

IDEA Fragrance Allergens Characterization workshop Brussels, Aug 2013

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What's the difference between these women??

A close-up photograph of a woman's face showing a severe allergic reaction. Her skin is bright red, swollen, and covered in small blisters. Her eyes are closed, and her lips are slightly swollen. She is wearing a blue t-shirt.

This one is clinically allergic

A close-up photograph of a woman's face with clear, healthy skin. She has dark hair pulled back and is looking directly at the camera. She is wearing a light pink shirt.

This one is clinically tolerant

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Why do some people become allergic??

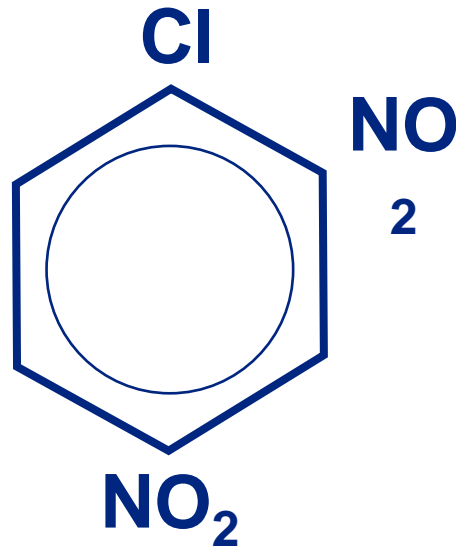
- ❖ **Exposure to allergen - quantity of exposure**
- ❖ **“Potency” of antigen - ?? some chemicals sensitise easily, some sensitise very few**
- ❖ **Individual “susceptibility”**

Human dose response studies with DNCB

- **Dose response of sensitization on fixed area**
- **Dose-responses of elicitation challenges**
- **Dose response of different areas**
- **Differences in susceptibility to sensitisation**

Human dose response studies with DNCB

- Sensitise normal volunteers with increasing doses of Dinitrochlorobenzene (DNCB)**

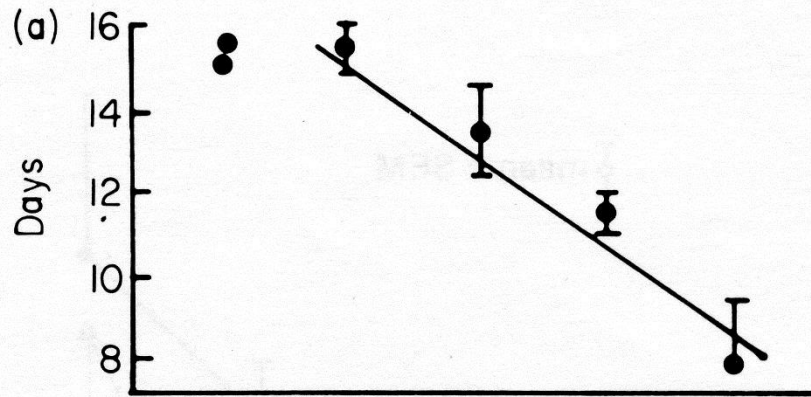


5 groups of normal

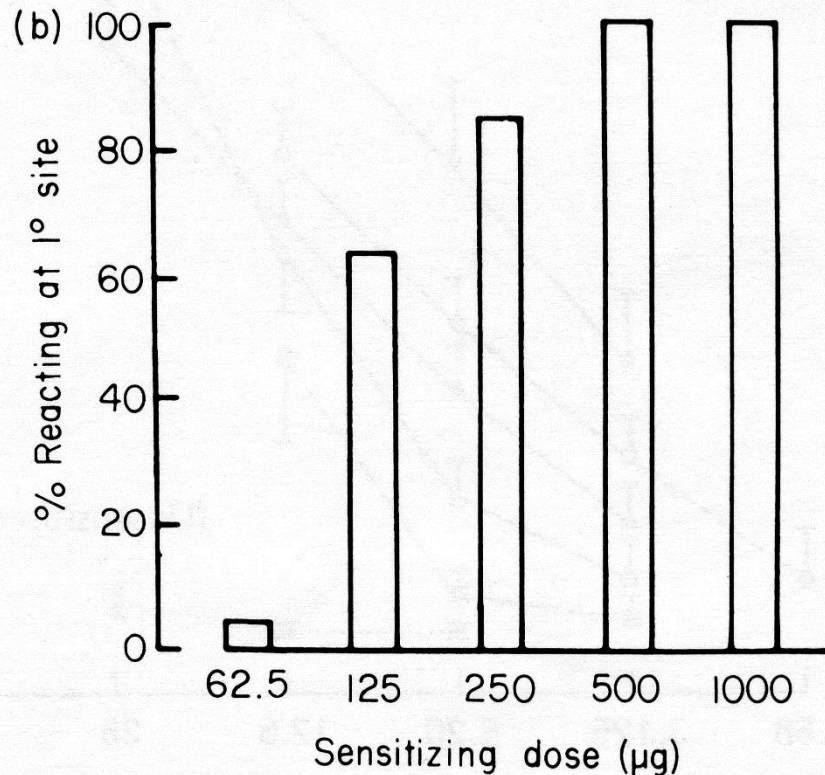
Each received a
Different sensitising dose:
62.5, 125, 250, 500, 1000µg
on a 3cm circle on forearm

4 weeks later challenge with
4 small doses

ALLERGIC CONTACT DERMATITIS

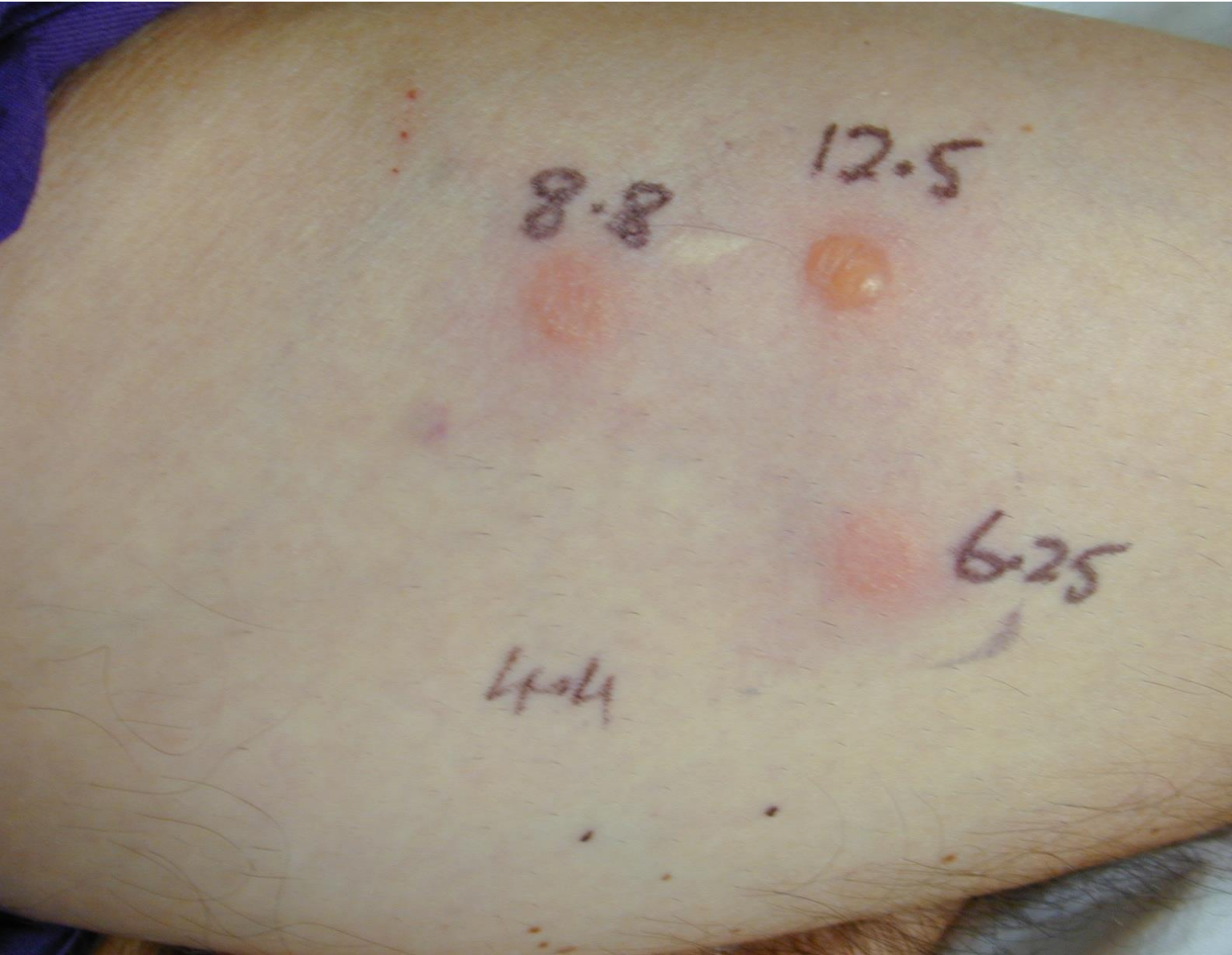


Time taken for delayed flare to appear at sensitising site

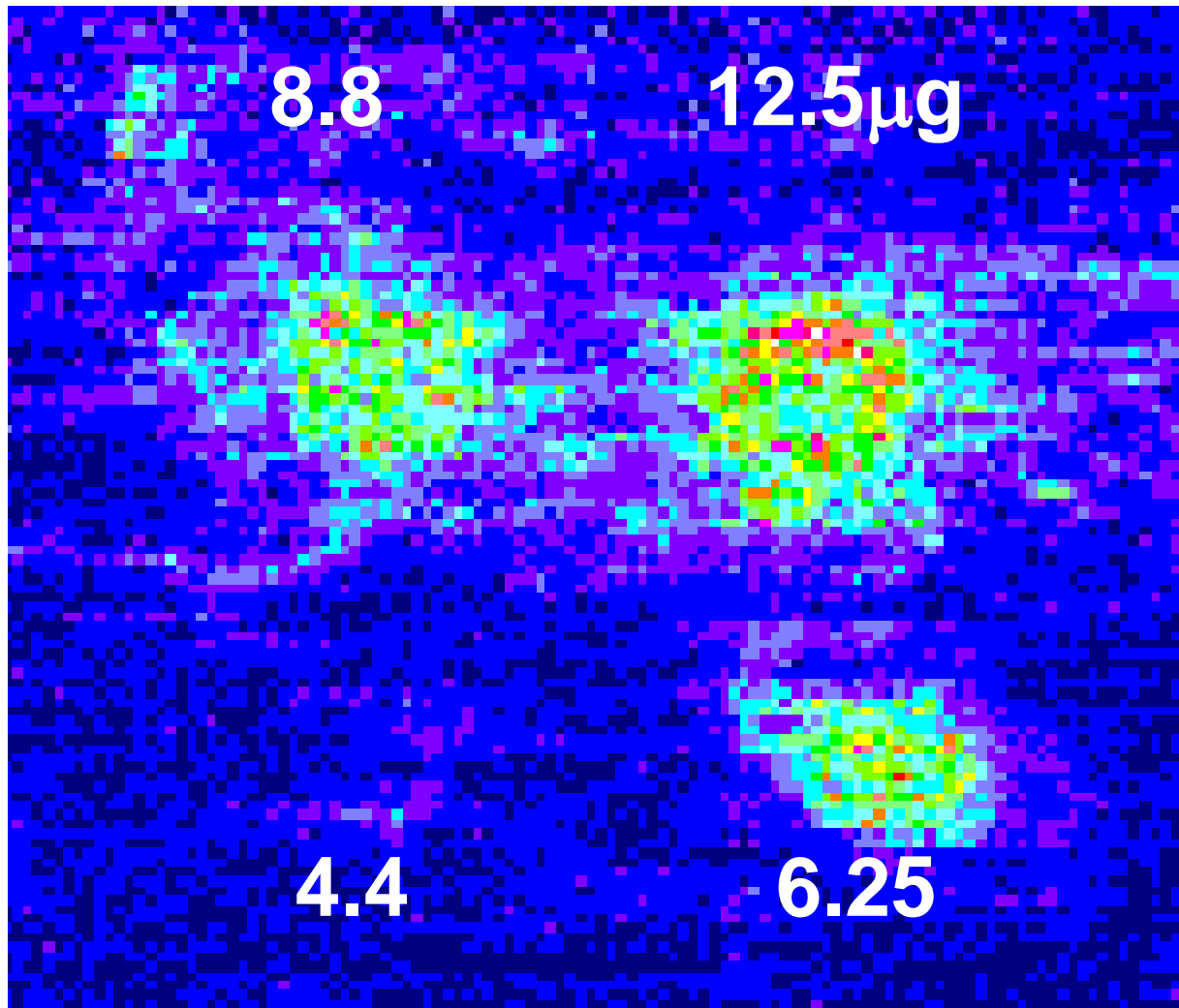


Proportion in each sensitisation group showing the delayed flare

Challenge with DNCB

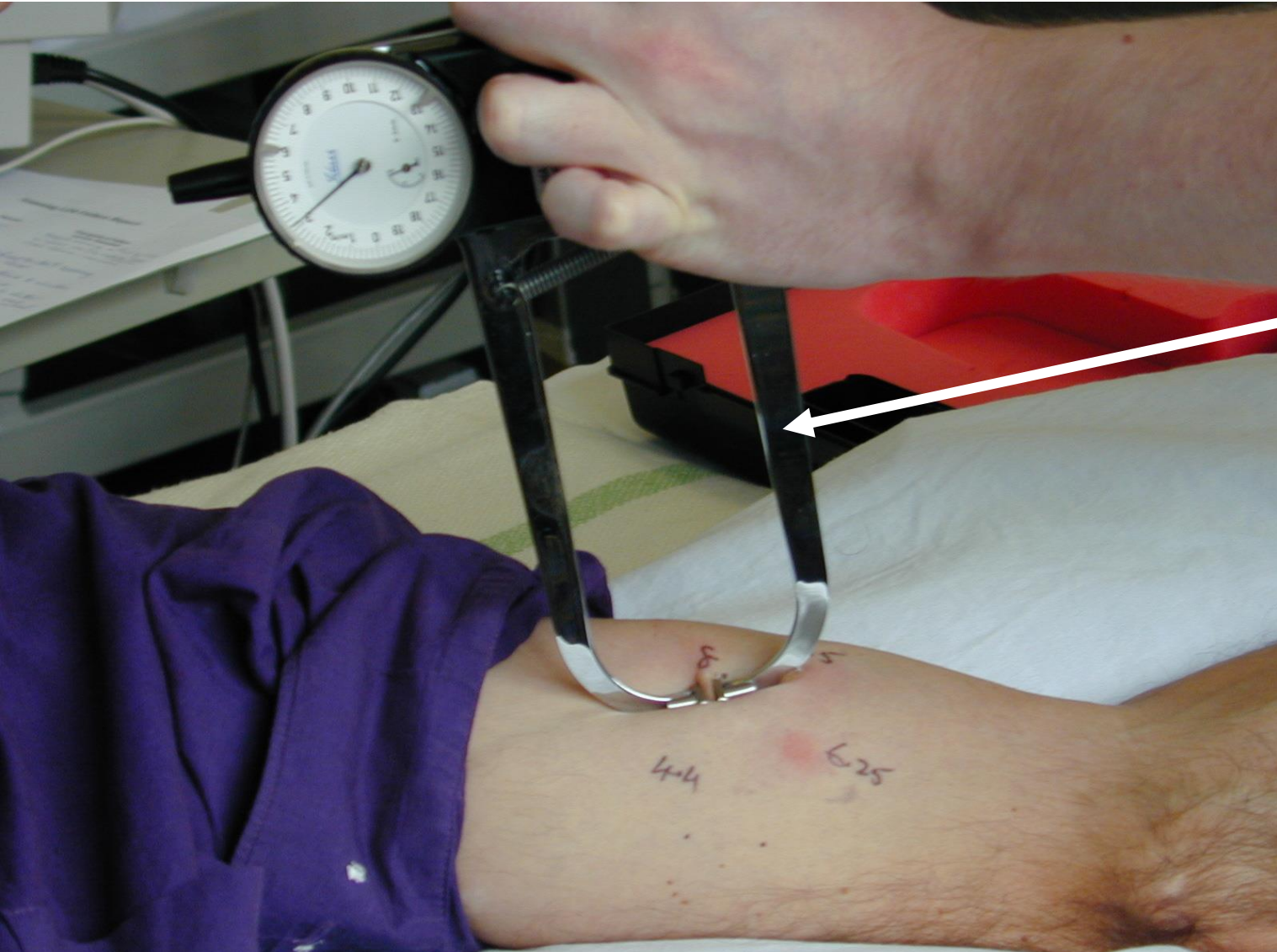


DNCB reactions read by laser Doppler flow meter

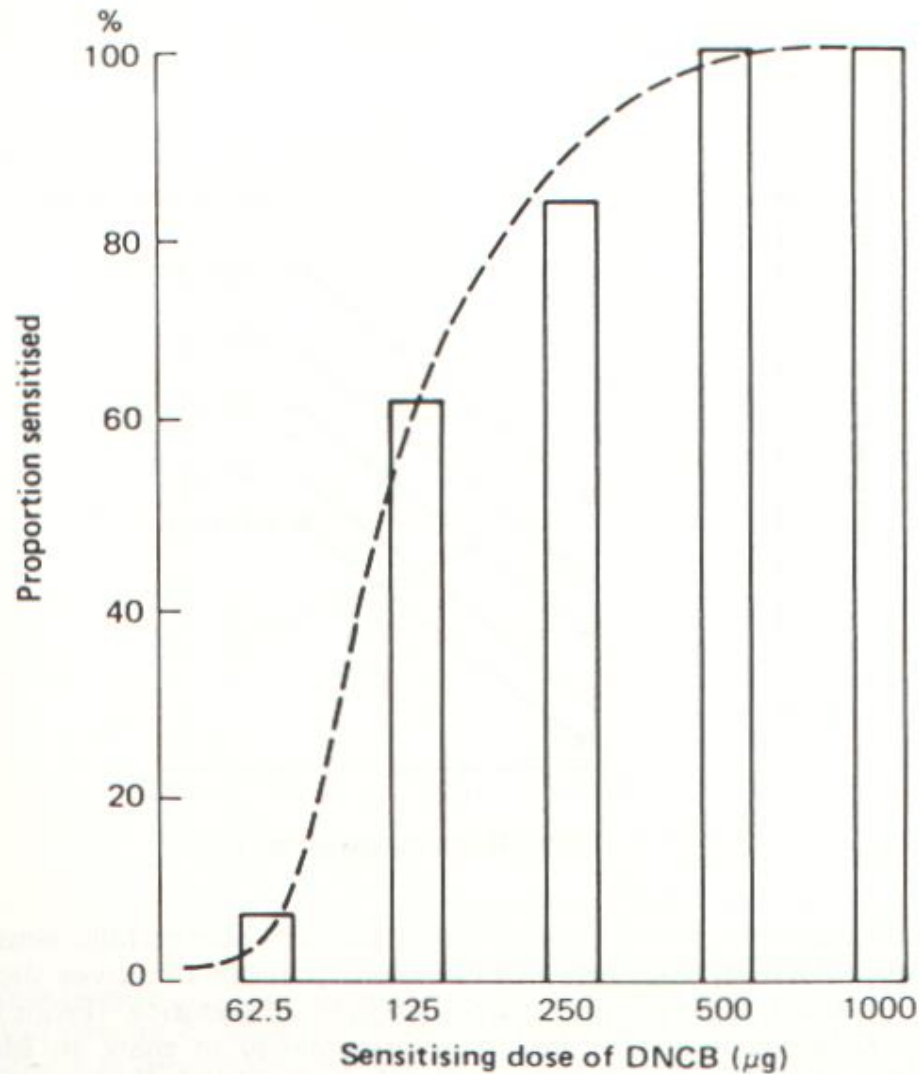


10 mm

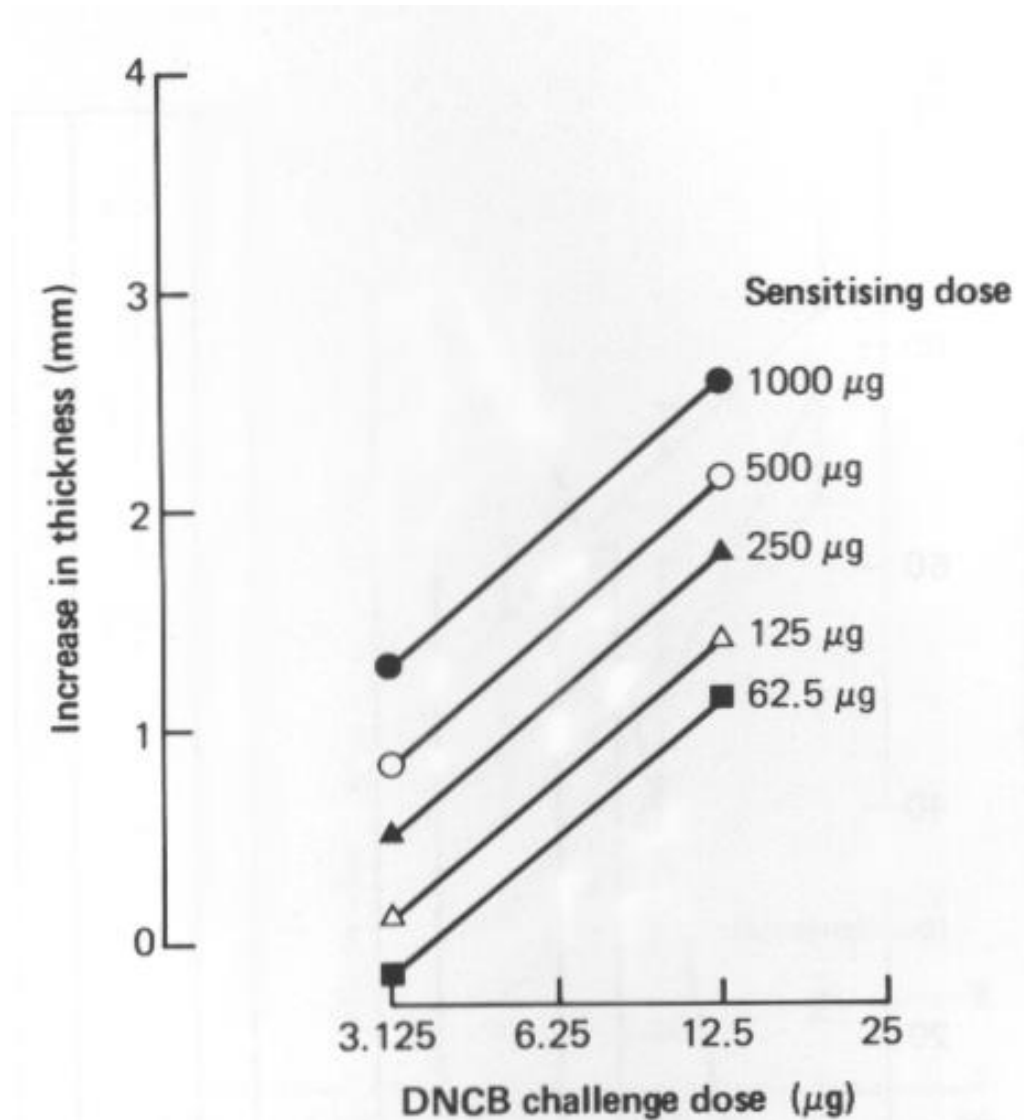
Measurement of responses to challenge with DNCB



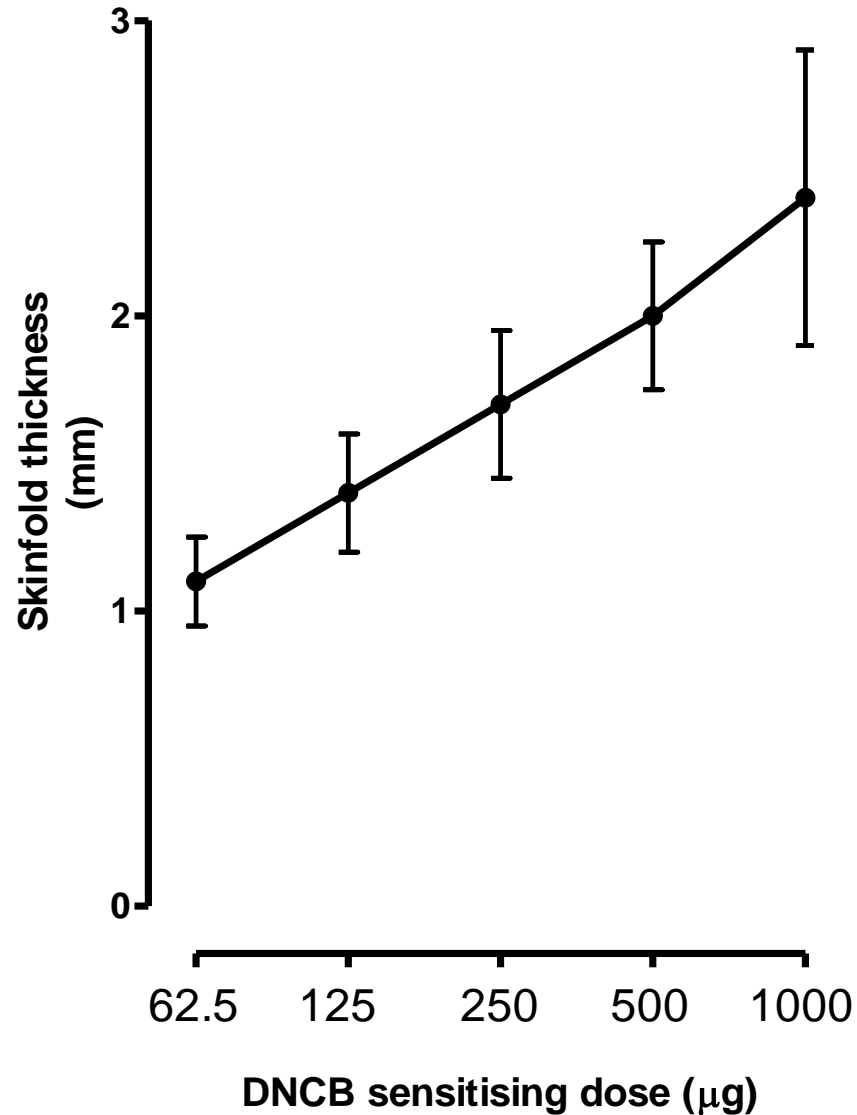
Proportions sensitised by increasing doses of DNCB



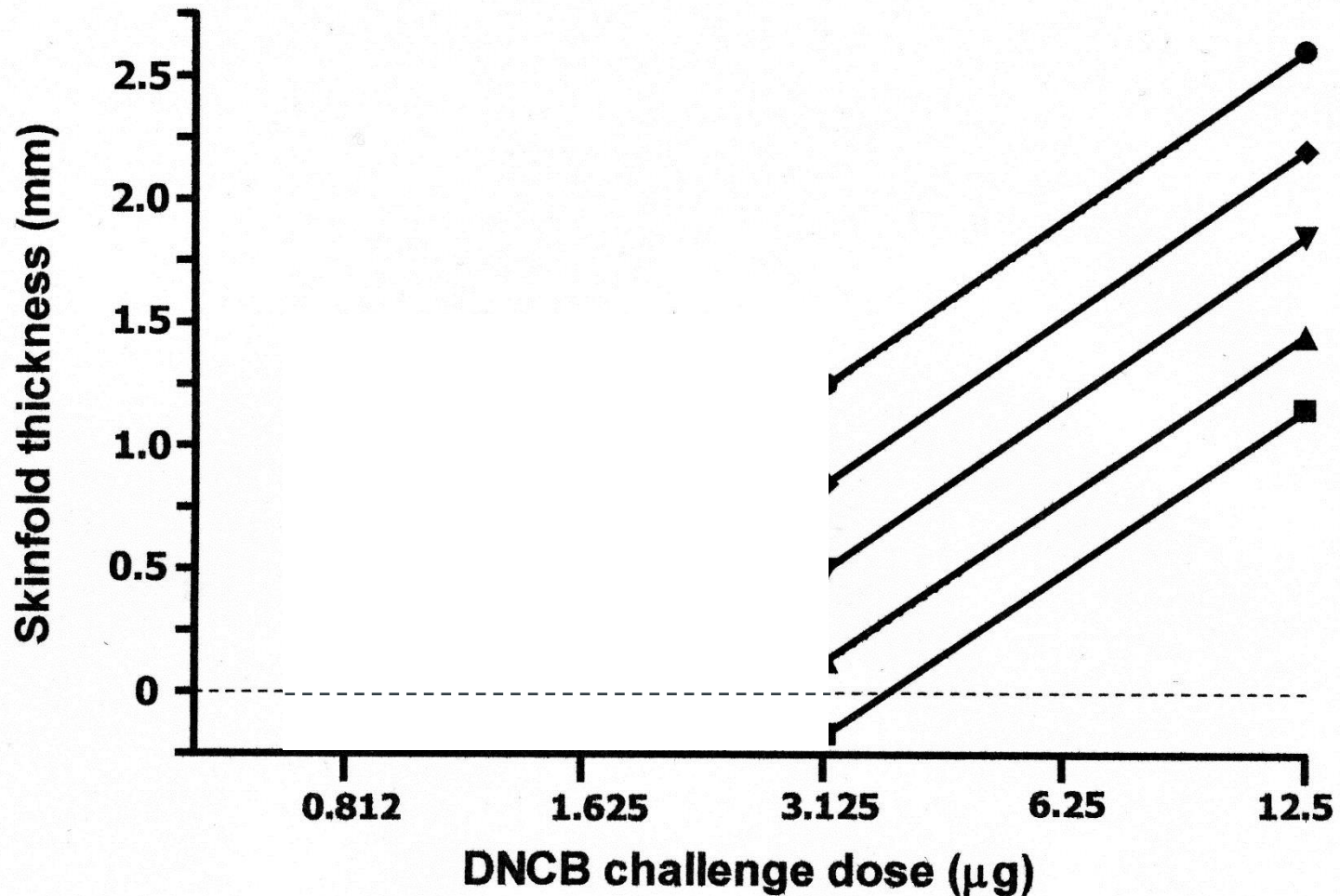
Increase in reactivity with rising sensitising dose of DNCB



Increase of sensitivity with increasing sensitising dose



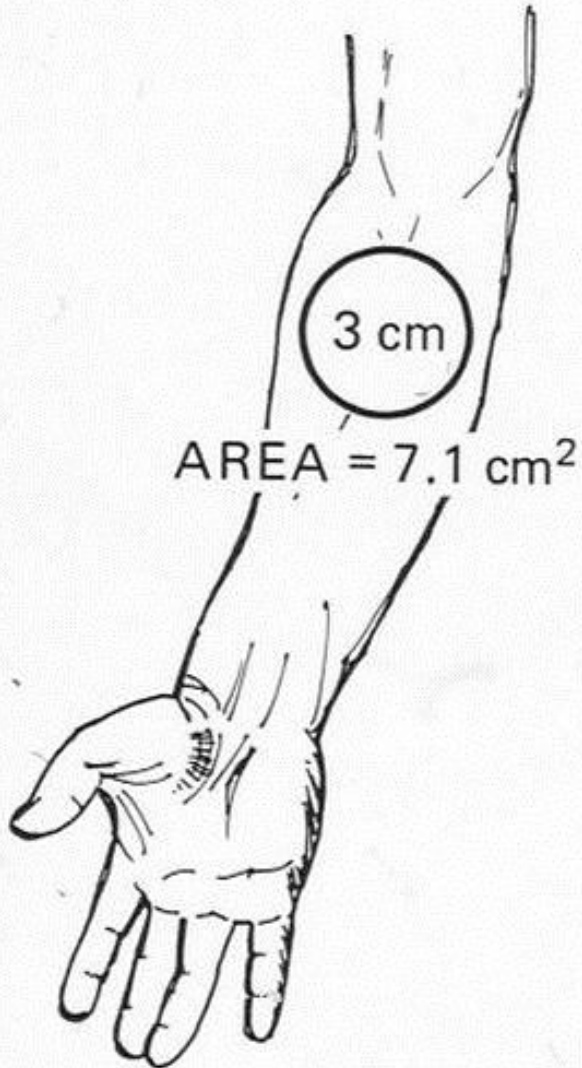
Threshold responses of DNCB sensitised individuals



Conclusion 1

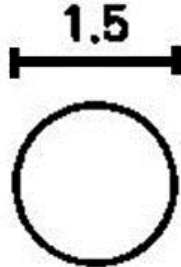
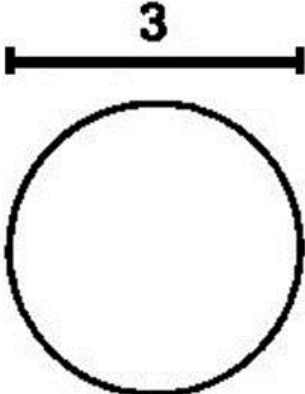
- ❖ **The immune response in humans exhibits very clear dose-response relationships**
- ❖ **The degree of sensitisation is proportional to the log of the sensitising stimulus**
- ❖ **Question: What is the effect of varying the area of application?**

Effects of area of sensitisation

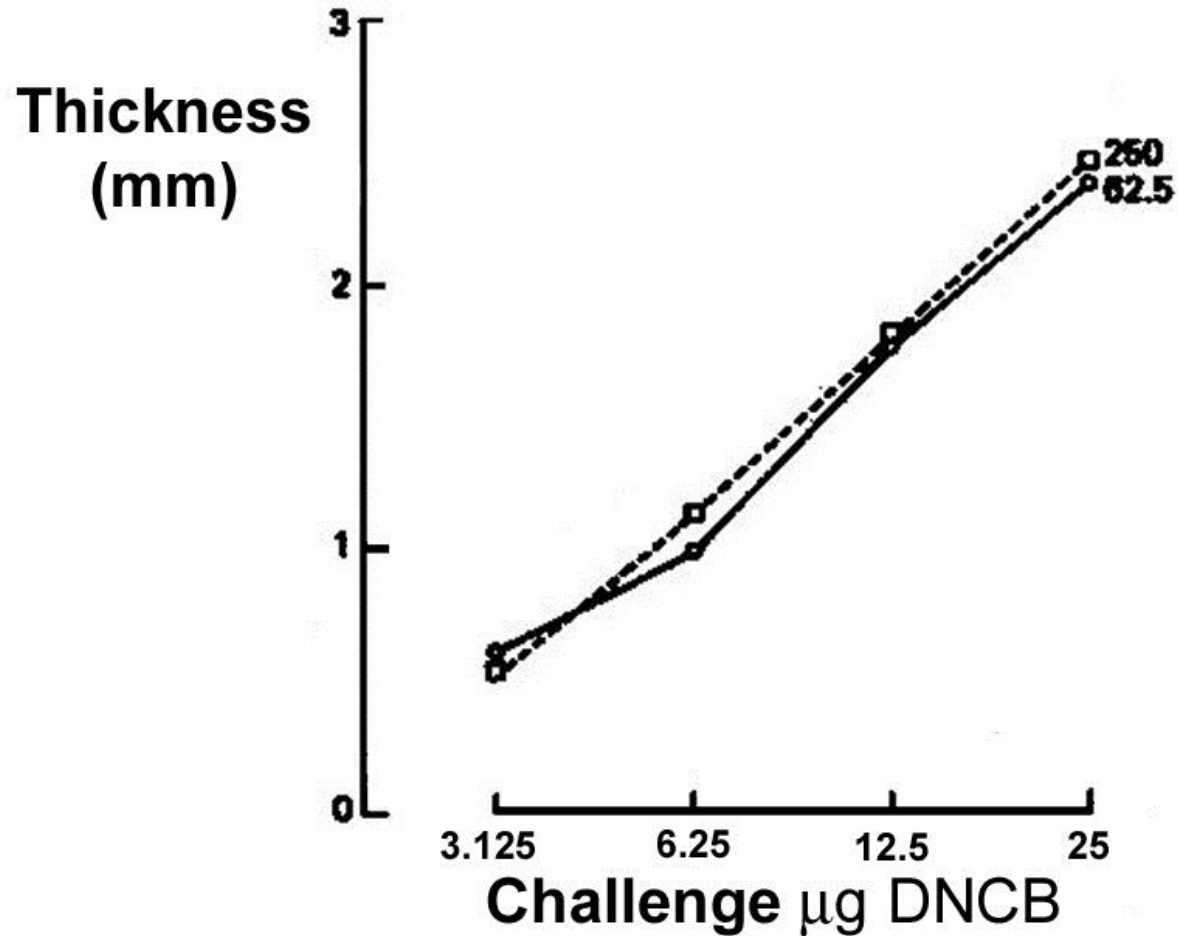


TOTAL DOSE μg	CONCENTRATION $\mu\text{g}/\text{cm}^2$
62.5	8.8
125	17.7
250	35.4
500	70.7
1000	141

Half diameter = quarter the area

DIAMETER cm	AREA cm ²	DOSE μg	CONCENTRATION μg/cm ²
	1.8	62.5	35.4
	7.1	250	35.4

Responses for subjects sensitised
with same concentration: 35.4mg/cm



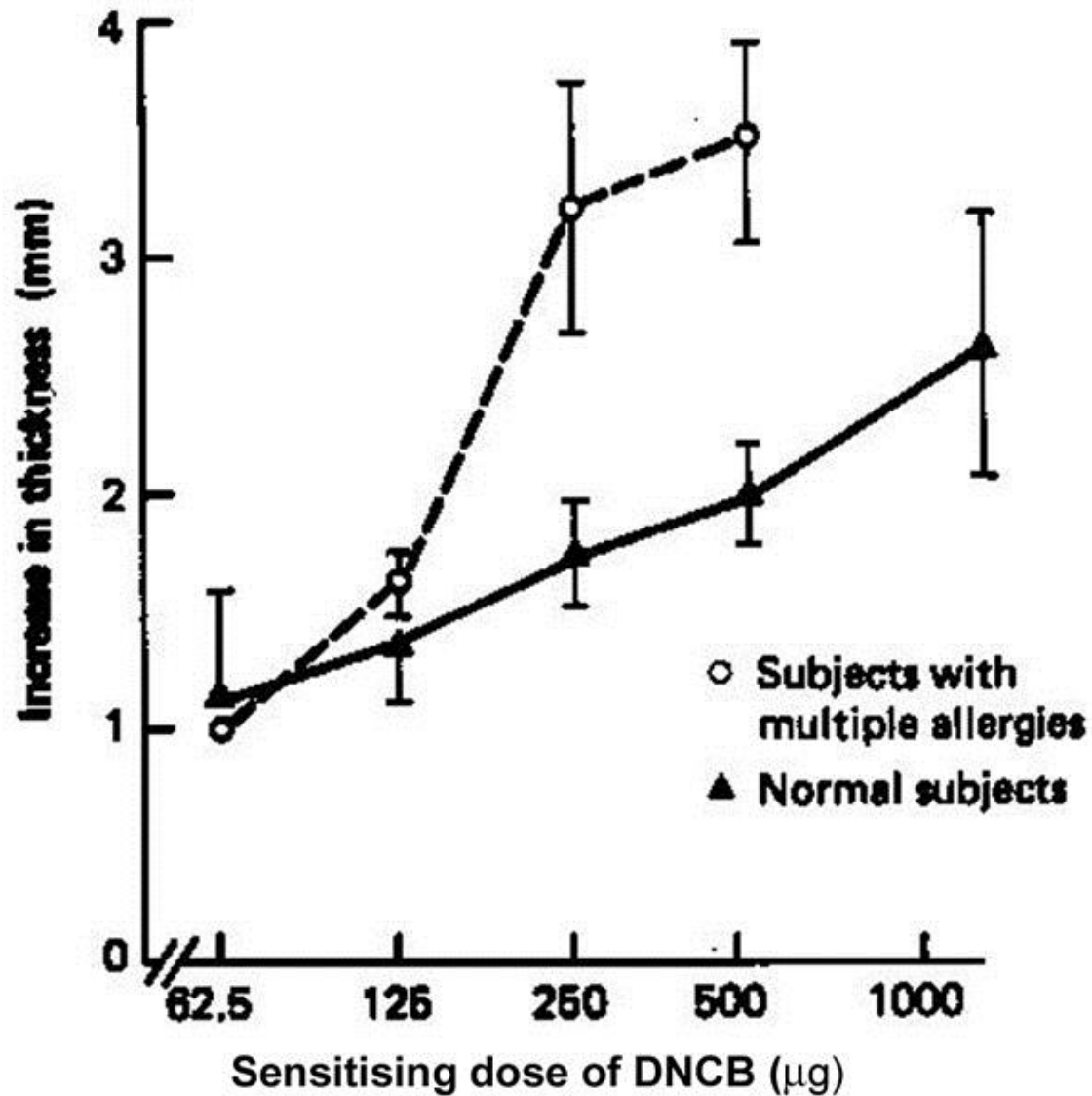
Conclusion 2

- ❖ **The major determinant of sensitisation is the dose per unit area ($\mu\text{g}/\text{cm}^2$).**
- ❖ **Above a certain level (about 1 cm^2), the area of application has no significant effect.**

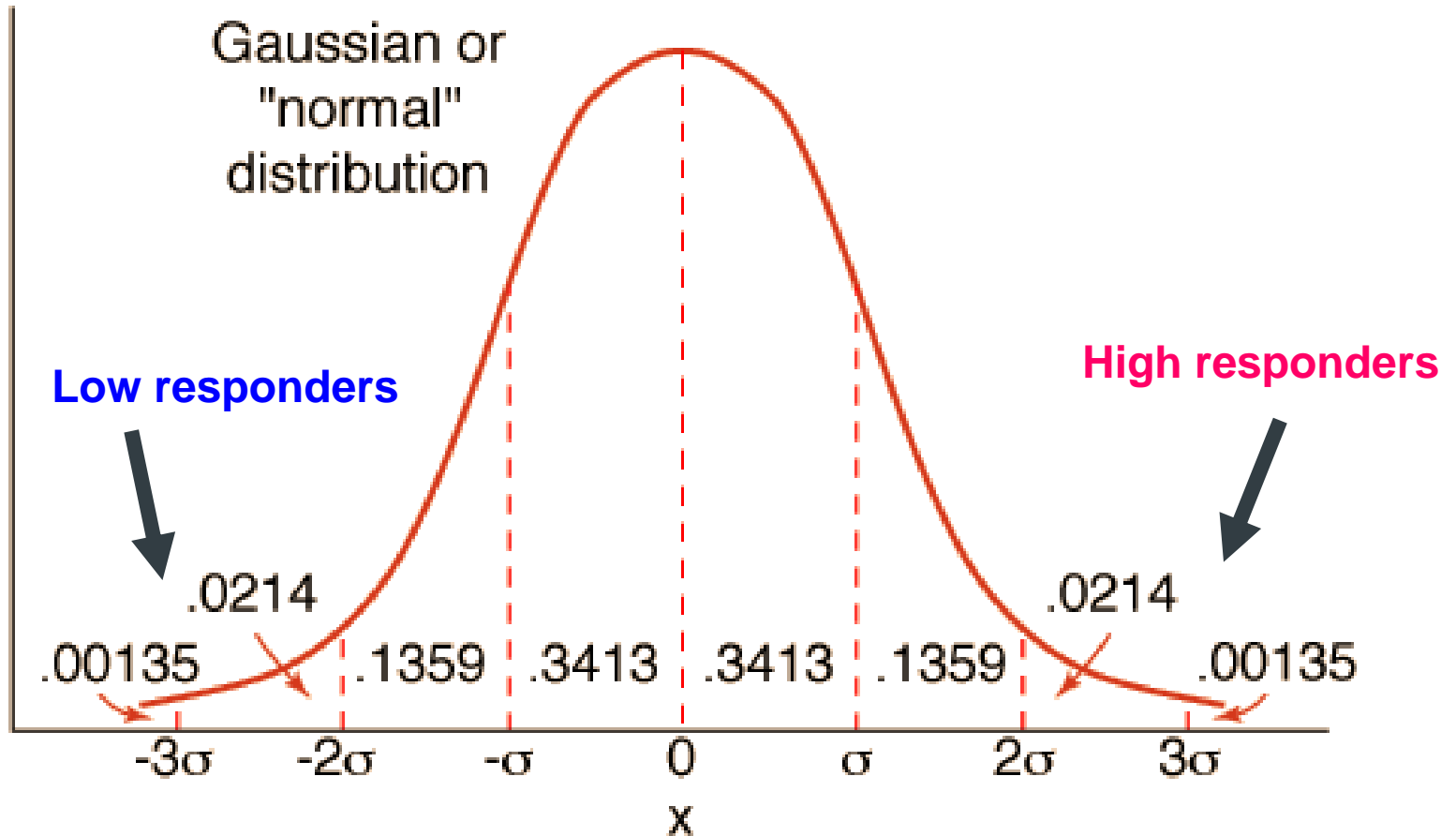
Do people differ in susceptibility to developing contact allergy?

- ❖ **People who develop multiple contact allergies**
- ❖ **Repeat basic sensitisation with different doses**
- ❖ **Measure responses to challenge**
- ❖ **Answer – yes they are more reactive to DNCB**

AUGMENTATION OF RESPONSIVENESS WITH INCREASING SENSITISING DOSE OF DNCB



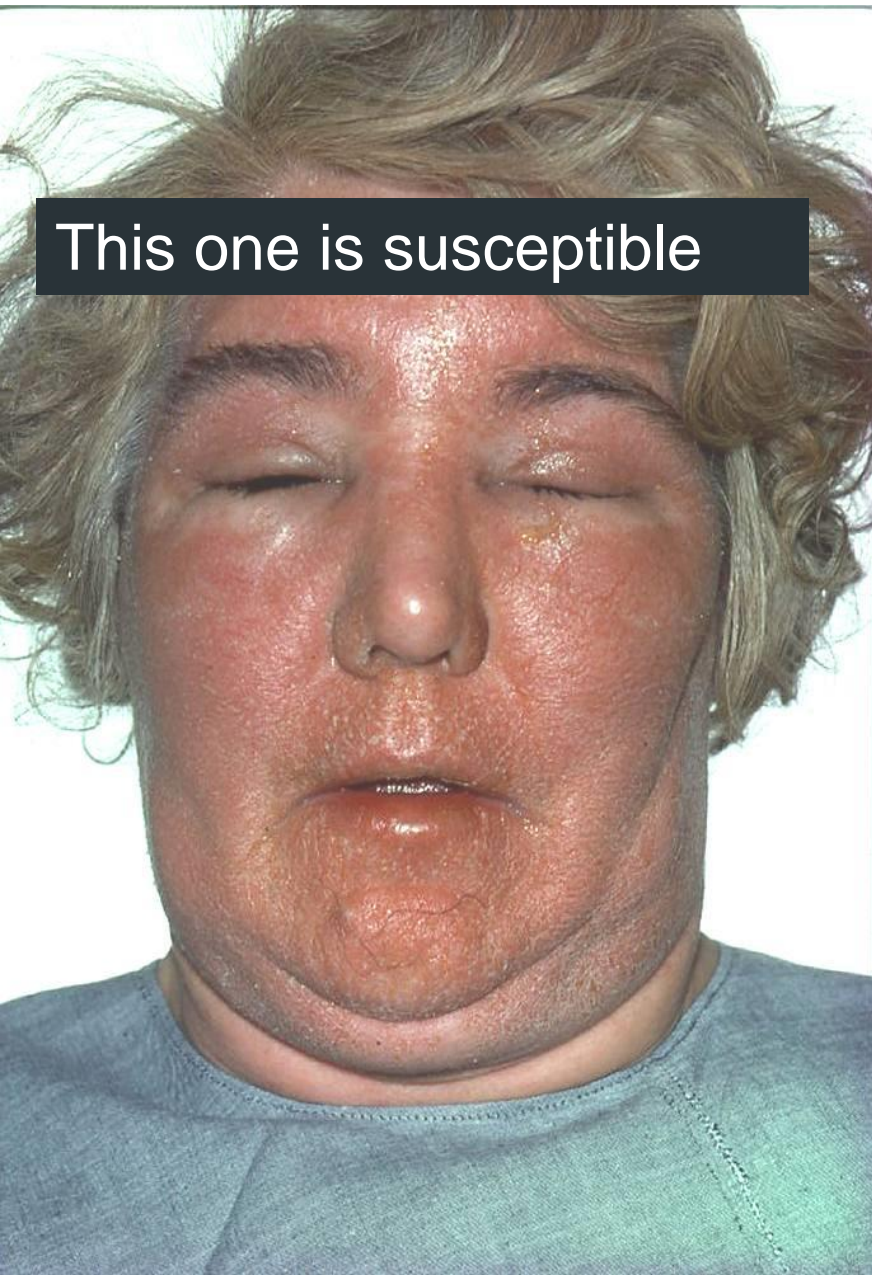
Who becomes allergic to contact sensitisers??



Conclusions 3

- ❖ **There are clear differences in susceptibility to contact allergy**

What's the difference between these women??



This one is susceptible



This one is resistant

What are the common ingredients that cause allergy?

❖ **Fragrances:**

➤ **Lyral**

➤ **Oak Moss (Atranol/Chloroatranol)**

❖ **Antimicrobials:**

➤ **Kathons (MCI/MI)**

➤ **Methyldibromoglutaronitrile (MDBGN)**

People are strongly allergic to additives: what are the lowest concentrations to which they react?

- ❖ Positive patch tests at 0.01%

 - (0.01% = 100 ppm = 0.1 µg/ml)

- ❖ ROATs elicit down to 0.0005% (5 ppm)

- ❖ Reaction to low dose of allergen means strongly allergic/sensitised?

Example ROAT Data

Ref	All'gen	ROAT conc %	Conc ppm	Volume μl	Area cm²	D/UA per applicn μg/cm²	No. of applics	Total D/UA μg/cm²	% +ve
1	Coloph -ony	20%	2x10⁵	5	3.1	318	9	2863	77
		1%	10⁴	5	3.1	15.9	9	143	31

Strongly sensitised people react to low concentrations of allergen

- ❖ **Many substances are only present at low concentrations – preservatives, anti-microbials**
- ❖ **Some people respond to very low concentrations**
- ❖ **How do people become strongly sensitised?**

Evidence on concentrations that sensitise

- MCI/MI occurs at around 45 ppm in paints, in personal products
7.5 – 15 ppm
- HRIPT **to sensitise**: x3/week, 9 applications; 3.5 x 3.5 cm patch = 12.25 cm². 1450 volunteers
- 5 concentrations used: 5, 10, 12.5, 20 ppm (=0.002%).
 - Actual doses up to 2.9 µg/ cm² / applicn.
- No sensitisation below 12.5 ppm
- 1 of 84 sensitised by 12.5 ppm
 - total dose = 16.1 µg/ cm²
- 2 of 45 sensitised by 20 ppm
 - total dose = 26.1 µg/ cm²
- **Cardin et al; Dose response assessments of Kathon biocide. (1986) Contact Derm; 15: 10-16**

Important conclusion

- **The dogma is that induction of sensitisation requires higher doses than elicitation.**
- **That may be correct but we must consider how the (?larger) sensitising dose is delivered.**
- **Repeated applications of low concentrations can clearly induce sensitisation (allergy)**

How do people become strongly sensitised?

1. By exposure to very potent allergen – e.g. DNCB, DPCP
2. By repeated exposure to sensitiser - ??low doses

Effect of repeated small exposures

- **Prediction of sensitisation uses HRIPT**
- **Unilever studies in Thailand show repeated use of hair colorants (PPD) generates contact sensitivity**
- **Kligman's evidence that increasing numbers of exposures increases sensitisation**
- **Friedmann study with DNCB**

Kligman's studies

- **In >2000 human “volunteers”**
- **Increasing numbers of exposures augmented sensitisation rates**
- **Repeated application to same site is more potent than the same number of exposures at scattered sites**
- **Addition of irritant to sensitisation exposure augmented sensitising potency**

Kligman's data

	3 apps	5 apps	10 apps	15 apps
TMTD 10%	0/25	0/25	2/22 9%	6/18 33%
Pen G 10%	1/25 4%	5/25 20%	10/21 48%	16/21 76%
Pen G 0.1% + SLS		3/23 14%	3/22 14%	10/24 42%

Aim of this study:

- **Use DNCB to compare sensitising potency of 2 regimens:**
- **Single dose of 60 $\mu\text{g}/\text{cm}^2$**
- **Six applications (weekly) of 10 $\mu\text{g}/\text{cm}^2$ to the same site**

Protocol

Single sensitising
Dose: 60 $\mu\text{g}/\text{cm}^2$



Elicitation challenge
with 4 DNCB doses
6.25, 8.8, 12.5, 17.7 μg

Measure
responses

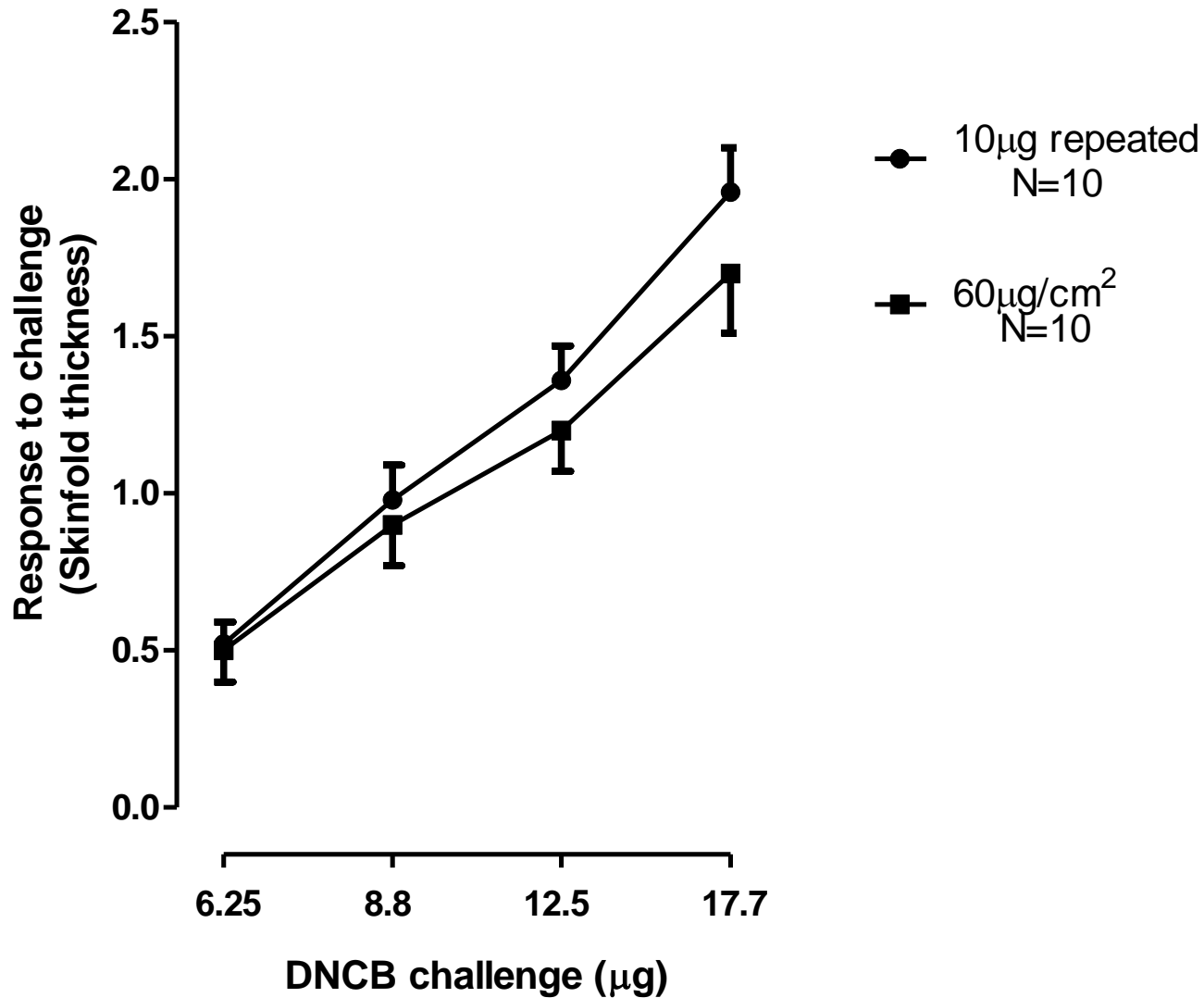
Elicitation challenge
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6.25, 8.8, 12.5, 17.7 μg

Measure
responses

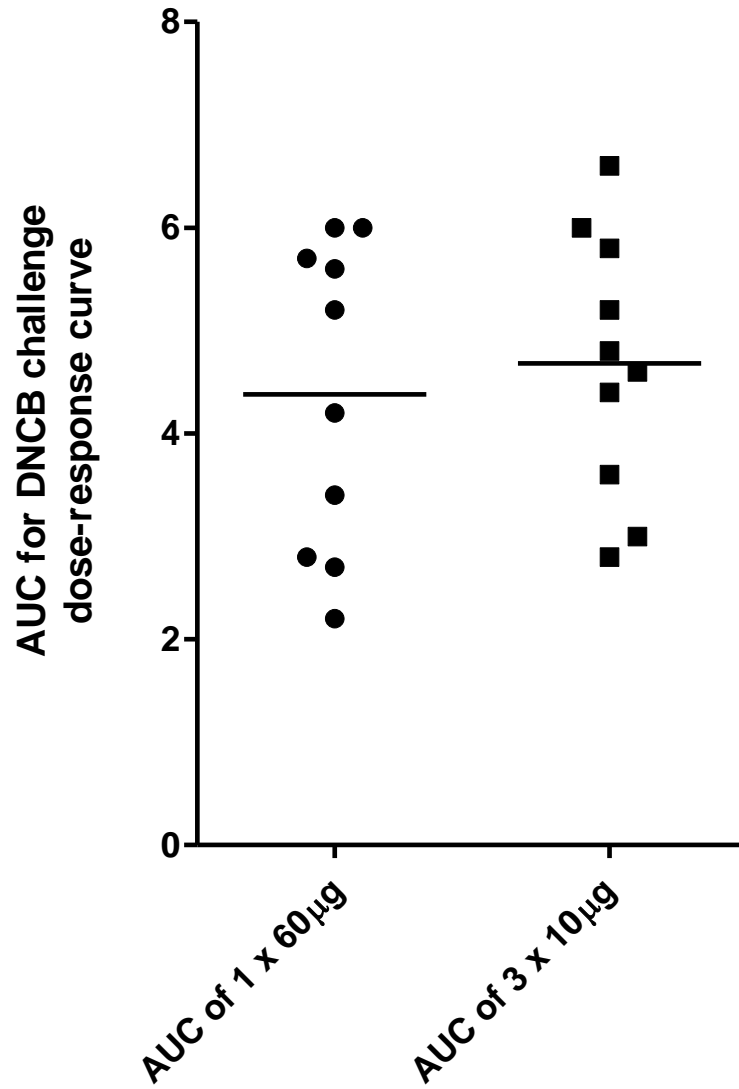


Weekly applicn
of 10 $\mu\text{g}/\text{cm}^2$

**Groups sensitised with DNCB
(60 $\mu\text{g}/\text{cm}^2$ or 3 repeats of 10 $\mu\text{g}/\text{cm}^2$)**



Area Under the Curve (AUC) for responses to DNCB of groups receiving different sensitising regimens



Conclusion so far

- **Repeated low dose exposures can be a more potent sensitising stimulus than a single high dose exposure**
- **So.....**

The question that concerns us in this workshop

- ❖ **Are the concentrations of additives present in personal products able to induce allergic sensitisation?**

- ❖ **relevant factors:**
 - 1. Dose effects**
 - 2. Individual susceptibility**

Dose-related considerations

- **There will be dose-response relationships for sensitisation by repeated low dose exposures – progressively reducing the dose will require more exposures**
- **The interval between exposures may be critical: daily vs less often**
- **For a given compound is there a threshold below which it won't sensitise?**

Dose-related considerations

- For “normal” allergens (nickel, hair dyes etc), at low doses tolerance develops
- The tolerance may be converted to “allergy” either with:
 - additional danger signals - injury (ear piercing) or concomitant irritant
 - sufficient dose (Kligman)

Individual susceptibility

- **Can everyone be made allergic to everything if given sufficient exposure?**
- **At “usual” exposure doses – NO**
 - **Analogy with drug allergy**
 - **Nickel – 10% become allergic – the rest are sensitised but clinically tolerant (Cavani)**
- **BUT Kligmans work suggests YES – given sufficient. 100% became allergic to PPD, 75% became allergic to penicillin**

Proper experiments are needed

- suggestion

- **Strongly sensitised people can in theory respond to 1 - 2 μ g/cm² DNCB**
- **Apply 1 μ g/cm² doses weekly to the same site for (say) 30 weeks or until a positive reaction develops.**
- **Quantify reactivity with formal dose-response challenge; compare with other sensitising doses (historic or concurrent)**
- **Three possible results:**
 1. **Everyone becomes allergised – means the dose is too high**
 2. **No-one becomes allergised – then we need to explore whether they are tolerant, not sensitised at all or sensitised sub-clinically**
 3. **A few become allergic – show these are individuals with high susceptibility (multiple spontaneous contact allergies).**

Caveats

- **There is NO evidence that DNCB ever induces “tolerance” although it can induce low-level (subclinical) allergic sensitisation.**
- **So it is different from “weaker” sensitisers**
- **Perhaps we need to take the dose down 10 or 100 fold lower?**

Conclusion

- **The human immune system exhibits classical dose-responses.**
- **We don't know anything about the lower end of the dose-response curves for induction of allergy. What dose-levels, what intervals of exposure**
- **Humans exhibit a normal distribution for susceptibility**
- **People who become strongly allergic to low concentrations are probably in the high responder tail of the normal distribution.**
- **This needs to be tested experimentally**