Bundesinstitut für Risikobewertung

Toxicological testing requirements to be developed: 3rd Expert discussion at the BfR on tattoo inks

Communication No 031/2022 of the BfR from 9 November 2022

On 29 June 2022, the 3rd international expert meeting took place at the German Federal Institute for Risk Assessment (BfR) on the prerequisites for safe tattoo inks. The meeting discussed the toxicological testing requirements.

The expert panels on tattoo inks have been held at regular intervals since June 2022. Experts from the field of analytics, state surveillance agencies, governmental organizations, tattoo ink manufacturers, and tattooists participated in the meetings.

1 Introduction of the BfR minimum requirements, test methods and a summary of the first two expert panels

The concept of reduction of risks and its implementation with the aid of the minimum requirements was presented. The goal is to achieve a constant chemical purity along with the identification of pigments suitable for tattooing based on selected toxicological testing. The legal status of the minimum test requirements was elucidated as being a non-binding guideline document, but rather having a recommending character to allow ink producers test their products for safety. This sets a framework for assessing tattoo ink ingredients having no harmonized classification.

Protocols of the first expert discussion on “Necessary specifications of tattoo ink ingredients” and the second expert discussion on “Operable minimum toxicological requirements” are available on the BfR website.[1]

Further, a study report on the applicability of the key event-based Organisation for Economic Co-operation and Development (OECD)Test Guideline (TG) 442D for *in vitro* skin sensitisation testing of nanomaterials was discussed. In the frame of this study, among many nanomaterials, tattoo inks were also tested. These included Carbon Black and Pigment Red 170. The study concluded that OECD TG442D is applicable for the testing of nanomaterials, although the limited *in vivo* data do not allow correlating the test results. Moreover, it was indecisive whether the test results are directly attributed to the effect of nanomaterials or the sensitizing potential of leachables. Hence, the dispersion protocol plays a critical role and requires the corresponding adaptation of the TG.

2 Feedback from participants on the minimum requirements

Participants raised concerns regarding the harmonized classification and the required expenses for identification of chemical groups, such as aldehydes, 1º amines, nitrosamines, and for quantification of different impurities for which concentration limits are set according to the REACH restriction.

It was mentioned that the BfR statement “Tattoo inks: minimum requirements and test methods” is quite comprehensive, however, its implementation is seen in a long-term framework.

[1] https://www.bfr.bund.de/cm/349/necessary-specifications-of-tattoo-ink-ingredients-expert-discussion-at-the-bfr.pdf
https://www.bfr.bund.de/cm/349/operable-minimum-toxicological-requirements-2nd-expert-discussion-at-bfr-on-tattoo-inks.pdf

More epidemiological data are considered necessary. Participants thanked BfR for the consideration of substances which are not classified according to REACH. Ink manufacturers expressed their interest in a long-term screening project. They recognized that toxicology of tattoo inks is critical. Manufacturers stated, that a transparent way to cooperate with regard to the identification of impurities is welcome. At the moment, working out an analytical strategy for identification of relevant concentrations of toxic moieties and elimination of these is considered necessary.

Tattoo ink manufacturers have the impression that the quality of the pigments is still not sufficient, however it might be improved by the use of pharmaceutical quality grade chemicals. Furthermore, the tattoo ink manufacturers expressed their dissatisfaction with the current REACH approach. Toxicological expertise is still lacking, however, tattoo ink manufacturers agreed that they would support research activities to improve the tattoo ink pigments. It was discussed, whether any activity is planned from manufacturer's associations to address the pending questions related to tattoo inks. So far, information is shared between the tattoo ink manufacturers. However, the focus is still on pigments with pharmaceutical quality available on the market. Additionally, costs for risk analysis pose a major challenge for manufacturers.

Tattoo ink manufacturers from the USA voiced interest in creating world-class products and cooperation with regulation. It is considered difficult to fulfil the REACH requirements, although U.S. tattoo ink manufacturers are to further improve in this direction. Furthermore, internal dose and systemic exposure which are linked to biokinetic studies need to be taken into account, including other approaches such as computational toxicology. The applicability and possible implementation of *in silico* predictions needs to be confirmed according to the participants. It was agreed that standards for *in vitro* tests are missing. Questions regarding the selection of appropriate parameters as well as regarding the implementation of lifelong exposure scenarios were considered urgent. Participants added the need for including systemic exposure scenarios in biokinetic modelling and establishing non-animal methods for tattoo ink pigments to predict internal exposure levels. ISO standards could help to guide the research community in this direction. The main goal is to understand the biokinetics of individual tattoo ink ingredients (e.g. preservatives, pigments, stabilizers, impurities etc.) and to precisely decipher which components causes health effects.

3 Identification of leachable components from pigments and application of a threshold of toxicological concern (TTC) concept for soluble substances

Experience from nanomaterial research regarding dissolution/leaching shall be considered for studies on tattoo ink pigments. Methods validated for testing the dissolution of nanomaterials in the frame of the Gov4Nano, GRACIOUS or PATROL projects were presented. The dissolution can be studied in different experimental setups such as a controlled reactor, a continuous flow system or a sequential dissolution system. The initial and long-term solubility for example, can be addressed by sampling from a reservoir of controlled temperature, pH and salinity. Pulmonary uptake may be simulated via a continuous flow setup, where the test substance is placed in a chamber and the dissolved constituents diffuse into the flow cell via a membrane of defined porosity. Gastro intestinal dissolution is best simulated in a sequential setup, where the decisive parameter is the acidity of the different compartments representing the mouth, stomach and the small intestine.

The kinetics of leachable components from tattoo pigments is critical for the assessment of relevant exposure. The simulation of the long-term residence of pigments in the dermis and their continuous dissolution may be investigated in a dynamic dissolution setup. Here, the pigments are placed in a silica-based matrix and treated with a continuous flow of a mobile

phase of choice. External factors such as temperature or UV radiation may be applied. Fractionation and the analysis of the samples follows.

As impurities may be seen as integral constituents of the pigments, it was pointed out that all toxicological assays should address the leachable components, even if not all of them can be identified. It is analytically very challenging to identify all leachables and degradation products. Different methods are required for different substance groups. The challenge further arises from the sensitivity requirements: Based on the expected very slow release kinetics, low concentrations of leachables are expected. Decomposition products under conditions similar to the skin are complex to investigate, as they are largely governed by external factors such as UV radiation but also by the mixture of pigments placed in the dermis. For instance, it was shown that TiO_2 present in tattoo mixtures could accelerate degradation of other pigments. The amount and spectrum of degradation products depends also largely on the kind of incoming radiation, namely UV-A or UV-B. The penetration depth and interaction with pigments should be taken into account. For instance, more aromatic amines are found after irradiation with UV-B alone. Moreover, it was pointed out that pigments of the same CI number but different sources may have different coatings and finishes, which may be crucial in terms of their stability. Therefore, participants called for the establishment of a protocol for irradiation studies taking into account the above-mentioned issues. This protocol shall simulate the situation in the skin. A solar simulator should be considered for covering the spectrum of UV-A, UV-B, and UV-Vis. Reference is given to Chapter 2.1.6 of the BfR minimum requirements, which provides guidance for stability and lightfastness testing of pigments. However, test methods are not yet confirmed experimentally. In terms of laser application, nano-, pico- and femto-second lasers are used in practice. Major non-linear effects were observed, attributed also to the used pulsation. Decomposition and re-aggregation of nanoparticles occurs during laser treatment based on the applied energy. The wavelength of the laser plays a critical role. The capability of molecules to absorb the energy depends also on the polarity of the surrounding environment.

Compliance with the Medical Devices Directive was discussed. The battery of tests described by the directive is suitable for tattoo inks as implemented in several countries as for example in Brazil. However, very high costs will result for the compliance. The testing of biocompatibility is very cost intensive. Hence, only selected requirements and tests according to the respective norm, DIN EN ISO 10993, standards for evaluating the biocompatibility of medical devices, should be considered.

The Threshold of Toxicological Concern (TTC) concept was presented as a tool for risk assessment of leachables having limited toxicological data, while considering its applicability domain. It can be assumed that the wound healing process, which takes about 14 days, can be distinguished from the final “steady state” by the kinetic parameters. In the absence of toxicokinetic data, which would elucidate the exposure parameters and the biokinetics of the pigments, two scenarios have to be considered: a) the acute phase - during tattooing and wound healing. Here the endpoints of skin irritation / corrosion, eye irritation / damage and skin sensitisation are the main focus. Acute, substance-specific toxicity is not expected from hardly soluble and inert pigment particles. This may be, however, relevant, if a substantial amount of hazardous impurities is present, leading to complications as e.g. an anaphylactic shock in this acute phase. b) The chronic phase - after the tattooed skin has healed (only low systemic release). Here, the endpoints sensitisation, genotoxicity and carcinogenicity are of major concern. After healing, only a very low systemic availability can be assumed, which may be below the TTC for non-genotoxic substances. The TTC concept is a pragmatic methodology for assessing the safety of substances with unknown toxicity. This, however, has to be verified for leachables, while considering a lifelong exposure.

4. Toxicokinetics of tattoo ink pigments

4.1 *In silico* test strategy for tattoo ink pigments

The applicability of *in silico* methods, e.g. quantitative structure-activity relationship (QSAR), analogy/grouping approaches, read-across, was presented. All available data based upon structure similarities of similar pigments should be considered. In this context, the advancement and progress made in the last decade regarding QSAR models in computational toxicology was briefly presented. Many commercial and publicly available *in silico* QSAR models already exist: e.g. DEREK/SARAH, OECD QSAR toolbox, VEGA, COSMOS (for cosmetics products), or EPI (Estimation Programs Interface) Suite™ from the US Environmental Protection Agency (EPA). Particularly, EPISuite™ and the OECD QSAR tool box are open access models suitable to predict tattoo pigment-related health hazards, e.g., skin sensitisation, eye/skin irritation, and genotoxicity. For automated read-across and grouping approaches of tattoo ink pigments, the EPA-sponsored Toxcast 21 database is one of the very advanced resources involving more than 10,000 chemicals with more than 300 biological pathways analyzed. These *in silico* models may be used for the risk assessment and toxicokinetic (TK) analysis of tattoo ink pigments, based on the structural information.

4.2 How can toxikokinetic data contribute to the risk assessment of tattoo inks?

To understand the biodistribution of tattoo ink pigments in the body, the processes of absorption, distribution, metabolism, excretion and toxicity (ADMET) need to be understood. Toxicokinetic-related ADMET profiling may contribute to the risk assessment of tattoo ink pigments. Particularly, ADMET profiling is useful for physiologically-based toxicokinetics (PBTK) modeling to establish quantitative descriptions of absorption, distribution, metabolism, and excretion of pigments. In addition, *in vitro* results in relation to human biokinetics can be applied for a quantitative *in vitro* to *in vivo* extrapolation (IVIVE) by *in silico* methods. This can be useful for adopting the operable minimum toxicological requirements. ADMET descriptors can be further useful in determining the threshold value for PBPK models, predicting the fraction absorbed or bound to plasma proteins while in systemic circulation, predicting the concentration maximum or the maximum concentration time point of a specific chemical in the blood plasma.

Transparency and data curation issues of existing QSAR models for tattoo ink pigment biokinetics prediction was further discussed. A huge amount of chemical and biological data that is available online in OECD QSAR databases can be easily retrieved to extract information related to tattoo pigments. Participants were asked for their preferences and feedback to select a specific QSAR toolbox, either commercial or open access, however, no consensus could be made.

In addition to widely known solute transporters, such as P-glycoprotein, which is involved in the elimination and disposition of chemicals and pharmaceuticals, participants emphasized the need to identify the dermal family of efflux transporters for injected tattoo pigments. Pigment particles, which can aggregate to larger particles inside the skin, undergo different transportation mechanisms, e.g. by dendritic cells, which may transport the particles to the lymph nodes. Immune cells and fibroblasts could be another versatile group of cells that is part of such kind of transportation. However, only few of these transportation mechanisms

are already considered in the databases. Thus, research regarding the development of transporter models is needed. Degradation of injected tattoo ink pigments is a lifelong process; therefore, research is needed to identify the main involved mechanisms.

Furthermore, during sunlight exposure of tattoos the UV radiation will lead to partial pigment degradation, resulting in transport to the lymph nodes. Details regarding that transport mechanism are still unclear though. It was also discussed whether the UV radiation additionally triggers the immune system and thereby affects the transportation or if it is a long-term effect.

4.2.1 NANO-QSAR for tattoo ink pigments

Considering the fact that tattoo ink suspensions contain pigments composed of nanoparticles, i.e., particles of sub-100 nm dimensions, NANO-QSAR likely is a more appropriate model for investigating the biokinetics of tattoo ink pigments in the skin compared to common QSAR models designed for soluble substances. Traditional QSAR is trained on soluble substances, not on nanoparticulates. For biokinetic-related analysis of tattoo ink pigments, nano-QSAR and subsequent modelling must in addition consider leachable components with extra compartments / fitting parameters in nano-physiologically based pharmacokinetic (PBPK) modelling.

Participants pointed out that for toxicokinetic modeling careful consideration has to be used in regard to the data required to develop and test a model for tattoo ink pigments. Especially, considering that such models shall be accepted by the authorities in the future.

It was proposed to implement the toxicity index for specific tattoo ink pigments based on the main physicochemical properties that contribute to the component's toxicity, e.g., percentage of hydrocarbons and hydrophilic/hydrophobic properties. Nonetheless, QSAR models need to be determined for designing such a toxicity index because of the multiparametric nature of pigments and the diversity of the tattooed population.

4.2.2 Reliability of *in silico* methods for assessment of tattoo ink pigment biokinetics

Few QSAR models, such as the OECD QSAR Toolbox, are specifically designed to help the user to fill data gaps. Chemical categories are built according to the OECD guidance on the grouping of chemicals. However, the transparency of the models varies.

Furthermore, regarding the reliability of *in silico* tools, the applicability domain of the respective *in silico* methods has to be considered. These tools are generally developed on a broad variety of chemicals, it has to be evaluated and validated how well they can be applied to tattoo ink pigments. Ultimately, the model is only as good as the underlying experimental data. Participants further suggested to develop new models for tattoo pigments based on structural similarities. The US EPA developed a tool called GENERA, which is a generalized read-across application embedded within the Toxcast dashboard. Participants also emphasized to develop new models based on the available data. So, the most important task is data collection. The second step would be to verify the quality of the data upon applying a variability analysis to generate a clean data set for tattoo ink pigments. Once a benchmark is established, it could be further used for regular assessments and prioritization of chemicals for further testing. The best would be to evaluate all available tools and develop a consensus model which again will require validation using experimental data.

4.2.3 Discussion regarding experimental data from human origin for use in grouping & read-across approaches

It was further discussed if human data should be prioritized for *in silico* approaches or if all available data (incl. non-human) should be used. Participants expressed that it will be difficult to find *in silico* methods relying on human experimental data alone. Therefore, current *in silico* practices in QSAR should consider all available data from rodents, mammals, humans, etc. Further differences between genders shall be considered for *in silico* assessments, but participants agreed that a gender-biased approach is not feasible. A gender-biased approach might be relevant for the epidemiological studies but seems not fit for the biokinetics. Participants further added that every prediction method has uncertainties which have to be considered in all data and that there is no single prediction model which can address all queries.

Ultimately, sufficient information within the chemical data space is needed to undertake a safety assessment considering QSAR and read-across approaches. Currently, a lot of data is already at hand from REACH regarding chemicals, pesticides or food ingredients. These may be used to improve the reliability of the *in silico* models in general but also for tattoo ink ingredients. A case study would help to evaluate the applicability and usefulness of *in silico* modeling.

4.2.4 Use of structural similarity as a parameter for QSAR predictions

Participants further discussed if the chemical structure of tattoo ink pigments should be used as sole basis for toxicity predictions. Furthermore, it was questioned, if grouping of tattoo ink pigments is a feasible way to reduce the number of experimental studies and to avoid repetitions. Moreover, pigment surface characteristics, and their influence on toxicity were a point of discussion. Computational experts pointed out that the principal of read-across or QSAR models is a structure-activity relationship. Therefore, the question arises how the structural similarities shall be selected and rated as currently several different approaches are used.

4.2.5 Modeling the cellular transport of particles

Regarding the question of tattoo pigment transport modelling, participants expressed concern. Pigments are particles of low solubility and are engulfed by immune cells, and transported to the lymph nodes where they may remain indefinitely. Furthermore, there are reports of excretion via urine, meaning some tattoo pigments reach and pass the kidney. Including such transport phenomena in *in silico* modelling based on structure-activity relationships could have significant impact on understanding the fate of intradermally injected tattoo inks.

Participants are in favor of not following only one approach. Mathematically modeling should be accomplished by *in vivo* data. Thus, practical studies and QSAR can complement each other.

4.2.6 Preferred animal models for the biokinetic method development

For tattooing, rodent models like mouse or rat are unfit due to fundamental differences in skin anatomy and physiology. For example, mouse skin differs from human skin in the lymphatic

system and particle biokinetics. Therefore, participants voiced the need to select the most suitable animal model which should have a similar skin architecture and physiology as humans. European participants highlighted the challenges regarding animal testing and shared their past experiences. Current databases in most QSAR models utilize mostly *in vivo* data from mouse or rat studies, thus, a shift to the use of human or more suited mammalian data is needed.

5 Concluding remarks and next steps

BfR thanked all participants for the fruitful discussion. BfR further highlighted the future roadmap and pointed out that the development of standard extraction methods as well as the characterization of leachable components and of pigment composition are technically very demanding but essential requirements. Regarding integrating laser treatment to *in vitro* experiments, protocols need to be developed. Some applicants stressed to use a solar simulator, nonetheless, participants did not agree on how to perform a standardized testing of UV and laser light application. The applicability of the medical device directive has also been discussed in reference to biocompatibility testing, however, it is seen as a financial burden by the manufacturers.

In reference to toxicokinetics of tattoo inks, a lifetime scenario and the (nano)particulate nature of tattoo pigments must be considered for the QSAR modelling. New QSAR models considering the nanoforms of tattoo ink pigments are needed and should not be based on the models applied for soluble substances.

Participants were invited to apply for the International BfR Committee for Tattoo Inks which is to be established.