Questions and answers on the study lead of BfR investigating the distribution of tattoo ink as nano-sized particles in lymph nodes

BfR FAQ, 12 October 2017

Scientists of the German Federal Institute for Risk Assessment (BfR) proved in the course of an international research project that nanometric pigments from tattoo inks can permanently accumulate in lymph nodes. The study has been published in the Scientific Reports magazine of the Nature Publishing Group on 12 September 2017 (https://www.nature.com/articles/s41598-017-11721-z).

By using X-rays from the particle accelerator in Grenoble (European Synchrotron Radiation Facility, ESRF) during the investigations it has been found out where the pigments accumulate in the tissue. Until now, the accumulation of pigments from tattooed persons has been known by optical colouring of the lymph nodes only, as they often had been of the same colour as the tattoo as such. Both skin and lymph nodes have now been characterized in terms of their chemical composition, pigment identity and size.

In the following, the BfR compiled frequently asked questions and answers on the study.

What has been studied in the experiment on the distribution of tattoo inks in the skin and lymph nodes led by the BfR?
We investigated skin and lymph node samples of deceased donors and used X-rays from the particle accelerator in Grenoble to study the elements and their distribution in the tissues. This method allowed us to investigate particle sizes down to 50 nm resolution and provided information on the elements in each particle. By doing this, we were able to investigate the particle sizes of different pigments in exactly the same skin and lymph node tissue areas.

How were the different samples chosen that were used for the research?
The samples were chosen for their diversity in color. By this, the translocation of different kinds of pigments could be proven. The exact sample number varied for each investigation method we used (please see research paper).

How long does it take for the (nano)particles from tattoo inks to reach the lymph nodes?
From studies with other particles of similar size it is known that particles reach the lymph nodes on short term. In the tattoo research community it is currently assumed that the main transport of pigments will occur within the first days and weeks after tattooing. This is the same time period necessary for wound healing. Transport of tattoo particles can either happen passively, along the lymphatic stream, or actively after incorporation by immune cells and their subsequent migration towards lymph nodes.

Why did the BfR look for lymph nodes specifically?
It is known from observations that the lymph nodes of tattooed people are packed with tattoo pigments. The BfR now aimed at characterizing these pigments and thus provide analytical proof of the deposition of different kinds of inorganic and organic pigments in the lymph nodes. Also, the BfR and cooperation partners from the ESRF could show the permanent deposition of toxic impurities in the lymph nodes.

What did the investigation of the biomolecular protein structure in the skin show?
The BfR and ESRF cooperation partners compared dermal regions without tattoo pigments with those containing a high load of pigments in order to investigate biomolecular changes in

terms of protein folding and lipid accumulation. In the skin with a high load of pigments we found more frequently a certain protein structure that is often associated with loss of function and considered as initiator of foreign body granuloma formation. Since our donors were healthy, it will be important to investigate which other factors contribute to granuloma formation in the skin in addition to the structural changes in proteins.

Which of the pigments found in the lymph nodes are organic/inorganic? Do they have the same toxicity?
In the cooperation project inorganic iron oxide and titanium dioxide were found. All organic pigments are hydrocarbons, e.g. copper phthalocyanine and azo pigments. Each pigment has different properties in terms of toxicity. By now, there is still a lack of data to clearly state their impact on human health after tattooing.

The difference in toxicity will depend on the chemical structure and impurities of the pigments used to create a certain pigment. Earth-toned iron oxide pigments are more likely contaminated with metals like nickel and chromium which are both sensitizing as well as carcinogenic when present in certain oxidation states. These elements are often impurities of the pigments or their synthesis. The effects of the different pigments deposited in the lymph nodes are unknown so far.

Organic azo pigments may release carcinogenic or sensitizing substances after sun light or laser irradiation as shown in previous studies. Red and pink color shades are often associated with allergic reactions of tattoos. Black colors are more often associated with granuloma formation.

Did you only find nanoparticles in the lymph nodes?
Nanoparticles are smaller than 100 nanometers (nm) in diameter. As shown in our study, titanium dioxide and iron oxide pigments are bigger in size but are still transported to the lymph nodes. The copper phthalocyanine green pigment contained particles as low as 50 nm which were preferably found in the lymph nodes. From other studies it is also known that especially carbon black pigments are generally smaller than 100 nm.

As we can state from our studies, all chemical types of pigments accessible with our methods were found in skin and lymph node tissue in one or multiple samples.

Can you be sure that the nanoparticles that you found in the lymph nodes come from tattoos?
It is unlikely that other sources, e.g. sun screens, would explain the high amounts found in this investigation. Increased amounts of titanium dioxide might be only expected in lung and hilar lymph nodes when respiratory exposure has been occurred.

Is there a higher concentration of particles in the lymph nodes when the body is tattooed a lot?
The size and degree of shading of the tattoo will determine the amount of ink injected into the body. Therefore, the amount of pigments will depend on the size of the tattoos. Whether there is an exact proportional relation to the size of the tattoos and the pigment deposition in lymph nodes has not been analyzed so far.

Does the amount of pigments in the lymph nodes depend on where the tattoo is located (near the lymph nodes, for example)?
By the data obtained in our study we cannot say whether the amount of pigments and their impurities might vary depending on the distance to the draining lymph nodes.

Can the nanoparticles be eliminated from the body?
Other studies of nanoparticles suggest that they are partially eliminated from the body after intravenous injection. Since bleeding is observed especially with tattooing of big areas, a similar kind of excretion of a portion of the tattoo pigments seems plausible. However, as the tattoo is present in skin for the whole lifetime, it is more likely that most of the pigments once transported to the lymph nodes will also stay there.

What is the risk of pigments when present in form of nanoparticles in the body?
The problem that comes with small-size particles is that the smaller the particles are, the easier they may also be transported to other organs like the liver. Liver enzymes were shown by other researchers to be capable of metabolizing pigments used in tattoo inks. These metabolites again may have their own toxicity. However, currently there is only little knowledge about this special issue of metabolic conversion of tattoo pigments.

What further research is needed?
In the future, the BfR will analyze further samples of patients who suffered from adverse effects in their tattoos together with other cooperation partners. We would like to find an association between the effects observed and the chemical and structural properties of the pigments used to create these tattoos.

The BfR will also be looking at pigment and heavy metal burden of other, more distant internal organs and tissues. The biodistribution, metabolism and possible excretion of substances are key points necessary to assess the toxicity of compounds. Especially when organic pigments will be transported to the liver, a higher metabolic turnover can be expected. The metabolites again have unknown toxicological properties.

Do the findings suggest that these may be linked to the development of cancer?
Some of the elements we found are categorized as carcinogenic and sensitizing substances by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (e.g. nickel and chromium if present as certain compounds). The question that can be raised is, to what extend these elements will increase the risk of developing cancer during lifetime. To quantify the risk, the average amount of these substances and likely an extrapolation of the risk by the route of exposure would be needed, amongst others. To this end, no full risk assessment of these compounds in terms of their application in tattoo inks has been carried out. Thus, the question to what extend the found elements may harm the health of tattooed individuals cannot be answered.

Do the findings suggest that getting tattoos could have long-term health risks that were previously unknown?
The deposition of elements in lymph nodes has never been investigated before. However, data on the exposure against toxic elements are necessary for risk assessment in terms of chronic diseases. Since we do not have such data yet, people should be aware of the unknown risks that might come along with tattooing rather than presuming that the colors are safe.

For general information about tattooing see the BfR-FAQ on tattoo inks
https://www.bfr.bund.de/en/faqs_on_tattoo_inks-201880.html

About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. It advises the Federal Government and Federal States (“Länder”) on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.