

# **Gaining *in vitro* and human data on tattoo ink toxicology**

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*(Online Version- some pictures have been removed)*

# Data gaps in tattoo toxicology

Summary of first BfR conference:

A medical-toxicological view of tattooing (*LANCET*, 2016, [http://dx.doi.org/10.1016/S0140-6736\(15\)60215-X](http://dx.doi.org/10.1016/S0140-6736(15)60215-X))

## Data gaps for full risk assessment..

- toxicological risks (acute & chronic)
- biokinetics

## How to assess the toxicological hazards

- In vitro (silico, chemico)
- In vivo, **animal testing\***
- In vivo, human data

\*Germany: Ethics on animal experiments for tattoo toxicology are in question (cf. ban for cosmetics)

**Many people are tattooed everyday, we should use all data that we can gain from this!**

# Hazard vs. Risk

Video:  
Lion = Hazard  
No Exposure = No Risk

<https://www.youtube.com/watch?v=maguIV1vxkA>

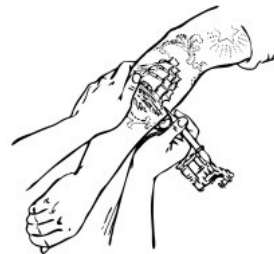
**What is the exposure?**

# Short-term biokinetics study for soluble ingredients

*In vivo in (hu)mans*



+ tracer substances



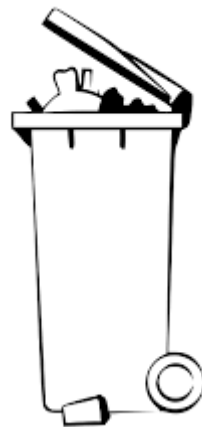
bioavailability of soluble tattoo ink ingredients

accurate determination of the amount of applied ink

screening for other ink substances in blood and urinary samples

biokinetic modelling for similar toxic substances

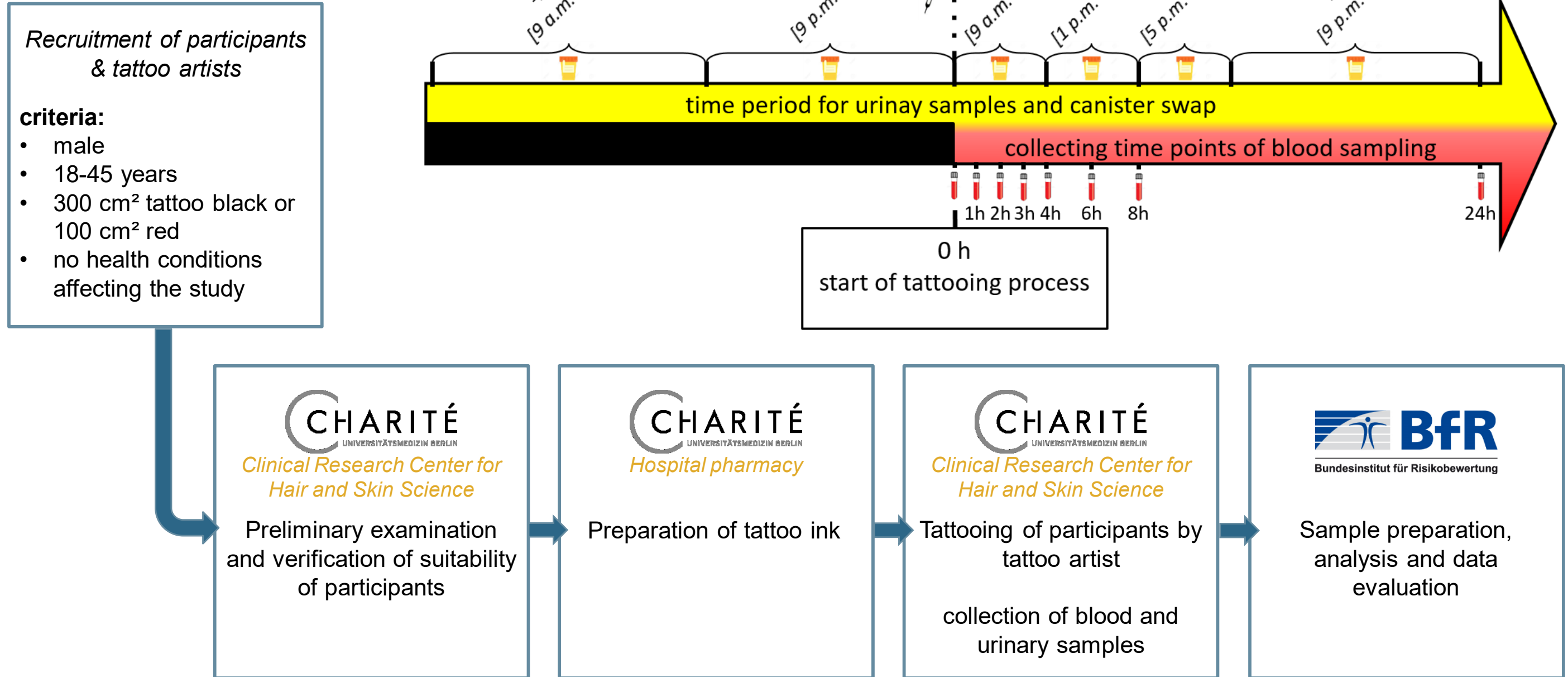
weight before / after usage



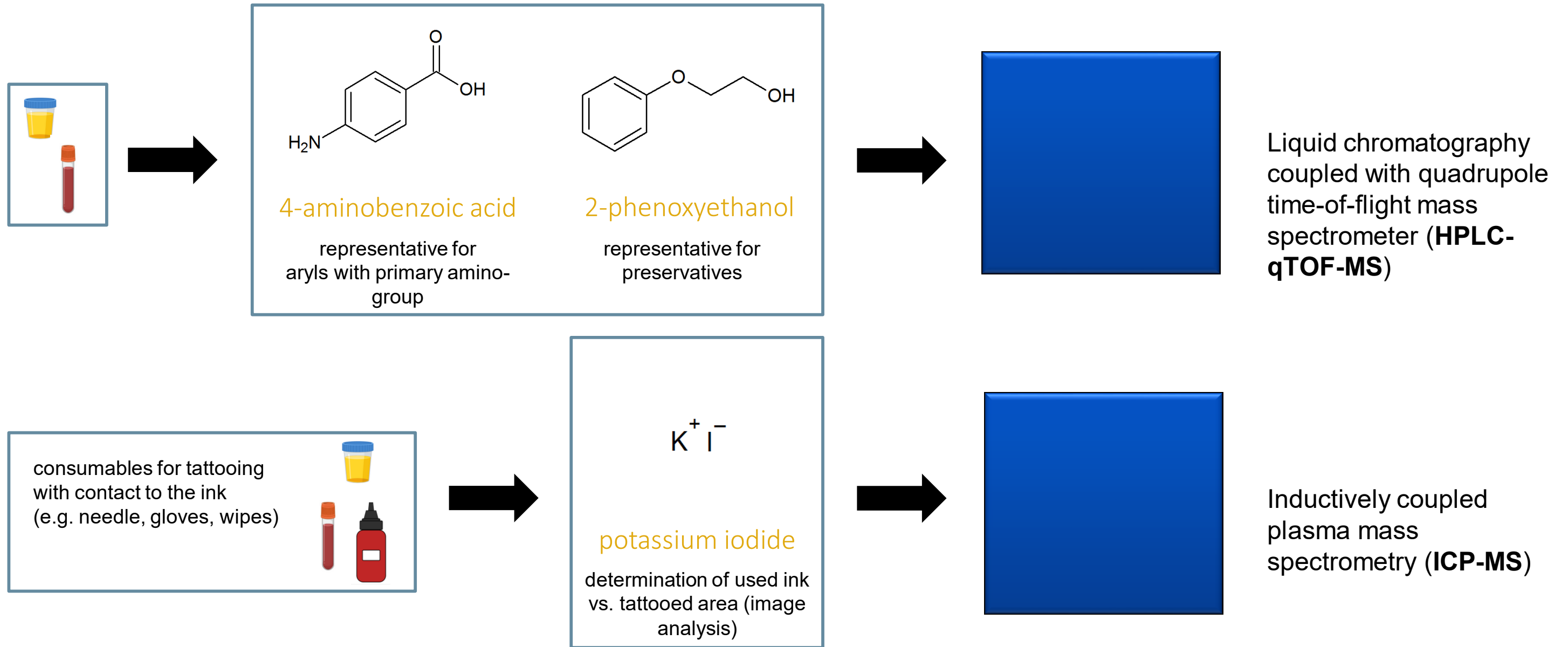
consumables with ink

Blood & urine collection

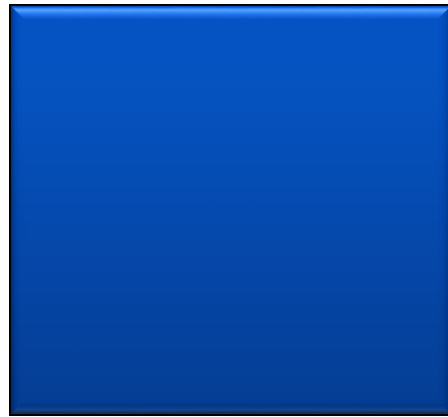
# What does the study look like (for a participant)?



# Analysis of tracer substances



# Size analysis of tattooed area



**Tattoo drawing**



52,14 cm<sup>2</sup>

**Estimated size (e.g. data from surveys!)**



22,44 cm<sup>2</sup>

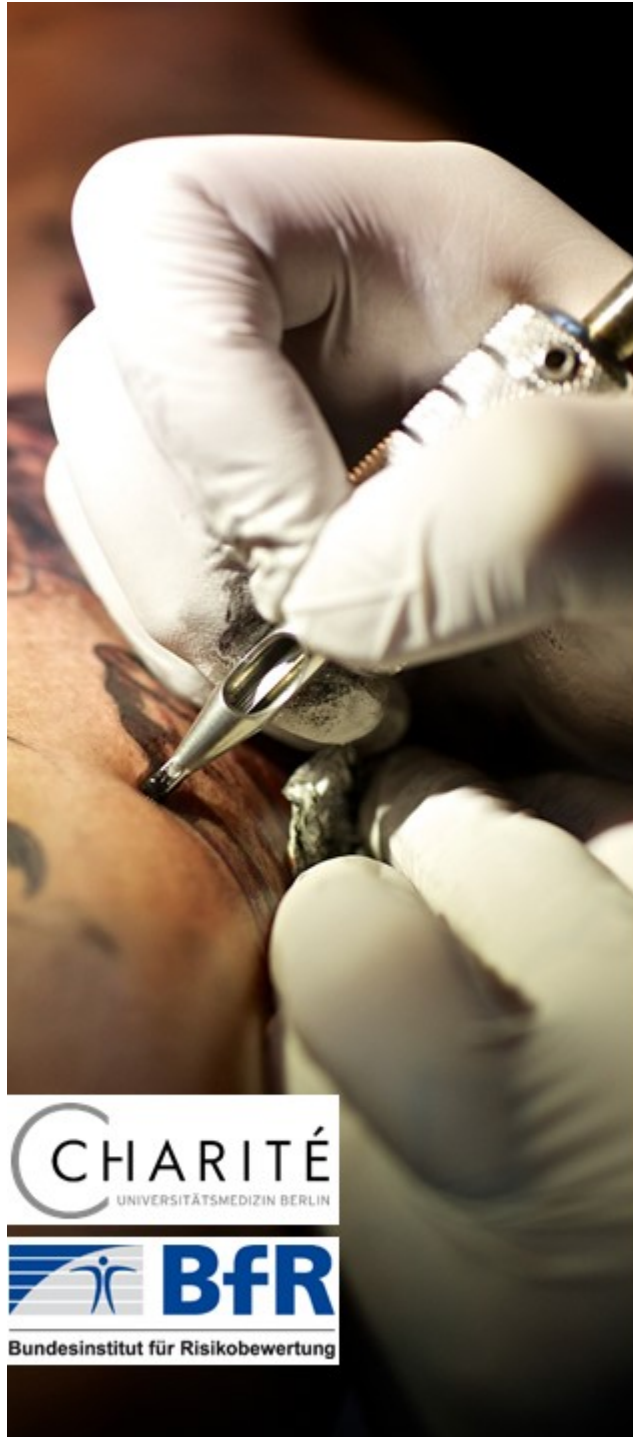
**Size with outlines**



11,50 cm<sup>2</sup>

**True tattooed area**

- tattooed area taken from surveys do not take the true tattooed area into account
- estimated tattooed area & thus the exposure per person is overestimated
- fully black tattooed person without any shadowing is unrealistic
- shadowing can be included in our analysis



## Wanted: Subjects for Tattoo-Study!

The German Federal Institute for Risk Assessment and the Clinical Study Centre for Hair and Skin Research at the Charité search:

Man between the ages of 18 and 45,

- who weigh between 60 and 100 kg,
- That want a large black tattoo (about 17 x 17 cm) **OR** a tattoo with a high proportion of red (about 10 x 10 cm),
- Who already have a minimum of one tattoo,
- who are willing to visit the study center (Charité Mitte, Berlin) three times within 2 weeks

### Study procedure:

- Your desired tattoo will be placed in the study center
- Blood samples and 48 hours of urine will be collected

### We offer:

- An expense allowance of 250 euros for the subjects and 100 euros for the tattoo artist

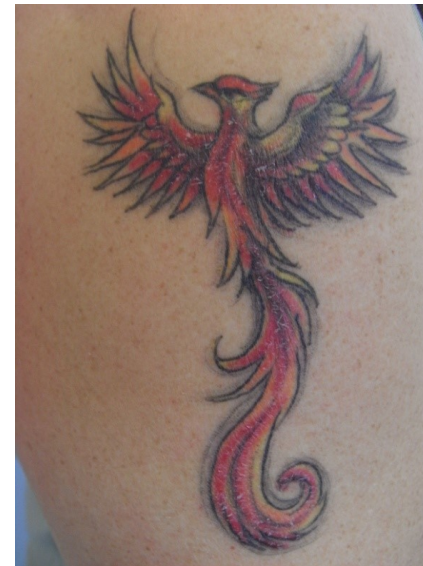
Please speak to your tattoo artist and register for the study at [tattoostudie@bfr.bund.de](mailto:tattoostudie@bfr.bund.de) without obligation. Further information can also be provided.



# Red Tattoo Allergies – identifying the pigment

## *Ex vivo human study*

Simultaneous reactions on multiple tattoos can occur:



Quelle: J. Serup

Question: Red allergies mostly occur within red color shades- but what are the molecules related to the allergy?

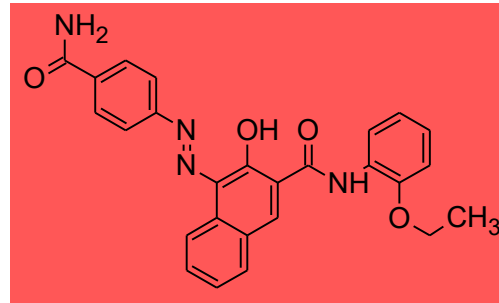
Analysis of 104 skin samples from the Bispebjerg Hospital in Copenhagen at the BfR:



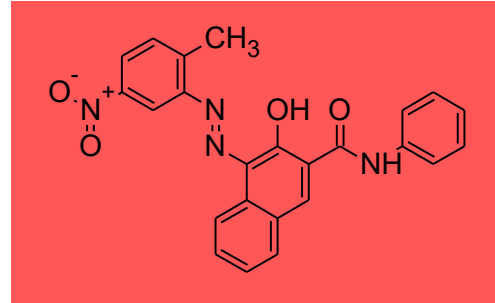
<http://estpresearch.org>

# Red Tattoo Allergies – identifying the pigment

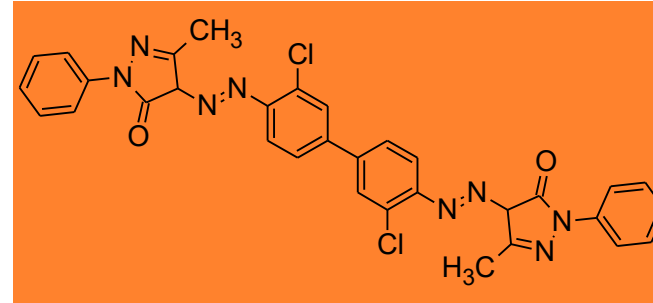
## Matrix-assisted laser-desorption/ionisation (MALDI-MS/MS)



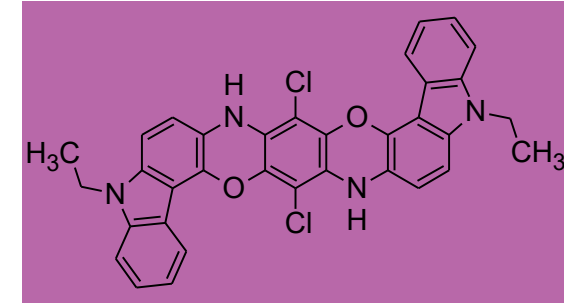
Red 170+210 (36%)  
C.I.12475&12477



Red 22 (35%)  
C.I.12315



Orange 13 (12%)  
C.I.21110



Violet 23 (8%)  
C.I. 51319

Other pigments		Pigment chemical class
no org. pigment identified	18%	-
Orange 16	2%	Diazo
Red 5	0.9%	Azo (Naphthol AS)
Violet 19	0.9%	Quinacridon
Red 112	0.9%	Azo (naphthol AS)

- **azo pigments** are the most common pigments identified
- most likely, the not the pigment but **impurities, additives** or UV/Laser **breakdown products** are the true allergen

Serup et al., Identification of pigments related to allergic tattoo reactions in 104 human skin, 2020, *Contact Dermatis.*, <https://doi.org/10.1111/cod.13423>

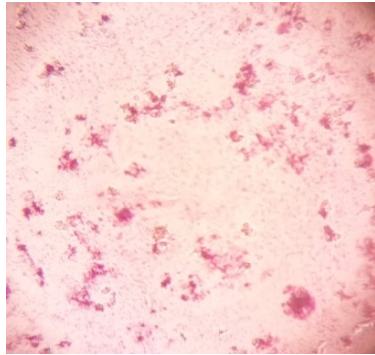
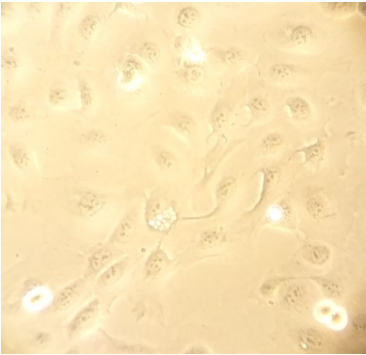
# Red Tattoo Allergies – identifying the allergen

*in silico / in chemico / in vitro*



digital prediction tools,  
protein-binding assays,  
cell-activation assays:

proof of sensitization  
potential of a substance



*in vivo in human*



patch-testing:

proof of sensitization in a  
patient

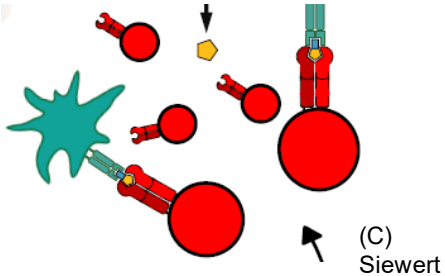


Picture from: Lundh et al, Contact Dermatitis, 2007



t-cell assay (K. Siewert, BfR):

proof of reactivity of the immune cells  
towards an allergen in the reactive skin

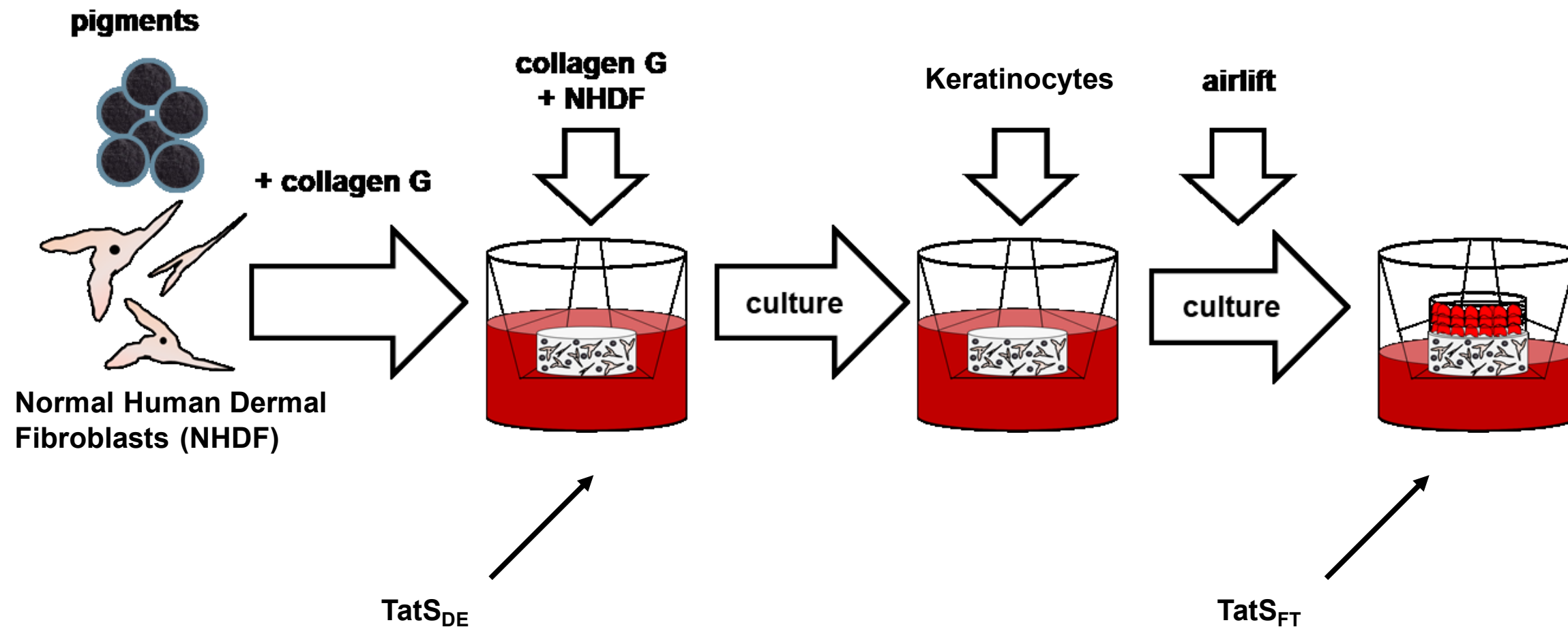


(C) Siewert

# TatS – Tattooed human skin models

## *In vitro*

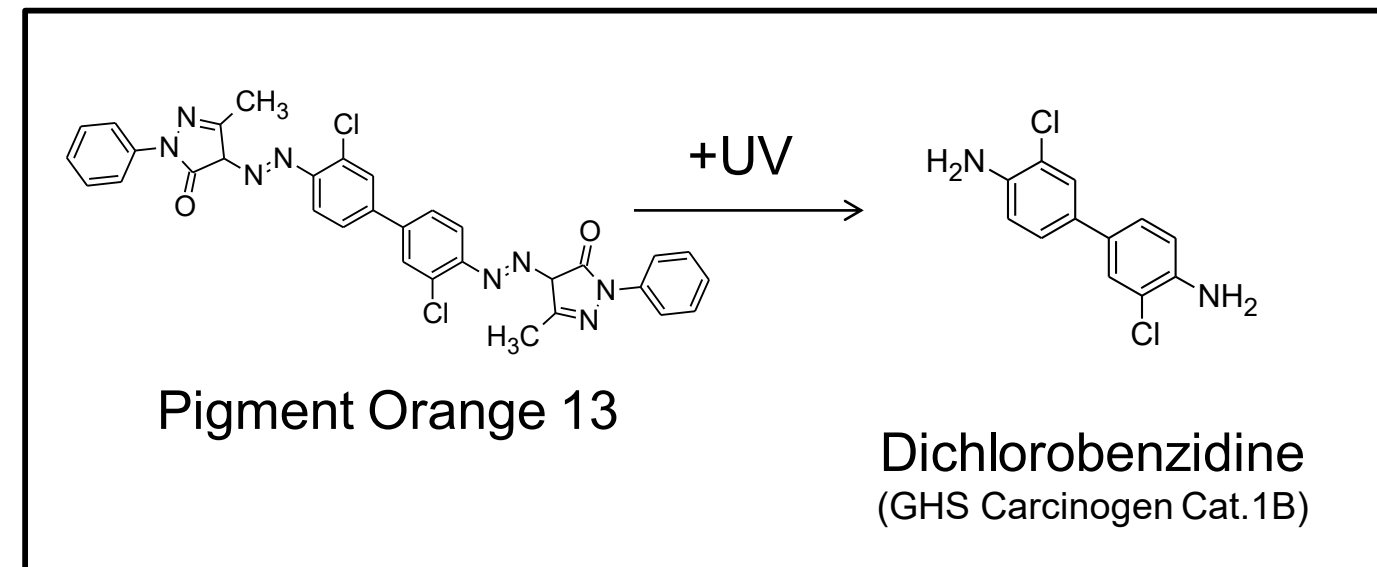
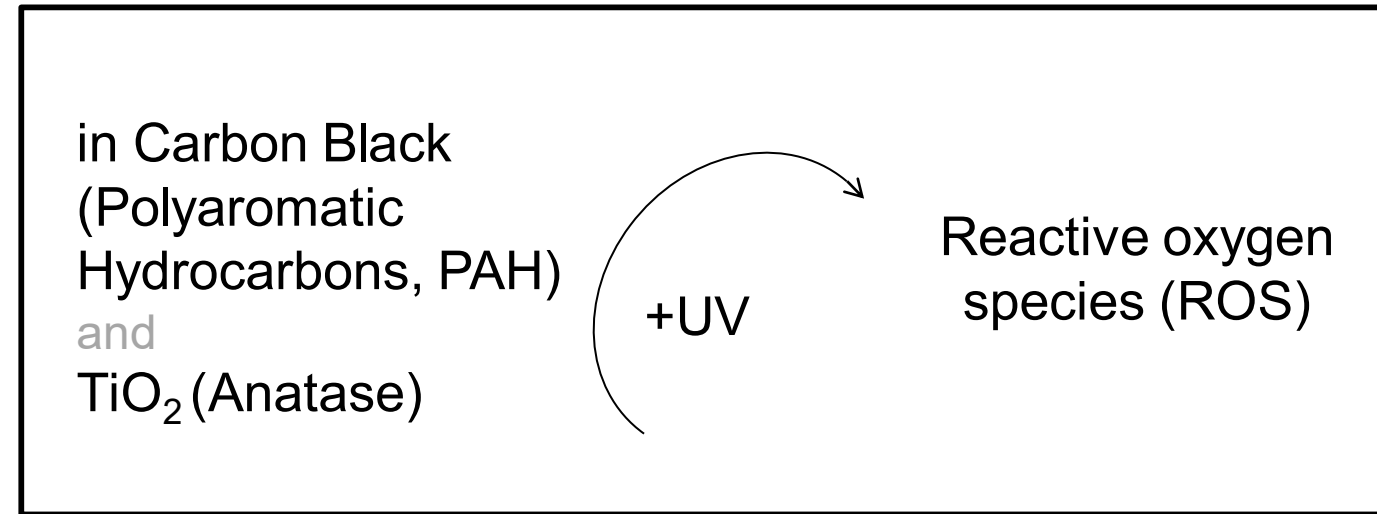
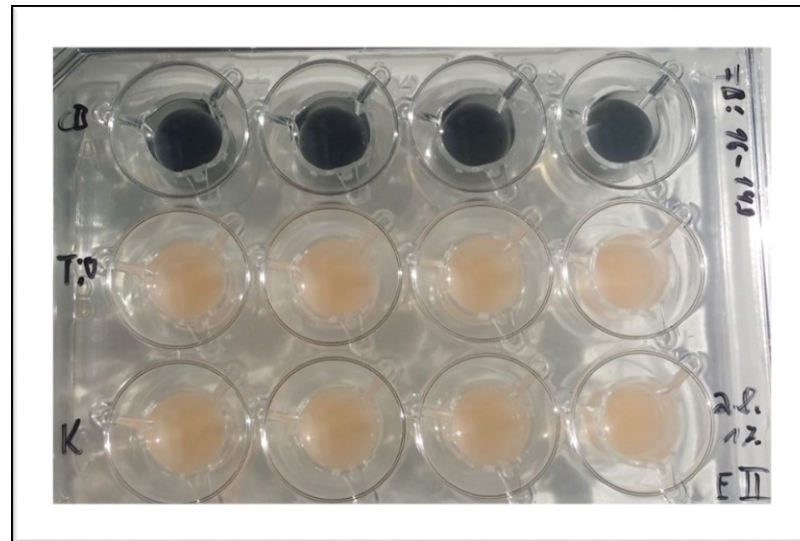
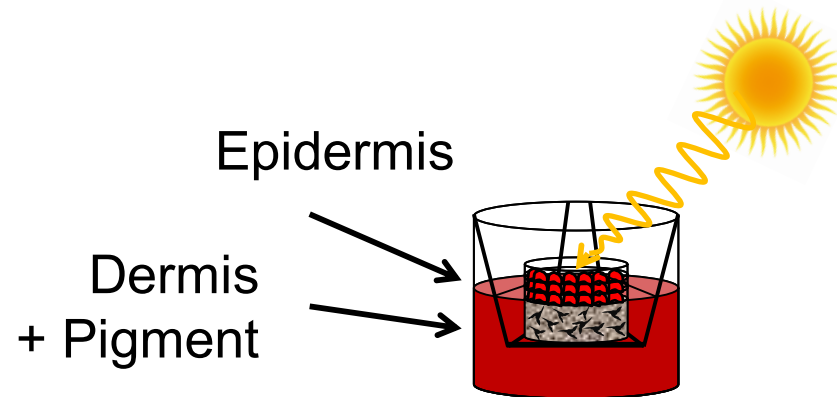
GOAL: Use to identify hazards & case of side effects -> (photo)toxicity testing



Hering et al., Arch Toxicol, 2020,  
<https://doi.org/10.1007/s00204-020-02825-z>

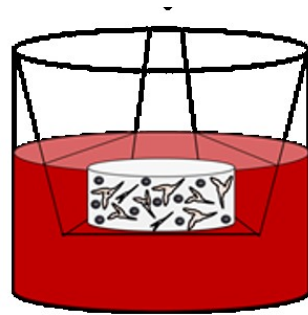
# TatS: Experimental set-up for phototoxicity testing

First endpoint: Frequent side effect, simple endpoint



from: Hering *et al.*, *JID*, 2018, <https://doi.org/10.1016/j.jid.2018.05.031> &  
*Arch Toxicol*, 2020, <https://doi.org/10.1007/s00204-020-02825-z>

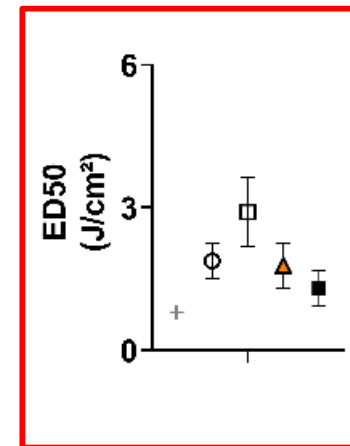
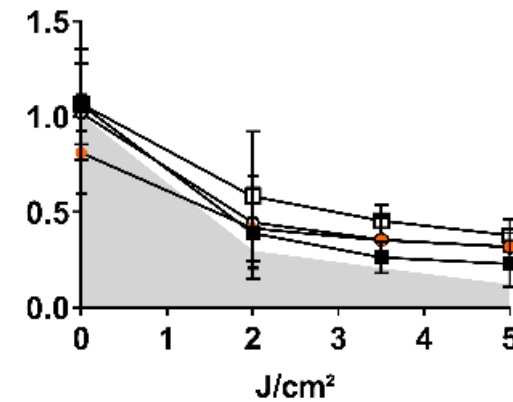
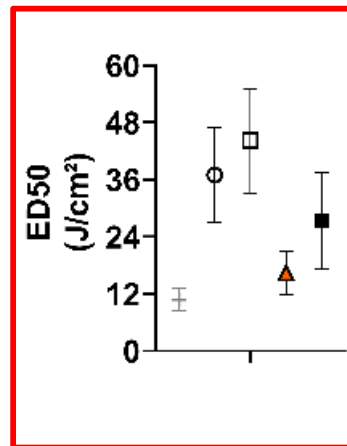
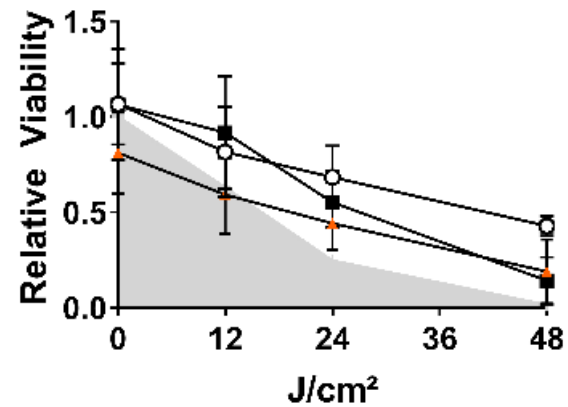
# Possible UV-protective effect of pigments in TatS<sub>DE</sub>



UVA

UVB

TatS<sub>DE</sub>

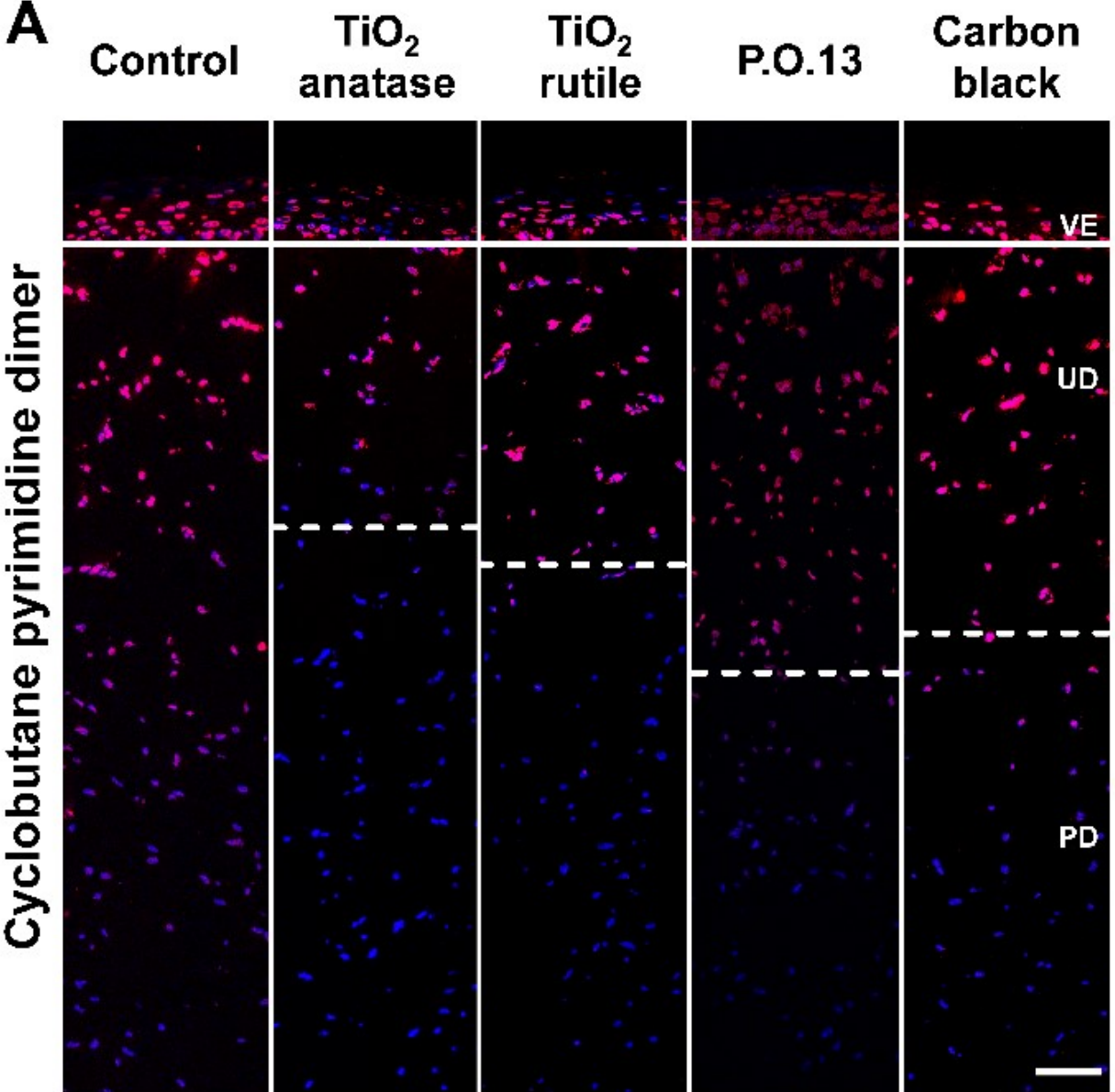


Control vs.	TiO <sub>2</sub> anatase	TiO <sub>2</sub> rutile	P.O.13	Carbon black
Interaction	ns	ns	ns	ns
Row Factor (UV)	***	***	***	***
Column Factor (pigment)	**	***	ns	*

Control vs.	TiO <sub>2</sub> anatase	TiO <sub>2</sub> rutile	P.O.13	Carbon black
Interaction	ns	ns	ns	ns
Row Factor (UV)	***	***	***	***
Column Factor (pigment)	*	**	ns	ns

+ Control    ○ TiO<sub>2</sub> anatase    ◻ TiO<sub>2</sub> rutile    ▲ P.O.13    ■ Carbon black

# Less DNA-damage below the pigment border in TatS<sub>FT</sub>



Hering et al., Toxicol, 2021, doi: 10.1016/j.tox.2021.152872

# Improving TatS: Immuno-competent models

- source of phototoxic effects have not been revealed
- Immune cells (macrophages) hold most of the pigments in tattooed skin
- macrophages will be incorporated into TatS
- do we see a protective effect if immune cells are present?

## First results:

successful incorporation of makrophages with pigment orange 13





# Limitations

.. of human data and in vitro experiments

- **cancer** development and toxicity to **reproduction** of single substances cannot be assessed
  - cancer rate increase may be visible in epidemiological studies (cf. talk of Dr. Milena Foerster)
- **biokinetic studies** of tattoo pigments cannot be assessed
  - initial dose, exact time until analysis and full deposit in organs must be known (not possible with postmortem analysis of human samples)

# Acknowledgement



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Dr. Marine Cotte



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Henrik Hering  
Susanne Kochs  
Sandra Schiewe  
Dimitra Eleftheriadou  
& many more..

# **Thank you for your attention**

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