

BfR-Symposium:  
"First International  
Conference on  
Tattoo Safety"



June 6–7, 2013



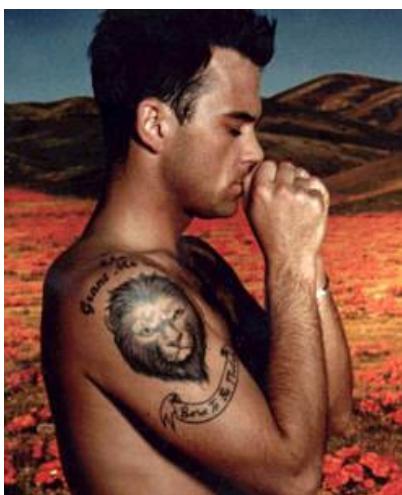
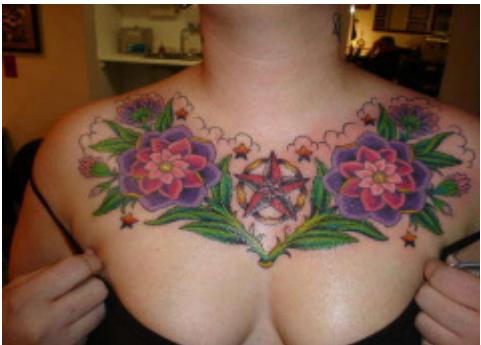
# The Fate of Tattoo Pigments in the Skin

Prof. Wolfgang Bäumler

Department of Dermatology  
University of Regensburg  
Germany



# Art...



# ... or medical problem ?

allergic, toxic, infections, ...

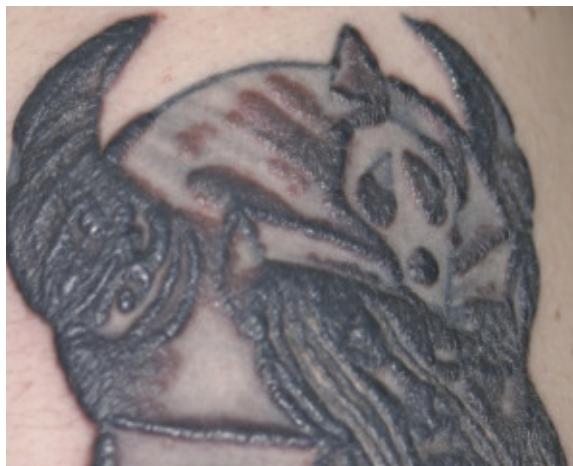


FIG.1 Acute allergic contact dermatitis on the tattoo site.

FIG.2 Erythema, edema and vesiculation on the tattoo site.

Images taken from different sources (Pubmed)

# How many tattoos ?

(% of population)

USA                    24 %                    (80 Mio.)

Sweden                7 %                    (0,6 Mio.)

United Kingdom      12 %                    (7 Mio.)

Germany               9 %                    (8 Mio.)

Germany               23 %                    (Age: 15-29)

Long et al., Clin Inf Dis, 1994, 18, 610-619

Laumann et al, J Am Acad Dermatol, 2006, 55, 413-421.

Survey, Allensbach, 2004

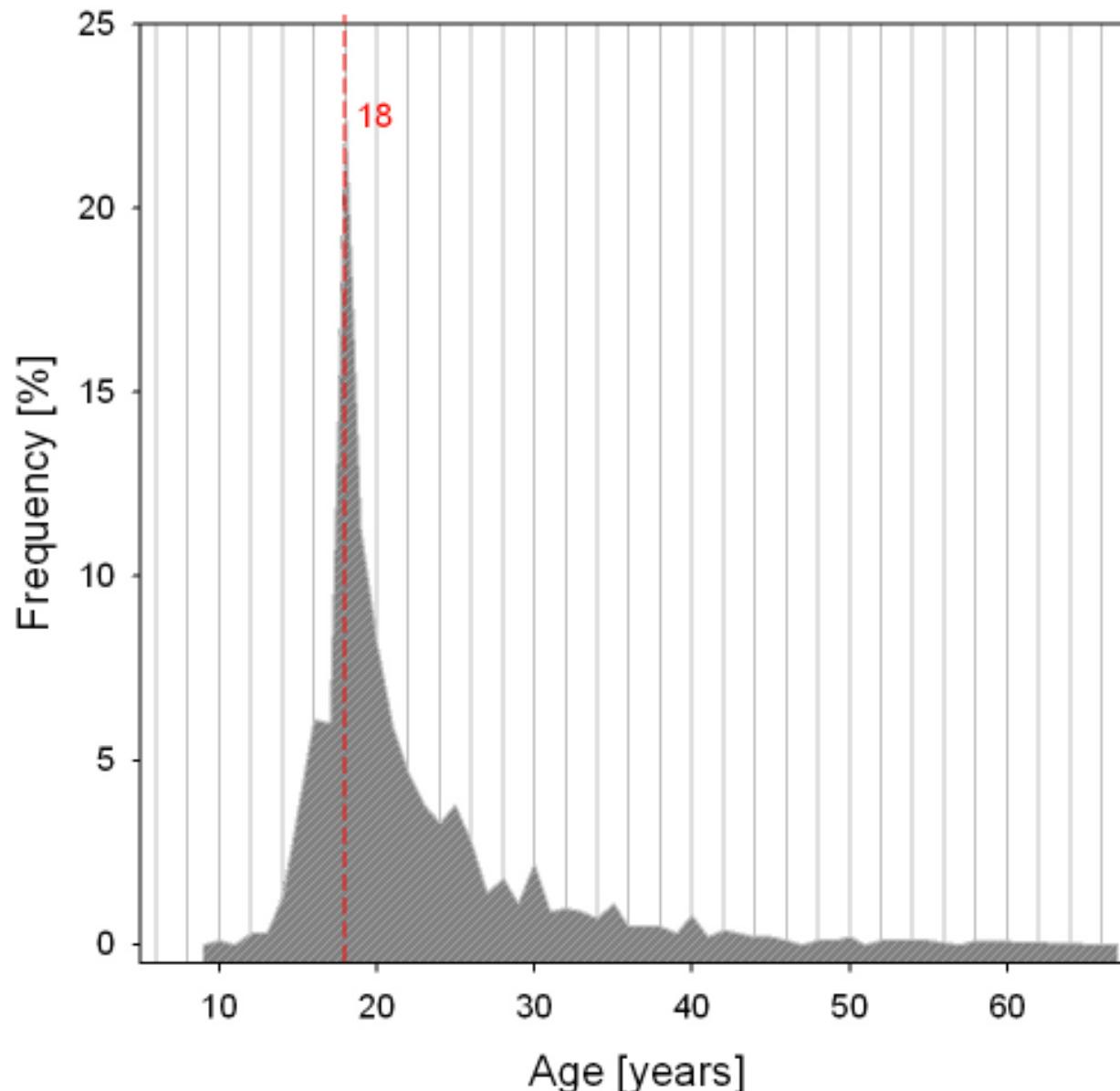
# Tattoo Survey

40 questions, 3411 participants, Germany, Austria, Switzerland

	<b>Study Population</b>
<b>Age (years), mean (SD)</b>	29.3 (8.6)
<b>Sex</b>	
Male, n (%)	1402 (41.1%)
Female, n (%)	2009 (58.9%)
<b>Type of tattoo color (n/a: n=6)</b>	
black	1995 (58.7%)
mixed-colored	1410 (40.1 %)
<b>Size of tattoo (n/a: n=2)</b>	
small (< 300 cm <sup>2</sup> )	1320 (38.8 %)
large ( $\geq$ 300 cm <sup>2</sup> )	2089 (61.1 %)
<b>Number of tattoos (n/a: n=6)</b>	
single	1187 (34.9 %)
multiple	2224 (64.9 %)

# Tattoo Survey

Age at first tattoo



# Tattoo Survey

## Problems

direct after tattooing

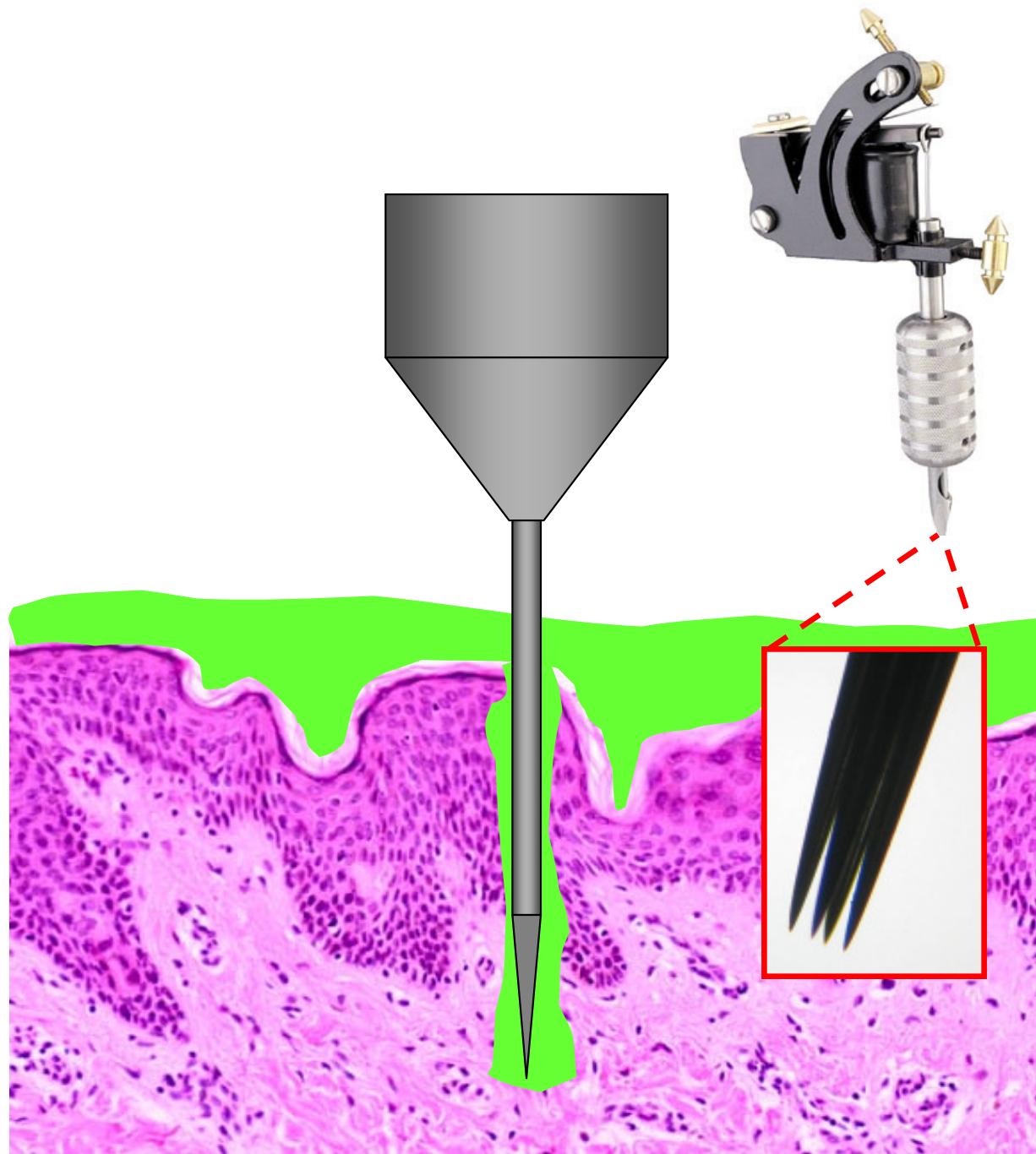
**skin (68 %):**   crusts, itching, edema, bleeding, blisters, pus, ...

**systemic (7 %):** dizziness, headache, nausea, fever, fatigue, ague, ...

persistent (9 %)

Scars, intermittent edema, light sensitive, itching, elevated skin, acne,  
skin papules, numbness, psychic problems, ...

# Making a tattoo

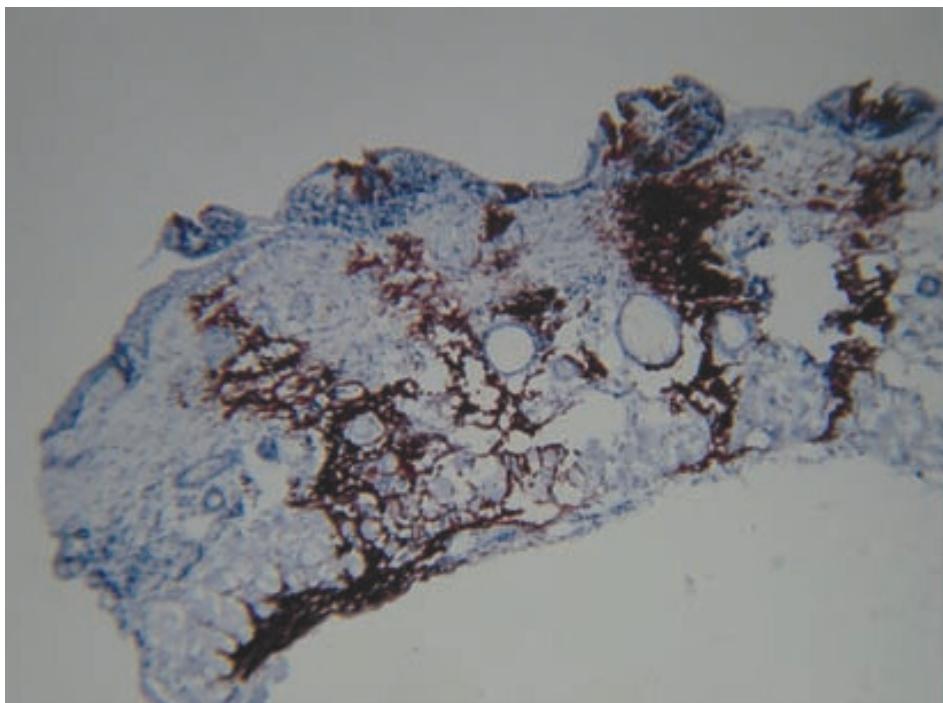


# Making a tattoo

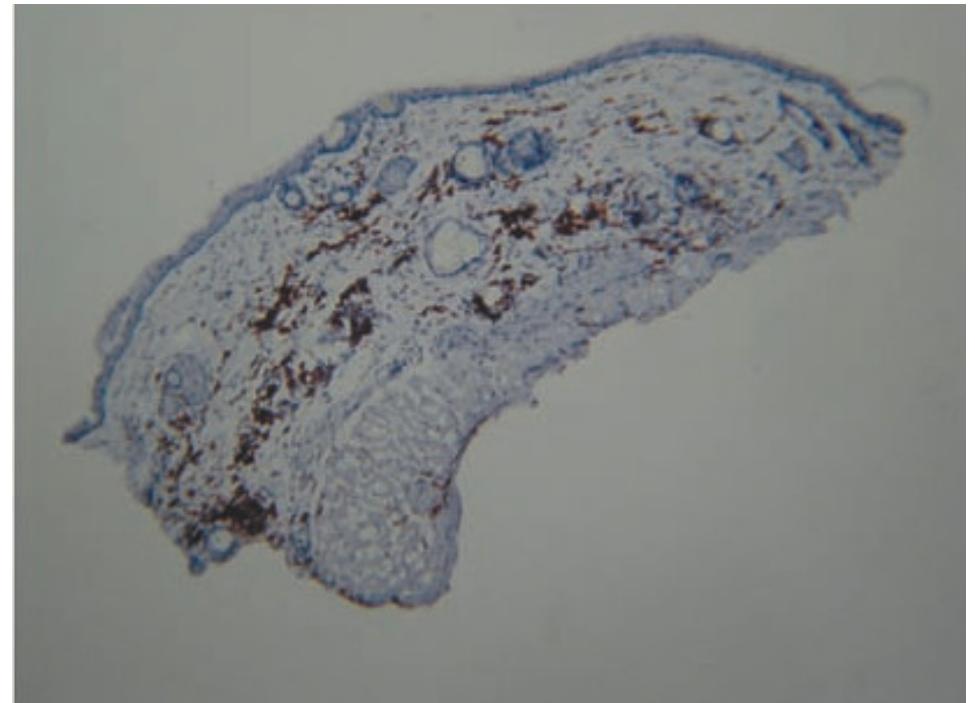
Mouse skin



1 day after tattooing



42 days after tattooing

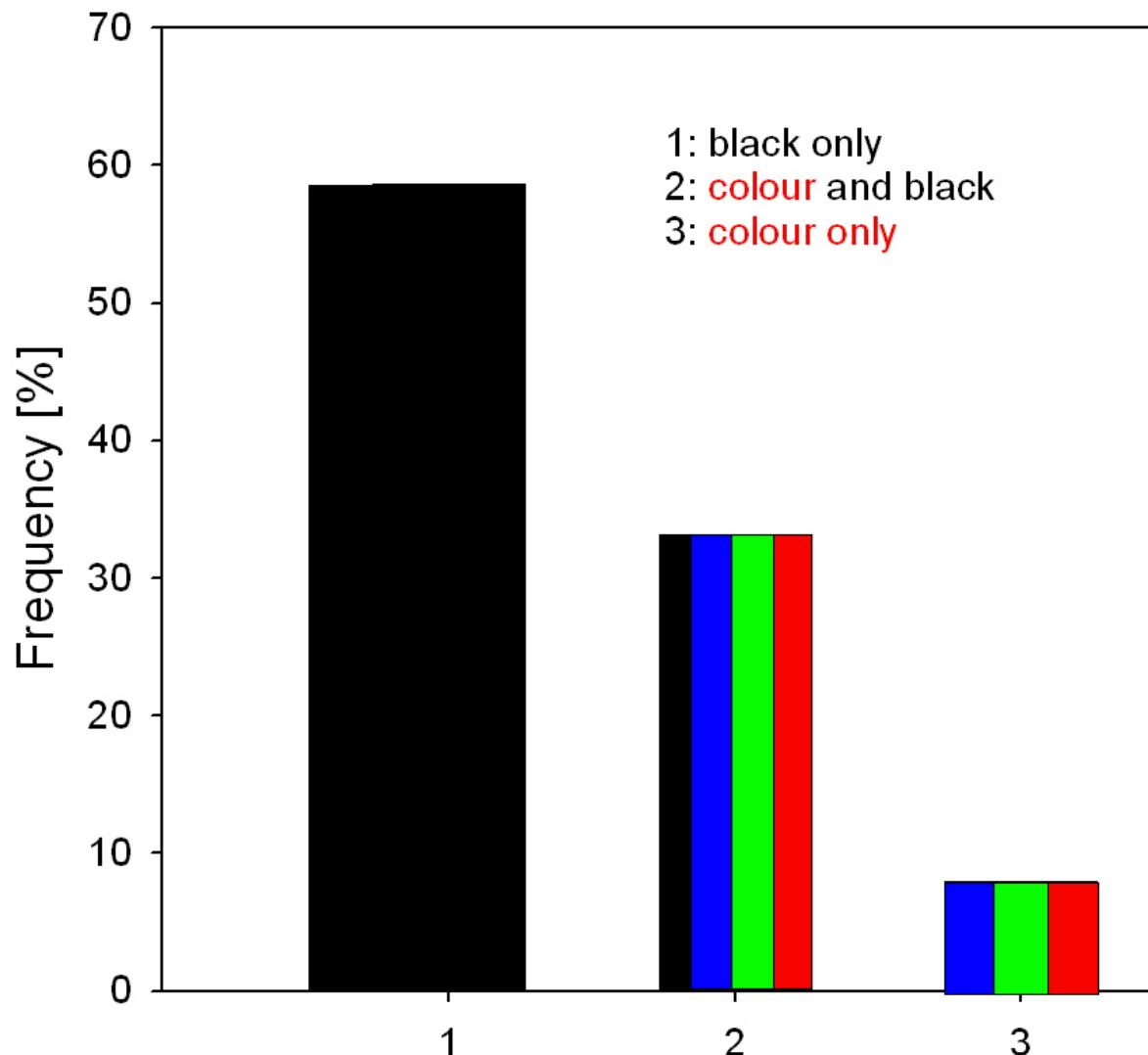


Tattooing of skin results in transportation and light-induced decomposition of tattoo pigments – a first quantification *in vivo* using a mouse model

Eva Engel<sup>1</sup>, Rudolf Vasold<sup>2</sup>, Francesco Santarelli<sup>1</sup>, Tim Maisch<sup>1</sup>, Neera V. Gopee<sup>3</sup>, Paul C. Howard<sup>3</sup>, Michael Landthaler<sup>1</sup> and Wolfgang Bäumler<sup>1</sup>

# Tattoo Inks

Data based on survey

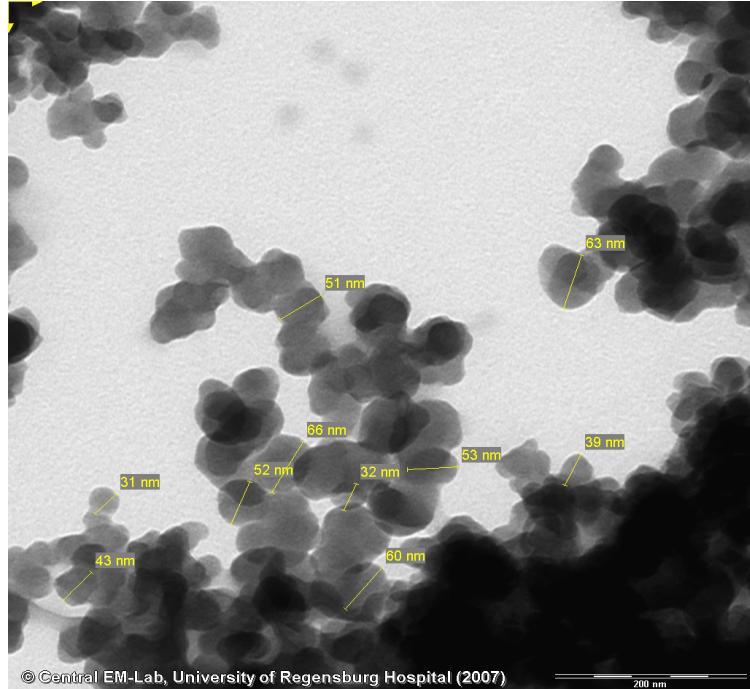


# Tattoo inks - Color

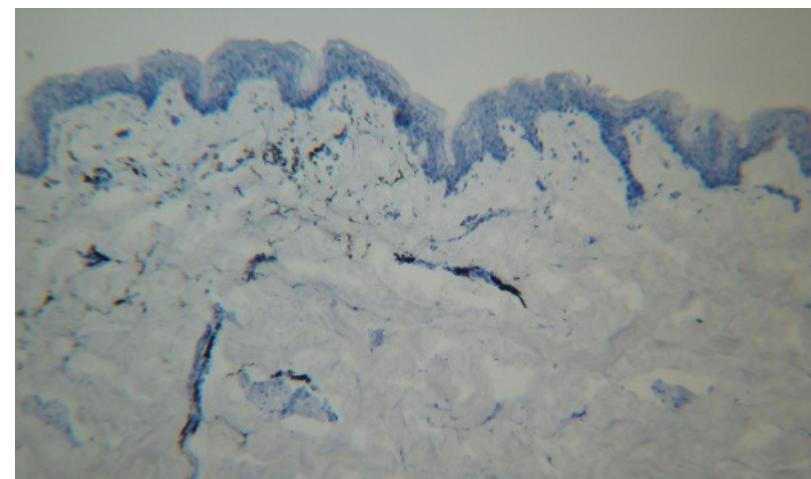
Number	Trade name	Pigment	Chemical structure
1	Canary Yellow	P.Y.14	Disazo-Diarylide
2	17	P.Y.55	Disazo-Diarylide
3	Dunkelgelb	P.Y.83	Disazo-Diarylide
	Golden Luv		
4	Zitronengelb	P.Y.74	Methine Pyrazolone
5	Sunset Yellow		
6	Orange, Navel Orange, Melon, P1		Disazo-Pyrazolone
7	Dunkelrot	P.R.15	NaphtholAS
8	I8	P.R.9	NaphtholAS
9	Red, Spanish Red, P8	P.R.22	NaphtholAS
10	Red, Red Velvet	P.R.112	NaphtholAS
11	P1	P.R.170	NaphtholAS
12	Burgandy, I5, Magenta	P.R.122	Quinacridone
13	I6, Pur Purple, True Purple, P3	P.V.23	Dioxazine
14	Permanent Blue, Navy Blue	P.B.15	Cu-Phthalocyanine
15	Permanentgrün, Waldgrün, Forest Green, I4, Fezan Blue Green, Permanent Green, P2	P.G.7	Cu-Phthalocyanine
16	Avocado Green	PG	Cu/Al-Phthalocyanine

# Tattoo inks - Black

Prior to tattooing: nano-particles

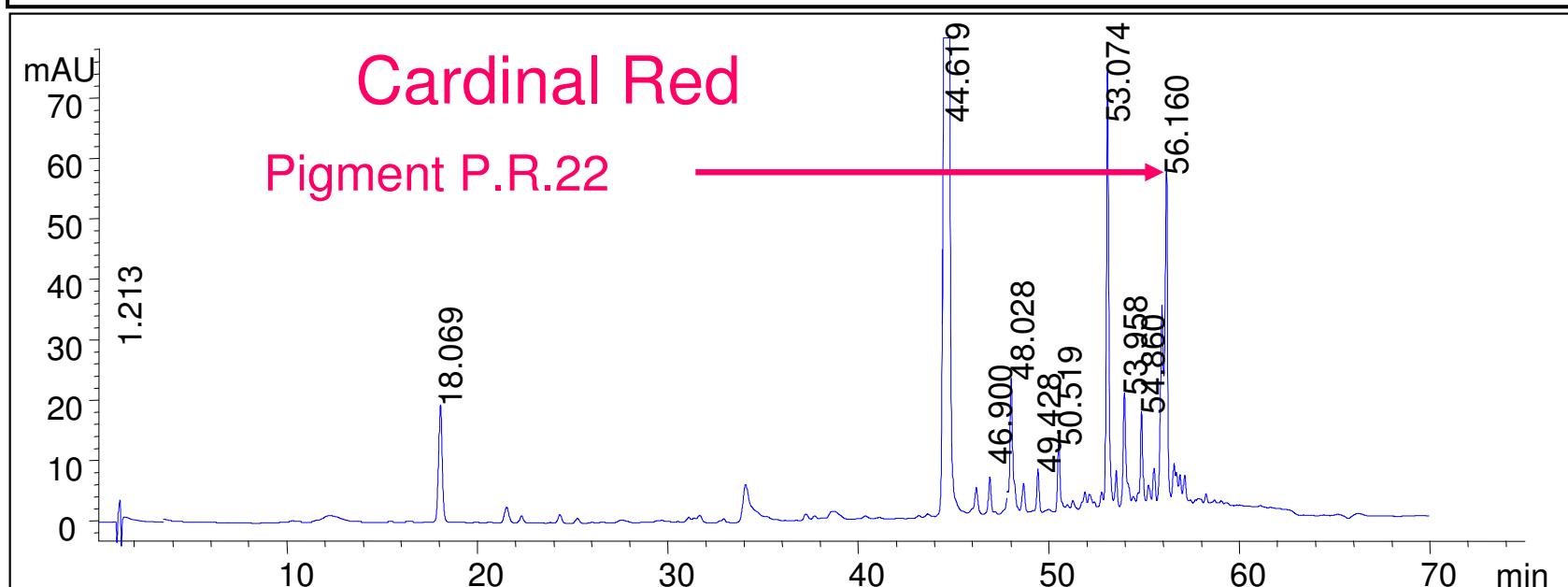
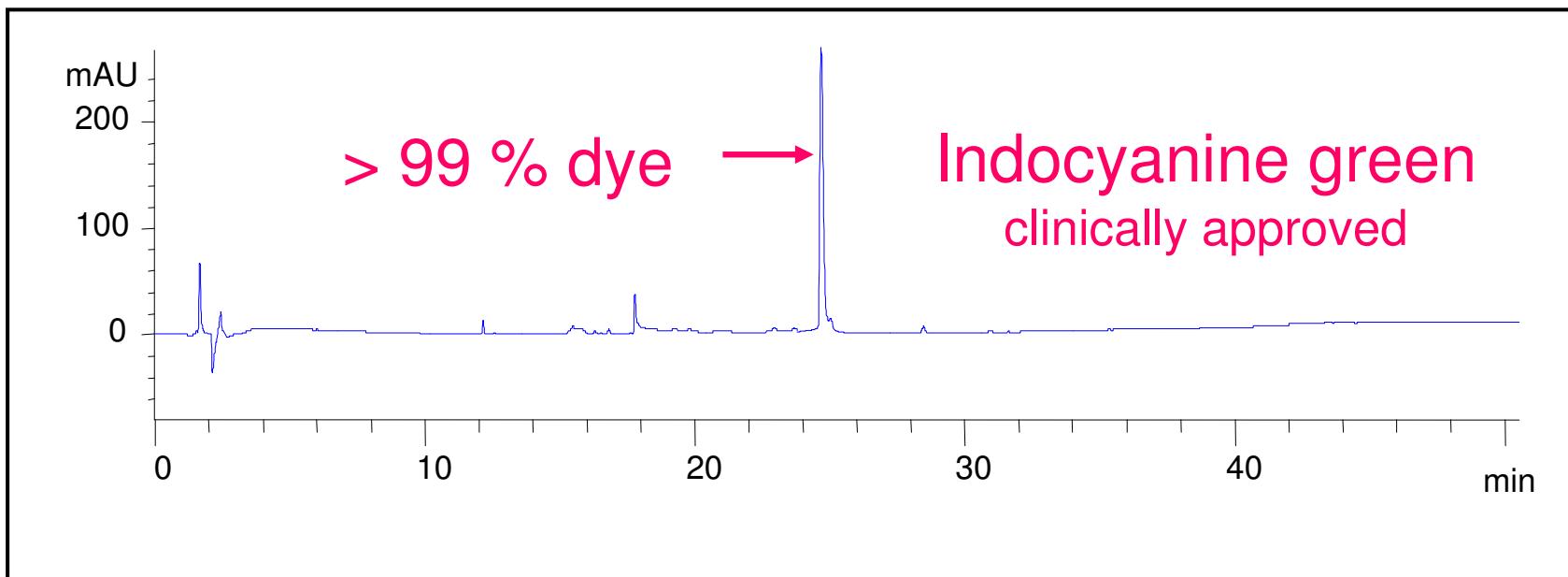


After tattooing:  
nano- and micro-particles



# Tattoo inks - Impurities

chemical analysis - HPLC



# Tattoo inks - Impurities

Detected so far...

## Dermatology

### Case and Review

Dermatology  
DOI: 10.1159/000346943

Received: December 29, 2012  
Accepted: December 24, 2012  
Published online: May 14, 2013

### Adverse Reactions after Tattooing: Review of the Literature and Comparison to Results of a Survey

Sabrina M. Wenzel Ines Rittmann Michael Landthaler Wolfgang Bäumler

Department of Dermatology, University of Regensburg Medical Center, Regensburg, Germany

Dermatology. 2013 May 14. [Epub ahead of print]

**Table 1.** Substances detected in tattoo inks

Compounds	References	Comments
Monoazopigment Disazodiarylide Naphthol-AS-pigment Quinacridone Dioxazine Cu-phthalocyanine Cu/Al-phthalocyanine-Br <sub>x</sub> Cl <sub>y</sub>	[11, 19]	pigment in color inks
Mercury Cadmium Cobalt Chrome	[62]	colored compounds in inks
Carbon black Iron	[13, 63, 64]	'color' of black, brown inks
Titanium dioxide Aluminium	[19, 63]	used for whitening of inks
Naphthol-AS 2-Methyl-5-nitroaniline 2,5-Dichloraniline 4-Nitro-toluene	[65]	synthesis educts and decomposition products of azo pigments
PAHs Phenol	[13]	residue of pyrolysis present in black inks
Diethyl-phthalate Hexachloro-1,3-butadiene Metheneamine Dibenzofuran Benzophenone 9-Fluorenone 3,6-Dimethyl-1 heptyn-3-ol 1,6-Hexandiole, oleamide 7-Hexyl-2-oxepanone 1,1'-Oxybis-2-propanol Carbitol cellosolve 1,2,3,4-Tetrahydro-1-phenyl-naphthalene	[12]	impurities in black inks

# Tattoo inks - Impurities

Causing reactions: allergic, toxic, infections, tumor (?), ...

## Case and Review

### Dermatology

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Department of Dermatology, University of Regensburg Medical Center, Regensburg, Germany





Pat.1



Pat.2



Pat.3

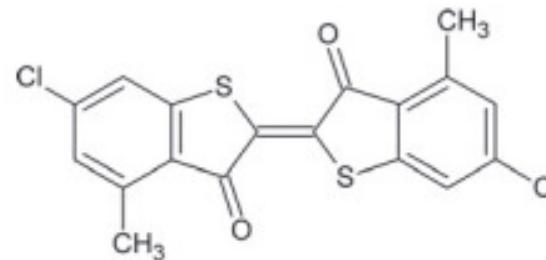


Pat.4

# Tattoo inks - Impurities

Permanent-Makeup = Tattoo

PMU colorant: Longtime Liner®  
Mainly iron oxides, however...



Pigment Red 181; CI 73360

Wenzel SM et al., Contact Dermatitis 2010; 63: 223-227

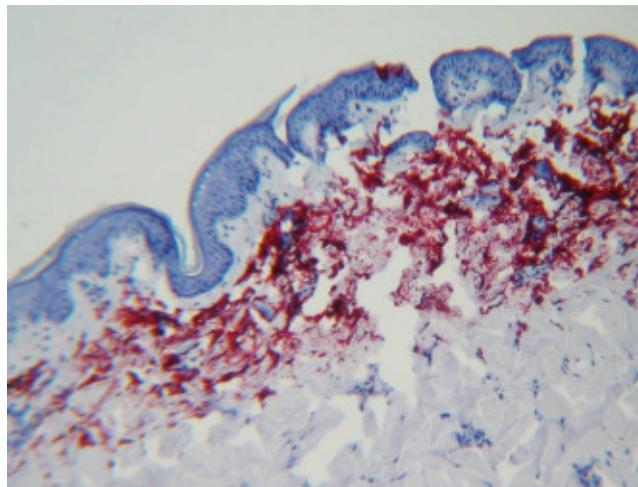
# The Fate of Tattoo Pigments

## Size of tattoos

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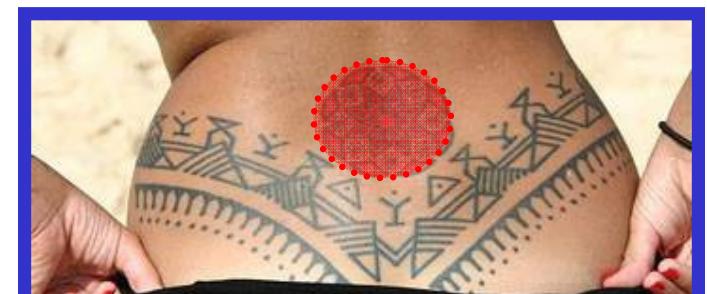
# The Fate of Tattoo Pigments

Determination of pigment concentration in the skin directly after tattooing  
(extraction, chemical analysis, HPLC)



excised skin specimen

$\sim 1 \text{ mg/cm}^2$



100 cm<sup>2</sup> Tattoo



Engel et al, 2006, Anal. Chem. 78, 6440-6447

Engel et al, 2008, Contact Dermatis, 58, 228-233

# The Fate of Tattoo Pigments

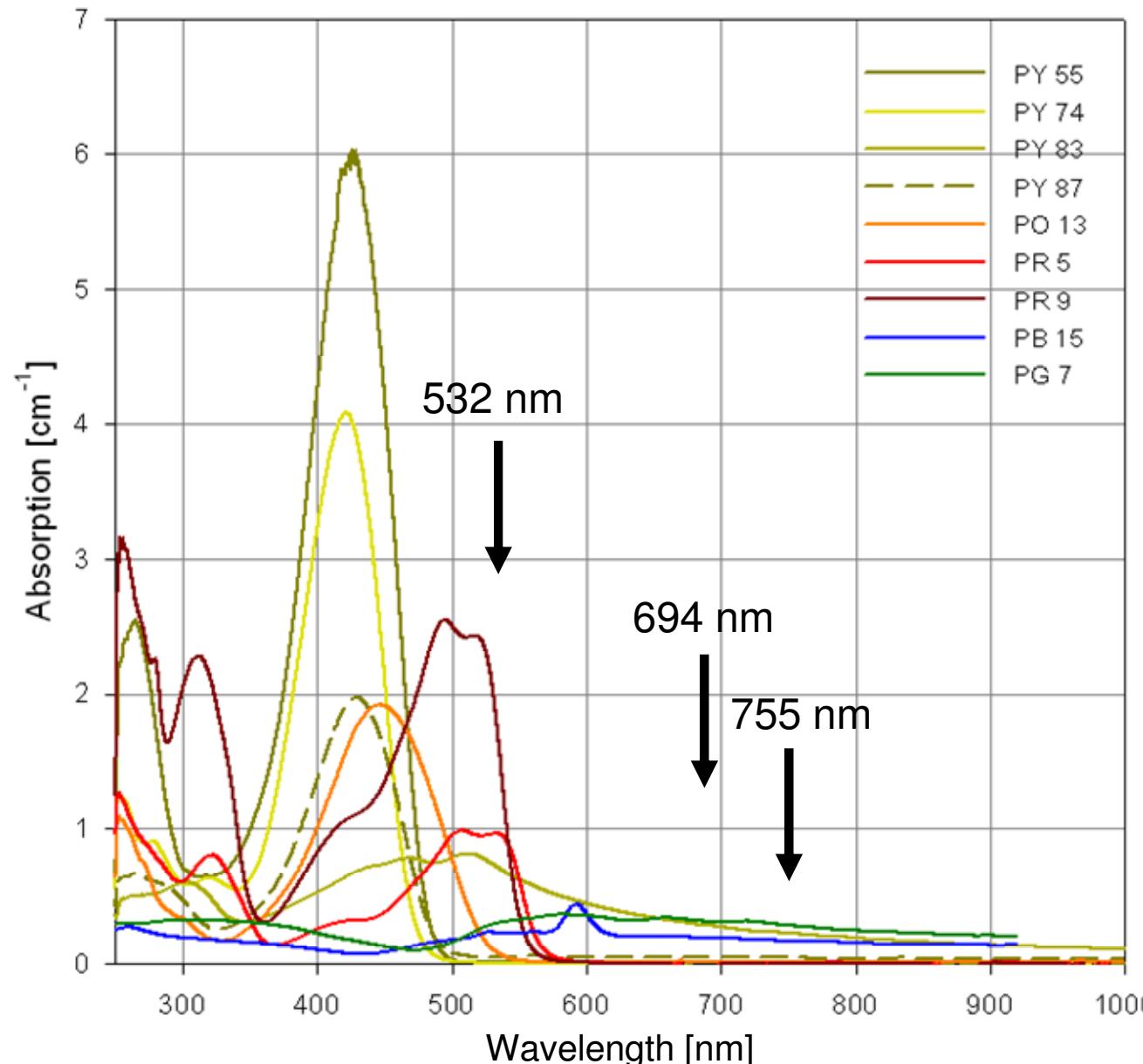
## Tattoo removal with lasers

Germany 10 Mio. with tattoos → 0.5 Mio patients ?  
USA 80 Mio. with tattoos → 4 Mio patients ?



# The Fate of Tattoo Pigments

## Tattoo removal with lasers

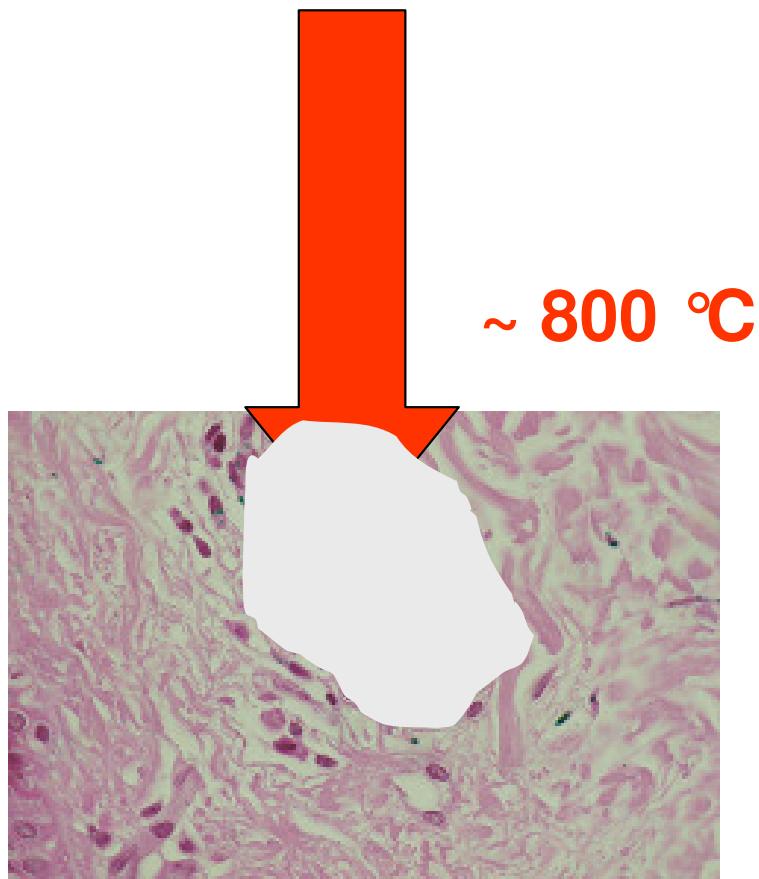


# The Fate of Tattoo Pigments

Tattoo removal with lasers

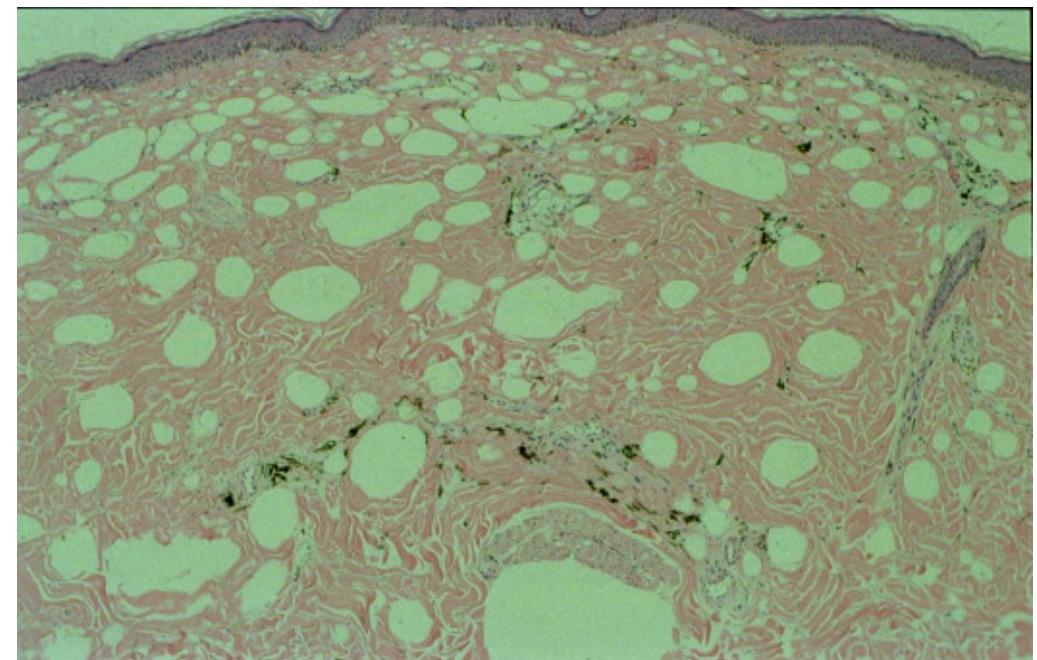
ultrashort pulse duration    10 ns

extremely high intensity     $10^8 \text{ W/cm}^2$



~ 800 °C

ruby laser (694 nm)



# The Fate of Tattoo Pigments

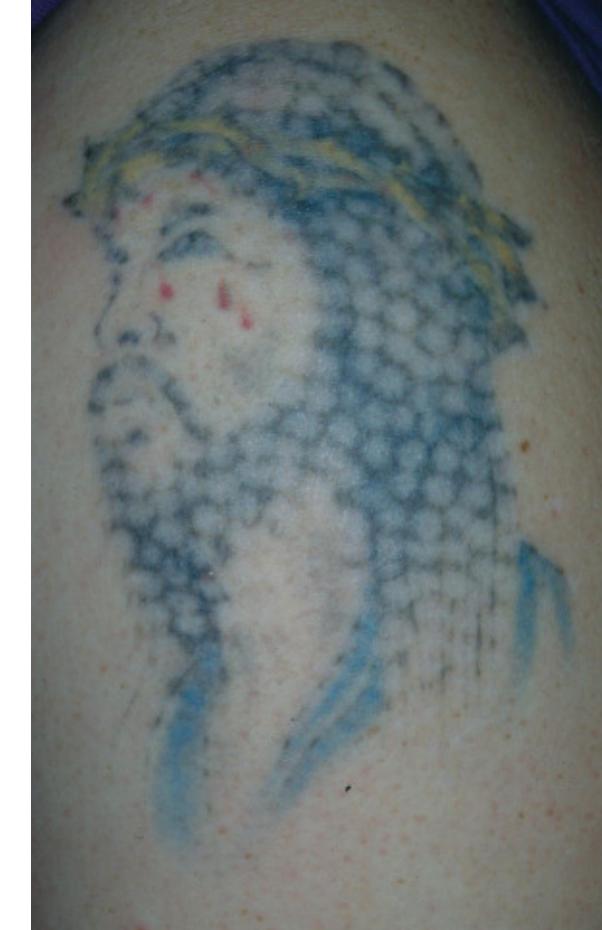
Tattoo removal with ruby laser



before laser therapy



5 min  
after laser therapy



4 weeks  
after laser therapy

# The Fate of Tattoo Pigments

Tattoo removal with ruby laser

Needs a series of treatments !



# The Fate of Tattoo Pigments

Tattoo removal with wrong device

Laser or IPL with ms pulses



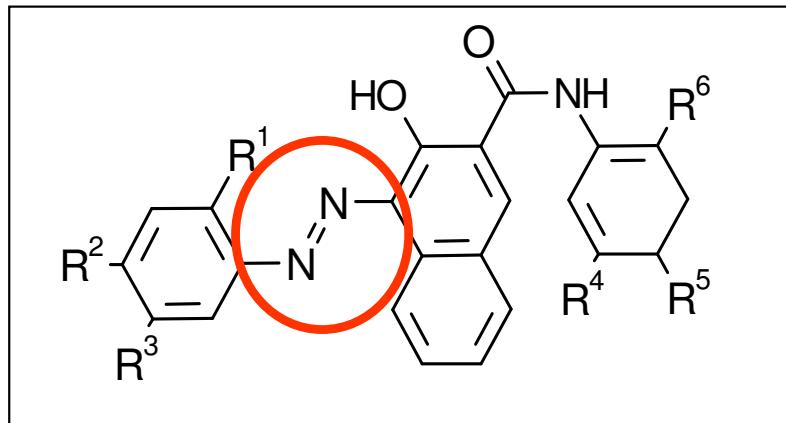
# The Fate of Tattoo Pigments

Tattoo removal – decomposition of azo pigments

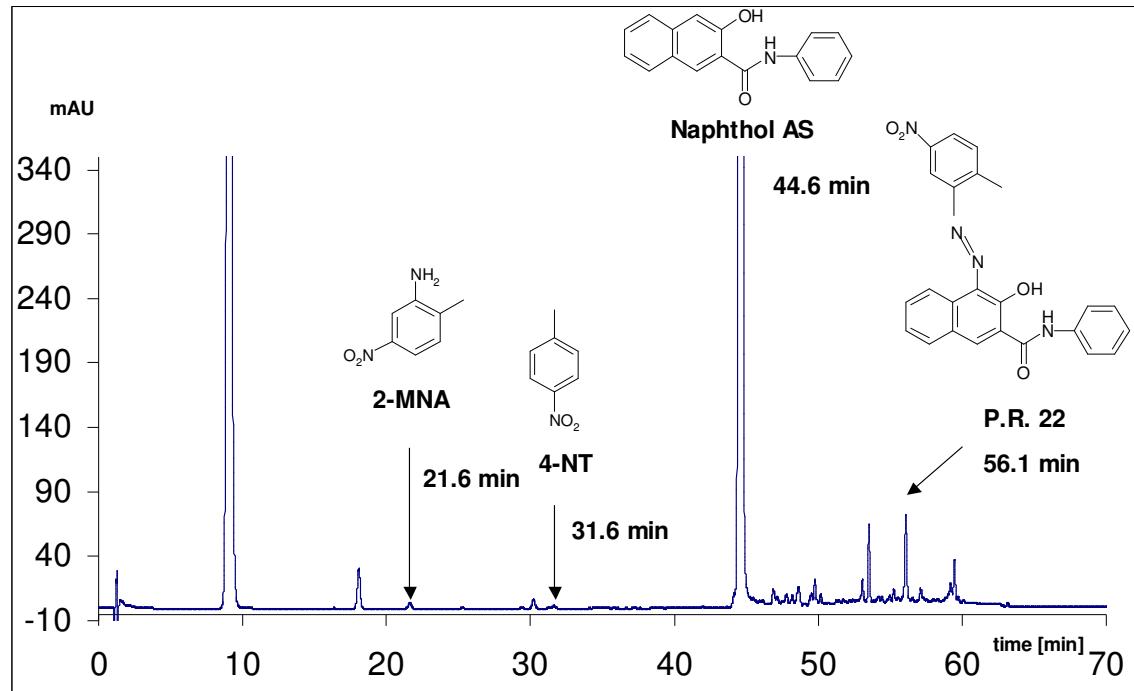
Laser irradiation: temperatures ~ 800 °C

Azo pigments

food industry: no temperatures above 200 °C

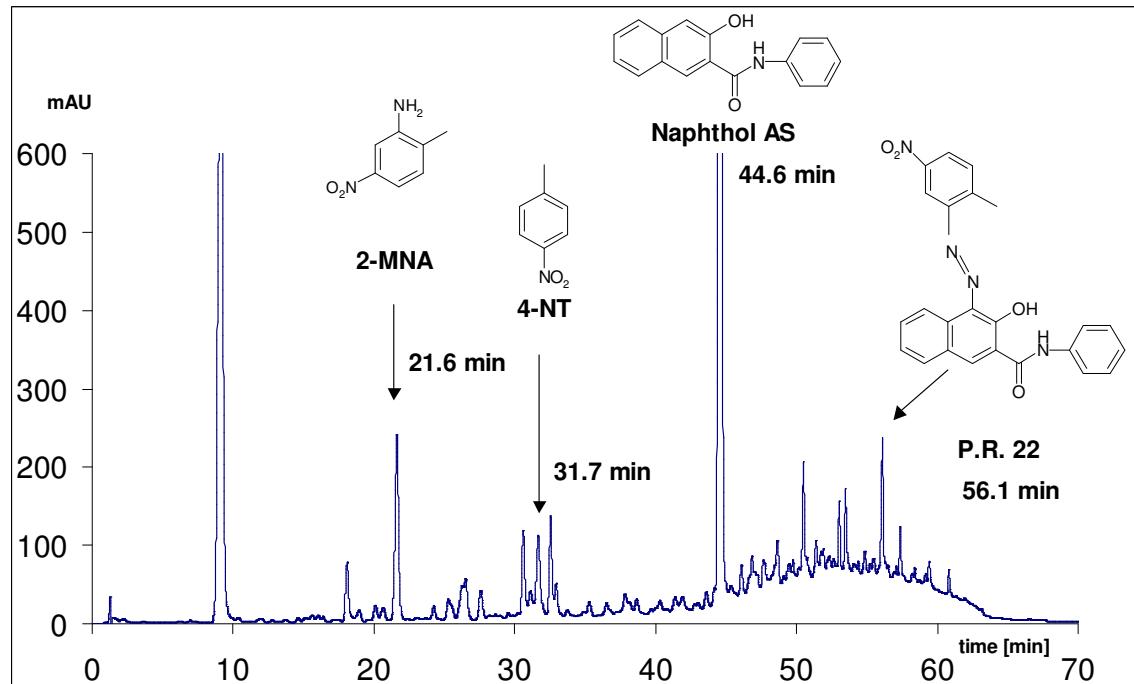


# The Fate of Tattoo Pigments



P.R.22

before  
laser irradiation

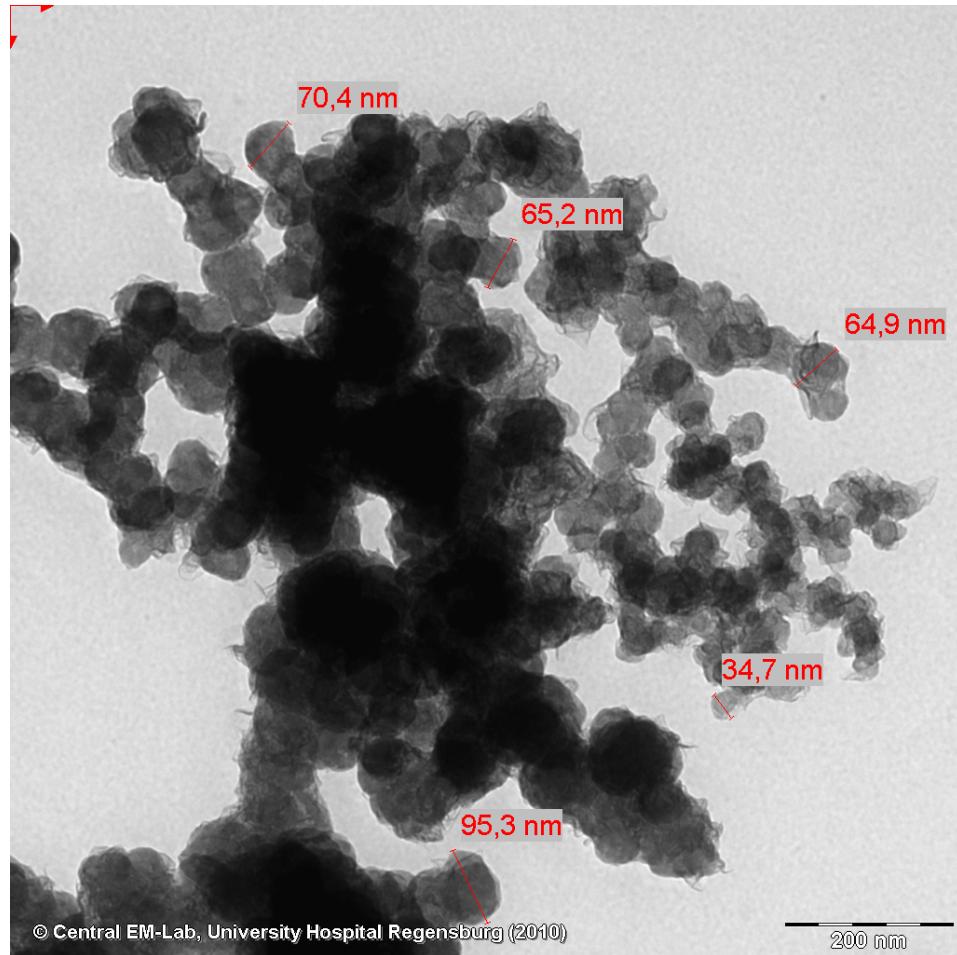


after  
laser irradiation

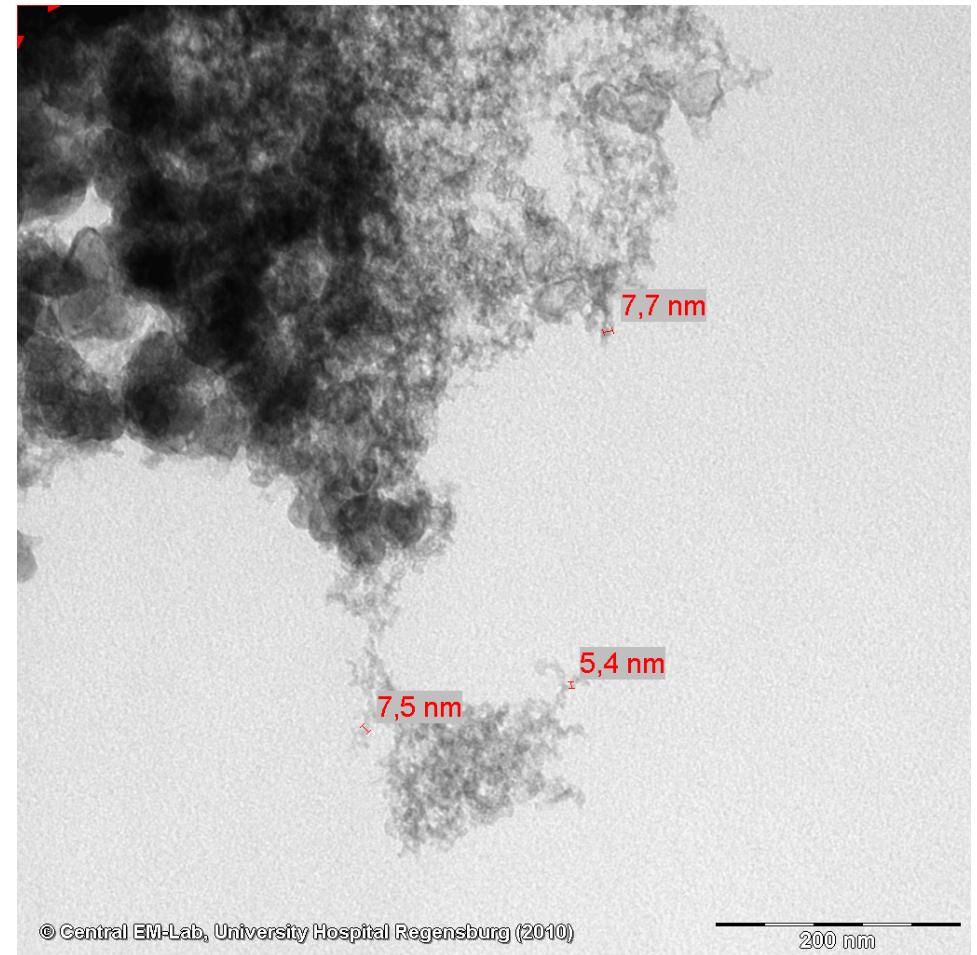
# The Fate of Tattoo Pigments

Tattoo removal – break down of carbon black particles

Before



after laser



Mean diameter of particles

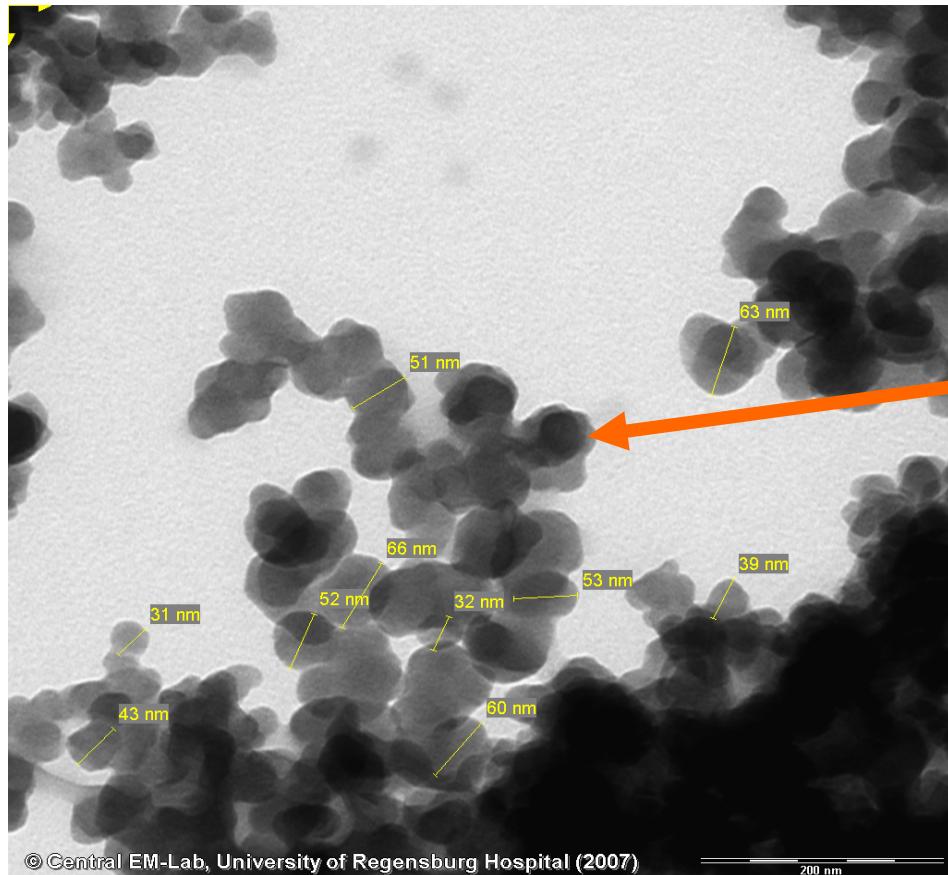
~ 50 nm

~ 6 nm

# The Fate of Tattoo Pigments

What about black tattoo inks ?

Simply Carbon black (soot) ?



**PAH**  
absorbed to the surface of nanoparticles  
and  
dibutyl-phthalate  
hexachloro-1,3-butadiene  
etc.

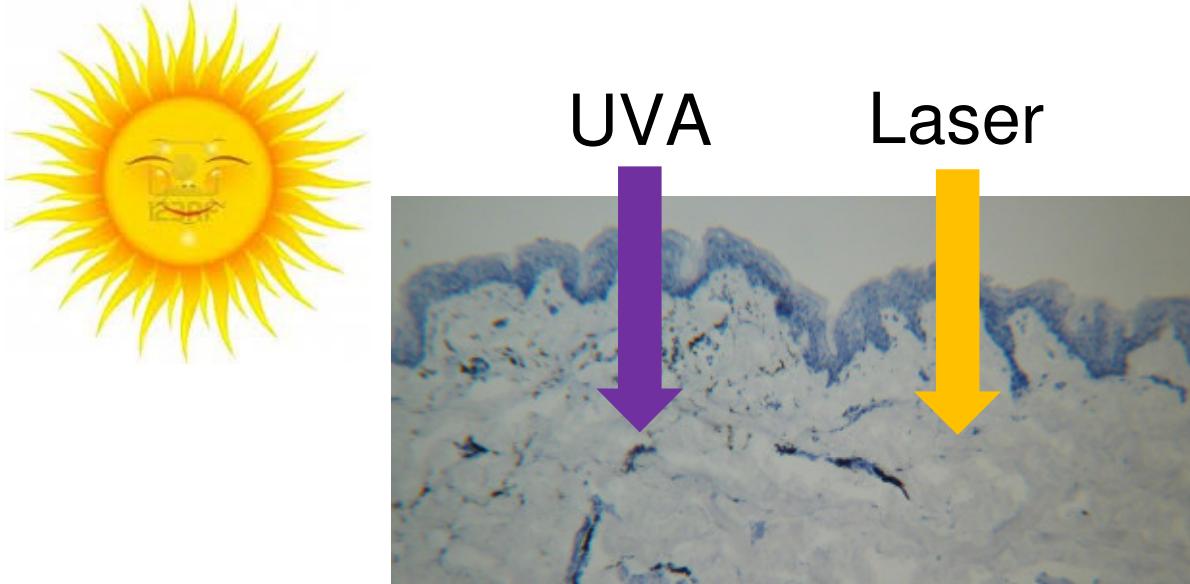
# Polycyclic Aromatic Hydrocarbons (PAH)



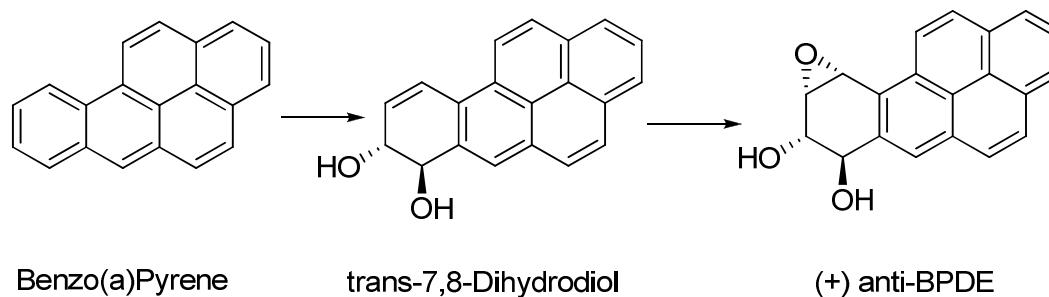
Up to 200 µg/g (total amount of PAH)

PAHs found in black tattoo inks				
PAHs*	mean value extracted [µg/g]	mean daily dietary intake (38) [µg/person]	carcinogenicity US-EPA (23)	Toxicity TEF (25)
phenanthrene (12)	24.5 ± 6.0	1.54	D	0.001
acenaphthylene (8)	14.5 ± 5.5	0.13	D	0.001
benzo[b]fluoranthene (2)	4.5 ± 4.3	<b>0.04</b>	<b>B2</b>	0.1
pyrene (12)	4.4 ± 0.8	0.35	D	0.001
anthracene (8)	3.3 ± 0.8	0.07	D	0.01
fluoranthene (14)	2.8 ± 1.0	0.35	D	0.001
chrysene (4)	1.7 ± 0.8	<b>0.11</b>	<b>B2</b>	0.01
benz[a]anthracene (6)	1.6 ± 0.2	<b>0.05</b>	<b>B2</b>	0.1
benzo[ghi]perylene (3)	1.2 ± 1.5	0.05	D	0.01
Indeno[1,2,3-cd]pyrene (2)	1.1 ± 1.0	<b>0.03</b>	<b>B2</b>	0.1
acenaphthene (8)	0.9 ± 0.3	0.98	-**	0.001
fluorene (6)	0.9 ± 0.2	0.59	D	0.001
benzo[k]fluoranthene (2)	0.4 ± 0.2	<b>0.01</b>	<b>B2</b>	0.1
benzo[a]pyrene (4)	0.3 ± 0.2	<b>0.04</b>	<b>B2</b>	1.0
naphthalene (7)	0.3 ± 0.1	-**	<b>C</b>	0.001
dibenzo[a,h]anthracene (1)	0.1 ± 0.1	<b>0</b>	<b>B2</b>	1.0

# The Fate of Tattoo Pigments



Formation of mutagenic diolepoxyde



## Irradiation of black tattoo inks (in solution)

UVA radiation:

QS-Laser radiation (532 nm)

diolepoxyde detected

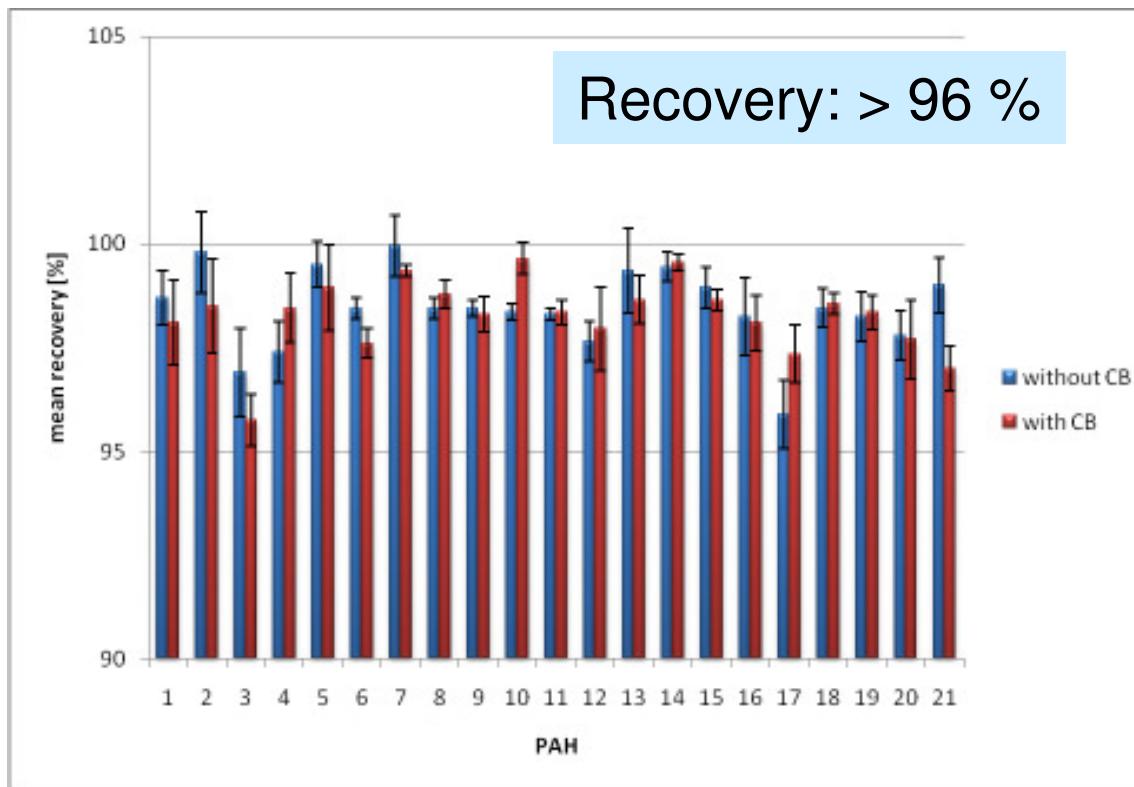
None

# The Fate of Tattoo Pigments

16 tattooed skin samples and corresponding regional lymph nodes



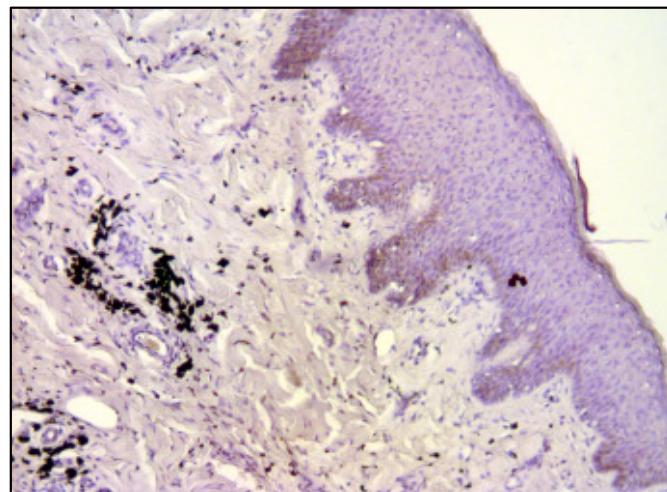
Extraction and quantification of 20 PAH and phenol at the same time



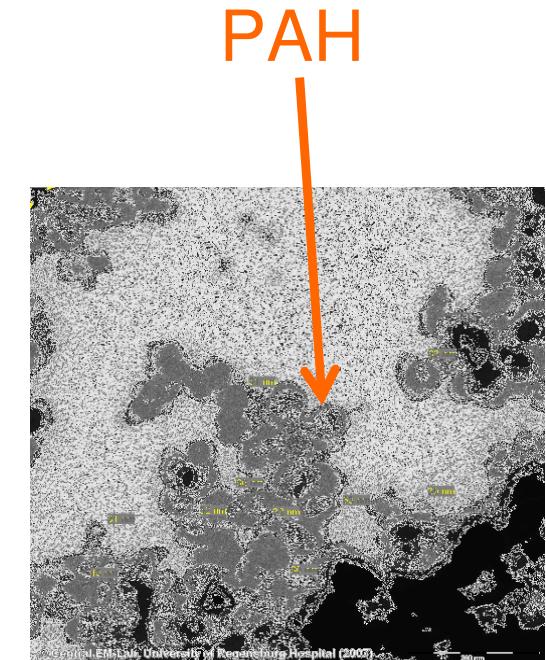
# The Fate of Tattoo Pigments

## 16 tattooed skin samples

PAH: 0 - 0.57  $\mu\text{g}/\text{cm}^2$  skin



Tattoo	Concentration of PAH $\mu\text{g}/\text{cm}^2$
# 1	0.08
# 2	0.57
# 3	n.d.
# 4	0.41
# 5	0.19
# 6	0.40
# 7	0.18
# 8	0.07
# 9	n.d.
#10	0.12
#11	0.08
#12	0.22
#13	n.d.
#14	n.d.
#15	0.14
#16	0.16



# The Fate of Tattoo Pigments

## 16 tattooed skin samples

Up to 200 µg of Carbon black per cm<sup>2</sup> skin

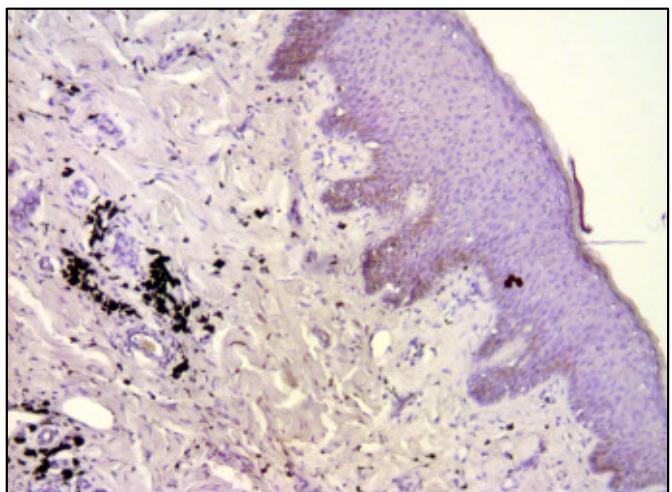


Tattoo	Concentration CB µg/cm <sup>2</sup>
--------	--

Tattooing: 1 mg/cm<sup>2</sup>

Up to 80 % of tattoo colorants disappeared

#5	152.0 ± 49.7
#6	194.9 ± 21.1
#7	157.0 ± 67.8
#8	89.4 ± 38.1
#9	82.8 ± 22.1
#10	105.3 ± 52.5
#11	94.3 ± 31.5
#12	42.1 ± 32.4
#13	157.3 ± 20.6
#14	167.4 ± 72.1
#15	167.5 ± 29.3
#16	87.6 ± 20.2



# The Fate of Tattoo Pigments

## transportation of inks in the body

**Tattooing of skin results in transportation and light-induced decomposition of tattoo pigments – a first quantification *in vivo* using a mouse model**

Eva Engel<sup>1</sup>, Rudolf Vasold<sup>2</sup>, Francesco Santarelli<sup>1</sup>, Tim Maisch<sup>1</sup>, Neera V. Gopee<sup>3</sup>, Paul C. Howard<sup>3</sup>, Michael Landthaler<sup>1</sup> and Wolfgang Bäumler<sup>1</sup>

<sup>1</sup>Department of Dermatology, University of Regensburg, Regensburg, Germany;

<sup>2</sup>Department of Organic Chemistry, University of Regensburg, Regensburg, Germany;

<sup>3</sup>National Toxicology Program Center for Phototoxicology, National Center for Toxicological Research, US Food & Drug Administration, Jefferson, AR, USA

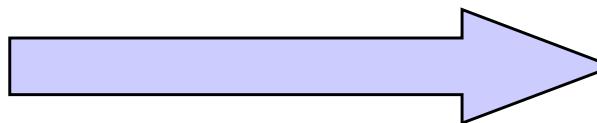
Quantification six weeks after tattooing:

~ 30 % of injected colorants is transported away from skin after tattooing

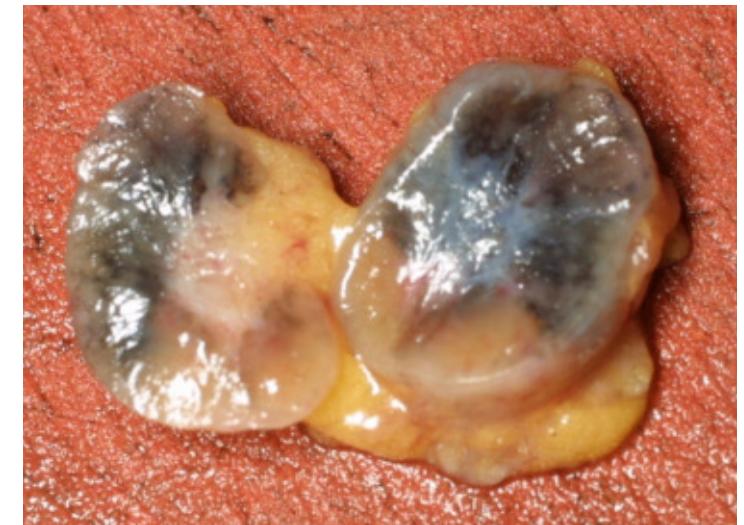
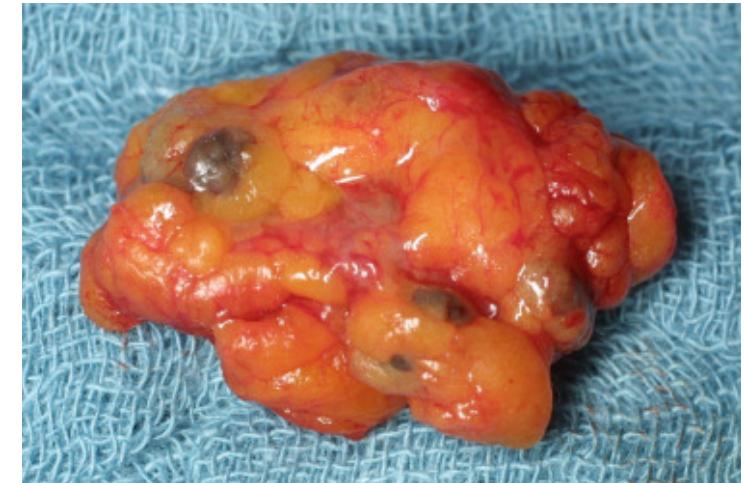


# The Fate of Tattoo Pigments

transportation of inks in the body



Human body



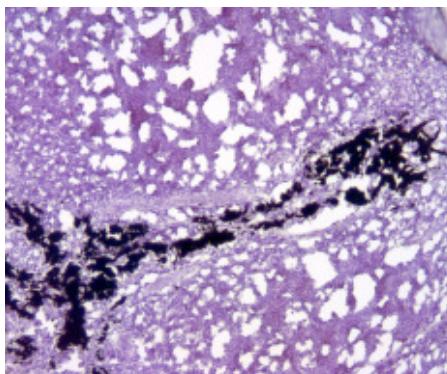
# The Fate of Tattoo Pigments

transportation of inks in the body

16 lymph nodes

Up to 17 mg of Carbon black per gram lymph node

Up to 11.8 µg PAH per gram lymph node



Lymph node	Concentration of CB [mg/LN]	Concentration of CB [mg/g]	Lymph node sample	Conc. PAH [µg/LN]	Conc. PAH [µg/g]
#1	0.80	13.30	# 1	0.02	0.05
#2	0.51	12.75	# 2	1.88	11.75
#3	0.06	1.20	# 3	n.d.	n.d.
#4	0.26	6.50	# 4	0.20	0.87
#5	0.64	12.80	# 5	0.39	2.29
#6	0.09	4.50	# 6	0.44	2.93
#7	0.01	0.11	# 7	0.52	3.06
#8	0.59	7.37	# 8	n.d.	n.d.
#9	0.19	4.75	# 9	n.d.	n.d.
#10	0.39	2.78	#10	0.10	0.17
#11	0.08	4.00	#11	0.74	10.57
#12	0.34	17.00	#12	0.61	10.16
#13	0.05	1.67	#13	n.d.	n.d.
#14	0.14	7.00	#14	n.d.	n.d.
#15	1.45	12.08	#15	0.10	0.43
#16	0.32	2.66	#16	0.46	2.19

# Some recommendations and ideas

- Inks without hazard impurities
- Remaining risk of adverse reactions
- Research regarding transportation processes in human body
  - Animal studies
- Interaction of UV or laser radiation with tattoo inks in skin
  - Animal studies
- High number of tattooed people: Need for epidemiologic studies