

# **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

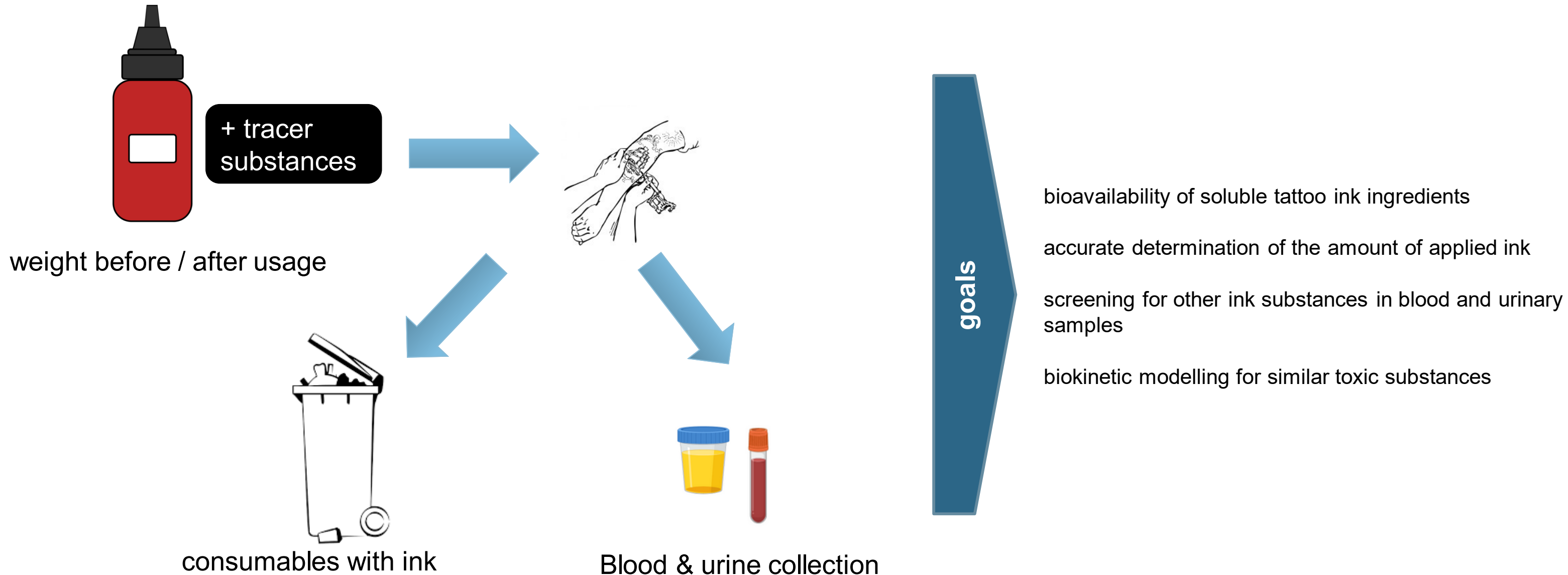
Video:  
Lion = Hazard  
No Exposure = No Risk

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

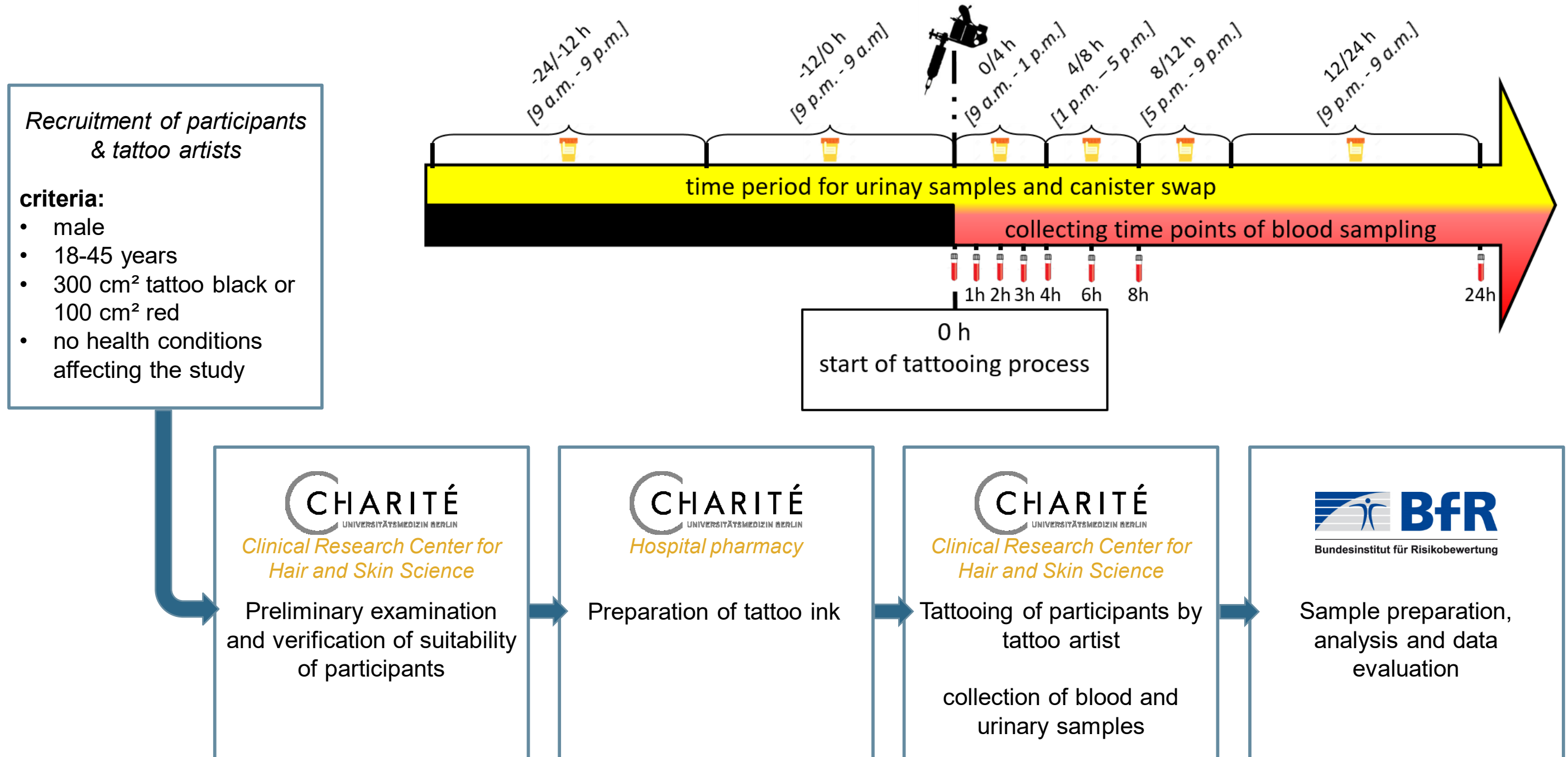
**What is the exposure?**

# Short-term biokinetics study for soluble ingredients

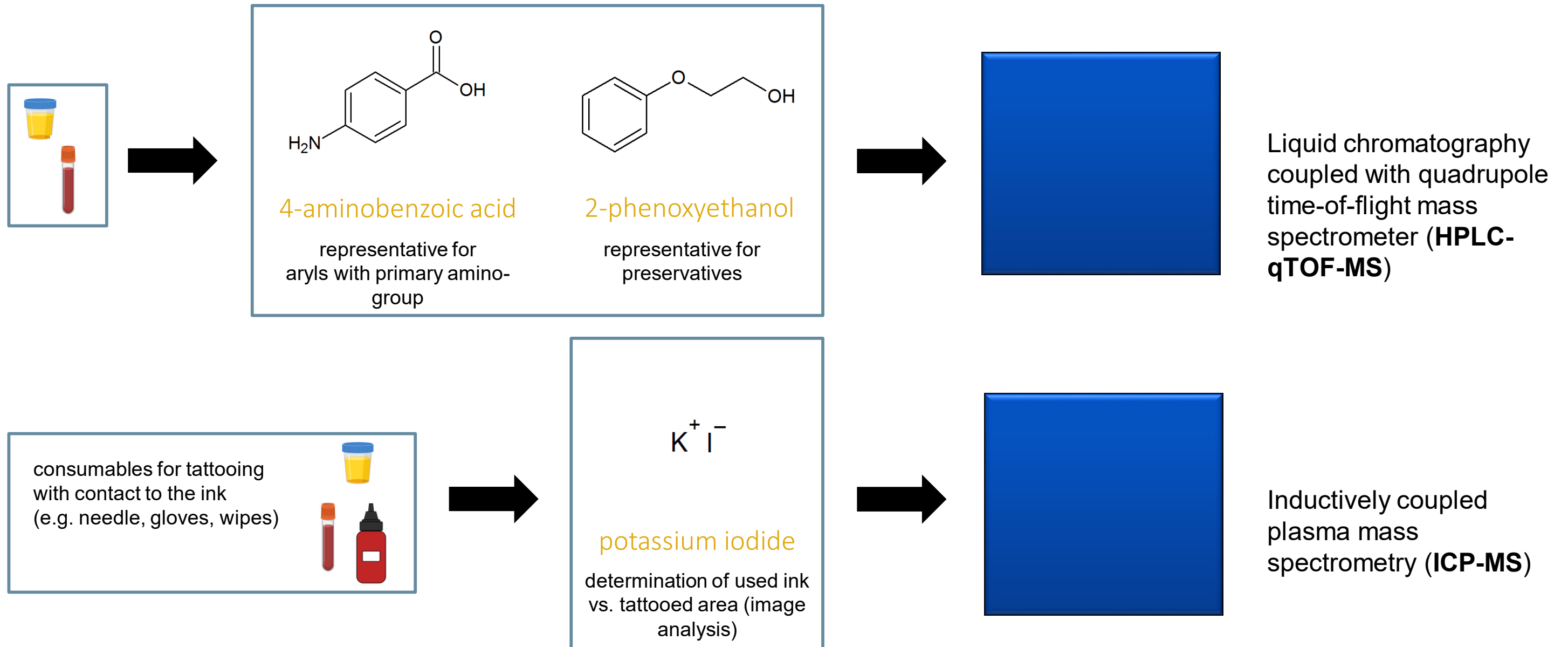
*In vivo in (hu)mans*



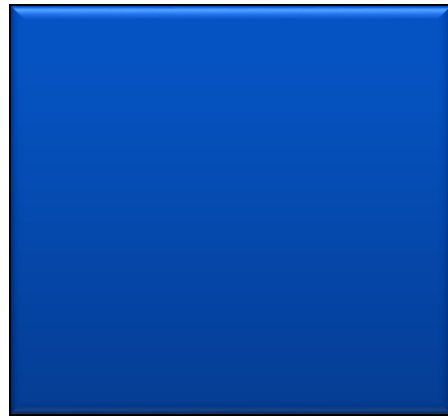
# What does the study look like (for a participant)?



# 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**

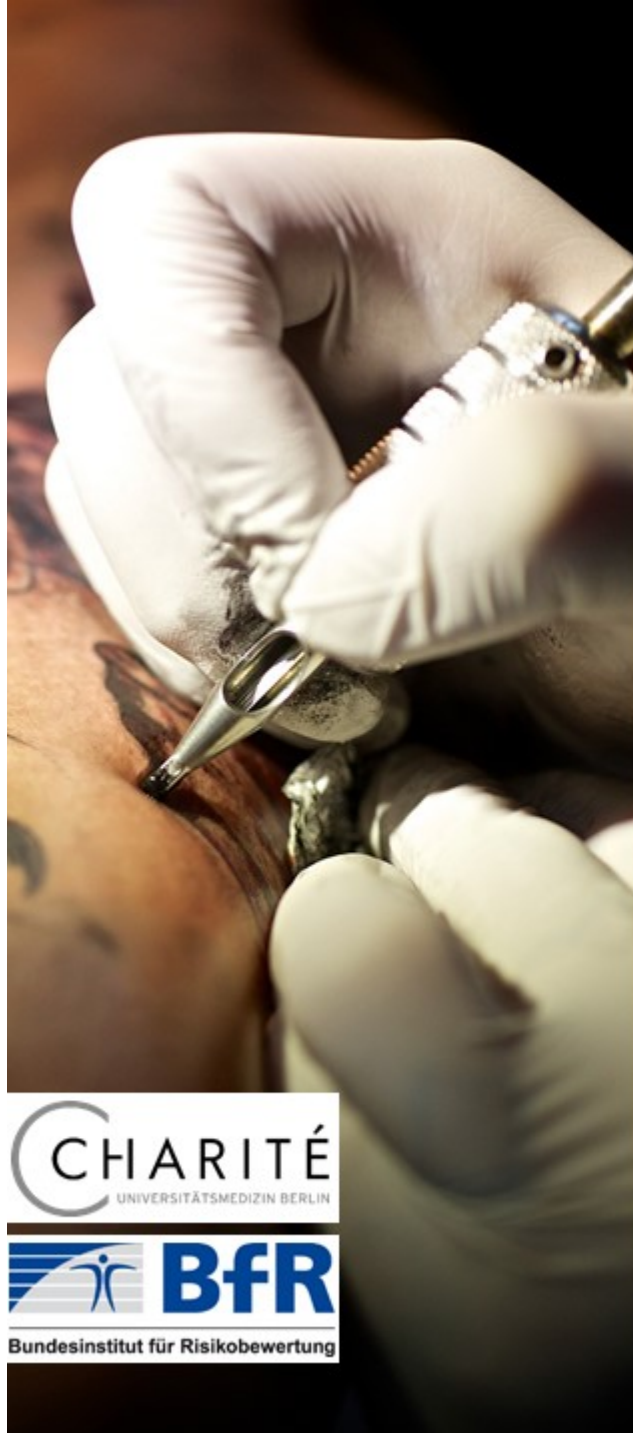


11,50 cm<sup>2</sup>

**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

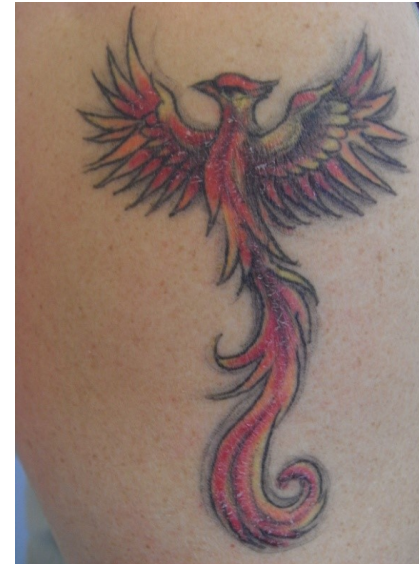
Please speak to your tattoo artist and register for the study at [tattoostudie@bfr.bund.de](mailto:tattoostudie@bfr.bund.de) without obligation. Further information can also be provided.



# Red Tattoo Allergies – identifying the pigment

## *Ex vivo human study*

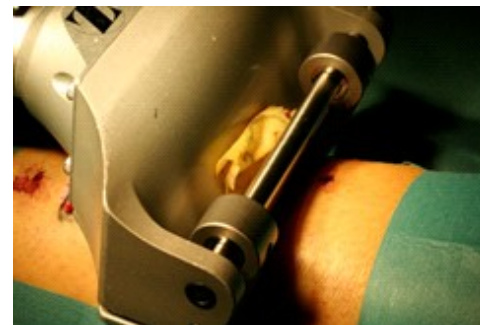
Simultaneous reactions on multiple tattoos can occur:



Quelle: J. Serup

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

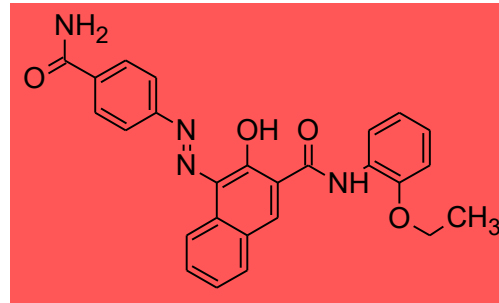
Analysis of 104 skin samples from the Bispebjerg Hospital in Copenhagen at the BfR:



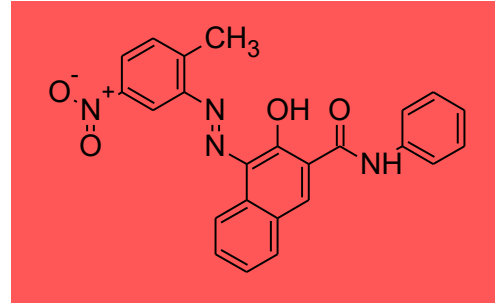
<http://estpresearch.org>

# Red Tattoo Allergies – identifying the pigment

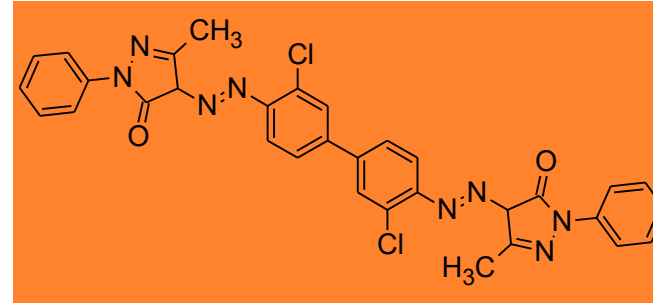
## *Matrix-assisted laser-desorption/ionisation (MALDI-MS/MS)*



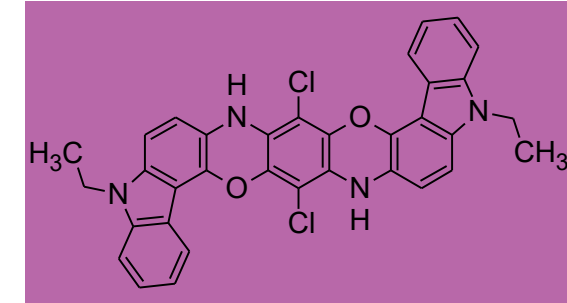
Red 170+210 (36%)  
C.I.12475&12477



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>

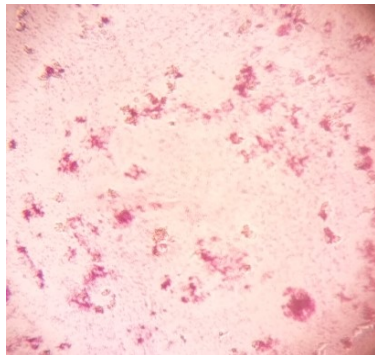
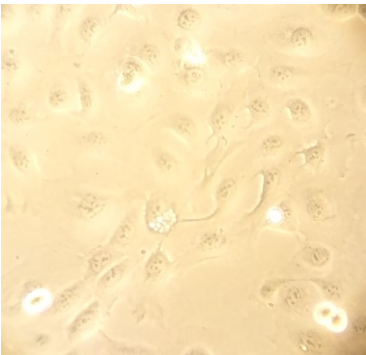
# Red Tattoo Allergies – identifying the allergen

*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

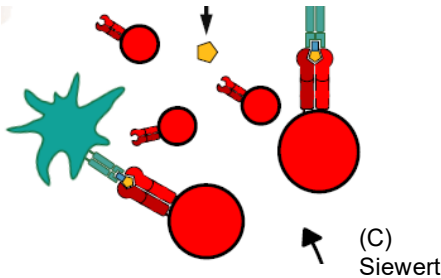


Picture from: Lundh et al, Contact Dermatitis, 2007



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

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

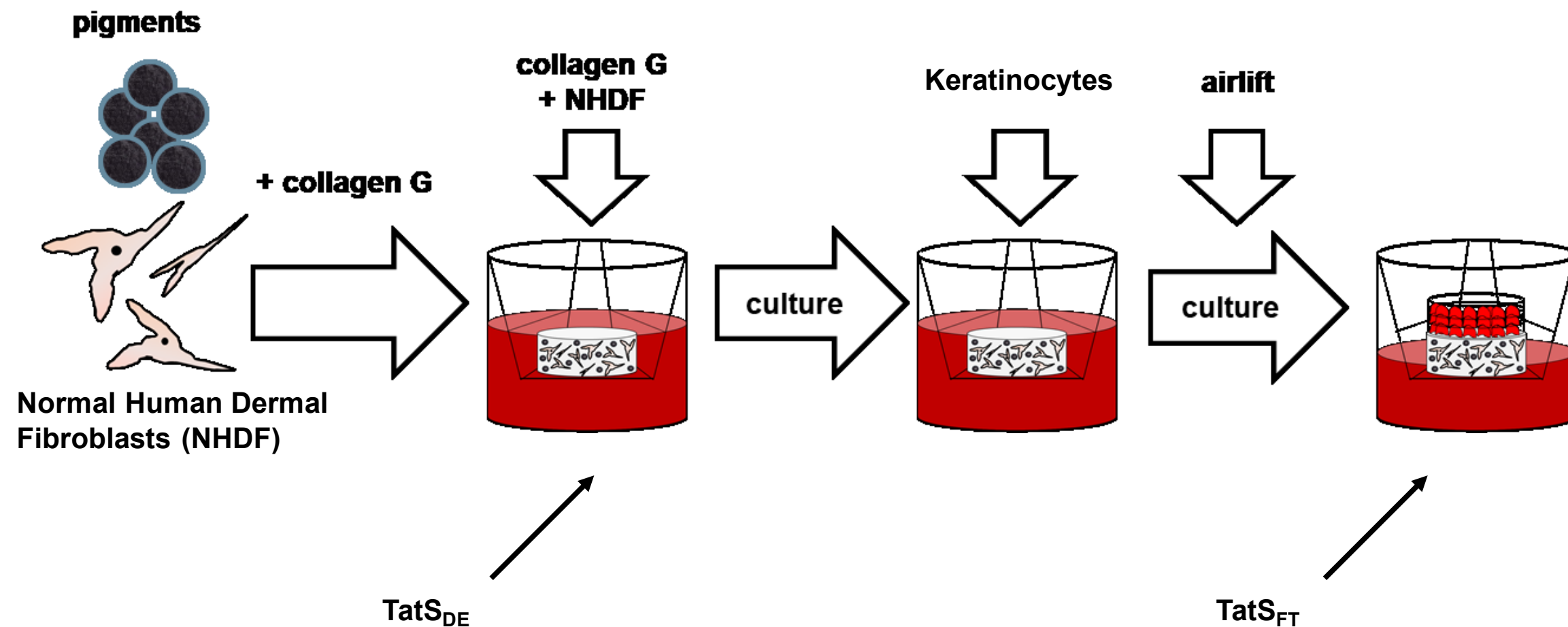


(C)  
Siewert

# TatS – Tattooed human skin models

## *In vitro*

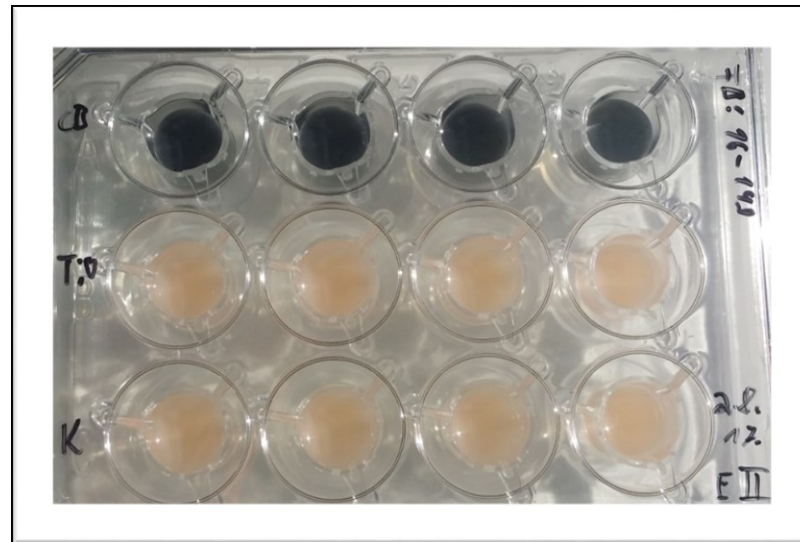
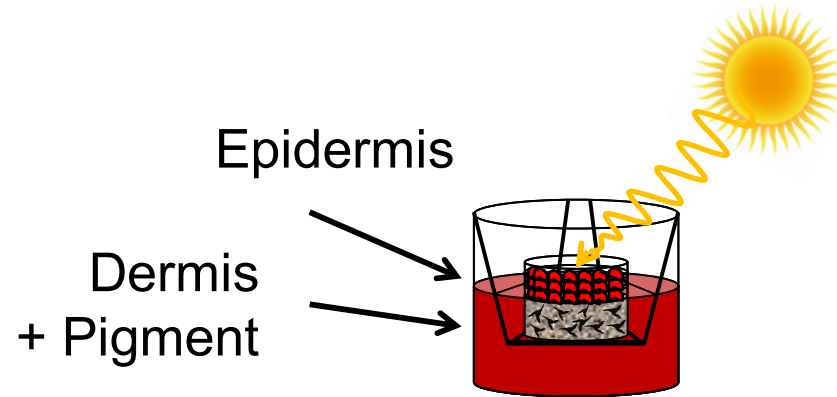
GOAL: Use to identify hazards & case of side effects -> (photo)toxicity testing



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

# TatS: Experimental set-up for phototoxicity testing

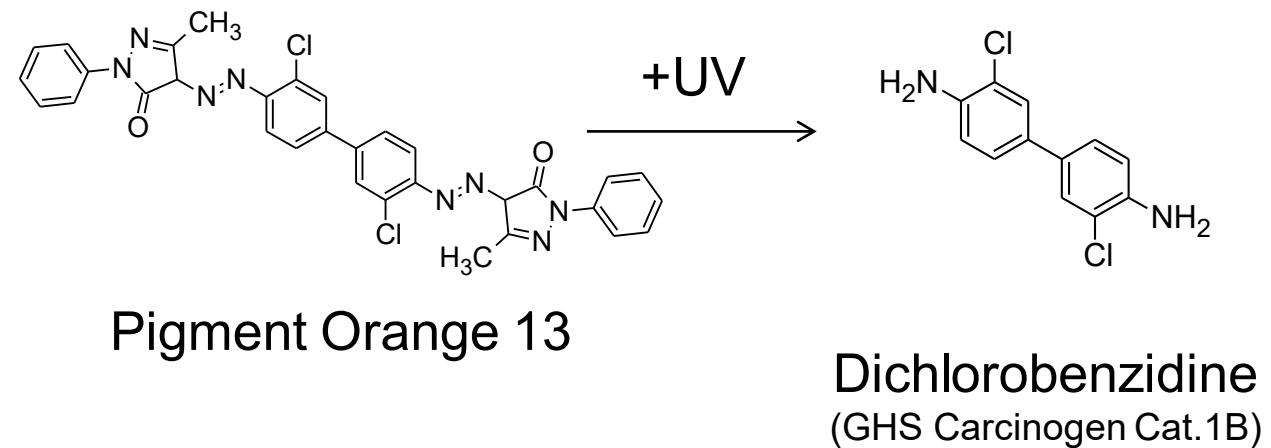
First endpoint: Frequent side effect, simple endpoint



in Carbon Black  
(Polyaromatic  
Hydrocarbons, PAH)  
and  
 $\text{TiO}_2$  (Anatase)

+UV

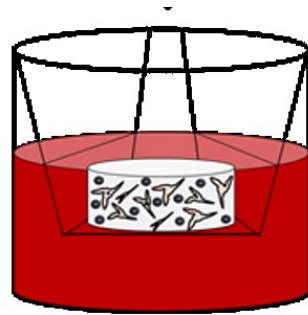
Reactive oxygen  
species (ROS)



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>



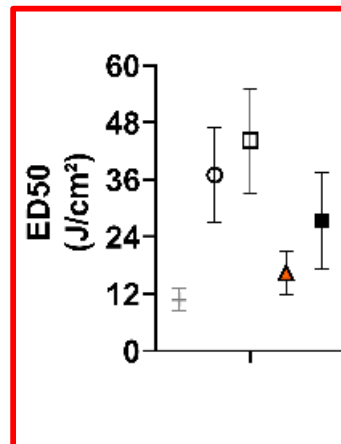
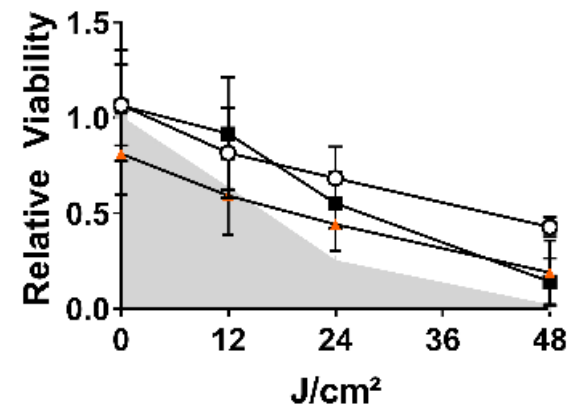
# Possible UV-protective effect of pigments in TatS<sub>DE</sub>



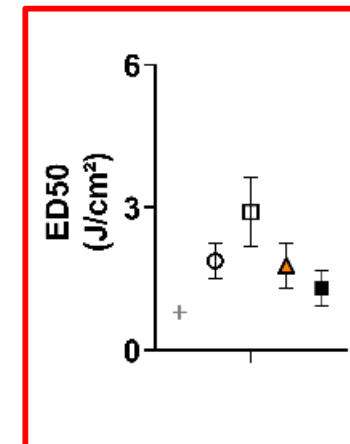
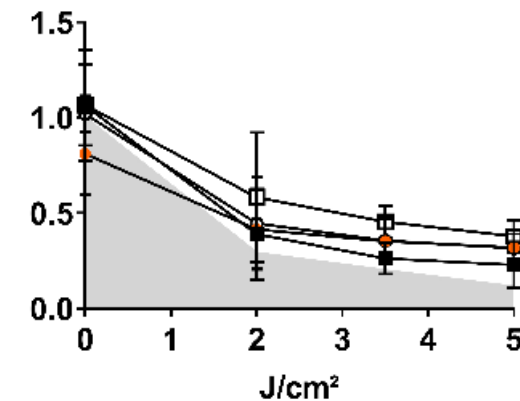
**UVA**

**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	*

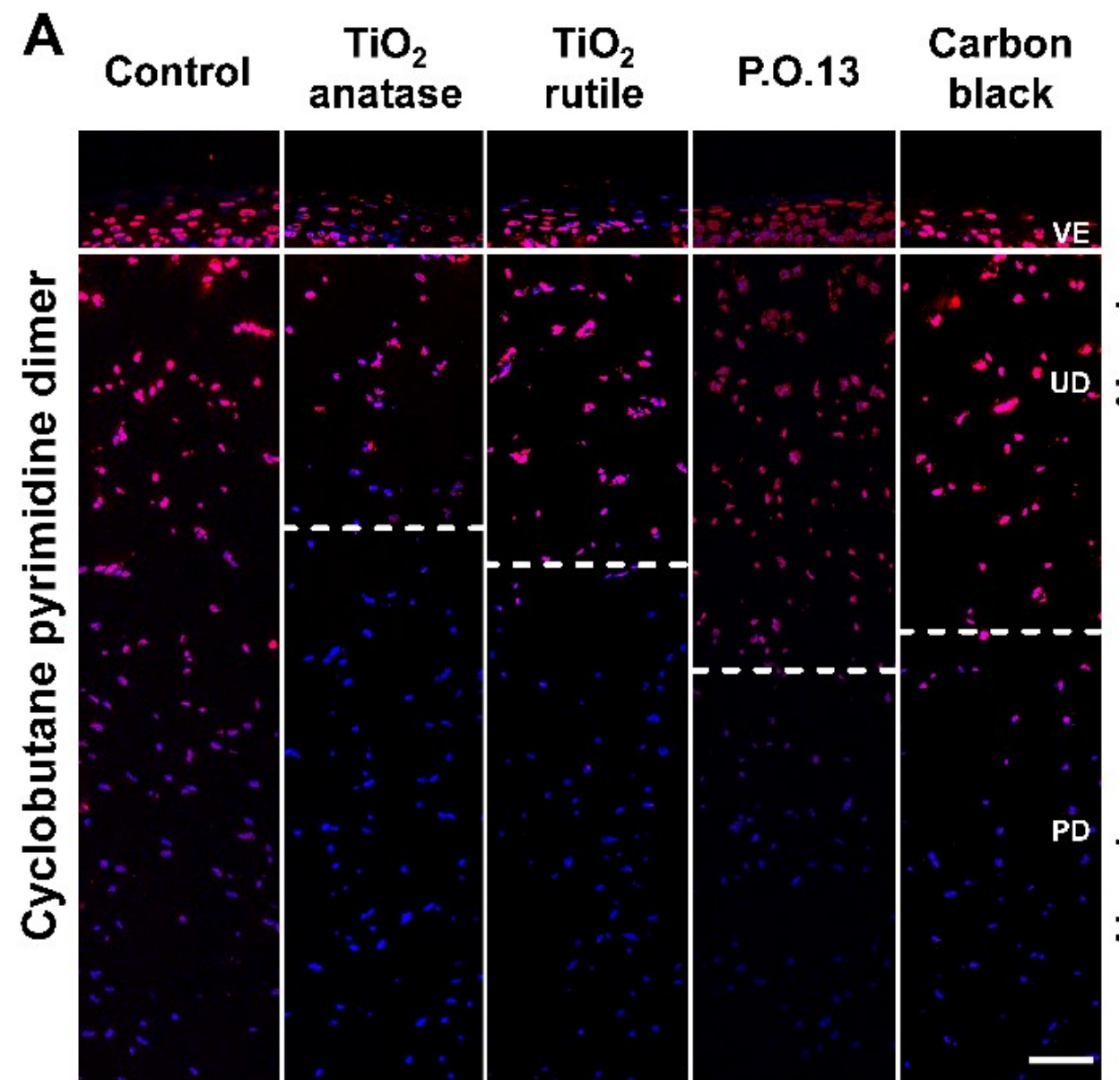
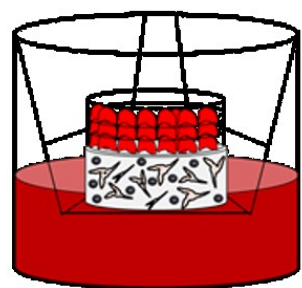


Control vs.	TiO <sub>2</sub> anatase	TiO <sub>2</sub> rutile	P.O.13	Carbon black
Interaction	ns	ns	ns	ns
Row Factor (UV)	***	***	***	***
Column Factor (pigment)	*	**	ns	ns

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

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

# 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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[International Agency for Research on Cancer](#)



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Dr. Lechner



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

# **Thank you for your attention**

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