Life cycle of tattooing pigments in the human body
Dr. Michael Giulbudagian
4th Joint Symposium on Nanotechnology, 30-31 May 2022
The spectrum of body modifications and their perception in society

What is tattooing?
…. a mixture “for tattooing purposes” means injection or introduction of the mixture into a person’s skin, mucous membrane or eyeball, by any process or procedure (including procedures commonly referred to as permanent make-up, cosmetic tattooing, micro-blading and micro-pigmentation), with the aim of making a mark or design on his or her body.
Are you wearing a tattoo?

YouGov; [ID 1253983](https://de.statista.com/statistik/daten/studie/1253983/umfrage/umfrage-in-deutschland-zu-tattoos-nach-altersgruppen/)

Survey in Germany on tattoos by age group 2021

<table>
<thead>
<tr>
<th>Age Group</th>
<th>No Tattoo</th>
<th>No but want a tattoo</th>
<th>Yes</th>
<th>More tattoos</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-24</td>
<td>73%</td>
<td>59%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>25-34</td>
<td>87%</td>
<td>59%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>35-44</td>
<td>70%</td>
<td>59%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>45-54</td>
<td>87%</td>
<td>59%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>55+</td>
<td>70%</td>
<td>59%</td>
<td>87%</td>
<td></td>
</tr>
</tbody>
</table>

Total

25-34 y/o 26%
Composition of tattoo inks

- **Inorganic** 15%
- **Organic** 81%
- **Unknown** 3%

**Pigments**
- Inorganic pigments: titanium dioxide, iron oxide, chromium oxide, carbon black, barium sulfate (filler)
- Organic pigments: e.g. azo pigments, polycyclic aromatic pigments, lake pigments precipitated with e.g., metal salts

**Binders** (prevent sedimentation)
- Polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), block copolymers (acrylates, poloxamers), shellac, sugars (traganth, gummi arabicum)

**Solvents**
- Water, simple alcohols, polyols

**Additives**
- Preservatives, thickening agents, surfactants

References:
What do we know about the distribution of tattoo pigments in the body?

- Pigments reside in macrophages or attached to membranes of dermal fibroblasts
- Role of red tattoos with respect to adversity better understood
- Transport to regional lymph nodes may occur passively or via phagocytes
- Not only pigments, but also abraded metallic particles from the tattoo needles may be transported to the regional lymph nodes
- Only a small fraction of the pigments remains at the site of injection
- Major questions with regard to systemic effects remain
What happens at the injection site?

- Pigments injected into the dermis reside in cells and connective tissue.
- Tattoo pigments injected into the mouse tail remained mainly in the dermal resident macrophages after endocytosis.
- Free pigment particles are readily internalised by newly forming macrophages.

What happens at the regional lymph nodes?

Migration of pigments to the regional lymph nodes

I. Schreiber et al., Scientific Reports (2017), 7 (1), 11395
What is known about the systemic distribution of tattoo inks?

- Direct contact of tattooing agents with blood and lymph fluids takes place.
- A rapid systemic distribution of the soluble ingredients can be assumed.
- Studies suggest that over 80% of the injected pigments are eliminated.
- In animal studies, pigments could be detected in the liver of mice one year after administration.
- No correlation could be found between tattoos and cancers or other systemic complications.

Minimum Testing Requirements

Specifications for Ingredients of Tattoo Inks

Minimum toxicological requirements - *in vitro/in chemico* testing
- Consider compatibility of tests with the physico-chemical properties of pigments
- Test according to OECD Test Guidelines and GLP

Skin irritation & corrosion
Eye irritation & damage
Skin sensitization
Phototoxicity
Mutagenicity/genotoxicity *incl.* photogenotoxicity

Tattoo pigments that meet the minimum toxicological requirements reduce possible risks according to the current state of science and technology.

https://www.bfr.bund.de/cm/349/tattoo-inks-minimum-requirements-and-test-methods.pdf

Michael Giulbudagian, 30.05.202, 4th Joint Symposium on Nanotechnology
Pigment particles in tattoo inks

### Asymmetric Flow Field Fractionation (AF4) and Multi-Angle Light Scattering (MALD-ICP-MS)

<table>
<thead>
<tr>
<th>Ink number (name)</th>
<th>TEM,(^a) nm</th>
<th>DLS,(^b) nm</th>
<th>AF4-MALS,(^d) nm</th>
<th>SP-ICP-MS,(^e) nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (Ice blue)</td>
<td>170 ± 64 (127–227)</td>
<td>421 (110–980)</td>
<td>42 (16–65)</td>
<td>109 ± 17 (Cu-phthalocyanine)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>269 (60–490)</td>
<td>183 ± 11 (Al₂O₃)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>441 ± 161 (TiO₂)</td>
<td></td>
</tr>
<tr>
<td>5 (Deep violet)</td>
<td>39 ± 10 (31–48)(^b)</td>
<td>31 (19–52)</td>
<td>56 (11–100)</td>
<td>427 ± 97 (TiO₂)</td>
</tr>
<tr>
<td></td>
<td>137 (60–281)</td>
<td>271 (100–490)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (Black outlining)</td>
<td>19 ± 11 (12–29)</td>
<td>152 (49–450)</td>
<td>137 (21–330)</td>
<td>109 ± 19 (CuO)</td>
</tr>
<tr>
<td>7 (Grasshopper green)</td>
<td>162 ± 51 (126–208)</td>
<td>277 (81–1350)</td>
<td>46 (18–56)</td>
<td>110 ± 17 (Cu-phthalocyanine)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>405 (50–1200)</td>
<td>225 ± 14 (Al₂O₃)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>466 ± 179 (TiO₂)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Diameter, median ± median absolute deviation (first-third quartile). \(^b\) Larger particles (100–450 nm) were also observed. \(^c\) \(D_h\), hydrodynamic diameter, mean (range). \(^d\) \(R_g\), radius of gyration (=diameter of gyration \((D_g)/2\)), mean (range). \(^e\) Diameter, mean ± standard deviation.

<table>
<thead>
<tr>
<th>Ink</th>
<th>Peak</th>
<th>Rt (min)</th>
<th>Rg (nm)</th>
<th>&lt; 100 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice blue</td>
<td>1</td>
<td>17-28</td>
<td>42 (16-65)</td>
<td>53%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>28-56</td>
<td>269 (60-490)</td>
<td>78%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15-32</td>
<td>56 (11-100)</td>
<td>78%</td>
</tr>
<tr>
<td>Deep Violet</td>
<td>2</td>
<td>32-54</td>
<td>271 (100-490)</td>
<td>77%</td>
</tr>
<tr>
<td>Black Outlining</td>
<td>1</td>
<td>17-50</td>
<td>137 (21-330)</td>
<td>77%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>16-27</td>
<td>46 (18-56)</td>
<td>32%</td>
</tr>
<tr>
<td>Grasshopper Green</td>
<td>2</td>
<td>27-56</td>
<td>405 (50-1200)</td>
<td>77%</td>
</tr>
</tbody>
</table>

In-vitro approach for investigation of pigment dissolution

- Long-term dynamic dissolution testing of tattoo pigments in skin simulant
- External factors like UV or temperature
- Fractionated sampling and analysis of pigments and/or metabolites
- Identification of pigments that release harmful substances under physiological conditions

Activities of the BfR on tattoo ink safety (selection)

7 SZ
Dermatotoxicology Study Centre
Dr. Katherina Siewert*
Dr. Ines Schreiver*

FAQ about tattoo inks

Updated BfR FAQ, 16 September 2019

In Germany, roughly 12 g of tattoo ink is applied to a person; in the future, the amount could be higher. Are there potential health risks? If so, what measures can be taken?

To date, there is no binding regulation governing the components used in tattoo inks at the European level. The EU Commission and member states are currently consulting on a proposal to create a regulatory framework for tattoo inks. A draft Regulation is currently under discussion.

Tattoo inks: risk assessment for Pigment Blue 15:3 and Pigment Green 7

BfR Opinion No 038/2020 issued 8 September 2020

To date, there is no binding regulation governing the components used in tattoo inks at the European level. The EU Commission and member states are currently consulting on a proposal to create a regulatory framework for tattoo inks. A draft Regulation is currently under discussion.
Thank you for your attention

Dr. Michael Giulbudagian
Michael.Giulbudagian@bfr.bund.de
German Federal Institute for Risk Assessment
Max-Dohrn-Straße 8-10 • 10589 Berlin, GERMANY
Phone +49 30 - 184 12 - 0 • Fax +49 30 - 184 12 – 99 0 99
bfr@bfr.bund.de • www.bfr.bund.de/en

https://www.philara.de/en/exhibitions/2021-02/timm-ulrichs