HUMAN SYSTEMIC EXPOSURE TO OXIDATIVE HAIR DYES

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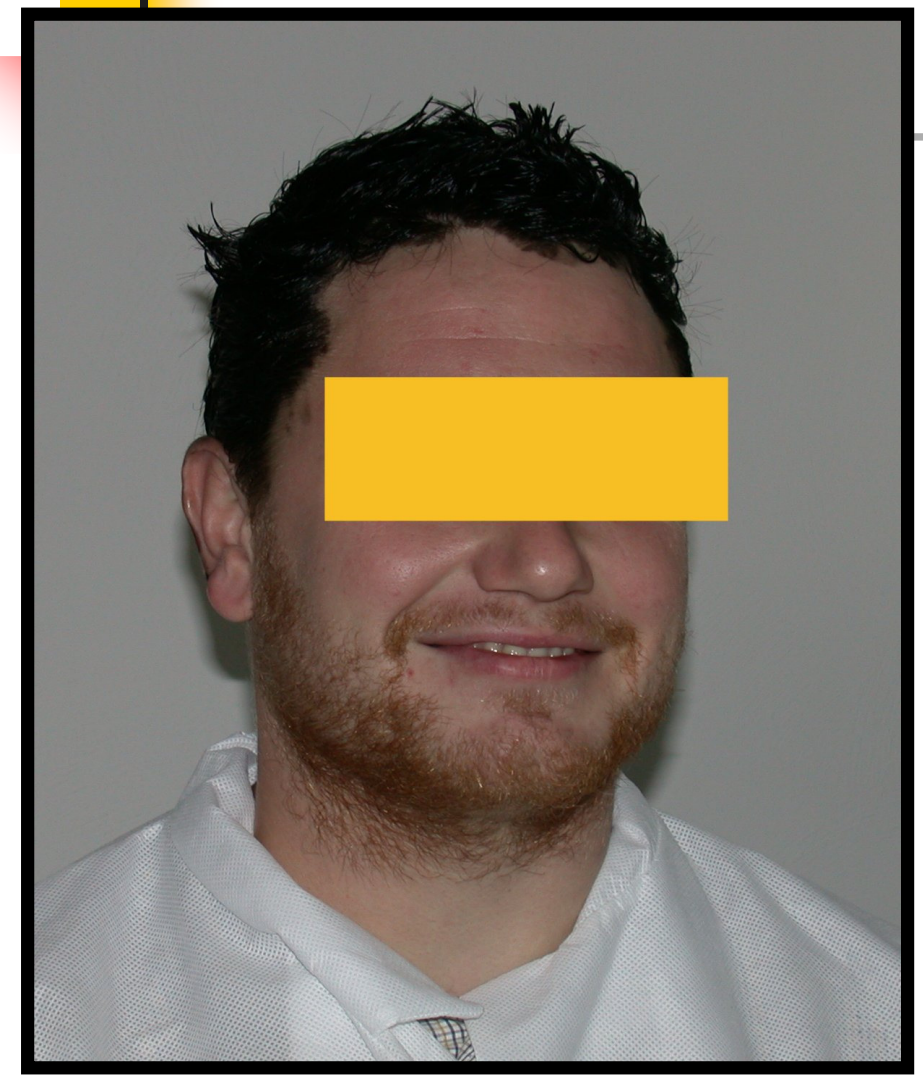
EPIDEMIOLOGICAL EVIDENCE ON HAIR DYES AND (BLADDER) CANCER: 1988 - 2008

- International Agency for the Research of Cancer (IARC, 2008):
  - *Personal use of hair colorants: inadequate evidence (Class 3)*
  - *Occupation as a hairdresser or a barber entails* ***exposures*** *that may be probably carcinogenic (Class 2A)*
- NB: In epidemiology, contact or profession are ***SURROGATE ENDPOINTS for EXPOSURE: If you work with the substance, you are (maybe?) exposed and (maybe?) systemically exposed***
- What is the human consumer and professional exposure to oxidative hair dyes?

Human skin penetration / systemic exposure to a [^{14}C]-PPD-containing hair dye *

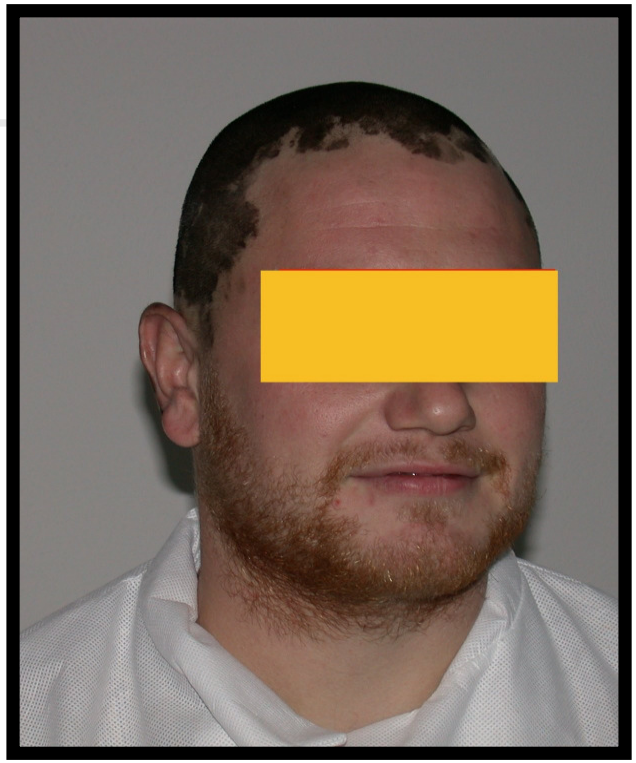


- Eight male volunteers (female volunteers hard to find)
- Treatment with 70 mL of a commercial hair dye containing 1.3 grams of [^{14}C]-PPD (contact: 30 minutes)
- Hair shampooed, washed, rinsed, dried, clipped, scalp washed after 24 hours
- [^{14}C] in hair, water, blood, urine and faeces for 144 hours after treatment
- Study conducted under GCP and GLP

* Hueber-Becker et al., 2004

Human systemic exposure to a [^{14}C]-PPD-containing hair dye: results *

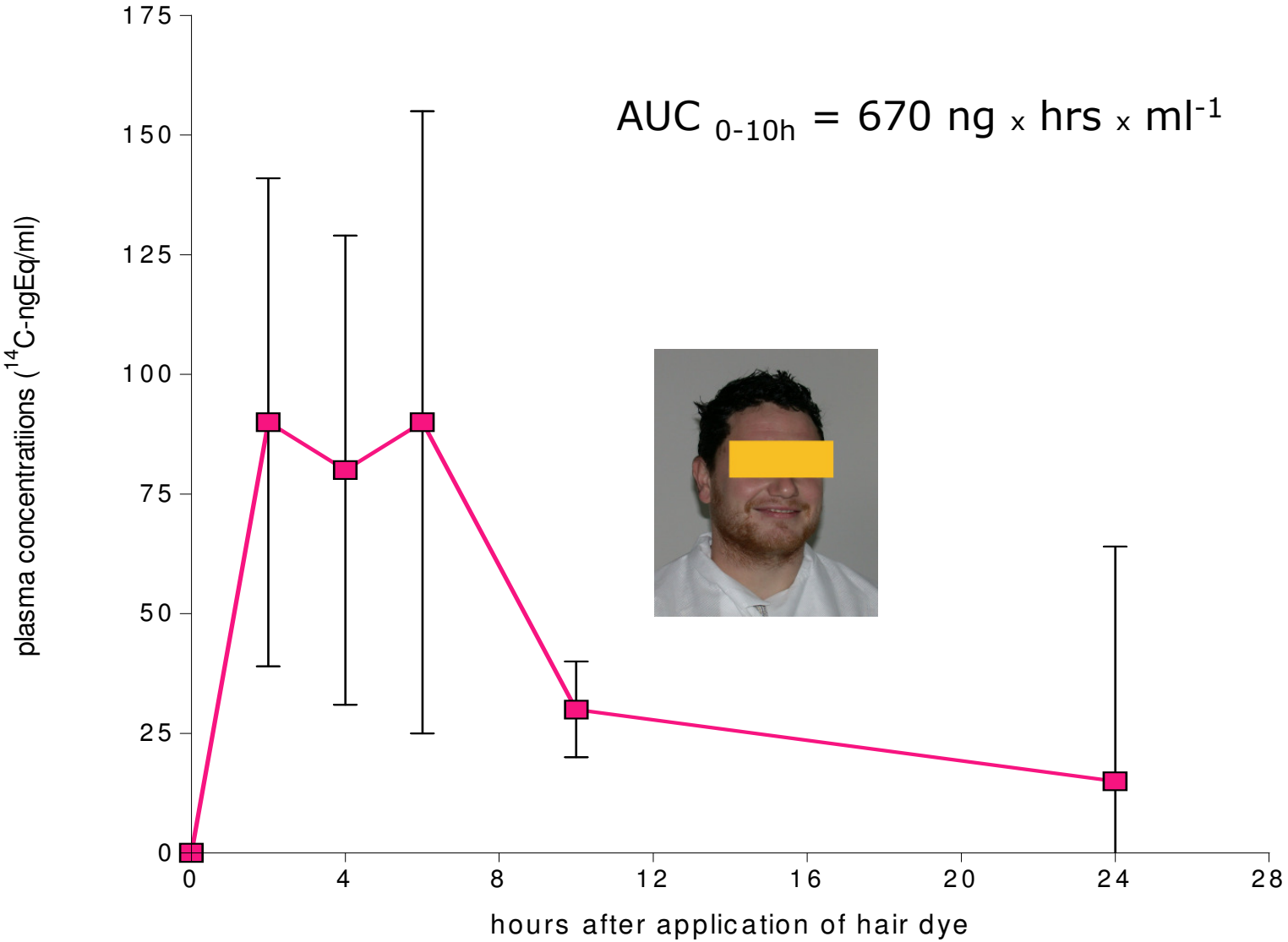
- Washing water: 81.7 ± 2.2% of radioactivity
- 24-Hour scalp wash: 0.41 ± 0.10%
- Hair: 13.0 ± 2.0%
- Urine: 0.50 ± 0.21% (mono- and di-acetylated PPD)
- Nearly quantitative excretion in 24 hours
- Total absorbed: 0.54 ± 0.25% (0.09 mg/kg)
- Urine: mono- and di-acetylated metabolites of PPD**



CONCLUSION: systemic exposure to hair dye components is minimal

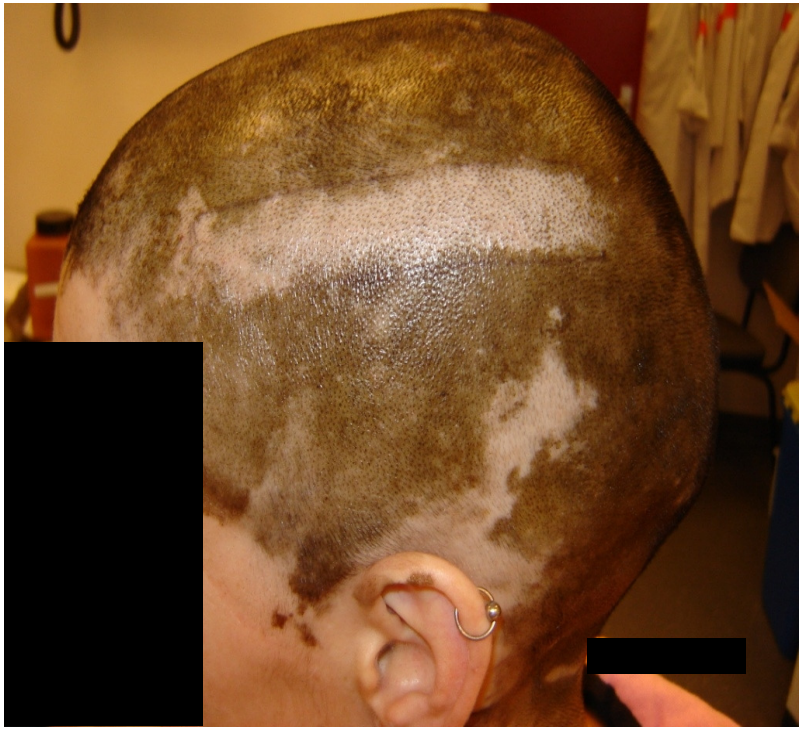
* Hueber-Becker et al., 2004; ** Nohynek et al., 2005

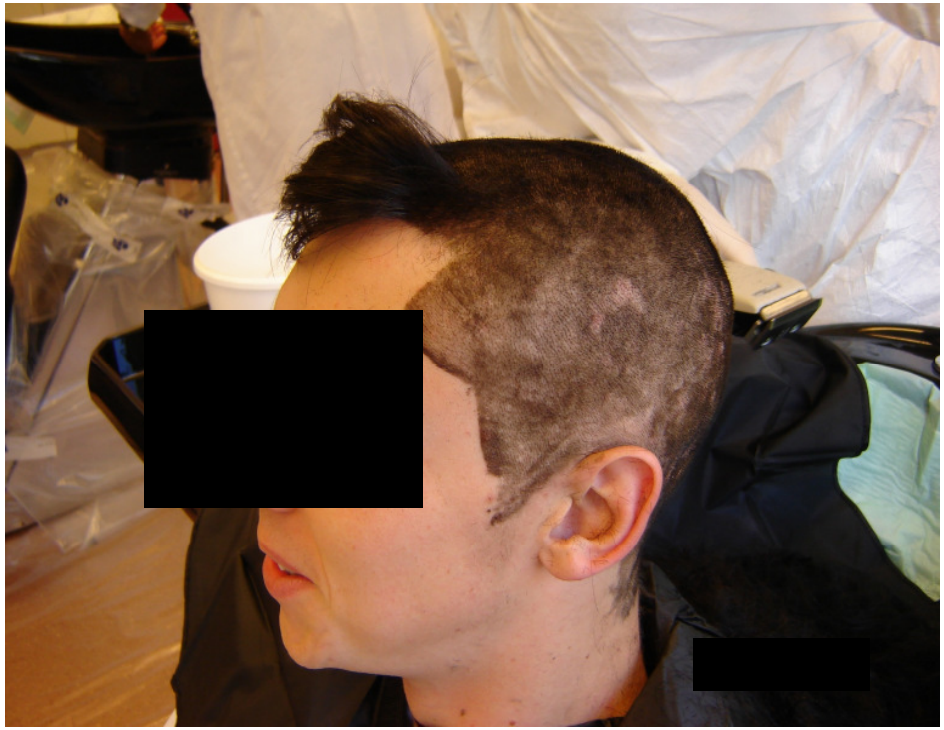
Plasma levels (ng ^{14}C-equivalents/ml) in human volunteers after hair dyeing with a dark-shade [^{14}C]-PPD hair dye (2004, max. exposure conditions) *



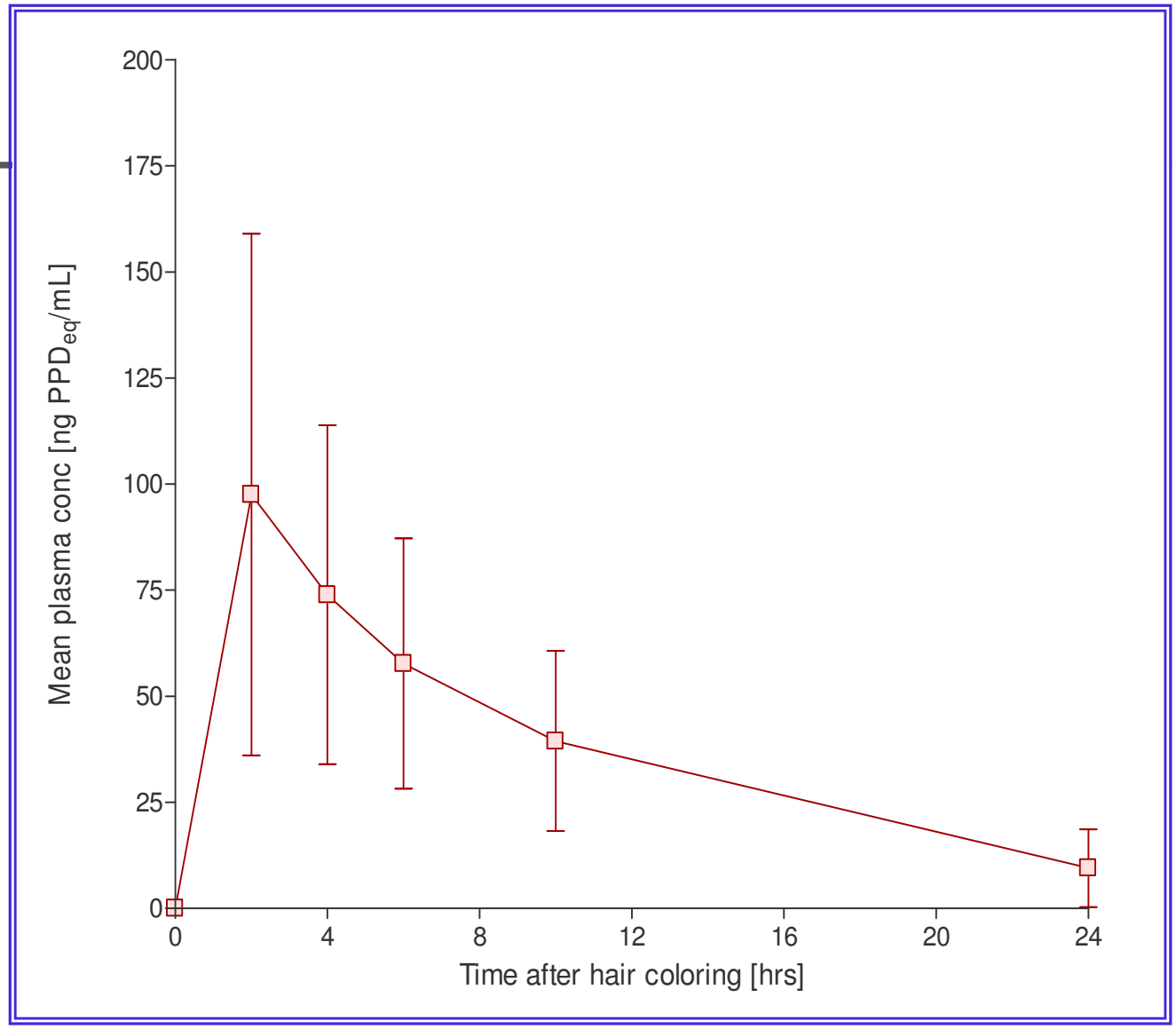
* Dark shade dye / short hair (Meuling et al., 2003; Hueber-Becker et al., 2004)

Consumer Exposure Study (2008) to a [^{14}C]-PPD-Based Oxidative Hair Dye (N = 18)





2008/2009 results: consumer syst. exposure ([^{14}C]- plasma equiv.) after exposure to a [^{14}C]-PPD-labelled oxidative hair dye

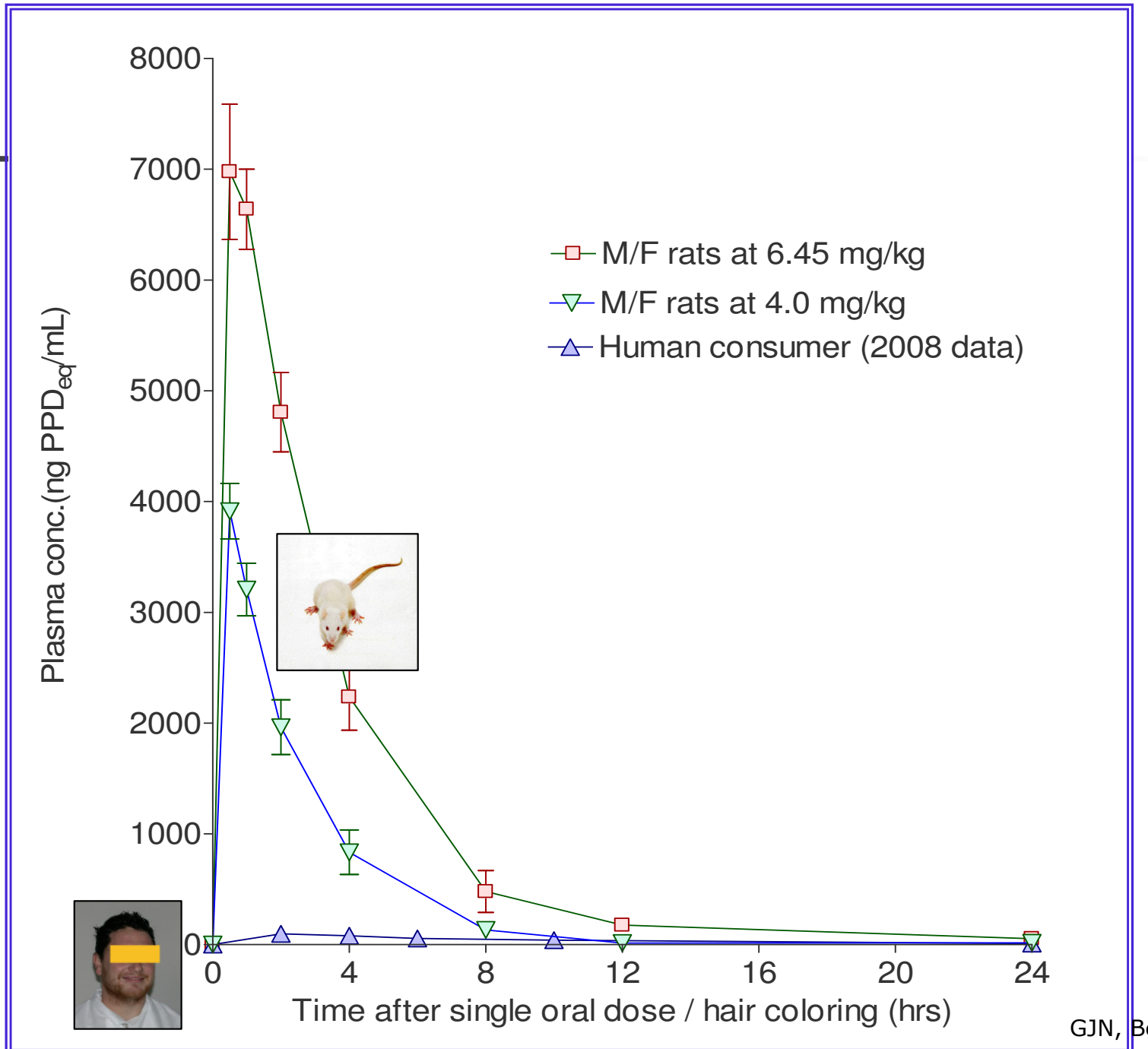


Consumer Expo Study (1.0% PPD)

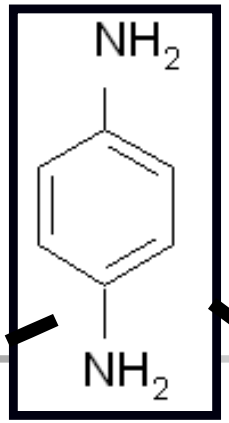
Mean $AUC_{0\text{-}24hrs}$: 711.5 ± 411 ng PPD_{eq} x hrs/mL

Mean Cmax: 98.9 ± 59.7 ng PPD_{eq}/mL

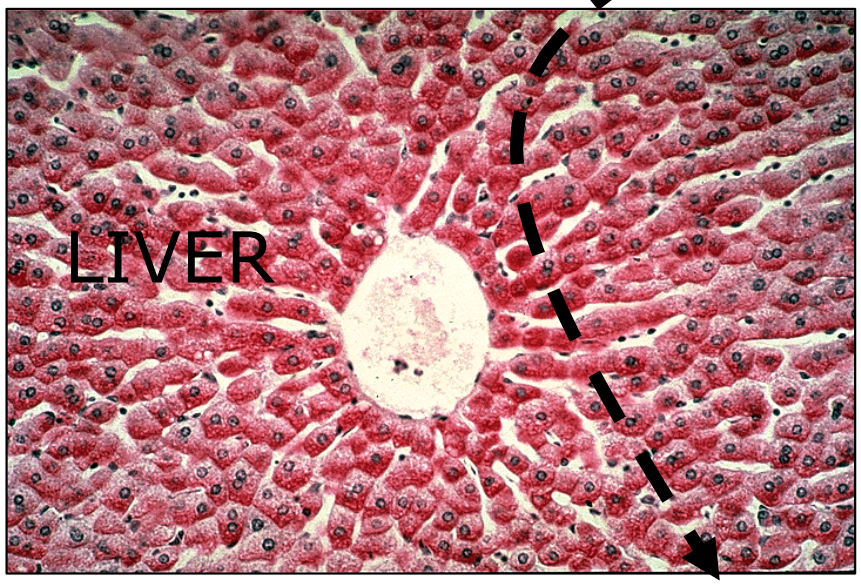
Consumer syst. exposure vs. that of rats at oral NOEL or <NOAEL (4.0 or 6.45 mg/kg/day, respectively): TK-based MOS = 16 to 45x

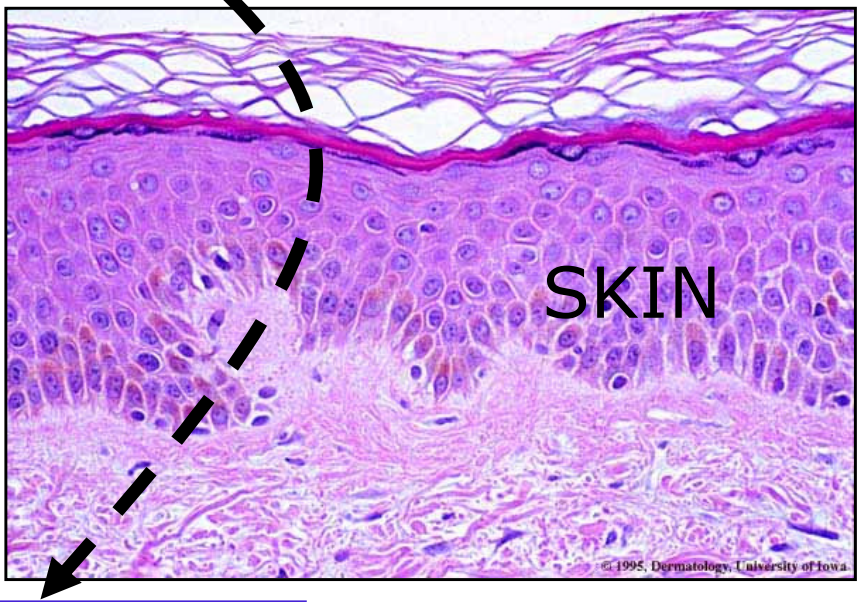


p-Phenylenediamine (PPD) is N-acetylated in human skin and hepatocytes, no N-hydroxylation*

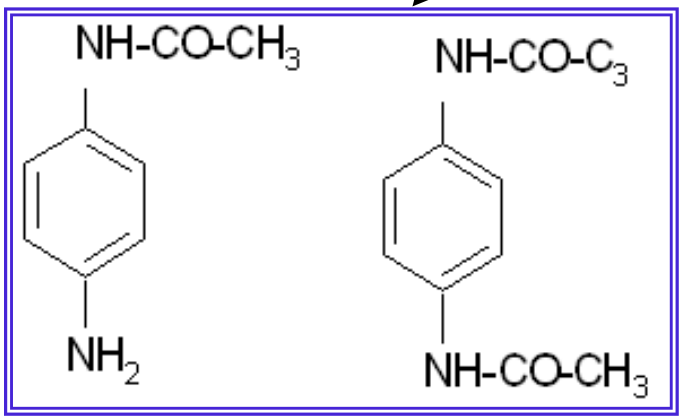


PPD





N.B.: SKIN AND LIVER HAVE DIFFERENT CAPACITY BUT SAME SPECIFICITY

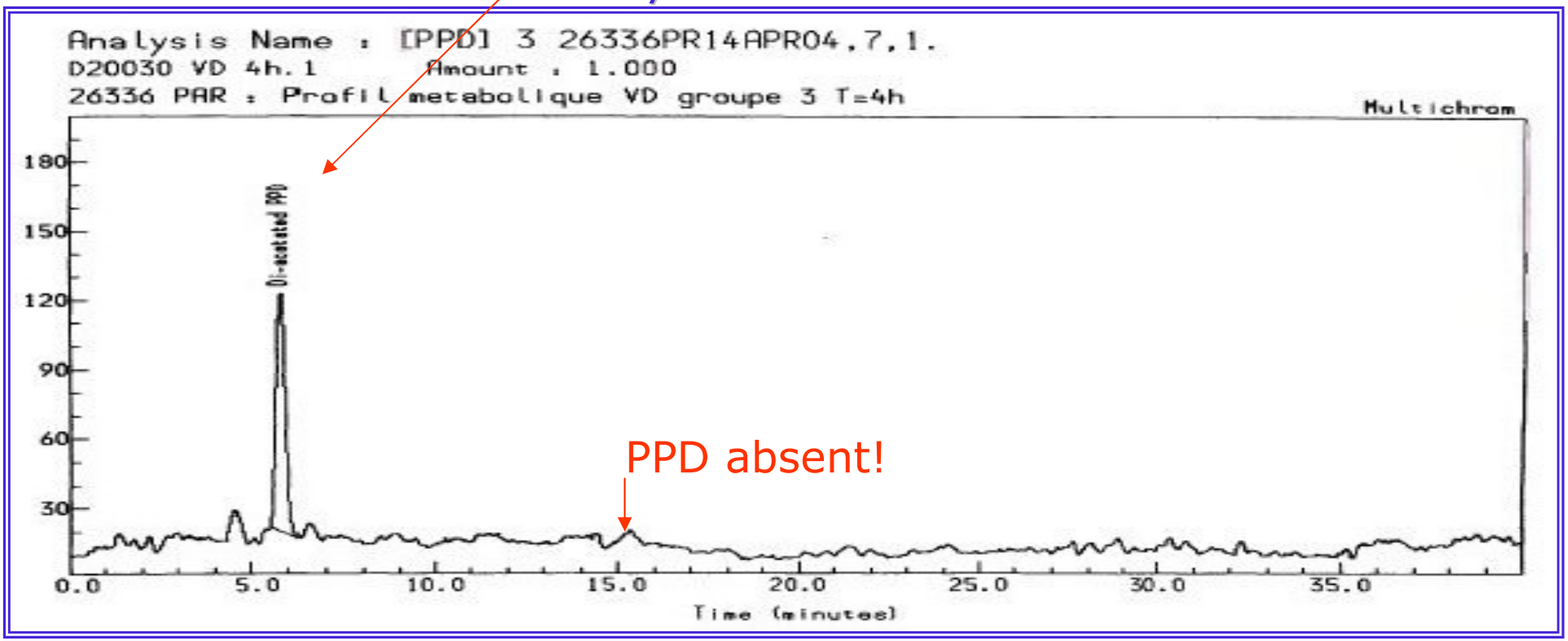


N,N'-diacetyl-PPD (DAPPD), some N-monoacetyl-PPD (MAPPD)

*Nohynek et al, *Toxicology Letters* (2005)

HPLC of rat plasma after dermal treatment with [^{14}C]-p-phenylenediamine (PPD) for 24-hours under occlusion * [1]

N,N'-diacetyl-PPD (DAPPD) only



DAPPD only metabolite found in human plasma (2009)

* Dressler and Appleqvist, *Food Chem. Toxicol*. 2006

DETOXIFICATION? - GENETIC TOXICITY OF N-MONO- and N,N'-DIACETYL-PPD *[1]

TEST	PPD	MAPPD	DAPPD
AMES TEST	+ (+S9)	-	-
MICRONUCLEUS TEST (Human Lymphocytes)	+ (+/- S9)	-	-

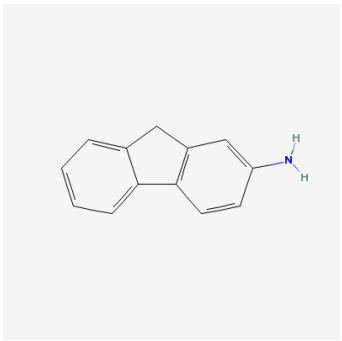
[1] N-acetylation of arylamines generally results in de-toxification, but there are exceptions (benzidine!)

* Garrigue et al., *Mut. Res*. 608, 58-71 (2006)

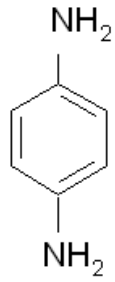
In contrast to the bladder carcinogen 2-aminofluorene (2-AF), human liver cells do not activate (N-hydroxylation) p-phenylenediamine (PPD) *

- Incubation of PPD with human hepatocytes, human S9, recombinant human P450 enzymes

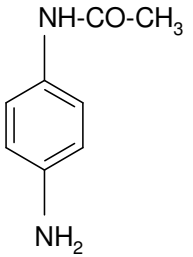
- Positive control substance: 2-aminofluorene (2-AF = carcinogenic aromatic amine)



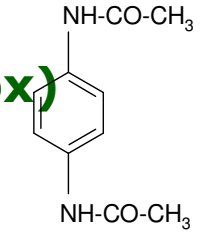
2-AF



PPD



MAPPD (detox)



DAPPD (detox)

HO-NH-AF
(Carcinogen)

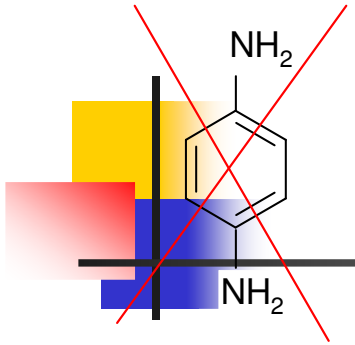
N-Acetyl-AF
(detox)

R-NH-OH

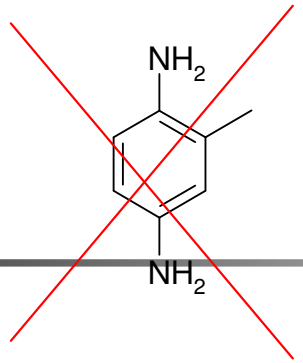
* (Stanley et al., Toxicology 2005)

Commonly Used Oxidative Hair Dye Ingredients

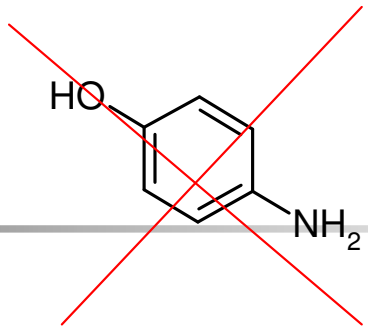
X = No Evidence for Formation of Activated, N-Hydroxylated Metabolites *



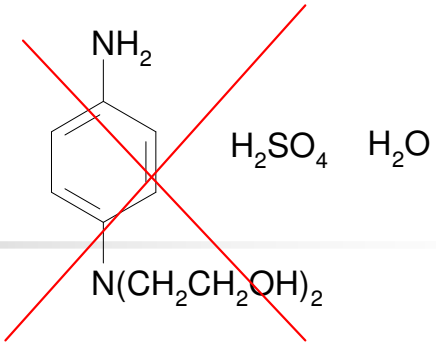
p-phenylenediamine



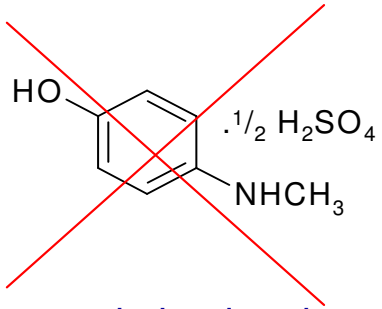
2,5-diaminotoluene



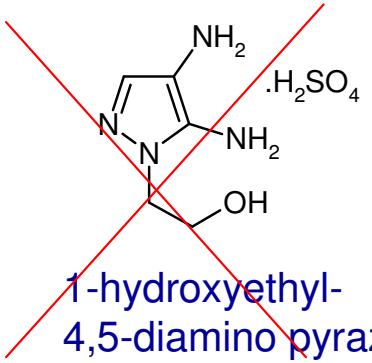
p-aminophenol



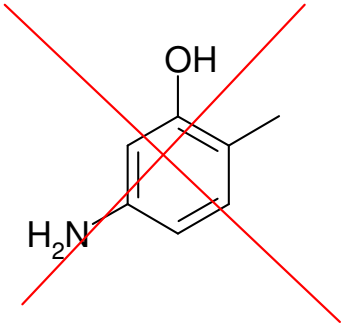
N,N-bis(2-hydroxyethyl)-
p-phenylenediamine



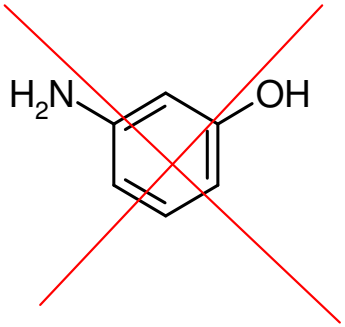
p-methylaminophenol



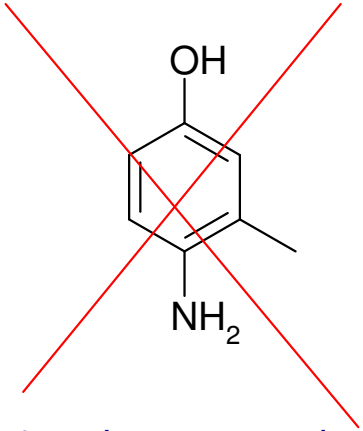
1-hydroxyethyl-
4,5-diamino pyrazole



4-amino-2-hydroxytoluene
(AHT)



m-aminophenol



4-amino-*m*-cresol

* Skare et al., *Xenobiotica*, 1-15, 2009

CONCLUSION

- Under maximum exposure conditions, human systemic exposure amounts to about 0.5% of the applied dye
- Human systemic exposure agent from PPD-containing hair dyes (and other arylamine-type hair dye ingredients) is N,N'-diacetylated PPD
- Metabolism takes places in the skin (NAT1)
- Metabolites are non-genotoxic and, therefore, detoxified
- Hair dye arylamines are unable to form potentially carcinogenic metabolites

PROFESSIONAL EXPOSURE TO HAIR DYES

- Little known about the exposure of hairdressers to oxidative hair dyes (2 published studies)

- Air levels of PPD in a hairdressing saloon (Gagliardi et al., 1992)
  - No PPD detected
  - Data insufficient evidence for exposure estimation

- Dermal exposure of hairdressers (Lind et al., 2005)
  - Non-controlled conditions (hairdressing salon)
  - Minute skin residues (ng/cm² range)
  - No difference in skin residues between glove-protected and non-protected hands (?)
  - No data on systemic exposure

OBJECTIVES AND METHODS

- Participants: 18 professional hairdressers with ample professional experience

- Application of a [^{14}C]-PPD-containing oxidative hair dye (commercial, dark-shade, containing 2% [^{14}C]-PPD / 1% resorcinol / 1% m-aminophenol on head).

- High quality training heads with natural human hair (length 30-35 cm, hair density similar to that of human hair)

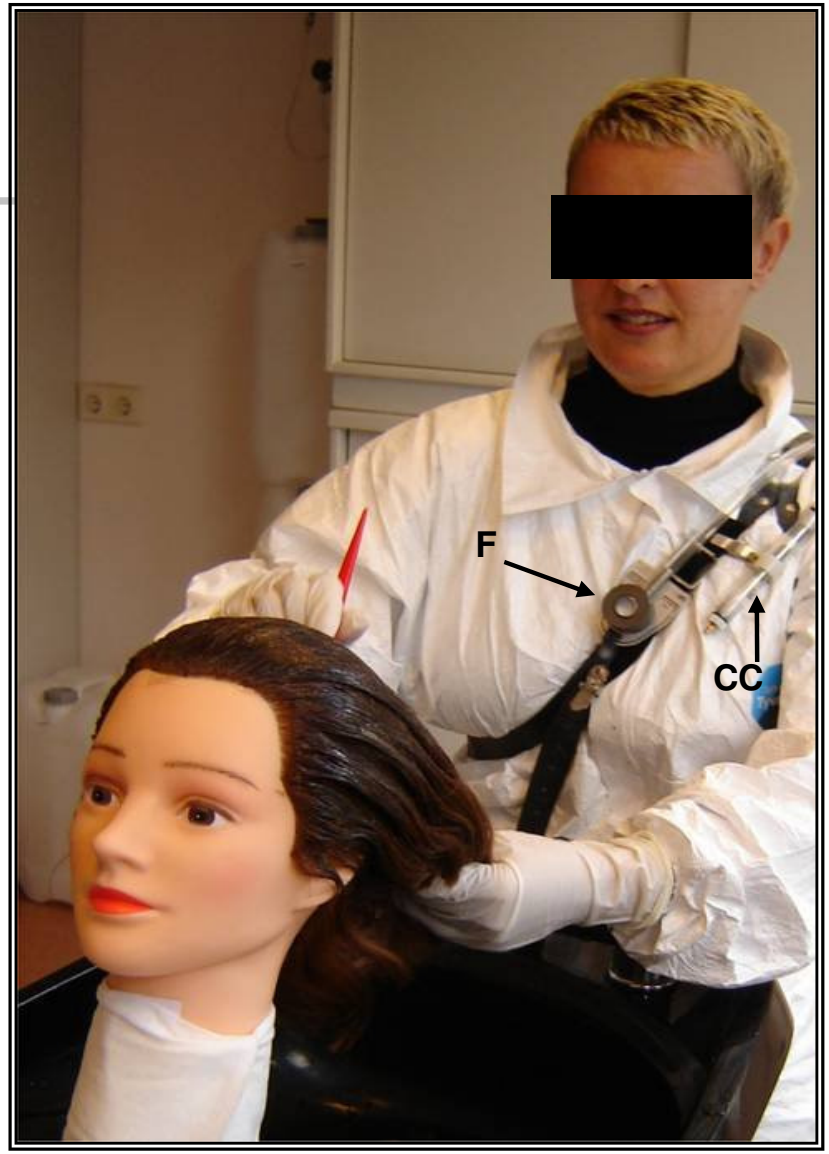
- Study performed under GLP / GCP conditions after ethical review and receipt of informed consent

SAMPLES FOR [^{14}C]-MONITORING

- Systemic exposure
  - Blood samples: pre-study, 4 h (end of morning shift), 8 h (end of afternoon shift), 24 h after start of exposure
  - Urine: quantitative, 4 samples up to 48 hours after start of exposure (urinary excretion = quantitative systemic exposure)
- External exposure
  - Hand washes: before and after each task
  - Air: area and personal monitoring, nasal rinses
- Mass-Balance (recovery)
  - All tools/materials, washing/rinsing liquids
  - Protective equipment (gloves, coats)
  - Training heads (shaved at the end of the study + scalp extraction)

PHASE A: Preparation and coloring

- Hair dyeing phase
  - Weighing and mixing of dye and developer (without gloves)
  - Application of hair dye product on roots/length of hair (gloves)
  - Cleaning of used material (gloves)



Personal Air Sampling device

F: Filter (particles)

CC: Charcoal Cartridge (vapours)

PHASE B:Rinsing, shampooing and conditioning

- Rinsing and shampooing of dyed hair (gloves)

- Application of conditioner, rinsing and drying of hair with absorbant paper (no gloves)

PHASE C: Hair cutting and styling

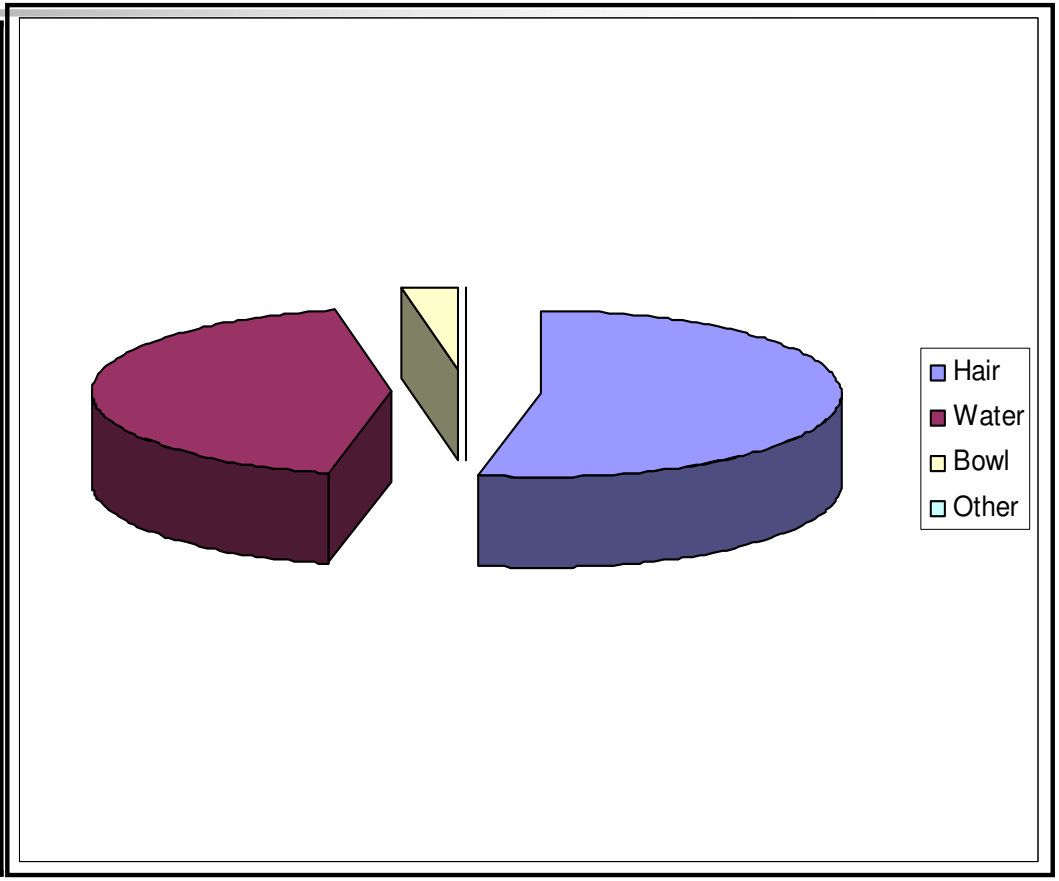
- Cutting of hair (no gloves)
- Drying with electric drier (no gloves)
- Brushing (no gloves)

RESULTS Of A WORKING DAY: INDIVIDUAL HAIR STYLES

SINKS OF RADIOACTIVITY

(mean recovery from 6 complete working days)

COMPARTMENT	MEAN ± S.D.
MIXING BOWLS	2.88 ± 0.54
CUT HAIR	53.46 ± 4.06
RINSING WATER	45.47 ± 2.95
TOTAL RECOVERY	**102.50 ± 2.2**



[^{14}C] IN BLOOD SAMPLES

- Blood at pre-exposure, 4 h (end of morning session), 8 h (end of afternoon session), and 24 h after start of exposure: no radioactivity detected above the limit of detection (<10 ng PPD_{eq}/mL) in any blood sample (n=72)

- **Perspective:** 1 baby antipyretic (acetaminophen = skin metabolite of p-aminophenol) results in a C_{MAX} of about 10.000 ng/mL

INHALATION EXPOSURE

- Vapours (charcoal filters)
  - BLQ for all samples during washing phase; some samples above the LoQ, mainly during dyeing phase
- Particles (acetate filters)
  - BLQ for all samples during washing and cutting phases; some samples from dyeing phase above the LoQ
  - Air levels at least one order of magnitude below those in charcoal filters
- All nasal rinsings and ambient air samples were BLQ

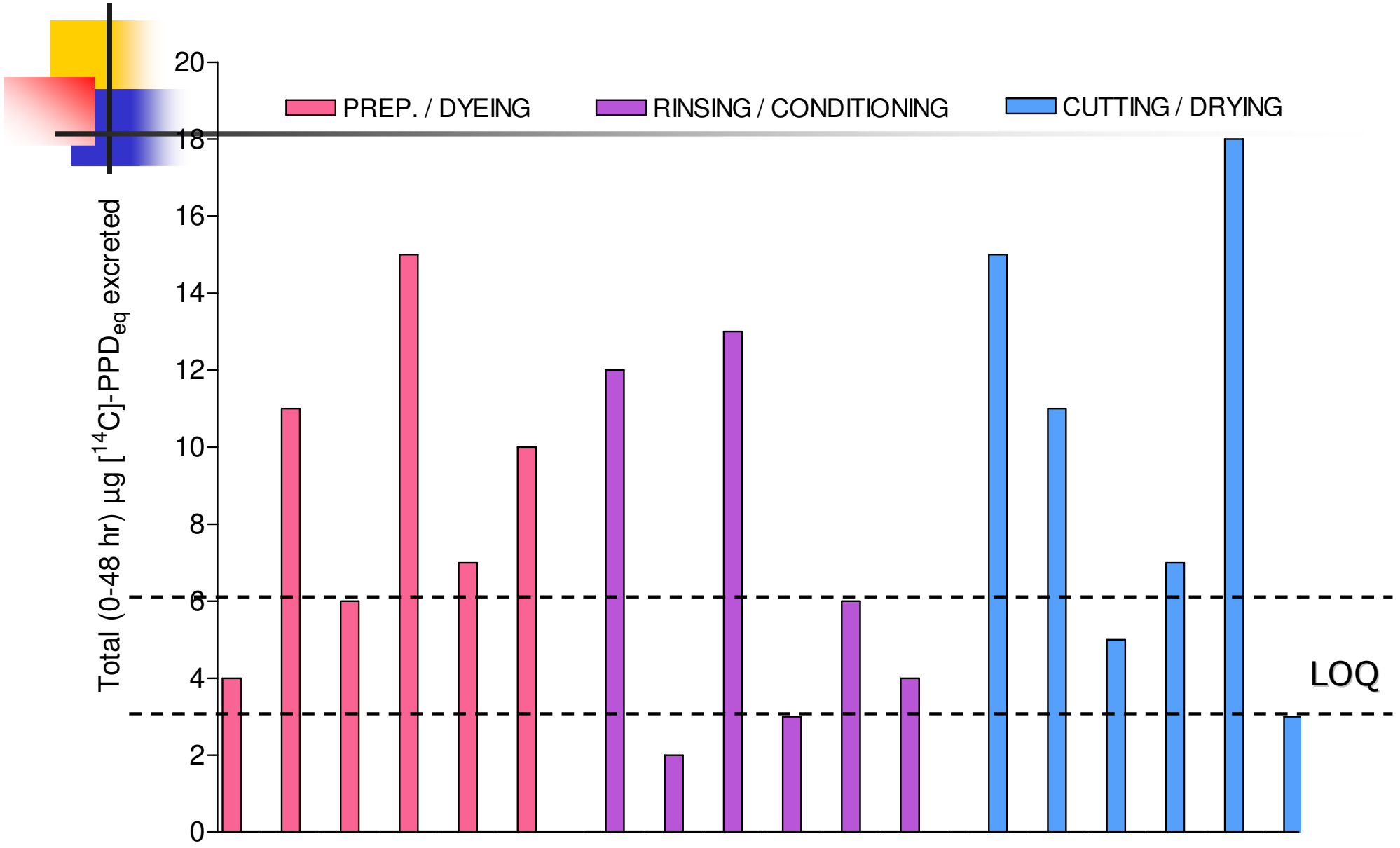
BLQ: Below Limit of Quantification
LoQ: Limit of Quantification

EXPOSURE OF HANDS (combined hand rinses per working day)

Study Phase	% of applied radioactivity	Skin exposure (mg PPD_{eq})	Skin area exposure dose (µg PPD_{eq}/cm^2)
Hair dye preparation/ application	0.0002 ± 0.0001	0.005 ± 0.002	0.006
Rinsing/shampooing	0.003 ± 0.001	0.061 ± 0.013	0.071
Cutting/drying	0.006 ± 0.002	0.128 ± 0.047	0.148

- Skin exposure highest at cutting/drying phase (no gloves)
- Results suggest that standard occupational precautions adequately protect against induction of PPD-mediated contact allergy: several µg/cm² skin of extreme sensitisers required to induce sensitisation

Individual 0-48hr Urinary Excretion after a single working day (µg [^{14}C]-PPD$_{eq}$)



SYSTEMIC EXPOSURE: [^{14}C]-URINARY EXCRETION IN PERSPECTIVE

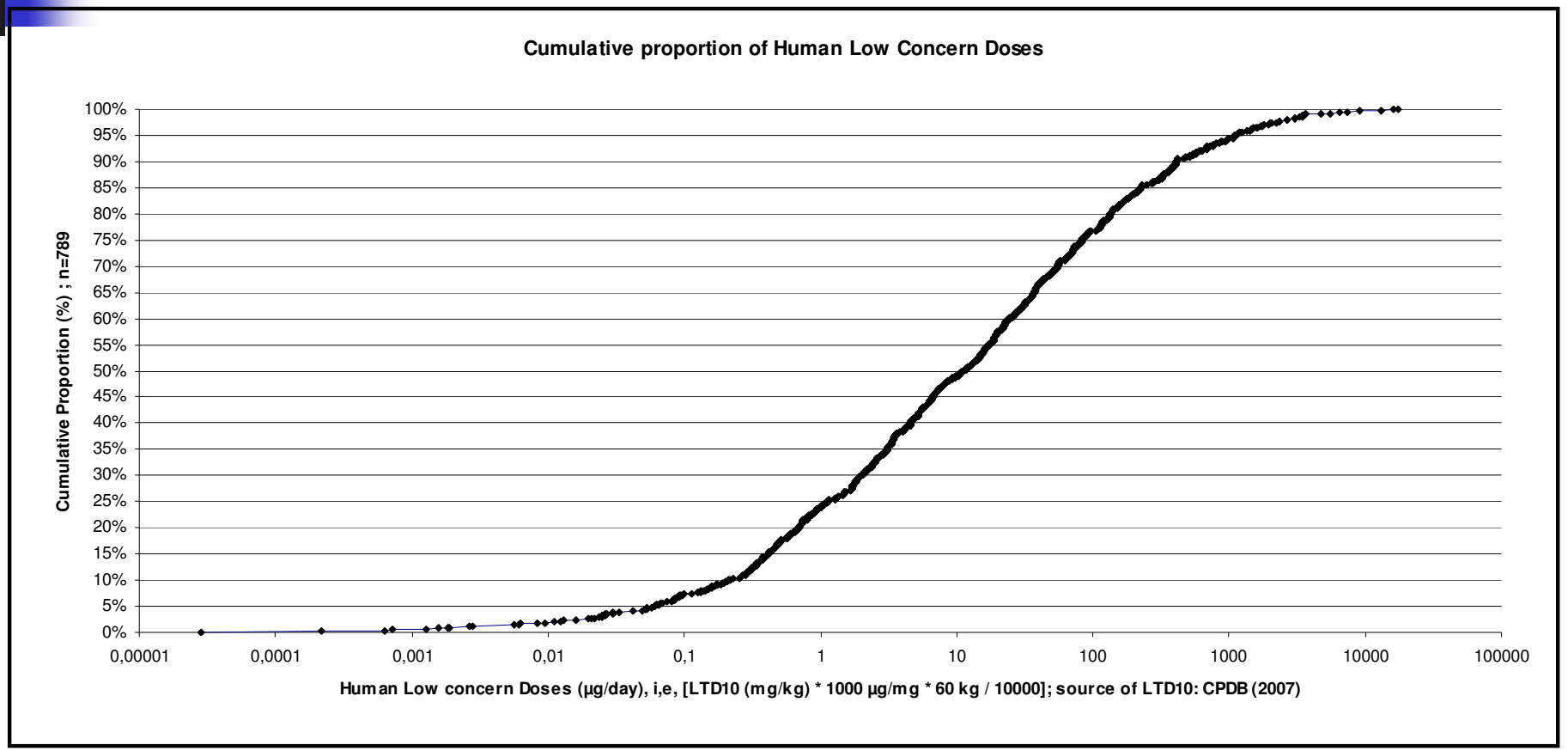
- Cumulative exposure (A+B+C study phases combined): **<0.36 µg** PPD_{eq}/kg bw/working day (<0.0012% of applied radioactivity)

- Worst case scenario:
  - 6 hair dyeing processes per day (heavy work load)
  - Darkest shade formulation, max. concentrations (the majority of US / EU shades used are blond!)
  - All BLQ-samples considered to be at LoQ

- It is improbable that such low exposure levels may produce significant systemic adverse health effects

- Corresponding to "low level of concern" - EU Food Safety Agency for most genotoxic carcinogens in food: daily human intake < BMD_{10} / 10.000

Human Virtually Safe Doses of Carcinogens (µg/day using EFSA approach, calculated with TD_{10} values of CPDB, Berkely, 2006)



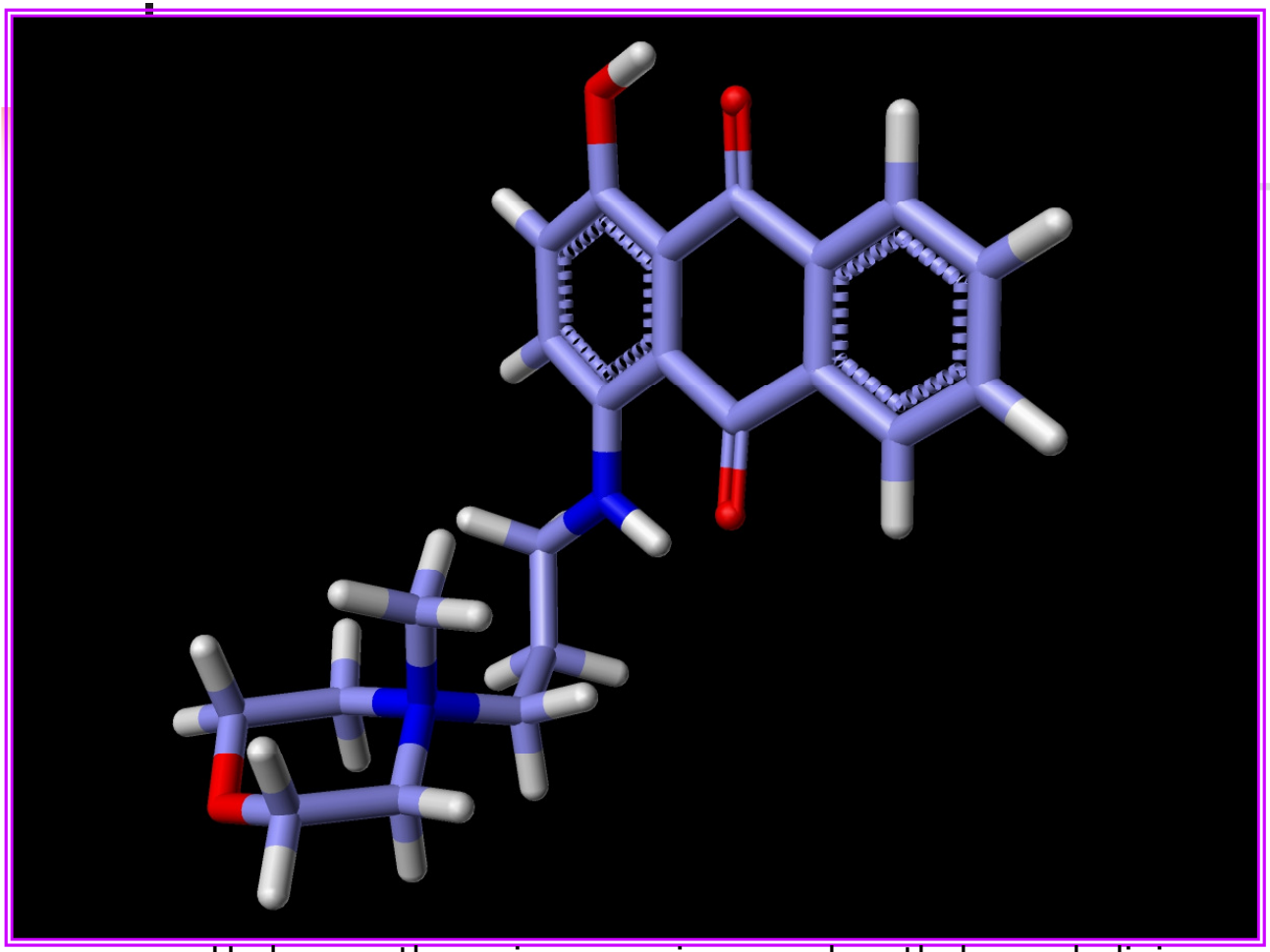
EFSA, 2005: a daily exposure to 0.36 µg/kg would be of « low concern » sfor most genotoxic carcinogens listed in the CPDB, when occurring in food

CONCLUSION

- Current epidemiological evidence suggests that hairdressers have no increased cancer risk *
- EVIDENCE that application of hair dye arylamines to the skin of humans, rats and pigs results in transformation to their acetylated metabolites, most likely by NAT1
- EVIDENCE that acetylated arylamines are detoxified
- EVIDENCE that acetylation is independent of human NAT2 status
- OVERALL EVIDENCE: NO SYSTEMIC HUMAN HEALTH RISK

* Czene et al. (2004): 45000 hairdressers

Skin absorption of hair dyes: Do in vitro skin penetration data always reflect human systemic exposure? *



- Hydroxyanthraquinone aminopropyl methyl morpholinium methosulphate (**HAM**, COLIPA C117)
- Empirical Formula: C22H25N2O4 - CH3SO4
- MW: 492.5
- Melting Point: 215° C
- Soluble in water and ethanol (>100 g/L at 20° C)
- Log Po/w: **-**2

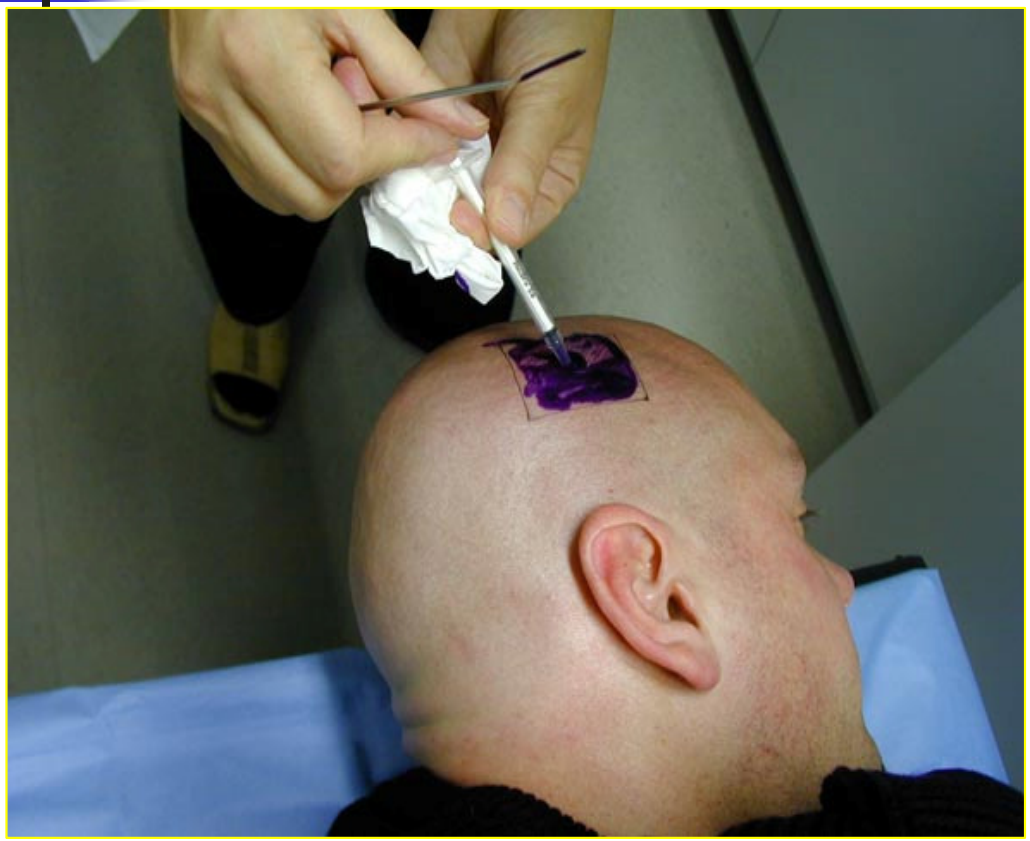
* Lademann et al., FCT, 2009

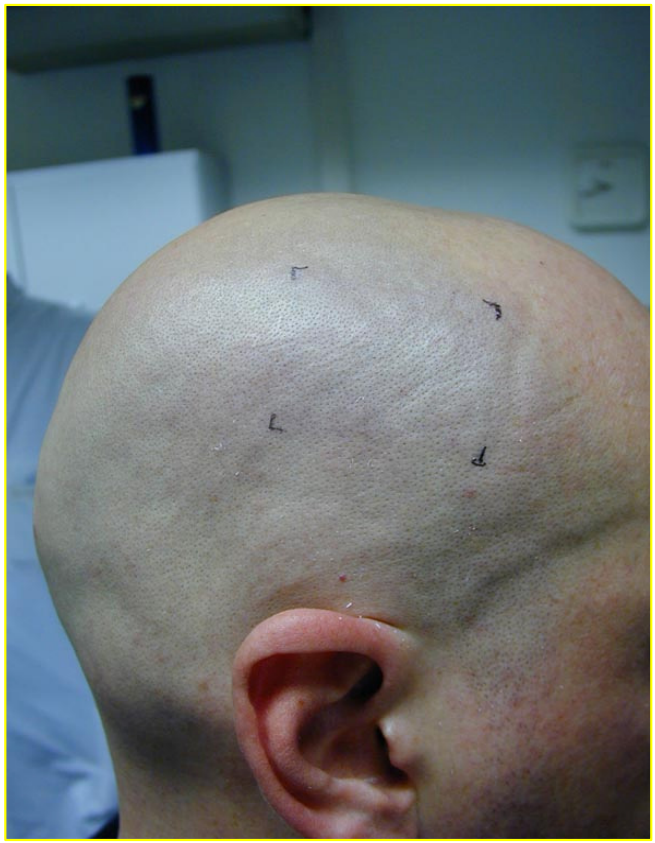
RESULTS *IN VITRO*

COMPARTMENT	% OF APPLIED DOSE (Mean ± SD)	µg/cm² (Mean ± SD)
HAM APPLIED	-	98.53 ± 0.18
SURFACE EXCESS	103.6 ± 2.4	102.21 ± 2.30
STRATUM CORNEUM	1.52 ± 0.36	1.50 ± 0.36
EPIDERMIS + DERMIS	0.87 ± 0.34	0.86 ± 0.34
RECEPTOR FLUID	<0.046 ± 0.03*	<0.045 ± 0.028*
RECOVERY	106.1 ± 2.1	104.4 ± 2.2
« BIOAVAILABLE » (epidermis/dermis/receptor fluid)	**0.92 ± 0.32**	**0.91 ± 0.32 (SED = 0.63 mg!)**

* Although 5/7 cell values were BLQ, presence at the LOQ was presumed

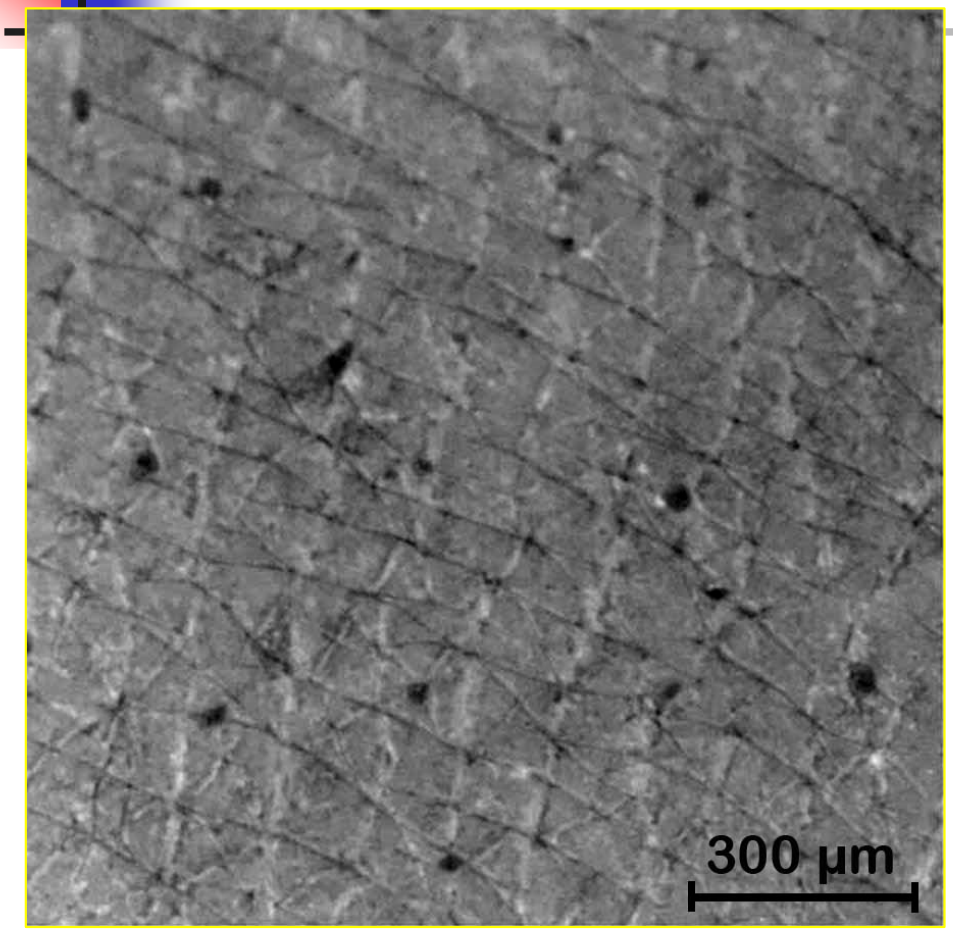
Results *in vivo*: skin rinsing appears to remove the dye from the treated skin site.

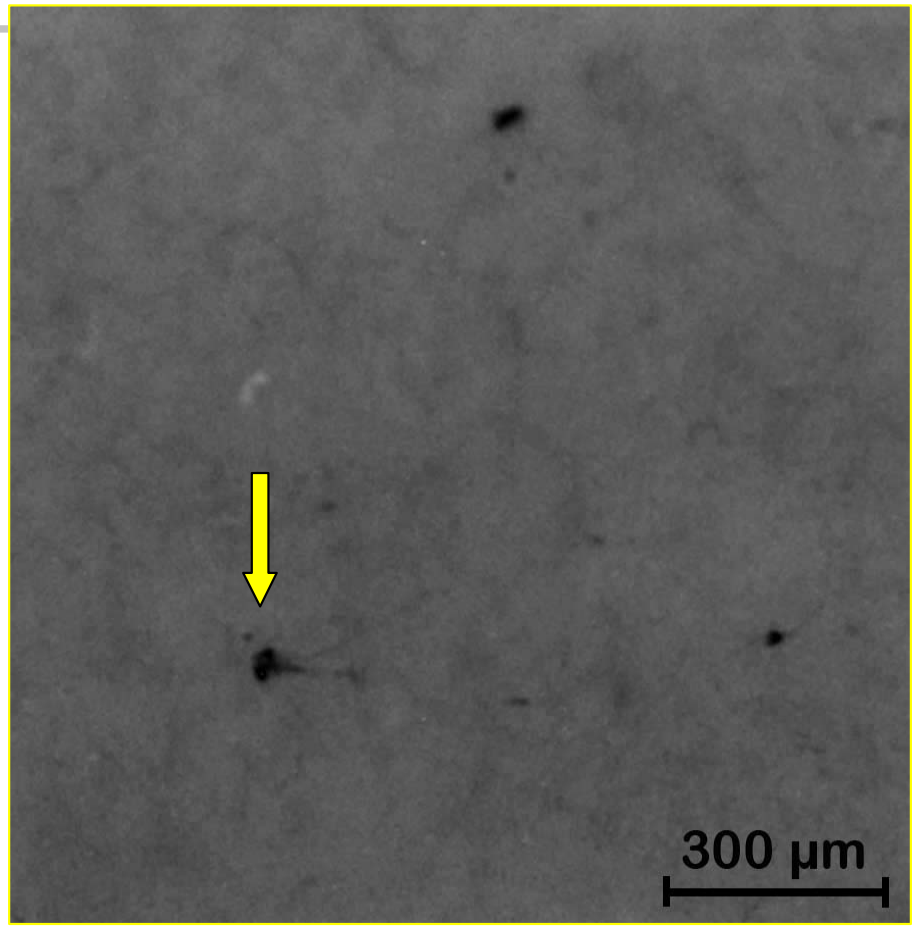




*A*pplication to human scalp skin *in vivo*

Light microscopy of human skin after application of HAM, followed by skin rinsing (a) and after five subsequent tape strips (b). Image (a) shows presence of dye in hair follicle openings and skin furrows. After tape stripping, visible dye residues remain in the follicular orifices (b).



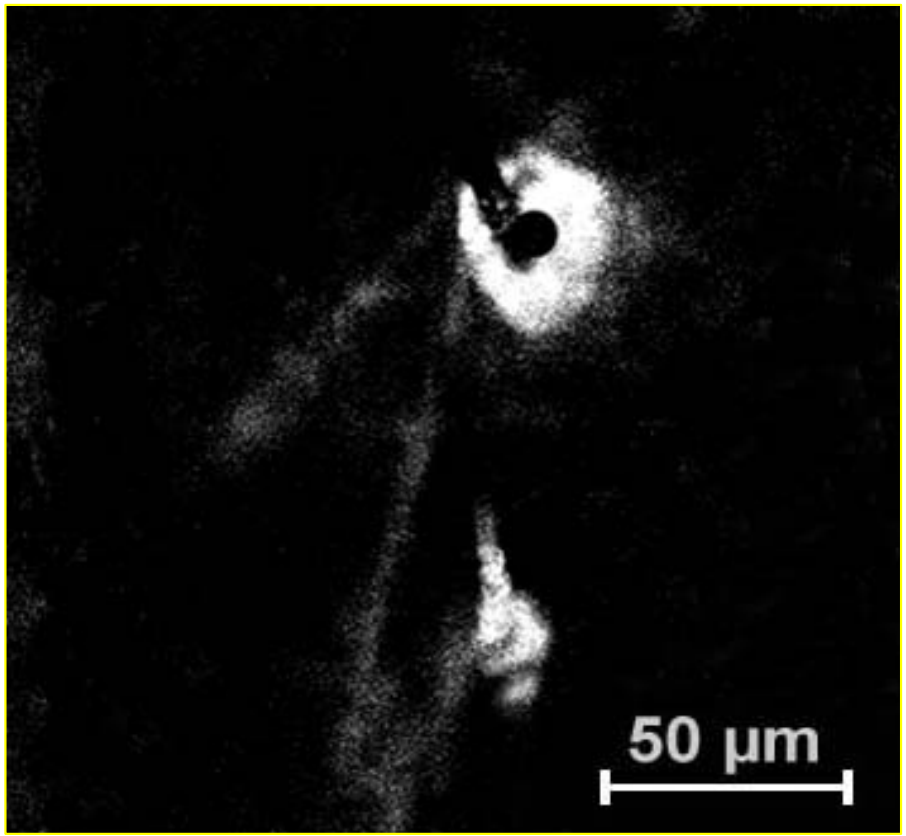


After rinsing and ....

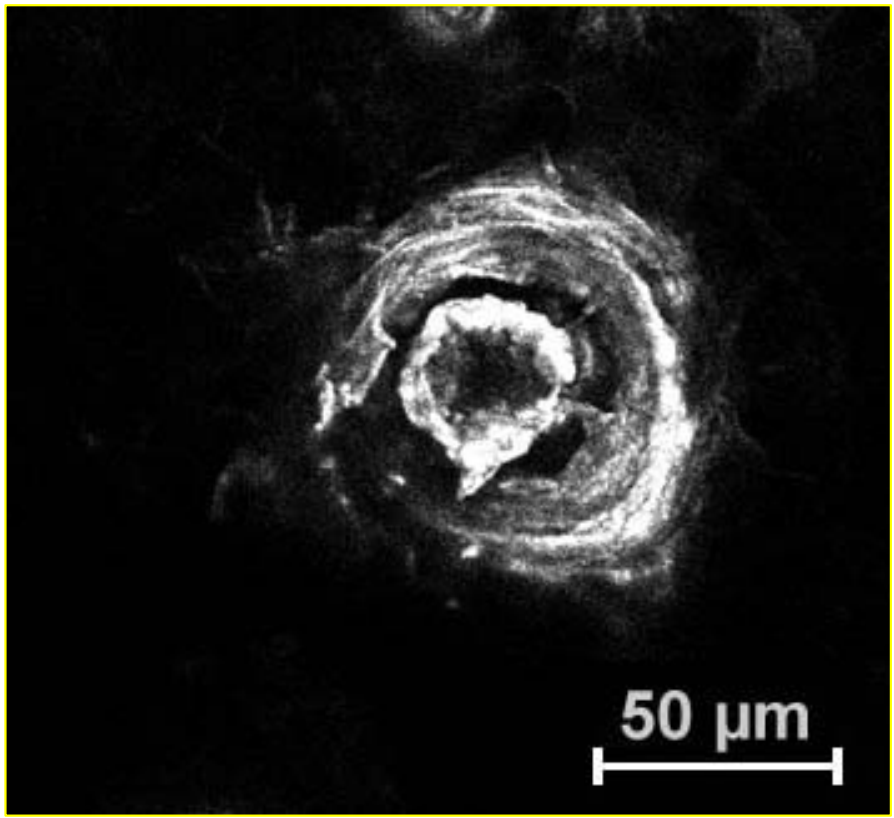
...tape stripping

Section (a) and topical view (b) of a cyanacrylate biopsy of the hair follicle opening (back and scalp).

Presence of fluorescence shows that HAM penetrates only into the upper part of the hair follicle orifices.

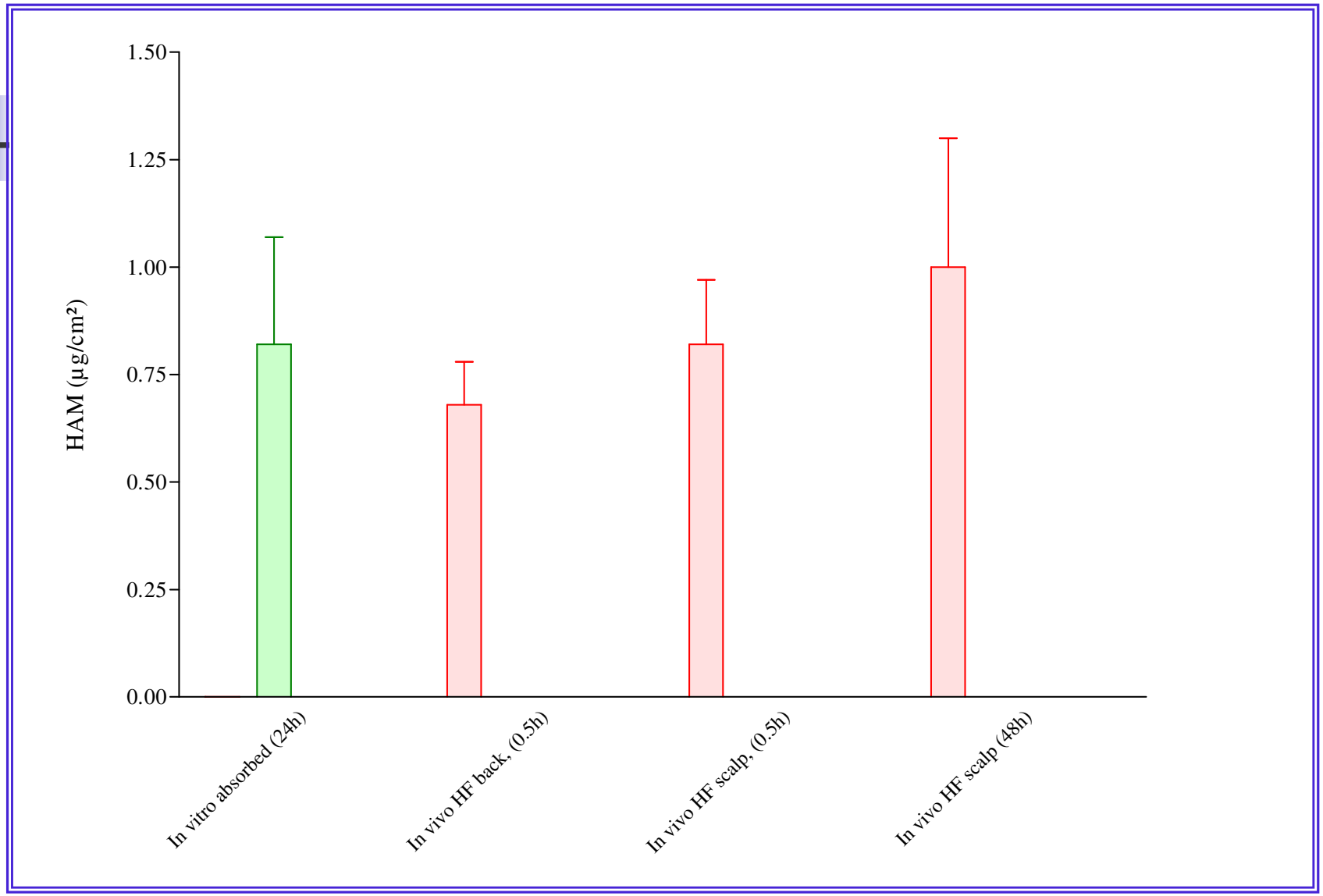


a)



b)

Overall skin residues: *in vitro* « absorbed » versus *in vivo* residues in non-living skin



N.B.: Values are similar or nearly-identical: *in vitro* artefacts

Combination of last-century mechanical method (skin stripping) with 21st-Century analytical methods…

- may produce artefactual residues that actually remain outside the living skin *in vivo*
- interpretation of such *in vitro* trace residues as a « *Human Systemic Exposure Dose* » **may result in unnecessary *in vivo* animal tests on substances that produce no systemic exposure in humans**
- **THEREFORE:**
  - For substances with low potential (phys./chem. properties) for significant skin penetration, residues in the epidermis/dermis compartments at <1.0 µg/cm² should be qualified as **negligible**
  - Need for a reasoned « Treshold of Skin Absorption » (TSA) defining a cut-off level for negligible percutaneous absorption/penetration: **<1.0 µg/cm²?**

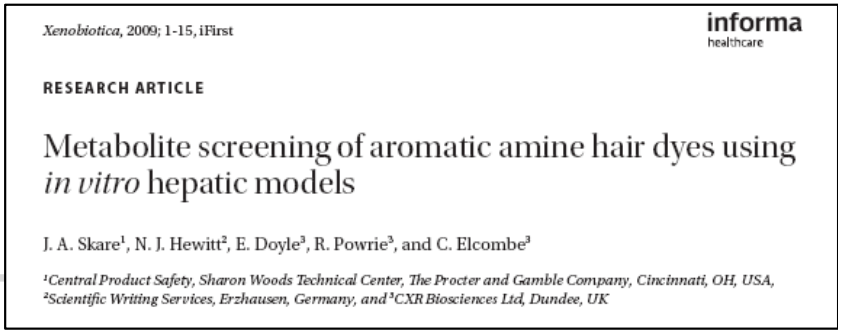
Final remark..

All models are wrong, some models are useful…

(George Box, 1959)

Useful when the interpretation of the model's data take into account its inherent limits…

Further reading



Xenobiotica, 2009; 1-15, iFirst

informa healthcare

RESEARCH ARTICLE

Metabolite screening of aromatic amine hair dyes using *in vitro* hepatic models

J. A. Skare[1], N. J. Hewitt[2], E. Doyle[3], R. Powrie[3], and C. Elcombe[3]

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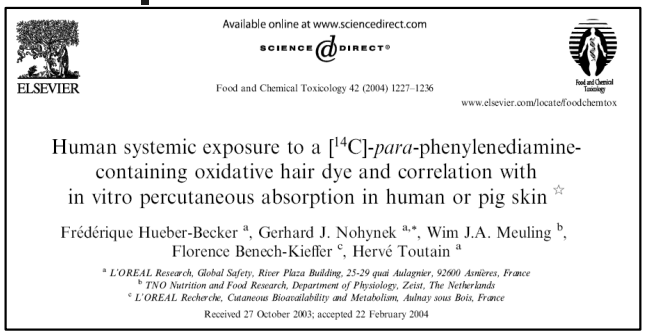

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Food and Chemical Toxicology 42 (2004) 1227–1236

www.elsevier.com/locate/foodchemtox

Human systemic exposure to a [^{14}C]-*para*-phenylenediamine-containing oxidative hair dye and correlation with in vitro percutaneous absorption in human or pig skin ☆

Frédérique Hueber-Becker [a], Gerhard J. Nohynek [a,*], Wim J.A. Meuling [b], Florence Benech-Kieffer [c], Hervé Toutain [a]

[a] *L'OREAL Research, Global Safety, River Plaza Building, 25-29 quai Aulagnier, 92600 Asnières, France*
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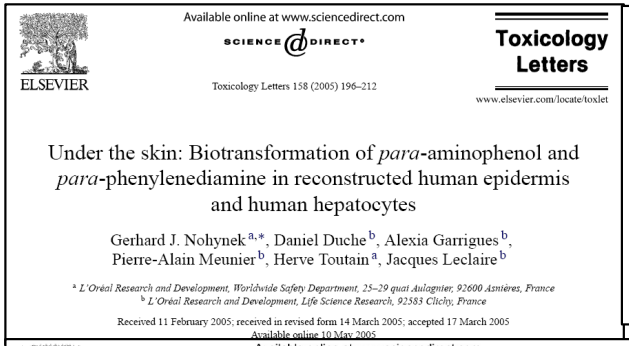

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Toxicology Letters

ELSEVIER

Toxicology Letters 158 (2005) 196–212

www.elsevier.com/locate/toxlet

Under the skin: Biotransformation of *para*-aminophenol and *para*-phenylenediamine in reconstructed human epidermis and human hepatocytes

Gerhard J. Nohynek [a,*], Daniel Duche [b], Alexia Garrigues [b], Pierre-Alain Meunier [b], Herve Toutain [a], Jacques Leclaire [b]

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Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

ELSEVIER

Human percutaneous absorption of a direct hair dye comparing *in vitro* and *in vivo* results: Implications for safety assessment and animal testing

J. Lademann [a], H. Richter [a], U. Jacobi [a], A. Patzelt [a], F. Hueber-Becker [b], C. Ribaud [c], F. Benech-Kieffer [c], E.K. Dufour [b], W. Sterry [a], H. Schaefer [a], J. Leclaire [c], H. Toutain [b], G.J. Nohynek [b,*]

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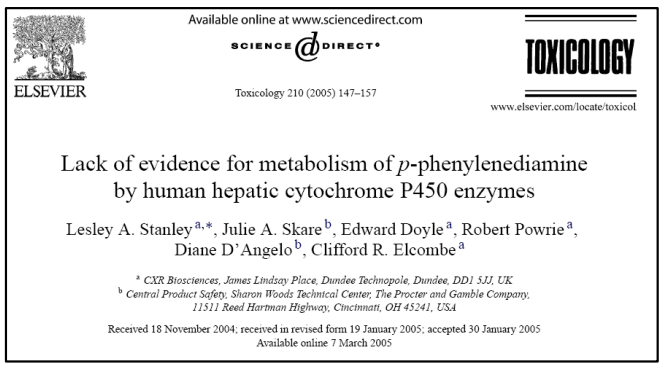
[c] *L'Oréal Research and Development, Life Science Research, 93600 Aulnay-sur-Bois, France*



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TOXICOLOGY

ELSEVIER

Toxicology 210 (2005) 147–157

www.elsevier.com/locate/toxicol

Lack of evidence for metabolism of *p*-phenylenediamine by human hepatic cytochrome P450 enzymes

Lesley A. Stanley [a,*], Julie A. Skare [b], Edward Doyle [a], Robert Powrie [a], Diane D'Angelo [b], Clifford R. Elcombe [a]

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[b] *Central Product Safety, Sharon Woods Technical Center, The Procter and Gamble Company, 11511 Reed Hartman Highway, Cincinnati, OH 45241, USA*

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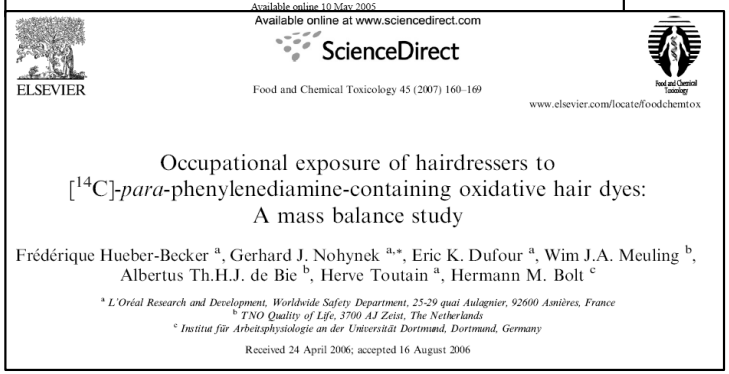

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Food and Chemical Toxicology 45 (2007) 160–169

www.elsevier.com/locate/foodchemtox

Occupational exposure of hairdressers to [^{14}C]-*para*-phenylenediamine-containing oxidative hair dyes: A mass balance study

Frédérique Hueber-Becker [a], Gerhard J. Nohynek [a,*], Eric K. Dufour [a], Wim J.A. Meuling [b], Albertus Th.H.J. de Bie [b], Herve Toutain [a], Hermann M. Bolt [c]

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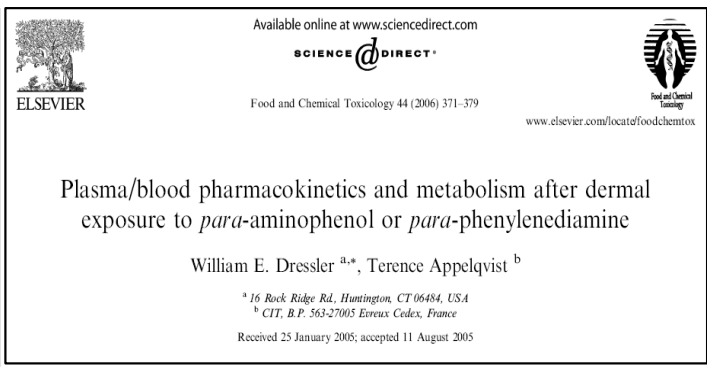

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Food and Chemical Toxicology 44 (2006) 371–379

www.elsevier.com/locate/foodchemtox

Plasma/blood pharmacokinetics and metabolism after dermal exposure to *para*-aminophenol or *para*-phenylenediamine

William E. Dressler [a,*], Terence Appelqvist [b]

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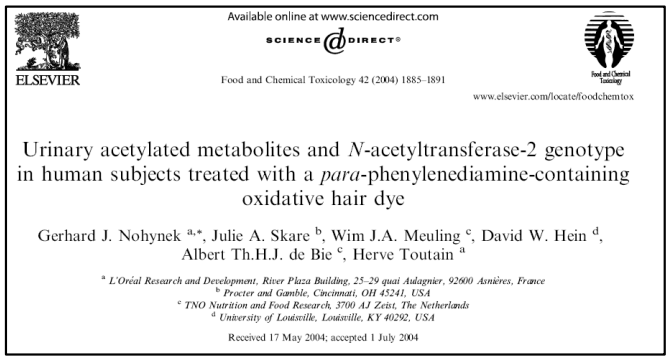
[b] *CIT, B.P. 563-27005 Evreux Cedex, France*

Received 25 January 2005; accepted 11 August 2005



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Food and Chemical Toxicology 42 (2004) 1885–1891

www.elsevier.com/locate/foodchemtox

Urinary acetylated metabolites and *N*-acetyltransferase-2 genotype in human subjects treated with a *para*-phenylenediamine-containing oxidative hair dye

Gerhard J. Nohynek [a,*], Julie A. Skare [b], Wim J.A. Meuling [c], David W. Hein [d], Albert Th.H.J. de Bie [c], Herve Toutain [a]

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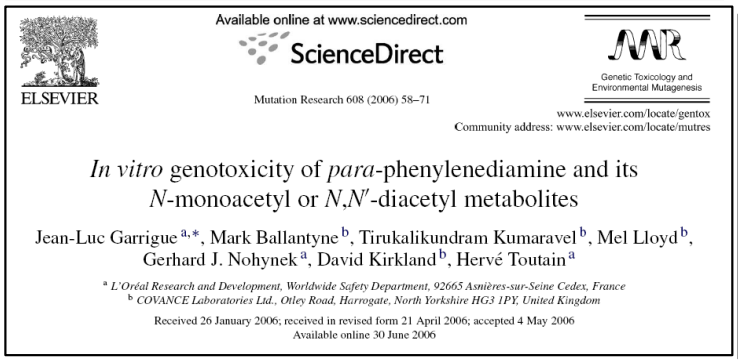

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Mutation Research 608 (2006) 58–71

Genetic Toxicology and Environmental Mutagenesis

www.elsevier.com/locate/gentox
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In vitro genotoxicity of *para*-phenylenediamine and its *N*-monoacetyl or *N*,*N*′-diacetyl metabolites

Jean-Luc Garrigue [a,*], Mark Ballantyne [b], Tirukalikundram Kumaravel [b], Mel Lloyd [b], Gerhard J. Nohynek [a], David Kirkland [b], Hervé Toutain [a]

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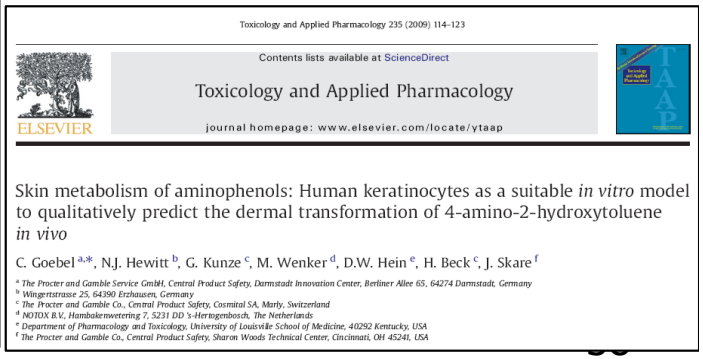

Toxicology and Applied Pharmacology 235 (2009) 114–123

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Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

ELSEVIER

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

C. Goebel [a,*], N.J. Hewitt [b], G. Kunze [c], M. Wenker [d], D.W. Hein [e], H. Beck [c], J. Skare [f]

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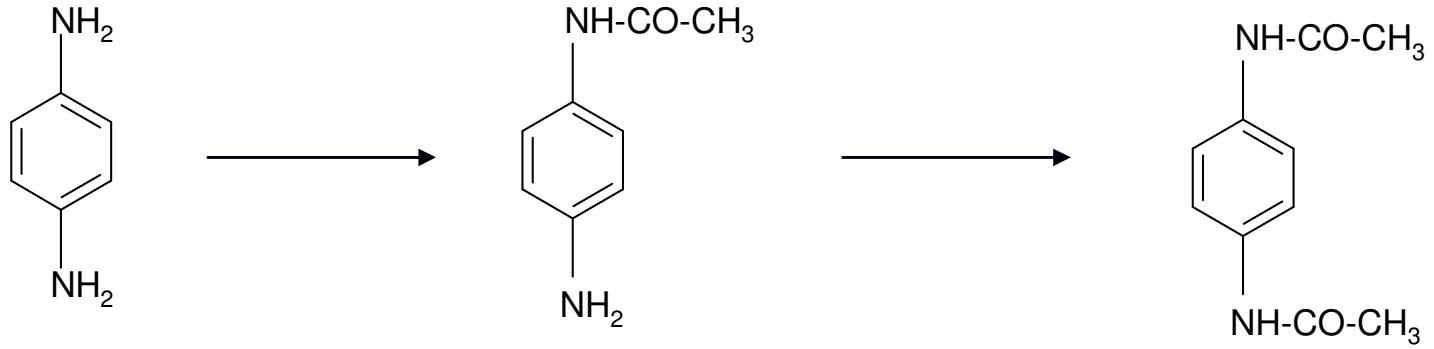
Metabolism of PPD in human epidermis and hpatocytes *



PPD

Mono-N-acetyl-PPD (MAPPD)

N,N'-Diacetyl-PPD (DAPPD)

CONCLUSION: human liver and skin transforms PPD to N-mono- and N,N'-diacetyl derivatives (NAT1).

Is the organism exposed to PPD or hair dye reaction products at all?

* Nohynek et al., *Toxicology Letters*, 2005