Kontaktallergie durch Haarfarben am Beispiel von PPD: Die Rolle von Oxidation, Hautmetabolismus und Exposition für die Induktion einer Immunantwort

(Contact allergy to hair dyes using PPD as example: The role of oxidation, skin metabolism and exposure for the induction of an immune response)

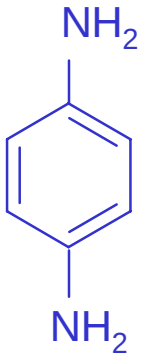
BfR-Symposium Haarfarben, 15. Oktober 2009

Carsten Goebel

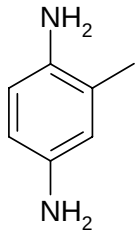
The Procter & Gamble Service GmbH, Wella, Darmstadt, Germany

Commonly used aromatic amine hair dye precursors

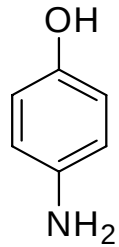
Primary Intermediates



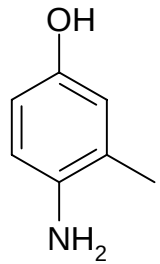
***p*-phenylenediamine (PPD)**



2,5-diaminotoluene

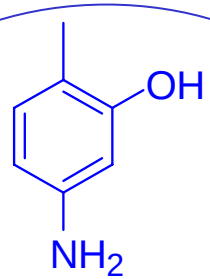


p-aminophenol

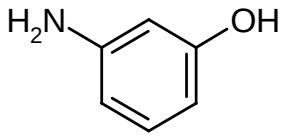


4-amino-*m*-cresol

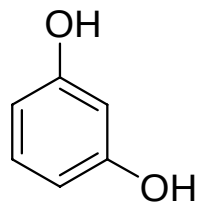
Couplers



4-amino-2-hydroxytoluene (AHT)



m-aminophenol

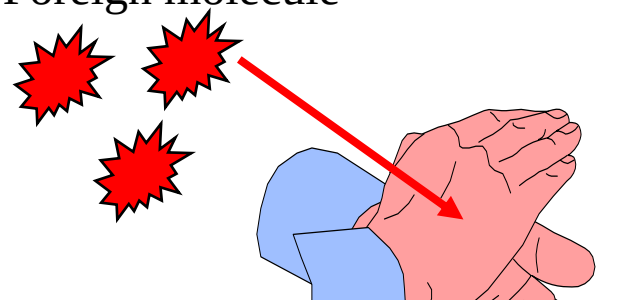


Resorcinol

How can a chemical induce contact allergy?

Initial Contact

Foreign molecule



No symptoms

Sensitization

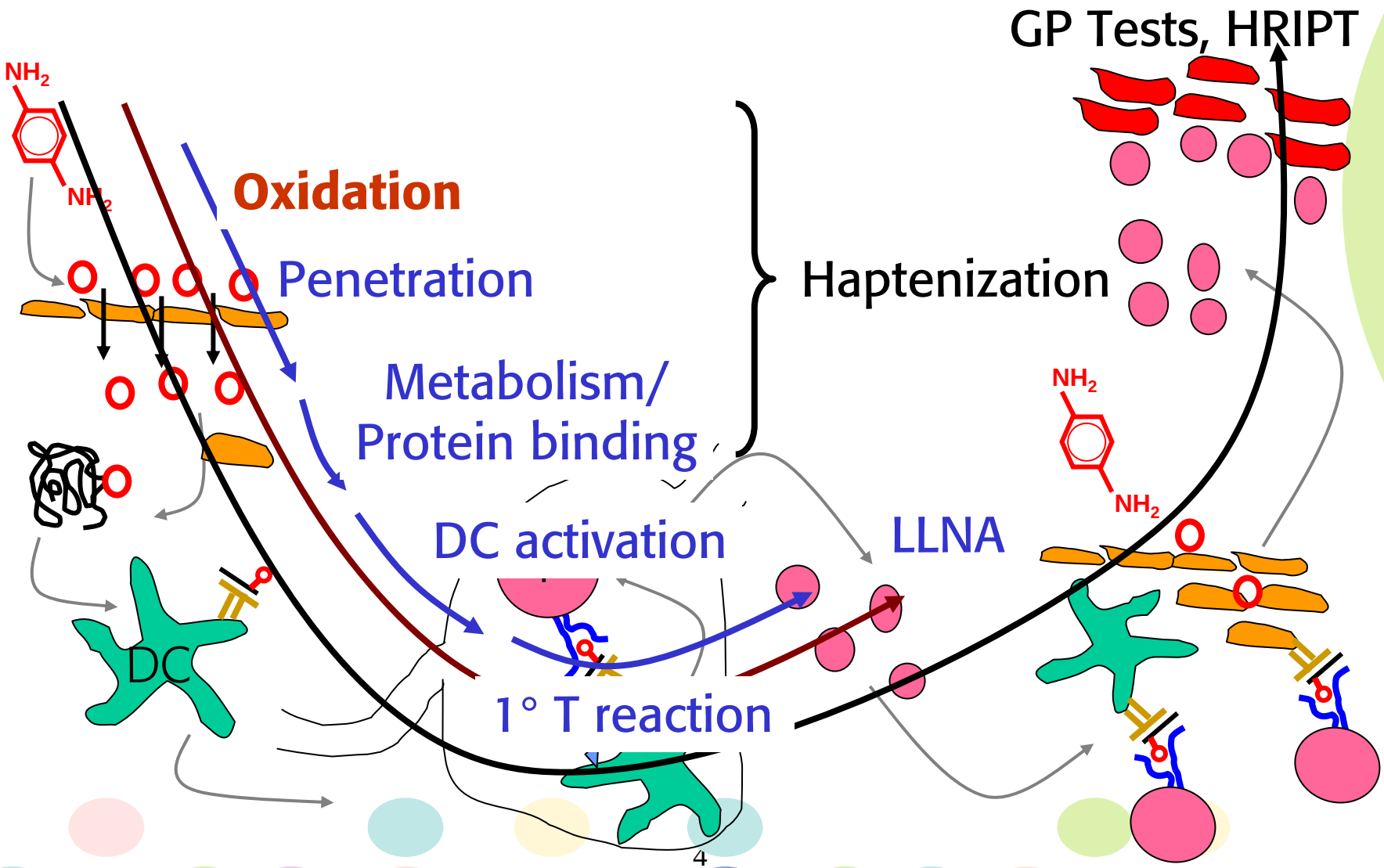
Repeated contact (Elicitation)

Redness
skin rush
urticaria

Contact allergy

Events occuring during Skin Sensitization

Induction | **Elicitation**

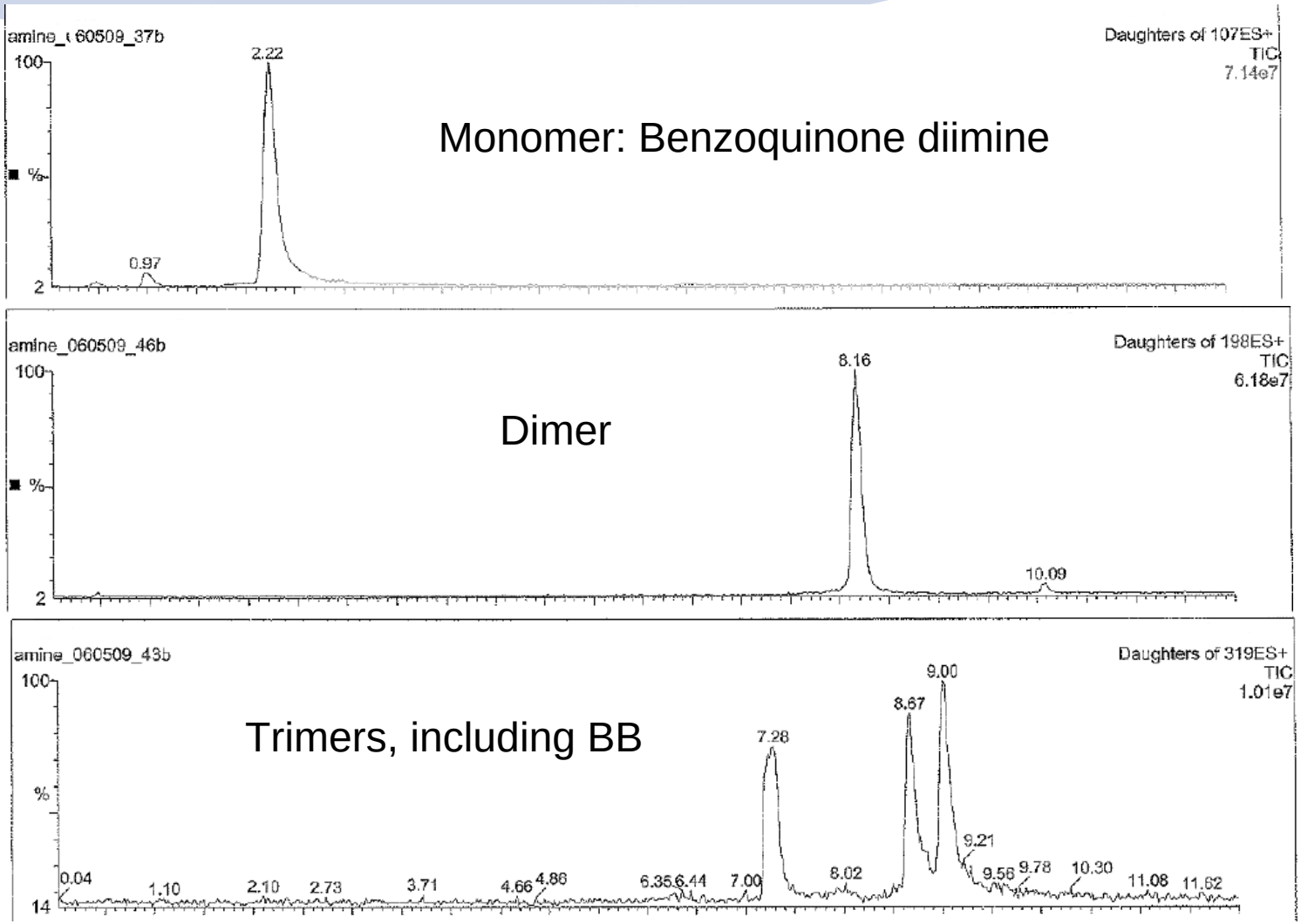


What happens to PPD on the skin under assessment conditions of sensitization/elicitation tests?

(e.g. LLNA, HRIPT, confirmatory clinical patch test)

To note: above conditions are different form use conditions of hair dyeing

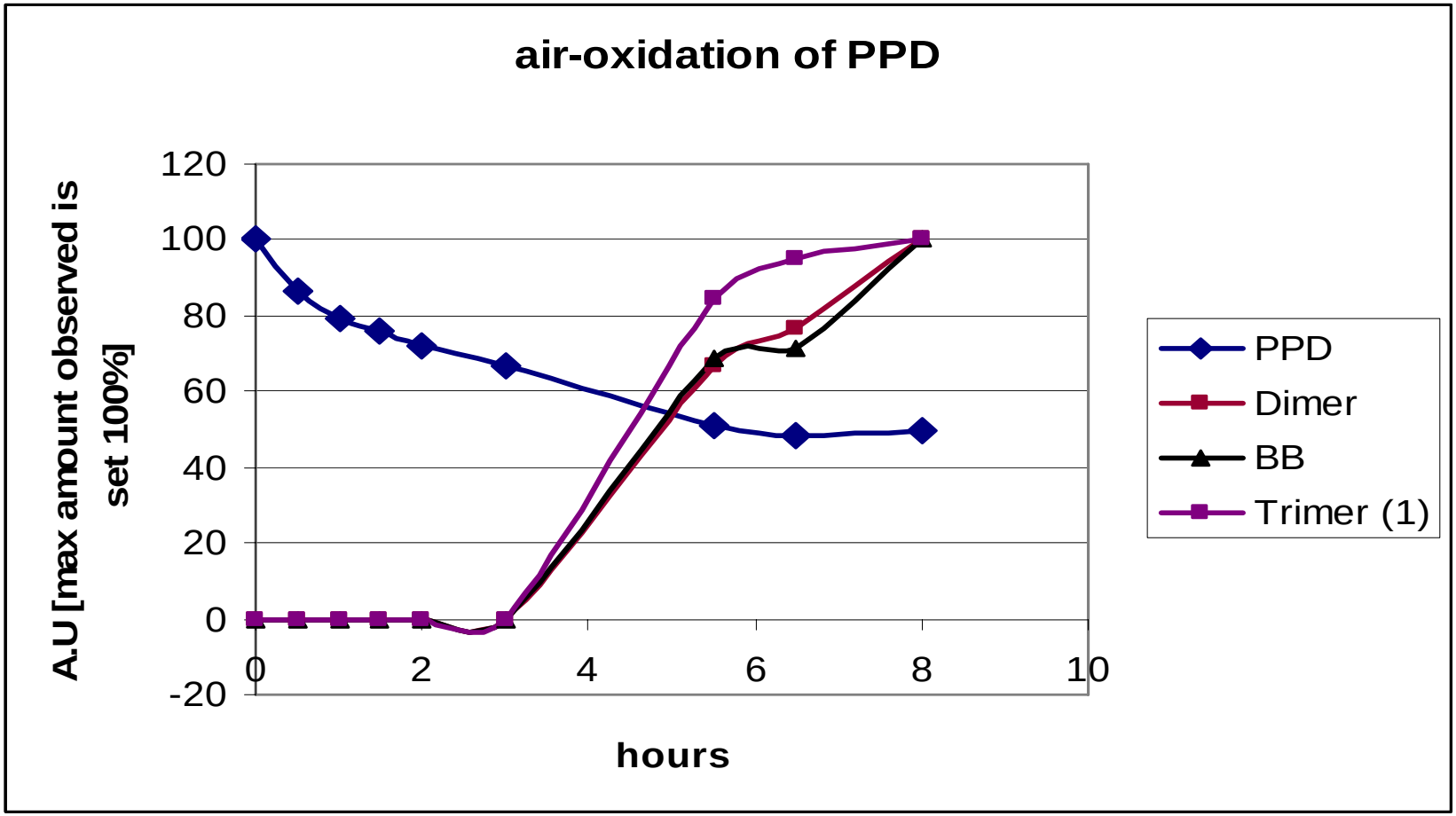
Oxidation of PPD by strong oxidizers



PPD after 30 min ferrocyanide exposure

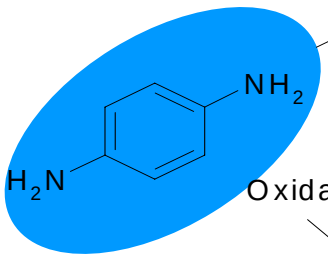
Top: potential monomeric species corresponding to benzoquinone diimine; Middle: dimeric species; Bottom: trimeric species; peak at 8.67 min corresponds to BB.

Oxidation of PPD by air oxygen



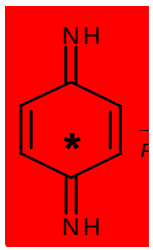
Typical spectrum of PPD auto-oxidation products

Auto-oxidation products of PPD



Oxidation

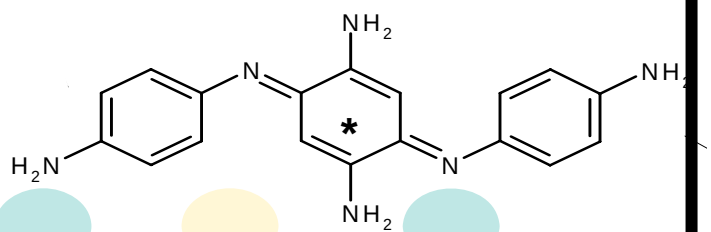
= **Fresh PPD**



Dimers/ Trimers

= **oxidized PPD**

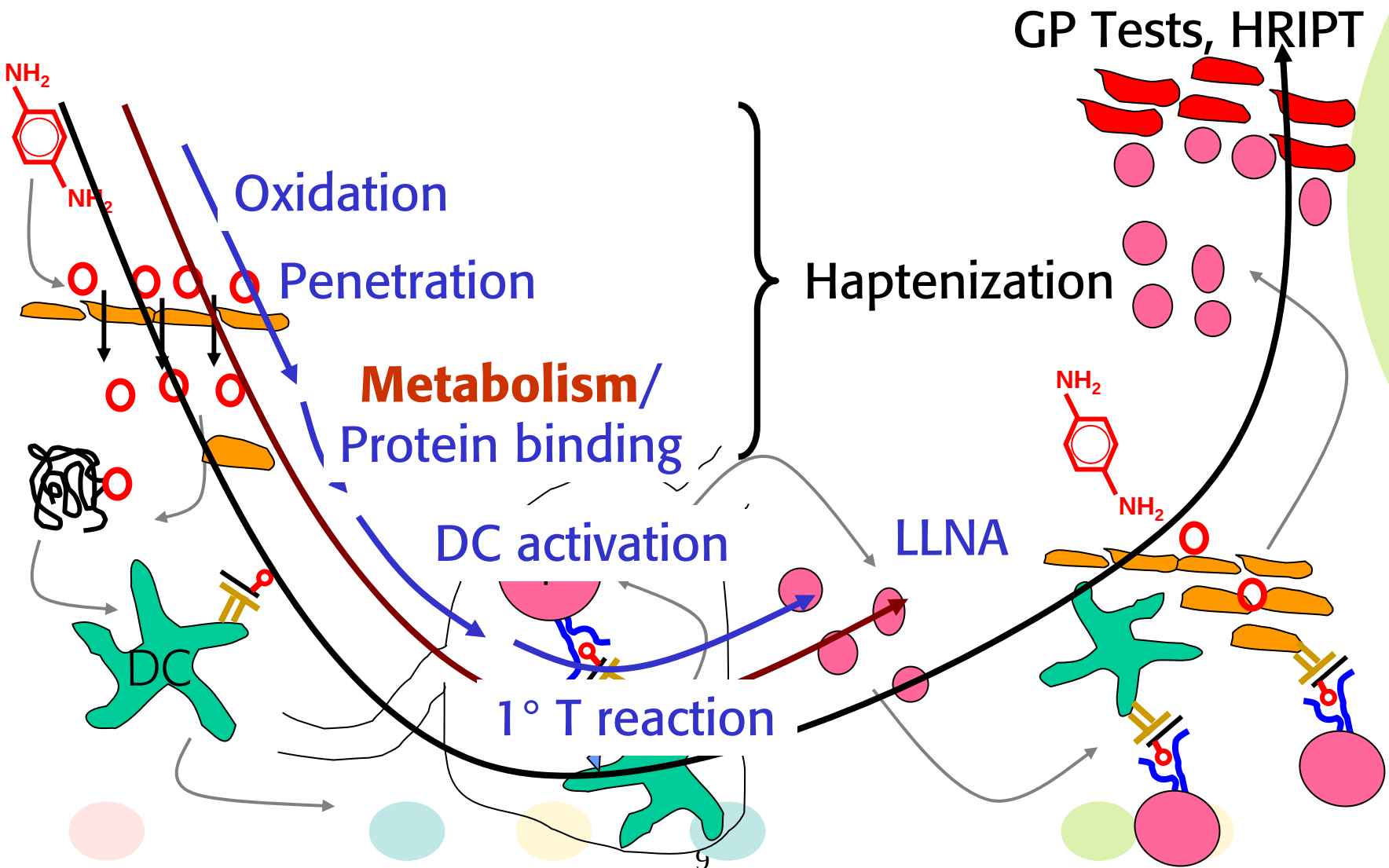
Bandrowski's Base



= **BB**

Events occuring during Skin Sensitization

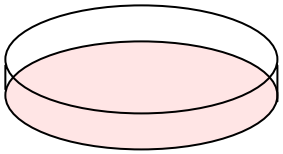
Induction | **Elicitation**



What happens to PPD when arrived in the skin, i.e. epidermis?

Metabolism

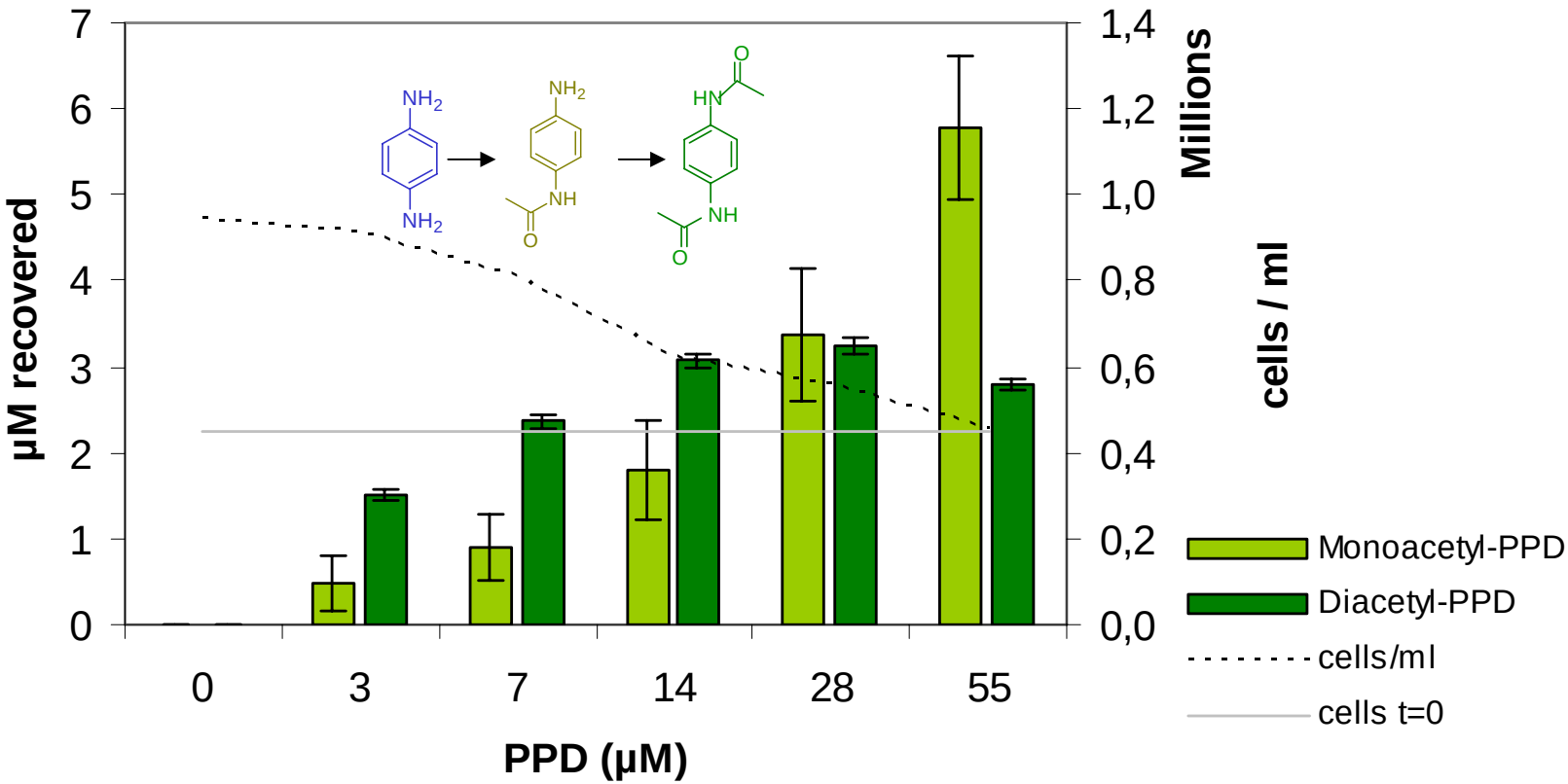
Dermal *N*-acetylation of PPD by keratinocytes (HaCaT) *in vitro*



keratinocyte cell line (HaCaT)

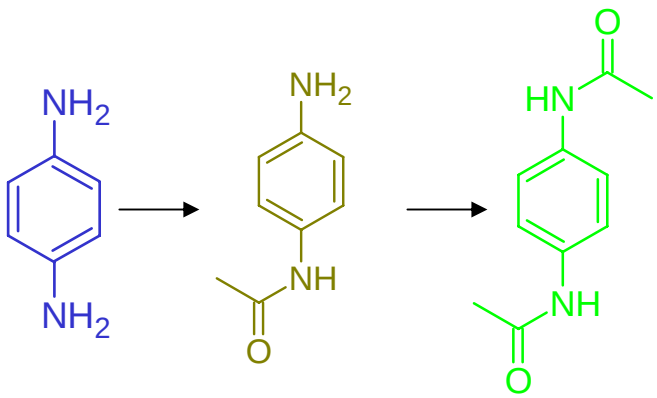
0 - 24 h

Detection of metablite(s)/parent by HPLC/MS



Major metabolic pathway in the skin is *N*-acetylation:

Human skin ex vivo results:



PPD **Mono-Acetyl-** **Di-Acetyl-**

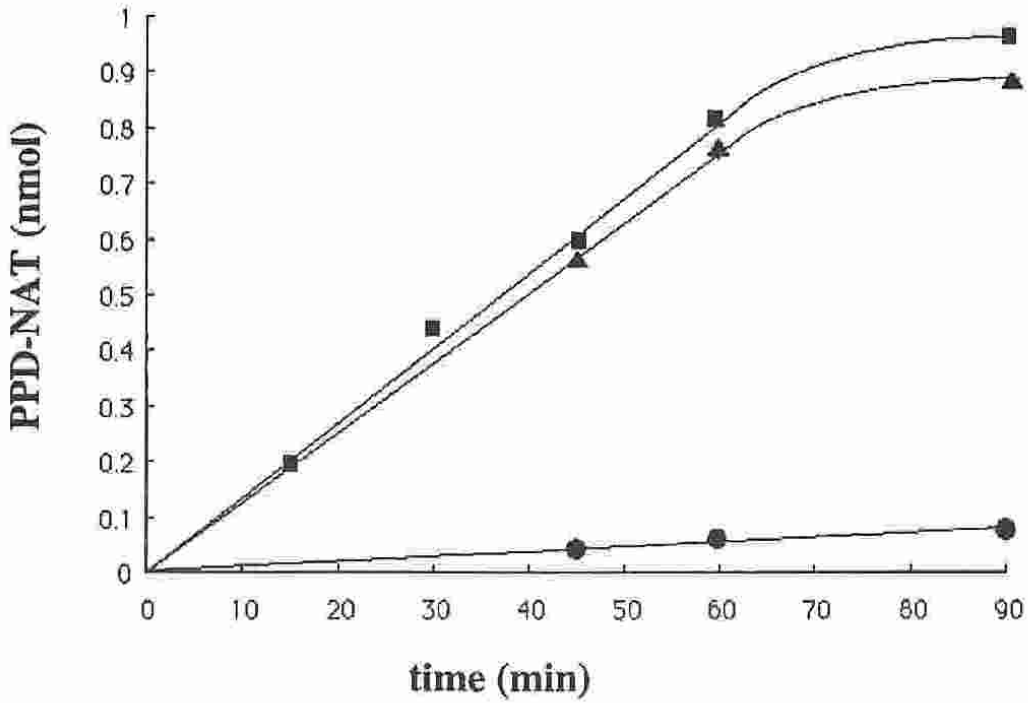
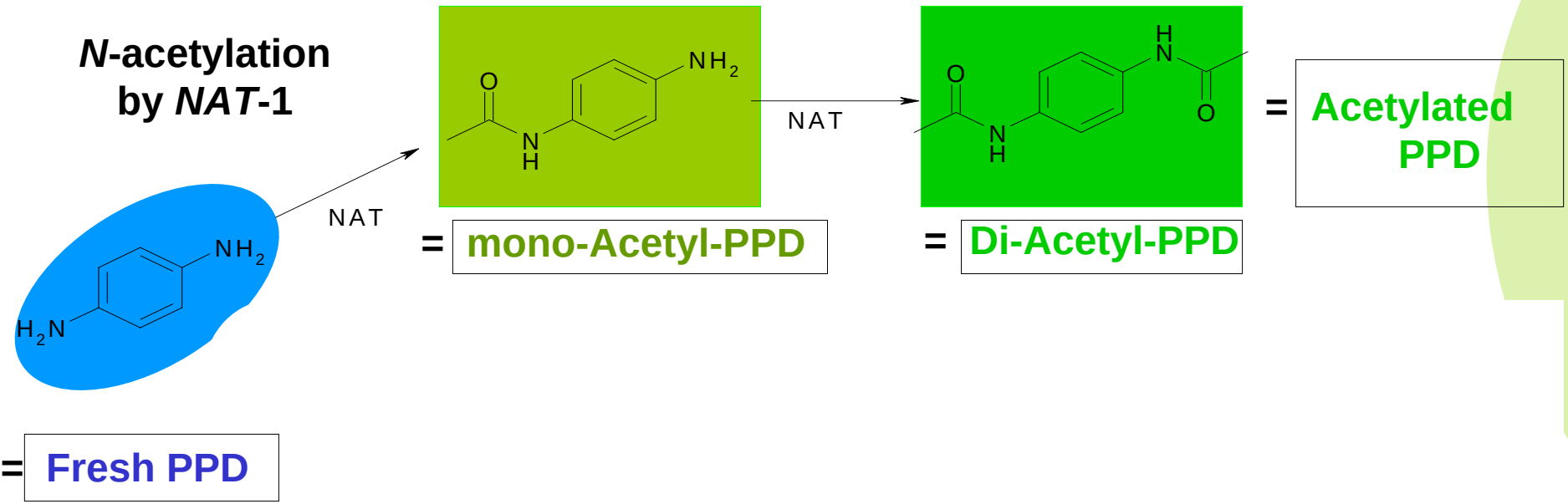


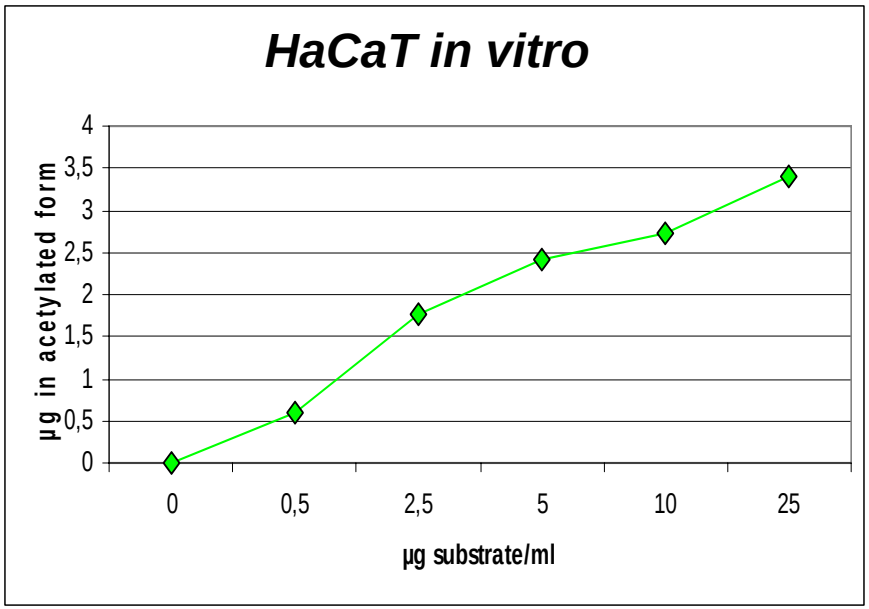
Fig. 1. Representative example of kinetic analysis of PPD acetylation in human skin cytosol (■). Time-dependent formation of MAPPD (▲) and DAPPD (●) was measured in the presence of 400 μM PPD as substrate. DAPPD was detected after a 45-min incubation.

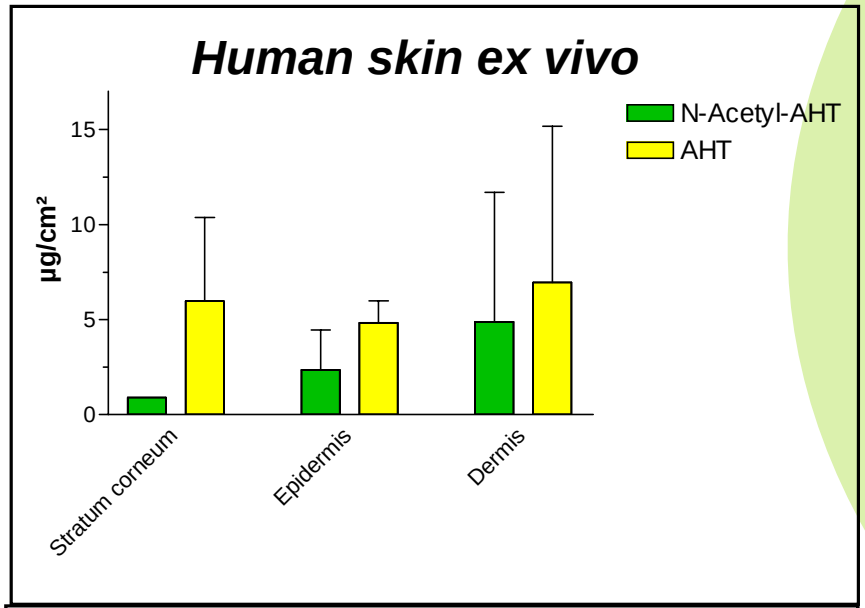
Kawakubo, et al., JPET 292:150-155 (2000)

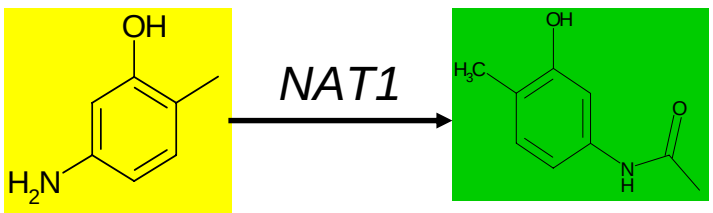
N-acetylated metabolites of PPD

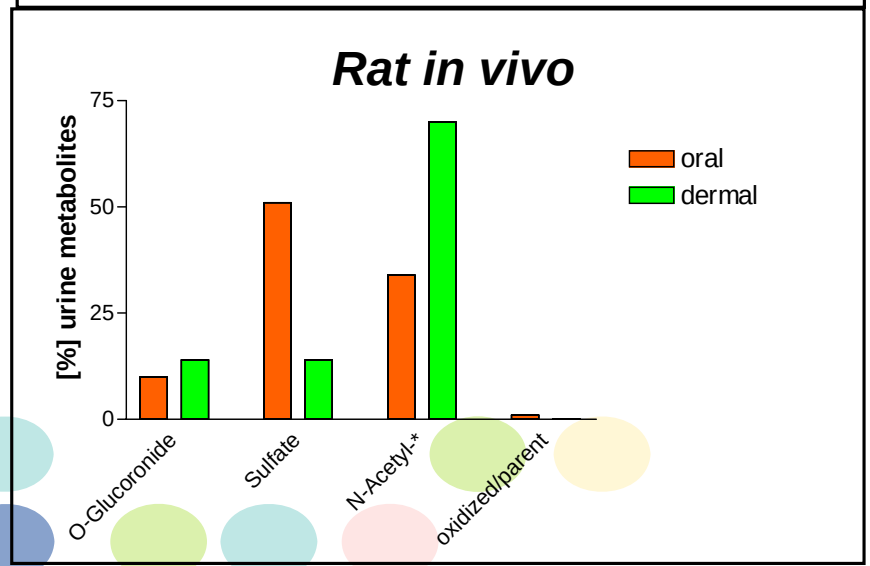


Epidermal N-acetylation of AHT (coupler)

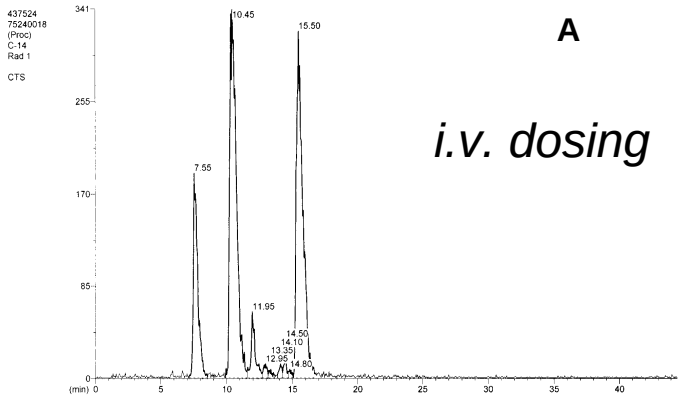


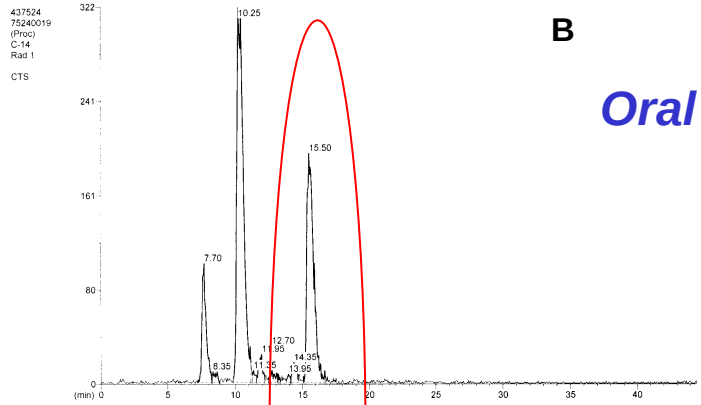


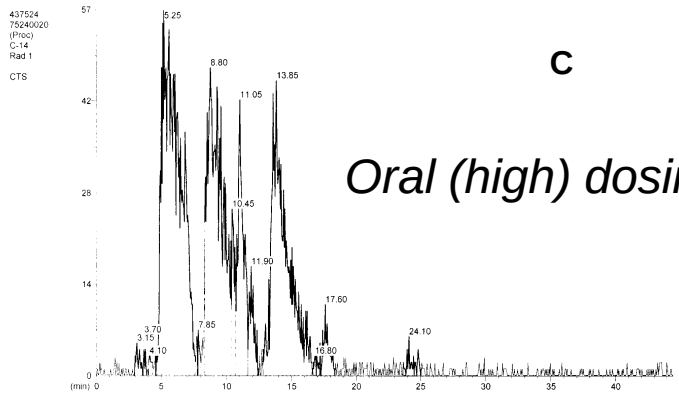


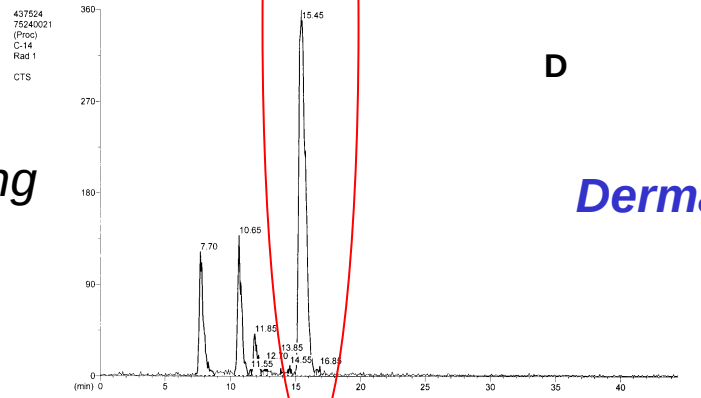


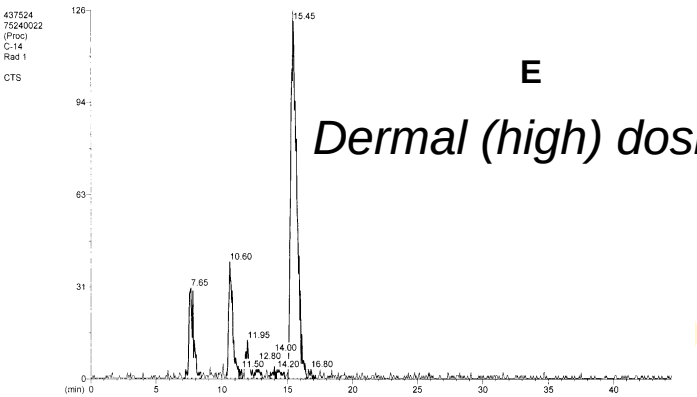
ADME study with AHT in the rat: Metabolism data: Urine





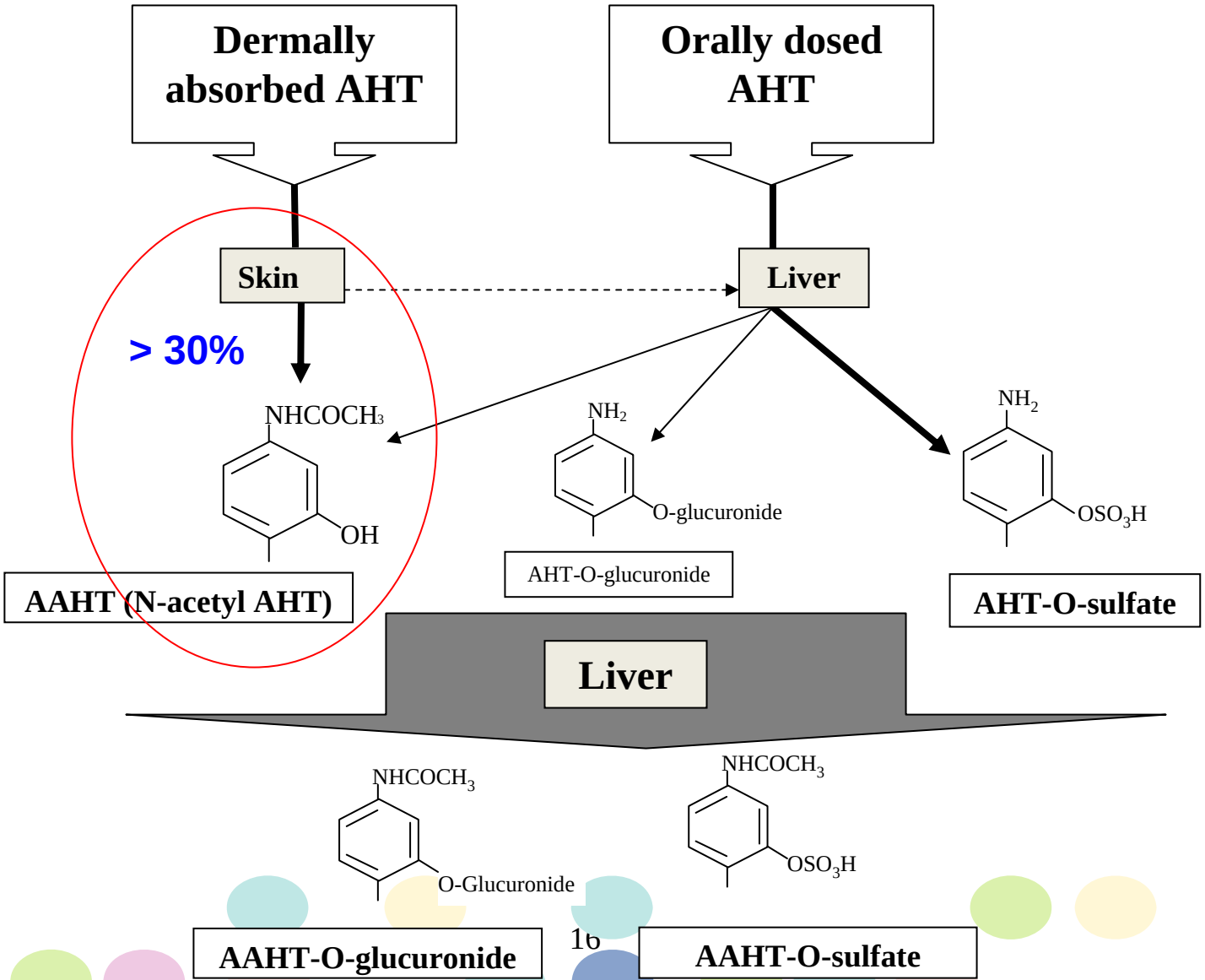






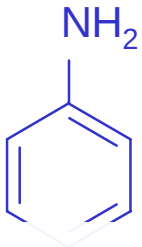
Radioactivity chromatograms after analysis of undiluted urine pools of group 1 (A) i.v., 2 (B) oral low, 3 (C) oral high, 4 (D) dermal low and 5 (E) dermal high (A, B, D= 12.5; C= 500; E = 37.5 mg/kg bw)

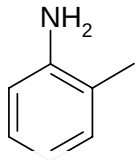
N-acetylation of AHT: A dermal first pass effect

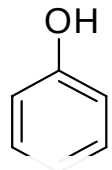


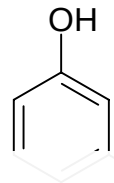
Commonly used aromatic amine hair dye precursors

Primary Intermediates



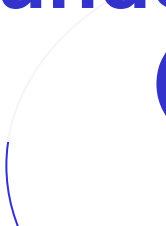




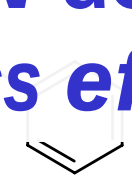


Conclusion on skin metabolism:

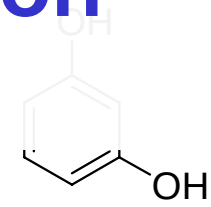
Aromatic amine hair dye precursors (PPD and AHT) undergo epidermal *N*-acetylation (*Dermal first pass effect)*



4-amino-2-hydroxytoluene (AHT)



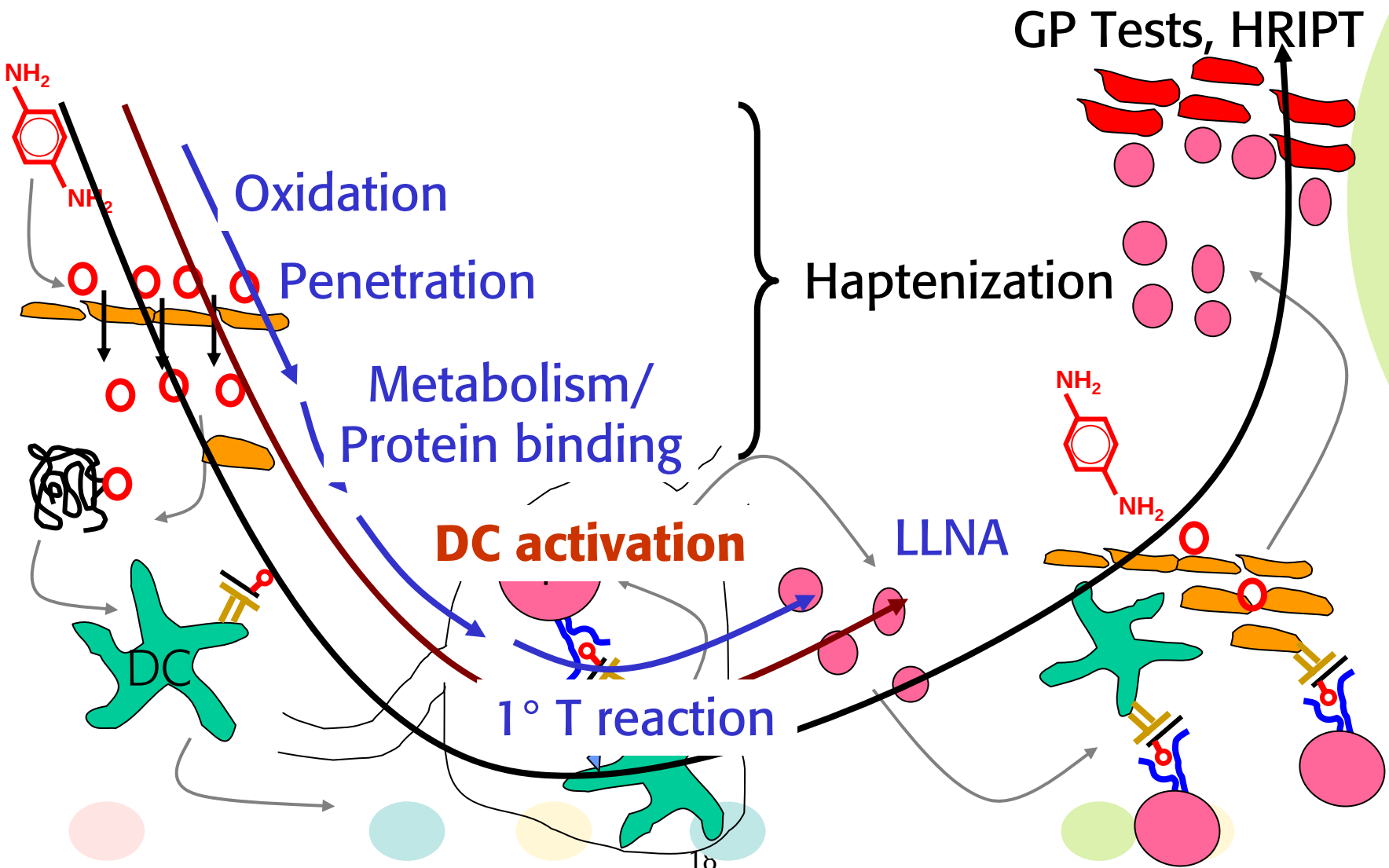
m-aminophenol



Resorcinol

Events occuring during Skin Sensitization

Induction | **Elicitation**

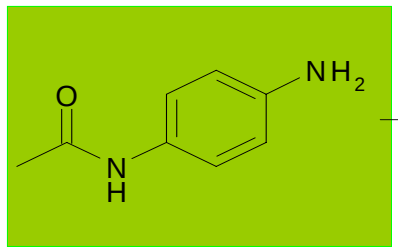


Effects of PPD and derivatives when arrived in the skin, i.e. epidermis?

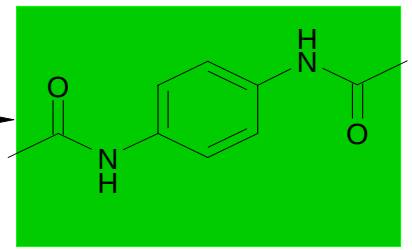
Activation of Dendritic cells (DC)

N-acetylated metabolites and auto-oxidation products of PPD

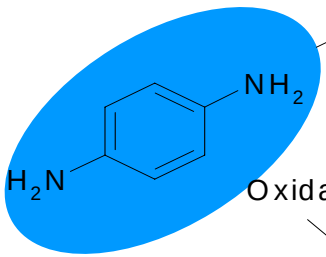
_N_-acetylation by _NAT_-1



NAT



= **Acetylated PPD**



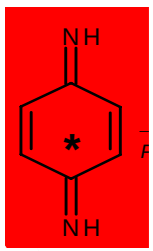
NAT

= **mono-Acetyl-PPD**

= **Di-Acetyl-PPD**

Oxidation

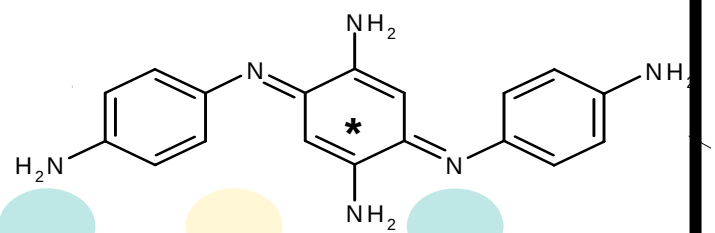
= **Fresh PPD**



Dimers/ Trimers

= **oxidized PPD**

Bandrowski's Base

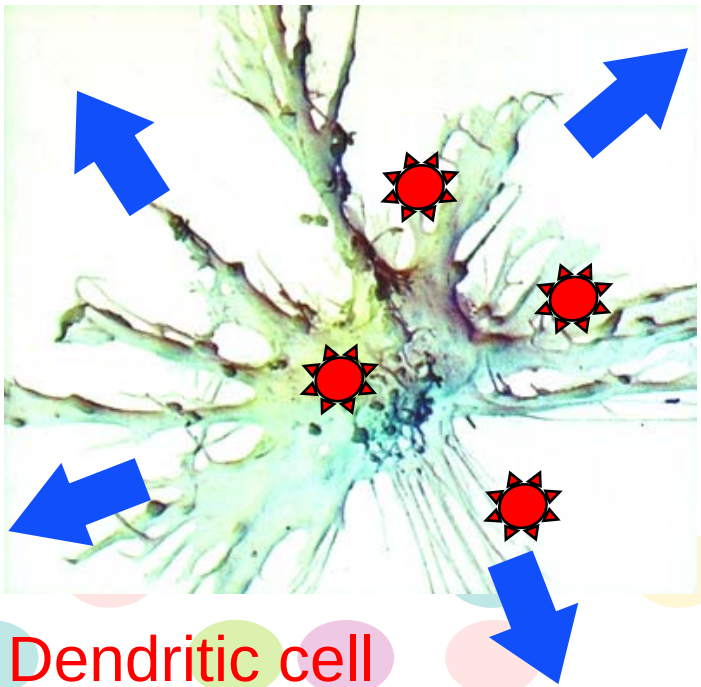


= **BB**

Principle of DC like cells activation

Pool of 4 donors → (IL-4 & GM-CSF, 4 days) → «Dendritic-like cells » → Exposure to chemical → (24 hours) → Analysis

Flow cytometric analysis



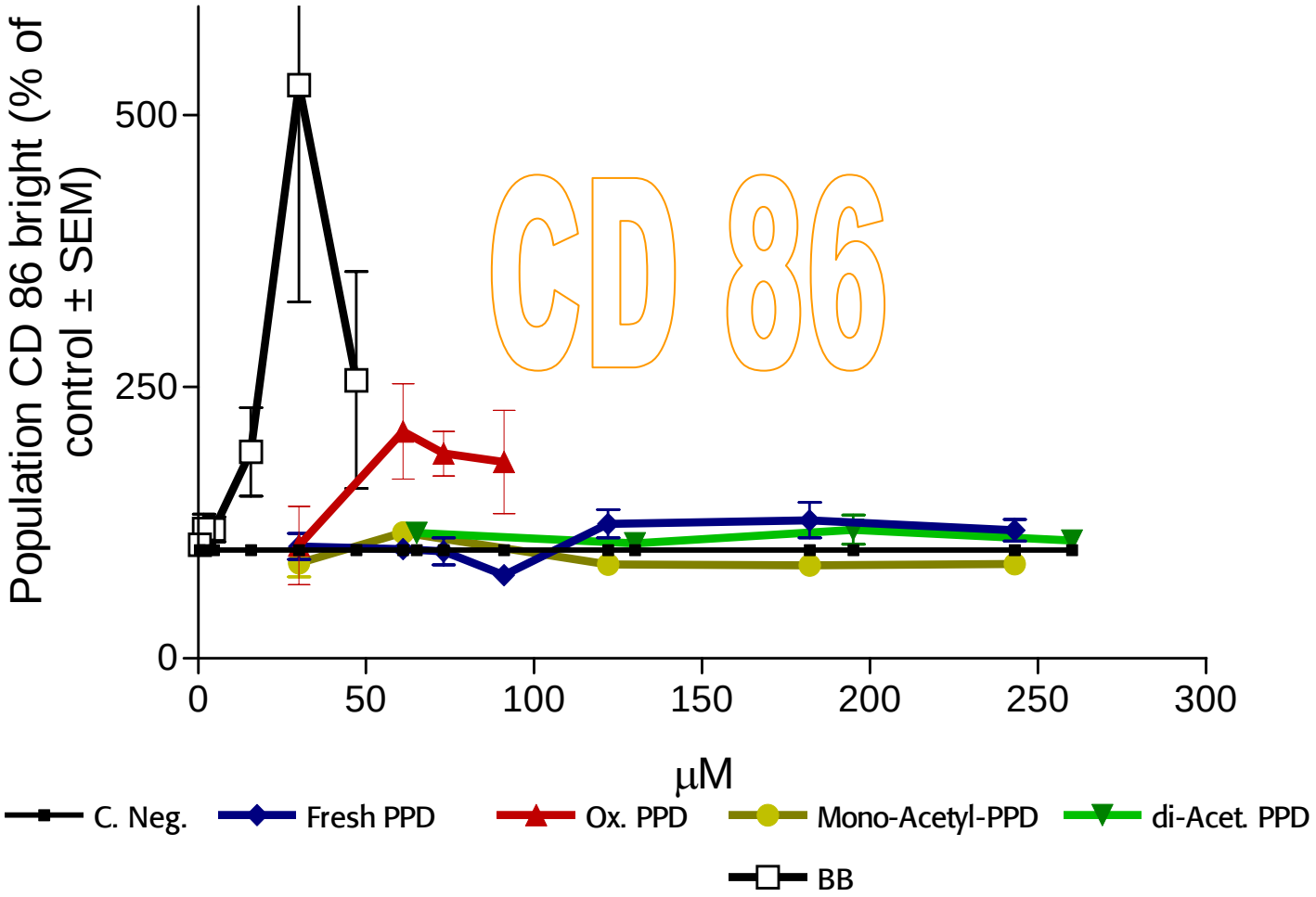
Dendritic cell

Activation markers

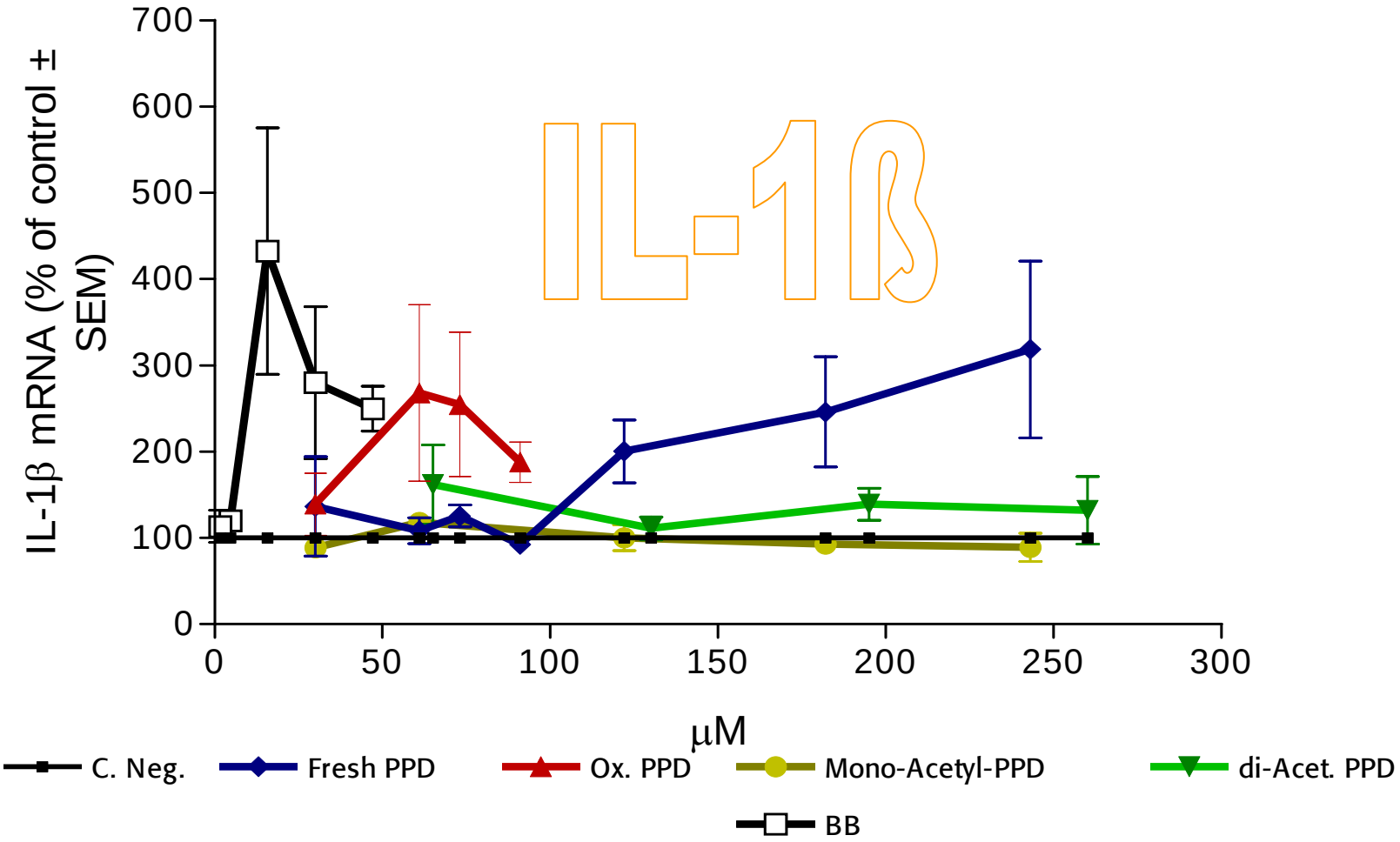
+
CD86 measurement by flow cytometry

+
IL-1β /IL-8 and AQP3 measurements by quantitative RT-PCR

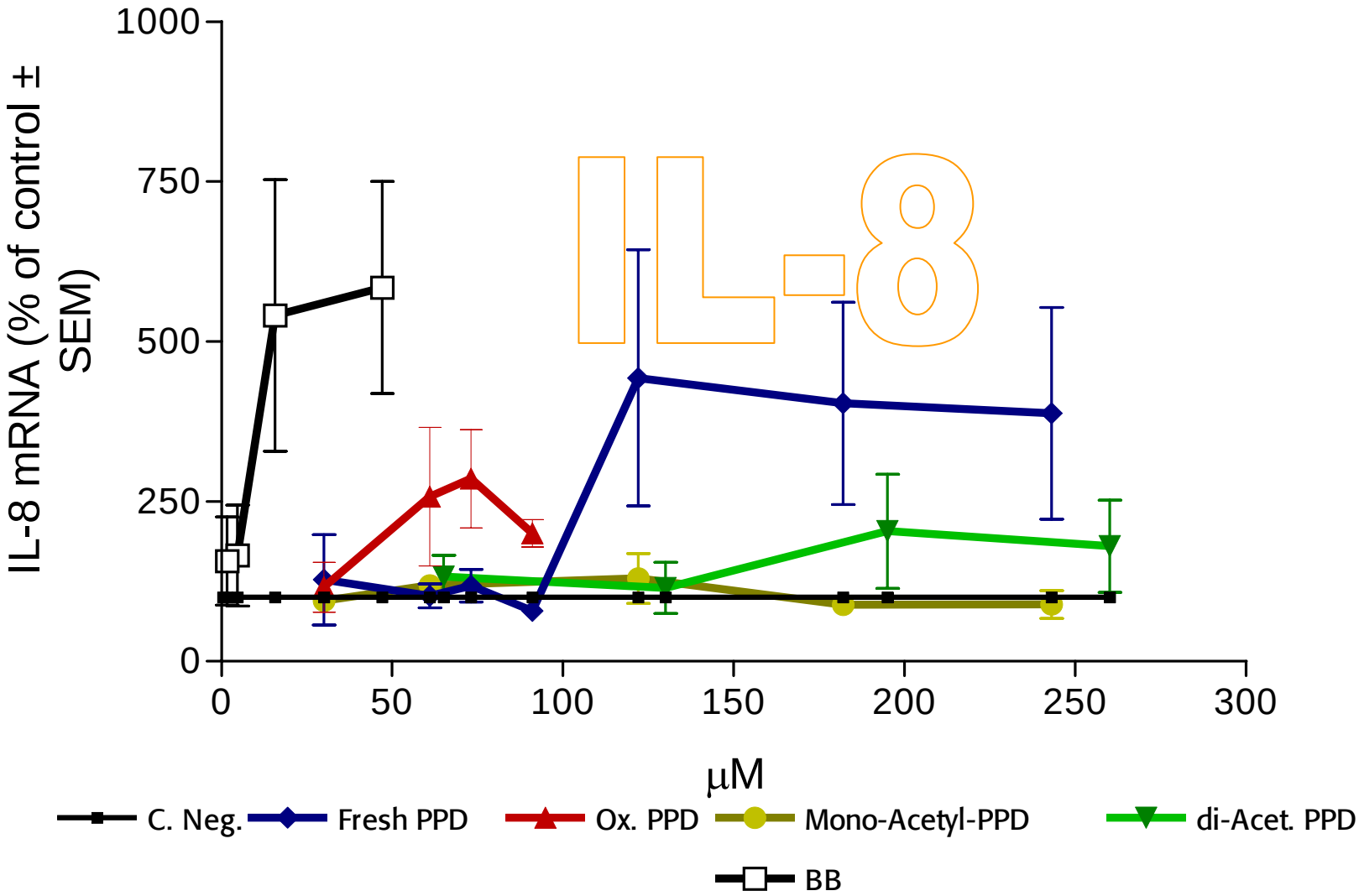
Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD



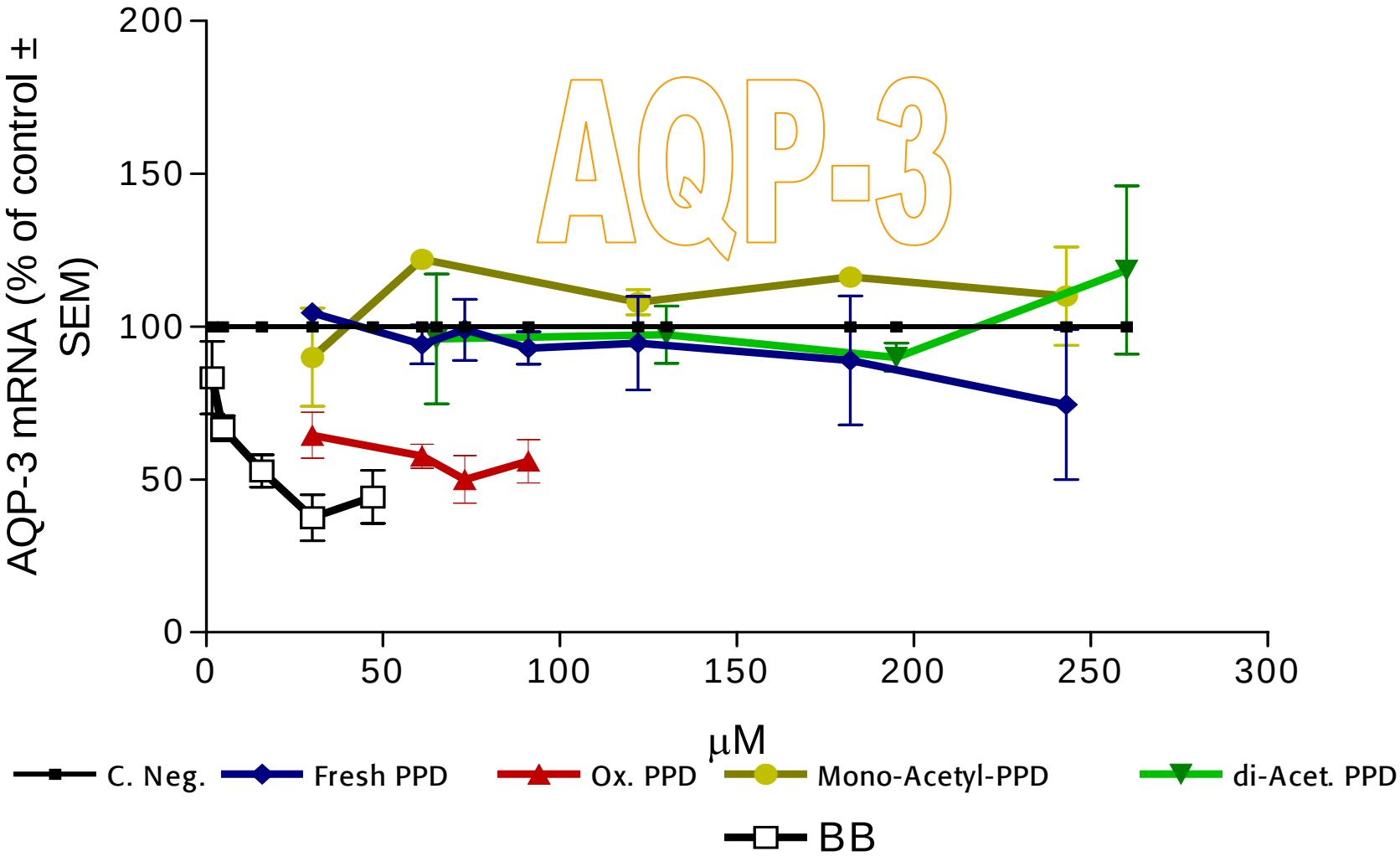
Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD



Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD

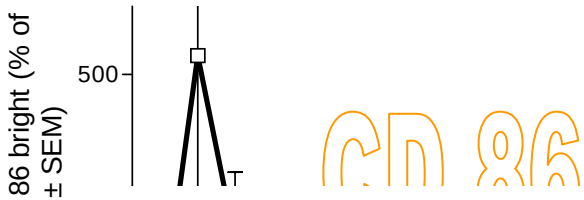


Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD



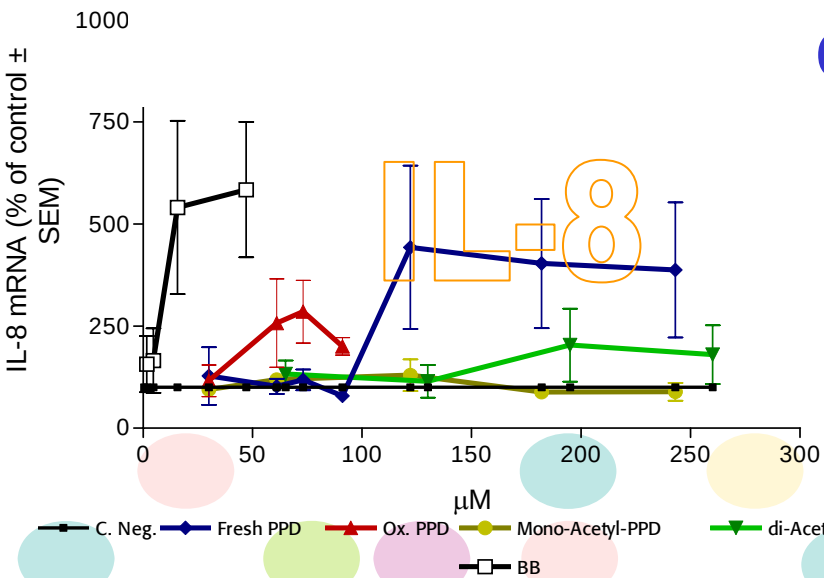
BB

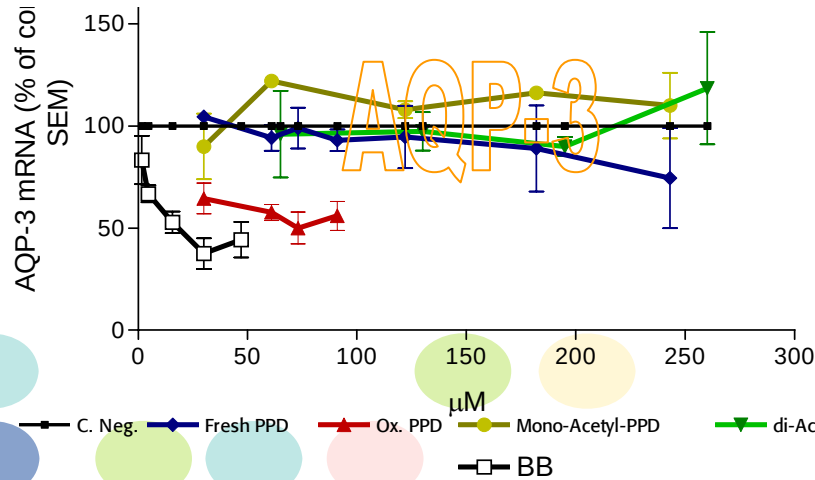
Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD



PPD oxidation is a precondition for DC activation/

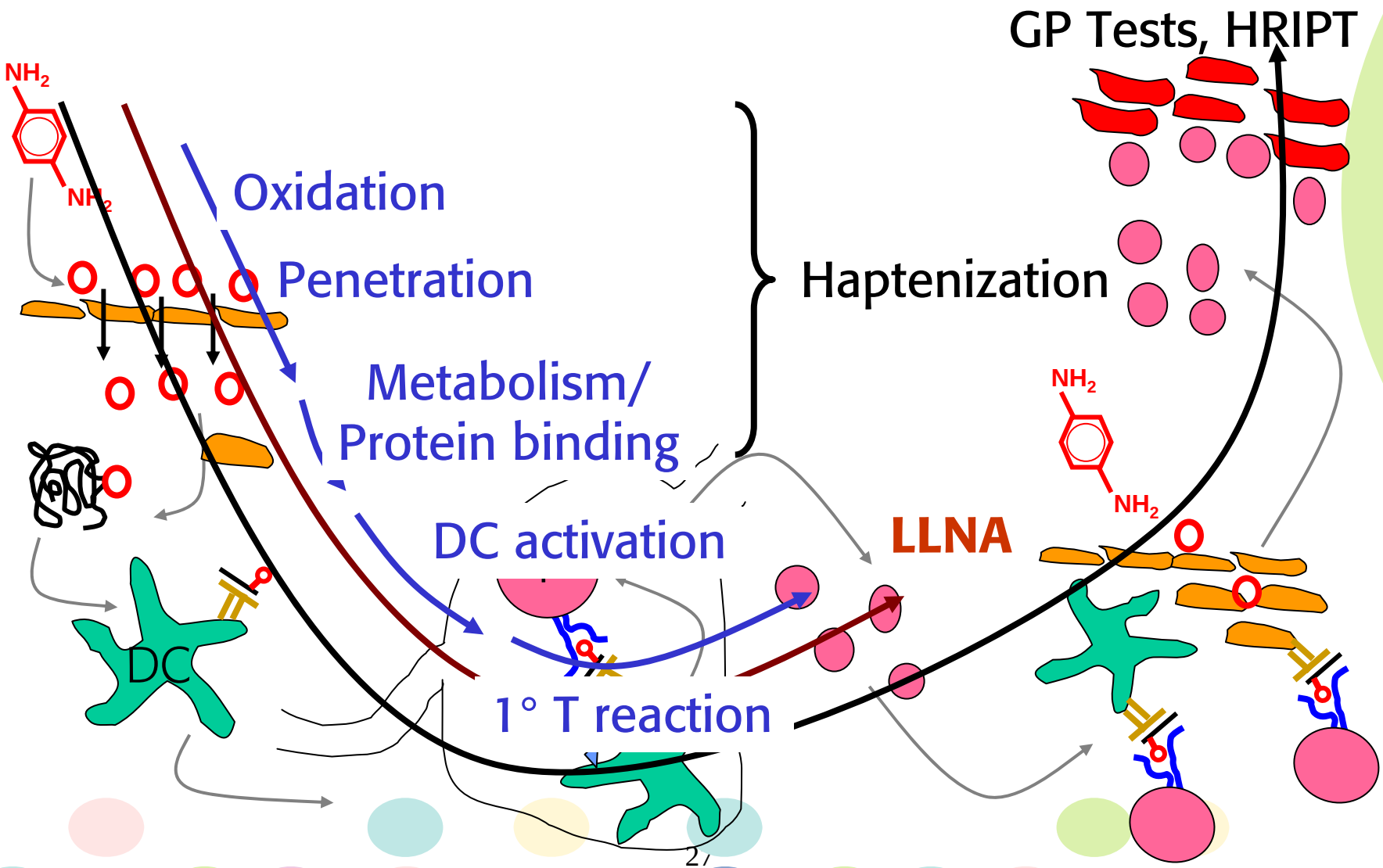
acetylated PPD has no activating effect





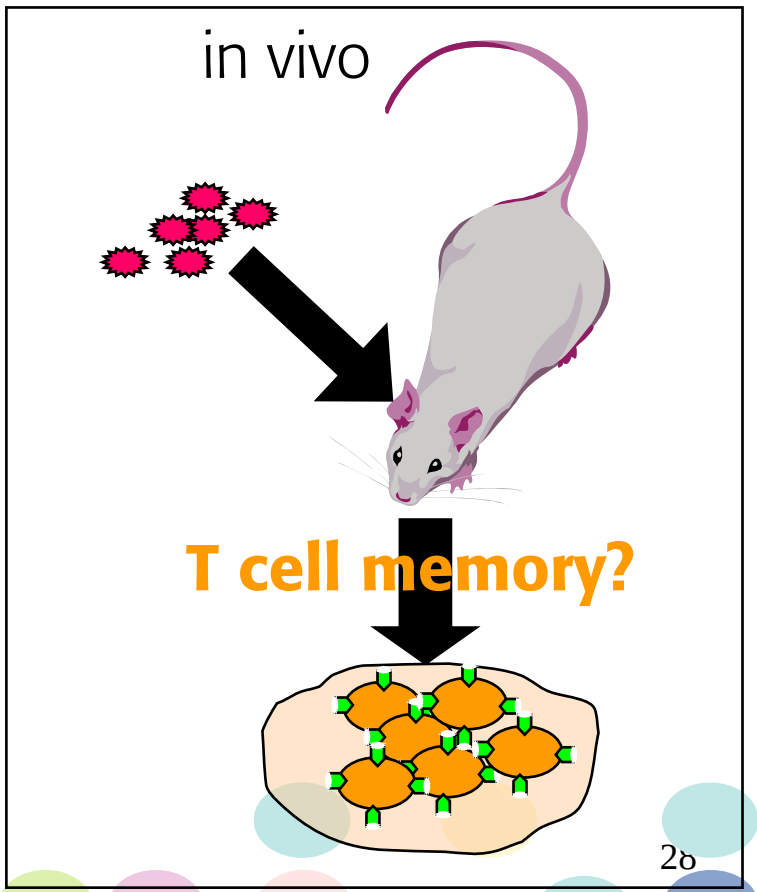
Events occuring during Skin Sensitization

Induction | **Elicitation**



Analysis of the skin sensitization potency of PPD/derivatives:

Local Lymph Node Assay (LLNA)

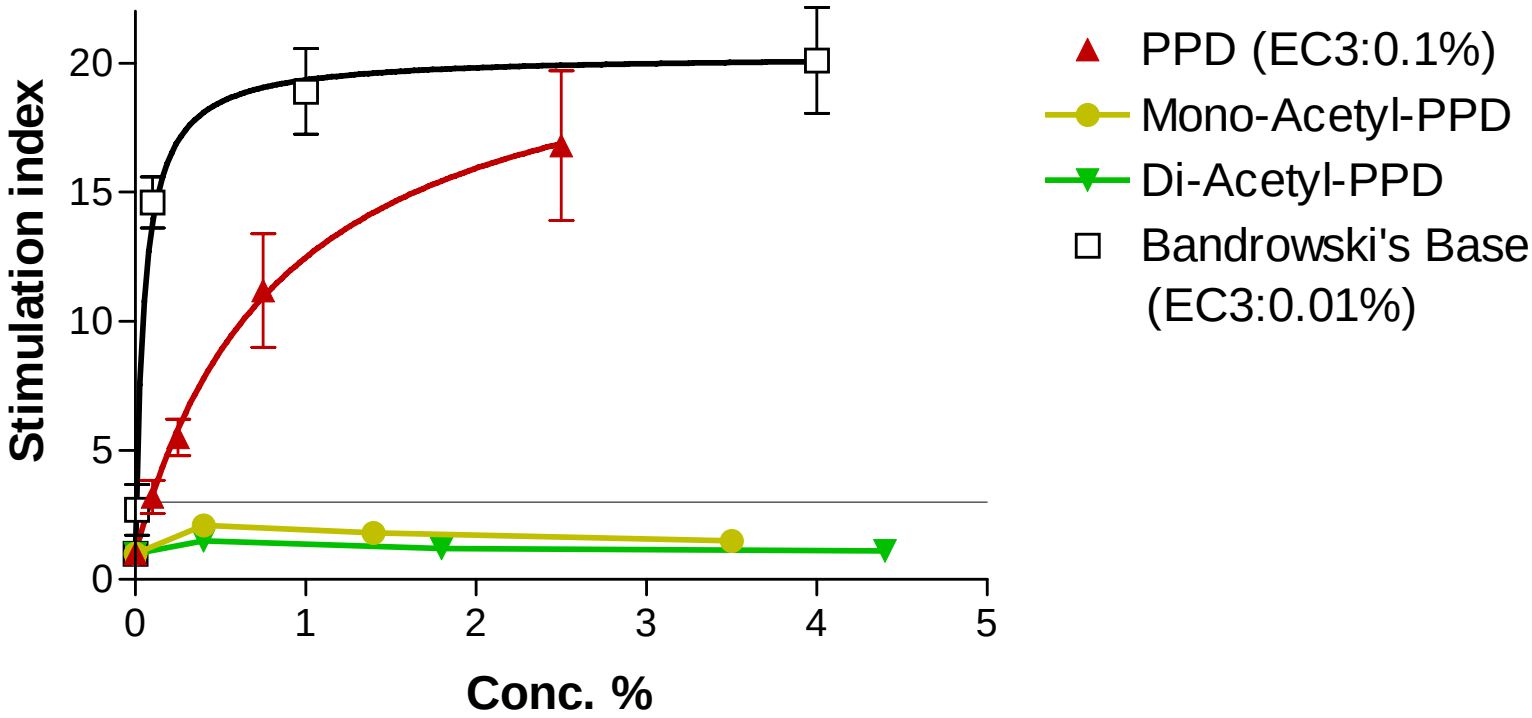


Exposure conditions support oxidation of PPD to oxidized PPD derivatives

In vivo (LLNA) effects of PPD, BB, Mono- and Di-Acetyl-PPD

Application (OECD 429):
3 daily applications of 250 µg/cm² (1%) PPD = 750 µg/cm² in DMSO/water (80:20)

Local lymph node assay (LLNA)



Based on all available LLNA data (summarized in Baketter 2008) as well as human data the Weight of Eivdence No Expected Sensitization Induction Level (WoE NESIL) is 0.1% ~ 25 µg/cm²

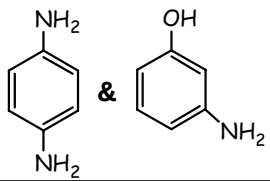
How do conditions of sensitization testing compare to PPD exposure during hair dyeing?

Exposure to PPD during hair dyeing: Basic chemistry of hair coloring

Commercial hair colorants are all based on similar chemistry:

Primary intermediates + couplers, oxidant, high pH, exposure for approx. 30 min

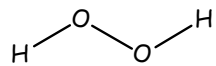
Dye Precursors



tint

+

Oxidant
Hydrogen Peroxide



developer

+

Alkaliser – pH 10

Ammonia (NH_3)
(Ethanolamine)

Chassis

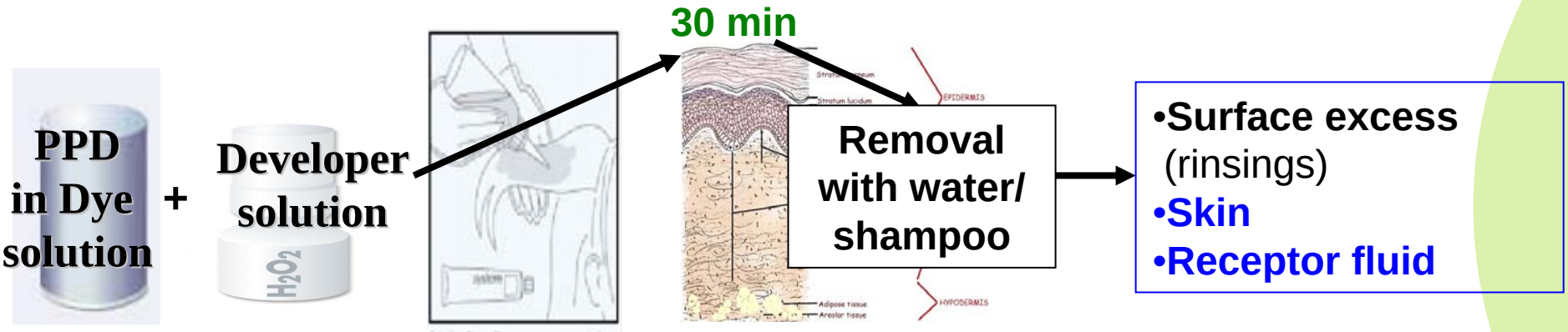
tint

Form coloured Chromophores + Bleach melanin & lighten hair

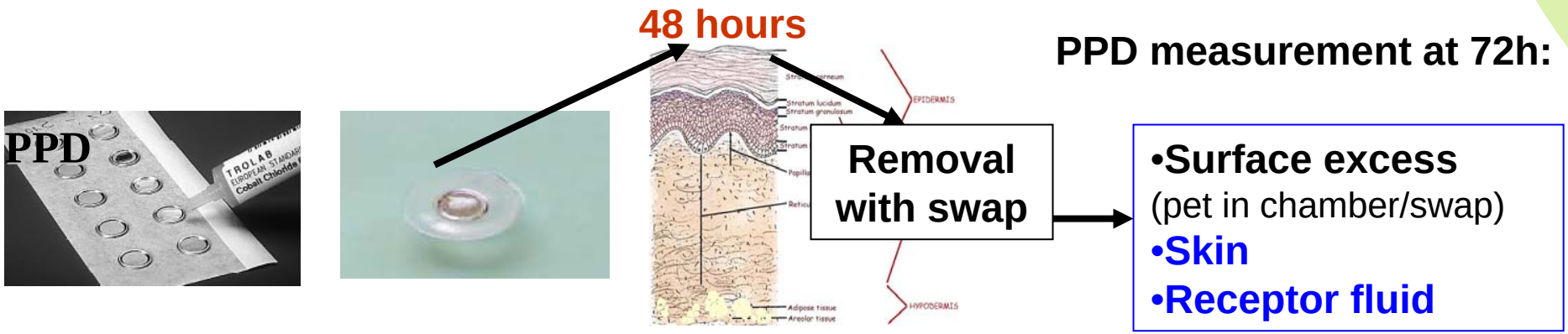
- ***Uncontrolled auto-oxidation of PPD is prevented***
- ***Reaction with coupler is chemically preferred***
- ***No benzoquinone diimine***
- ***Exposure time approx. 30 min***

Skin penetration study to measure actual PPD exposure

Permanent hair dye usage conditions: Test Product F



Diagnostic patch testing conditions: Test Patch H

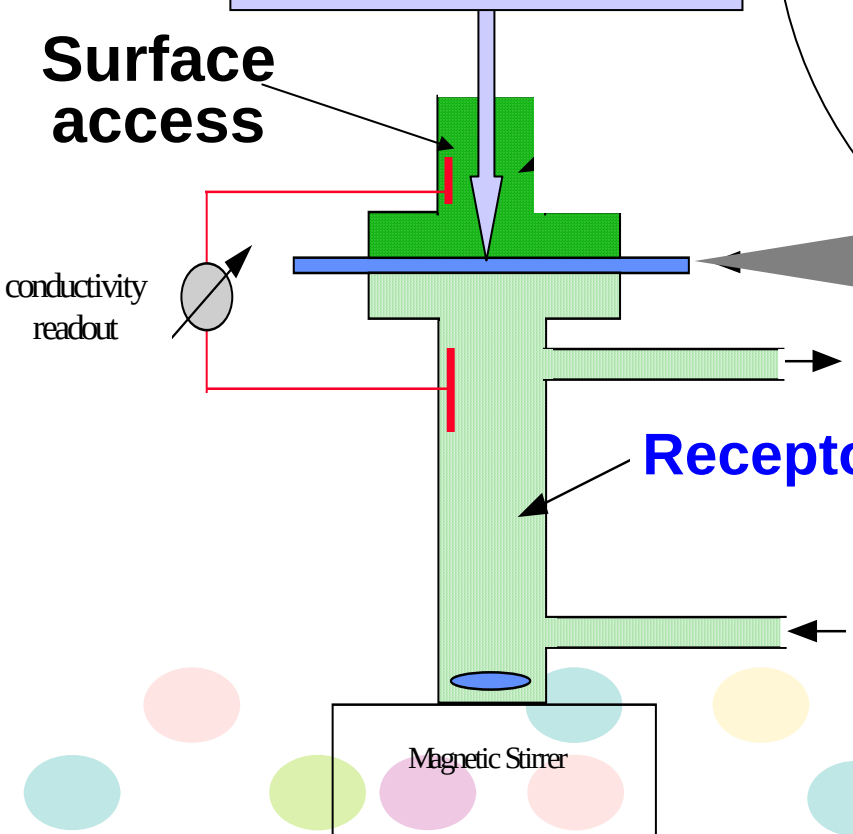


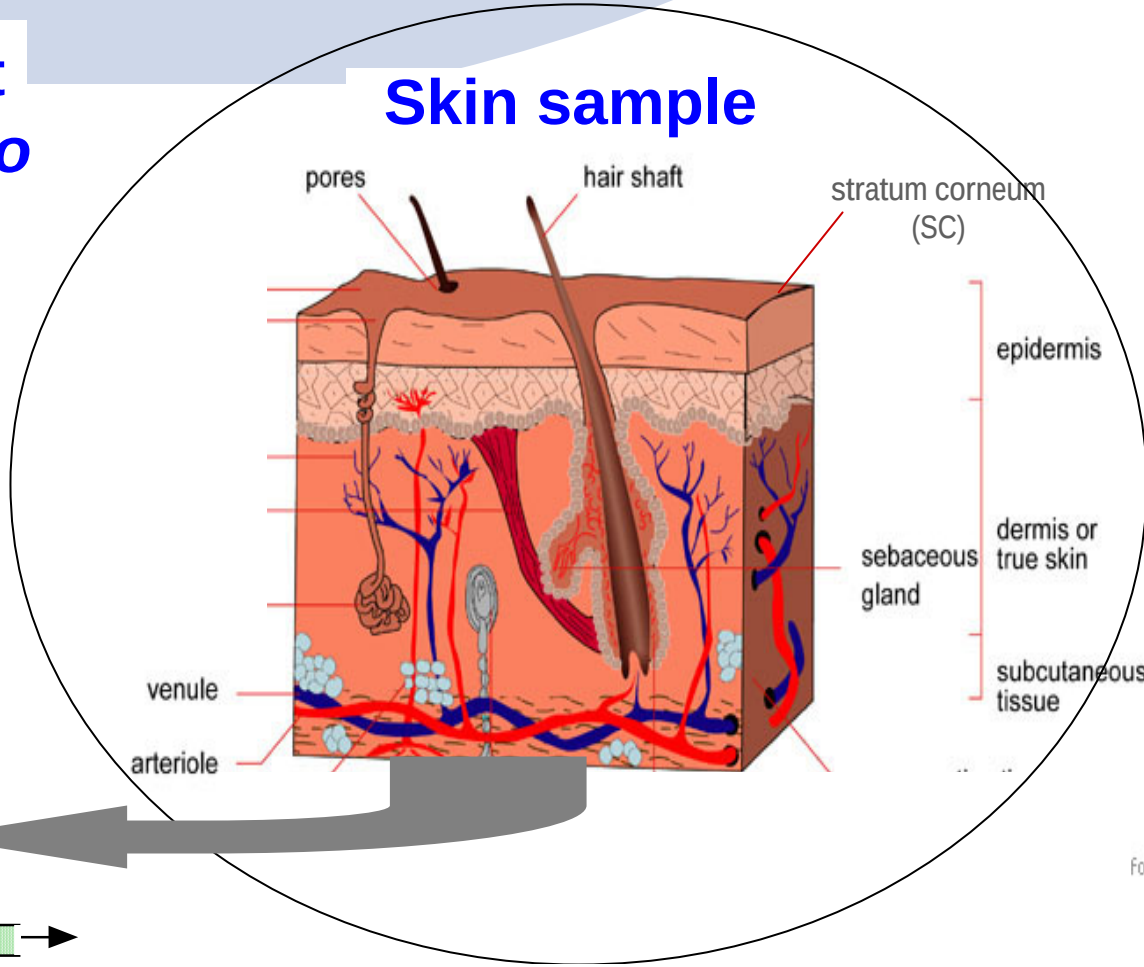
Applied dose - surface excess = Measured exposure level (MEL)

Exposure measurement with skin samples *in vitro* (OECD 428)

Application of hair dye product containing 2% PPD + coupler for 30 min and rinse off

Surface access





Receptor fluid (systemic circulation)

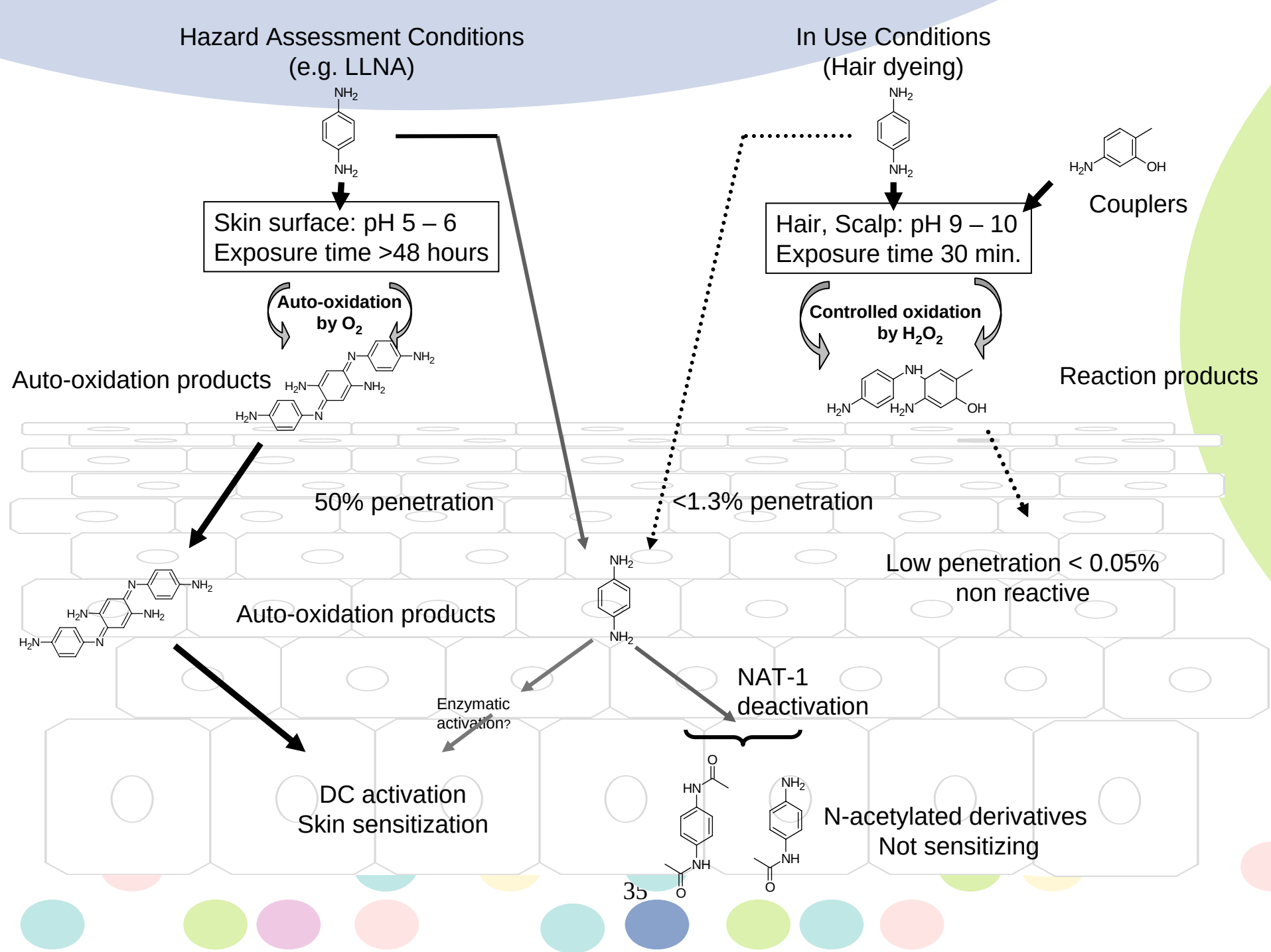
Measured PPD exposure level

Determination of Measured exposure level (MEL) for PPD under hair dyeing and diagnostic patch test conditions

	Diagnostic patch: Test patch **H (1% PPD)**	Test Product **F (2% PPD)**	Product[1] (2% PPD)
Applied dose [µg/cm²]	**400**	**3000**	**400**
Exposure time [h]	**48**	**0.5**	**0.5**
Surface excess/rinsings (%)	49	95	95
Measured Exposure level (**MEL**) [µg/cm²]	**206**	**7**	**15** (22)

[1]from Hueber-Becker et al 2004,

Patch test exposure (MEL) more than 10 fold higher


Hazard Assessment Conditions
(e.g. LLNA)
In Use Conditions
(Hair dyeing)
Couplers
Skin surface: pH 5 – 6
Exposure time >48 hours
Hair, Scalp: pH 9 – 10
Exposure time 30 min.
Auto-oxidation
by O2
Controlled oxidation
by H2O2
Auto-oxidation products
Reaction products
50% penetration
<1.3% penetration
Low penetration < 0.05%
non reactive
Auto-oxidation products
NAT-1
deactivation
Enzymatic
activation?
DC activation
Skin sensitization
N-acetylated derivatives
Not sensitizing

Conclusions on PPD sensitization potency

- **PPD (non-oxidized) is a weak activator of DC *in vitro* (CD86, Il-1ß, Il-8, AQP3)**
- **Auto-oxidation is a precondition for relevant DC activation (danger)**
- **Current (*in vivo*) assays predominantly consider sensitization potency of PPD auto-oxidation**
- **Hair dyeing conditions block PPD auto-oxidation and significantly reduce exposure to PPD**
- **Skin actively *N*-acetylates PPD to non sensitizing derivatives**
- **Equilibrium between dermal *N*-acetylation and oxidative activation of PPD is critical for individual risk of induction of PPD allergy**

Publications

PPD data:

Skin sensitization to *p*-Phenylenediamine: The diverging roles of oxidation and *N*-acetylation for dendritic cell activation and the immune response

Aeby P, Sieber T, Beck H, Gerberick GF, and Goebel C (2008)
J Invest Dermatol; doi:10.1038/jid.2008.209 (online)

AHT data:

Skin metabolism of aminophenols: Human keratinocytes as a suitable *in vitro* model to predict the dermal transformation of 4-amino-2-hydroxytoluene *in vivo*

Goebel C, Hewitt NJ, Kunze G, Wenker M, Hein D, Beck H, and Skare J
Submitted to Pharmacl. Appl. Toxicol.

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