657 solubility), 3-chloropropionitrile (high volatility and cytotoxicity), and sodium dehydroacetate
658 (cytotoxicity). JaCVAM provided test substances to the three participating laboratories.
659 Import/export restrictions prevented JaCVAM from supplying either TEA or bovine fetal serum to
660 Korea, Biotoxtech Co., Ltd, which obtained these two substances from a local supplier in Korea.
661 Since it was not possible for all three participating laboratories to use reagents from a single
662 manufacturing lot, the VMT decided that the purpose of Phase I would be to assess transferability
663 only.
664 Three runs of four test substances were performed in Phase I. The mean and standard deviation for
665 IC_{50} of the relative control at 1382.8 ±33.3 at Lab A, 1529.3 ±132.7 at Lab B, and 1898.1 ±350.3
666 at Lab C (Table 8.1).
667 Discrepancies in the test results led the VMT to direct Lab C to repeat tests for all four test
668 substances.
669 The means and standard deviations for IC_{50} of the relative control for all three participating
670 laboratories are shown in Table 8.1. The coefficient of variation of the relative controls was 2.4%
671 at Lab A, 8.7% at Lab B, and 18.5% at Lab C. Variation of IC_{50} was small except at Lab C. The
672 mean for the positive controls was 82.0 at Lab A, 87.0 at Lab B, and 170.9 at Lab C, and standard
673 deviation range from 1.9% to 4.3%, indicating a small variation. The quality of the
674 SIRC-CVS:TEA cytotoxicity test was confirmed per the six criteria specified in Section 3.2.8
675 *Quality Control*.
676
677 **4.2 Phase II study**
678 Phase II was carried out using twenty coded-test substances and divided into two parts: five test
679 substances in Phase IIA and fifteen in Phase IIB. After obtaining permission to ship TEA to Korea
680 from the Chemical Weapon and Drug Materials Control Policy Office of the Japanese Ministry of
681 Economy, Trade and Industry, JaCVAM procured and shipped twenty coded test substances as
682 well as TEA to all three participating laboratories. Bovine fetal serum from a single lot was
683 procured from Gibco International Co. Ltd in the USA, which shipped directly to Biotoxtech Co.,

684 Ltd in Korea and to JaCVAM in Japan. JaCVAM then shipped to Bozo Research Center and
685 Nihon Kolmar in Japan. All three participating laboratories were able to perform the
686 SIRC-CVS:TEA test without departing from the six quality control criteria stipulated in chapter
687 3.2.8 of this method..
688 Phase II comprised three runs per set for each of three sets of test substances. The means of IC_{50} of
689 the relative control were between 1232 μg/mL and 1605 μg/mL, while those of the positive control
690 were between 85 μg/mL to 92 μg/mL (Table 8.2). These coefficients of variation were small.
691
692 **4.3 Phase III study**
693 Phase III was designed to validate inter-laboratory reproducibility and predictive capacity of the
694 SIRC-CVS:TEA test method using one hundred coded test substances. JaCVAM provided each
695 participating laboratory with forty coded test substances, comprising one set of ten common test
696 substances and one set of thirty unique test substances. During Phase III, JaCVAM received
697 complaints from the study directors at two of the three participating laboratories regarding eight test
698 substances, all of which were liquid and high volatile compounds. A significant quantity of these test
699 substances was lost during storage and transportation, because the bottles were not sealed properly
700 prior to distribution. Upon receipt of these complaints, JaCVAM re-distributed these substances in
701 properly sealed bottles, and testing at the two laboratories was performed with no difficulty.
702 Phase III was designed so that a third run was needed only when the results of the first two runs did
703 not match. Lab C, however, followed the procedure used in Phase IIB and conducted three runs for
704 all forty test substances. Due to this mistaken procedure, our analysis of data from Lab C ignores the
705 third run when the results of the first and second runs match.
706 All data obtained in Phase III satisfied the six quality control criteria stipulated in chapter 3.2.8 of
707 this method. Data for test substance 3,3-dithiodipropionic acid (P3-023) was excluded from analysis
708 because of precipitation occurring within 72 hours of culturing and for test substance calcium
709 thioglycolate trihydrate (P3-066), because IC_{50} could not be calculated. Additionally, data for test
710 substances hexyl cinnamic aldehyde (P3-052), citric acid (P3-067) and potassium sorbate (P3-068)

was excluded from analysis of predictive capacity due to a mismatch with GHS categories for eye irritation. Quality of the SIRC-CVS:TEA test method was confirmed per the six criteria stipulated in Section 3.2.8 *Quality Control*.

The mean IC_{50} was between 1120 μg/mL and 1359 μg/mL for the relative control and between 89 μg/mL to 123 μg/mL for the positive control at all three participating laboratories, as shown in Table 8.3. The coefficient of variation was 5.5–14.0% for the relative control and 2.3–10.0% for the positive control.

Thus, just as in Phase I and Phase II, variation in both the relative and positive controls for Phase III was found to be small.

4.4 Transferability

The data from Phase I shown in Table 9.1 and Fig. 5 indicates that variation for each test substance was small in Labs A and B but relatively large at Lab C. All three laboratories classified ethyl-2-methyl acetoacetate and Safflower oil as non-irritants and 3-chloropropionitrile as an ocular irritant, as shown in Table 9.2 and Table 9.3. The lead laboratory also obtained similar results for these substances. Sodium dehydroacetate, however, was classified as an ocular irritant by Labs A and B but as a non-irritant by Lab C. The lead laboratory also classified sodium dehydroacetate as an ocular irritant. Accordingly, sodium dehydroacetate was retested at Lab C to identify the reason for this discrepancy. As a result of retesting, all three participating laboratories classified sodium dehydroacetate as an ocular irritant. Moreover, variation of the reference controls during the retest was much smaller at Lab C than in the first test, as shown in Table 9.1. Thus, transferability of the SIRC-CVS:TEA test method was validated.

4.5 Intra- and inter-laboratory reproducibility

4.5.1 Intra-laboratory reproducibility

In Phase II, a common set of twenty coded test substances was tested by the three participating laboratories. Data from Phase II is shown in Table 10.1. Variation for these twenty test substances,

738 the relative controls, and the positive controls was small in each laboratory. Classification for eye
739 irritation potential was in complete agreement for all three sets (three runs per set) at all three
740 participating laboratories, as shown in Tables 10.2 to 10.4, and the results satisfied the acceptance
741 criteria of 80%. Thus, intra-laboratory reproducibility for Phase II was considered to be excellent.
742
743 **4.5.2 Inter-laboratory reproducibility**
744 In Phases II and III, a common set of thirty test substances were tested by all three participating
745 laboratories to validate inter-laboratory reproducibility. Variation for the thirty test substances was
746 small in each laboratory. Data from Phase II is shown in Table 10.5, and data from Phase III is
747 shown in Tables 11.1 to 11.3. Classification for eye irritation potential of the twenty test substances
748 from Phase II was in complete agreement (20/20) at all three participating laboratories, as shown
749 in Table 12, indicating excellent inter-laboratory reproducibility. Agreement on classification of
750 eye irritation potential in Phase III, however, was 7/10, as shown in Tables 13 and 14. Thus,
751 overall inter-laboratory reproducibility was 27/30 or 90%, indicating a high degree of
752 inter-laboratory reproducibility and satisfying the acceptance criteria of 80%. The solvents used in
753 this validation study were 10% FBS-medium, DMSO, and ethanol , but there were no effects on
754 the assessment of test substances for eye irritation potential that could be ascribed to the solvents.
755
756 **4.6 Predictive capacity**
757 As shown in Table 14, the results from the testing of twenty test substances in Phase II and
758 ninety-five test substances in Phase III or a total of 115 test substances was analyzed to correlate *in*
759 *vitro* and *in vivo* data from a variety of perspectives in evaluating the predictive capacity of the
760 SIRC-CVS:TEA test method Table 15.
761 The SIRC-CVS:TEA test method was developed primarily to identify non-ocular irritants in a
762 bottom-up approach. Analysis in a top-down approach for identifying GHS Category 1-2 eye
763 irritants was also performed as a part of this validation study to compared results from a bottom-up
764 approach to those from a top-down approach, as shown in Table 16. Used in a top-down approach,

765 the SIRC-CVS:TEA test method demonstrated an accuracy of 53.9% (62/115), a sensitivity of
766 71.4% (20/28), and a specificity of 48.3% (42/87), which is similar to results when used in a
767 bottom-up approach.
768 Since the SIRC-CVS:TEA test method demonstrated low predictive capacity for identifying
769 non-irritants, further analysis was performed to determine if predictive capacity could be improved
770 by defining the applicability domain.
771
772 **4.7 Applicability domain**
773 **4.7.1 Chemical class**
774 Tables 18.1- 18.6 shows these results of an analysis of chemical class. Chemical classes employed
775 as applicability domains for the analysis are shown in the following tables:
776 Table 18.1: surfactants and halogen compounds, Table 18.2: heterocyclic compounds and phenols,
777 Table 18.3: organic salts and thiol compounds, Table 18.4: esters and hydrocarbons, Table 18.5:
778 ethers and carboxylic acids, and Table18.6: alcohols and ketones.
779 Surfactants had 0% (0/5) false negatives and an accuracy of 85.7% (7/6). Similarly, halogen
780 compounds had 0% (0/5) false negatives and an accuracy of 63.6% (11/7), as shown in Table 4.7.1.
781 In contrast, ketones, alcohols, and carboxylic acids all showed a high rate of false negatives. Thus,
782 the predictive capacity for surfactants was high.
783
784 **4.7.2 Properties of interest**
785 Analysis of predictive capacity based on physicochemical properties is shown in Table 19.1 – 19.7.
786 The following properties of interest were identified: phase, molecular weight, purity, water solubility,
787 Log D, and vapor pressure. This approach, however, demonstrated only a poor rate of false
788 negatives at 40.6% (28/69) and an accuracy of only 54.8% (63/115), as shown in Table 4.6.1.
789 Further analysis, however, showed that false negatives could be reduced to a mere 4.8% (1/21) and
790 accuracy increased to 71.5% (30/42) by excluding test substances with a molecular weight of less
791 than 180, as shown in Table 19.2. A further improvement to 0% (0/16) false negatives with an

accuracy of 75.0% (24/32) was achieved by excluding test substances with a molecular weight of less than 220 (data not shown).

As can be seen from the following data, molecular weight was the only property of interested to demonstrate and improvement in false negatives and accuracy.

Table 19.1: Liquids and Solids, Table 19.3: Water solubility (1.0 g/L), Table 19.4: Water solubility (10.0 g/L), Table 19.5: Water solubility (100.0 g/L), Table 19.6: log D (2.88), Table 19.7: log D (1.70), and Table 19.8 :Vapor pressure (6.0 kPa).

5. Discussion

5.1 Validation of SIRC-CVS:TEA test method

In an earlier study* performed in Japan, reproducibility and the predictive capacity of the SIRC-CVS test method was validated on the basis of assessing eye irritation potential for solutions or suspensions at a 10% concentration. In the present study, the SIRC-CVS:TEA test method validated on the basis of assessing undiluted substances by using TEA as a relative control. TEA was selected as a suitable control substance after a reanalysis of previous studies that discriminated between GHS No Category (non-irritants) and other categories, such as Category1 and 2 (irritants). The test substances were selected from chemicals for which individual Draize scores could be confirmed, and so that chemicals from Category 1, 2, and No Category were represented appropriately. The 20 test substances selected for analysis of intra-reproducibility comprised three from GHS Category 1, seven from Category 2, and 10 from No Category. The 30 test substances selected for analysis of inter-reproducibility comprised five from Category 1, 11 from Category 2, and 14 from No Category. The 115 test substances selected for the analysis of predictive capacity comprised 28 from GHS Category 1, 41 from Category 2, and 46 from No Category. Although testing by the three participating laboratories could not be performed in in strict accordance with Good Laboratory Practice (GLP), it was performed in the spirit of GLP. The test data record sheets were all checked by the Record Management Group.

819 **5.2 Transferability**

820 Transferability was validated per testing of three runs at the three participating laboratories using
821 four non-coded test substances. The four test substances can be characterized as (1) water soluble,
822 (2) oil soluble, (3) highly volatile and cytotoxic, and (4) cytotoxic near the relative control. The three
823 participating laboratories each achieved similar results similar to that of the lead laboratory for the
824 identification of substances that were ocular non-irritants (GHS No Category), although Lab C was
825 required to retest after they acquired proficiency in the procedure. The mean IC_{50} and SD for the
826 relative control were 1382.8 ± 33.3 at Lab A, 1529.3 ± 132.7 at Lab B, and at Lab C 1280.8 ± 61.3,
827 while those of the positive control were 82.0 ± 3.6 at Lab A, 87.0 ± 1.7 at Lab B, and 84.6 ± 1.5 at
828 Lab C. This data corresponded well enough to the background data to validate transferability of
829 SIRC-CVS:TEA test method based on data obtained from the four test substances and two control
830 substances at the three participating laboratories.
831
832 **5.3 Intra- and inter-reproducibility**

833 The study of intra-reproducibility was performed by three runs of three laboratories using twenty
834 substances in the validation phase II. Three runs' results of each laboratory were the same in all the
835 substances. The index of the intra-reproducibility was 100% (20/20) and it satisfied the criteria of
836 80%. The study of intra-reproducibility was performed by the results of three laboratories using
837 twenty substances in the phase II and common ten substances in the phase III. Three of thirty
838 substances had the different predicting results in three laboratories. Those were dipropyl disulfide,
839 n,n-dimethylguanidine sulfate and polyethylene hydrogenated caster oil (40E.O.), that had IC50
840 comparatively near the IC50 of the relative control and had in vivo data of no category in the UN
841 GHS classification. The index of the inter-reproducibility was 90% (27/30) and it satisfied the
842 criteria of 80%.
843 The results indicate that SIRC-CVS:TEA test method has good intra- and inter-laboratory
844 reproducibility in the evaluation for the identification of substances which are not ocular irritants.
845

5.4 Predictive capacity /Relevance

The predictive capacity of the eye irritation by the SIRC-CVS:TEA test method was analyzed by various angles using the data of 115 substances. When the prediction of THE UN GHS classification on the basis of IC50 was performed by the comparison with that of TEA as a Bottom-up approach, the accuracy, sensitivity and specificity were 54.8%(63/115), 59.4%(41/69) and 47.8%(22/46), respectively. If the cut off value of IC50 was 1600 μg/mL, those predictive indexes was 58.3%(67/115), 69.6%(48/69) and 41.3%(19/46), respectively. Both were much the same manner. When the prediction of EPA classification on the basis of IC50 was performed by the comparison with that of TEA, the accuracy, sensitivity and specificity were 53.9%(62/115), 56.8%(50/88) and 44.4%(12/27), respectively. The predictive capacity on the basis of the UN GHS and EPA were much the same manner. The results show that the predictive capacity of the SIRC-CVS:TEA test method on the basis of bottom-up approach was not enough in any analyses using all the substances. Though the same analyses were performed on the basis of top-down approach, the predictive capacity was not also enough good. The examination of applicability domain was determined to need for the improvement to the predictive capacity of the SIRC-CVS:TEA test method.

5.5 Applicability domain

Improvement to the predictive capacity was examined on the basis of chemical class, physical state, molecular weight, purity, water solubility, distribution coefficient (log D), and vapor pressure. It was particularly worth noting that predictive capacity was best for test substances with a molecular weight of 180 or higher, which demonstrated an accuracy of 71.4% (30/42), sensitivity of 95.2% (20/21), and a specificity of 47.6% (10/21) with a false negative rate of 4.8% (1/21). Thus, limiting the applicability domain based on molecular weight virtually eliminates false negatives. Existing in vivo eye irritation data for alcohols shows there is an inverse relationship between chain length and eye irritation potential, so that the higher the molecular weight, the lower the eye irritation potential. Our findings suggest that SIRC-CVS:TEA test method is suited to distinguishing test substances

872 with a molecular weight of 180 or higher used for cosmetic ingredients that are not ocular irritants
873 from those that are irritants under GHS.
874
875 **5.6 Example of use of the test method**
876 The SIRC-CVS:TEA test is useful in distinguishing test substances GHS No Category non-irritants
877 from Category 1 and 2 irritants. The SIRC-CVS test method was previously validated for assessing
878 eye irritation at a concentration of 10%*. The Japanese draft guidance states that, if a test substance
879 is found to be a non-irritant on the basis of an alternative methods (such as the SIRC-CVS) alone
880 and will not be formulated into a product at a concentration in excess of 10%, it may be classified as
881 a non-irritant without additional animal testing*. Furthermore, polyoxyethylene sorbitan
882 monolaurate (20E.O.) was recommended as the relative control in such cases. Hagino et al. reported
883 that the SIRC-CVS test method was useful in classifying test substances as non-irritants at a
884 concentration of 10%*. In the present study, we demonstrate that a test substance judged to be a
885 non-irritant on the basis of the SIRC-CVS:TEA test method alone can be formulated into products
886 without limiting concentration. Furthermore, Nihon Kolmar Co., Ltd. has in-house in vitro data
887 evaluating the formulations of 10 marketed leave-on cosmetic products, all of which were negative
888 for eye irritation. A further 10 marketed rinse-off cosmetic products were evaluated, some of which
889 were negative and some of which were positive for eye irritation, but none of these 20 products have
890 been reported by consumers to cause irritation. Thus, the SIRC-CVS:TEA is expected to be a useful
891 means of evaluating eye irritation potential of cosmetic ingredients.
892
893 **6. Conclusion**
894 This validation study of the SIRC-CVS:TEA test method was performed using a wide variety of 120
895 test substances. It was implemented at three participating laboratories in the spirit of GLP to validate
896 intra- and inter-laboratory reproducibility as well as usefulness for distinguishing non-irritants from
897 irritants in a bottom up approach.
898 The results showed 100% (20/20) intra-laboratory reproducibility at all three laboratories and an

899 excellent 90% (27/30) inter-laboratory reproducibility. Unfortunately, predictive capacity for
900 distinguishing non-irritants from irritants per UN GHS categories in a bottom-up approach was not
901 as favorable. After considerable review of the data, however, it was determined that, for test
902 substances with a molecular weight of 180 or greater, the SIRC-CVS:TEA test method
903 demonstrated an accuracy of 71.4% (30/42), sensitivity of 95.2% (20/21), and specificity of 47.6%
904 (10/21) with a low false-negative rate of 4.8% (1/21).
905 From the above described results, we concluded that the SIRC-CVS:TEA test method demonstrated
906 excellent intra- and inter-laboratory replicability and that, with a carefully defined applicability
907 domain, it is a useful alternative to the Draize test for distinguishing cosmetic ingredients that are
908 ocular non-irritants from those that are irritants.
909
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951

Figures for SIRC-CVS:TEA validation version 7.7

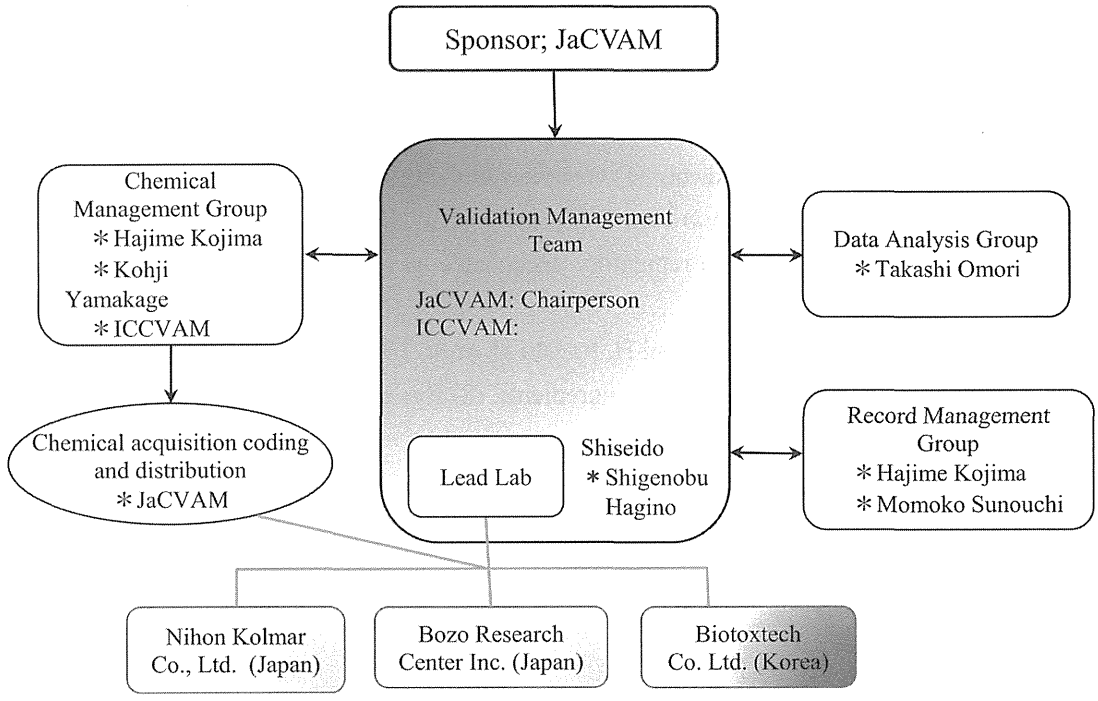


Fig. 1. Study organization for SIRC-CVS:TEA validation study

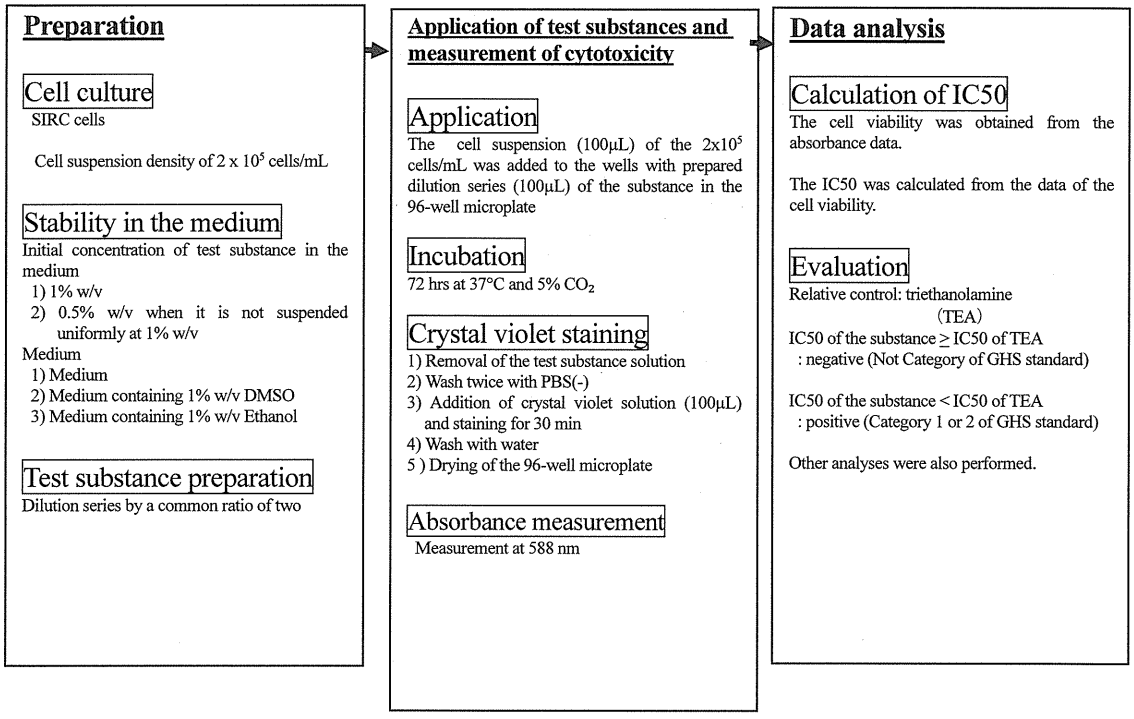


Fig. 2. SIRC-CVS:TEA test procedures

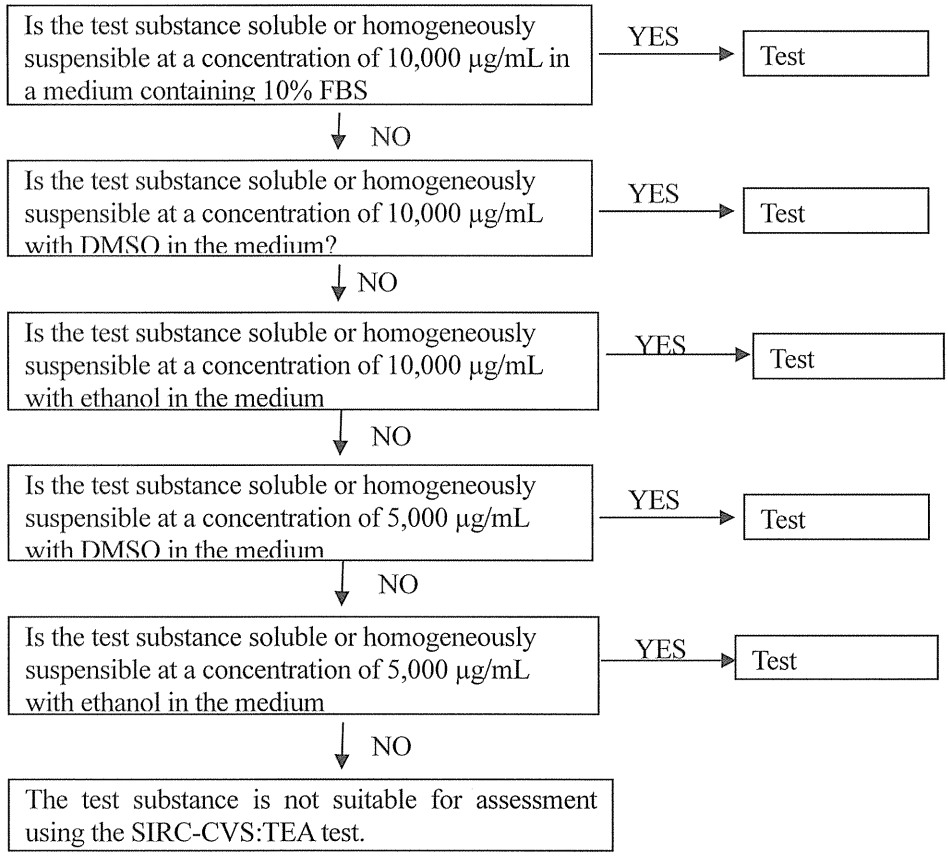
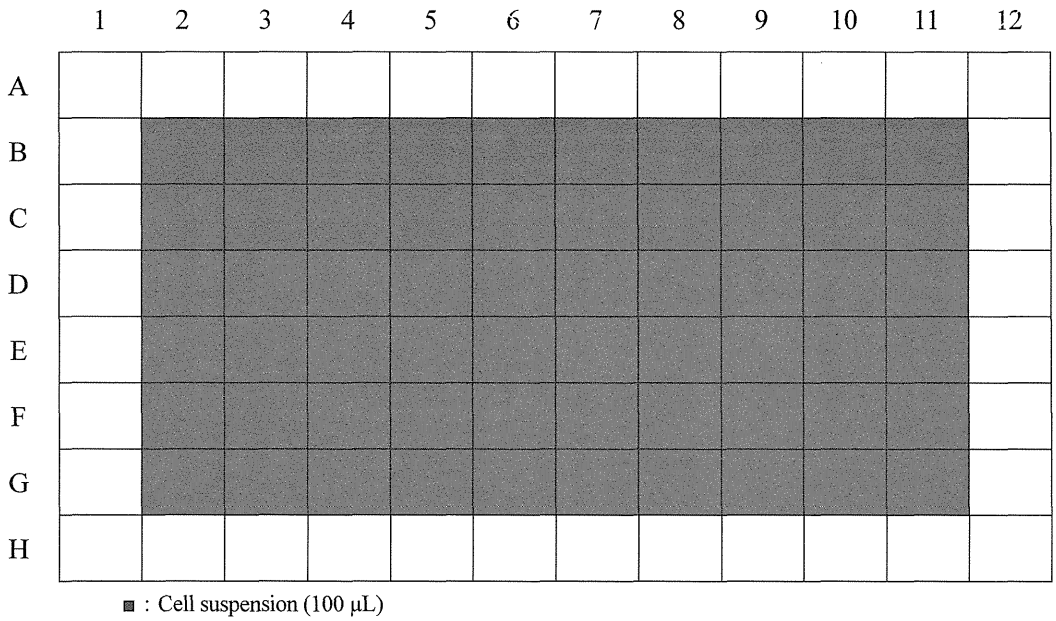


Fig. 3. Flow chart of examination of stability for the substance in the medium

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
B	PBS	NC	S1	S2	S3	S4	S5	S6	S7	S8	NC	PBS
C	PBS	NC	S1	S2	S3	S4	S5	S6	S7	S8	NC	PBS
D	PBS	NC	R1	R2	R3	R4	R5	R6	R7	R8	NC	PBS
E	PBS	NC	R1	R2	R3	R4	R5	R6	R7	R8	NC	PBS
F	PBS	NC	P1	P2	P3	P4	P5	P6	P7	P8	NC	PBS
G	PBS	NC	P1	P2	P3	P4	P5	P6	P7	P8	NC	PBS
H	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS

Fig. 4.1. Layout of 96-well microplates

PBS: 200 μL of PBS(-)
NC: Medium, 10,000 μg/mL DMSO-medium solution or 10,000 μg/mL ethanol-medium solution of 100 μL
S: A 1:1 serial dilution (by adding 100 μL)
R: A 1:1 serial dilution of the relative control (by adding 100 μL)
P: A 1:1 serial dilution of the positive control (by adding 100 μL).
The dilution series of the test substance was made using medium, 10,000 μg/mL DMSO-medium solution or 10,000 μg/mL ethanol-medium solution. The dilution series of positive control and relative control was made using medium.



■ : Cell suspension (100 μL)

Fig. 4.2. Addition of cell suspension

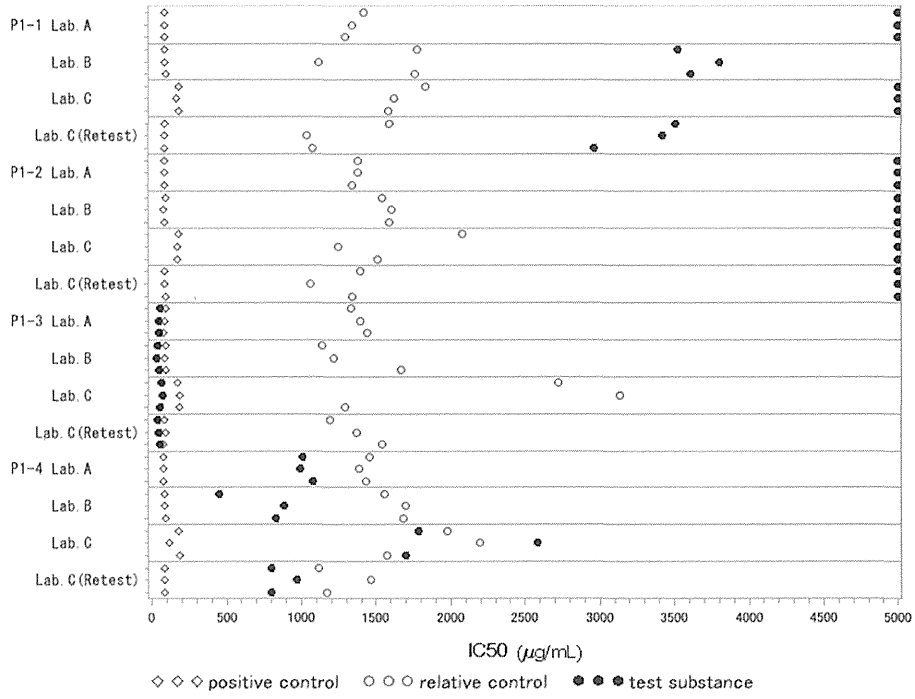


Fig. 5. A comparison of test substances, reference control, and positive control at the three participating laboratories.

P1-1: ethyl-2-methyl acetoacetate, P1-2: safflower oil,
P1-3: 3-chloropropionitrile, P1-4: sodium dehydroacetate

Tables for SIRC-CVS:TEA validation version 7.7

Table 1. Members of SIRC-CVS:TEA Validation Management Team (VMT)

Name	Organization	Duties
Momoko Sunouchi	JaCVAM, NIHS Japan	Chairperson Quality Assurance Record management
Hajime Kojima	JaCVAM, NIHS Japan	JaCVAM Chemical Management Record management
Warren Casey	ICCVAM, NIH USA	NICEATM Chemical Management
Takashi Omori	Doshisha University, Japan	Data Analysis
Kohji Yamakage	Food and Drug Safety Center, Hatano Research Institute, Japan	Chemical Management
Shigenobu Hagino	Shiseido Research Center, Japan	Lead laboratory

Table 2. Distribution of 100 test substances used in phase III study

Test substances	Lab A	Lab B	Lab C
10 common test substances	☑	☑	☑
30 unique test substances	☑		
30 unique test substances		☑	
30 unique test substances			☑

Table 3. Breakdown of substances used in the SIRC-CVS:TEA validation study

Phase	No. of test substances	No. of sets	No. of runs per set	Area of Validation	
I	4 non-coded	1	3	Transferability	
IIA	5 coded	3	3	Intra- and inter-laboratory reproducibility	Predictive capacity
IIB	15 coded	3	3		
III	A total of 100 coded test substances: 40 at each laboratory, including 10 common and 30 unique substances.	1	2 or 3	Inter-laboratory reproducibility	

Table 4. Substances used to confirm transferability

Substance	IC_{50} (μg/mL)	Result	n
sodium dodecyl sulfate	102 ± 26[1]	Positive	144
TEA	1614 ± 356[1]	-	152
ethyl-2-methyl acetoacetate	3194.7[2]	Negative	2
safflower oil	2215.5[2]	Negative	2
3-chloropropionitrile	48.7[2]	Positive	2
sodium dehydroacetate	919.9[2]	Positive	2

1: The data are the means ± standard deviations.

2: The data are the averages of two IC_{50} results.

Table 5. Twenty substances for phase II study

NO.	Chemical Name	CAS	Physical state	Supplier	GHS	Source
001	2,5-dimethylhexaediol	110-03-2	Solid	Sigma-Aldrich	1	STE review
002	1-naphthaleneacetic acid	86-87-3	Solid	Wako Pure	1	ECETOC 12
003	sodium oxalate	62-76-0	Solid	Sigma-Aldrich	1	ECETOC 14
004	ammonium nitrate	6484-52-2	Solid	Sigma Aldrich	2A	NICEATM 6
005	cyclopentanol	96-41-3	Liquid	Sigma Aldrich	2A	ECETOC 57
006	propylene glycol propyl ether	1569-01-3	Liquid	Sigma Aldrich	2A	NICEATM 1
007	camphene	79-92-5	Solid	Sigma-Aldrich	2B	STE review
008	ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropionate	96568-04-6	Solid	Sigma-Aldrich	2B	NICEATM 10
009	isobutyraldehyde	78-84-2	Liquid	Sigma-Aldrich	2B	STE review
010	1-(2-propoxy-1-methylethoxy)-2-propanol	29911-27-1	Liquid	Sigma-Aldrich	2B	STE review
011	1-bromo-4-chlorobutane	6940-78-9	Liquid	Sigma-Aldrich	No	STE review
012	1-bromohexane	111-25-1	Liquid	Sigma-Aldrich	No	STE review
013	ethyl thioglycolate	623-51-8	Liquid	Sigma-Aldrich	No	NICEATM 13
014	4,4'-methylenebis(2,6-di-tert-butylphenol)	118-82-1	Solid	Sigma-Aldrich	No	ECETOC 26
015	2-phospho-L-ascorbic acid trisodium salt	66170-10-3	Solid	Sigma	No	BASF 6
016	piperonylbutoxide	51- 03- 6	Liquid	Sigma Aldrich	No	US-EPA 8
017	potassium tetrafluoroborate	14075-53-7	Solid	Sigma-Aldrich	No	ECETOC 71
018	propyl 4-hydroxybenzoate	94-13-3	Solid	Sigma-Aldrich	No	LNS 1
019	sodium hydrogensulfite	7631-90-5	Solid	Sigma-Aldrich	No	NICEATM 17
020	3,4,4'-trichlorocarbanilide	101-20-2	Solid	Sigma-Aldrich	No	Cosing 36

Table 6. The 100 substances for the phase III study

No.	Chemical Name	CAS	Physical state	Supplier	GHS	Source
001	dodecanoic acid	143-07-7	Solid	Sigma-Aldrich	1	ECETOC 8
002	tetraethylene glycol	17831-71-9	Liquid	Sigma-Aldrich	1	TSCA
003	2-amino-3-hydroxy pyridine	16867-03-1	Solid	Sigma-Aldrich	2A	Cosing 14
004	gamma-butyrolactone	96-48-0	Liquid	Sigma-Aldrich	2A	STE review
005	diethyl toluamide	134-62-3	Liquid	Sigma-Aldrich	2B	US-EPA 2
006	4-nitrobenzoic acid	62-23-7	Solid	Sigma-Aldrich	2B	NICEATM 9
007	n,n-dimethylguanidine sulfate	598-65-2	Solid	Sigma-Aldrich	No	STE review
008	dipropyl disulfide	629-19-6	Liquid	Sigma-Aldrich	No	STE review
009	2-(2-ethoxyethoxy)ethan e	111-90-0	Liquid	Sigma-Aldrich	No	Cosing 25
010	polyethylene hydrogenated caster oil (60E.O.)	61788-85-0	Solid	Sigma-Aldrich	No	STE review
011	3-(2-aminoethylamino)pr opel]trimethoxysilane	1760-24-3	Liquid	Chemo's	1	Envoi 1
012	benzenamine,4,4'-(4-aimi no-3-methylphenyl)(4-im ino-3-methyl-2,5-cyclohe xadien-1-ylidene)methyl-2-methy HCL	3248-91-7	Solid	Sigma-Aldrich	1	Cosing 3
013	1,2-benzisothiazol-3(2H) -one	2634-33-5	Solid	Wako Pure	1	Cosing 5
014	benzyl alcohol	100-51-6	Liquid	Sigma-Aldrich	1	STE review
015	butanol	71-36-3	Liquid	Wako Pure	1	STE review
016	calcium thioglycollate	5793-98-6	Solid	TCI	1	STE review
017	cetylpyridinium bromide	140-72-7	Solid	Sigma-Aldrich	1	STE review
018	cyclohexanol	108-93-0	Liquid	Sigma-Aldrich	1	STE review
019	disodium 4,4'-Bis(2-sulfonatostyry l)biphenyl	27344-41-8	Solid	Wako Pure	1	Ciba 5
020	distearyldimethylammon ium chloride	107-64-2	Solid	TCI	1	STE review
021	imidazole	288-32-4	Solid	Sigma-Aldrich	1	STE review
022	isobutyl alcohol	78-83-1	Liquid	Sigma-Aldrich	1	STE review
023	lactic acid	50-21-5	Liquid	Wako Pure	1	STE review
024	methoxyethyl acrylate	3121-61-7	Liquid	Wako Pure	1	STE review
025	2-methylbuthyric acid	116-53-0	Liquid	Sigma-Aldrich	1	STE review
026	methylthioglycolate	2365-48-2	Liquid	Sigma-Aldrich	1	ECETOC 1
027	monoethanolamine	141-43-5	Liquid	Sigma-Aldrich	2 B	NICEATM
028	4,4'-(4,5,6,7-tetrabromo-1,1-dioxido-3H-2,1-benz oxathiole-3,3-diyl)bis[2, 6-dibromophenol]	4430-25-5	Solid	Sigma-Aldrich	1	Coiling 2
029	1,2,4-triazole,sodium salt	41253-21-8	Solid	Sigma-Aldrich	1	ECETOC 9
030	m-phenylenediamine	108-45-2	Solid	TCI	1	STE review
031	promethazine hydrochloride	58-33-3	Solid	Sigma-Aldrich	1	STE review
032	pyridine	110-86-1	Liquid	Sigma-Aldrich	1	STE review
033	sodium hydroxide	1310-73-2	Solid	Wako Pure	1	STE review