EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

ECVAM
SCIENTIFIC
ADVISORY
COMMITTEE
(ESAC)

ESAC OPINION

based on the ESAC Scientific Peer Review of the ECVAM Eye Irritation Validation Study (EIVS) and a related Cosmetics Europe study on HPLC/UPLC-photometry as an alternative endpoint detection system for formazan reaction product

ESAC Opinion Nr.	2014-03
Relevant ESAC request Nr.	2013-03
Date of opinion	17.11.2014

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17 November 2014

Summary of the ESAC opinion

The ESAC was requested (see Annex 2) to conduct a scientific peer review of and provide an ESAC opinion on

(1) the EURL ECVAM Eye Irritation Study (**EIVS**); co-sponsored by EURL ECVAM and Cosmetics Europe) evaluating the EpiOcular Eye Irritation Test (EIT) and SkinEthic Human Corneal Epithelium (HCE), both based on Reconstructed human Tissue (RhT); with the ESAC review addressing the study design, results and conclusions. The review examined the scientific basis of the assays, their reproducibility and transferability as assessed during the study, as well as their predictive capacity. The latter was examined in the context of the test methods' envisaged use within an *in vitro* testing strategy (Scott et al., 2010) based on strategic combinations of alternative test methods to replace the Draize eye test, either as the initial step in a Bottom-Up Approach or a second or subsequent step in a Top-Down Approach to discriminate chemicals (substances and chemical mixtures) not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling as Category 1 and Category 2 (UN 2011; EU 2008). The term "strategic combinations" here refers to the placement of methods in a sequential manner in view of either identifying first negatives (high sensitivity: bottom-up approach) or first positives (high specificity: top-down approach).

(2) The usefulness, based on a study conducted by Cosmetics Europe and submitted to EURL ECVAM, of HPLC/UPLC-photometry as an alternative endpoint detection system in particular for highly coloured chemicals interfering with the conventional formazan endpoint measurement by OD-photometry.

EIVS

The ESAC concludes that the study was generally well planned and executed. Briefly, reproducibility, transferability and predictive capacity of both test methods were assessed in a three laboratory ring trial testing 104 chemicals plus an additional 8 solids for assessing the optimised EpiOcular™ EIT protocol. The chemicals represented a wide range of irritancy scores and chemical and use classes. The aims of the study were well described and clear target criteria for test method performance had been predefined. Using appropriate provisions for retesting, the study generated almost complete data sets as a solid basis for evaluating test method performance. However, the ESAC does not agree with the approach chosen by the Validation Management Team (VMT) to calculate the predictive capacity based on the individual test runs: the ESAC believes that confidence limits based on the number of test chemicals are more appropriate. The ESAC believes that the approach chosen by the Validation Management Team (VMT), over-estimated the sample size, produced overly narrow confidence limits, and resulted in a seemingly high degree of certainty with respect to the point estimates of sensitivity and specificity. In addition rather than relying only on single test runs to calculate accuracy, the ESAC believes that, in case single runs are used for analysing predictive

capacity, reliance is better placed on bootstrapping or worst-case/best-case scenarios of predictive capacity.

EpiOcular™ EIT

The EpiOcular™ EIT test method was found to be highly reproducible within laboratories, irrespective of the protocol used. The ESAC considers that the VMG predictive capacity criteria are deemed to have been met with the 60% cut-off value using protocol V8.0. This version was the result of optimisation phase during the validation study aiming to improve results for solids while maintaining the accuracy obtained for liquid test materials.

The ESAC concludes that the EIVS study findings with respect to transferability, reproducibility and predictive capacity, taking account of the supplementary analysis undertaken by the ESAC WG, justify the EpiOcular™ EIT test method (SOP V 8.0) being considered for use within a test strategy to determine the eye irritation potential of chemicals, specifically to detect non-irritants as part of a Top-Down or Bottom-Up approach (Scott et al, 2010).

However, the ESAC believes that confidence in the test method would be further increased if supplementary studies confirm that the test method correctly classifies a small but representative sample of labelled products or mixtures as defined by REACH and from various sectorial use classes: for products containing mixtures of substances, such as plant protection products or cosmetic products, the testing of the mixture is considered more representative of the expected exposure situation in actual practice than testing its constituent substances separately.

SkinEthic™ HCE

The ESAC agrees with the VMG's view that this test method does not meet the performance criteria that would be required to consider it as part of a test strategy. ESAC also agrees that with the VMG that there is potential for improvement but that the necessary work is beyond the scope of the validation study.

The Cosmetics Europe Report

The Cosmetics Europe Report is not a Validation Study Report (VSR) relating to a predictive alternative test method, but is a report of a study which evaluated the performance of a bio-analytical endpoint measurement system that overcomes a limitation of the conventional OD-photometry measurements.

The ESAC believes the evidence presented tends to confirm that for chemicals compatible with these RhT methods, the measurements obtained of formazan values by OD-photometry and the suitably qualified HPLC/UPLC endpoint detection systems are comparable. ESAC appreciates that an advantage of the HPLC/UPLC endpoint detection measurement is that it can also be used in the case of strongly coloured chemicals incompatible with formazan measurement by OD-photometry (e.g. colorants used in cosmetics and other consumer products). The report also provides plausible arguments that this endpoint measurement system is suitable for use with other RhT test methods relying on measurement of the same endpoint.

The ESAC concludes that the use of HPLC/UPLC would overcome the current limitation of RhT test methods with respect to problems encountered with strongly-coloured chemicals which interfere with the measurement of formazan levels by OD-photometry.

1. Mandate of the ESAC

The 38th ESAC plenary (19 June 2013) established an ESAC Working Group (WG)[1] to undertake a detailed scientific review of an EURL ECVAM/Cosmetics Europe Eye Irritation Validation Study (EIVS) evaluating two *in vitro* test methods based on Reconstructed human Tissue (RhT) models for assessing acute eye irritation caused by chemicals: the EpiOcular™ EIT and the SkinEthic™ HCE.

A revised EURL ECVAM Request for ESAC advice (Annex 2) was adopted at the 39th ESAC plenary (11/12 March 2014) extending the ESAC mandate to include a scientific review of a Cosmetics Europe study of HPLC/UPLC-photometry as an alternative formazan endpoint measurement system.

2. Detailed opinion of the ESAC

Taking into account (a) the report of the detailed review undertaken by the ESAC WG; (b) the information made available to ESAC by EURL ECVAM (Including the EIVS VSR, the Cosmetics Europe report, and other supporting documentation), and; (c) the ECVAM request for ESAC advice (Annex 2) - the ESAC has adopted the following opinion.

2.1 Background, regulatory and scientific rationale

Both the EIVS and the Cosmetics Europe reports describe the potential role of the RhT-based test methods, and the endpoint measurement system, in the context of the regulatory testing.

EIVS

The ESAC considers that the VSR provides plausible arguments supporting the use of these RhT test methods, when combined with other *in vitro* methods, within a tiered approach using strategic combinations of alternative test methods. This tiered approach (Scott et al, 2010) is supposed to be integrated into future more complete "Integrated Testing Strategies" (ITS) or "Integrated Approaches to Testing and Assessement" (IATA) to replace the Draize eye test. The tiered approach is argued to have the potential to be at least as relevant and reliable as the current *in vivo* test system for the identification of non-irritants, either as the initial step in a Bottom-Up Approach or a second or subsequent step in a Top-Down Approach test to discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling (UN GHS Category 1 and Category 2).

Taking account of the probably rather small prevalence of chemicals inducing serious eye damage (Adriaens et al, 2014) RhT test methods validated for this purpose would significantly reduce animal testing by identifying the large number of chemicals not requiring classification.

[1] The WG was established according to the ESAC Rules of Procedure and included experts nominated by international partner organisations of EURL ECVAM collaborating under the ICATM (International Collaboration on Alternative Test Methods) framework.

SkinEthic™ HCE

The tissue component of the test kit is manufactured by controlled culture of immortalised human corneal epithelial cells to produce a multi-layered epithelium resembling *in vivo* corneal epithelium. Although derived from cultured human corneal cells the cells used have been transformed and immortalised. The ESAC offers no opinion on whether these cells are inherently more biologically relevant in the context of eye irritation testing than other epithelial cells (e.g. RhT models derived from normal untransformed human keratinocytes).

EpiOcular™ EIT

The EpiOcular™ EIT tissue construct is prepared from normal human-derived epidermal keratinocytes grown under defined conditions. The resulting 3D tissue is a non-keratinized multi-layered and stratified (but non-cornified) epithelium intended to model properties of human corneal epithelium.

The Cosmetics Europe Report

The Cosmetics Europe Report is not a Validation Study Report (VSR): the study evaluated the performance of a bio-analytical endpoint measurement system (which is not itself a test method).

The Scientific Committee on Consumer Safety (SCCS) of the European Commission Directorate General for Health and Consumers previously advised that *"...for coloured substances, a different endpoint, not involving Optical Density (OD) quantification, should be envisaged. Analytical methods such as HPLC/UPLC might be more appropriate to detect formazan in the in vitro assay..."* (McNamee *et al*. 2009). An alternative endpoint detection measurement for RhT/MTT reduction product test methods that overcomes this current limitation would extend the applicability domain of these RhT tests.

The Cosmetics Europe study proposes the use of suitably qualified HPLC/UPLC endpoint measurement in this context. The ESAC believes The Cosmetics Europe Report describes a bio-analytical system that has the potential to overcome a current limitation on the use of these and other RhT test methods.

2.2 Design and conduct of the study

2.2.1 Definition of the study objectives

EIVS

The ESAC believes these are clearly defined.

The Cosmetics Europe Report

The ESAC considers the study objectives to have been sufficiently clearly defined.

2.2.2 Study design for the prospective part

EIVS

The ESAC concludes that, subject to specific qualifications set out elsewhere in this Opinion, the study design was generally appropriate and sufficiently robust with respect to determining the relevance and reliability of these test methods for the purposes defined in the VSR.

SkinEthic™ HCE

The protocol for the SkinEthic™ HCE test method described the following testing strategy**:**

(1) The first step was an "Eye irritation Peptide Reactivity Assay" (EPRA).
(2) For EPRA reactive chemicals the SkinEthic™ HCE Short-time Exposure protocol (HCE SE).
(3) For EPRA non-reactive chemicals the SkinEthic™ HCE Long-time Exposure protocol (HCE LE).

However, for the purposes of the EIVS study all chemicals were tested using both the HCE SE and HCE LE protocols.

104 coded chemicals were blind-tested using both the SE and LE protocols in three runs with three replicate tissues per run, in three laboratories. A test strategy combining, in a sequential manner, the Eye Irritation Peptide Reactivity Assay (EPRA) with both the SE and LE variants of the SkinEthic™ HCE test method was also assessed.

EpiOcular™ EIT

In the ring trial 104 coded chemicals were blind-tested (three runs with two replicate tissues per run, in three laboratories) using EpiOcular™ EIT SOP (V 6.0), using separate protocols for liquid and solid chemicals, and two prediction models (50% and 60% cell viability).

With the original EpiOcular™ EIT protocol (SOP V6.0) all of the VMG acceptance criteria were met for liquid chemicals: however, some of the VMG predictive capacity acceptance criteria were not met for solid chemicals.

The VMG considered that test method performance might be improved if a. Test optimisation was undertaken by the test developer within EIVS.

To improve the performance of the test methods with solid chemicals, test optimisation was undertaken within EIVS to produce a balanced increase in sensitivity offset by some decrease in specificity using 11 of the most challenging EIVS solid chemicals. An amended optimised for solid chemicals protocol, V 8.0, was used for a post-optimisation validation study of the EpiOcular™ EIT optimised in one laboratory with 60 solid chemicals. The post-optimisation findings are incorporated and analysed in the VSR, and form the basis of the VMG conclusions on the performance of the EpiOcular™ EIT.

The Cosmetics Europe Task Force Eye Irritation Report

The ESAC believes the Cosmetics Europe study was well-designed, and is satisfied that a sufficient number of RhT MTT-reduction product test methods, chemicals, and laboratories were used.

In the first phase of the study, three laboratories qualified HPLC/UPLC systems using appropriate qualification criteria in accordance with the May 2001 US Food and Drug Administration (FDA) guidance for industry "Bioanalytical Method Validation".

Next, three laboratories tested up to 26 chemicals using one of three RhT test methods measuring three different toxicological endpoints (EpiOcular™ EIT as per the EIVS protocol v6.0 for eye irritation, SkinEthic™ RhE as per OECD TG 431 for skin corrosion, and the EpiSkin™ as per OECD TG 439 for skin irritation). The three laboratories with the suitably qualified HPLC/UPLC systems for tested the samples and applied the Prediction Models using both endpoint measurements.

Chemical selection:

EIVS

The ESAC believes the method by which the test chemicals were selected, and the number and nature of the test chemicals, were appropriate.

The Cosmetics Europe Report

The ESAC believes that the 26 chemicals selected were adequate for this study, and represent the almost the full dynamic range of the relevant toxicological endpoints.

2.2.3 Study design for the retrospective part

Not applicable.

2.2.4 Statistical analysis used in both part of the study

The ESAC believes that whist the data management systems for both studies were robust, elements of the statistical analysis were not best practice.

EIVS

Almost complete datasets are available for both RhT test methods, but ESAC has some concerns about some of the statistical methods applied - see 2.3.3 below.

The Cosmetics Europe Report

The ESAC believes that all of the reference and new data relied upon are of sufficient quality.

However, the statistical method by which the formazan measurements by OD-photometry and HPLC/UPLC were correlated in the study report is not ideally suited to situations where the true value of the variable of interest in unknown, and in producing this Opinion the ESAC has taken account of an alternative approach developed for use in these situations (Bland and Altman, 1986).

2.3 Study results and conclusions

2.3.1 Standardised use

EIVS

The ESAC considers that the SOPs used in the EIVS validation study are sufficiently detailed and complete, with SOP V 8.0 being considered the definitive protocol for EpiOcular™ EIT.

The Cosmetics Europe Report

The ESAC considers the qualification process and parameters described for suitably qualified HPLC/UPLC to be appropriate and sufficiently clear.

2.3.2 Within- and between laboratory reproducibility

Within-Laboratory Reproducibility (WLR)

EIVS

Both RhT test methods proved to be highly reproducible within laboratories, satisfying the acceptance WLR criteria established by the VMG.

EpiOcular™ EIT

The possible short-comings of the VMG WLR calculations are described elsewhere in this Opinion. The ESAC WG, having re-analysed the data set on the basis of the number of chemicals tested estimated the 95% Confidence Interval (95%-CI) for the WLR using the 60% cut off to be 89.0% to 94.4%, satisfying the VMG acceptance criteria (WLR ≥ 85%).

The WLR of the EpiOcular™ EIT optimised for solid chemicals protocol (SOP V8.0) at the one laboratory that conducted the supplementary testing satisfied the VMG acceptance criteria.

The Cosmetics Europe Task Force Eye Irritation Report

During the Phase 1 (qualification) reproducibility was addressed with both intra- and inter-day evaluations of the formazan calibration reproducibility calculated by regression analysis. Whilst the ESAC accepts that variations on this method of assessing agreement between two test methods are commonly used, the use of correlation for this purpose can be misleading and make it difficult to

recognise differences, and the significance of the differences, between the findings with different measurement systems when the true value of the variable of interest is unknown. The ESAC takes account of an alternative approach (Bland and Altman, 1986), developed for use in these situations, which also confirms the limits of agreement are good.

Within laboratory reproducibility (WLR) of formazan measurement by HPLC/UPLC was evaluated in one laboratory: the results obtained were 100% concordant.

Between-Laboratory Reproducibility (BLR)

EIVS

The ESAC notes that all of the BLR values reported in the VSR are significantly above the acceptance criteria set by the VMG (BLR ≥ 80), but that there was no BLR study conducted using the EpiOcular™ EIT optimised protocol for solids (SOP V 8.0).

The Cosmetics Europe Task Force Eye Irritation Report

There was 100% concordance between OD-photometry and HPLC/UPLC formazan endpoint measurements for non-interfering chemicals.

2.3.3 Predictive Capacity

EIVS

The ESAC believes that the VMG's determination of confidence limits for the predictive capacity based on the number of test runs, rather than the number of chemicals tested, produces confidence limits which may be inappropriately narrow, and has taken account revised calculations based on the number of chemicals tested.

Furthermore, the ESAC considers that accuracy figures should reflect the way decisions will be made in practice. Specifically, as chemical classification will be based upon one qualifying test run, the study results should have been evaluated to produce an accuracy range determined by the best- and worst-cases based on data from single runs rather than aggregating data from different test runs with the same chemical. Additional analysis was undertaken along these lines.

SkinEthic™ HCE

The SkinEthic™ HCE, SE and LE protocols, failed to meet the 'definitely acceptable' criteria for sensitivity and overall accuracy defined by the VMG (specificity ≥ 60%; sensitivity ≥ 90%, overall accuracy ≥ 75%).

The SkinEthic™ HCE sensitivity, as judged by the VMG criteria (specificity < 50%; sensitivity < 80%, overall accuracy < 65%), was 'definitely unacceptable' [2].

EpiOcular™ EIT

ESAC believes that with SOP V 8.0 and a 60% cut off all of the VMG acceptance criteria can be deemed to have been met.

When used for liquid chemicals, the original EpiOcular™ EIT SOP V6.0 protocol the VSR reported results satisfying all of the VMG predictive capacity acceptance criteria. A 60% cut-off resulted in a better sensitivity. For solid chemicals the results with protocol version (V6.0) did not satisfy all of the VMG acceptance criteria: six chemicals were under-predicted, one of which (misclassified by two of the laboratories) is classified *in vivo* as Category 1.

When the solid chemicals were tested in one laboratory by the protocol optimised for solid chemicals (SOP V 8.0) and a 60% cut-off all of the VMT definitely acceptable values were achieved.

The ESAC notes that with respect to accuracy using the best-case/worse-case calculation model even the worst-case values satisfy VMG criteria for sensitivity and accuracy, and that the worst-case specificity is very close to the VMG acceptance criterion. As the ESAC considers that the true predictive capacity falls somewhere between the best- and worst-case calculations based on all test runs, the VMG predictive capacity criteria are deemed to have been met with the 60% cut-off value.

The Cosmetics Europe Task Force Eye Irritation Report

Where comparisons can be made between HPLC/UPLC data and *in vivo* classification data, the HPLC/UPLC data did not under-estimate the *in vivo* classification. In case of EpiOcular™ EIT data, for test substances that do not exhibit colour interference or direct MTT reduction, the tissue viability values obtained are almost identical whether using absorbance (OD-photometry) or HPLC/UPLC, with the OD-photometry values being consistently, marginally above the values obtained with the HPLC/UPLC test method.

2.3.4 Applicability and possible limitations

EIVS

The EpiOcular™ EIT and SkinEthic™ HCE test methods were not developed, designed, or evaluated to differentiate between UN GHS / EU CLP Category 1 (serious eye damage) and Category 2 (eye irritation) classifications. There is limited information available on the performance of the test methods with chemical mixtures.

[2] The VMG defined a three tier system for judging the acceptability of performance of the assays by using a three category system – “definitely acceptable” and “definitely unacceptable”, with the values between these figures being borderline acceptable. The same approach had been chosen by EURL ECVAM for validating the skin corrosion assays (Fentem et al., 1998).

EpiOcular™ EIT

The ESAC believes that the available evidence on the **relevance** and **reliability** of the EpiOcular™ EIT supports its consideration for use within an integrated, tiered approach using strategic combinations of alternative test methods to replace the Draize eye test, either as the initial step in a Bottom-Up Approach or a second or subsequent step in a Top-Down Approach, to discriminate chemicals (substances and chemical mixtures) not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling (Category 1 and Category 2) according to the UN GHS Classification and Labelling of Chemicals (UN GHS) and as implemented by the EU CLP regulation (EU CLP).

With respect to the chemicals that were misclassified when the EpiOcular™ EIT findings were judged against the Draize eye test reference data, the ESAC has identified no specific additional limitations or exclusions.

SkinEthic™ HCE

The ESAC believes that the reported predictive capacity of the SkinEthic™ HCE test method does not justify it being considered for regulatory use.

The Cosmetics Europe Task Force Eye Irritation Report

The ESAC believes that the evidence presented suggests that for chemicals compatible with the three RhT methods used within this study comparable classifications are obtained using both OD-photometry and HPLC/UPLC endpoint detection systems; that with these RhT test methods HPLC/UPLC endpoint detection measurement can be used in the case of strongly coloured chemicals which are incompatible with formazan measurement by OD-photometry; and that there are plausible and reasoned arguments for why this endpoint measurement system might be applicable to other RhT test methods relying on measurement of the same endpoint.

2.3.5 Identified gaps between study design and study conclusions

EIVS

On the basis of the EIVS data the VMG concluded that the use of 2 tissue replicates in similar or modified RhT/MTT-based test method aiming at identifying chemicals not requiring classification for serious eye damage/eye irritation is statistically and scientifically justified. However, the ESAC does not accept that this can be generalised to all RhT test methods on the basis of the available evidence.

The Cosmetics Europe Task Force Eye Irritation Report

Nil of note.

2.4 Potential regulatory use of the test method

EIVS

EpiOcular™ EIT

The ESAC believes that the available evidence on the **relevance** and **reliability** of the EpiOcular™ Eye Irritation Test (EIT) supports its consideration for use within an integrated, tiered approach using strategic combinations of alternative test methods to replace the Draize eye test, either as the initial step in a Bottom-Up Approach or a second or subsequent step in a Top-Down Approach, to discriminate chemicals (substances and chemical mixtures) not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling (Category 1 and Category 2) according to the UN GHS Classification and Labelling of Chemicals (UN GHS) and as implemented by the EU CLP regulation (EU CLP).

SkinEthic™ HCE

The ESAC believes that the predictive capacity of the SkinEthic™ HCE test method reported in the EIVS VSR does not currently justify it being considered for regulatory use.

The Cosmetics Europe Task Force Eye Irritation Report

The ESAC judges that the Cosmetics Europe Report provides evidence, and sets out plausible and reasoned arguments, for in the case of regulatory testing this endpoint measurement system being considered for RhT test methods relying on the quantification of formazan.

2.5 Recommendations

EIVS

SkinEthic™ HCE

ESAC agrees with the VMG and the test method developer's decision to not advance the SkinEthic ™ **HCE** test method in this validation on the basis of insufficient predictive capacity (in particular specificity) for considering the test method, at present, for a bottom up test strategy within a tiered approach for inclusion into an Integrated Testing Strategy (ITS) / Integrated Approach to Testing and Assessment (IATA). ESAC understands that the test method developer is currently optimising the test method protocol before re-entering the validation process. ESAC supports the decision for optimisation, including considering developing and evaluating different protocols for liquid chemicals and solid chemicals.

EpiOcular™ EIT

1. The ESAC recommends that the EpiOcular™ EIT test method SOP V 8.0 using the 60% cut off be considered for use within an integrated, tiered approach using strategic combinations of

alternative test methods to replace the Draize eye test, either as the initial step in a Bottom-Up Approach or a second or subsequent step in a Top-Down Approach, to discriminate chemicals (substances and chemical mixtures) not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling (Category 1 and Category 2) according to the UN GHS Classification and Labelling of Chemicals (UN GHS) and as implemented by the EU CLP regulation (EU CLP).

2. Based on the data generated during validation, the ESAC recommends that EpiOcular™ EIT be considered applicable to a wide range of liquid and solid chemicals. However, confidence in the ability of the EpiOcular™ EIT test method to detect solid irritants (using SOP version 8.0) would be increased if more chemicals were tested under blind conditions by the other two laboratories.

3. The ESAC recommends that the SOP should be amended to better define the protocol to be used for test chemicals with an unclear physical state, specifically that all viscous, waxy and gel-like chemicals are placed in a water bath for 15 minutes at 37°C before deciding if they should be tested with the liquids or the solids protocol; and that the test chemical should be applied directly from the water bath and should not be brought to room temperature before testing.

General

4. The ESAC recommends that a range of labelled chemical mixtures be identified for use as test substances within future eye irritation validation studies.

The Cosmetics Europe Task Force Eye Irritation Report

5. The ESAC recommends that the use of suitably qualified HPLC/UPLC systems for the detection and quantification of formazan be proposed for inclusion in relevant regulatory guidelines to extend the applicability domain of RhT test methods to include coloured chemicals interfering with formazan measurement by OD-photometry. However, the HPLC/UPLC provided marginally but systematically lower values for formazan levels than OD photometry. This may impact on some of the prediction models which currently rely on formazan quantification by OD-photometry

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Annex 1 ESAC and ESAC Working Group charged with the scientific review

ECVAM Scientific Advisory Committee (ESAC)

- Prof. Jürgen BORLAK
- Dr. Neil CARMICHAEL
- Dr. Edward CARNEY
- Dr. Harvey CLEWELL
- Prof. Lucio G. COSTA
- Dr. Kristina KEJLOVÁ
- Prof. David John KIRKLAND
- Prof. Annette KOPP-SCHNEIDER
- Dr. Renate KRÄTKE
- Prof. Claus-Michael LEHR
- Dr. José Maria NAVAS
- Prof. Aldert PIERSMA
- Dr. Jonathan RICHMOND
- Dr. Erwin L ROGGEN
- Dr. Dorothea SESARDIC

ESAC Working Group*:

- Dr. Jon RICHMOND (ESAC Member, WG Chair)
- Prof. Annette KOPP-SCHNEIDER (ESAC Member)
- Dr. Renate KRÄTKE (ESAC Member)
- Dr. Kristina KEJLOVA (ESAC Member)
- Dr. Tohru MARUNOUCHI (Fujita-Health University; ICATM nomination by JaCVAM)
- Dr. Jill MERRILL (FDA; ICATM nomination by NICEATM/ICCVAM)

*) One member of the ESAC WG (JM) had been the ICCVAM liaison to the EIVS VMG during the validation study. She had not been a member of the core Validation Management Group (VMG). Although all decisions during the validation study were taken by the core VMG, JM advised and commented on some elements of the study design. The ESAC WG does not believe that this liaison role constitutes a significant conflict of interest with respect to the work of the ESAC WG.

EURL ECVAM

- Dr. Claudius GRIESINGER (ESAC Coordinator)
- Dr. Michael SCHÄFFER