SIRC-CVS Test Method

Report of the Peer Review Panel

on

a JaCVAM coordinated study programme addressing the validation status of the SIRC-CVS test method for the prospective identification of eye irritating substances

Report completed by the Peer Review Panel on August 19, 2019.

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Executive Summary

The Statens Seruminstitut Rabbit Cornea–Crystal Violet Staining Cytotoxicity Test Method with Triethanolamine (SIRC-CVS Test Method) has been proposed as an *in vitro* test method to discriminate eye irritating chemicals from non-eye irritating chemicals. It could contribute to replacing the Draize eye irritation test as being used as the first test method in a bottom-up approach. The test method main advantages are the use of a rabbit cornea cell line and that it is relatively simple to perform. Coordinated and sponsored by the Japanese Center for the Validation of Alternative Methods (JaCVAM), the SIRC-CVS Test Method was prospectively validated by a Validation Management Team (VMT).

The peer review panel (PRP) found the Validation Management Team's report presented the necessary information for an independent review. The PRP concluded that the SIRC-CVS Test Method was, with the exception of the applicability domain, sufficiently well defined, with a clear protocol and criteria for data interpretation. Both within and between laboratory reproducibility information were considered to be satisfactory. However, the predictive capacity could not be assessed, as the applicability domain of the test method was adjusted several times without providing clear mechanistic insights and justification of the proposed domain restrictions. Accordingly, the PRP concluded that, based on the provided information, the scientific validity of the SIRC-CVS Test Method could not be demonstrated for its use as a part of an integrated testing strategy for distinguishing chemicals classified from chemicals not classified according to the UN GHS classification system.

Peer Review Panel Composition

Sebastian Hoffmann (chair)	seh consulting + services, Paderborn, Germany
Chantra Eskes	Swiss 3R Competence Centre, Bern, Switzerland (until August 2018 SeCAM, Agno, Switzerland)
Pertti Hakkinen	National Institutes of Health, Bethesda, USA
Tae Cheon Jeong	Yeungnam University, Gyeongsan, South Korea
Tadashi Kosaka	Institute of Environmental Toxicology, Ibaraki, Japan
Jill Merrill	U.S. Food and Drug Administration, Silver Spring, USA
Sanae Takeuchi	P&G Innovation Godo Kaisha, Kobe, Japan

Background

The SIRC-CVS Test Method was evaluated in a prospective validation study by a Validation Management Team (VMT), which was chaired, coordinated and sponsored by the Japanese Center for the Validation of Alternative Methods (JaCVAM). The study was designed to assess the usefulness of the SIRC-CVS Test Method as an alternative to the *in vivo* Draize test method to identify ocular non-irritants in a bottom-up testing strategy approach according to the UN GHS classification scheme (Scott et al., 2010).

The PRP was assembled at the end of 2014 and met in March 2015 to discuss the outcome of the validation study with the VMT, and to review, in the absence of the VMT, the validation study of the SIRC-CVS Test Method. The peer review was conducted based on 14 evaluation criteria. As these criteria were derived from the modular approach (Hartung et al., 2014), the PRP review is structured by the seven validation modules. Upon repeated requests for clarifications by the PRP on a number of elements, but most importantly on the applicability domain, the VMT provided replies that were discussed via several teleconferences and meetings from 2015 to 2018. In March 2019, the PRP held a final telephone conference. With the provision of all of the amended, updated and additional material, including the final VMT report, this PRP report was prepared.

SIRC-CVS Test Method Definition

The PRP concluded that the SIRC-CVS Test Method has been fully described in the report of the Validation Management Team (VMT) and in the associated detailed test protocol. Protocol amendments made during and after the validation study were traceable over the various versions. The validation report adequately stated the need for the assay in the current regulatory context. Furthermore, a rationale for the test method has been given and helpfully included references to existing *in vitro* methods for eye irritation that have been validated and adopted into OECD guidelines. The PRP agreed that the mechanistic basis of the method and how it related to eye irritation was sufficiently described in the report although further details would have been appreciated.

The PRP agreed that this method is intended to contribute to the replacement of animal usage for eye irritation assessment and that, when compared to other *in vitro* cell-based methods, which aim at discriminating irritating from non-irritating chemicals, it is likely to offer time, throughput, and cost benefits. Furthermore, the PRP was of the opinion that the use of corneal cells in the SIRC-CVS Test Method can present mechanistic advantages as compared to other cell types for predicting ocular irritation.

Within Laboratory Reproducibility

Based on information provided in the VMT report, the PRP agreed that the results demonstrated a good within-laboratory reproducibility. The PRP achieved this conclusion on the basis of the data of Phase II of the validation study, in which 20 substances were tested three times in three laboratories, regardless of any applicability domain considerations. As all substances were consistently predicted in the laboratories, resulting in a within-laboratory reproducibility of 100%, the success criterion of a within-laboratory reproducibility of at least 80% set by the VMT was met.

In addition, the results provided in the validation report indicate that bovine serum and TEA from different manufacturing lots have no effect on the reproducibility.

Transferability

The PRP noted that the fact that the three participating laboratories were all naïve and that no practical training was provided is a good indication of the robustness of the test method.

Between Laboratory Reproducibility

As with within laboratory reproducibility, the PRP based its evaluation of the between-laboratory reproducibility on the information provided in the VMT report. The PRP agreed that the results demonstrated a good between-laboratory reproducibility. The PRP achieved this conclusion on the basis of the data of Phase II and of 10 substances of Phase III of the validation study, in which 30 substances were tested in three laboratories, regardless of any applicability domain considerations. As 27 substances were consistently predicted across the three laboratories, resulting in a between-laboratory reproducibility of 90%, the success criterion of a between-laboratory reproducibility of at least 80% set by the VMT was met.

Predictive Capacity

Demonstration of a test method's performance should be based on the testing of representative, preferably coded, reference chemicals. The PRP concluded that the validation study used an appropriate level of test chemical coding to ensure fully blinded evaluation. With respect to predictive capacity, the PRP confirms that a suitable balance of stronger, weaker, and non-classified (according to the GHS classes of 1, 2A, 2B and no class) test chemicals was selected. The PRP agreed that the rabbit was considered as the target species, noting that prediction of

human eye irritation is the ultimate goal and acknowledging that high quality human data are in general not available. The PRP notes that *in vivo* data have been reviewed by ICCVAM. Additionally, the majority of test chemicals were also used in other validation studies (e.g. of the RhCE and STE). Finally, the majority of tested chemicals differed from those used in initial method development.

The PRP agreed that the predictive capacity for an unrestricted applicability domain was based on a sufficiently large and representative set of test chemicals. However, the accuracy of 55% (64/116) did not meet the VMT success criteria of 80%, and the false negative rate of 40% (28/70) did not meet the success criterion of <5%.

The PRP understood that this poor predictive performance triggered the test developer to explore various physical and chemical properties to identify possible reasons for misclassification. Various restricted applicability domains, all of which resulted in the exclusion of a substantial amount of substances, but also in improved predictive performance, were presented to the PRP. However, the VMT failed to provide a clear mechanistic justification for the restrictions. Consequently, the PRP could not conclusively assess the predictive capacity of the SIRC-CVS Test Method.

Applicability Domain

Due to unsatisfactory predictive capacity with an unrestricted applicability domain, the VMT explored different potential reasons for misclassification, including chemical classes and physicochemical properties, but not *in vivo* drivers of classification as suggested by the PRP (Adriaens et al., 2014). The proposals presented to the PRP restricted the applicability, for example using a combination of molecular weight and chemical classes, the dissociation constant (pKa) and the distribution coefficient (log D). However, the VMT failed to provide a clear mechanistic justification for any of these. Consequently, the PRP concluded that the applicability domain of the SIRC-CVS Test Method was not sufficiently defined.

Performance Standards

Because the assay does not include components, equipment, or other scientific procedures that are covered by (or pending) intellectual property rights, the PRP initially agreed that performance standards are not mandatory at this stage, but could be useful if similar or modified test methods become available. However, due to the lack of a clearly justified applicability domain, performance standards are not required.

Additional Comments

The PRP concluded that the validation study management and conduct met the criteria set out in OECD GD 34 (2005). However, based on the information provided to the PRP, including a dedicated discussion at a PRP meeting, the PRP concluded also that it is unclear whether the study was conducted in accordance with the principles of GLP.

The PRP notes that during the conduct of the review, access to the full raw data files associated with SIRC-CVS Test Method validation work was provided.

Conclusions and Recommendations

The PRP concluded that, based on the provided information, the scientific validity of the SIRC-CVS Test Method could not be demonstrated for its use as a part of an integrated testing strategy for distinguishing chemicals classified from chemicals not classified according to the UN GHS classification system.

Acknowledgements

The PRP is grateful to the members of the VMT for their hard work and patience and to JaCVAM for their support in setting up and hosting meetings in Japan, as well as for the setting up of several telephone conferences.

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Annex: Short professional bios of the member of the peer review panel

Dr. Sebastian Hoffmann (PhD, ERT)
is running the independent consultancy 'seh consulting + services' in Paderborn, Germany, since 2009, specialised in validation and assessment of new approach methods (NAM) and test strategies as well as in the regulation of chemicals (REACH) and cosmetic ingredients. Being statistician by training, he received a Ph.D. from the University of Konstanz (Germany) in 2005 for his thesis 'Evidence-based *in vitro* toxicology'. He has being working for the European Centre for the Validation of Alternative Methods of the European Commission for five years, contributing inter alia to the management of various validation studies of NAM. His research activities focus on methodological challenges in the assessment of *in vitro* tests, the construction and evaluation of integrated testing strategies and in exploring the application of evidence-based approaches to toxicology. He is an appointed member of the European Commision's Scientific Committee on Occupational Exposure Limits and member of the Board of Trustees of the Evidence-based Toxicology Collaboration.

Dr. Chantra Eskes (PhD)
is director of the Swiss 3R Competence Centre (www.swiss3rcc.org). With a background in food sciences, *in vitro* neurotoxicity and topical toxicity, Chantra has over 20 years of experience in the development, optimization, validation, peer-review and regulatory acceptance of alternative methods to animal toxicity testing conducted in Europe and abroad. Her activities contributed to the formal validation and international acceptance of a number of test methods, and to the development of a number of international test guidelines and guidance documents. Chantra currently acts as the chairwoman of the EURL-ECVAM Scientific Advisory Committee (ESAC), and as a Swiss Nominated Expert for the Organisation for Economic Co-operation and Development (OECD). In the past she has been the president of the European Society of Toxicology In Vitro and Executive Secretary of the Animal Cell Technology Industrial Platform on the production of biopharmaceuticals (ACTIP).

Dr. Pertti Hakkinen (PhD, S-FRA)
is the Senior Toxicologist and the Toxicology and Environmental Health Science Advisor at the (U.S.) National Institutes of Health's National Library of Medicine. His job includes providing leadership in the development of new resources in exposure science, toxicology, risk assessment, and risk communication, and enhancements to existing resources in these fields. In addition, he is the project leader for the Chemical Hazards Emergency Medical Management (CHEMM) tool and the recently updated and enhanced ToxTutor educational resource. Further, he is an Adjunct Associate Professor in Preventive Medicine & Biostatistics in the F. Edward Hébert School of Medicine at the Uniformed Services University of the Health Sciences (USU) in Bethesda, and a co-leader of the Environmental Health Sciences graduate level course offered by the Foundation for Advanced Education in the Sciences at the NIH (FAES@NIH). Dr. Hakkinen earned a B.A. in Biochemistry and Molecular Biology from the University of California, Santa Barbara, and received a Ph.D. in Comparative Pharmacology and Toxicology from the University of California,

San Francisco. He is a member of the Society of Toxicology, a Councilor of the International Society of Exposure Science, and a Fellow of the Society for Risk Analysis. Recent publications include a) Exploring Global Exposure Factors: Resources for Use in Consumer Exposure Assessments and b) New Studies About Everyday Types of Chemical Exposures: What Readers Should Consider. He is a co-editor of an upcoming (mid-2019) book on the Practice of Consumer Exposure Assessment.

Prof. Tae Cheon Jeong (PhD)
is a professor of toxicology in College of Pharmacy at Yeungnam University, Gyeongsan, Republic of Korea, since 2000. He majored in pharmacy at Sungkyunkwan University in 1986 and received his Master and Ph.D. degree (1992) at the Korea Advanced Institute of Science and Technology (KAIST), majoring toxicology. As a post-doctoral fellow, he worked with Dr. Michael P. Holsapple in the Department of Pharmacology and Toxicology at the Medical College of Virginia, located in Richmond, Virginia, USA. Thereafter, he worked for the Korea Institute of Toxicology (KIT) as a senior scientist for 6 years. His research area has been the toxicology in liver and immune systems and the development of alternative testing methods. He has published 227 research papers since 1992. He is the president of the Korean Society for Alternatives to Animal Experiments (KSAAE) at present.

Dr. Jill C. Merrill (PhD, DABT)
is a senior reviewer for the Division of Dermatology and Dental Products, Center for Drug Evaluation and Research (CDER), USFDA. She received her doctorate in toxicology from Texas A&M University and was a postdoctoral fellow at the University of Texas Southwestern Medical Center and at Monsanto Co. In 1990 she joined the Gillette Medical Evaluation Laboratories as a product safety toxicologist. Under Gillette's no animal testing policy she coordinated the company's first use of the BCOP assay to further the development of innovative products otherwise sidelined by ocular irritation concerns. Subsequently she served as Study Director (2000-2003) at the Institute for In Vitro Sciences, Inc., specializing in alternative ocular and dermal irritation assays conducted under contract for pharmaceutical and personal care companies. Since 2003 she has evaluated the significance of results from both nonclinical and alternative toxicology studies conducted in support of dermal drug products prior to first-in-human studies. Dr. Merrill proactively supports CDER's use of alternative test methods.

Sanae Takeuchi (MSc)
joined Procter & Gamble Far East Inc. (current: P&G Innovation Godo Kaisha) in 1992 after getting her master's degree of science in biology from Ochanomizu University in Tokyo. After working in Clinical Development department for years, she has been working for Central Product Safety function in Global Product Stewardship, since 2001, supporting Beauty Care sector.
From 2008, she has been working with JaCVAM (Japanese Center for the Validation of Alternative Methods) as a member of Editorial Committee of alternatives for ocular irritation testing. She is a member of International Committee and Council for The Japanese Society for Alternatives to Animal Experiments, and also a member of the Japan SOT.