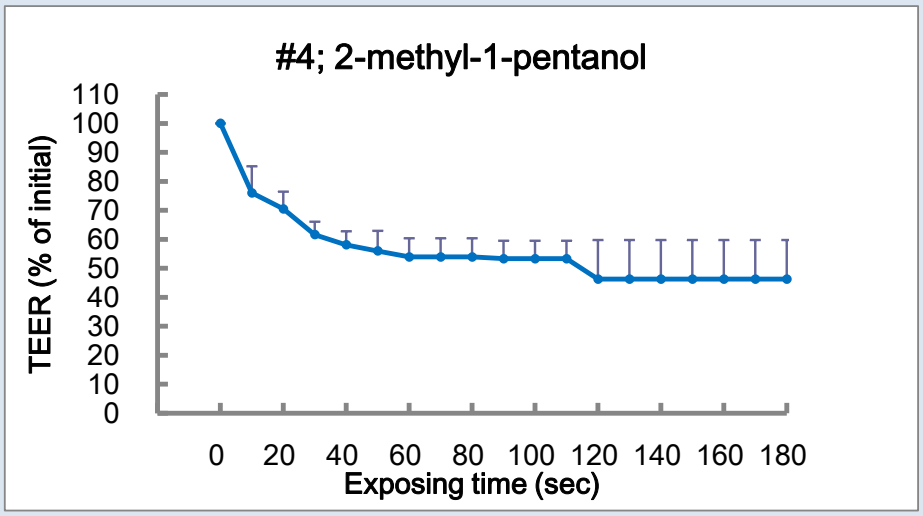
Test #	1	2	3
Model #	11090932	11090933	11090934
Initial	111	115	106
0	111	115	106
10	101	96	98
20	98	95	94
30	94	92	88
40	92	92	85
50	92	91	83
60	90	91	82
70	90	91	82
80	90	91	82
90	90	90	82
100	90	90	82
110	90	90	82
120	89	88	75
130	89	88	75
140	89	88	75
150	89	88	75
160	89	88	75
170	89	88	75
180	89	88	75

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.77	I
Plateau level	46	I



試験日	2011/10/20
実施者名	yamaguchi
被験物質名	#3; 2,5-dimethyl-2,5-hexandiol

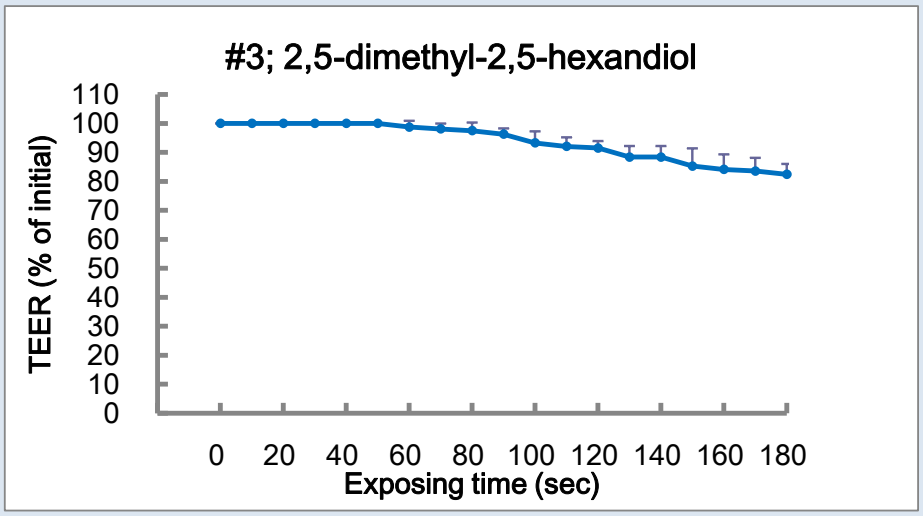
Test #	1	2	3
Model #	11101401	11101402	11101403
Initial	104	107	104
0	110	119	123
10	110	119	123
20	110	119	123
30	110	119	123
40	110	119	123
50	110	119	123
60	110	117	123
70	109	117	123
80	109	116	123
90	108	116	122
100	107	113	121
110	106	113	120
120	106	113	119
130	102	113	118
140	102	113	118
150	99	112	117
160	99	111	116
170	99	111	115
180	99	110	114

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	80	I
Intensity	0.15	I
Plateau level	18	I



試験日	2011/9/15
実施者名	yamaguchi
被験物質名	#2; 2-ethyl-1-hexanol

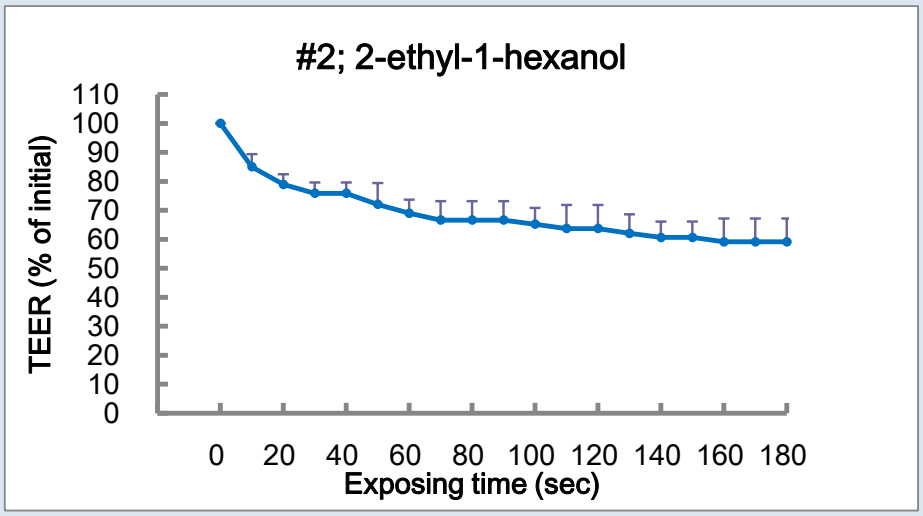
Test #	1	2	3
Model #	11090920	11090921	11090923
Initial	104	86	103
0	104	86	85
10	100	82	81
20	96	80	81
30	96	80	79
40	96	80	79
50	95	80	77
60	93	79	77
70	92	79	76
80	92	79	76
90	92	79	76
100	92	78	76
110	92	78	75
120	92	78	75
130	90	78	75
140	90	77	75
150	90	77	75
160	90	77	74
170	90	77	74
180	90	77	74

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	0	I
Intensity	0.23	I
Plateau level	41	I



試験日	2011/11/3
実施者名	yamaguchi
被験物質名	#1; 1-octanol

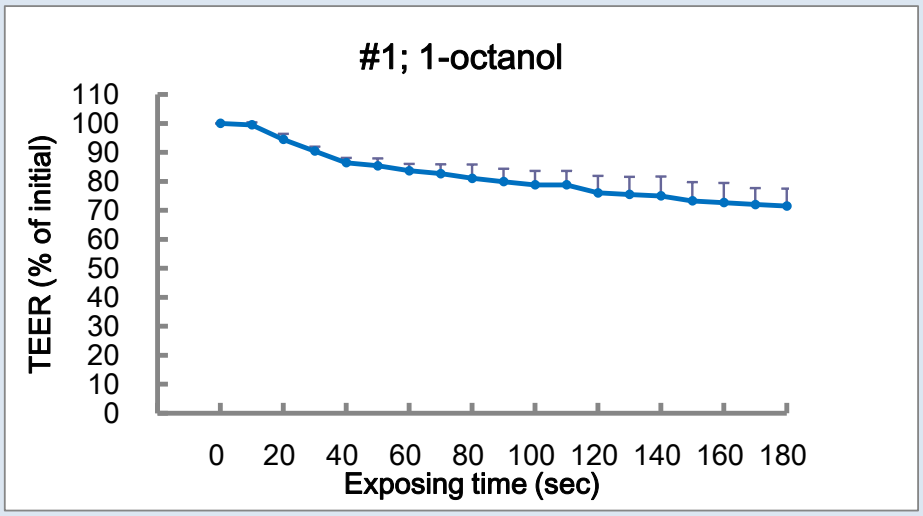
Test #	1	2	3
Model #	11102801	11102802	11102803
Initial	111	115	117
0	114	121	131
10	114	121	130
20	112	118	126
30	110	115	124
40	108	112	122
50	108	111	121
60	107	110	120
70	107	110	118
80	107	109	116
90	106	109	115
100	106	107	115
110	106	107	115
120	105	106	112
130	105	105	112
140	105	105	111
150	104	104	110
160	104	103	110
170	103	103	110
180	103	102	110

判定結果

I

I	刺激性
NI	非刺激性
ERROR	再試験

Index	Score	Calss
Time lag	10	I
Intensity	0.16	I
Plateau level	29	I



1. Background

At the Vitrigel VMT meeting, Dr. Sozu, our biostatistician, expressed his opinion about the statistical validity of the number of test chemicals used for the ECVAM validation study for skin sensitisation.

2. Regarding the statistical validity of the number of test chemicals

2.1 Overview

As part of the ECVAM validation, a statistical power was calculated to determine the number of test chemicals necessary to determine within-laboratory reproducibility (WLR) and between-laboratory reproducibility (BLR). Unfortunately, as is described below, this cannot be considered a suitable approach for the purposes of that validation. Just as in the alternative proposal by the ESAC Working Group, the approach more suitable for the purpose of the validation is to consider the estimation accuracy for each indicator as the basis of the statistical validity of the number of test chemicals. Yet even this is not fully sufficient and the results obtained in such an approach should be considered only as a reference point.

2.2 Why it was not suitable to design the ECVAM validation by basing the required number of test chemicals on statistical power

In general, null and alternative hypotheses are assumed if the sample size (or, in this case, the required number of test chemicals) is determined based on a statistical power. The approach taken in the ECVAM validation was to determine the required number of test chemicals based on statistical power for hypothesis testing with following null and alternative hypotheses. This example shows the approach for determining WLR, but the approach for BLR is the same.

Null hypothesis: $\pi_W \leq \pi_0$

The true value of WLR (π_W) is equal to or less than the acceptable value (π_0).

Alternative hypothesis: $\pi_W > \pi_0$

The true value of WLR (π_W) is greater than the acceptable value (π_0). The purpose of statistical hypothesis testing is to argue in favor of the alternative hypothesis by rejecting the null hypothesis based on the obtained data. It is difficult to see, however, how this approach achieves the purpose of the validation. Therefore, demonstrating the validity of the required number of test chemicals based on statistical power does not achieve the purpose of the validation.

When this method is used, the required number of test chemicals decreases as the acceptable value π_0 decreases. Conversely, the required number of test chemicals increases as the acceptable value π_0 increases. Thus the required number of test chemicals is highly dependent on the acceptable value π_0. Moreover, rather than being determined statistically, the determination of the acceptable value π_0 is based on the fact the test method is judged to be have no utility (no value) when the true value of WLR is less than the acceptable value π_0. In the ECVAM validation, π_0 was set to 0.65, but this value should be chosen after considering a wide range of factors and is not something that can be made arbitrarily.

2.3 Designing the required number of test chemicals based on estimation accuracy

The method for designing the required number of test chemicals based on the estimation accuracy for each indicator, as recommended by the ESAC WG, is a basic approach based on a binomial distribution. Just as with design of the required number of test chemicals based on statistical power, however, this requires the assumption that all events X_{ij}, in which WLR is achievable at laboratory i for multiple tests of test chemical j, are mutually and independently distributed as a Bernoulli distribution with success probability of π_W.

During actual validations, although the assumption that all X_{ij} are mutually independent is likely to hold true, it is also highly unlikely that all probabilities π_W would be identical. This is because it is natural that the potential to achieve repeatability will strongly depends on the strength of the test chemicals. For example, although substances with extremely strong or weak sensitization have good reproducibility, substances with moderate sensitization have poor reproducibility. Therefore, even when designing the required number of test chemicals based on estimation accuracy, the study must be based on this kind of mathematical assumption. As previously mentioned, the results obtained in such an approach should be considered only as a reference point. Given these assumptions, the rationale for the design of the number of required test chemicals based on estimation accuracy is as follows.

Assuming n to be the total number of test runs (the denominator for the reproducibility indicators) the variance of the estimator of WLR is given by the following formula.

$$\frac{\pi_W(1-\pi_W)}{n}$$

The ESAC WG uses the following formula, i.e., the half-width of the 95% confidence interval of the WLR, as the measure of estimation accuracy, and shows the required number of test chemicals n so that the estimation accuracy is no greater than an arbitrary value c.

$$\left[\left(1.96\times\sqrt{\frac{\pi_W(1-\pi_W)}{n}}\right)\right]\times 100\ (\%)$$

A simple conversion yields a formula that can be used to find the smallest integer equivalent to the required number of test chemicals n when the half-width of the 95% confidence interval is no greater than an arbitrary value c.

$$n \geq \pi_W(1-\pi_W)\left(\frac{1.96\times 100}{c}\right)^2$$

For example, when c is 5.0%, the required number of test chemicals n is 139. When c is 7.5%, n is 62; when 10.0%, it is 35; and when 12.5%, it is 23. In fact, ESAC WG use these values in its examples. For Phase I of this validation, since $n = 10$ test chemicals $\times$ 4 facilities $= 40$, the level of uncertainty is 9.3% for $\pi_W = 0.9$ or 12.4% for $\pi_W = 0.8$. As I have mentioned repeatedly, however, the results obtained in such an approach should be considered only as a reference point.