HOW FAR DO CURRENT IN VIVO AND IN VITRO METHODS INFORM ON THE TRANSFORMATION OF PRE/PRO HAPTENS TO HAPTENS?

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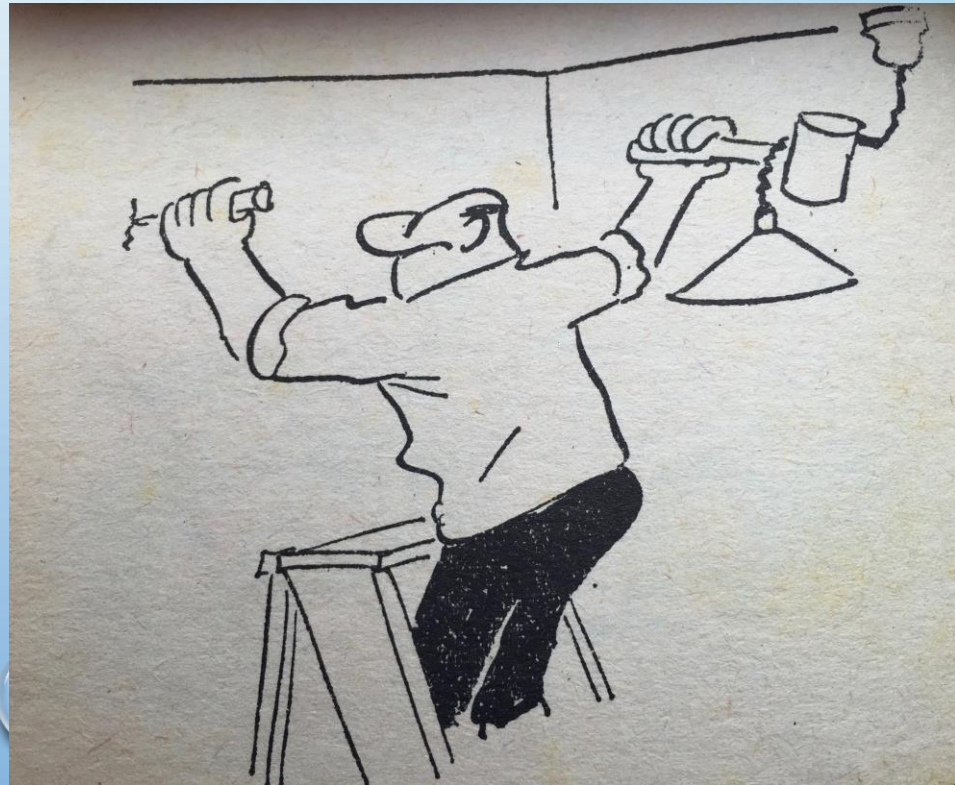
ARE PRE/PRO HAPTENS AN ISSUE?

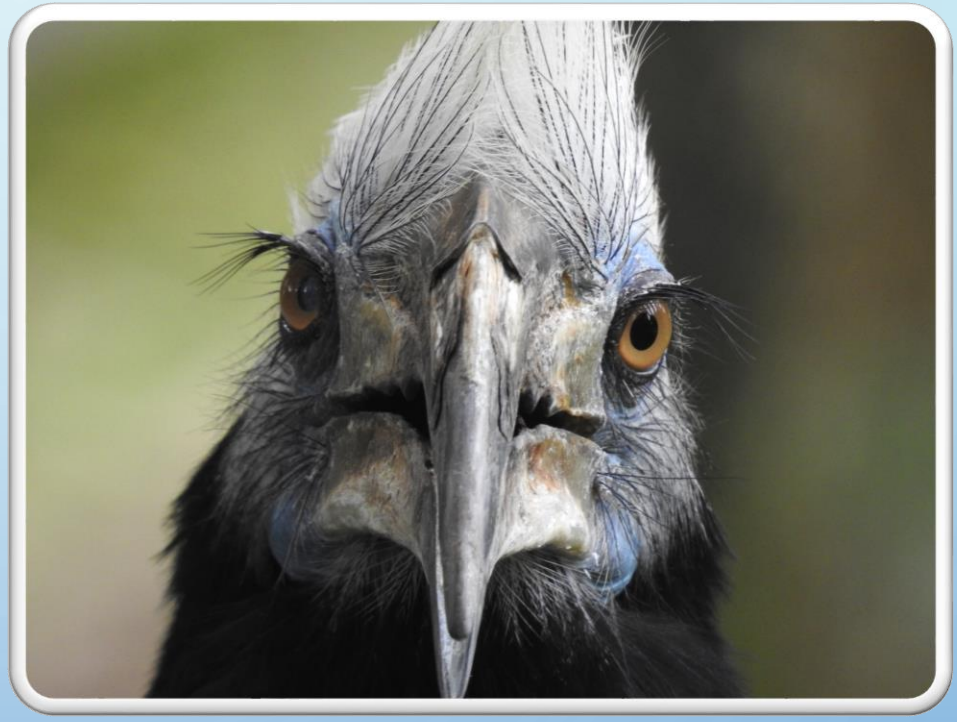
- GUINEA PIG METHODS
  - SUBSTANCES ORIGINALLY DESCRIBED AS “PRO-HAPTENS” WERE IDENTIFIED VIA POSITIVE DATA FROM “NON-REACTIVE” CHEMICALS
- MURINE LLNA
  - “ALL” SUPPOSED PRE/PRO HAPTENS HAVE BEEN FOUND POSITIVE
- IN VITRO
  - A FAIR PROPORTION OF PRE/PRO HAPTENS ARE POSITIVE, SUCH THAT SEVERAL PROs ARE NOW SUSPECT PREs

- IN VIVO – POSITIVE – PERFORM RISK ASSESSMENT
- IN VITRO – POSITIVE – USE RISK ASSESSMENT ABOVE
- ALL MANAGED WITHOUT ANY KNOWLEDGE OF THE TRUE HAPTEN

EUGENOL



LET'S EXAMINE THE PERFORMANCE OF THE LLNA



- 319 SUBSTANCES (GERBERICK ET AL, 2005 AND KERN ET AL, 2010)

- OF THESE 60 (19%) ARE REPORTED AS PRE OR PRO HAPTENS…

- …AND OF THESE, ALL EXCEPT TWO WERE POSITIVE (97% ACCURACY)

- I'VE DELIBERATELY NOT NOTED WHICH THEY WERE TO ENCOURAGE FOCUS ON SUCCESS RATHER THAN FAILURE!

Table 3. Chemicals That Are Pro-electrophiles or Pre-electrophiles*

Chemical Name	*CAS No.*
Aniline	62-53-3
Anisyl alcohol	105-13-5
Atranol	526-37-4
trans-Anethole	104-46-1
Bandrowski's base	20048-27-5
(+/−) Linalool	78-70-6
1,2-Dibromo-2,4-dicyanobutane	35691-65-7
1,3-Phenylenediamine	108-45-2
1,3-Bis-(2,4-diaminophenoxy)-propane	74918-21-1
1,4-Phenylenediamine	106-50-3
1-Amino-2-nitro-4-bis-(2-hydroxyethyl)-amino-benzol	29705-39-3
1-Naphthol	90-15-3
2-Amino-6-chloro-4-nitrophenol	6358-09-4
2-Aminophenol	95-55-6
2-Mercaptobenzoxazole	2382-96-9
2-Methoxy-4-methylphenol	93-51-6
2-Methyl-5-hydroxyethylaminophenol	55302-96-0
2-Nitro-p-phenylenediamine	5307-14-2
2,4-Diaminophenoxyethanol dihydrochloride	66422-95-5
2,5-Diaminotoluene sulfate	615-50-9
2,5-Diaminotoluene	95-70-5
3,5-Diamino-2,6-dimethoxypyridine-dihydrochloride	56216-28-5
3-Aminophenol	591-27-5
3-Bromomethyl-5,5-dimethyl-dihydro-2(3H)-furanone	154750-20-6
3-(Dimethylamino)propylamine	109-55-7
3-Methylisoeugenol	186743-29-3
3-Methyleugenol	186743-26-0
4-Allylanisole	140-67-0
4-Amino-3-methyl phenol	2835-99-6
4-Amino-3-nitrophenol	610-81-1
5-Amino-2-methyl phenol	2835-95-2
5-Methyleugenol	186743-25-9
4-Nitro-benzene-1,2-diamine	99-56-9
4-([2-Hydroxyethyl]amino)-3-nitrophenol	65235-31-6
4-(N-ethyl-N-2-methan-sulfonamido-ethyl)-2-methyl-1,4-phenylenediamine	25646-71-3
6-Methylisoeugenol	13041-12-8
6-Methyleugenol	186743-24-8
7,12-Dimethylbenz(a)anthracene	57-97-6
Abietic acid	514-10-3
Benzo(a)pyrene	50-32-8
Cinnamyl alcohol	104-54-1
Chloroatranol	57074-21-2
Diethylenetriamine	111-40-0
Dihydroeugenol	2785-87-7
Ethylenediamine	107-15-3
Eugenol	97-53-0
Geraniol	106-24-1
HC Red No. 3	2871-01-4
Hydroquinone	123-31-9
Hydroxytyrosol	10597-60-1
Isoeugenol	97-54-1
Isopropyl isoeugenol	186743-30-6
Lauryl gallate	1166-52-5
Metol	55-55-0
N,N-Dibutylaniline	613-29-6
Pentachlorophenol	87-86-5
Resorcinol	108-46-3
R(+)-Limonene	5989-27-5
R-Carvoxime	(Not known)

Table 3. Continued

CAS = Chemical Abstracts Service.
*Collated from both local lymph node assay data sets.

Kern et al, 2010, Dermatitis, 21, 8-32

IN VITRO

- UNTIL THE ECVAM REVIEW THERE WAS NO INDEPENDENT/SYSTEMATIC ANALYSIS
- HOWEVER, A RANGE OF COMMONLY REPORTED PRE AND PRO HAPTENS HAVE BEEN TESTED
- FOR EXAMPLE, NATSCH ET AL, IN 2014 REPORTED ON 145 SUBSTANCES: OF 22 SUSPECTED PRE/PROHAPTENS 17 (77%) WERE POSITIVE USING THE "DEMOCRACY" MODEL
- (REMINDER: ANALYSIS OF INDIVIDUAL ASSAYS IS ENCOURAGED **ONLY** FOR UNDERSTANDING APPLICABILITY DOMAIN COMPLEMENTARITY!)

THE ECVAM WORK (1 YEAR AGO)

ARTICLE IN PRESS

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Workshop report

Can currently available non-animal methods detect pre and pro-haptens relevant for skin sensitization?

Grace Patlewicz [a, *], Silvia Casati [b], David A. Basketter [c], David Asturiol [b], David W. Roberts [d], Jean-Pierre Lepoittevin [e], Andrew P. Worth [b], Karin Aschberger [b]

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THE ECVAM WORK (1 YEAR AGO)

- THE CONCLUSION WAS THAT THE IN VITRO APPROACHES WORK.
- THIS AGREES WITH URBISCH ET AL, 2016 IN CHEM RES TOXICOL.

ABSTRACT

Predictive testing to characterize substances for their skin sensitization potential has historically been based on animal tests such as the Local Lymph Node Assay (LLNA). In recent years, regulations in the cosmetics and chemicals sectors have provided strong impetus to develop non-animal alternatives. Three test methods have undergone OECD validation: the direct peptide reactivity assay (DPRA), the KeratinoSens™ and the human Cell Line Activation Test (h-CLAT). Whilst these methods perform relatively well in predicting LLNA results, a concern raised is their ability to predict chemicals that need activation to be sensitizing (pre- or pro-haptens). This current study reviewed an EURL ECVAM dataset of 127 substances for which information was available in the LLNA and three non-animal test methods. Twenty eight of the sensitizers needed to be activated, with the majority being pre-haptens. These were correctly identified by 1 or more of the test methods. Six substances were categorized exclusively as pro-haptens, but were correctly identified by at least one of the cell-based assays. The analysis here showed that skin metabolism was not likely to be a major consideration for assessing sensitization potential and that sensitizers requiring activation could be identified correctly using one or more of the current non-animal methods.

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"...sensitisers requiring activation could be identified correctly..."

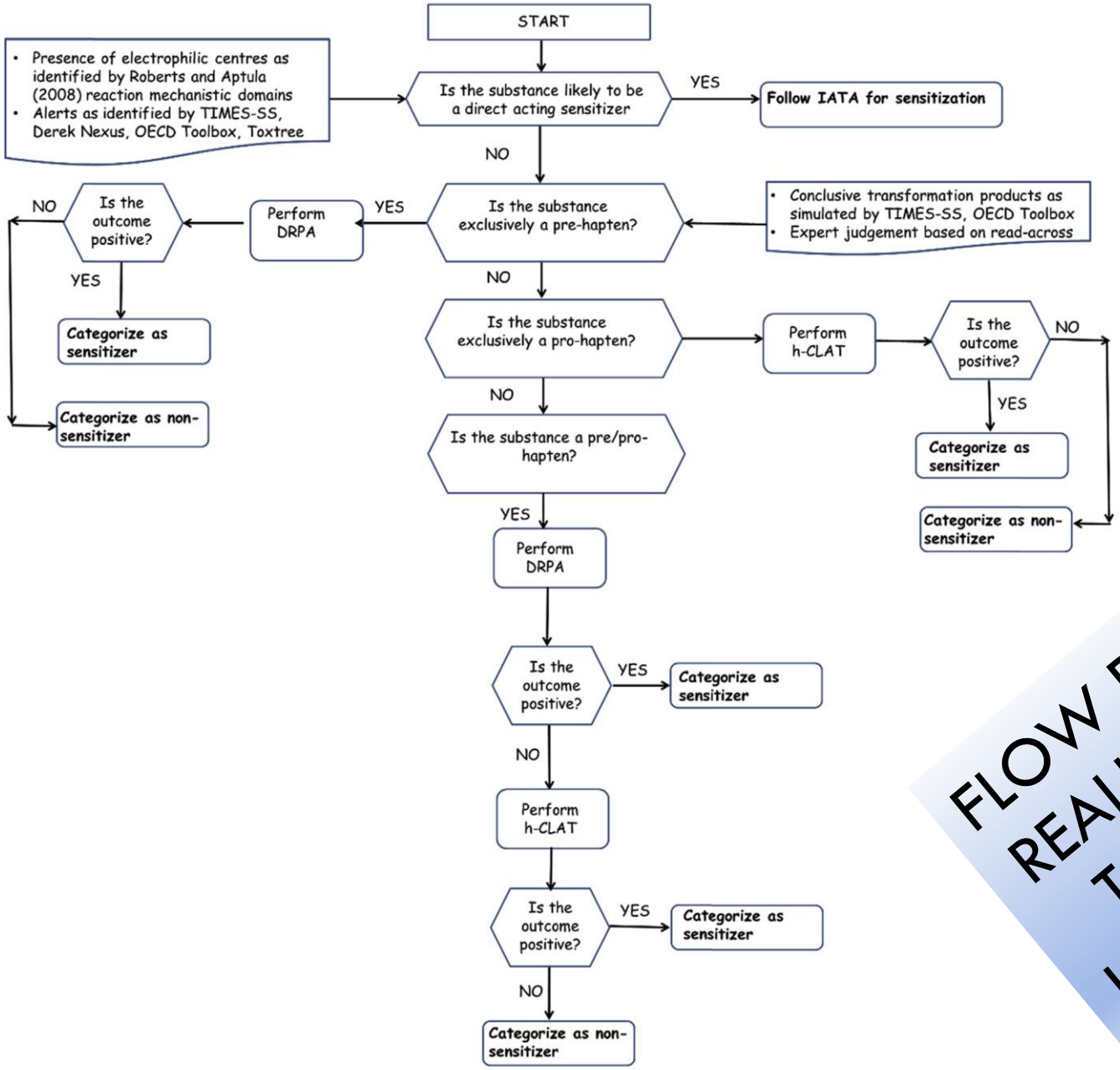

Fig. 1. Workflow summarizing possible testing and assessment strategies to address indirectly acting sensitizers.

FLOW DIAGRAMS REALLY DO MAKE TOXICOLOGY LOOK COMPLEX!!

Chemical Research in Toxicology

Article

pubs.acs.org/crt

Assessment of Pre- and Pro-haptens Using Nonanimal Test Methods for Skin Sensitization

Daniel Urbisch,[†] Matthias Becker,[†] Naveed Honarvar,[†] Susanne Noreen Kolle,[†] Annette Mehling,[‡] Wera Teubner,[§] Britta Wareing,[†] and Robert Landsiedel*[,†]

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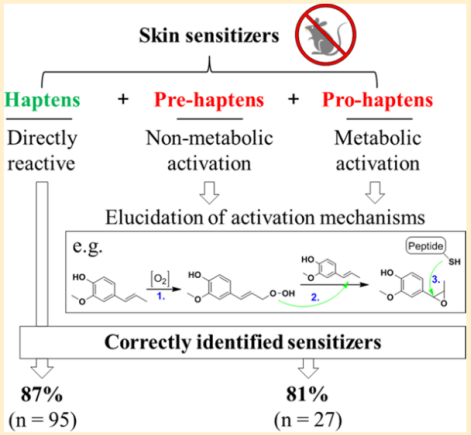
Supporting Information

ABSTRACT: Because of ethical and regulatory reasons, several nonanimal test methods to assess the skin sensitization potential of chemicals have been developed and validated. In contrast to *in vivo* methods, they lack or provide limited metabolic capacity. For this reason, identification of pro-haptens but also pre-haptens, which require molecular transformations to gain peptide reactivity, is a challenge for these methods. In this study, 27 pre- and pro-haptens were tested using nonanimal test methods. Of these, 18 provided true positive results in the direct peptide reactivity assay (DPRA; sensitivity of 67%), although lacking structural alerts for direct peptide reactivity. The reaction mechanisms leading to peptide depletion in the DPRA were therefore elucidated using mass spectrometry. Hapten−peptide adducts were identified for 13 of the 18 chemicals indicating that these pre-haptens were activated and that peptide binding occurred. Positive results for five of the 18 chemicals can be explained by dipeptide formations or the oxidation of the sulfhydryl group of the peptide. Nine of the 27 chemicals were tested negative in the DPRA. Of these, four yielded true positive results in the keratinocyte and dendritic cell based assays. Likewise, 16 of the 18 chemicals tested positive in the DPRA were also positive in either one or both of the cell-based assays. A combination of DPRA, KeratinoSens, and h-CLAT used in a 2 out of 3 weight of evidence (WoE) approach identified 22 of the 27 pre- and pro-haptens correctly (sensitivity of 81%), exhibiting a similar sensitivity as for directly acting haptens. This analysis shows that the combination of *in chemico* and *in vitro* test methods is suitable to identify pre-haptens and the majority of pro-haptens.

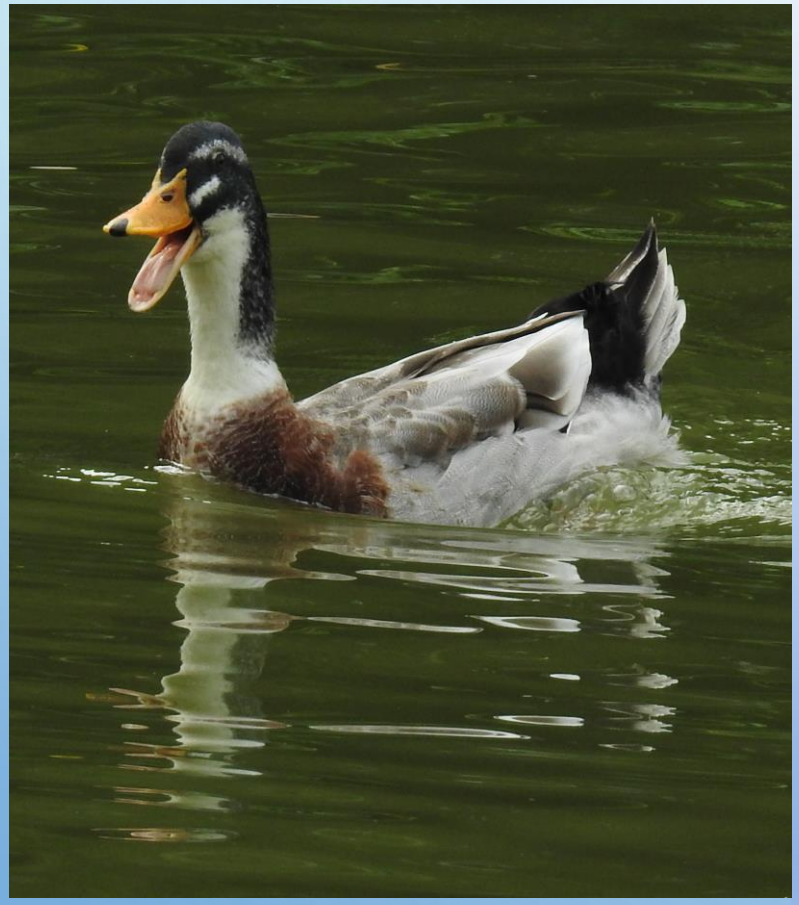


77%

79%

81%

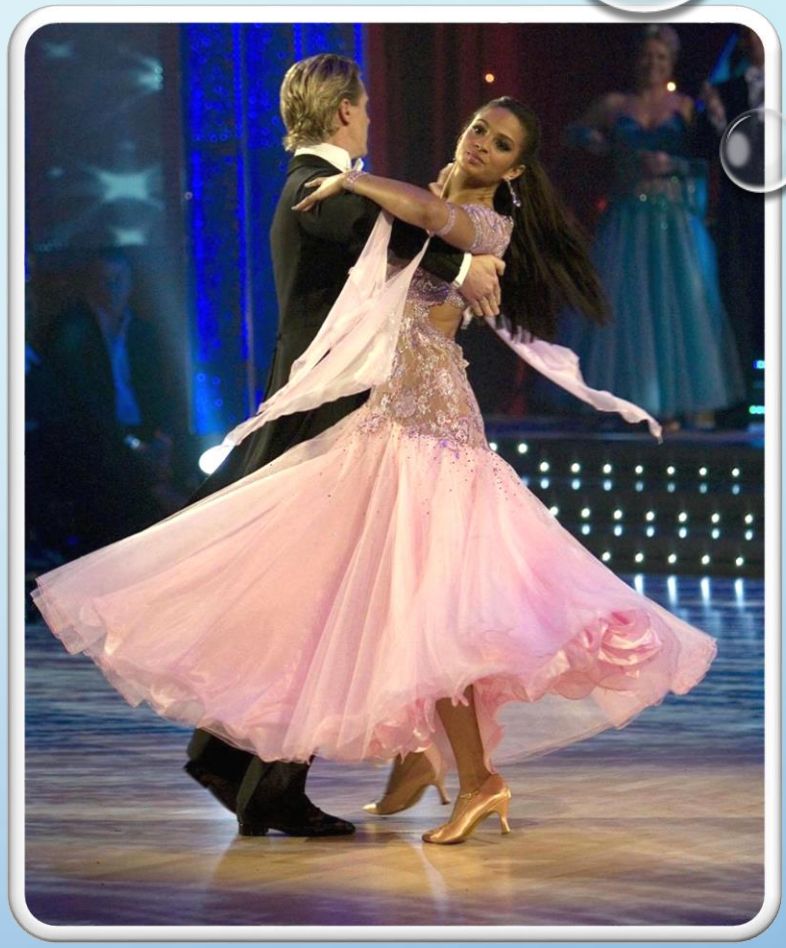
"INFORM?"



- USED IN ISOLATION, MAMMALIAN TESTS TELL US NOTHING – THEY DO NOT INFORM US ABOUT POTENTIAL PRE AND/OR PRO HAPTEN STATUS

- I CONCLUDE SIMILARLY FOR IN VITRO METHODS, EXCEPT:
  - A NEGATIVE DPRA IN ASSOCIATION WITH TWO POSITIVE CELL TESTS *COULD* SUGGEST A PRO HAPTEN
  - THERE ARE 3 EXAMPLES IN PATLEWICZ ET AL, 2016, INCLUDING ETHYLENEDIAMINE, DMAPA AND DIHYDROEUGENOL
  - A POSITIVE DPRA WITH A NON-ELECTROPHILE COULD BE FURTHER EXPLORED TO IDENTIFY ADDUCTS

SLOW, SLOW, QUICK QUICK, SLOW….

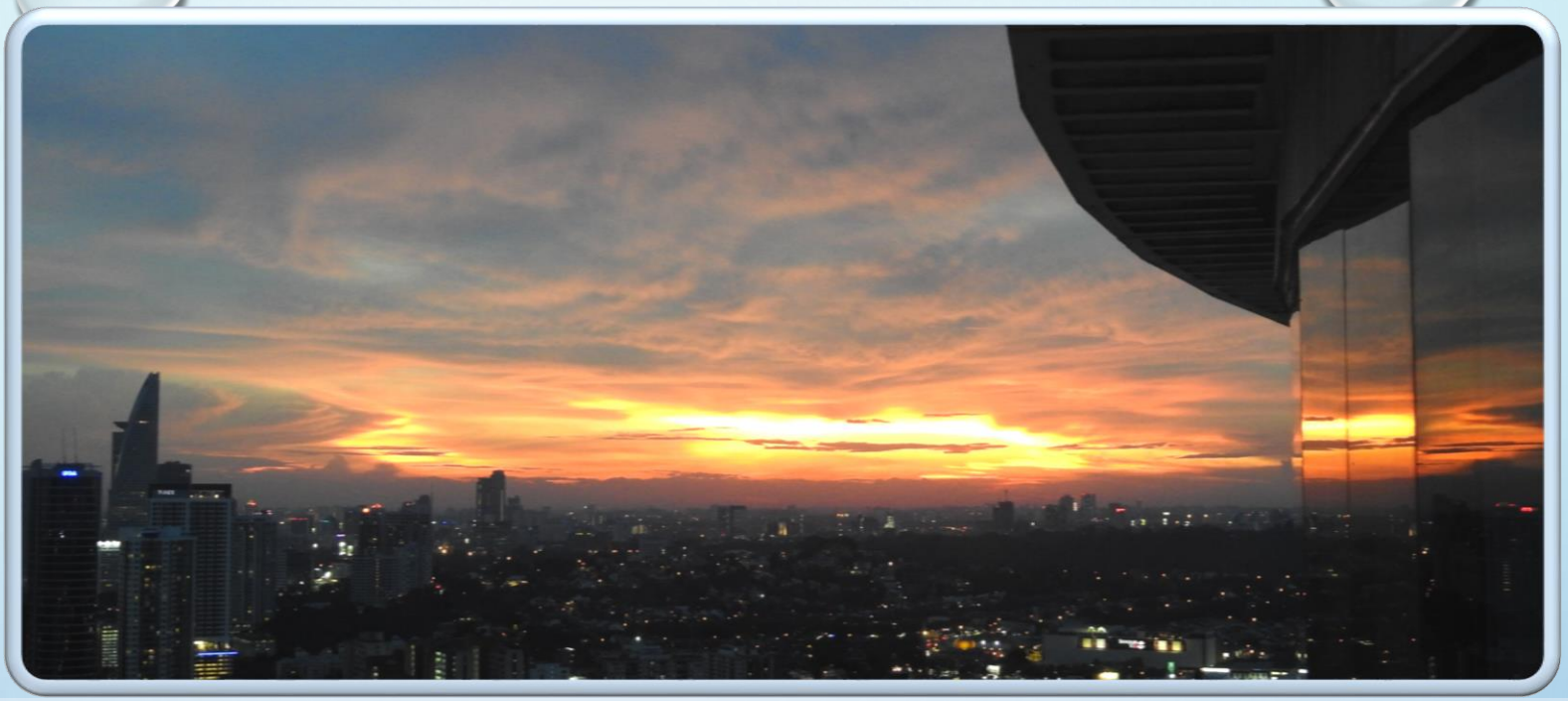


- SUBSTANCES THAT OXIDISE QUICKLY TO PRODUCE SKIN SENSITISERS ARE IDENTIFIED IN PREDICTIVE TESTS *(AT LEAST WITH THE AID OF AN IATA)*

- SUBSTANCES WHICH OXIDISE SLOWLY TO GIVE SENSITISING SPECIES MAY BE IMPORTANT CLINICALLY, BUT WE LACK A SYSTEM FOR THEIR PREDICTIVE IDENTIFICATION…

- …WHICH MEANS THAT WE MUST IDENTIFY THESE MATERIALS FROM CLINICAL C(L)UES AND THEN ***USE*** THE INFORMATION TO REFINE OUR SCIENCE AND/OR RISK MANAGEMENT

- **PERHAPS IT'S THE SLOW OXIDISERS THAT ARE THE REAL PROBLEM TO BE FACED BY RISK ASSESSMENT**



WHAT MIGHT WE CONCLUDE?

1. NON-ANIMAL IATAs CAN IDENTIFY PRE/PRO HAPTENS
2. WITHOUT OTHER INPUTS, THE FACT THAT CHEMICALS MAY BE PRE AND/OR PRO HAPTENS IS OCCULT
3. SLOWLY OXIDISING HAPTENS REMAIN AN ISSUE
4. WE MUST DECIDE IS WHETHER THE STATUS QUO IS OK