Getting under your skin:

Health risks through tattoo inks

Carcinogenic substances, allergies, infections – a wide range of health risks are being discussed in connection with tattoo inks. Up to now it has been mostly unclear which substances have which effects. Various BfR projects are now producing results which are attracting attention all over the world.

They go by the name of Dotwork, Blackout or Double Exposures – the trend techniques of the tattoo scene. Something new hits the market just about every year: watercolour techniques, anatomical motifs, white ink or black light images that illuminate in the dark. What used to be the domain of sailors and criminals turned into a popular mass phenomenon in the 1990s. The trend towards tattooing has remained unbroken ever since, as a current study conducted by the University of Leipzig shows. One in five Germans already has a tattoo and in the age group between 25 and 34, it's even the half of all women. Despite being so widespread, however, tattoos have rarely been discussed up to now in connection with health risks.

What risks are we talking about?

Tattoos can have various undesired effects on health (see chart). Just like other open wounds, freshly tattooed skin can occasionally become infected through poor hygiene or inks contaminated with bacteria, viruses or fungi. In addition to this, the constituents of tattoo inks can trigger undesired reactions in the body, such as allergies and other complaints. The possibly carcinogenic effect of certain substances is also under discussion, and UV or laser beams can also alter the health effects of the pigments. Sunbathing as well as tattoo removal using laser technology can therefore pose a health risk.

The experts, at least, have been aware of the large number of possible health risks that tattoos can cause for quite some time now, but many questions have remained open up to now when assessing which dyes, ingredients and techniques are of particular concern from a health point of view. The reasons for the uncertainty are of a legal and scientific nature.

How are tattoo inks regulated?

The tattooing agent regulation contains a negative list of substances which may not be used, while additionally prohibiting other substances on the basis of the cosmetics regulation. The problem is that not all of the dangerous substances that can occur in tattoo inks are regulated here. Moreover, the scientific data required to make the corresponding safety assessment is often missing. A restriction proposal for tattoo inks is currently being prepared within the scope of the European regulation concerning the Registration, Evaluation, Authorisation and Restriction of chemicals (REACH, see page 30) in order to exclude substances whose hazardous properties have already been identified from use in tattoo inks.

The scientific data basis: uncertainty prevails

Certain constituents such as dyes and preservatives must be authorised in accordance with cosmetics law. According to European law, however, tattoo inks are not cosmetics as they are applied under and not on top of the top layers of skin, so that the manufacturers are not obliged to prepare toxicological safety reports for their products. Animal experiments of tattoo inks have not been permitted in Germany up to now for ethical reasons, and no epidemiological studies exist.

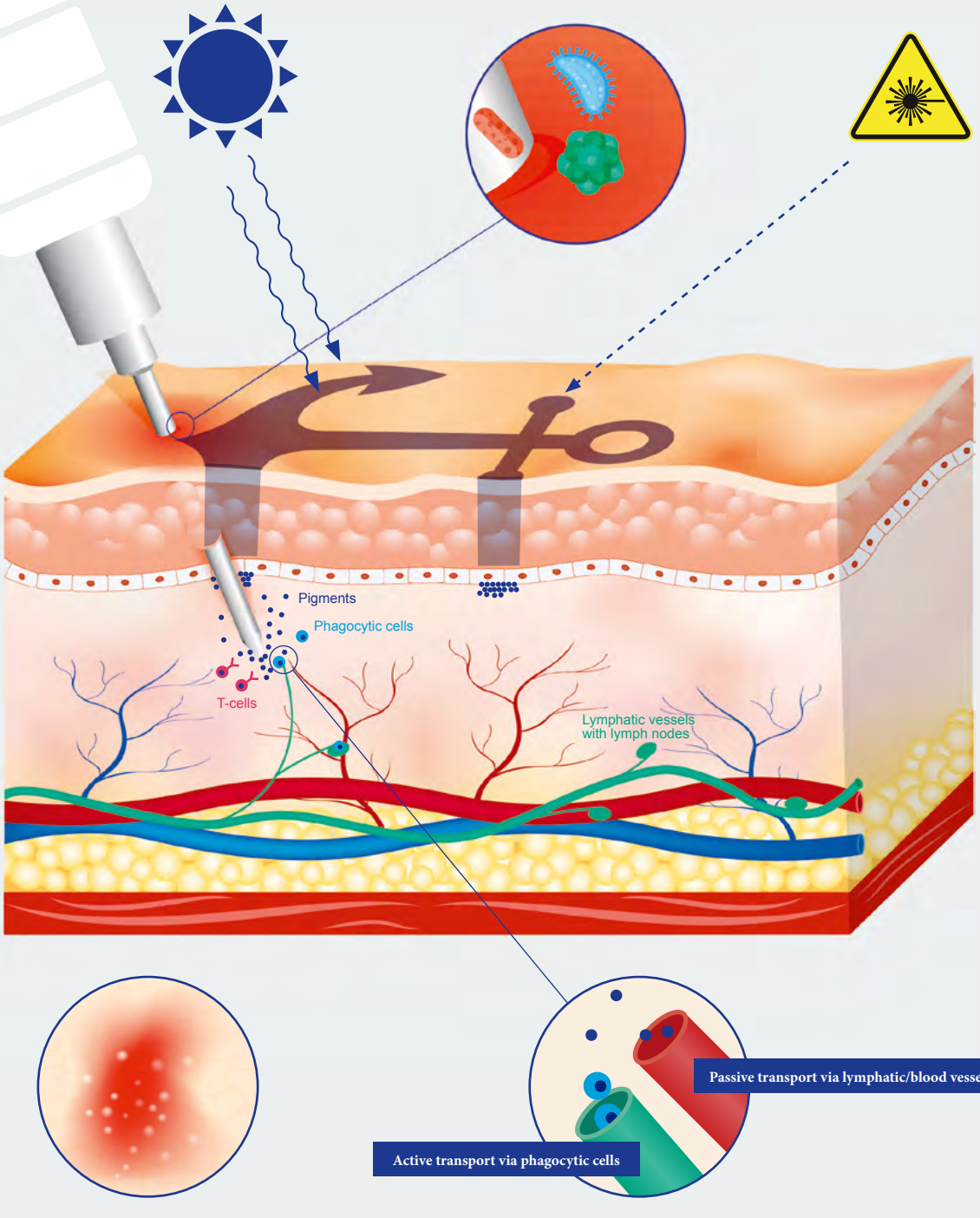
The health effects of tattoo ink ingredients such as colour pigments are being examined at the BfR.

Risks of tattooing

UV light

Infections
through bacteria, viruses and fungi

Laser irradiation
for tattoo removal. The colour pigments decompose into other, sometimes toxic, substances.



Allergies /
allergic reactions to ingredients

Spread of toxic substances in the body

Colour pigments are filled into test tubes for thermal treatment by mean of pyrolysis.

All of this has had the result that the scientific data basis for the health assessment of tattoo inks is currently insufficient.

A further challenge from a scientific point of view is above all the large number of substances used in tattoo inks. There are organic and inorganic pigments with various chemical structures, additives such as binding agents and preservatives. All of these agents can ultimately be contaminated with elements or other substances, and with each additionally used substance, it becomes more difficult from a scientific point of view to assess the health effects that the agents can bring about.

Where toxicology is concerned, the long-term effects of tattoo inks are of particular importance. "The chronic risks could only be examined by means of the corresponding animal experiments or epidemiological studies conducted on a large number of humans, but animal experiments are not permitted for tattoo inks", explains Professor Dr. Dr. Andreas Luch, head of the Chemical and Product Safety department at the BfR. "And no epidemiological studies have been conducted yet. All we have is an uncoordinated experiment which all people with tattoos conduct on themselves in principle – with open results".

Tattoo removal per laser – a health risk

Within his department, Professor Luch consolidates various research projects on the subject of tattooing. In 2013, he organised a symposium on the safety of tattoo inks together with Dr. Peter Laux, head of the Product Safety and Nanotechnology unit, after which the BfR started its first experimental examinations. Dr. Ines Schreiver, who today heads the BfR junior research group on tattoo ink research (see interview on page 12), joined the BfR back then as a doctoral candidate. In her work she wanted to identify the substances into which colour pigments decompose when they are lasered during tattoo removal. At that time, this had only been examined for a few pigments using *in vitro* methods and a red pigment on mice. "The animal's skin was tattooed, then lasered after a few weeks before being extracted and ultimately analysed – a very elaborate process", recalls Schreiver. "We were looking for a simpler method."

And they found one. The idea was based on the observation that the intense heat that develops when lasering leads to the decomposition of the pigments. To simulate this process, the pigment powder was heated to 800 degrees Celsius by means of pyrolysis. The substances produced in this way could then be separated in the usual way by means of gas chromatography and identified per mass spectrometry. A new and successful method for simulating toxicological aspects through laser radiation had been found, because pyrolysis had only been used for pigment identification up to then.

The results showed that when the 36 pigments examined are lasered, some substances that are undesired from a health point of view are produced, such as primary aromatic amines, some of which have a carcinogenic effect. In particular copper phthalocyanine, a blue, particularly lightfast and therefore very popular pigment, was the focus of the study. It decomposed through pyrolysis and after laser irradiation into hydrogen cyanide and benzene among other things – substances with a high toxic potential. Once the study had been published, there were increased reports in the media on the health risks of tattoo removal.

Where do the pigments migrate to in the body?

The next project at the BfR was dedicated to toxicokinetics. The original question was: What happens to the pigments under the skin? It was known from tests on mice that as much as 30 percent of the pigments disappear from the skin after approx. 42 days. But where are they then? The mouse study and observations in the clinic had shown that the lymph nodes of people with tattoos are often enlarged and coloured. To verify this transport scientifically and characterise the particle structure and composition, the BfR received skin and tissue samples from deceased persons with tattoos

from its forensic medicine cooperation partners in Munich for research purposes. Examination of the samples showed that a majority of the pigments accumulate in the adjacent ("regional") lymph nodes. The small, nano-size particles in particular are very mobile. In this way, the BfR analytically proved for the first time what science had long presumed: the toxic elements of tattoos do not remain locally restricted to the skin, they accumulate in the lymph nodes. This news attracted attention all over the world. The study appeared in Scientific Reports journal, a Nature Publishing Group publication, and was one of the most read contributions there in 2017. TV stations and newspapers from Europe, Latin America and the USA, including the BBC several times, reported on the research results.

Risk perception has to change

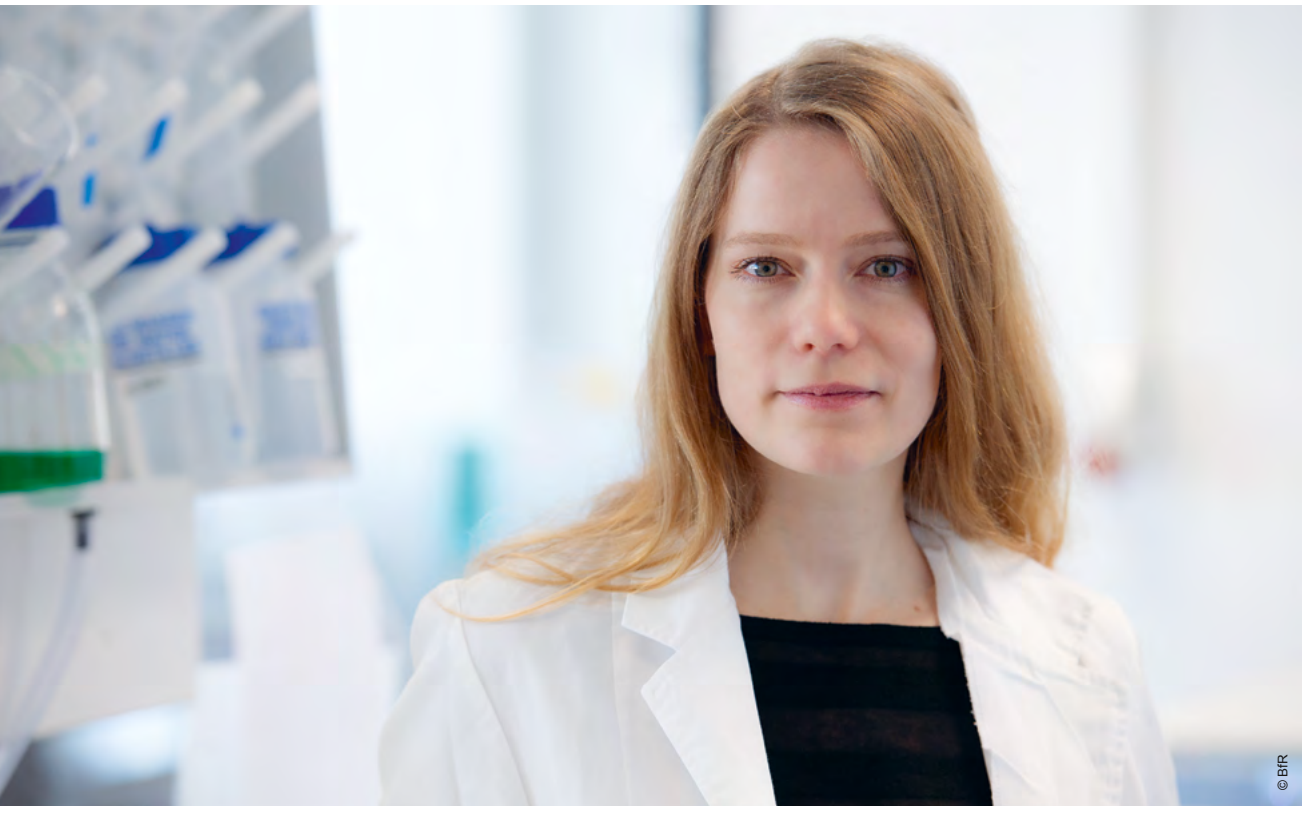
The BfR is regarded in the meantime as one of the world's most important institutions for the research of the health risks of tattoo inks. A corresponding junior research group was established at the Institute in 2017 (see page 12) to continuously advance experimental research in this field. The BfR is planning a representative population survey in 2018 dedicated solely to the risk perception of tattoo inks. It will be seen in the coming years to what extent scientific findings on tattoo inks contribute towards a change in how risks are perceived by the general public. "The decisive thing is communication. It should be clear to every consumer that it is linked with a health risk which people take voluntarily", says Professor Luch. ◘

More information
www.bfr.bund.de/en > A-Z-Index: tattoo

> **Colour pigments migrate in the body – nanoparticles in particular are very mobile**

Tattoo inks are tested for their toxic effect.

When a tattoo is applied, the natural barrier function of the skin is switched off. Hygienic work in the tattoo studio is therefore particularly important.



"We want to identify as many allergens as possible"

Dr. Ines Schreiver is head of the new BfR junior research group on tattoo ink research.

Dr. Schreiver, what is your junior research group working on?
We are currently working on two projects. The first one deals with the allergenic risk of tattoos, starting off with the observation made by dermatologists that many tattoos from the red colour spectrum cause allergies. Medical people lump together pink, purple and orange under the heading "red", even though these substances are completely different from a chemical point of view. We want to find out which pigments have a particularly allergenic effect.

Why do you believe it is the pigments and not the co-formulants of the tattoo inks that cause the allergy?
Both are possible in theory, but the co-formulants are usually water-soluble, which means that they are excreted after a few days, whereas the insoluble pigments remain in the body. So when we talk about allergies which do not occur in the first few days but only after years, this can only be triggered by the pigments or the substances into which they decompose. As the allergies often only occur after many years, we suspect that the decomposition substances are the actual cause.

What method do you use in your study?
We have received over 100 tattooed skin samples from allergy patients from our medical cooperation partners. During the analysis of these, we have identified four to six pigments which were used repeatedly and two to three of which are very dominant. We are now conducting tests to find out which substances could have been cleaved off. There are several *in vitro* tests with which this can be analysed. My long-term goal is

BfR junior research groups

Since 2017, there have been five junior research groups at the BfR which were set up to promote research in selected areas of main emphasis at the BfR, while furthering the scientific career of young scientists at the same time. The groups are dedicated mainly to research and are headed by qualified junior staff shortly after they have obtained their doctorate. They run for a period of three years with the option of extending to a total period of five years. The junior research group on the health risks of tattoo inks started up on 1 September 2017. It currently consists of one leader, two doctoral candidates and a technical assistant.

The junior research group on tattoo ink research headed by Dr. Ines Schreiver started up in 2017.

> **We want to find out which pigments have a particularly allergenic effect.**

to identify as many substances as possible which can play a role in the development of allergies.

What does your second project deal with?
The second project examines how pigments react to UV radiation. We are conducting *in vitro* tests here, too.

Does that mean that you expose a skin model cultivated in a Petri dish to UV light?
Yes, but we don't use a conventional skin model here. The challenge with this issue is that the tattooing agent is always applied beneath the epidermis, the top skin layer, into the dermis, the thicker skin layer below. This means that the cells of the epidermal cells lie between the dermal cells with the pigments and the UV radiation. It is customary, however, in *in vitro* tests without skin models, to simply place the cells next to one another. By doing so, it cannot be seen how the pigments farther down react to the radiation and how cells in their proximity behave, because the interaction between the cells works differently in space than on a flat surface. That is why we are developing a three-dimensional skin model for our study.

How are we to envisage this?
To begin with, you mix pigments and cells from the human dermis with collagen to produce a model for the tattooed dermis. Cells from the epidermis are seeded over this which can then form a horny layer. The result is that the skin model is then about 1 to 3 millimetres high and the surface area about the same as a little fingertip. Effects can then be examined on this little cylinder which can only be depicted in three dimensions.

A personal question to finish off with. Everyone in your group is aged around 30, which makes them the main target group for tattoos. Does this make itself noticed?
As far as can be seen, none of us has a tattoo at the moment but we have had some colleagues with tattoos behind their ears or on their arms. Tattoos are to be found all through society these days. That's also the reason why research in this area is as relevant as never before.

Many thanks for the discussion, Ms. Schreiver. ◘

More information:
Schreiver et al. 2017. Synchrotron-based ν-XRF mapping and μ-FTIR microscopy enable to look into the fate and effects of tattoo pigments in human skin. Sci Rep. 7: 11395, doi:10.1038/s41598-017-11721-z.

Schreiver et al. 2016. Identification and hazard prediction of tattoo pigments by means of pyrolysis-gas chromatography/mass spectrometry. Arch Toxicol. 90: 7, 1639–1650. doi: 10.1007/s00204-016-1739-2 (Open Access)