Questions and Answers From REACH Webinar 2: Skin Irritation and Corrosion

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1. For a substance or mixture with no irritation data, and if an *in vitro* skin corrosion test was conducted – can the result be used for eye irritation classification?

   CR: Only if the result is positive and the mixture is classified as H314 (causes severe skin burns and eye damage).

2. Could we confirm that the test substance can cause corrosion or irritation by testing a single test, either skin corrosion or skin irritation *in vitro* methods?

   CR: The question is not clear. If it is about when just one test may conclude on the classification of the substance, the answer is yes if the result is "skin corrosive" after the first step of the top-down approach or if the result is "not irritant" after the first step of the bottom-up approach.

3. Are there clear regulatory circumstances in which *in vivo* test methods are still always essential? What are they? Could the use of *in vivo* in these situations be replaced at some point?

   CR: As far as I know, there are no circumstances limiting the applicability of the *in vitro* set of tests more than *in vivo* tests. However, in some countries the *in vitro* approach is still not fully accepted and the *in vivo* test is still necessary to market a substance.

4. Can you speak more to how well these assays can be applied to poorly water soluble substances and high Kow substances? Is there a reasonable Kow limit for these assays?

   GEC: The assays based on reconstructed tissue models can accommodate water-insoluble materials. If the products are solids, they can be tested as such unless they need to be diluted to become the test article of interest. An alternative solvent can be considered in which the products are soluble; in this case, a solvent control should be used. I am not aware of any studies investigating the impact of various Kow values on the performance of the assays.

5. In OECD 439, if the test shows non-irritation, thus not GHS Category 2, does that also imply not Skin Irritation Category 3?

   CR: Unfortunately, Category 3 is not included. It should be derived in a weight of evidence approach with additional data.

6. I would like to know about the use of RHE assays to predict finished product irritation as cosmetics or personal care.

   GEC: The regulatory assays based on reconstructed tissue models can be used to assess the safety of cosmetics products, as the guidelines allow the use of the assays for mixtures. That said, I need to emphasise that the outcome of the regulatory assays is in the form of a yes/no answer regarding irritation potential; therefore, on comparison between formulations is possible (moderate, mild, etc.).

7. And for Corrositex - if it could be applicable only to extreme pH values that change the color – which anyway could cause corrosion on skin – in that case what is the use of the model?

   GEC: Not all extreme pH mixtures and formulations are corrosive. The final corrosive outcome or prediction of the assay can be induced by the pH, the ingredients in the formulation, the acid or alkali reserve, any synergistic effects, etc.

8. Could you talk about the false positive or negative during *in vitro* test compare with *in vivo* test?

   CR: That was extensively evaluated in the validation process. False negative and positive results may derive from the *in vivo* study as well (ALTEX. 2016;33(2):123-134. doi: 10.14573/altex.1510053)

9. To use rat discs (OECD 430), do we need to consider the physiological differences between rat vs. human skin in terms of metabolism and structure?

   GEC: Interspecies differences exist; however, the validation was performed using paired *in vivo—in vitro* data and led to the prediction model that aligned the paired data with the highest accuracy. Although the animal model does not always predict the human response, in the absence of human data, the paired *in vivo—in vitro* data approach provides a prediction close enough to the human response.

10. In the OECD 430 model, is it not possible to categorize the corrosives based on the classification of the reduction in the TER?

    GEC: The assay allows the identification of non-corrosive and corrosive test chemicals in accordance with the UN GHS. However, it cannot sub-categorise corrosive substances and mixtures.

11. I would like to know if there are other considerations when substances could be used not only in skin but also as food or oral supplements.

    CR: We are talking only about skin irritation. Acute toxicity evaluation requires a completely different approach. If your concern is irritation of the oral cavity, there are other human epithelium models available.

12. Bath bomb question - the handling prior to placing in water is different to after the reaction with water? Should it not be classified prior to applying to water – is the application in water relevant to cosmetic safety assessments?

    GEC: It depends on the goal of the testing: To assess the hazard (workers safety, any accidental exposure to an end-user in an unintended way), the neat product (undiluted in water) should be tested; alternatively, an end-user dilution may be tested if the package provides the end-user with instructions on the product-to-water ratio. To assess the risk to the end-user, testing the dilution makes more sense though; as a cautious approach, the neat product and the dilution should probably be tested. I assume, this type of product may contain surfactants, in which case there is even more reason to test the undiluted and diluted product as different types of surfactants have different irritation potentials when tested neat or diluted. Therefore, testing both the neat and diluted product should cover all the bases.

13. For DG Class 8, is there an obligation to test on metal if the OECD skin corrosion test is negative?

    CR: The classification as H290 (may be corrosive to metals) has no relationship with the skin irritation label. It should be assessed independently

14. Is it feasible to perform high throughput screening test for skin irritation/corrosion?

    GEC: I assume the question refers to a 96-well plate format? For the moment, the assays have been validated for inserts that fit a 12-well plate, and I am not aware of efforts to validate a 96-well format assay.

15. Are there online databases of skin irritation testing results that contain animal and human data? If so, could you please provide their URL addresses? Thank you.

    CR: As far as I know, there is nothing specific. The validation reports of the skin irritation tests contain some tables. You can retrieve other information from eChemPortal or from the ECHA database, but in both cases the query is manual.

16. Could you provide the reference for the book chapter and paper please?

    GEC:

    a. European Commission Joint Research Centre. Explanatory Background Document to the OECD Draft Test Guideline on *In Vitro* Skin Irritation Testing. http://www.oecd.org/chemicalsafety/testing/43670220.pdf.

b. European Centre for Ecotoxicology and Toxicology of Chemicals. Skin Irritation and Corrosion: Reference Chemicals Data Bank. Technical Report No 66. March 1995. http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-066.pdf.
c. Eskes C, Cole T, Hoffmann S, *et al*. The ECVAM international validation study on in vitro tests for acute skin irritation: selection of test chemicals. *Altern Lab Anim*. 2007;35(6):603-619. (MID: 18186668)
d. Spielmann H, Hoffmann S, Liebsch M, *et al*. The ECVAM international validation study on in vitro tests for acute skin irritation: report on the validity of the EPISKIN and EpiDerm assays and on the Skin Integrity Function Test. *Altern Lab Anim*. 2007;35(6):559-601. (PMID: 18186667)
e. Fentem JH, Briggs D, Chesné C, *et al*. A prevalidation study on in vitro tests for acute skin irritation. results and evaluation by the Management Team. *Toxicol In Vitro*. 2001;15(1):57-93. (PMID: 11259870)
f. Kandárová H, Liebsch M, Gerner I, *et al*. The EpiDerm test protocol for the upcoming ECVAM validation study on in vitro skin irritation tests – an assessment of the performance of the optimised test. *Altern Lab Anim*. 2005;33(4):351-367. (PMID: 16185104)
g. Kandárová H, Hayden P, Klausner M, Kubilus J, Kearney P, Sheasgreen J. In vitro skin irritation testing: Improving the sensitivity of the EpiDerm skin irritation test protocol. *Altern Lab Anim*. 2009;37(6):671-689. (PMID: 20105002)
h. Basketter D, Jírova D, Kandárová H. Review of skin irritation/corrosion hazards on the basis of human data: A regulatory perspective. *Interdicip Toxicol*. 2012;5(2)98-104. (PMID: 23118595)
i. Jírová D, Basketter D, Liebsch M, *et al*. Comparison of human skin irritation patch test data with in vitro skin irritation assays and animal data. *Contact Dermatitis*. 2010;62(2):109-116. (MID: 20136894)
j. Basketter DA, York M, McFadden JP, Robinson MK. Determination of skin irritation potential in the human 4-h patch test. *Contact Dermatitis*. 2004;51(1):1-4. (PMID: 15291823)
k. Walters RM, Gandolfi L, Mack MC, *et al*. In vitro assessment of skin irritation potential of surfactant-based formulations by using a 3-D skin reconstructed tissue model and cytokine response. *Altern Lab Anim*. 2016;44(6):523-532. (PMID: 28094534)
l. Costin G-E, Norman KG. (2015) Application of in vitro methods in preclinical safety assessment of skin care products. In: Farage M, Miller K, Maibach H, eds. *Textbook of Aging Skin*. Springer, Berlin, Heidelberg. https://link.springer.com/referenceworkentry/10.1007/978-3-642-27814-3_130-1.

17. Is there a way to get a list of labs in the US that do corrosive/irritant testing on chemicals?

    Chemical Watch: Chemical Risk Manager will provide this in its test methods database.

18. How do the *in vitro* and *in vivo* test methods compare in terms of cost?

    Chemical Watch: In case you manage to correctly plan your testing strategy and you manage to perform only one test, *in vitro* methods can be cheaper than *in vivo*

methods. If you have to perform more than one test, *in vitro* testing is more expensive than *in vivo* testing (at least in the EU).