ICCVAM Responses to ECVAM Recommendations[1]

Direct Peptide Reactivity Assay

- **ECVAM Recommendation (SEE THE FULL RECOMMENDATION AT http://ihcp.jrc.ec.europa.eu/our_labs/eurl---ecvam/eurl---ecvam---recommendations/files---dpra/EURL_ECVAM_Recommendation_DPRA_2013.pdf):**
    - DPRA can be used to support the identification of sensitizers/non---sensitizers, the DPRA may also be able to contribute to the assessment of sensitizing potency. DPRA data should always be considered in combination with other information in the context of integrated approaches such as Weight of Evidence (WoE) or Integrated Testing Strategies (ITS)
- **Draft ICCVAM Statement**: Based o the demonstrated performance of the DPRA in an interlaboratory validation study, ICCVAM concurs with the ECVAM recommendation that DPRA can be used as part of an integrated testing strategy to identify potential skin sensitizers. However, ICCVAM emphasizes that, because of concerns over the false negative rate, the identified limitations for DPRA (see below), and the fact that it accounts for only one event in the mechanistically complex process of skin sensitization (i.e., haptenation), DPRA is not sufficient as a standalone method for the identification of potential skin sensitizers or to determine skin sensitization potency categories.
    - **Identified limitations**: Metal compounds are outside of the applicability domain for DPRA. In addition, DPRA is designed to detect peptide reactivity associated with lysine and cysteine residues only, and it may not detect substances that require metabolic or abiotic activation.

Zebrafish Embryo Toxicity Test (ZFET)

- **ECVAM Recommendation (SEE THE FULL RECOMMENDATION AT http://ihcp.jrc.ec.europa.eu/our_labs/eurl---ecvam/eurl---ecvam---recommendations/public---comments/comments---ZFET):**
    - ZFE provides information on acute fish toxicity that can be considered comparable to that derived from standard acute fish toxicity tests (e.g. OECD TG203; 71 OECD 1992).
        - Additionally, the ZFET can be used for range---finding tests to determine the appropriate concentration range for higher tier tests (e.g. chronic fish toxicity tests [see OECD TG210, OECD 287 2013b]), thus avoiding the use of juvenile or adult fish for this purpose.
- **Draft ICCVAM Statement**: ZFE has now been adopted by OECD as *Test Guideline 23 "Fish embryo acute toxicity test"*[2] As a result, and in accordance with policies at individual agencies and the OECD Mutual Acceptance of Data system, ICCVAM regulatory agencies will consider ZFE results for acute fish toxicity evaluations.

[1] Links to the final ECVAM recommendations and supporting documents for each of these 5 methods are at http://ihcp.jrc.ec.europa.eu/our_labs/eurl---ecvam/eurl---ecvam---recommendations/eurl---ecvam---recommendations

[2] Available at http://www.oecd---ilibrary.org/docserver/download/9713161e.pdf?expires=1400006043&id=id&accname=guest&checksum=454E86397B026FB14B0648FFF3A6E070

Keratinosens

- **ECVAM Recommendation (see http://ihcp.jrc.ec.europa.eu/our_labs/eurl---ecvam/eurl---ecvam---recommendations/file---kerati/JRC_SPR_Keratinosens_Rec_17_02_2014.pdf):**
    - Nrf2–dependent luciferase induction measurements in the KeratinoSens assay when combined with information from other non---animal methods in the context of Weight---of---Evidence (WoE) approach or Integrated Testing Strategy (ITS) may provide useful information about the sensitization potential of chemicals
- **Draft ICCVAM Statement**: Based o the demonstrated performance of the KeratinoSens assay in an interlaboratory validation study, ICCVAM concurs with the ECVAM recommendation that it can be used as part of an integrated testing strategy to identify potential skin sensitizers. However, ICCVAM emphasizes that, because of concerns over the false negative rate, the identified limitations for the KeratinoSens assay (see below), and the fact that it accounts for only one event in the mechanistically complex process of skin sensitization (i.e., activation of the Keap1---Nrf2---ARE pathway), it is not sufficient as a standalone method for the identification of potential skin sensitizers or to determine skin sensitization potency categories.
    - **Identified limitations**: Only chemicals soluble in DMSO or water can be tested in the KeratinoSens assay Pro---haptens requiring P450 activation are reported not to be identified by the assay; chemicals with selective reactivity towards nucleophiles other than cysteine sulfhydryl groups may not be reliably identified

Cell Transformation Assay based on the Bhas 42 cell line

- **ECVAM Recommendation (see http://ihcp.jrc.ec.europa.eu/our_labs/eurl---ecvam/eurl---ecvam---recommendations/files---bhas/EURL_ECVAM_Recommendation_Bhas---CTA_2013.pdf):**
    - The Bhas 42 CTA, may provide useful information about possible genotoxic and non---genotoxic carcinogenicity potential for use in conjunction with other data to generate supporting information for hazard identification that can eventually contribute to a risk assessment.
- **Draft ICCVAM Statement** Based on the demonstrated performance of the Bhas 42 CTA in an interlaboratory validation study, ICCVAM concurs with the ECVAM recommendation that it can be used as part of an integrated testing strategy to detect potential carcinogens, but not to specifically discriminate between initiators and promoters. ICCVAM emphasizes that, because of concerns over the false negative rate, the identified limitations for the Bhas 42 CTA (see below), and the complexity of the mechanisms associated with tumor formation, it is not sufficient as a standalone method for the identification of potential carcinogens.
    - ~~o~~ **Identified limitations**: the limited metabolic activity of the Bhas 42 cell line should be taken into account when interpreting negative results from this assay.

3T Neutral Red Uptake (3T3 NRU) Cytotoxicity Assay for the Identification of Substances not Requiring Classification for Acute Oral Toxicity

- **ECVAM Recommendation (see http://ihcp.jrc.ec.europa.eu/our_labs/eurl---ecvam/eurl---ecvam---recommendations/files---3t3/ReqNo_JRC79556_lbna25946enn.pdf):**
    - The 3T3 NRU test method could prove a valuable component of a weight---of---evidence or integrated testing strategy approach for supporting hazard identification and safety assessment in agreement with the EU CLP Regulation and international regulatory schemes implementing the upper threshold of UN GHS Category 4 as the cut---off for non---classification of substances.
- **Draft ICCVAM Statement:** ICCVAM acknowledges the utility of the 3T3 assay in a weight of evidence approach to support hazard classification and labeling according to regulatory schemes that use the upper threshold of LD50 > 2000 mg/kg b.w. as the cut---off for non---classification of substances (i.e., GHS Category 4). However, U.S. hazard classification systems may use different cut---off dose levels and users of the 3T3 should be aware of agency---specific relevant policies.