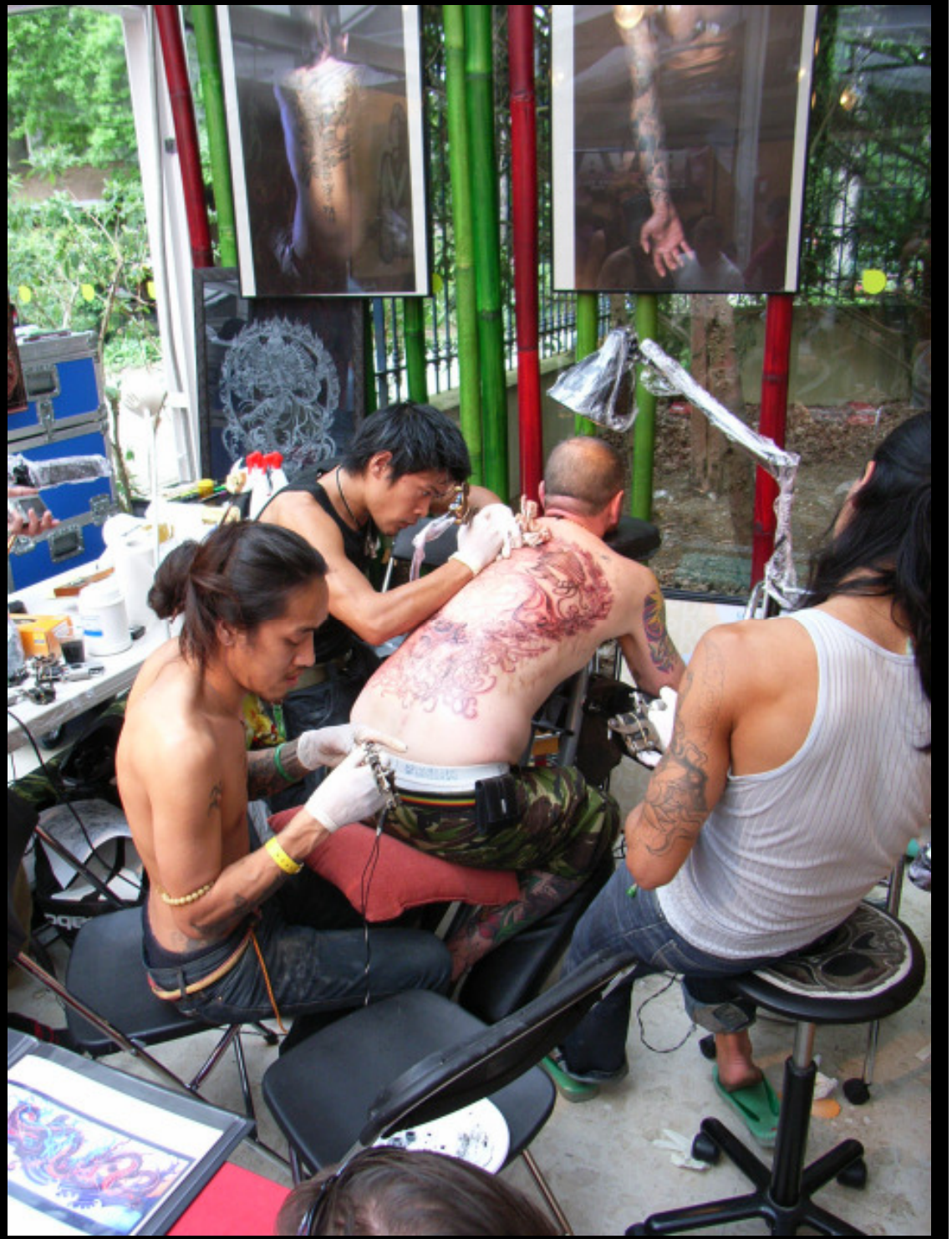
Tattooing, inks & cancers: Facts and Controversies



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Conflicts of interest

- ANSM (French drug agency)
  - Former Member of the *ad hoc* expert group on tattoo inks France/EU (N, Dk, No)

- Contacts on specialized forums
  - Dermatologists, physicians
  - Tattooists
  - Tattooed customers

- Honorary member of the french tattoo union (SNAT)

- Former free lance journalist for a tattoo magazine
- 9 tattoos

« Death to the Kings »

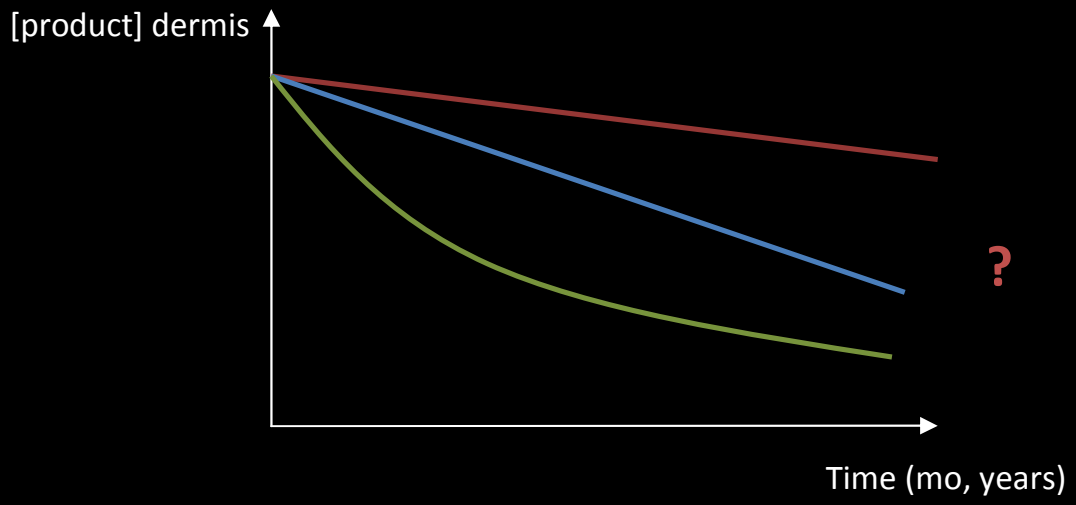
Tattoo and cancer is not a new issue

- Goldstein N. Tattoos today. From eyelids to ankles and some in '3-D'. Arch Dermatol. 1985;121:604-5

- Prediction of an increased rate of skin cancers with the increased prevalence of tattooing

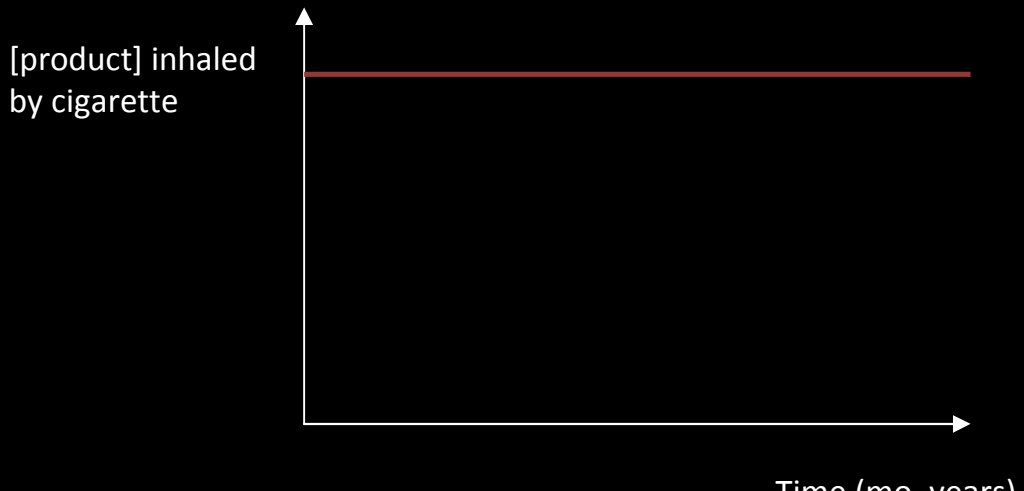
Tattooing is a unique toxicologic model

- Persistance in the dermis during the whole life of the bearer
- « bolus » of high concentration of pigment in a single shot
- Lymph node transport
- +/- rest part of the body (?)



One/several tattoos for a lifetime

Smoking every day for a lifetime



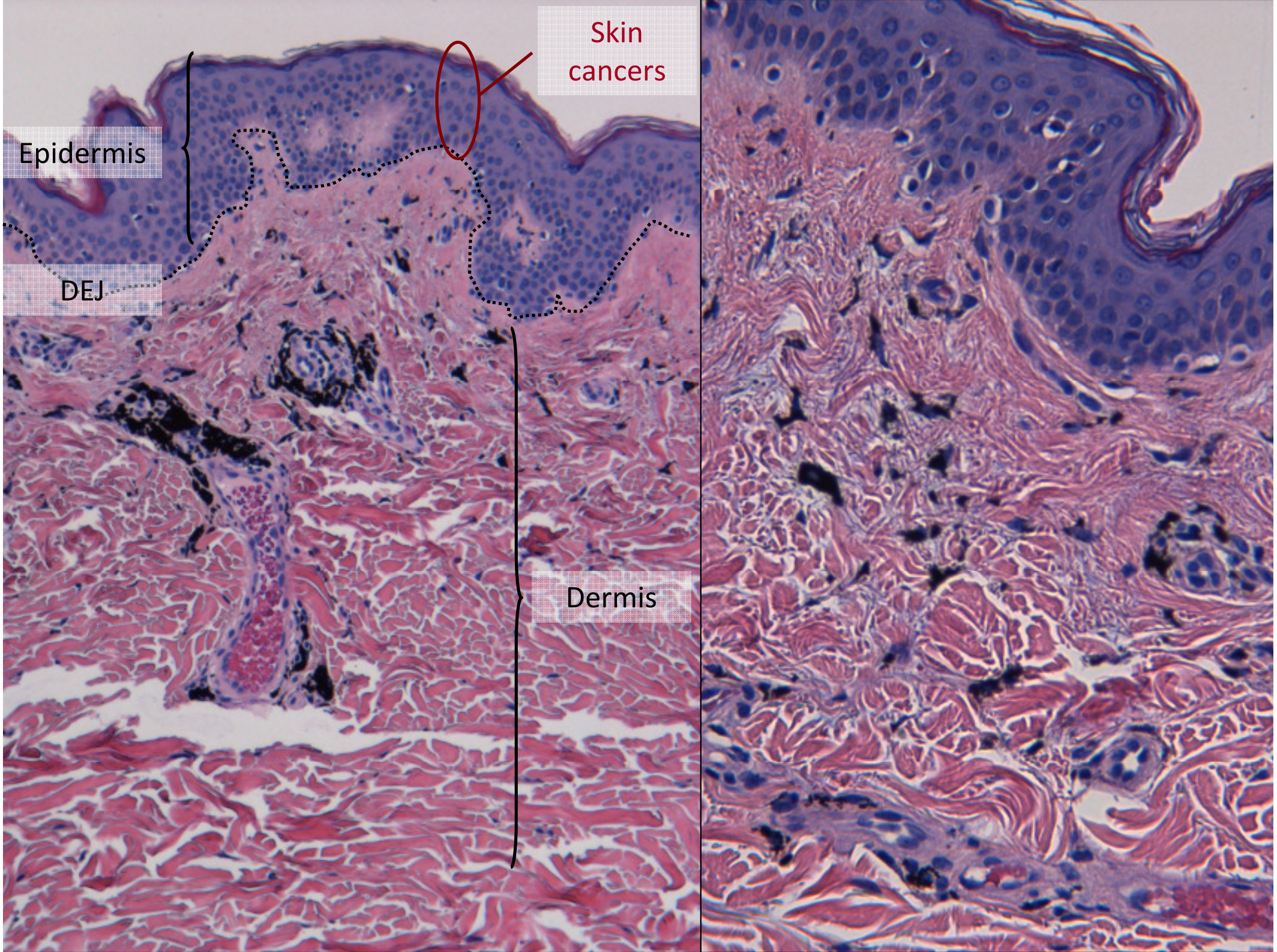
Tattoos and cancers

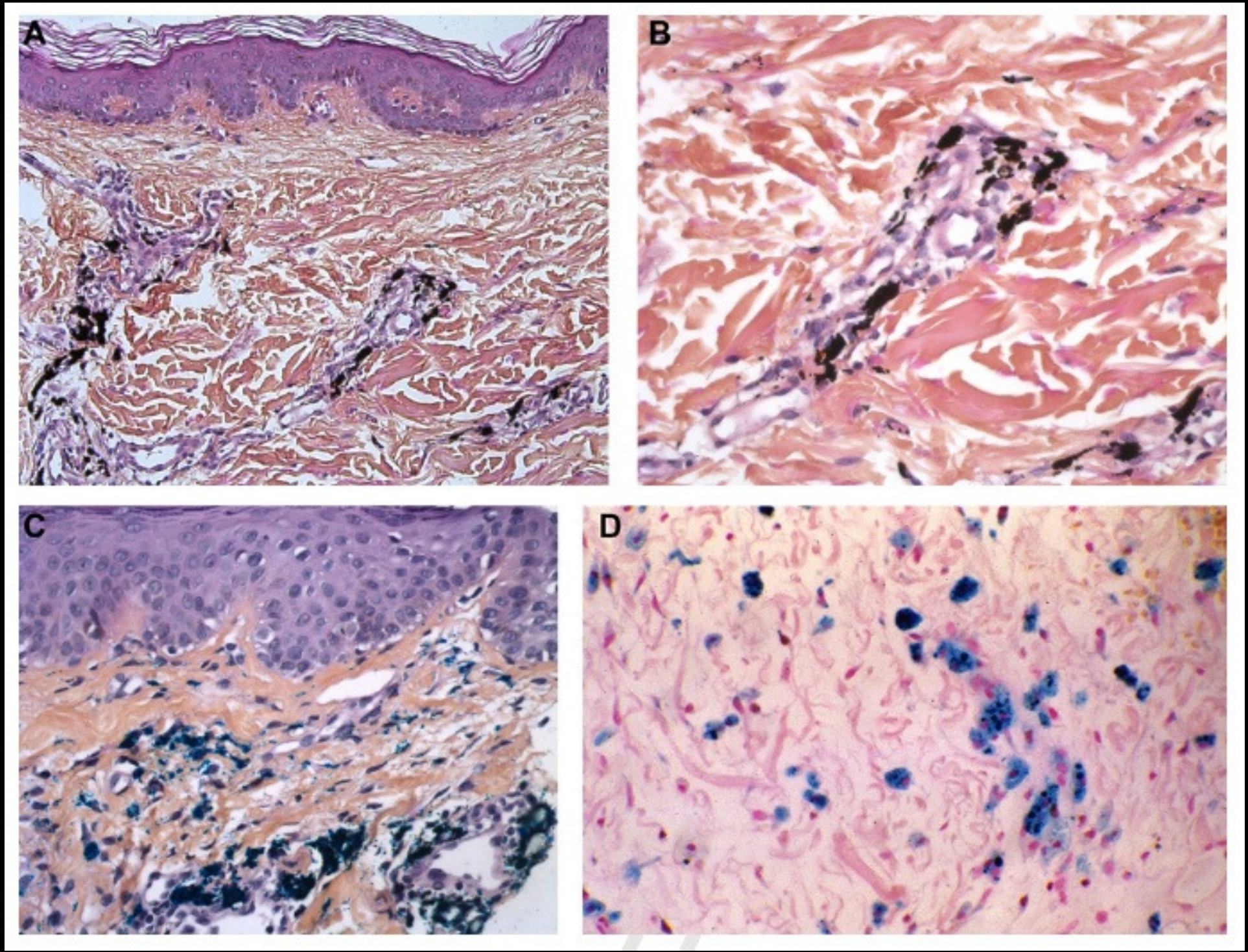
- Can tattoos give rise to skin cancers ?
  - Do epidemiology, clinical and histopathology support that hypothesis ?
  - How ?
  - Which type of skin cancer ?

- Can tattoos give rise to other cancer ?
  - Can tattoo pigments or by-products go in to blood flow ?
  - Can they deposit in other organs ?

Kluger N, et al. Lancet Oncol 2012;13:e161-168

Same risks ?


Skin cancers
Epidermis
DEJ
Dermis



Kluger N et al. Ann Dermatol Venereol 2011;138:146-54

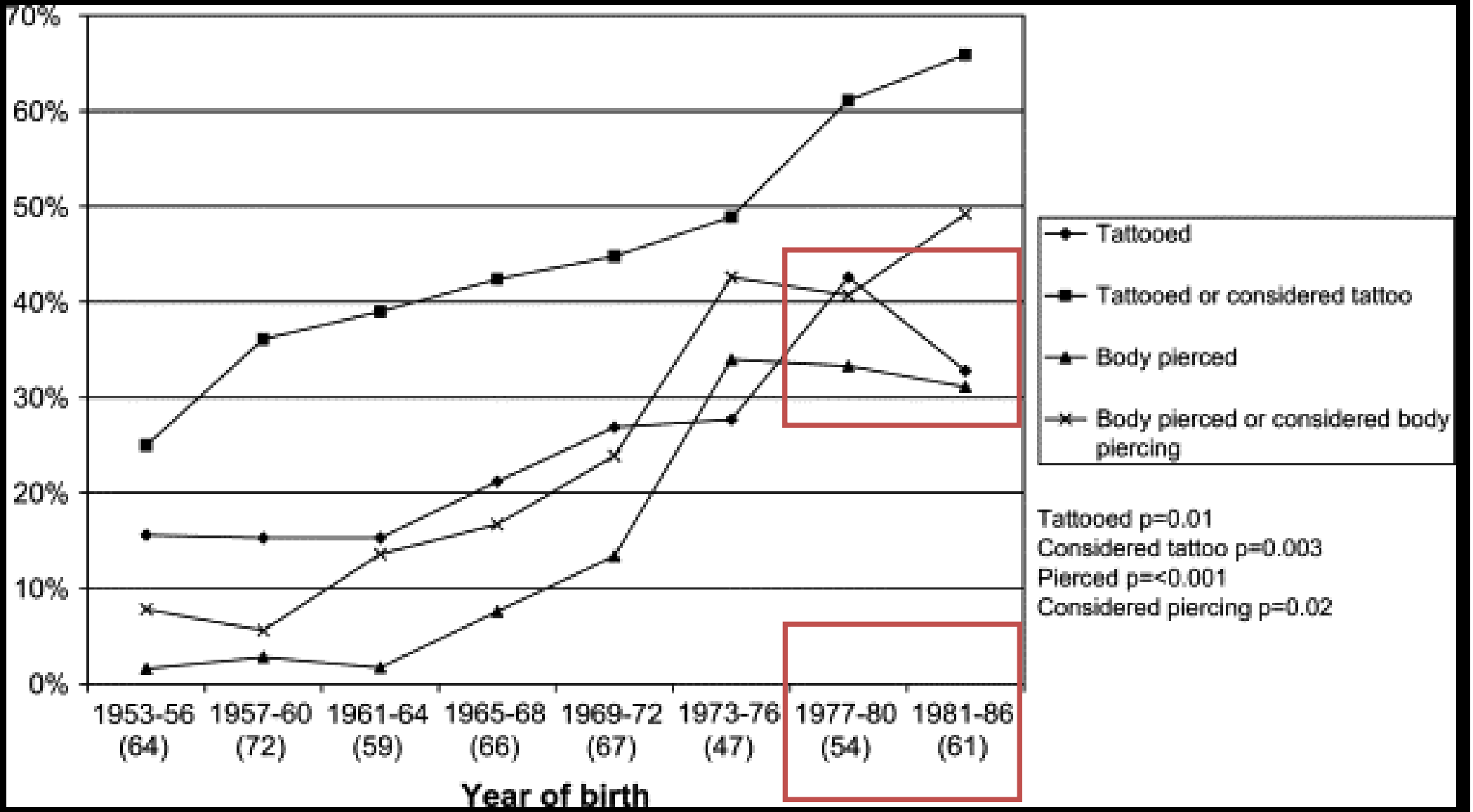
Tattoo inks and skin cancers: a clinical view

- Malignant tumors
  - Melanoma
  - Squamous cell carcinoma
  - Basal cell carcinoma
- Eruptive or isolated keratoacanthoma
- Pseudo-epitheliomatous hyperplasia
- Anecdotal tumors
  - Cutaneous lymphoma (1)
  - Leiomyosarcoma (1)
  - Dermatofibrosarcoma protuberans (2)
- Benign tumors
  - Traumatized naevus, seborrhoeic keratoses, histiocytofibroma, epidermal cysts, milia

A popularity among the young…

- USA (*Laumann AE, et al. J Am Acad Dermatol 2006*)
  - 24% (n = 500, 18 – 50 yo, Illinois)
- Germany (*Stirn A, et al. J Psychosom Res 2006*)
  - 8,5% (n > 2000, 14 - 93 yo)
  - 15% among the 14 – 44 yo
  - 22% among the : male 25 – 34 yo

- France 10%, and 25% between 25-34 yo *(ifop 2010)*
- Finland 15%, among the 20-30 yo *(nuorisobaromeetri 2009)*



Laumann AE, Derick AJ. Tattoos and body piercings in the United States: a national data set. J Am Acad Dermatol. 2006;55:413-21.

- Germany
  - 3,411 tattooed individuals
  - July 2007 – March 2008

Table 2. Features of tattoos in the total study population

	Females	Males	Total
Number of tattoos (n/a: 6; 0.2%)			
1	757 (37.8)	430 (30.7)	1,187 (34.9)
2–3	812 (40.5)	470 (33.6)	1,282 (37.7)
4–5	250 (12.5)	202 (14.4)	452 (13.3)
6–9	132 (6.6)	132 (9.4)	264 (7.8)
≥10	54 (2.7)	166 (11.9)	220 (6.5)
Age group at first tattoo (n/a: 12; 0.4%)			
Minor (<18 years)	422 (21.0)	178 (12.7)	12.7 (17.6)
Adult (≥18 and <35 years)	1,509 (75.1)	1,127 (80.4)	80.4 (77.3)
Adult (≥35 years)	69 (3.4)	94 (6.7)	6.7 (4.8)
Age group at most recent tattoo (n/a: 3; 0.1%)			
Minor (<18 years)	137 (6.8)	44 (3.1)	181 (5.3)
Adult (≥18 and <35 years)	1,704 (84.8)	1,115 (79.5)	2,819 (82.6)
Adult (≥35 years)	159 (7.9)	240 (17.1)	399 (11.7)
All following data refer to the most recent tattoo			
Localization (n/a: 43; 1.3%)			
Trunk			(45.2)
Arms			(32.8)
Legs	391 (19.4)	223 (15.9)	614 (18.0)
Head/neck	58 (2.8)	34 (2.4)	92 (2.7)
Type (n/a: 3; 0.1%)			
Professional	1,947 (96.9)	1,339 (95.5)	3,286 (96.3)
Amateur	33 (1.6)	59 (4.2)	92 (2.7)
Permanent makeup	25 (1.2)	3 (0.2)	28 (0.8)
Medical tattoo	2 (0.1)	0 (0)	2 (0.1)
Size (n/a: 2; 0.1%)			
25 cm^2	232 (11.5)	61 (4.4)	293 (8.6)
50 cm^2			
100 cm^2			
150 cm^2			
300 cm^2	509 (25.3)	339 (24.2)	848 (24.9)
900 cm^2	311 (15.5)	350 (25.0)	661 (19.4)
>900 cm^2	232 (11.5)	332 (23.6)	564 (16.5)

1 to 3 tattoos

Adult 18 - 35 yo

> 300 cm2 : 61%

Klügl I, et al. Dermatology 2010;221:43-50

	Age (years) at diagnosis and sex	Duration of tattoo at presentation	Delay of onset after tattooing/prior to diagnosis*	Tattoo location	Colour within affected area	Chronic sun exposure	Other skin cancer
Sharlit (1938)[21]	9, M	3 months	NA/NA	Forehead	Indelible pencil puncture	NA	NA
Allen (1967)[22]	NA	NA	NA	Arm	NA	NA	NA
Kirsh (1969)[23]† Kirsh (1972)[24]†	52, M	27 years	26 years/1 year	Right arm	Blue	NA	No
Wolfort et al (1974)[25]	55, M	29 years	24 years/5 years	Right arm	Red	NA	NA
Bartal et al (1980)[26]	52, F	2 years	2 years/NA	Breast (radiotherapy field marking tattoo)	Black (Indian ink)	NA	No
Bartal et al (1980)[26]	34, NA	6 years	6 years/NA Two metastatic malignant nodules from a melanoma of the left forearm localisation	Back (radiotherapy field marking tattoo)	Black (Indian Ink)	NA	No
Lee and Craig (1984)[27]	44, M	>20 years	>16 years/4 years	Chest	Blue	NA	No
Kircik et al (1993)[28]	36, F	10 years	9 years/1 year	Right scapula	Dark blue-green	NA	NA
Soroush et al (1997)[29]	47, M	20 years	16 years/4 years	Abdomen	Black	Outdoor recreational and occupational sun exposure, no sunburn	No
Khan et al (1999)[30]	44, M	25 years	NA/NA	Right arm	Black, blue	Outdoor recreational and occupational sun exposure, no sunburn	No
Stinco et al (2003)[31]	26, M	NA	Pre-existing naevus, modification for 3 years	Left scapula	..	NA	NA
Paradisi et al (2006)[32]	36, M	10 years	Prexisting naevus, modification for 1 year	Left scapula	Dark blue	Outdoor recreational and occupational sun exposure, no sunburn	No
Shariff et al (2006)[33]	48, M	30 years	30 years/3 months	Right arm	NA	NA	NA
Singh et al (2007)[34]	56, M	NA	NA/NA, recent change in colour	Right arm	NA	NA	NA
Kluger et al (2008)[35]	70, M	>40 years	NA/NA	Left arm	Black, dark blue	Farmer with chronic sun exposure	Developed a second melanoma on tattoo-free area
Varga et al (2011)[12]	28, M	5 years	4 years/pre-existing naevus, modification for 1 year after a trauma	Right arm	Black	NA	No

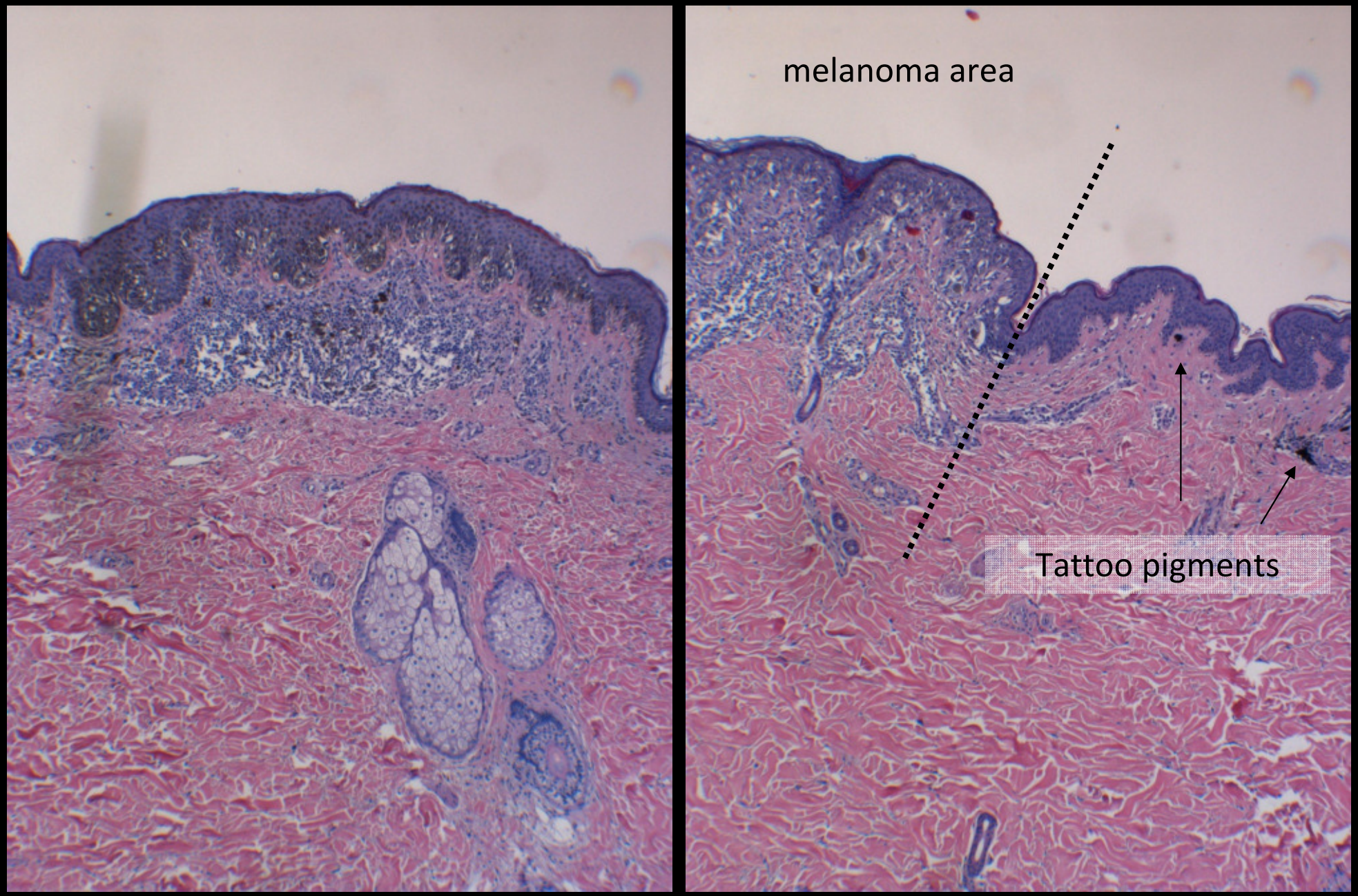
Kaskel et al (2000)[13] also mentioned a case of melanoma on the arm over a 10-year-old tattoo without other detail. M=male. NA=data not available specifically. F=female. *Delay of onset after tattooing=time span between tattoo application and occurence of skin tumour in the tattoo. Delay prior to diagnosis=time span between first noted occurence of skin lesion in the tattoo by the patient and the medical consultation that confirmed diagnosis. †Double publication of the same case.

Table 1: **Main characteristics of reported cases of melanoma arising in tattoos**

Vraga E, et al. J Cutan Pathol 2011

Collection Pr L Thomas, Lyon
Kluger N et al. Dermatology 2008

Kluger N, et al. Acta Dermato Venereol 2013, under press



Superficial spreading melanoma

Superficial spreading melanoma + nodular evolution

Kluger N, et al. Acta Dermato Venereol 2013, under press

Basal - cell carcinoma

	Age (years) at diagnosis and sex	Duration of tattoo at presentation	Delay of onset after tattooing/prior to diagnosis*	Tattoo location	Colour within affected area	Chronic sun exposure	Other skin cancer
Bashir (1976)[36]	60, F	20 years	17 years/3 years	Temple (therapeutic tattoo)†	Dark pigment (carbon)	NA, but most likely (southern Iraq)	NA
Bashir (1976)[36]	52, M	15 years	11 years/4 years	Temple (therapeutic tattoo)†	Dark pigment (carbon)	NA, but most likely (southern Iraq)	NA
Earley (1983)[37]	76, M	57·5 years	55 years/2·5 years	Shoulder	Red	NA	NA
Earley (1983)[37]	64, M	40 years	40 years/NA	Hand	Dark black pigment	NA, most likely	NA
Wiener and Scher (1987)[38]	64, M	46 years	NA/NA	Left arm	Blue-green	Yes (merchant seaman), but no other lesion related to sun exposure	No
Doumat et al (2004)[39]	35, M	1 year	1 year/NA	Left scapula	NA	No (applied sunblock regularly)	No
Birnie et al (2006)[40]	28, F	6 years	6 years/NA	Central back	Black	No	No
Kluger et al (2008)[35]	40, M	7 years	2–3 years/4–5 years	Shoulder and back	Blue	Yes	Several BCCs and one melanoma
Lee et al (2009)[41]	60, F	5 years	3 years/2 years	Eyebrow (cosmetic tattoo)	Black	NA‡	No
Omidian and Emad-Mostofi (2009)[42]	64, F	30 years	29 years/14 months	Upper lip§	Blue-black	NA‡	No
Omidian and Emad-Mostofi (2009)[42]	72, F	NA	NA/1 year	Upper lip§	Blue-black	NA‡	No

F=female. NA=data not available specifically. M=male. BCC=basel-cell carcinoma. *Delay of onset after tattooing=time span between tattoo application and occurence of skin tumour in the tattoo. Delay prior to diagnosis=time span between first noted occurence of skin lesion in the tattoo by the patient and the medical consultation that confirmed diagnosis. †Applied as part of traditional medicine for treatment of headache. ‡Chronic sun exposure not mentioned, but most likely according to the precise location of tattoos. §Traditional religious Iranian tattoos.

Table 2: **Main characteristics of reported cases of basal-cell carcinomas arising in tattoos**

Kluger N, et al. Lancet Oncol 2012;13:e161-168

Birnie AJ, et al. Clin Exp Dermatol 2006; 31: 820–21.
Lee JS, et al. Ann Dermatol 2009; 21: 281–84.
Omidian M, et al. Arch Iranian Med 2009; 12: 198.

	Age (years) at diagnosis and sex	Duration of tattoo at presentation	Delay of onset after tattooing/prior to diagnosis*	Diagnosis (KA or SCC)	Tattoo location	Colour within affected area	Chronic sun exposure	Other skin cancer
MacQuarrie (1966)[63]	NA	21 years	NA	SCC	Breast	Red	NA	NA
Cipollaro (1973)[63]	24, M	6 months	4 months/2 months	KA†	Arm	Red	NA	NA
Goldstein (1979)[64]	NA	NA	NA	KA	NA	Red	NA	NA
Goldstein (1979)[64]	NA	NA	NA	KA	NA	Red	NA	NA
Pitarch et al (2007)[44]	35, M	10 years	10 years/4 months	Well differentiated SCC	Right arm	Black	NA	No
Pitarch et al (2007)[44]	30, F	10 months	NA/NA ("recent") NA/NA	Well differentiated SCC KA	NA	Black Red and black	NA	No
Kleinerman et al (2007)[65]	43, F	9 years	9 years/1 month	KA	Right calf	Red	NA, African American origin	No
Chorny et al (2007)[67]	56, M	3 weeks	3 weeks/NA, patient consult occurred soon after	Eruptive KA (x4)	Left forearm	Red, black, and yellow	NA	2 BCCs
Tan-billet et al (2007)[45]	51, M	4 weeks	4 weeks/NA	SCC with verrucous features	Right forearm	NA	Yes, several actinic keratoses	No
Kluger et al (2008)[66]	41, F	1·5 months	6 weeks/1 month	KA	Scapula	Red	NA	NA
Goldenberg et al (2008)[68]	38, M	1 months	1 month/NA	Eruptive KA (x5)	Left forearm	NA, many colours	NA	No
Ortiz and Yamauchi (2009)[46]	47, F	7–10 days	7–10 days/NA	SCC (KA-like feature)	Upper lip (cosmetic tattoo)	Red (permanent make-up ink)	No	No
Fraga and Prossick (2010)[70]	54, M 55 56	NA	NA/4 weeks NA/NA	KA KA KA (x2)	Right leg Arm Right shin	Red and yellow Red Red	NA, but most likely	Actinic keratoses and melanoma in situ of the scalp
Fraga and Prossick (2010)[70]	62, F	NA	NA	KA	Ankle	Red	NA	NA
Fraga and Prossick (2010)[70]	66, M	NA	NA	KA	Leg	Red	NA	NA
Fraga and Prossick (2010)[70]	36, F	NA	NA	KA	Ankle	Red	NA	NA
Fraga and Prossick (2010)[70]	53, M	NA	NA	KA and SCC	Arm	Red	NA	NA
Fraga and Prossick (2010)[70]	52, M	NA	NA	KA	Arm	Red	NA	NA
Fraga and Prossick (2010)[70]	50, M	NA	NA	KA	Arm	Red	NA	NA
Fraga and Prossick (2010)[70]	49, M	NA	NA	KA	Arm	Red	NA	NA
Vitiello et al (2010)[48]	39, M	3 months	1 month/2 months	Eruptive KA (x8)	Left leg	Red	NA, African American origin	NA
Gon Ados et al (2009)[69]	60, F	4 month	3 months/1 month	KA	Right leg	Red	NA	NA
Sarma et al (2010)[47]	79, M	>50 years	NA/NA	Poorly differentiated SCC	Left forearm	Black	Yes, homeless and sun-damaged dermis	No

Keratoacanthoma(s)

Chorny JA et al. Arch Dermatol 2007

Pseudo-epitheliomatous hyperplasia

Kluger N et al. Am J Clin Dermatol 2008

Keratacanthomas

Vitielo M, et al. J Clin Aesth Dermatol 2010

Squamous cell carcinoma

Pitarch G et al. JAAD 2007

Pseudo-epitheliomatous hyperplasia

Dr R. Fournier, Nïmes

Issues with keratoacanthoma, pseudoepitheliomatous hyperplasia and squamous cell carcinoma within tattoos: a clinical point of view

Journal of
Cutaneous Pathology

- Distinguishing PH, KA and SCC can be challenging
  - KA is nowdays considered as a variant of SCC
  - Full-thickness biopsies and/or surgical removal of the entire lesion + thorough histological examination
  - Follow-up of the patient in case of KA or SCC on tattoo
- PH and KA occur rapidly after the procedure (< 1 year) ++
- SCC occur usually within years after ++

- PH and KA *versus* SCC on tattoos are ”different” physiopathologic processes ?

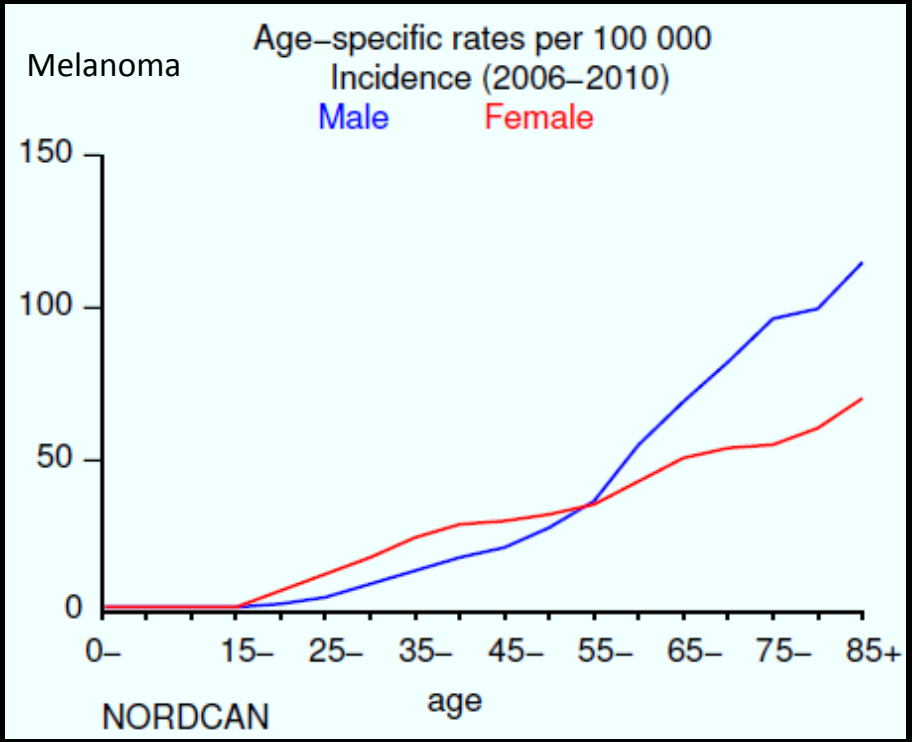
Kluger N. J Cutan Pathol 2010;37:812-3.

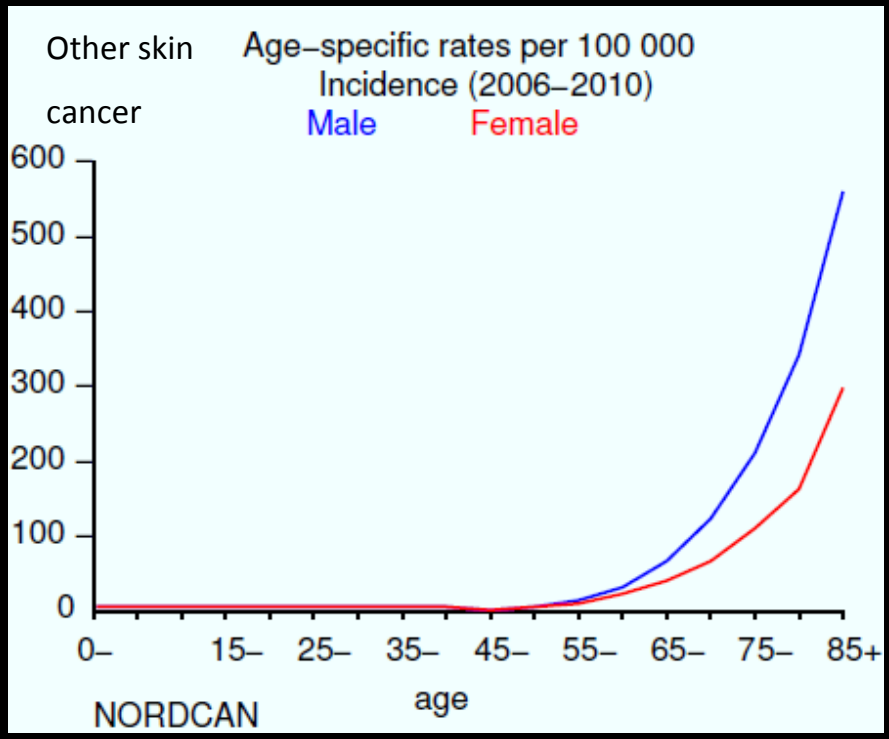
Pros	Cons
Trauma induced by the procedure	
KA and PH +++, CBC, CSC	Debatted for MM
Inflammation	
Acute inflammation	Chronic ? (macrophages)
UV exposure	
Photo-reaction in 21%	Sunscreen use in 76%
Genetic background	
Tattoo inks	
In vitro data	In vivo data ?

Kluger N, et al. Dermatology. 2008;217:219-21; Hutton Carlsen K, Serup J. J Eur Acad Dermatol Venereol. 2013. doi: 10.1111/jdv.12093.

Increase incidence of skin cancers

- The probability of having a fortuitous lesion increase with the tattooed surface





Kluger N, et al. Dermatology. 2008;217:219-21; NORDCAN (association of Nordic Cander Registries)

Keratoacanthoma / PH	Squamous cell carcinoma
Red +++	Red
Occurs within days to a year	Occurs within several years
Rapid growth	Slow growth
Trauma +++ Inflammation +++ Tattoo inks	Tattoo inks Other factors (UV...)

Basal cell carcinoma	Melanoma
Dark color	
Occurs within several years	
Slow growth	
Lack of detection, delay in diagnosis, UV, trauma (?)...	

2 lesions at 2 different sites on the same tattoo :

1) PH and 2) KA SCC

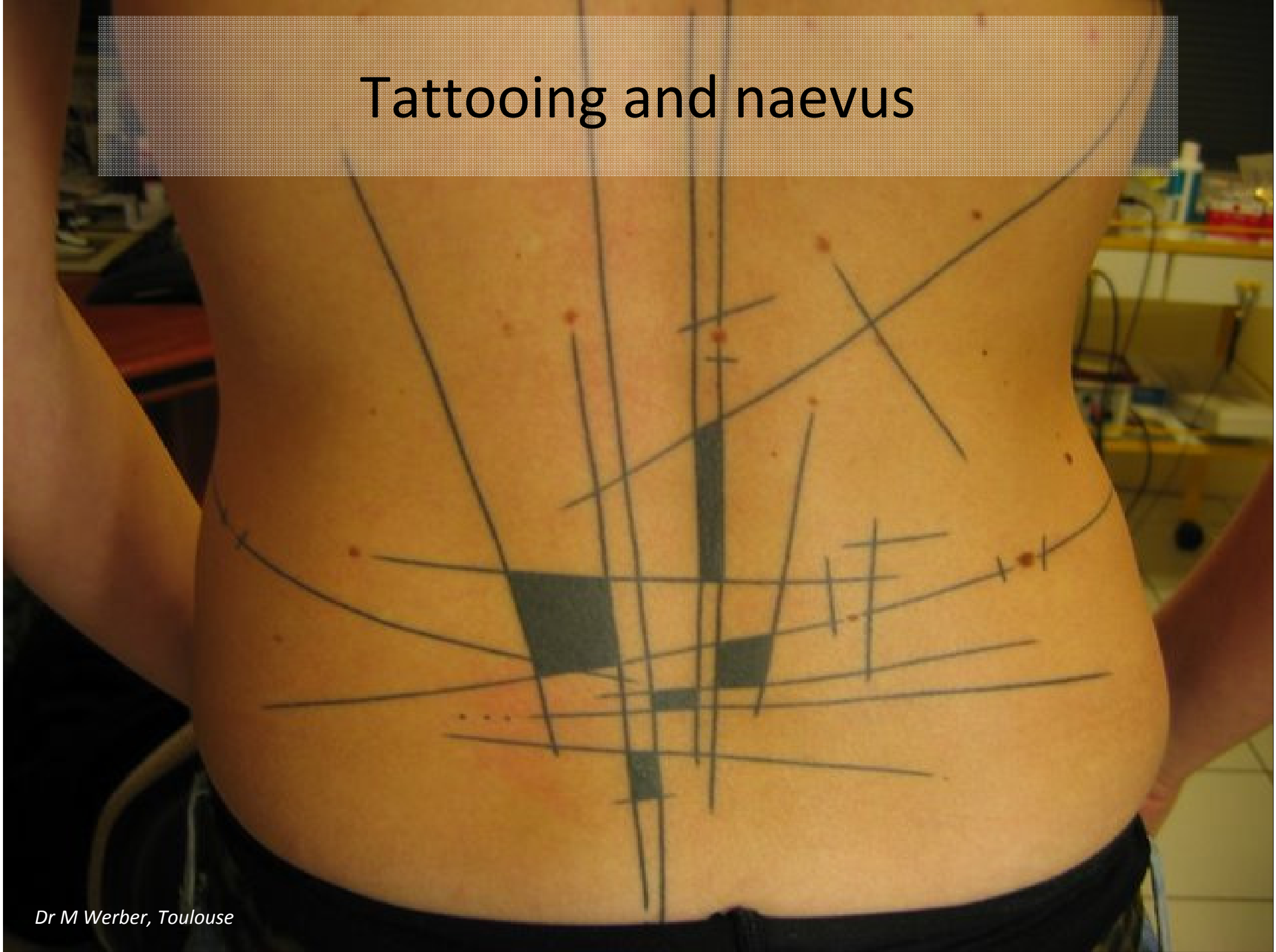
2 lesions at 2 different sites on the same tattoo :

1) KA and 2) SCC

No case of ”double” melanoma on one tattoo so far

Ortiz A, et al. J Am Acad Dermatol 2009;.60:1074-1075

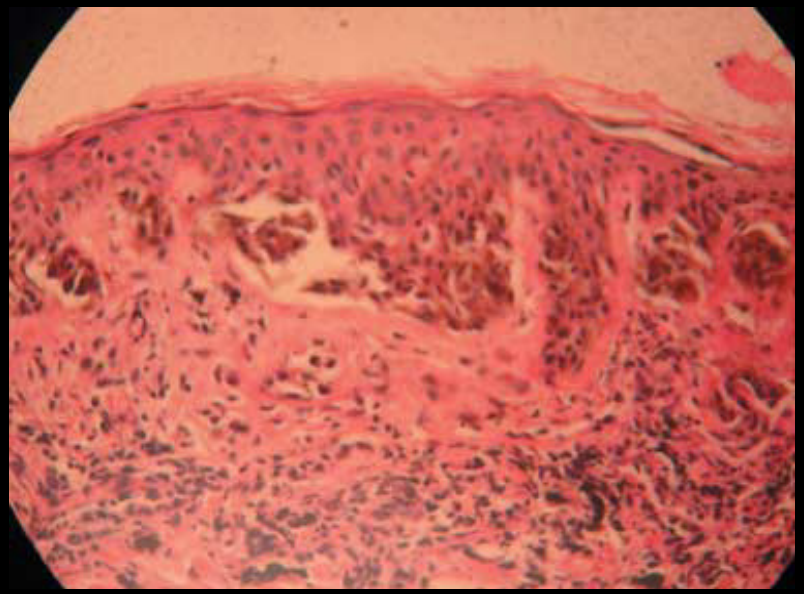
Tattooing and naevus



Dr M Werber, Toulouse

Naevus trauma during tattooing

- Melanocytic nevus is the most common skin lesion
- In case of trauma, it can display worrisome clinical appearance that prompts the realization of a full-excision of the lesion



Kluger N, et al Naevus and tattooing: a matter of concern. J Eur Acad Dermatol Venereol. 2008;22:767-8.


Vida Loca

Dr S Reberga, Toulouse

Dr G Colonna, Porto Vecchio

Differential diagnosis:
Seborrheic keratosis

Kluger N, et al. Nouvelles Dermatologiques 2010; 29:3-4

Dr G Colonna, Porto Vecchio

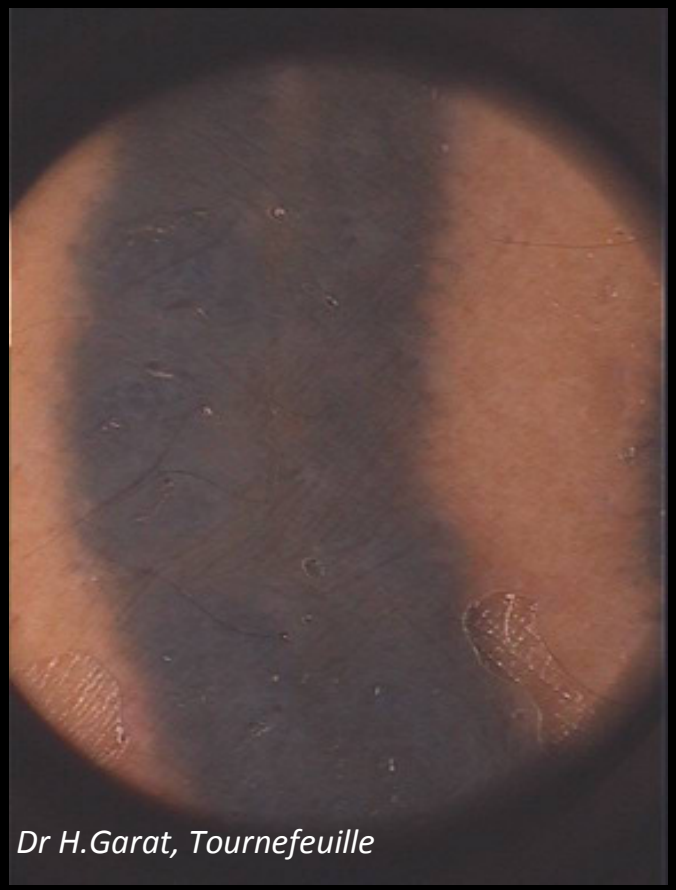
Follow-up

- Atypical mole syndrome
- Personnal history of melanoma
- Difficult follow-up

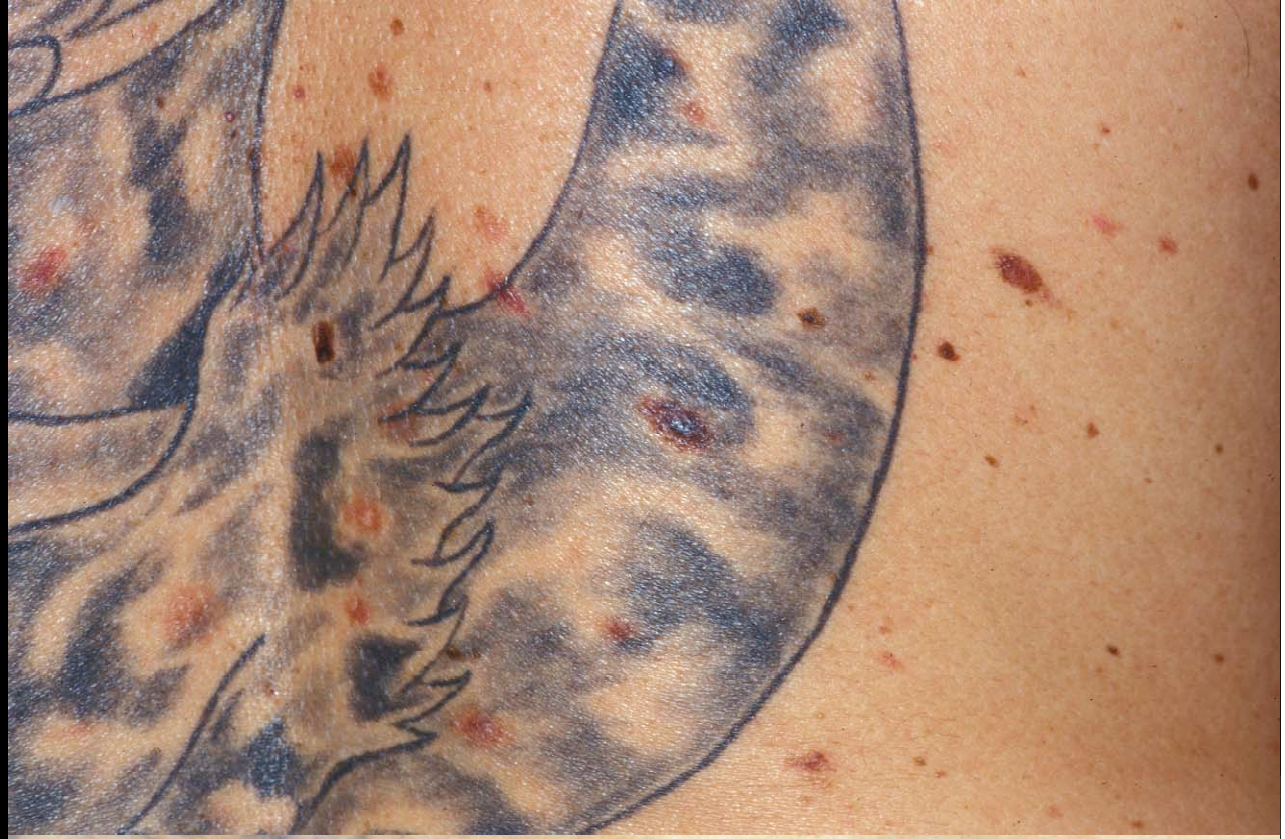
*Kluger N, Thomas L. Arch Dermatol 2008**Dermatol**. 2008;144:948-9.*
Gall N, et al. J Dtsch Dermatol Ges. 2007;5:1120-1.

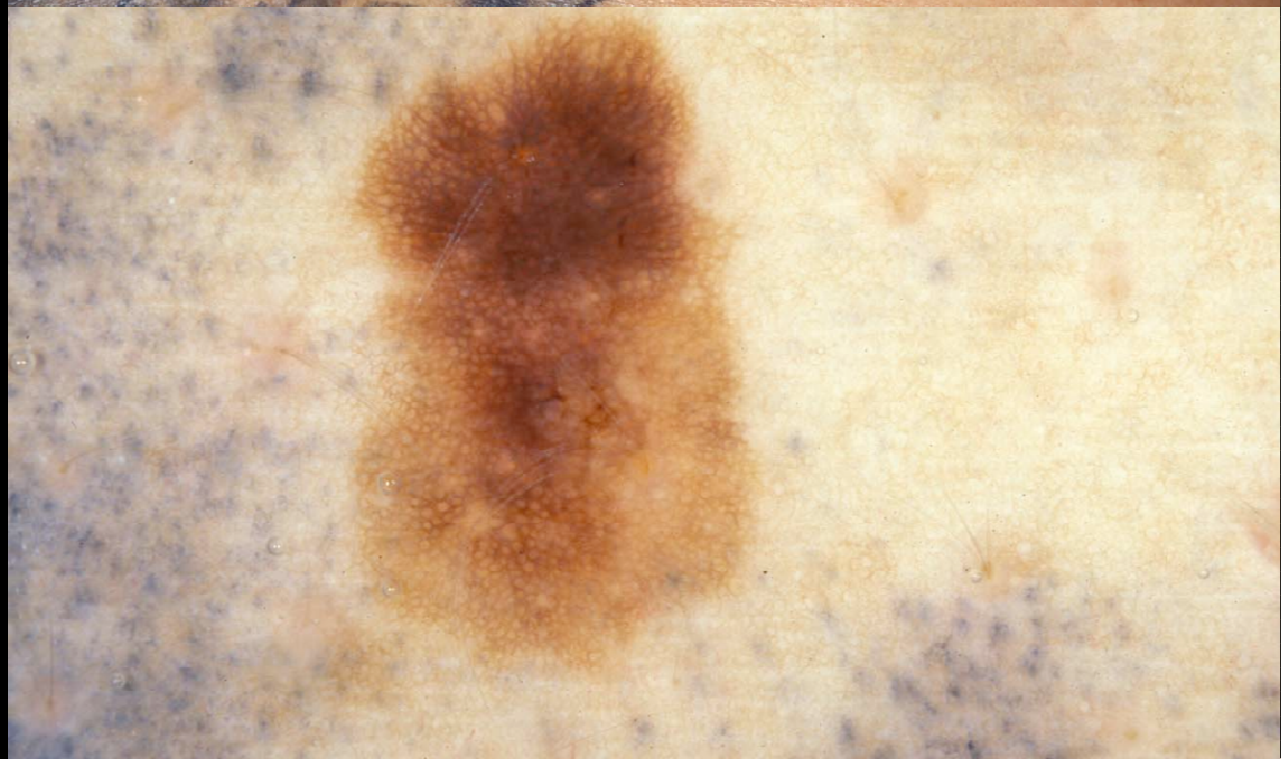
Follow-up

- Dermoscopy (!?!)
- Dark blue globules



Dr H.Garat, Tournefeuille





Kluger N, et al. Arch Dermatol 2008

Naevus: what to do

- Personnal history of melanoma
  - No tattoo

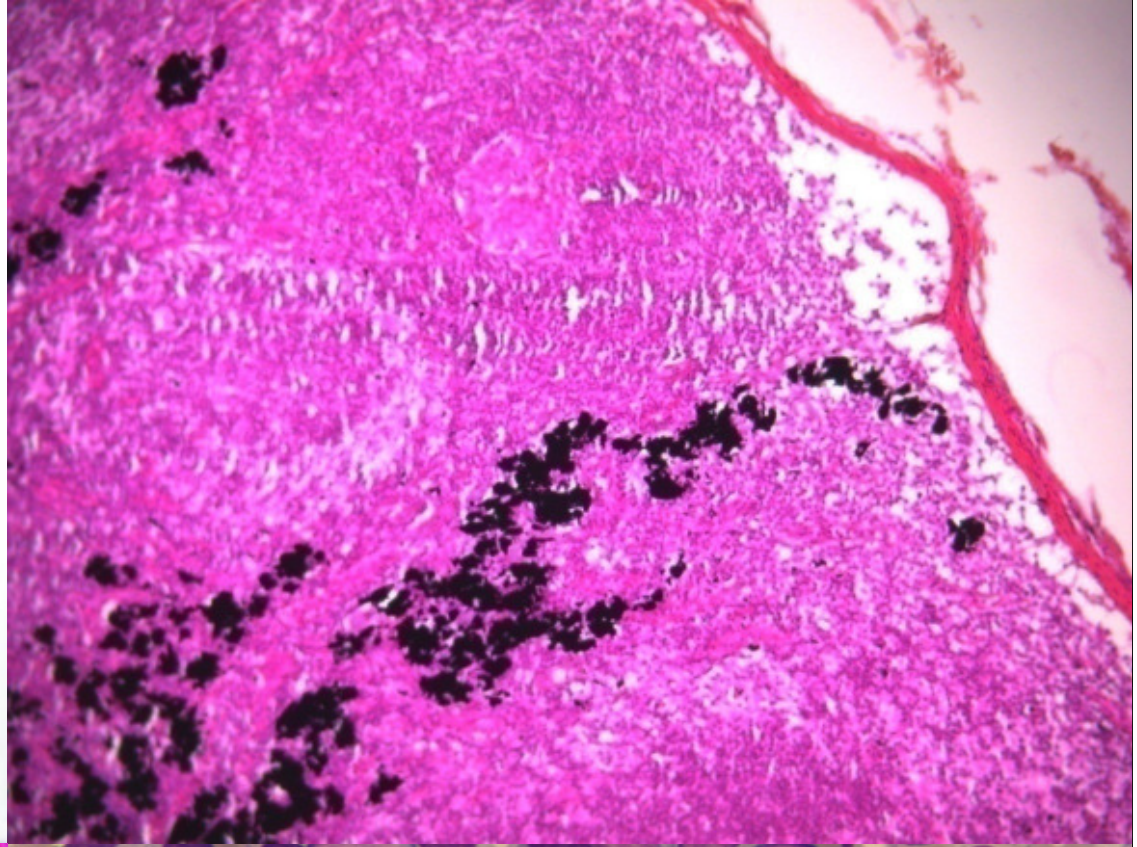

Dr F. Trouche, Rodez

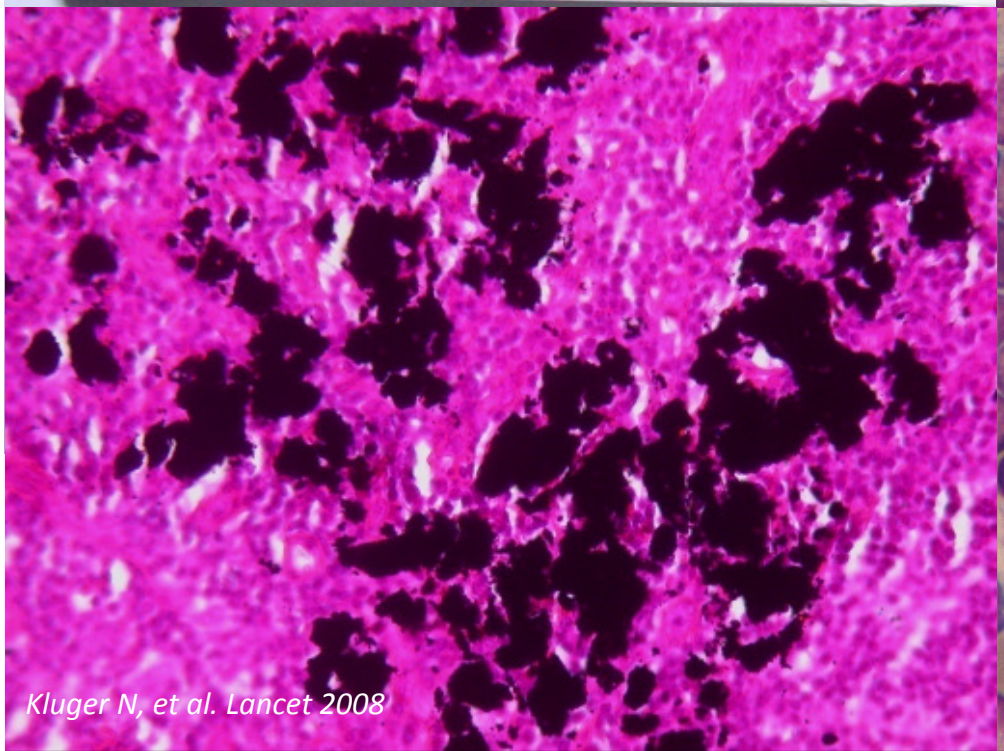
- Patients with familial history of melanoma, atypical mole syndrome or numerous naevi
  - Wait to be aged 20 – 25 years (all the naevi have appeared)
  - Avoid areas full of naevi
  - Choose a « clear » design, small size, light colors
  - Tattooist should avoid tattooing on naevi

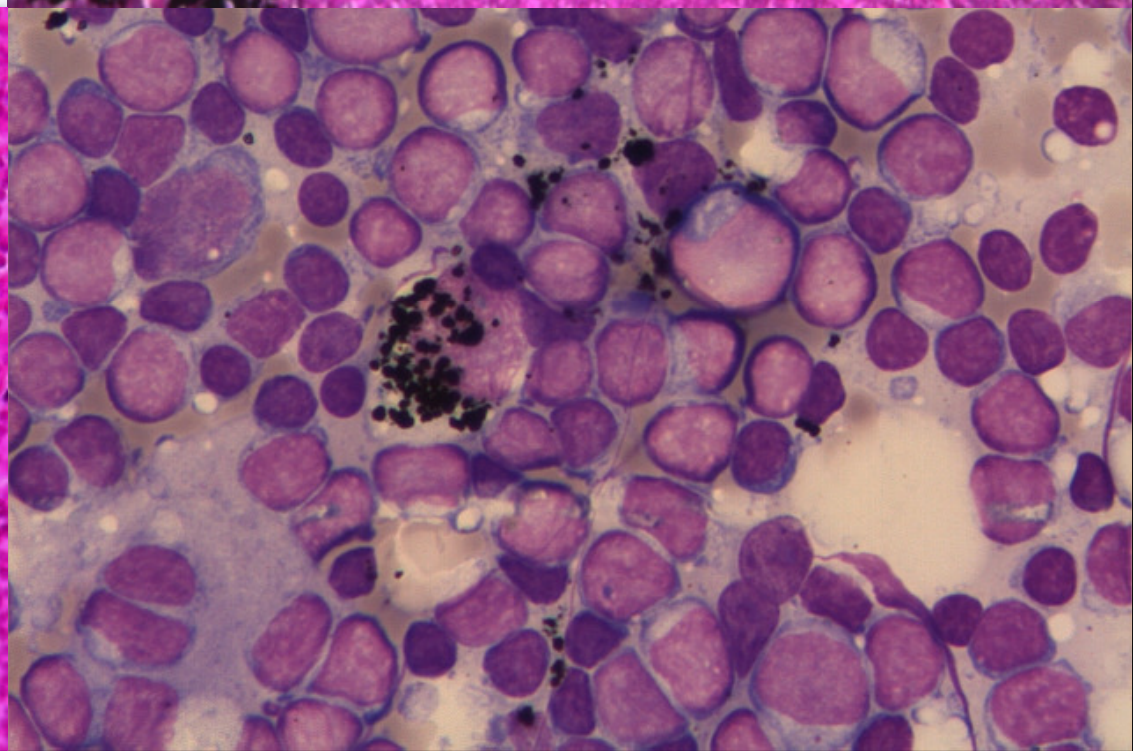
Kluger N, Thomas L. The dragon with atypical mole syndrome. Arch Dermatol 2008

Dr V Blatière, Montpellier

Tattoo inks and systemic risk of cancer







Kluger N, et al. Lancet 2008

Consequences ?

- Do pigments within the lymph node may disturbe the anatomo-pathologist ?
  - Stainings (Fontana, iron...)
  - By masking tumoral cells ?
- What are the long term consequences of pigments in the lymph nodes ?
  - Alteration of immune defenses ?
  - Chronic overstimulation ?
  - ...

Nam H el al. A pitfall of 18-fluorodeoxyglucose-PET in a patient with a tattoo. Lancet Oncol 2007;8:1147-48

Metallic salts chronic intoxication ?

- To date, no *convicing* case report suggests so
  - One case report rather ”debattable”
- The concentration and exposure remain low
- Even if metallic salts are introduced in the dermis, there is no proof of they are released in the circulation
- If so, the exposure will be different to ”conventionnal” chronic exposure

- Toxicological studies in heavily tattooed individuals are waranted +++

Fircanis S, et al. The girl with the iron tattoo. Virulence. 2012 Oct 17;3(7).

CLINICAL AND LABORATORY INVESTIGATIONS

BJD
British Journal of Dermatology

Tattoo inks in general usage contain nanoparticles

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None.

Conflicts of interest
None declared.

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Summary

Background To our knowledge tattooing has never been thought of as a method of introducing nanoparticles (NPs) into the human body by the intradermal route, and as such it has never been a topic of research in nanotoxicology. The content of NPs in tattoo inks is unknown.

Objectives To classify the particle sizes in tattoo inks in general usage.

Methods The particle size was measured by laser diffraction, electron microscopy and X-ray diffraction.

Results The size of the pigments could be divided into three main classes. The black pigments were the smallest, the white pigments the largest and the coloured pigments had a size in between the two. The vast majority of the tested tattoo inks contained significant amounts of NPs except for the white pigments. The black pigments were almost pure NPs, i.e. particles with at least one dimension < 100 nm.

Conclusions The finding of NPs in tattoo inks in general usage is new and may contribute to the understanding of tattoo ink kinetics. How the body responds to NP tattoo pigments should be examined further.

A critical view on the risk assessment by toxicologists

- **Lots of pigments have other uses than tattooing**
- **None have been approved so far by the FDA for tattooing**
- **Resolution ResAP(2008)1**

  Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up (superseding Resolution ResAP(2003)2 on tattoos and permanent make-up)

- **No regulation in Europe**
  - Starting (Switzerland, France…)


Ingredients
C.i. 77491
Glycerin; distilled water;
Isopropanol
INTENZE
Deep Indigo
Dark Salmon
Dark Green
Dark Chocolate
Dark Brown
Creamsicle
advanced tattoo ink formula
Disclaimer


Waverly
COLOR COMPANY
Magenta
Waverly
COLOR COMPANY
Med. Blue

Table 1. Percentage of Elemental Composition of Tattoo Pigments Obtained by X-ray Diffraction*

Stock No.	Color	Element† Titanium (22)	Aluminum (13)	Silica (14)	Copper (29)	Chromium (24)	Iron (26)	Chlorine (17)	Sulfur (16)	Carbon (6)	Oxygen (8)	Magnesium (12)
8001	Black	...	0.25	...	...	...	85.95	...	0.29	...	13.51	...
8007	White	98.55	1.45	...	...	...	...	...	...	...	...	...
8016	Fire red	...	26.21	...	...	...	...	9.68	10.76	23.29	30.06	...
8022	Flesh No.1 (tan)	74.27	1.75	...	...	...	23.98	...	...	...	...	...
8031	Indian brown	41.98	0.76	...	...	...	57.26	...	...	...	...	...
9001	Crimson red	...	...	17.2	...	...	...	...	...	49.36	22.25	11.19
9002	Devil's red	27.65	2.6	0.53	...	...	...	...	...	51.67	17.55	...
9008	Lotus (red)	40.17	3.2	...	...	...	...	...	...	17.71	38.92	...
9009	Venetian brown	...	0.38	1.73	...	...	79.85	...	...	...	18.04	...
9014	Florida orange	...	...	...	...	...	...	...	...	84.35	15.65	...
9017	Lemon yellow	53.06	1.86	...	...	...	...	...	...	23.9	21.18	...
9022	White	96.41	3.59	...	...	...	...	...	...	...	...	...
9023	Black	...	...	...	...	...	87.98	...	...	...	12.02	...
9024	Permanent green	...	0.89	...	13.32	...	...	51.55	...	34.24	...	...
9025	Emerald green	5.45	0.49	...	...	72.66	...	4.15	...	...	17.25	...
9026	Pine green	44.34	4.08	...	8.27	...	...	6.71	...	14.64	21.96	...
9029	Parrot green	58.57	2.51	...	...	...	...	4.61	...	17.12	17.19	...
9036	Sky blue	37.95	1.9	...	11.06	...	...	2.16	...	35.41	11.52	...
9061	Blue green	...	1.14	...	14.46	...	...	52.24	...	32.16	...	...
9090	Cerise (red)	51.51	2.18	...	...	...	15.34	...	...	9.81	21.16	...
9091	Yukon white	94.98	5.02	...	...	...	...	...	...	...	...	...
9092	Misty green	51.5	2.51	...	...	...	...	3.59	...	18.43	23.97	...
9093	Misty blue	94.82	4.13	...	...	...	...	1.05	...	...	...	...
9094	Tulip yellow	27.29	2.29	0.22	...	...	...	2.29	0.3	37.3	30.31	...
9095	Peony (pink)	63.77	2.38	...	...	...	...	...	...	13.85	20	...
9096	New blue	50.93	2.36	...	8.54	...	...	1.39	...	18.21	18.57	...
9097	Blush (orange)	58.06	2.25	...	...	...	...	2.87	...	14.39	22.43	...
9098	Wild violet	65.29	2.88	...	...	...	...	...	...	9.56	22.27	...
9099	Tulip red	...	0.19	13.4	...	...	...	...	...	52.29	25.12	9
Not applicable	India ink	...	...	...	...	...	...	...	...	92.19	7.81	...

*The numeric values in the table represent the elemental percentage in the composition of the pigment. Ellipses indicate absence.
†The parenthical number below the element represents its atomic number.

Timko AL et al. Arch Dermatol 2001;137:143-147; Clarke Beute T, et al. Dermatol Surg 2008;34:508-516

Tabelle 1: Elementanalytische Zusammensetzung nativer Tattoofarbstoffe. Zusammenfassung der EDS-Analysen verschiedener Farbtonproben.

Table 1: Elemental composition of tattoo dyes. Summary of results obtained by EDS analysis of different colors.

Schwarz	(hauptsächlich C), P und S, wenig Si und Al
Violett	Al, P, S, Cl, Ti, Cr
Grün	Al, P, Cl, Ti, Cu, einige Töne auch Cu-frei
Rot	Al, Si, S, Cl
Gelb	Si, Al, Ti, S
Blau	Na, Al, Si, P, S, Cl, Ti, Cu, K, Ca

Schmitz I, et al. JDDG 2004;2:350-353

Market survey on toxic metals contained in tattoo inks

Giovanni Forte [a,*], Francesco Petrucci [a], Antonio Cristaudo [b], Beatrice Bocca [a]

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[b] Istituto Dermatologico S. Gallicano, Via Elio Chianesi 53, 00144 Rome, Italy

- 56 tested inks (4 brands), spectrometry
  - Aluminium, baryum, cooper, iron, strontium +++
- Allergenic metallic salts
  - Chromium (35/56), Nickel (9/56) and Cobalt (1/56)
- Toxic metallic salts
  - Cadmium, manganese, lead, antimony
  - But no mercury…

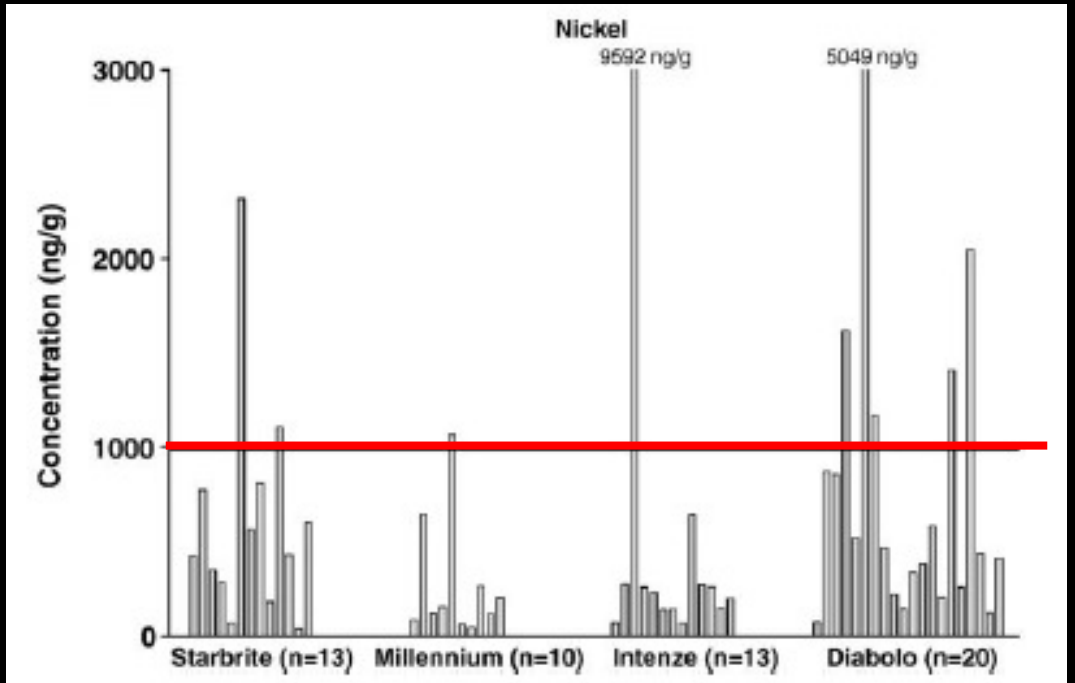


Fig. 1. Tattoo ink concentration of Cr and Ni compared to the safe allergological limit of 1 ppm (horizontal line).

Forte G, et al.. Sci Total Environ. 2009 407:5997-6002.

2 caps of black
1 cap of red
1 cap of grey
1 cap of white
1 cap = 0,3 to 2,8 ml

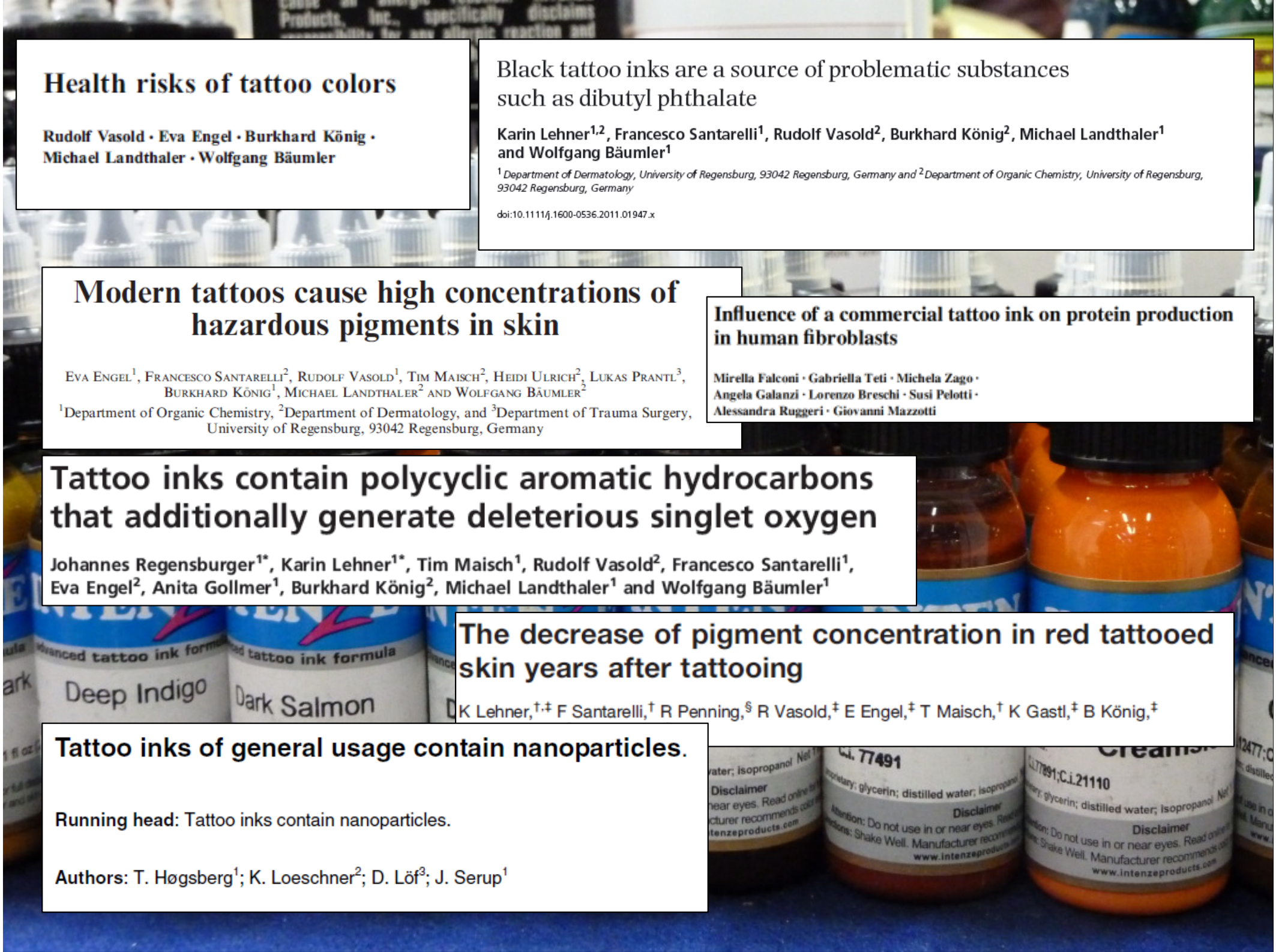
[Pb] = 31 to 2127 ppm = 31 to 2127 pg/mg
[As] = 18 to 8977 ppm = 18 to 8977 pg/mg

Ink in a 16 ml/cap weights approx 0,20 g
(HelsInki, 02.2013)

	1 Cap 16 mL	
Pb	6200 pg	0,0062 µg
Pb	425 400 pg	0,4254 µg
As	3600 pg	0,0036 µg
As	1 795 400 pg	1,795 µg

[Pb] tap water = 10-50 µg/1000mL

Unpublished data



Health risks of tattoo colors

Rudolf Vasold · Eva Engel · Burkhard König · Michael Landthaler · Wolfgang Bäumler

Black tattoo inks are a source of problematic substances such as dibutyl phthalate

Karin Lehner[1,2], Francesco Santarelli[1], Rudolf Vasold[2], Burkhard König[2], Michael Landthaler[1] and Wolfgang Bäumler[1]

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doi:10.1111/j.1600-0536.2011.01947.x

Modern tattoos cause high concentrations of hazardous pigments in skin

Eva Engel[1], Francesco Santarelli[2], Rudolf Vasold[1], Tim Maisch[2], Heidi Ulrich[2], Lukas Prantl[3], Burkhard König[1], Michael Landthaler[2] and Wolfgang Bäumler[2]

[1]Department of Organic Chemistry, [2]Department of Dermatology, and [3]Department of Trauma Surgery, University of Regensburg, 93042 Regensburg, Germany

Influence of a commercial tattoo ink on protein production in human fibroblasts

Mirella Falconi · Gabriella Teti · Michela Zago · Angela Galanzi · Lorenzo Breschi · Susi Pelotti · Alessandra Ruggeri · Giovanni Mazzotti

Tattoo inks contain polycyclic aromatic hydrocarbons that additionally generate deleterious singlet oxygen

Johannes Regensburger[1*], Karin Lehner[1*], Tim Maisch[1], Rudolf Vasold[2], Francesco Santarelli[1], Eva Engel[2], Anita Gollmer[1], Burkhard König[2], Michael Landthaler[1] and Wolfgang Bäumler[1]

The decrease of pigment concentration in red tattooed skin years after tattooing

K Lehner,[†,‡] F Santarelli,[†] R Penning,[§] R Vasold,[‡] E Engel,[‡] T Maisch,[†] K Gastl,[‡] B König,[‡]

Tattoo inks of general usage contain nanoparticles.

Running head: Tattoo inks contain nanoparticles.

Authors: T. Høgsberg[1]; K. Loeschner[2]; D. Löf[3]; J. Serup[1]

Toxicity ?

- **Photodecomposition** of monoazo compounds (Pigment Red 22 and P.R. 9) induce the production of
  - 2-methyl-5-nitroaniline (2-MNA, hepatic dysfunction, **mutagen** for *salmonella*)
  - 4-nitro-toluene (4-NT, **genotoxic** on lymphocytes)
  - 2,5-dichloroaniline (2,5-DCA, **nephrotoxic** in the rat)
  - 1,4-dichlorobenzene (1,4-DCB, **kidney cancer** in the rat, **liver cancer** in the mouse)

Engel E, et al. Anal. Chem 2006; 78:6440-47

Vasold R, et al. Anal Bioanal Chem. 2008;391:9-13

Photoinstability of tattoo inks after laser

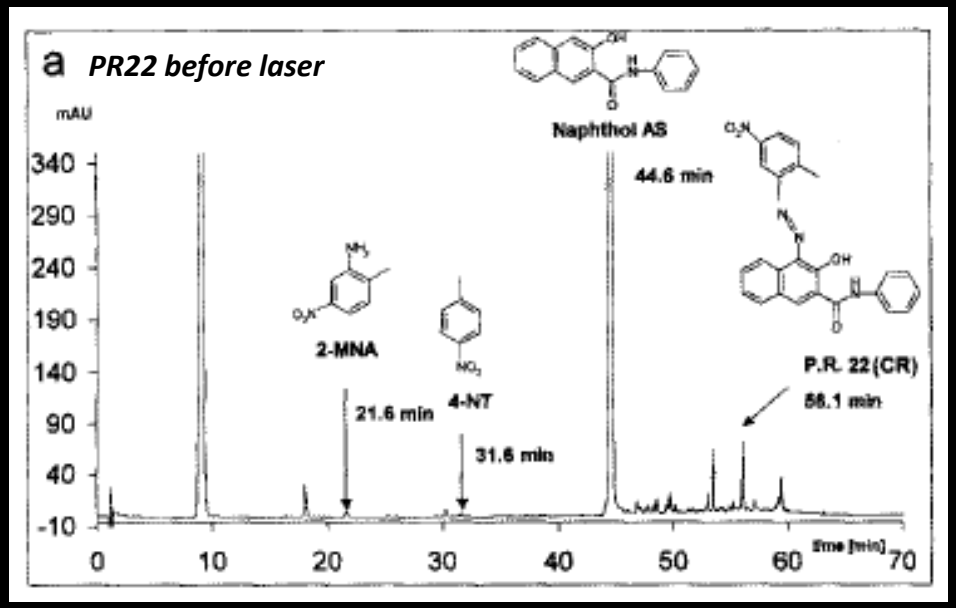
- Irradiation of P.R. 22 and P.R.9 by a Nd:Yag laser
- Increase of byproducts
  - 2-methyl-5-nitroaniline
  - 2-5-dichloraniline
  - 4-nitro-toluene
  - 1,4-dichlorobenzene

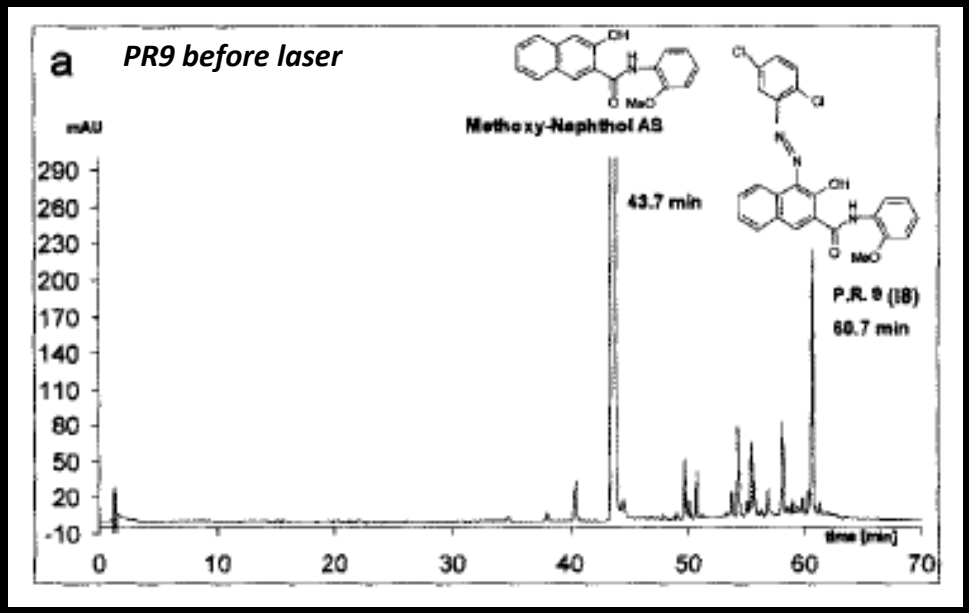
Table 1 Amounts (μg mL^{-1}) of decomposition products before and after laser irradiation of the pigments Cardinal Red (CR) or I8. The products found were 2,5-dichloroaniline (2,5-DCA), 1,4-dichlorobenzene (1,4-DCB), 2-methyl-5-nitroaniline (2-MNA), and 4-nitrotoluene (4-NT) [29]

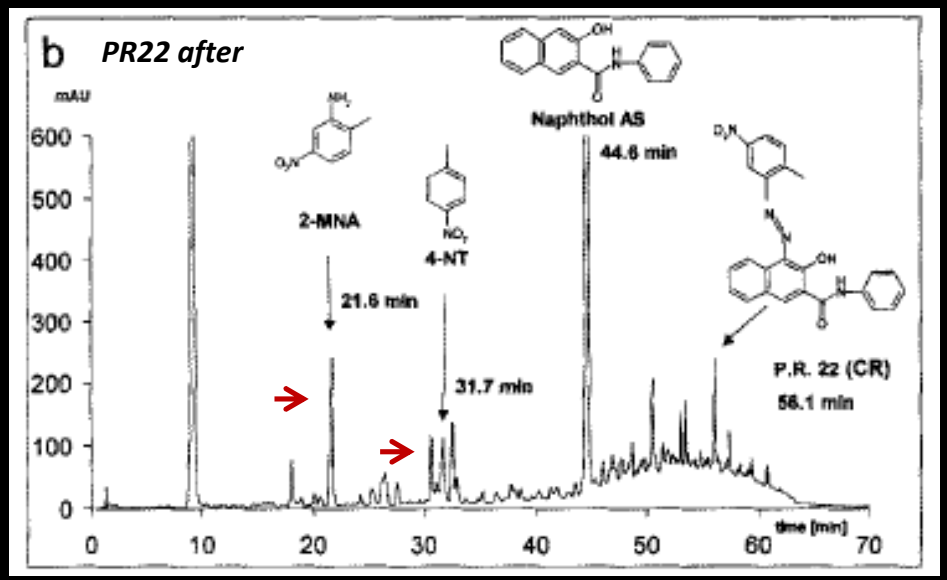
	CR before irradiation	CR after irradiation	I8 before irradiation	I8 after irradiation
μg/ml				
2-MNA	1.6±0.3	53.1±10.1	–	–
4-NT	1.0±0.2	44.7±8.2	–	–
2,5-DCA	–	–	11.8±0.3	79.6±1.4
1,4-DCB	–	–	< 0.5	32.6±0.4

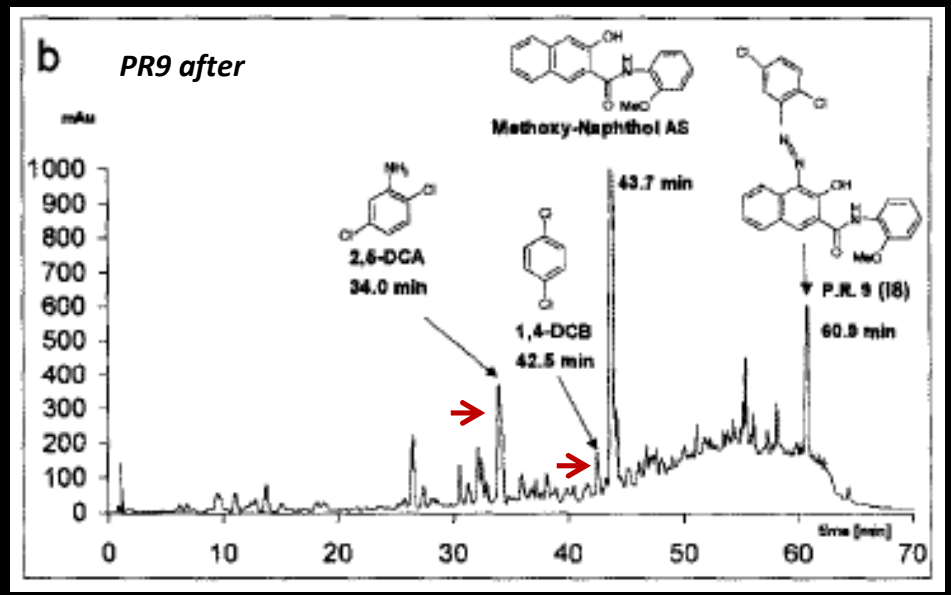
- Products potentially toxic or carcinogenic
- What are the risks in real life after laser removal ?
- UVB and natural light on P.R. 22: detection of naphthol AS

Vasold R et al, Photochem Photobiol 2004;80:185-90
Engel E et al, JGSD 2007;5:583-89









Vasold R et al, Photochem Photobiol 2004;80:185-90

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

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

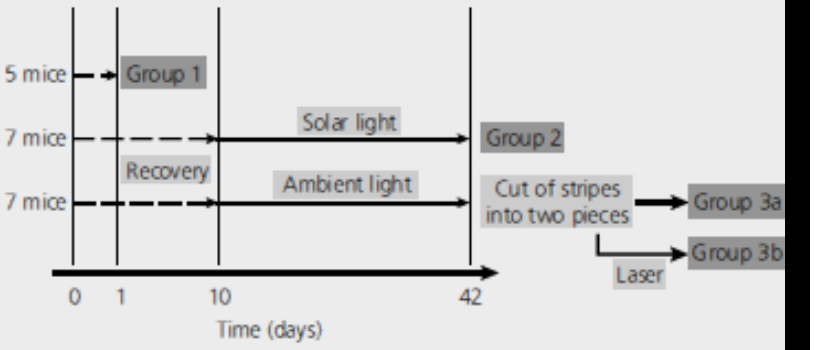
- In vivo study of UV decomposition and transportation of PR22
- 4 groups of mice
  - G1: Sacrificed at day 1
    - Extraction of the pigments
  - G2: Sacrificed at day 42
    - At D10, solar radiation 32 days
    - Total 44,8 SED (Standard erythemal dose)
    - Extraction of the pigments
  - G3a & G3b: Sacrificed at day 42
    - At D10, normal light exposure 32 days
    - G3a : Extraction of the pigments at D42
    - G3b : irradiation Nd:YAG 532 nm, 2,5J/cm²/pulse, total : 165 J/cm²
    - Extraction of the pigments

Figure 2. Mice were tattooed with highly pure synthesized PR 22 (25% w/v) as shown by the four single pass tattoo 'stripes'(top). PR 22 has been transported to the lymph nodes causing a reddish coloration (bottom 2 panels).

- Day 1
  - 36,5 µg PR22/punch
  - Estimation 584,0 µg PR22/mouse
- Day 42 (group 3a)
  - 24,9 µg PR22/punch
  - Estimation 398,4 µg PR 22/mouse
  - Diminution of 32%
- Day 42 (group 2)
  - 9,9 µg/punch; 158,4 µg/mouse
  - Diminution of 60%
  - No carcinogens (2,5MNA, NT)
- Day 42 + laser (group 3b)
  - Detection of 2,5 MNA, 6-NT, NAS

Table 1. The values show the amount of PR 22 extracted 1 day after tattooing (group 1), 42 days after tattooing and exposure to solar radiation (group 2), or 42 days with room light followed without (group 3a) or with (group 3b) laser light irradiation. The respective standard deviation of the values (SD) is included in the table

Groups (conditions)	Amount per punch[1] (mean) [µg]	Amount per animal (mean) [µg]	Standard deviation (SD) [%]	Loss of pigment [%]
Group 1 (1 day after tattooing)	36.5	584.0	26	
Group 2 (42 days after tattooing + solar radiation)	9.9	158.4	64	Corr. to group 3a 60 $P = 0.002$[2]
Group 3a (42 days after tattooing, ambient light only)	24.9	398.4	24	Corr. to group 1 32 $P = 0.04$[2]
Group 3b (42 days after tattooing + laser)	12.3	196.8	16	Corr. to group 3a 51 $P = 0.002$[2]



[1]Weight per punch: 35 mg.
[2]Students *t*-test, two-sided.

Engel E, et al. Exp Dermatol 2009:19:54-60

- « Natural » diminution of pigment concentration: 32%
  - Elimination through the epidermis (during the healing phase)
  - Transport in the rest of the body
    - Lymph node: yes
    - Further in the body: ?
  - Cutaneous *in situ* decomposition (UV, enzyme degradation) ?
- Diminution after laser: 51%
  - only 8% of PR22 is found as 2,5-MNA and 4-NT
  - Other not extracted/analyzed products ?
- Diminution after UV: 60%
  - No decomposition product
    - *In situ* metabolism?
    - Spreading in the body ?
    - Other photochemical mechanism ?

Table 2. The values of the amount of the laser-induced decomposition products NAS, 2,5-MNA and 4-NT that were detected

Decomposition product	Amount per punch[1] (mean) [μg]	Amount per animal (mean) [μg]	Standard deviation (%)
NAS	0.1	1.6	28
2,5-MNA	0.3	4.8	27
4-NT	0.1	1.6	28

[1]Weight per punch: 35 mg.
The respective standard deviation (SD) of the values is indicated in this table.

Engel E, et al. Exp Dermatol 2009:19:54-60

- To date, no reported case of skin cancer on priorly tattooed skin area which underwent laser removal
  - But, is it easy to make the link ?
  - One case of dermato-fibrosarcoma on a tattoo
  - *”The original tattoo was placed (...) 15 years prior, and was partially removed with QS Nd:Yag 1024 nm 5 years after placement. Another tattoo was placed in the same area 7 years prior to his presentation”*

Reddy KK, et al. J Drugs Dermatol. 2011 Aug;10(8):837-42.

Anal. Chem. **2006,** *78,* 6440−6447

Establishment of an Extraction Method for the Recovery of Tattoo Pigments from Human Skin Using HPLC Diode Array Detector Technology

Eva Engel,[†] Francesco Santarelli,[‡] Rudolf Vasold,[†] Heidi Ulrich,[‡] Tim Maisch,[‡] Burkhard König,[†] Michael Landthaler,[‡] Neera V. Gopee,[§] Paul C. Howard,[§] and Wolfgang Bäumler*[,‡]

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Modern tattoos cause high concentrations of hazardous pigments in skin

Eva Engel[1], Francesco Santarelli[2], Rudolf Vasold[1], Tim Maisch[2], Heidi Ulrich[2], Lukas Prantl[3], Burkhard König[1], Michael Landthaler[2] and Wolfgang Bäumler[2]

[1]Department of Organic Chemistry, [2]Department of Dermatology, and [3]Department of Trauma Surgery, University of Regensburg, 93042 Regensburg, Germany

- Determination of [pigment] in the skin
  - Tattooing of human and pig skins
  - Performed by the researchers and tattoo artists
  - PR22 ink with a purity of 98%
    - On the market, purity < 80%
  - Mean concentration 2,53 mg/cm² (0,6 to 9,42 mg/cm²)
  - Difference between purified and commercial PR 22

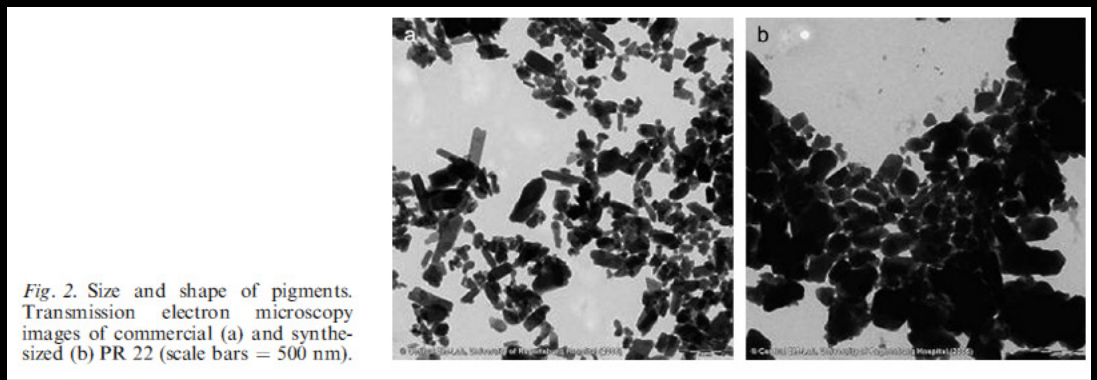
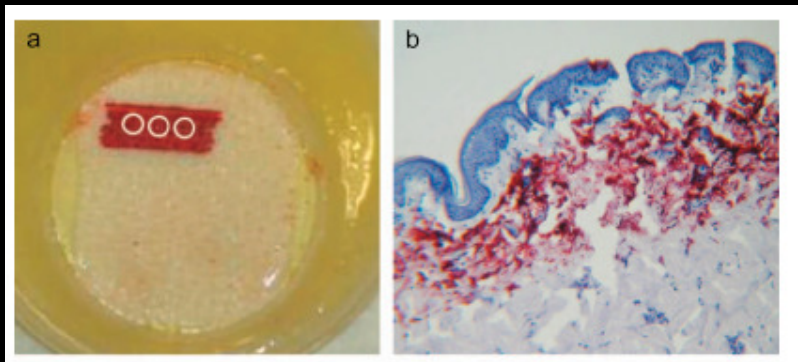


Fig. 2. Size and shape of pigments. Transmission electron microscopy images of commercial (a) and synthesized (b) PR 22 (scale bars = 500 nm).



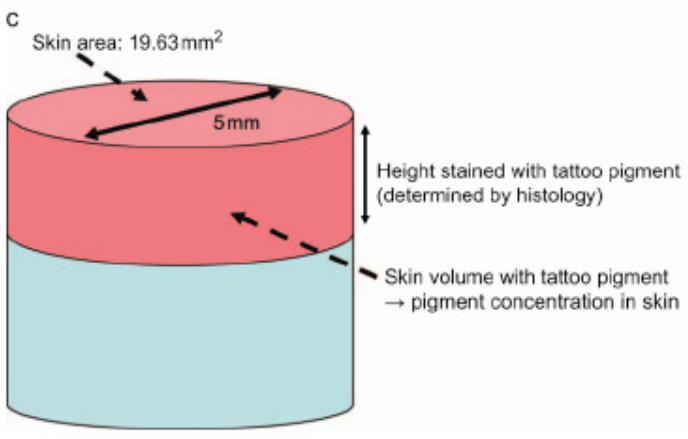


Table 1. Concentrations of pigments in skin[a]

Method	Needle size	Applied concentration (w/v) (%)	Amount per tattooed area (mg/cm^2)	RSD (%)
A	8R	10	0.63	13.5
	8R	25	1.42	7.8
	4R	10	1.75	5.9
	4R	25	5.19	15.8
	8F	10	1.02	30.0
	8F	25	2.60	21.6
	4F	10	2.49	4.9
	4F	25	3.44	13.4
B	8R	10	1.90	32.9
	8R	25	3.59	14.1
	4R	10	2.90	45.3
	4R	25	9.42	11.8
C	9R	25	0.60	14.7
D	8R	25	0.95	23.9
E	8R	25	1.69	7.4
Mean value			2.53	17.9

RSD, relative standard deviation.
[a]The amount of PR 22 deposited in 1 cm^2 pigskin and human skin each. Researchers obtained concentration values in experi-

Method A : purified PR22, pig skin, researchers
Method B : commercial PR22 , pig skin, researchers
Method C : purified PR22, pig skin, tattooists
Method D: commercial PR22, human skin, researchers
Method E: purified PR22, human skin, researchers

« 2,53 mg/cm² » : an overestimation ?

- Limitations
- *In vitro* data ; Pig skin ; study only on PR22
- [PR22] obtained right after tattooing
  - [PR22] after tattoo healing ?
  - Transepidermal elimination ? Lymphatic clearance ?
- No real discussion on the weak concentration obtained by the tattooists (versus the researchers)
  - In vivo, tattooists do it better
  - Unexperienced « trainees » may tattoo more inks than experienced tattooists

Tattoo inks contain polycyclic aromatic hydrocarbons that additionally generate deleterious singlet oxygen

Johannes Regensburger[1*], Karin Lehner[1*], Tim Maisch[1], Rudolf Vasold[2], Francesco Santarelli[1], Eva Engel[2], Anita Gollmer[1], Burkhard König[2], Michael Landthaler[1] and Wolfgang Bäumler[1]

- 19 commercial tattoo inks (HPLC, spectrometry)
  - Quantification of 20 PAH
  - 16 PAH detected
  - 7 PAH found were carcinogen 2B
  - [HAP] mean total max = 201,1 +/- 19,5 µg/g
    - 0,1 µg/g (dibenzo[a,h]anthracene)
    - 24,5 µg/g (phenanthrene)
  - [phenols] max = 385 µg/g

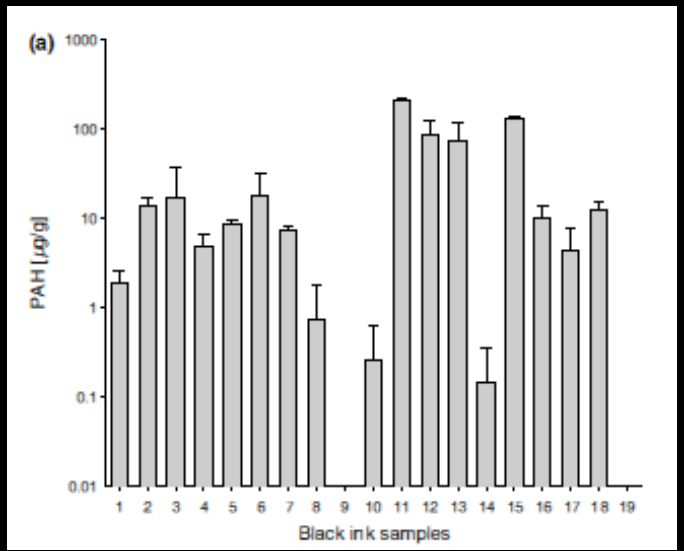
- Estimation for a 400 cm^2 tattoo
  - 2,53 mg/cm^2
  - Injection de 402 µg (PAH), 770 µg (phenol)

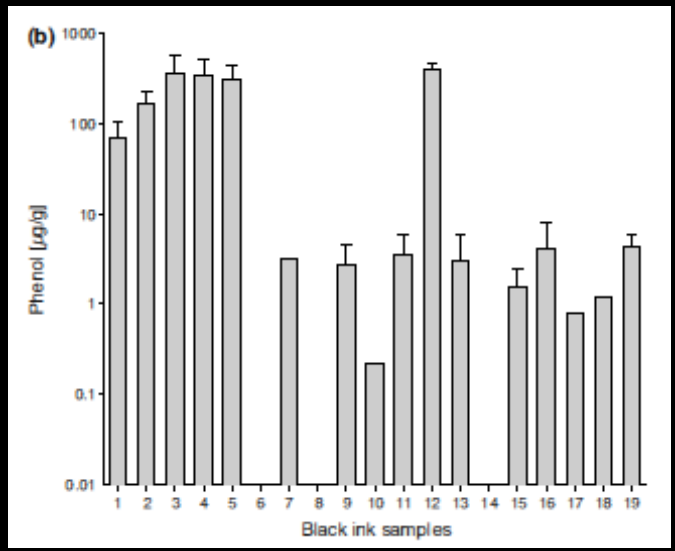
Regensburger J, et al. Exp Dermatol 2010:19:e275-281

Table 1. PAHs found in black tattoo inks

PAHs[1]	Mean value extracted [μg/g]	Mean daily dietary intake (49) [μg/person]	Carcinogenicity USEPA (7)	Toxicity TEF (35)
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	**0.04**	**B2**	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	**0.11**	**B2**	0.01
Benz[a]anthracene (6)	1.6 ± 0.2	**0.05**	**B2**	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	**0.03**	**B2**	0.1
Acenaphthene (8)	0.9 ± 0.3	0.98	-[2]	0.001
Fluorene (6)	0.9 ± 0.2	0.59	D	0.001
Benzo[k]fluoranthene (2)	0.4 ± 0.2	**0.01**	**B2**	0.1
Benzo[a]pyrene (4)	0.3 ± 0.2	**0.04**	**B2**	1.0
Naphthalene (7)	0.3 ± 0.1	-[2]	**C**	0.001
Dibenzo[a,h]anthracene (1)	0.1 ± 0.1	**0**	**B2**	1.0

[1]The number in brackets indicate the total number of inks in which the respective PAH was found.
[2]Data not available.
The bold characters highlight the probable or possible carcinogenic PAHs.





Carcinogen 2B = *possibly* carcinogenic to human

Cell phones

Furan

Caffeic acid

Some approved treaments in medicine,...

Nickel, Cobalt...

http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf

Tattoo inks contain polycyclic aromatic hydrocarbons that additionally generate deleterious singlet oxygen

Johannes Regensburger[1*], Karin Lehner[1*], Tim Maisch[1], Rudolf Vasold[2], Francesco Santarelli[1], Eva Engel[2], Anita Gollmer[1], Burkhard König[2], Michael Landthaler[1] and Wolfgang Bäumler[1]

- Incubation of human keratinocytes with 3 inks extracts and various [PAHs]
- Irradiation with UVA (330-400 nm), 4-8 J/cm²
- Phototoxicity with one ink
- Consequence *in vivo* in case of a black tattoo and repeated UV expositions ?
- Limitations
  - In vitro
  - In vivo, keratinocytes are not in direct contact with the inks (dermis)

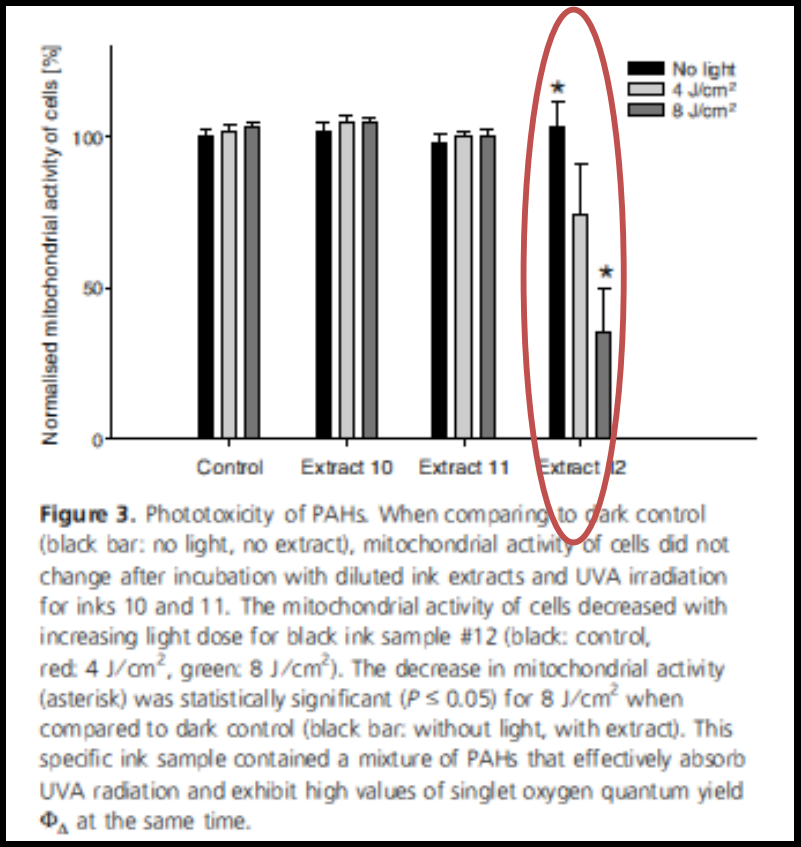


Figure 3. Phototoxicity of PAHs. When comparing to dark control (black bar: no light, no extract), mitochondrial activity of cells did not change after incubation with diluted ink extracts and UVA irradiation for inks 10 and 11. The mitochondrial activity of cells decreased with increasing light dose for black ink sample #12 (black: control, red: 4 J/cm², green: 8 J/cm²). The decrease in mitochondrial activity (asterisk) was statistically significant ($P \leq 0.05$) for 8 J/cm² when compared to dark control (black bar: without light, with extract). This specific ink sample contained a mixture of PAHs that effectively absorb UVA radiation and exhibit high values of singlet oxygen quantum yield Φ_Δ at the same time.

Regensburger J, et al. Exp Dermatol 2010:19:e275-281

Conclusions

- The risk of skin cancer is today fortuitous
- The risk of systemic toxicity is currently unknow and overstated by *in vitro* data

Conclusion

- Is there a risk related to UV exposure and tattoos ?
- Is there a risk after tattoo laser removal ?
- Is there a risk of chronic intoxication by metallic salts ?
- Is there a risk of cancer of for the heavilly tattooed individuals ?
- Necessity of follow – up of large cohort of tattooed individuals (blood analysis, biopsies, cancer registries) and in vivo studies in the skin of tattooed individuals

The tattooist's choice…

The most common complication is…


STRASBOURG
Mother
Cartoon Convention
COPENHAGEN 2000
Second Place
US/EURO - TRADITIONAL