Skin Sensitisation - Chemical Applicability Domain of the Local Lymph Node Assay (LLNA)

Anne Marie Api[a], Jon F Lalko[a], Grace Y Patlewicz[b], David W Roberts[c]

a, Research Institute for Fragrance Materials, USA; b, DuPont Haskell Global Centers, USA; c, Liverpool John Moores University, UK

Abstract

Whilst the LLNA has been through an extensive validation relative to the incumbent assay of the time, the Guinea Pig Maximisation Test (GPMT), to date there has been no systematic evaluation of the chemical applicability domain of the LLNA, i.e. an exercise to define in chemical terms which classes of chemicals are well predicted, which are liable to be wrongly predicted and which are unpredictable by this *in vivo* method. "Wrongly predicted" in this context is being defined to encompass false negatives, false positives, as well as false **relative potencies.** The chemical domain of the LLNA is particularly important when considering outcomes of new *in vitro* test methods for sensitisation in order to be able to determine whether a given result is reasonable or whether the outcome is impacted by uncertainties in the LLNA which is typically used as a basis of comparison. The aim of this project therefore is to define the range of chemicals for which the LLNA result can be considered a reliable (quantitative) indicator of the chemical's potential to sensitise, can therefore be carried forward for subsequent risk assessment purposes and/or can be relied on as the benchmark when developing or evaluating new *in vitro* assays. Here we present a work programme, divided into 4 tasks, aimed at defining the chemical applicability domain of the LLNA, and report early progress on the first task

Task 1. Identify and evaluate structure-potency trends based on human data and/or on guinea pig (GP) data and compare these structure-potency trends to those in the LLNA.

Purpose of this task: For classes of chemicals where similar structure-potency trends apply for both LLNA, human (if available) and GP data, LLNA data can be confidently used for benchmarking new non-animal approaches as well as for risk assessment. For classes of chemicals where this does not apply, LLNA data should not be used for benchmarking new non-animal approaches, and for risk assessment purposes the LLNA data alone should not be relied upon.

Programme The published literature on chemistry-potency trends (including SAR and QSAR) for human and GP sensitisation will be reviewed, and in each case a check will be made as to which if any of the chemicals have also been tested in the LLNA. Having these LLNA data, chemistry-potency trends in humans and GP will be compared with those found in the LLNA. Where the trends are dissimilar, the implications for the LLNA applicability domain will be presented. In the course of this exercise it is likely that "chemical space gaps" will be identified – i.e. classes of chemicals whose potency is well characterised in the GP, and possibly also in humans, but for which no LLNA data have been reported. For new chemicals in these classes, the LLNA will not be fully reliable.

Relative Human Skin Sensitising Potency vs LLNA

Basketter et al. defined 6 human potency classes ranging from 1 (most potent) to 6 (non sensitiser). We have started to compare these classes with EC3 ranges for chemicals with both LLNA and human potency data.

There is a trend between NOEL and LLNA, and there is clearly a trend between human class and EC3, but with a substantial spread and overlap of EC3 values between human classes. Nevertheless, the mean EC3 value in each human class is an increasing function of the human class.

Basketter et al. (2014) Dermatitis 25(1), 11-21.

Task 2. Characterise quantitatively the mechanistic domains (as defined by Aptula and Roberts, 2006) that have been already established qualitatively. For each of the mechanistic domains that are covered in the LLNA validation dataset, the range of hydrophobicity and reactivity (where possible to identify) will be determined.

Purpose of this task: A new chemical whose properties establish it as within the ranges of one the established mechanistic domains covered in the LLNA validation dataset can be considered well modelled, in terms of sensitisation potency, by the LLNA, and consequently well modelled by a non-animal model that correlates with the LLNA for that mechanistic domain.

Programme The first action under this objective will be to perform a mechanistic applicability domain classification of the original dataset used for the validation of the LLNA in 1999. This will identify the range of reaction mechanistic classes, and the range of relevant physicochemical parameters for each mechanism, that are covered in the original validation dataset. This having been done, the next stage will be to analyse the currently published "gold standard" LLNA datasets (Gerberick et al, 2005, Kern et al, 2010, Natsch et al, 2009; Emter et al, 2010), making an assessment of the extent to which the new datasets are inside or outside the original validation ranges.
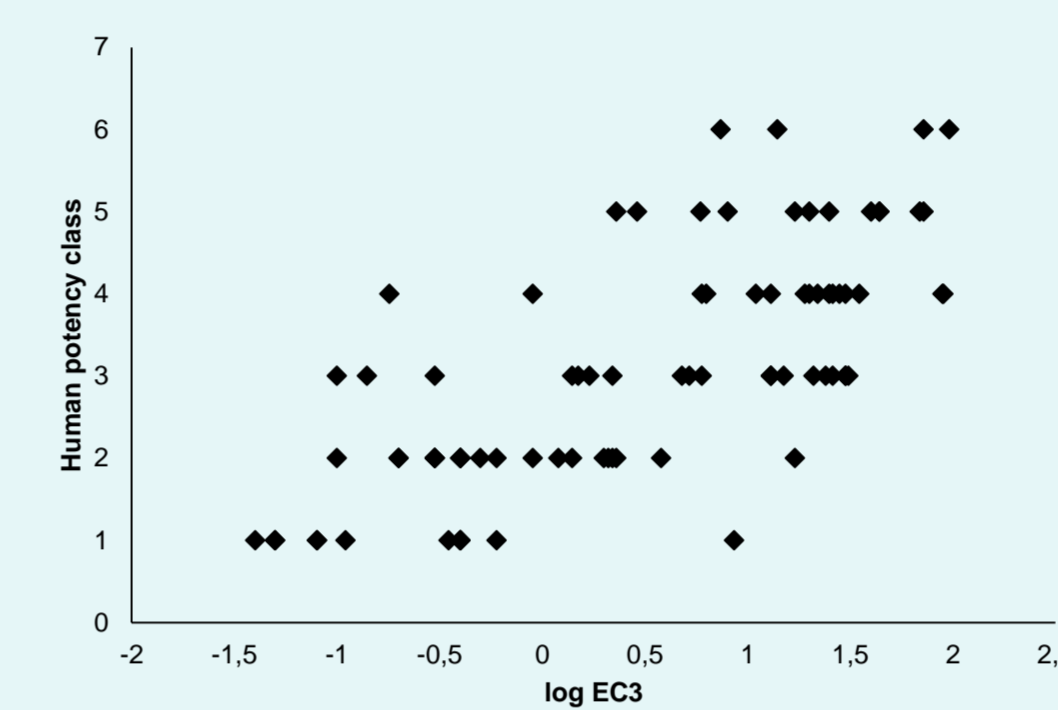
Importance of Chemical Domains

Skin sensitisers act by initial covalent reaction with protein nucleophiles. Most skin sensitisers therefore act as electrophiles or become activated to electrophiles in the skin.

There are 5 major reaction mechanistic domains, as shown here.

Depending on the domain various quantitative relationships between potency and chemistry can be found.

Task 3. Review how well the known bioactivation processes are represented in the LLNA and how they are aligned with human and GP data.

Purpose of this task: The major bioactivation processes known in skin sensitisation are oxidative, and these are expected to apply to LLNA, human and GP data. However, confirmation of this expectation is required. There are other bioactivation processes that also need to be considered – in particular dehydrohalogenation, sulphation etc.
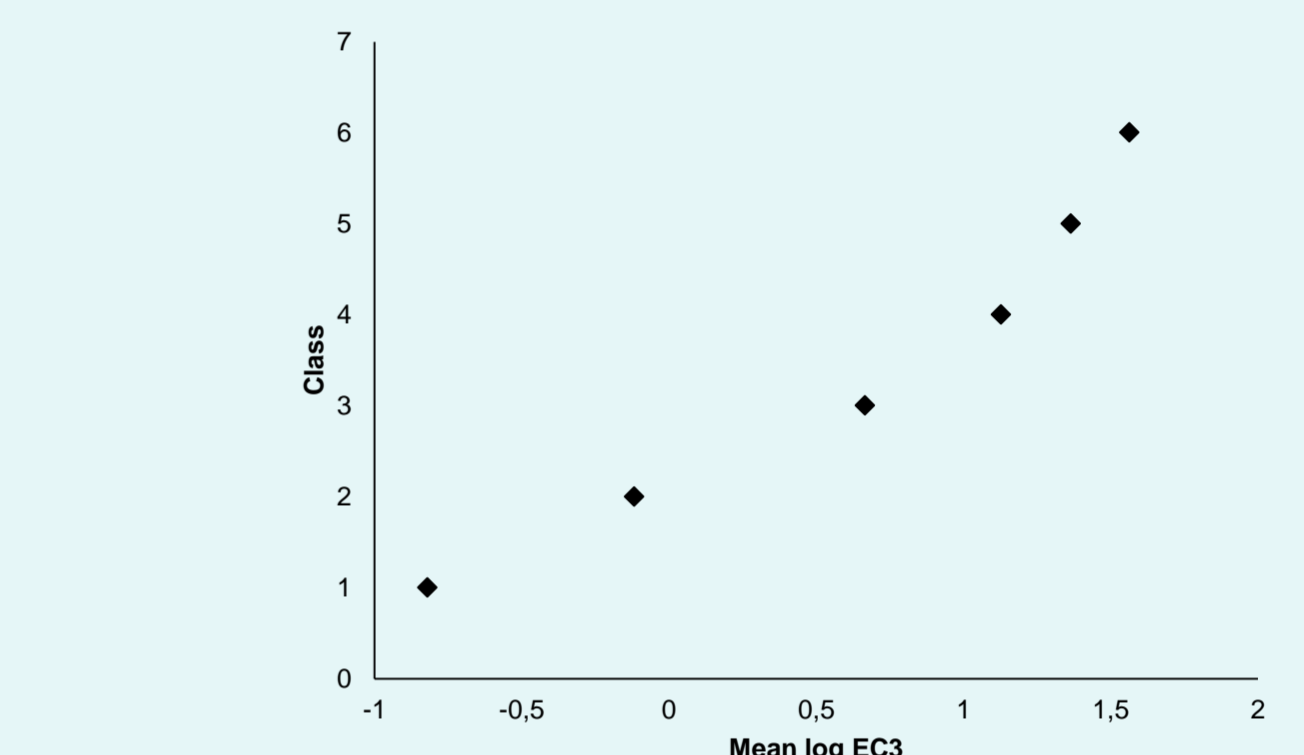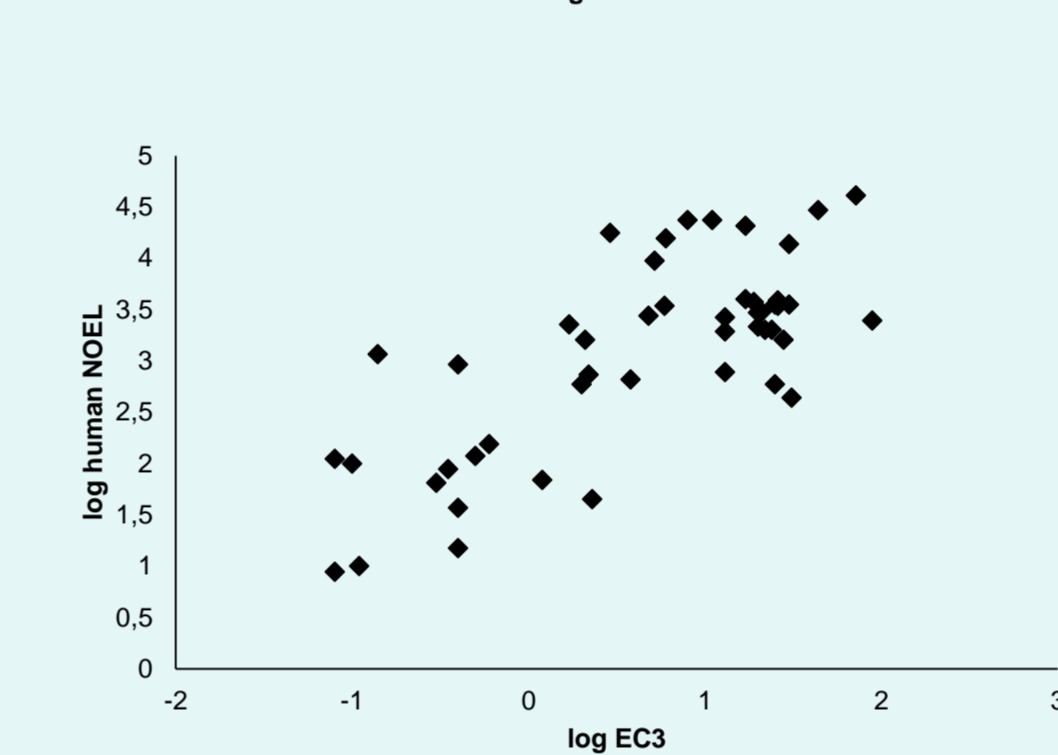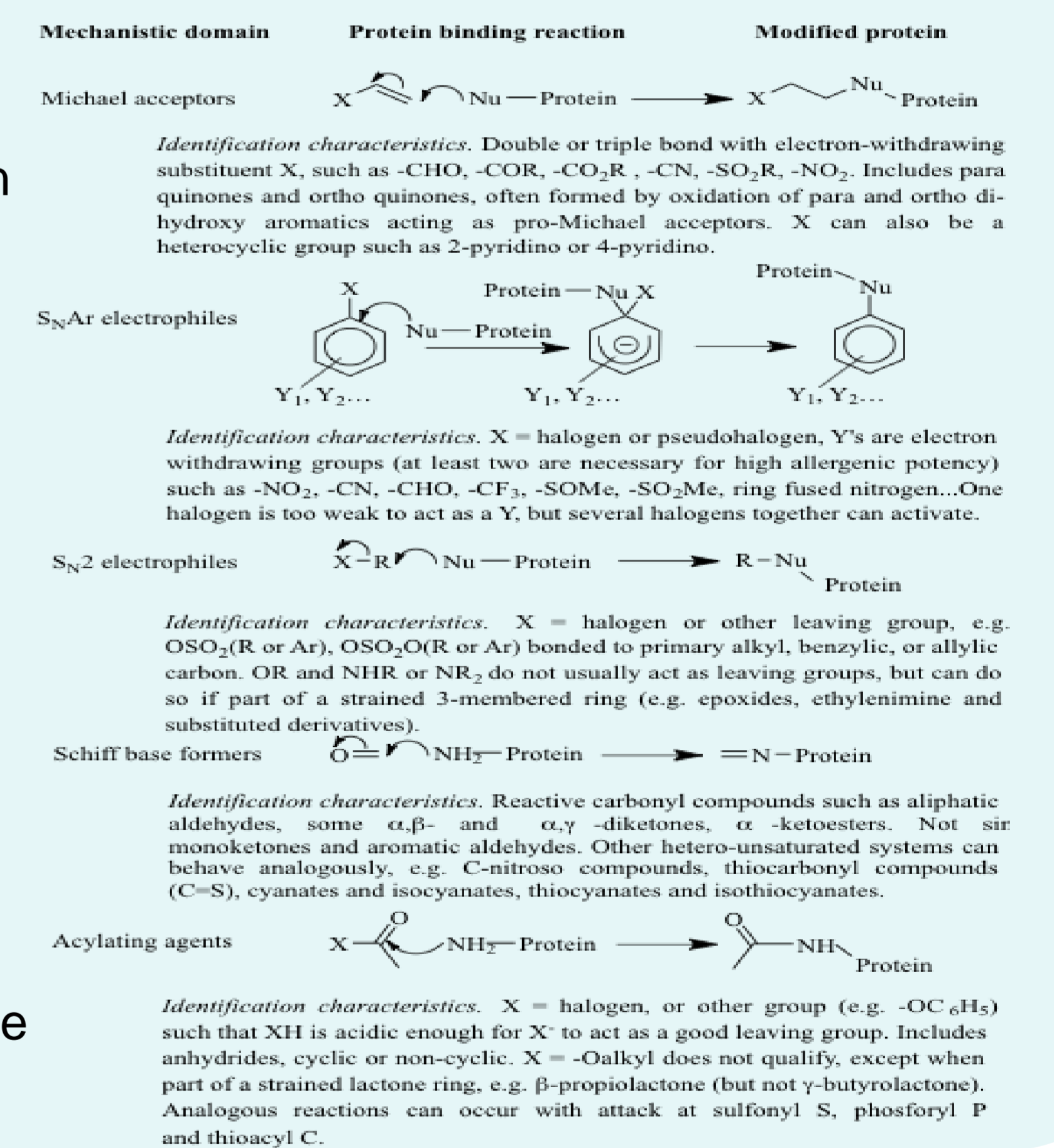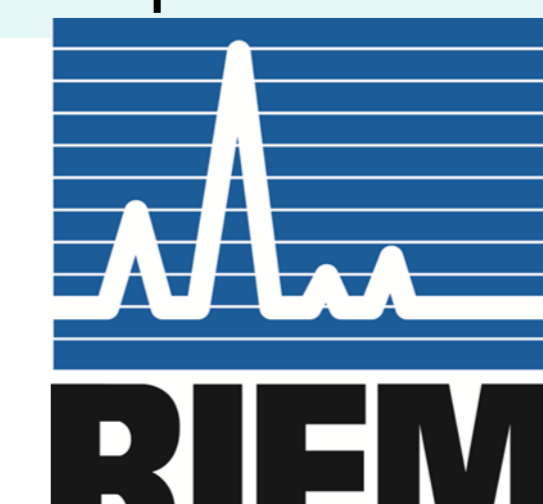
Programme This relates to the pro-hapten type of sensitisers, i.e. chemicals that are not directly reactive but can be converted in cutaneo to protein reactive species. In previous published reaction mechanistic domain classification exercises such compounds have been assigned to the mechanistic domains of their activated derivatives (e.g. hydroquinone is included in the Michael acceptor domain because it can be activated in cutaneo to benzoquinone, which is known to react as Michael acceptor). To address this task, the literature will be analysed to search for commonalities and differences for chemicals that are not directly reactive but are sensitisers in at least one test system.

Task 4. Evaluate the ranges of key physicochemical properties that are covered by the LLNA, in particular hydrophobicity values as modelled by LogP (octanol/water). Molecular Weight (MW) is often considered to be important for sensitisation potential, and recent publications on this topic will be considered in this exercise.

Purpose of this task: New chemicals that are outside these ranges are strictly outside the validation range of the LLNA and consequently the results of LLNA testing or prediction for such compounds will be less reliable. However, it is possible that new data (e.g. from the ECHA REACH dissemination database) may enable us to extend the range. The effects of volatility and other properties that may impact sensitisation potential will also be considered.

Programme A search will be made using the OECD Toolbox with particular focus on the ECHA REACH dissemination data that has been made available within the Toolbox in order to identify other substances for which both LLNA and human and/or GP data are available. Depending on the availability of data, this may facilitate an extension of the range of physicochemical properties for which the LLNA is applicable, refining the scope of the reaction chemistry mechanisms addressed by the LLNA or the bioactivation processes evaluated in tasks 2 and 3.

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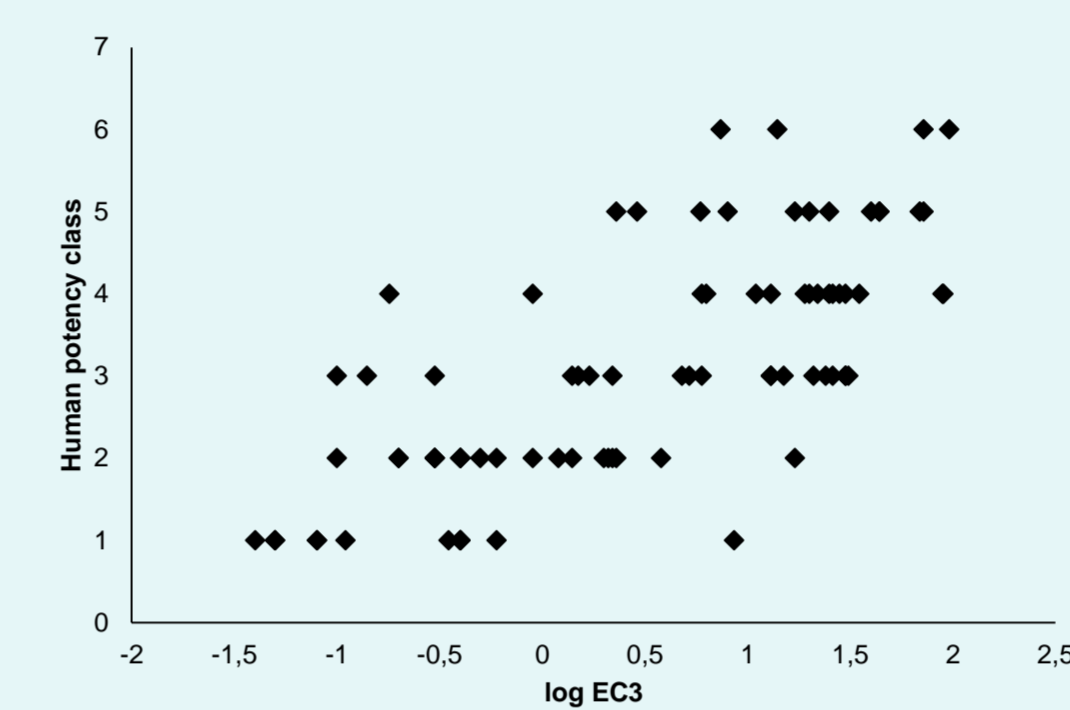
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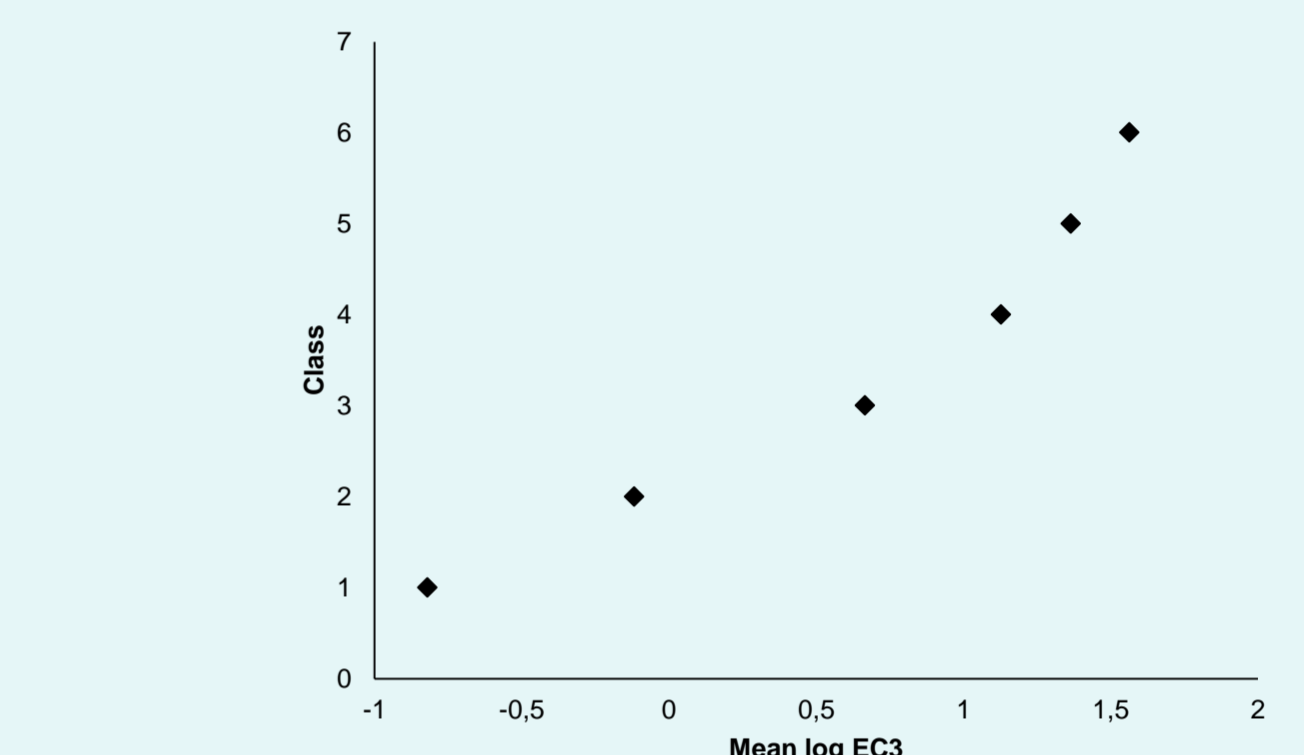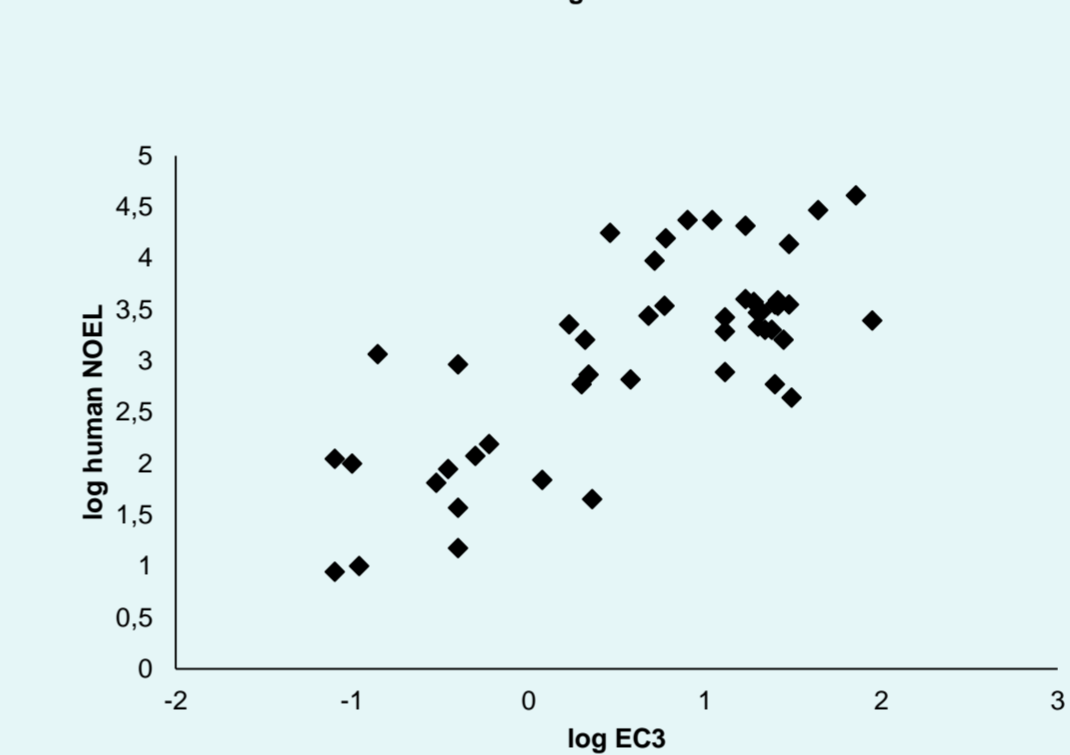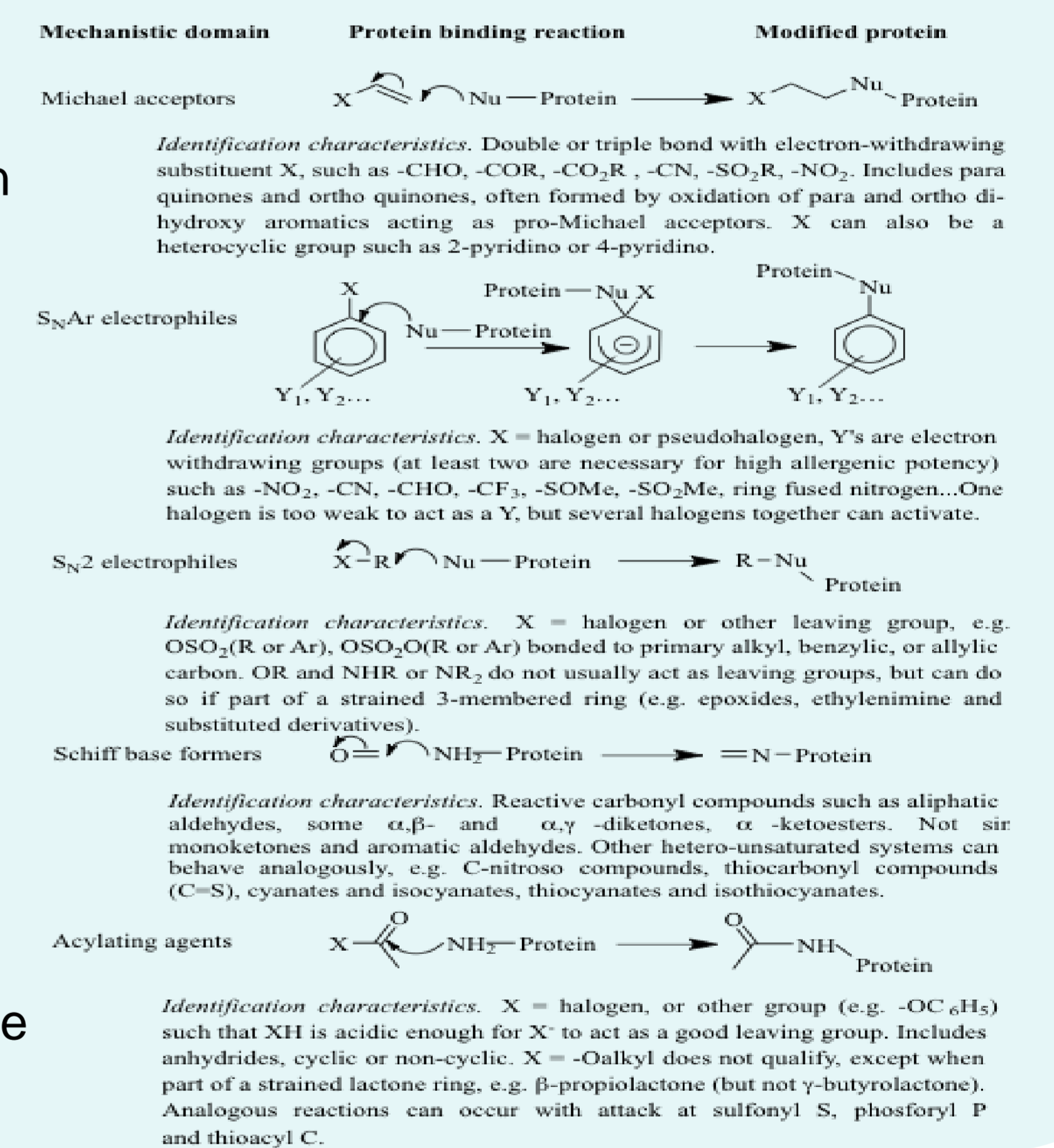
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