Variability in data from skin sensitization (LLNA) and reactivity (DPRA) testings

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INTRODUCTION

Skin sensitization is an endpoint of significant health concern. Until March 2013, the potency of chemicals to elicit skin sensitization was evaluated through data from animal testing, such as the local lymph node assay (LLNA). To replace these tests, new hazard and risk assessments based upon *in silico, in chemico* and *in vitro* methods are developed. Skin sensitization depends, amongst others, on the ability of chemicals to form covalent bonds with skin proteins. In this respect, the *in chemico* approaches that measure the reactivity of chemicals toward model nucleophiles called direct peptide reactivity assays (DPRA), could be used as alternative surrogate to animal testing.Studies on the correlation between the skin sensitization (SS) potential of chemicals and their reactivity towards model nucleophiles date back to the early 1930s [1]. Recently, an extensive research on the relationship between SS potency and reactivity of chemicals has been undertaken by Gerberick *et. al.* [2,3]. However, to build *in silico* prediction models for skin sensitization, one key issue concerns the reliability of the experimental data, used as reference or learning sets.

The aim of the current work is to analyze the variability of experimental data from *in vivo* LLNA (i.e. EC3, %) and *in chemico* DPRA (i.e.% of reactivity for Cysteine and Lysine peptide) assays and to take it into account for a more robust classification of chemicals.

MATERIALS AND METHODS

The variation of experimental data was estimated based on the confidence interval measured by the standard deviation (SD).

s_ε - Sample Standard Deviation

$$s_\varepsilon = \sqrt{\frac{\sum_{n=1}^{N}\left(x_n - \bar{x}\right)^2}{N-1}}$$

The data used to analyze the variability of EC3 % and DPRA experimental results was collected from different public sources or provided by industry. The Confidence Interval (ΔEC3, ΔDPRA) based on SD was calculated for each chemical.

ANALYSIS OF VARIABILITY IN EC3 % AND DPRA DATA

Variability in EC3 % data

The variability of EC3 % data was analyzed based on 26 chemicals having at least five available values. The confidence interval based on ΔEC3 approximation is calculated and illustrated in Fig. 1. A linear relationship between the variance and mean EC3 % values was observed in the range of EC3 values from 0.0 to 6.0%. The variance of EC3 data seemed independent from the mean skin sensitization potency in the range of 6.0 to 20.0%.

$$\Delta EC3 = a + bEC3$$

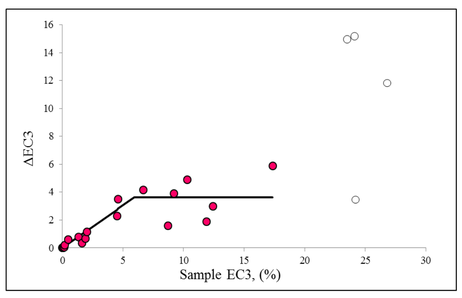


Fig.1. Confidence interval for EC3 data

$$EC3 = Sample\ EC3 \pm \underbrace{t(\upsilon,\alpha)s_\varepsilon/\sqrt{N}}_{\Delta\ EC3}$$

α – level of confidence
N – number of *EC3* values
$t(\upsilon,\alpha)$- Student distribution – probability distributions that arises when estimating the mean of a normally distributed population
Sample EC3- sample mean of *EC3* value
s_ε - sample standard deviation

Has been found that:

- $\Delta EC3 = 0.6 EC3$ *for* $EC3 \in [0, 6]$
- $\Delta EC3 = 3.6$ for $EC3 \in [6, 23]$

Variability in DPRA data

The variability of DPRA data was analyzed based on 29 chemicals with cysteine peptide (Cys) and 27 chemicals with lysine peptide (Lys) reactivity having at least four available values. The confidence intervals based on ΔDPRA approximation are calculated separately for Lys and Cys data and illustrated in Fig. 2.a,b. A parabolic relationship between the variance of Cys or Lys and their mean reactivity values has been found.

$$\Delta DPRA = a + b * SampleDPRA + c * SampleDPRA^2 \qquad \begin{matrix} a = 0 \\ b = -100c \end{matrix} \qquad \begin{matrix} \text{c – depends on the} \\ \text{level of confidence} \end{matrix}$$

$$\Delta DPRA = -100c * SampleDPRA + c * SampleDPRA^2$$

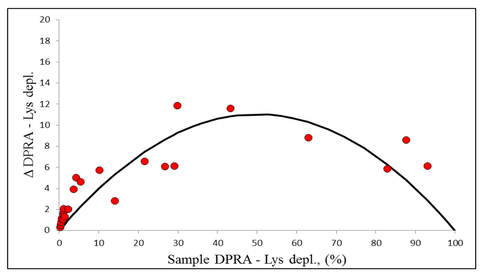


Fig.2a. Confidence interval for DPRA Lys data

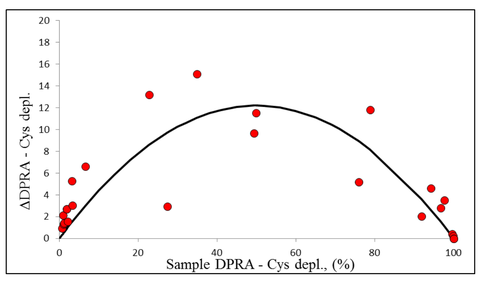


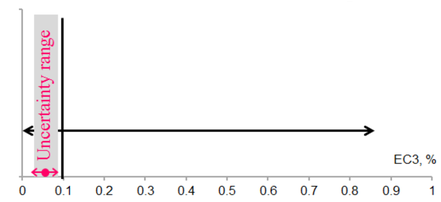
Fig.2b. Confidence interval for DPRA Cys data

Examples of EC3 % data uncertainty for robust classification :

From our analysis in Fig. 1 it seems that for a classification **accounting for** EC3 variation with very high 95% confidence:

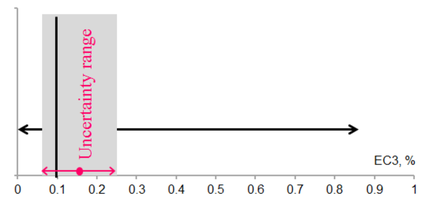
- ***If EC3 < 6% then EC3 = EC3 ± 0.6*EC3***
- ***If 6 ≤ EC3 ≤ 23% then EC3 = EC3 ± 3.6***

Example 1
Obs. *EC3* = 0.063%; Accounting for variation
EC3 = 0.063 ± 0.6*0.063 or *EC3*∈[0 .025÷ 0.095]



univocal classification at the 0.1% EC threshold with confidence of 95%

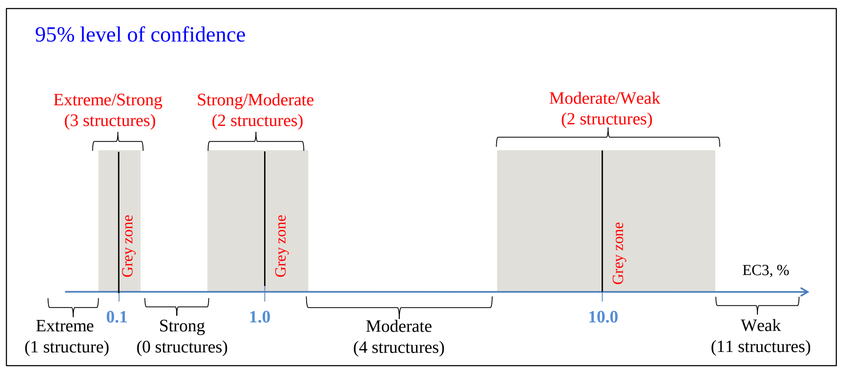
Example 2
Obs. *EC3* = 0.150%; Accounting for variation
EC3 = 0.150 ± 0.6*0.150 or *EC3*∈[0 .060÷ 0.240]

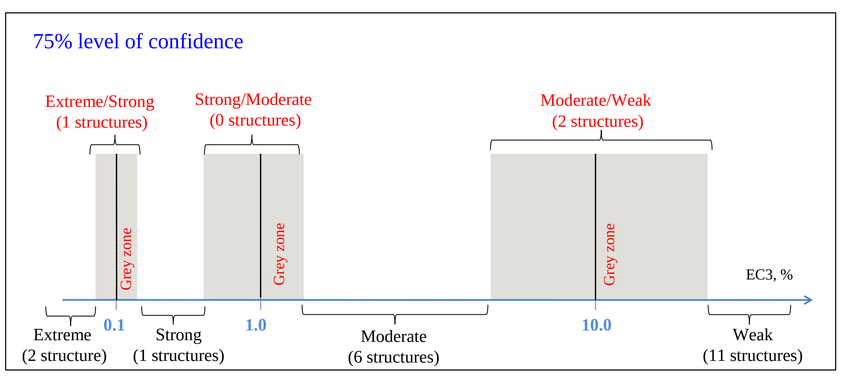


No univocal classification at the 0.1% threshold with confidence of 95%

Examples of the classification of chemicals according to EC3 % thresholds at different levels of confidence :

In turn, the confidence intervals could also be used to derive "grey" zones associated with EC3 or DPRA classification thresholds. These grey zones are adapted according to a varying level of confidence (ex. 95 and 75%).





SUMMARY

- Confidence intervals have been defined for EC3 and DPRA data variation from different sources.
- These ranges of uncertainties could be associated with observed EC3 and DPRA data for a more robust classification of chemicals, according to their skin sensitization and reactivity potencies determined by different sources.
- In turn, the confidence intervals could also be used to derive "grey" zones associated with EC3 or DPRA classification thresholds.

References:

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