PRO-HAPTENS: ONE VIEW

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BACK IN THE GOOD OLD DAYS...

- In the 1930s, Landsteiner, Chase and Jacobs worked with directly acting haptens to explore a variety of aspects of skin sensitisation, contact allergy and ACD.

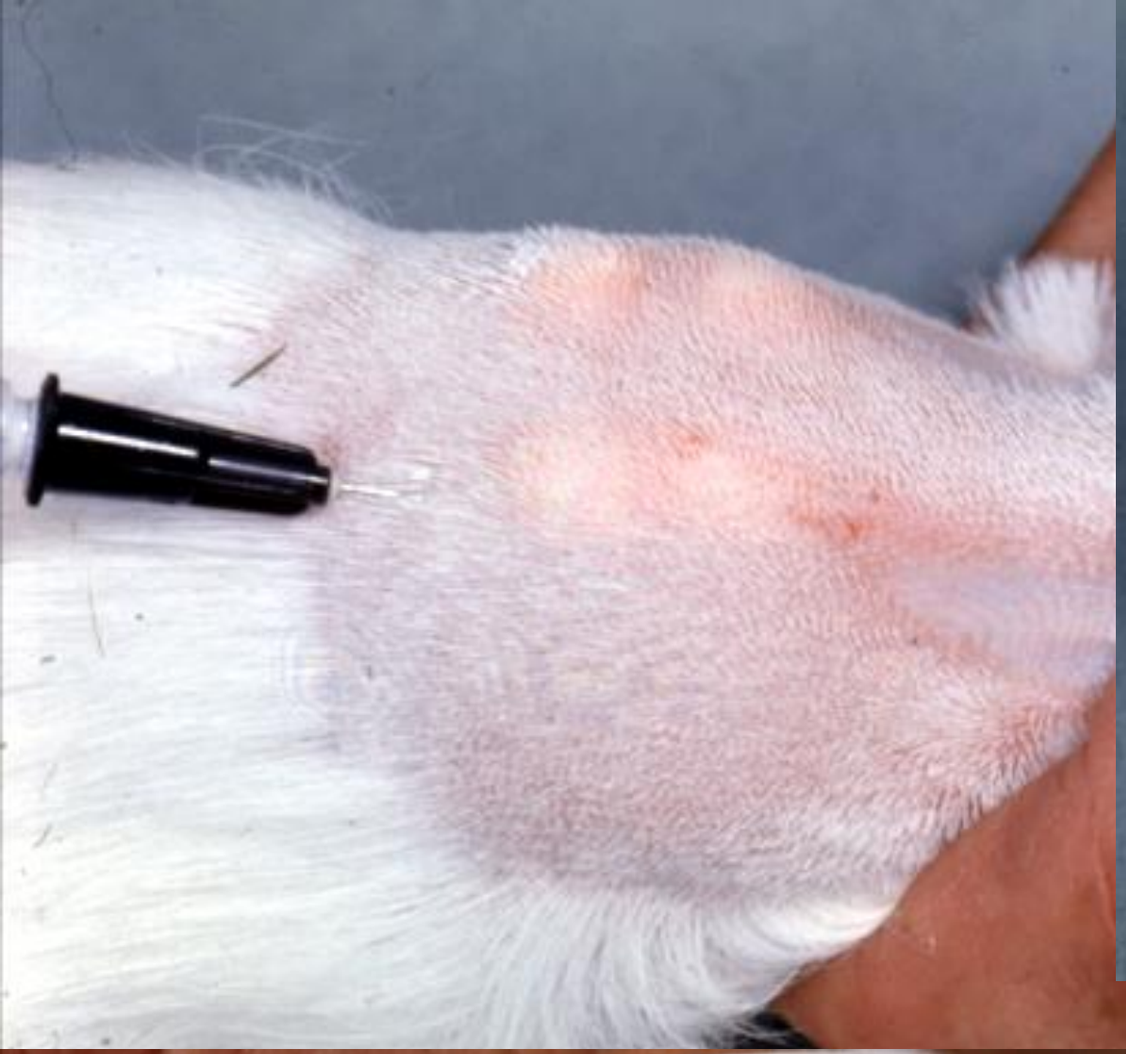
- In the 1954, Mayer penned a huge treatise on quinones which mentioned in vivo oxidation/reduction as a means to produce active sensitising species.

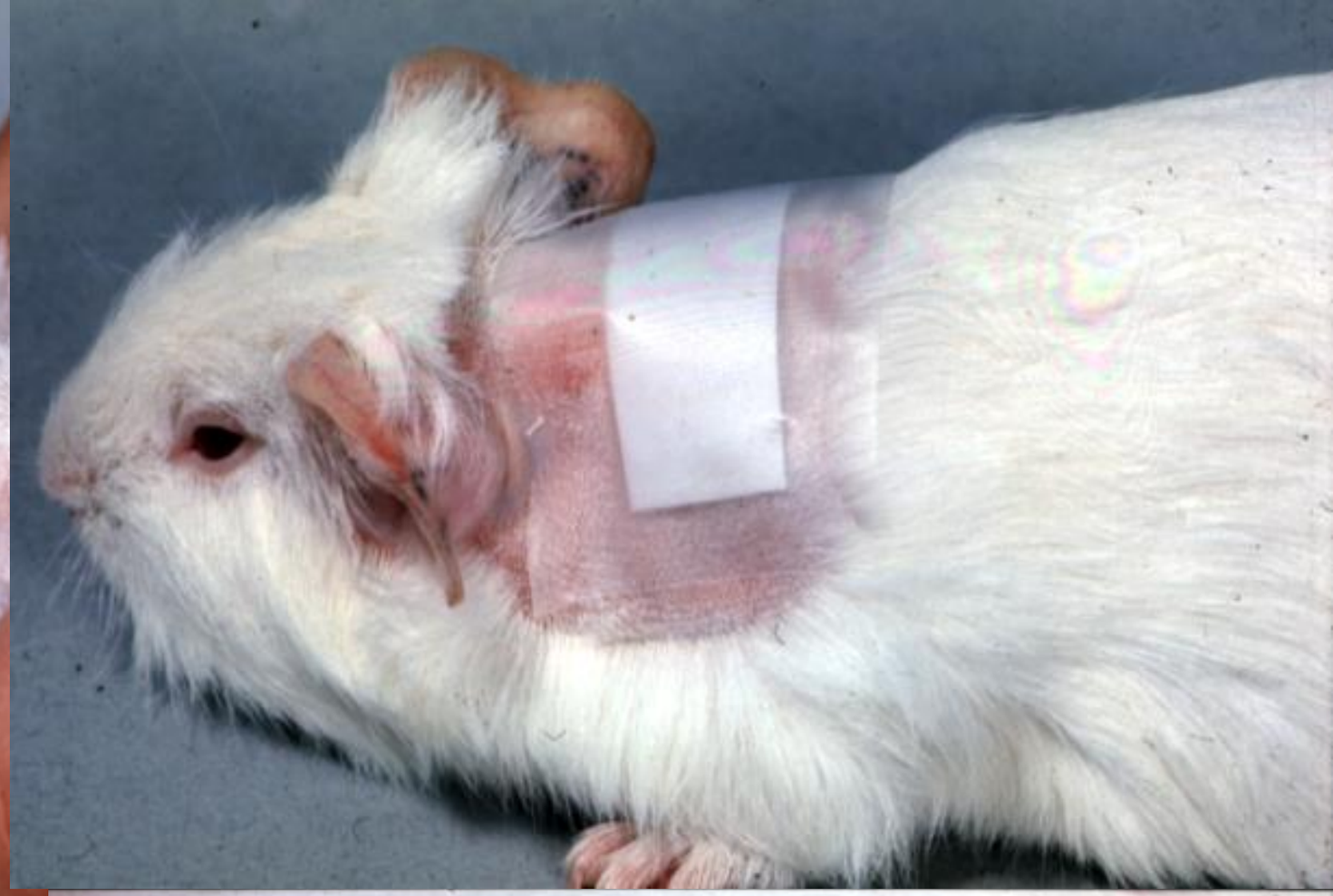
- Also in 1954, Baer published a review on cross sensitisation which also discussed in vivo transformation of topically applied chemicals.

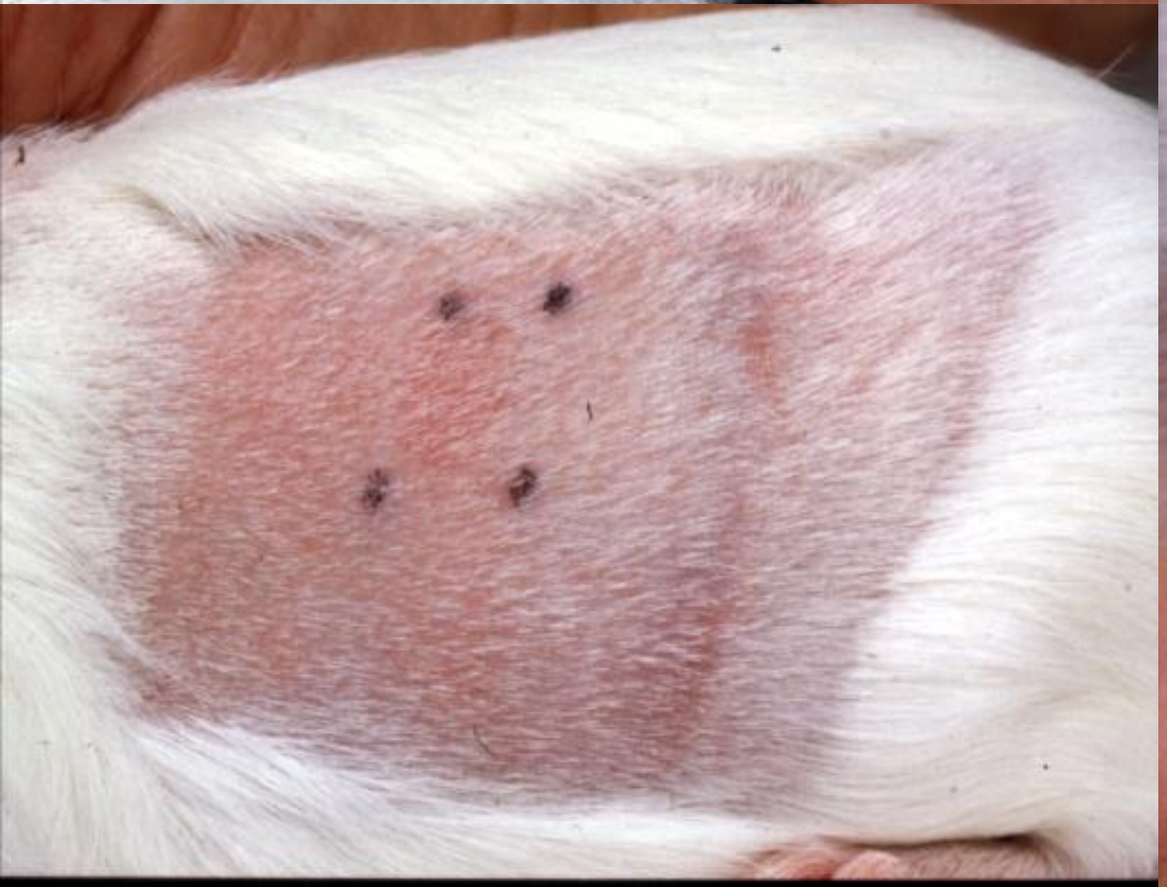
SO, PROHAPTENS ARE NOT EXACTLY A “NEW” IDEA!

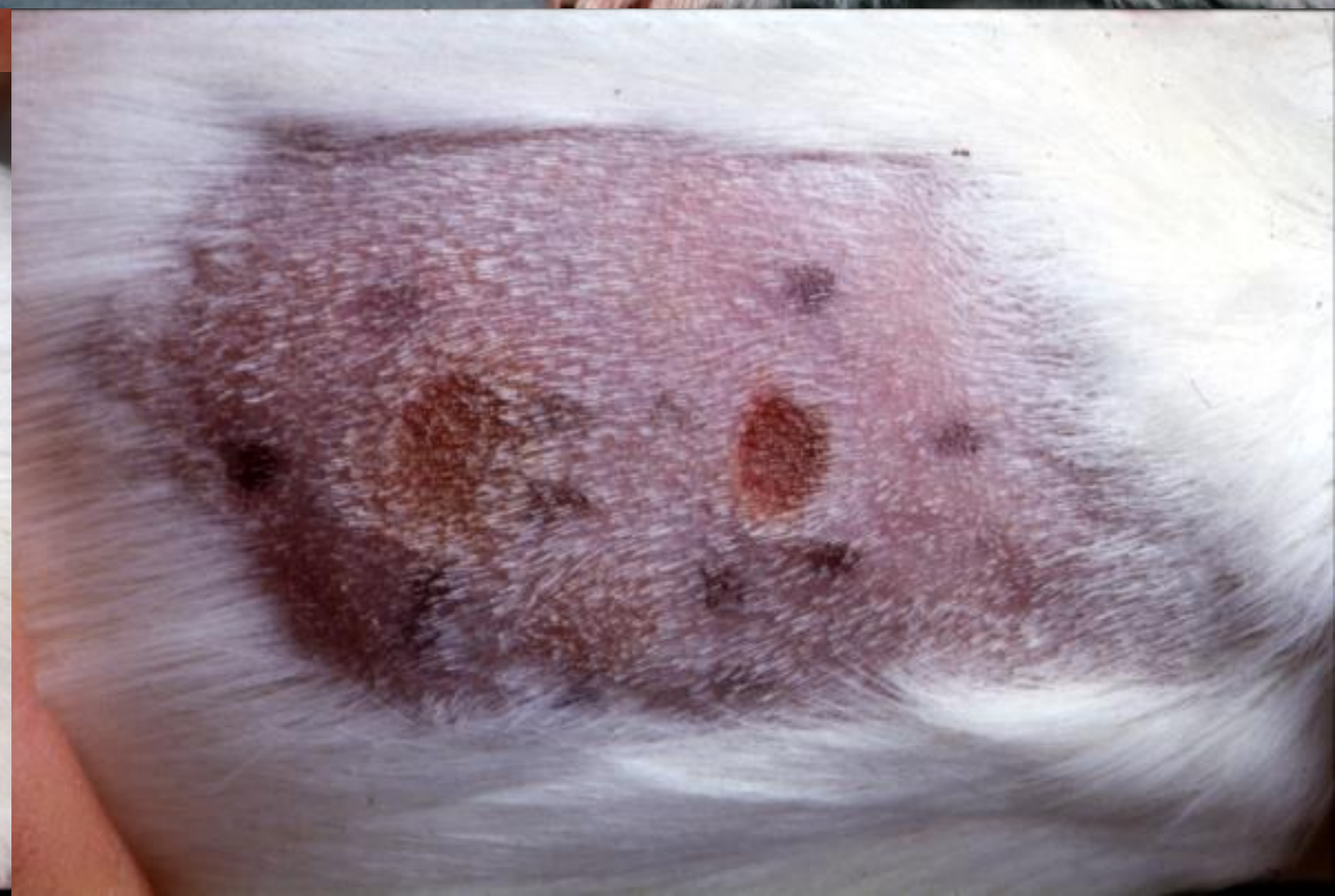
NOT QUITE SO LONG AGO

- In 1982, Dupuis and Benezra published a great book “ACD to Simple Chemicals: A Molecular Approach
- Chapter 6, pp 66-76 details the prohapten concept, gives potential examples and set me thinking about some of them









WHAT IDENTIFIES A CHEMICAL SENSITISER AS A PROHAPTEN

- Evidence/opinion that it is not a direct (re)acting chemical, i.e. an electrophile
- Evidence/opinion that is not susceptible to air oxidation to produce an electrophilic species
- Comparison with known activation systems, e.g. carcinogens, typically based on liver metabolism data
- An absence of any other explanation for its action

Characterisation of a contact allergen as a "prohapten" is a "diagnosis of exclusion"

THE QUESTION I ASKED ORIGINALLY AND STILL ASK TODAY IS "WHAT DO WE KNOW ABOUT THE REALITY IN MAN, AND HOW CAN WE INVESTIGATE THE TOPIC?"

AT THE TIME, MY IMPRESSION WAS THAT **ALL OF** OUR APPARENT KNOWLEDGE WAS BASED ALMOST ENTIRELY ON CHEMICAL THEORY.

THIS CENTURY

- COLIPA/Cosmetics Europe funded a work programme which delivered…Allergic Contact Dermatitis: Chemical and Metabolic Mechanisms, Smith and Hotchkiss, 2001
- J-PLeP and colleagues with studies on PPD
- ATK and colleagues, including new work on oximes and epoxy alcohols
- Efforts in relation to improved accuracy of in vitro methods

FROM THEORY…

- chemical reactivity
- liver enzymes (incl HLMs)
- skin enzymes (maybe)
- random human data

TO EXPERIMENT

A (MY) FIRST FAILURE

- To avoid substances of particular interest to my employer (Unilever), I elected to study 1,4-phenylene diamine (PPD).
- Theory said that oxidation led to 1,4-benzoquinone (BQ).
- However, studies in the guinea pig showed almost no cross reaction between PPD and BQ.
- Humans with contact allergy to PPD did not respond to BQ.
- (Humans also do not react to Bandrowski’s base.)

AND A SLIGHT SUCCESS

- The obvious mechanism for cinnamic alcohol to behave as a hapten is conversion by alcohol dehydrogenase in the skin to cinnamal.
- Evidence for conversion from the alcohol to the aldehyde was demonstrated using an epidermal homogenate.

...PLUS A SECOND FAILURE

- Investigations of the putative prohaptens eugenol and isoeugenol were undertaken in the mouse.
- The hypothesis was that inhibition of P4501A would reduce eugenol allergy, leaving isoeugenol unaffected, so efforts were made to modulate cytochromes P450.
- However, inhibition of P4501A distinctly enhanced the response to isoeugenol markedly compared to eugenol and potassium dichromate.

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...which maybe unsurprising since we now know that P450 enzymes are about 100 fold lower in skin compared to other enzymes. (van Eijl et al, 2012)

?HOW MANY PROHAPTENS?

- We used to suggest 30% (ACD: Chemical and Metabolic Mechanisms, Smith and Hotchkiss, 2001)
- Increasing evidence of the relative importance of air oxidation lowers this figure (e.g. work on geraniol from Karlberg and colleagues)
- Also, the evidence that some supposed “prohaptens” are positive in reactivity tests forces us to rethink
- Perhaps 10% is closer

IN VIVO PREDICTIVE TESTS

- In the guinea pig methods, it was simply assumed that the animal was a good model – the only real debate was on test sensitivity.
- With the mouse (LLNA), validation demonstrated that predictivity for the human hazard was acceptable (85%), but again no specific consideration was applied regarding metabolic differences.
- Both species did detect many substances believed not to be direct acting haptens.

WHAT IS THE CURRENT STATUS?

- A search on PubMed for "prohaptens and skin" yields only 25 hits; add "metabolism" and its falls to just 21
- Of these, just 2 involve research on which enzymes are involved in murine/human skin (in)activation of prohaptens
- Enzymes implicated: NADPH-dependent reductase and N-acetyl transferase
- NAT role is supported by work in 2009 from Blomeke and colleagues on NAT1 and 2 genotypes (fast v slow)

WHAT IS THE CURRENT STATUS?

- Other searching yields these:

  - Götz C, Pfeiffer R, Tigges J, Blatz V, Jäckh C, Freytag EM, Fabian E, Landsiedel R, Merk HF, Krutmann J, Edwards RJ, Pease C, Goebel C, Hewitt N, Fritsche E, Xenobiotic metabolism capacities of human skin in comparison with a 3D epidermis model and keratinocyte-based cell culture as in vitro alternatives for chemical testing: activating enzymes (Phase I). Exp Dermatol. 2012: 21: 358-363.

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  - van Eijl S, Zhu Z, Cupitt J, Gierula M, Götz C, Fritsche E, Edwards RJ. Elucidation of xenobiotic metabolism pathways in human skin and human skin models by proteomic profiling. PLoS One. 2012: 7(7): e41721.

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So, we have now a much better knowledge of the metabolic capabilities of skin, but that does not tell us which prohatpens are activated, nor how!

PROHAPTENS – THE HISTORY

chemistry of haptens

liver metabolic knowledge

reality

in vivo + clinical data

skin metabolic capability

PROHAPTENS - THE OPPORTUNITY

chemistry of haptens

adduct formation

reality

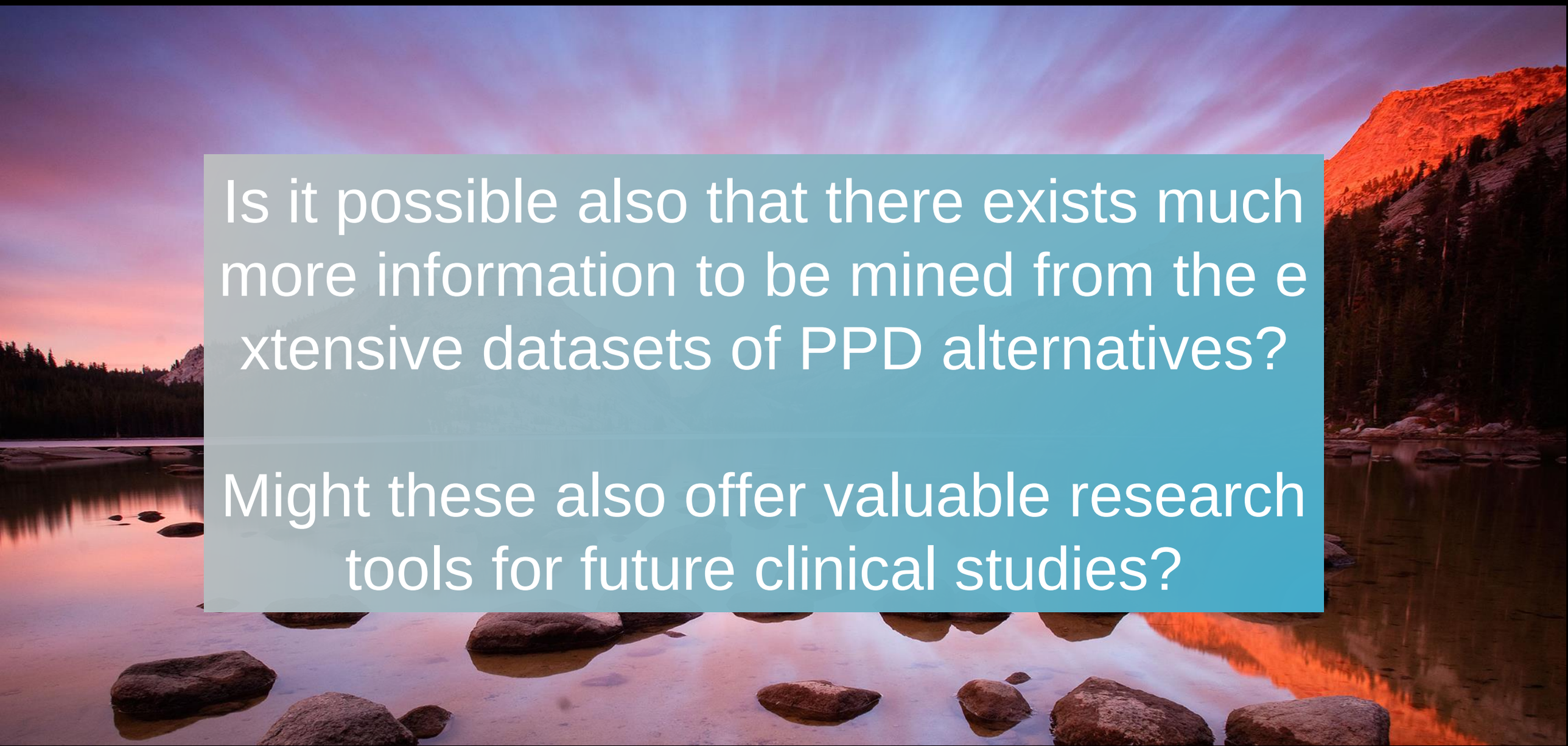
in vivo + ***clinical data***

skin metabolic capability

CROSS REACTIONS: WHAT CAN WE LEARN?

- **Cinnamal and cinnamic alcohol: positivity to both could be concomitant contact allergy…**
- **…but very few dual positives to eugenol and isoeugenol occur, suggesting these do not share a common in vivo hapten**
- **Failure to cross react may be important**

N-acetylation detoxifies PPD, but in reality the true in vivo hapten(s) remain unknown, as is the relative importance of air versus metabolic oxidation. This knowledge derives very largely from human data.



Is it possible also that there exists much more information to be mined from the e xtensive datasets of PPD alternatives?

Might these also offer valuable research tools for future clinical studies?

- A final random thought: can we learn anything from dermal metabolism of topically applied drugs which also turn out to be skin sensitisers?

WHAT DOES IN VITRO ALTERNATIVES WORK SAY ABOUT METABOLISM?

- S9 is the commonly used metabolic activator, eg in genetic toxicology
- The effect of S9 on prohaptens is mixed: sometimes it activates, other times it inactivates (Gerberick, Karlberg)
- A proposed “skin mix” might be more effective (Karlberg), but…
- …simply using peroxide/peroxidase also appears to function well (Lepoittevin/Gerberick)

…but how much of this is based on knowledge of what happens in man ?

It is legitimate to ask
whether a
confirmatory HRIPT is
the only way to check
that a prohapten has
not been missed.